بسم الله الرحمن الرحيم

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مركز أبحاث للجينات والتقنية البيولوجية
http://aecenar.com/institutes/megbi

MEGBI-APP (Antibiotics Production Pilot Plant) Final Report (Period 2016 - 2020)

Introduction to Biotechnological upstream and downstream processing

Basics for Penicillin and Ampicillin Production

Feasibility Study and Business Plan

Manufacturing Manual

- Bioreactor (Design & Manufactoring)
- Plant System Design
- Bioreactor Automation
- Plant Automation, Plant System Testing

Operations Manual

- Materials & Consumables
    - Lab scale penicillin production
    - large scale penicilllin production
- Experimental Laboratory scale production of penicillin
- Plant Automation

Quality Assurance

- Determination of penicillin (quantitive diagnostic)

Last Update: 28.07.2020 03:00

Based on the following research reports:

[MEGBI-VPP 2012] Samir Mourad, Rihab El Merheb, Layal Chbib, *" MEGBI Vaccine Pilot Plant – 1st Project Report (Feb 2012 – Jan 2013)", مدخل تطبيقي الى البيوتكنولوجيا (Introduction to Biotechnological upstream and downstream processing)*

[MEGBI-APP 2016] Samir Mourad, Mariam Mourad, Maryam El Khodor, Bilal Mourad, *"MEGBI-APP, 4th Project Report (2016)"*

[MEGBI-APP 2017] Samir Mourad, Rami Nassouh, Fatima Antar, Razan Kalaoun, Abdurrahman Mourad, Asia Mourad *"MEGBI-APP, 5th Project Report (Jan 2017 - Mar 2018)"*

[MEGBI-APP 2018] Fatima Antar, Mariam Mourad, Asia Mourad, Samer Youssef, Samar Youssef, Samir Mourad *"MEGBI-APP, 6th Project Report (Apr 2018 - Feb 2019)"*

[MEGBI-APP 2019] Fatima Antar, Mariam Ied, Abdullah Mourad, *MEGBI-APP 7th report (Mar 2019 - Dec 2019)*

[MEGBI-APP PCS 2020] AQ, *Control System of Antibiotics Production Pilot Plant, Version 2020, Developers & Operation Manual*

Content in short

Content

مدخل تطبيقي الى البيوتكنولوجيا (Introduction to Biotechnological upstream and downstream processing)

From: **MEGBI Vaccine Pilot Plant – 1st Project Report (Feb 2012 – Jan 2013)**

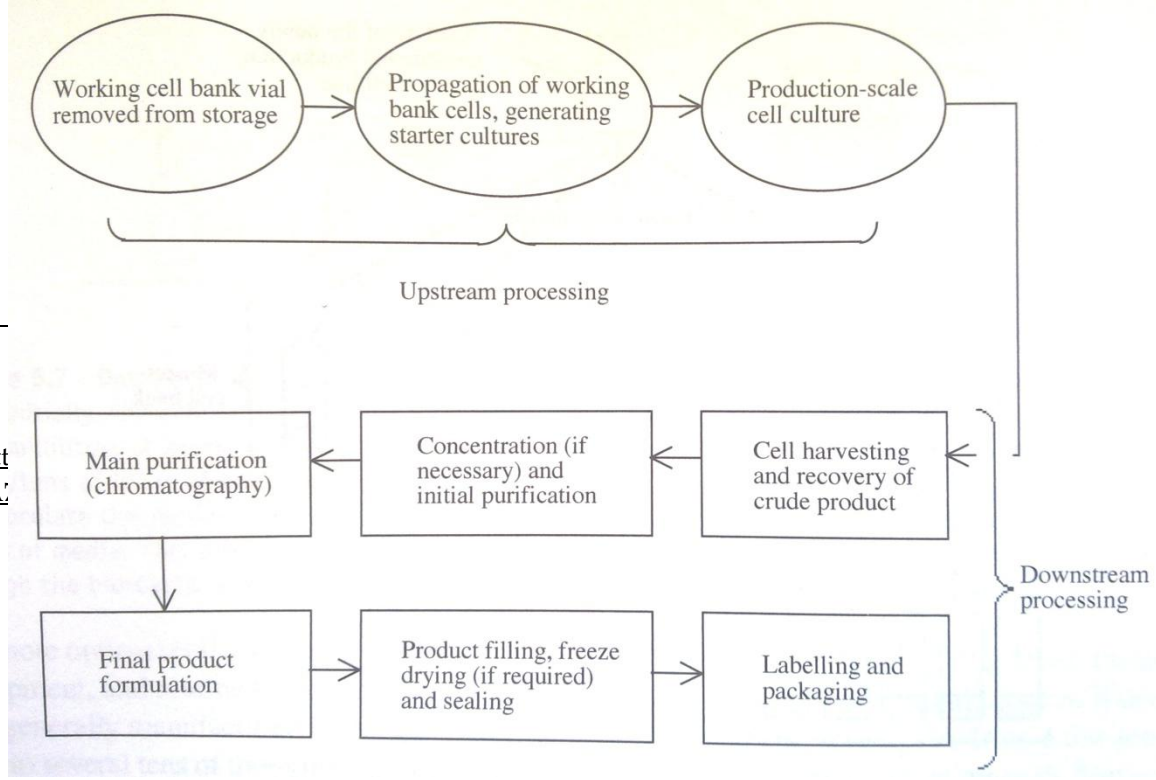
1 نظرة عامة في عملية انتاج بروتينات علاجية في البيوتكنولوجيا

ان انتاج الجزيئات الحيوية ينقسم الى معالجة المنبع (upstream) و المصب (downstream).

المنبع عبارة عن جزء من العملية الحيوية (upstream bioprocess)وهو أول خطوة لنمو الجزيئات الحيوية عن طريق المفاعلات الحيوية التي تحدث بواسطة خطوط الخلايا البكتيرية أو الحيوانية. وعندما تصل إلى الكثافة المطلوبة يتم حصدها ثم نقلها إلى المصب (downstream) وهي قسم آخر من العملية الحيوية.

المعالجة المتعاقبة للنواتج النهائية لعملية التخمر (downstream processing) : هي تنقية المواد الكيميائية، والمستحضرات الصيدلانية والمكونات الغذائية الناتجة عن التخميرأو الاصطناع في الأنسجة النباتية والحيوانية، مثل المضادات الحيوية، وحمض الليمون، وفيتامين(E) ، والأنسولين.[1]

الصورة مأخوذة في الاسفل مأخوذة من [Walsh 2007].



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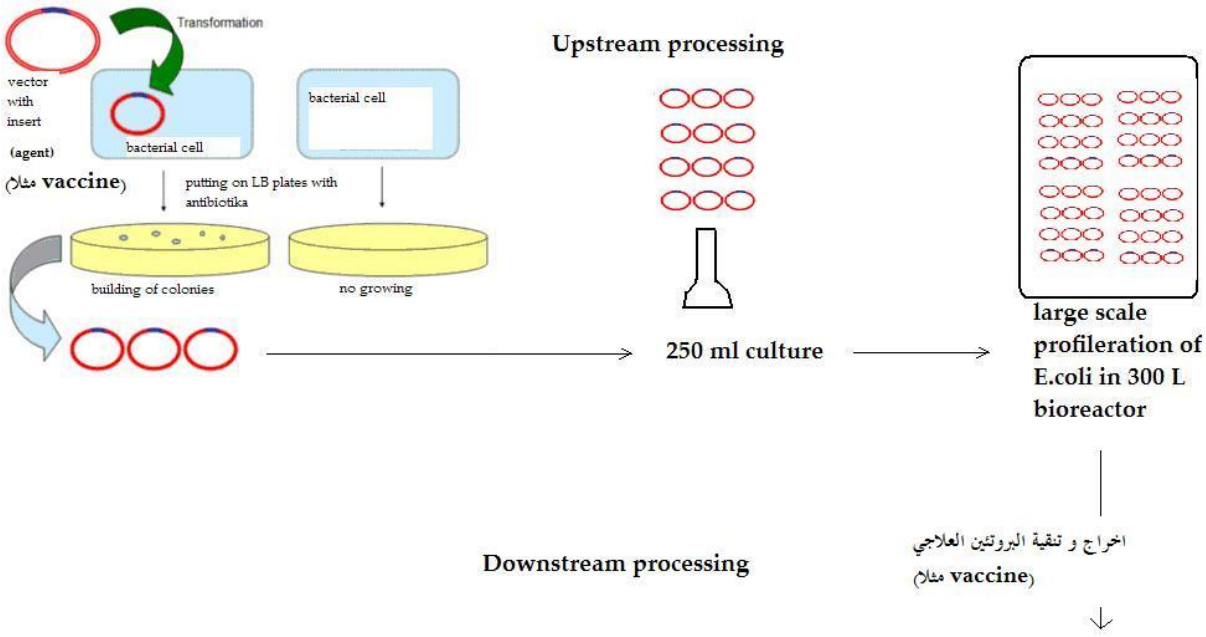


الصورة فوق: مثال

1.1 المنبع (upstream processing)

From [Walsh 2007], Ch 5

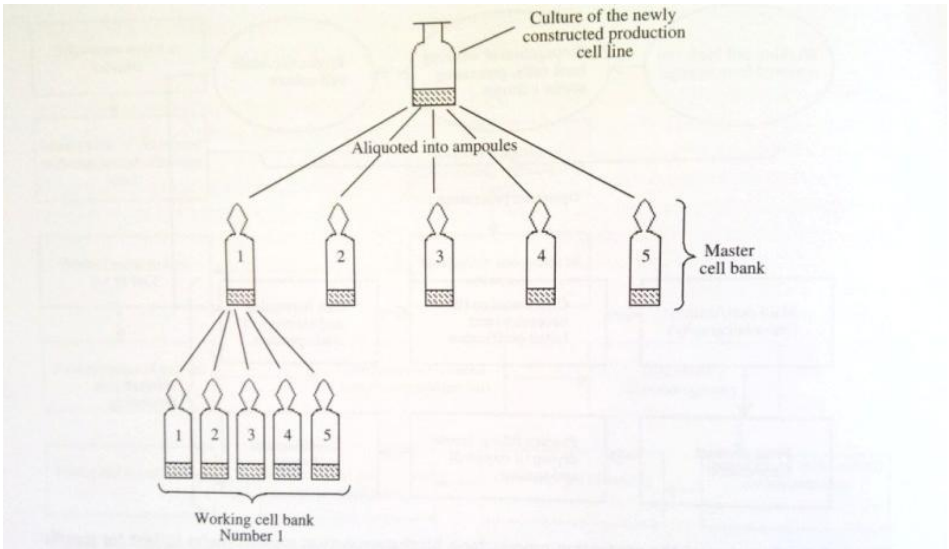


Figure 5.6 The master cell bank/working cell bank system. For simplicity, each bank shown above contains only five ampoules. In reality, each bank would likely consist of several hundred ampoules. Working cell bank number 2 will be generated from master cell bank vial number 2 only when working cell bank number 1 is exhausted and so on

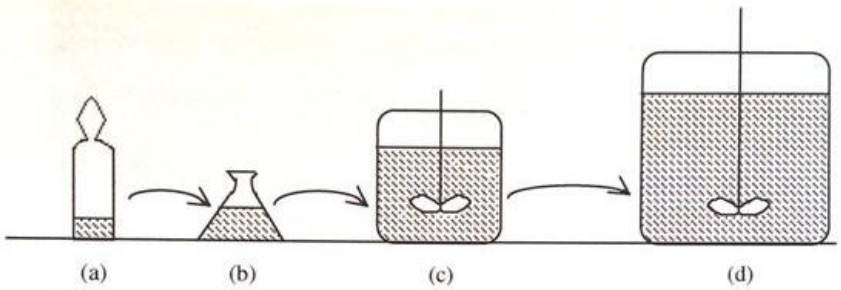
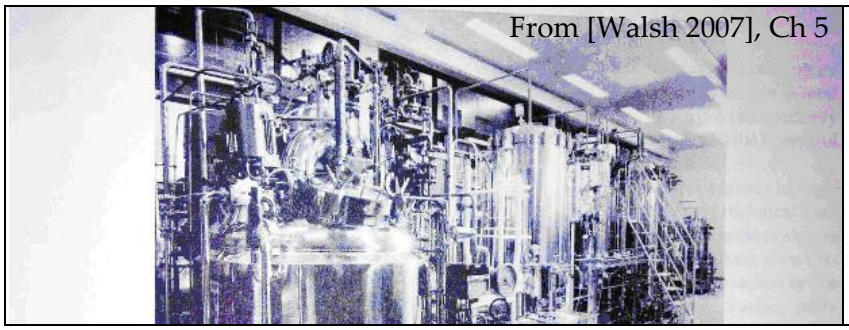
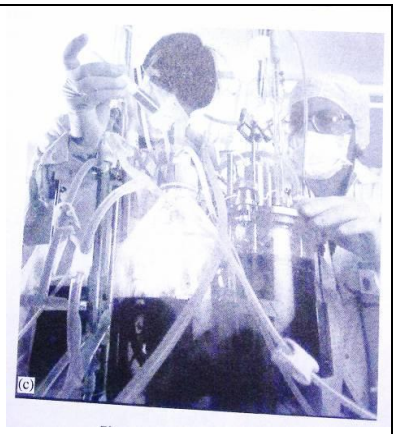


Figure 5.7 Outline of the upstream processing stages involved in the production of a single batch of product. Initially, the contents of a single ampoule of the working cell bank (a) are used to inoculate a few hundred millilitres of media (b). After growth, this laboratory-scale starter culture is used to inoculate several litres/tens of litres of media present in a small bioreactor (c). This production-scale starter culture is used to inoculate the production-scale bioreactor (d), which often contains several thousands/tens of thousands litres of media. This process is equally applicable to prokaryotic or eukaryotic-based producer cell lines, although the bioreactor design, conditions of growth, etc., will differ in these two instances

From [Walsh 2007], Ch 5





1.2 مدخل الى تنقية البروتيئينات (protein purification) المنتوجة داخل خلايا (downstream processing)

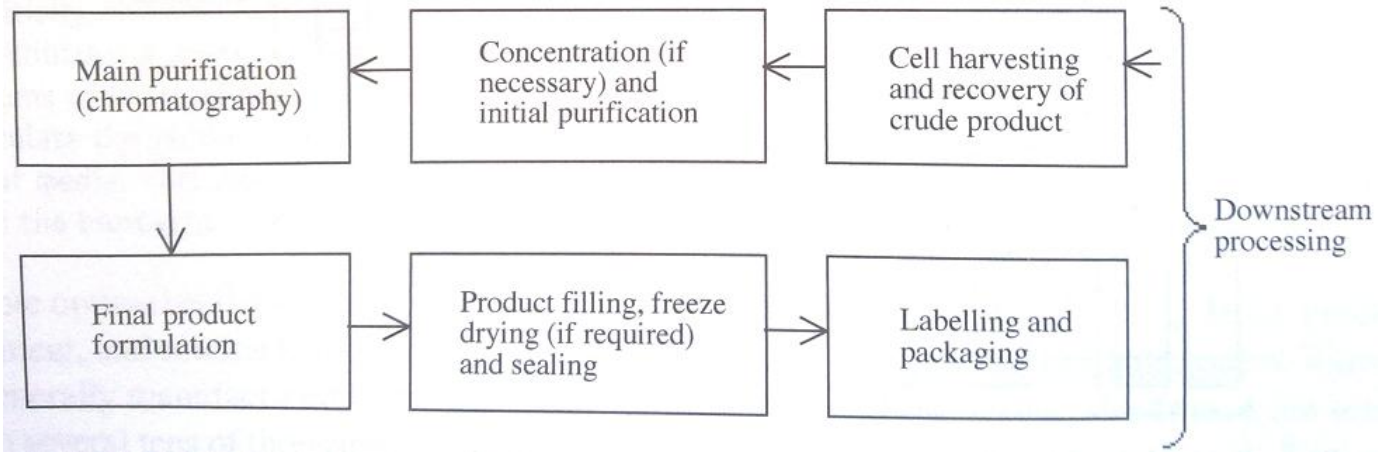


Fig.: From [Walsh 2007]

تنقية البروتين هو عبارة عن سلسلة من العمليات تهدف إلى عزل نوع واحد من البروتين من خليط معقد.

تنقية البروتين هي عملية مؤلفة من عدة مراحل : اخراج أولي , ترقيد بالاختلاف , غسيل ,كروماتوغرافي (chromatography) للأنواع المختلفة ,وغيرها

1.3 المبادئ الأساسية للتنقية

احد الطرق لتدمير الغلاف الخارجي للباكتيريا هو من خلال الصدمة الحرارية وذلك بوضعه في الثلاجة (-20°C) ومن ثم في درجة حرارة الغرفة (25°C) حتى يذوب السائل وبعدها مباشرة في الثلج مرة اخرى و تكرر الخطوات ثلاث مرات.

ومن بعدها نقوم بالتنبيذ (centrifugation) على درجة 3000 دورة في الدقيقة (rpm) فنحصل على البروتين المطلوب ولكنه موجود مختلط مع بقايا الخلية المكسورة وغيرها من مكونات البكتيريا

وهنا تبدأ التنقية الفعلية للبروتين من خلال استعمال الكبريتات الأمونيوم (ammonium sulfate) $(NH_4)_2SO_4$ كمحفز لترقيد (precipitation) البروتين وذلك من خلال جذبه للبروتين أكثر من المياه وباختلاف تركيز (concentration) الكبريتات الأمونيوم (ammonium sulfate) سيختلف البروتين المرقد وذلك بسبب جذبه المختلف وبذلك نفصل البروتين عن الأشياء الكبيرة الموجودة

ومن المفضل استعمال مرحلة تليها وهي الاستشراب (chromatography).

أحيانا بين هذه المراحل نستعمل الdialysis لنتخلص من الكبريتات الأمونيوم (ammonium sulfate) $(NH_4)_2SO_4$

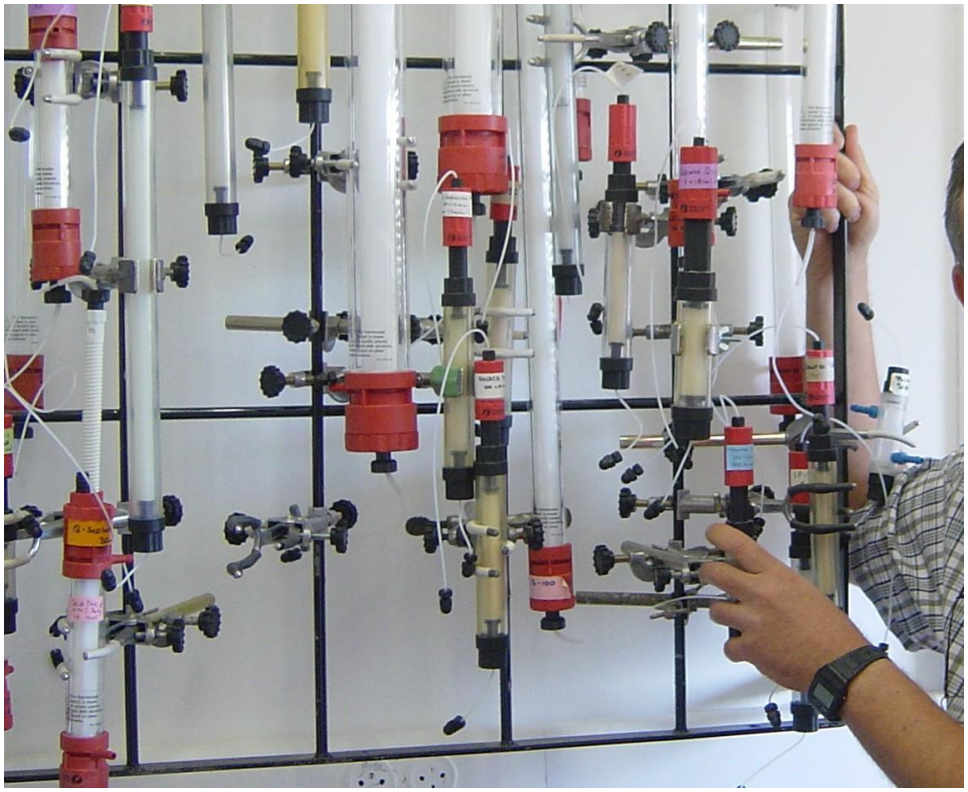
1.4 بعض الاشياء الذي يجب الانتباه اليها

الأهم في كل هذه المراحل هو الحفاظ على بنية البروتين وذلك لا يتحقق الا من خلال أن نخلق له محيط مشابه للمحيط البيولوجي الذي كان يتواجد فيه كالرقم الهيدروجيني (pH value).

ولهذا السبب سنضيف اليه NaCl 0.85 %. يعطي جو مائي قريب لذلك الذي كان متواجدا داخل الخلية. كما يجب المحافظة على الرقم الهيدروجيني pH 7.4 تقريبا وذلك من خلال اضافة PBS ("phosphate buffered saline"). ومن المهم جدا أن لا نعرض البروتين للهواء لأنه يتسبب بالأكسدة للبروتين .

ال sepharose هو مادة جاذبة توضع على chromatography column.

مثال لِ chromatography columns في نطاق مختبري (laboratory scale).



من: http://wolfson.huji.ac.il/purification/index.html

مثال لِ chromatography columns في نطاق انتاجي (production scale).

من:

GE Healthcare

Bedienungsanleitung BioProcess LPLC- und MPLC-Säulen

GE Healthcare

Data file 18-1115-23 AD BioProcess Column

BPG Columns 100, 140, 200, 300 and 450 series

BPG™ columns are glass chromatography columns designed for industrial applications which demand high standards of hygiene. The columns are constructed from component materials of the highest quality and withstand the harsh conditions used for cleaning in place of packed separation media. An overview of column characteristics is shown in Table 1. The columns are characterized by:

- Hygienic design and operation. Microbial attachment and growth is hindered through the use of calibrated precision glass, high grade electropolished stainless steel and an absence of dead pockets.
- Easy, efficient packing and running with the singlescrew adapter.
- Operating pressures matching most BioProcess™ Media.
- All polymeric materials meet the requirements for USP class VI, described in USP <88> Biological Reactivity Tests, *In Vivo*.

Fig 1. BPG column family.

1.4.1 تحكم للتنقية عن طريق الكومبيوتر

data file
BioProcess systems

Now part of GE Healthcare

BioProcess MPLC/HPLC Systems

BioProcess™ MPLC/HPLC systems comprise a family of stainless steel, liquid chromatography systems for use in process-scale applications where high pressures (20–80 bar) are required. Reliable 24 hour-a-day unattended operation contributes to cost-effective processing, all the way from feed introduction to final fractionation. BioProcess MPLC/HPLC systems simplify chromatographic procedures and offer:

- UNICORN™ software that meets GMP requirements, including electronic signatures and records
- Precise control of gradient with feedback (Type II system only)
- Compact, modular and sanitary design
- Multi-product processing, prepared for automated CIP
- Compatible MPLC and HPLC columns

Fig 1. BioProcess MPLC and HPLC systems allow cost-saving, unattended operation in biopharmaceutical processing.

General system description

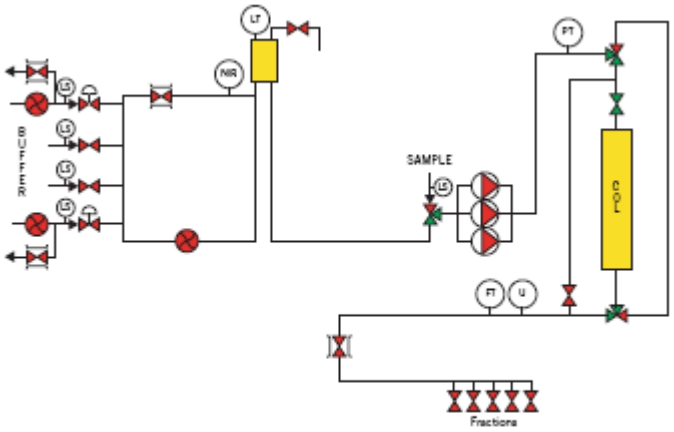
BioProcess MPLC/HPLC system – Type

The BioProcess MPLC/HPLC system – Type II is an advanced gradient system designed to blend solvents continuously. Control of the blending system is based on conductivity, NIR, or refractive index of the solvents; this results in very accurate and reproducible gradients. The inclusion of a bubble trap in the gradient system ensures that the mobile phase is free of air.

The standard configuration includes 4 inlet lines, gradient
blending loop with conductivity measurement or NIR detection,
bubble trap, pressure transmitter, flow meter after the column,
UV detector, and 5 fractionation valves. All systems are
controlled by UNICORN software.
Several additional features/components are available to ensure
that systems match specific needs. These options include:

- 2 extra inlet buffer lines
- 5 extra fractionation valves
- magnetic coupling of circulation pump
- temperature control before or after the column
- conductivity meter in gradient blending system or after the column
- pressure transmitter after the column
- pH meter before and after the column
- refractive index detector for fractionation or

gradient blending system
- valve feedback
- filter module
- injection loop
- heat exchanger



3. Piping and instrumentation diagram of a BioProcess LC/HPLC system – Type II.

UNICORN control

UNICORN control software provides a single familiar interface for both chromatography and membrane separations. It provides efficient control of the process as well as flexible method programming, extensive data evaluation and powerful reporting functionality. In addition, UNICORN is compliant with FDA 21 CFR Part 11, satisfying the regulatory requirements for electronic records and signatures.

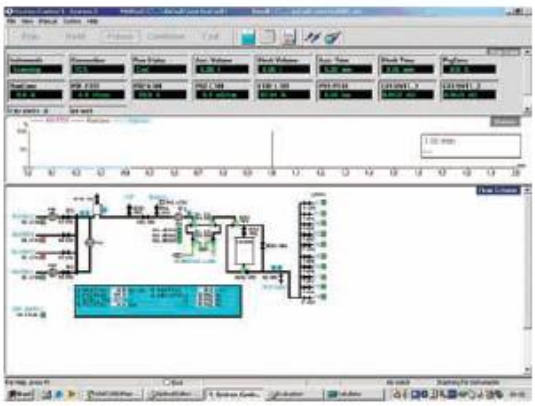


Fig 4. Same familiar interface for both chromatography and membrane system

BioProcess HPLC systems and HPLC columns are especially
for purifying small biomolecules with media such as SOURCE.
system and column combinations are also available for
applications.

Basics for Penicillin and Ampicillin Production

2 Penicillin[2]

2.1 What is penicillin and semi-synthetic penicillin?

2.1.1 Definition

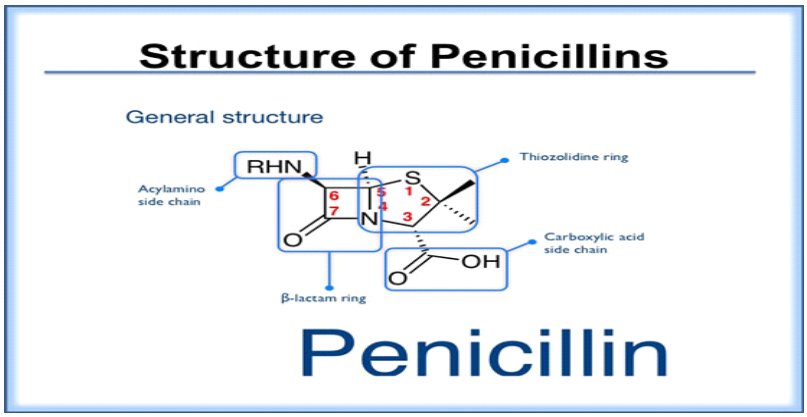
Antibiotics are a type of antimicrobial drug. They are one of the secondary metabolites produced by some fungi and bacteria.

They are pharmaceutical products that have an important role in health of living organisms. They used in the treatment and prevention of bacterial infection.

Penicillin is a group of antibiotics. It is the first medications to be effective against many bacterial infections caused by staphylococci and streptococci , it still widely used today though many types of bacteria have developed resistance following extensive use.

2.1.2 The structure of the penicillins:

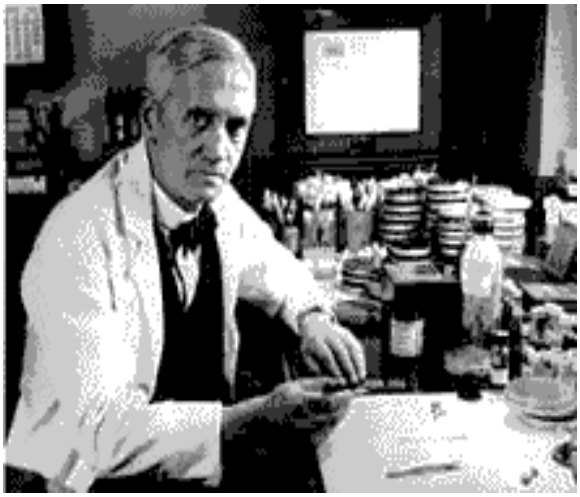
consists of a thiazolidine ring connected to a beta-lactam ring, which is attached to a side chain. All penicillins are derived from 6-amino-penicillanic acid.



2.1.3 History:

In 1928, the Scottish scientist" *Alexander Fleming*" discovered the penicillin. In his laboratory, *Fleming* put a petri dish containing staphylococcus that has been mistakenly left open. After a few days, a visible growth was formed which is the result of a contamination by blue-green mould from an open window. 32

[2] from [MEGBI-APP 2016]



In the petri dish, there was a halo of inhibited bacterial growth around the mould. *Fleming* concluded that the mould released a substance that repressed the growth and caused lysing of the bacteria. 30

Then, he grew a pure culture and discovered it was penicillium mould, now known to be *Penicillium Notatum.*

2.1.4 Strains of penicillium:

In the early days of penicillin production (1928)

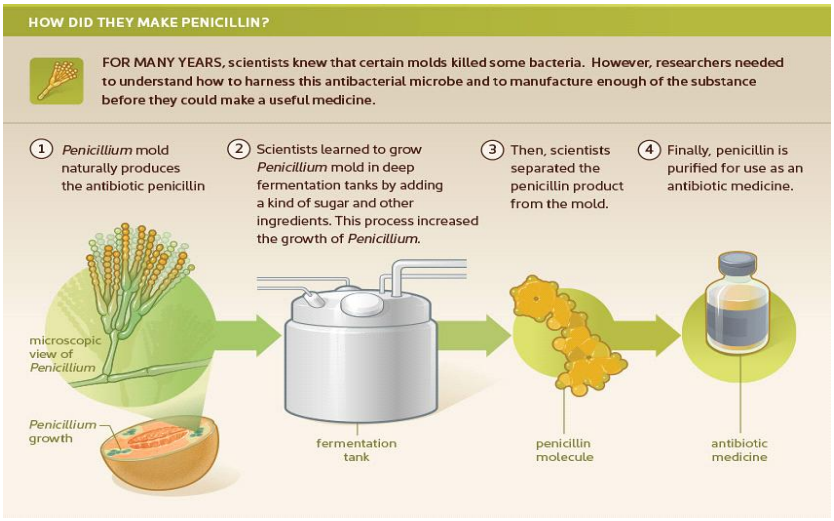
Penicillium Notatum strain was employed. After a few years, a new strain of Penicillium Chrysogenum discovered in 1943 was employed for penicillin production.

This strain gave a penicillin yield of up 250 oxford units

(1oxford units = 0.5988 of sodium benzyl penicillin) which was 2 to 3 times more than given by *Penicillium Notatum.*

2.1.5 Penicillin production

Penicillin is produced by fermentation. The penicillium cells are grown using a technique called Fed-batch culture, in which the cells are subject to stress that is required for induction of penicillin production and it is not produced during active growth.



Fermentation medium for the penicillin production should be containing: - carbohydrate as a source of glucose.

- Beet molasses as source of lactose .
- Corn steep liquor as source of nitrogen.
- Calcium carbonate or phosphate as a buffer.
- Automatic addition for H2SO4 or NaOH as necessary.
- Phenyl acetic acid as a precursor for penicillin production.
- PH in the medium: 6.8-7.4

It can divided penicillin fermentation into 3 phases:

First phase: *trophophase* where there is a rapid growth of penicillium, the mycelia are produced in a temperature between 30-32°c for 30 hours.

Second phase: idiophase where there is a low growth of penicillium and high production of penicillin in a temperature 24°c, it can take from 5 to 7 days.

Third phase: when the amount of the carbon and nitrogen decreased, the mycelia lysed, the antibiotic production ceased, the ammoniac released into the medium and the PH increased.

2.1.6 Production of semi synthetic penicillin:

Semi- synthetic Beta- lactamic antibiotics are the most used anti bacteria agents. They are usually produced by the hydrolysis of natural antibiotics (penicillinG). They are created through modifications that can be made in a laboratory. Chemists can obtain new forms of penicillin by the modification of side chains. In other meaning, they extract natural penicillin, remove R group, and attach wanted group.

Semi- synthetic penicillins can be further modified to increase the efficiency of inhibiting bacterial growth.

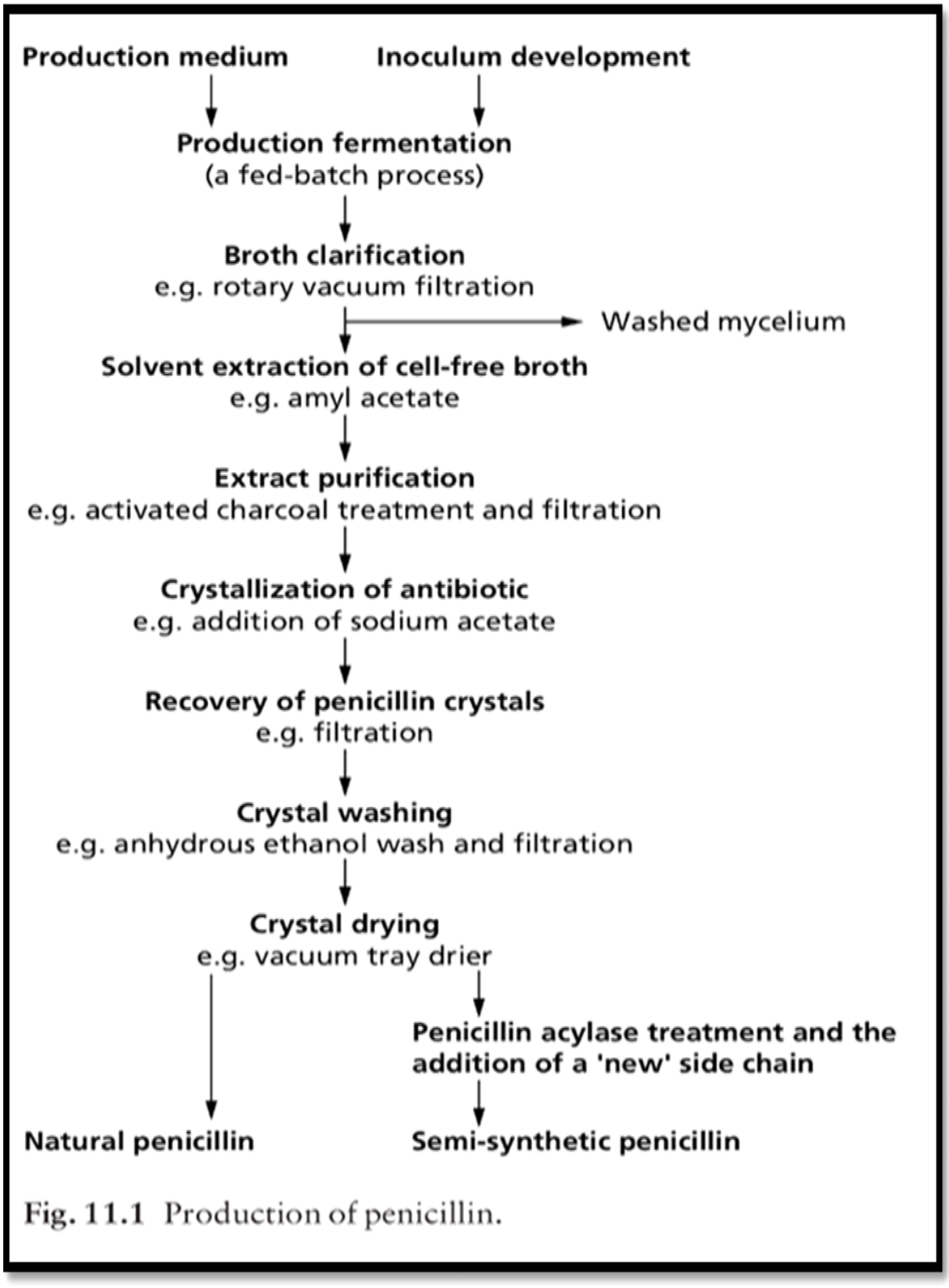


Fig. 11.1 Production of penicillin.

2.1.7 classification of penicillin:

The various penicillins differ in their side chain structure.

Penicillins are divided into several members:

- Natural penicillin:

  - penicillin G
  - Penicillin V

    *This member has a limited range of activity.

    * is highly susceptible to beta lactamase which are produced by many staphylococci and gram- bacteria.

    *it is inactivated by gastric acid.

    * efficacious only against gram+.

- B lactamase- resistant(penicillinase resistant penicillins)

  -Methicillin

  -Naficillin

  -Oxacillin

  - cloxacillin

  - dicloxacillin

  *This member was developed by adding substituents onto the aromatic ring of penicillin to sterically inhibit beta lactamases.

  * Methicillin was the first semi synthetic penicillin developed .

    *Is poorly absorbed orally due to gastric acid instability and is not very potent.

    *effective against gram+ beta lactamase producing bacteria.

- Aminopenicillins: (broad spectrum penicillins)

  -ampicillin

-amoxicillin

-hetacillin

-bacampicillin

- metampicillin

- talampicillin

- epicillin

* Very important group of drugs due to their activity against both gram+ and some gram-.

* susceptible to penicillinase.

* Stable in gastric acid.

- Carboxypenicillins (antipseudomonas and extended-spectrum penicillin):

-carbenicillins

- ticarcillin

* More active against pseudomonas and some Anaerobes.

*they are inactivated by beta lactamases and gastric Acid.

2.1.8 Mechanism of action:

Beta- lactam antibiotics inhibit the formation of peptidoglycan an essential part of the cell wall.

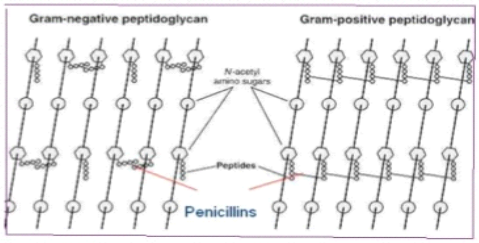
All penicillins work in the same way:

They interfere with cell wall synthesis by binding to penicillin-binding proteins (PBPs) which are located in bacterial cell walls, and by activating other enzymes to break down the protective wall of the microorganism. Then, inhibition of PBPs leads to inhibition of peptidoglycan synthesis then, inhibition a new cell formation. Without a cell wall, bacterial cell is vulnerable to outside water and molecular pressures, and quickly dies.

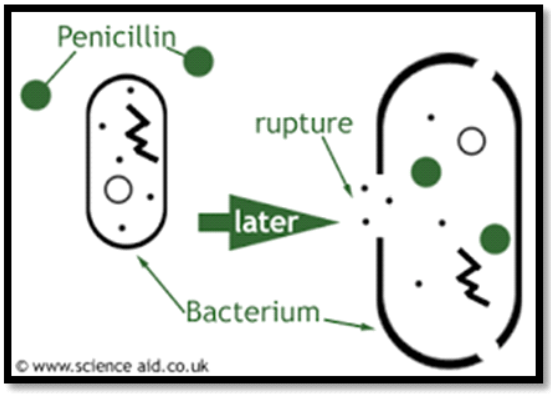
Since human cells do not contain a cell wall, penicillin treatment results in bacterial cell death without affecting human cells.

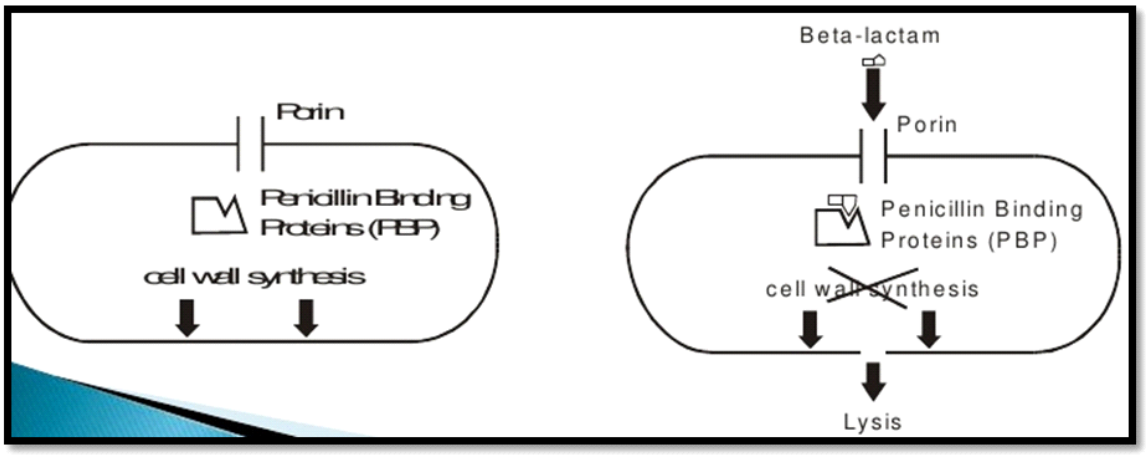
Gram positive bacteria have thick cell walls containing high levels of peptidoglycan, while gram negative bacteria are characterized by thinner cell walls with low levels of peptidoglycan. The cell wall of gram negative bacteria is surrounded by a lipopolysaccharide (LPS) layer than prevents antibiotic entry into the cell. Therefore, penicillin is most effective against gram positive bacteria.

Mechanism of action



Mainly interferes with cell wall synthesis of bacteria. These drugs inhibit the enzyme transpeptidase which is responsible for cross linkage of peptidoglycan during bacterial cell wall synthesis.





2.1.9 Resistance to beta lactams :

Bacteria reproduce quickly and are prone to genetic mutations when growing in the presence of environmental pressures, such an antibiotic.

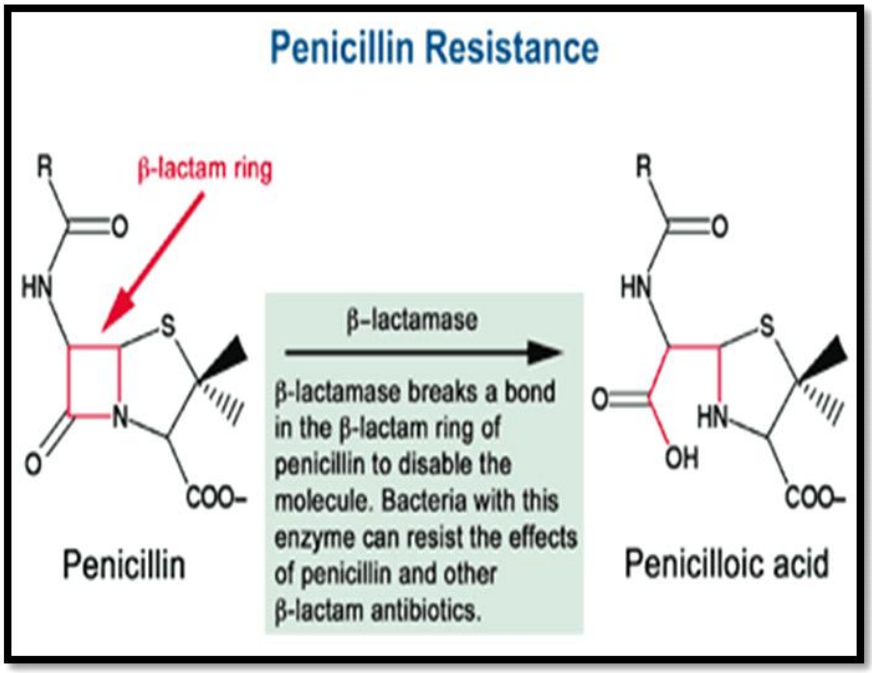
Bacteria are constantly finding ways to counteract antibiotics, one of the most important bacterial defense mechanisms is the production of enzymes B lactamase.

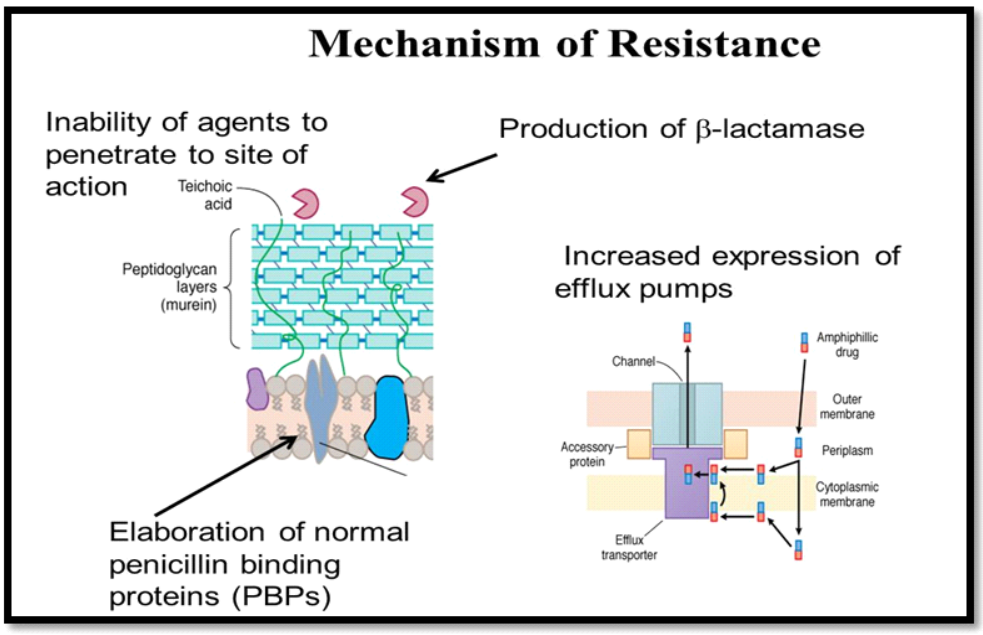
Organisms that produce B lactamase are resistant to penicillin by hydrolyses beta-lactam ring.

Example:

Some strains such as staphylococcus have developed a specific resistance to the nature penicillins.

These bacteria either produce B lactamase (penicillinase), an enzyme that disrupts the internal structure of penicillin and thus destroys the antimicrobial action of the drug, or they lack cell wall receptors for penicillin. Then this enzyme reduces the ability of the drug to enter bacterial cells.





2.1.10 Beta- lactamase inhibitors:

One way to overcome penicillin resistance is to combine penicillin drug with molecule that protects the penicillin such as clavulanic acid, sulbactam or tazobactam, this diminishes or impedes beta-lactamase activity.

These molecules inactivate beta-lactamases and are used to enhance the antibacterial actions of beta-lactam antibiotics. They are inhibitors of many but not all bacterial beta-lactamases and can protect hydrolysable penicillins from inactivation by the enzymes

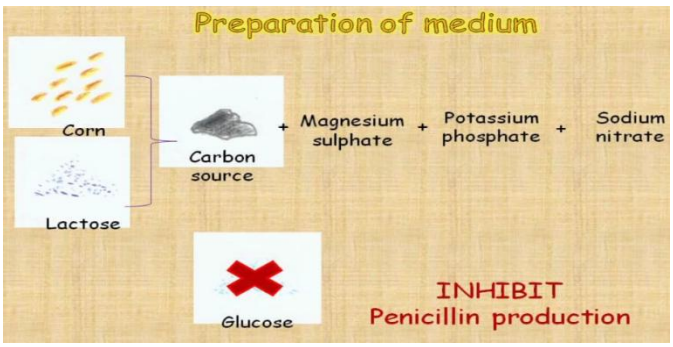
2.2 Production of Semisynthetic Penicillins

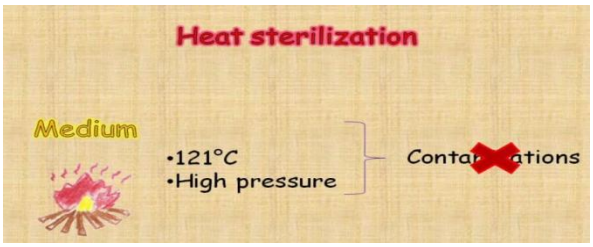
Semisynthetic penicillins:

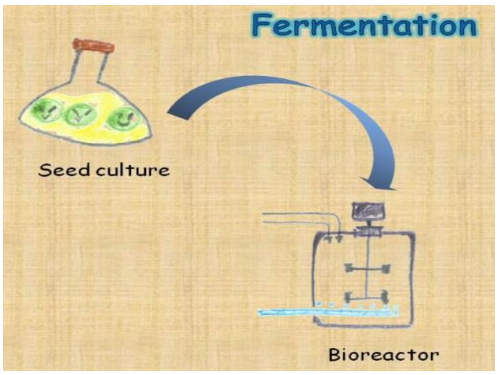
Ampicillin

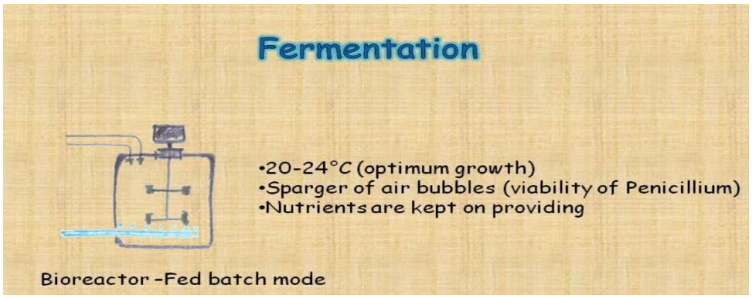
Amoxillin

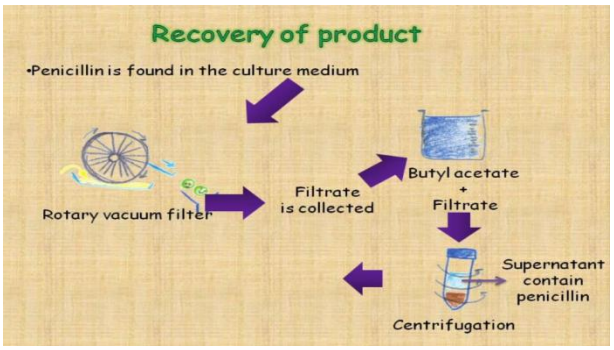
2.2.1 Penicillin in culture

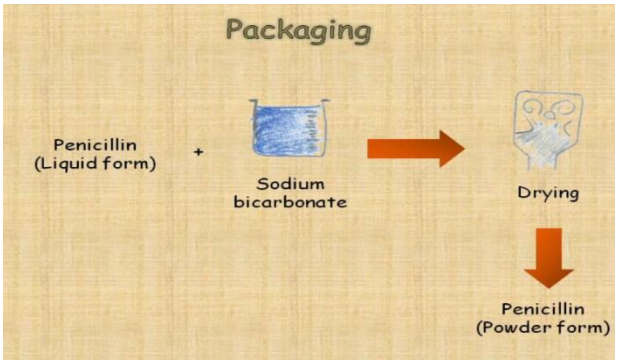


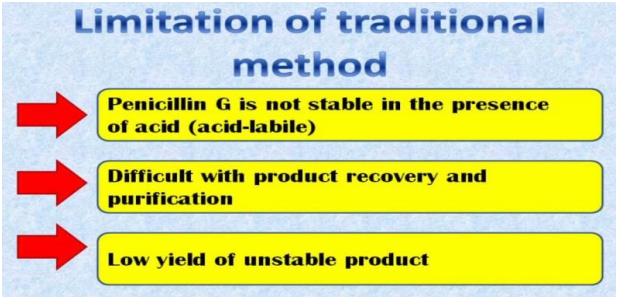




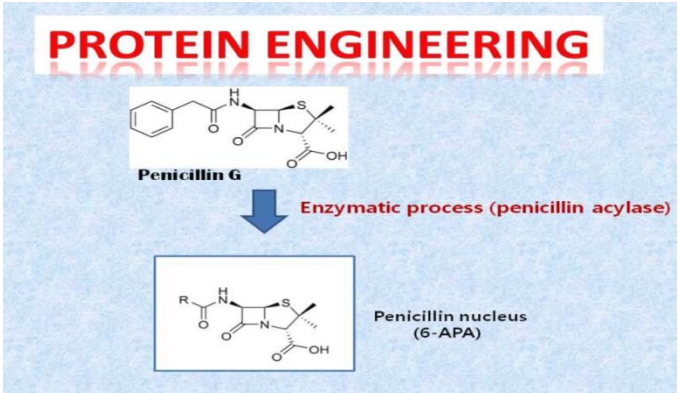


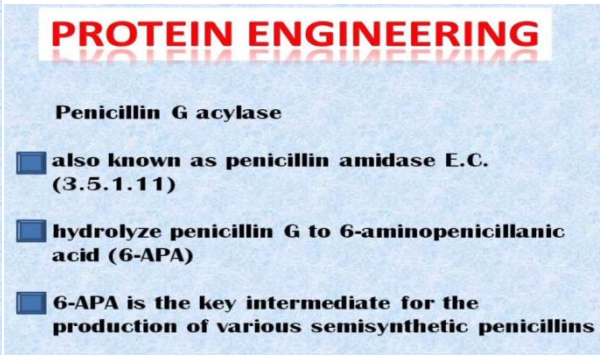


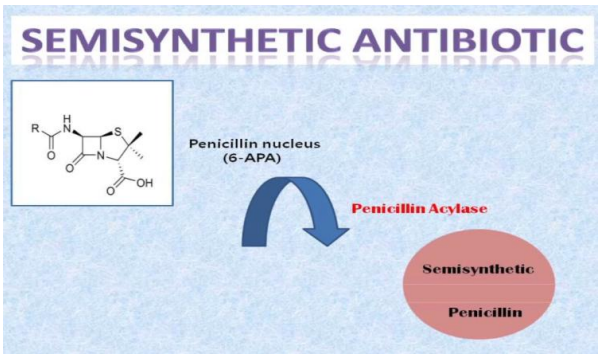


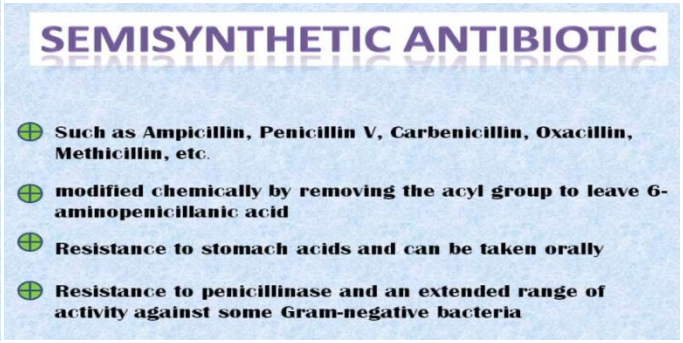


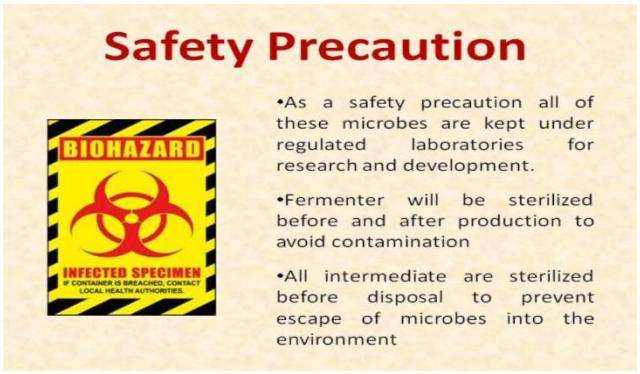
2.2.2 Production of semisynthetic penicillins



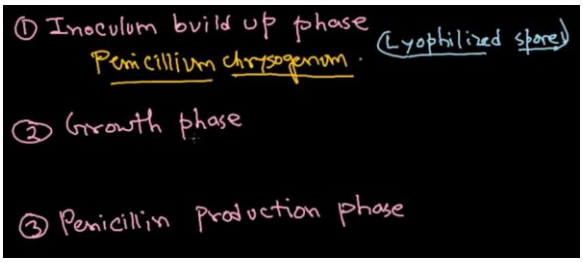


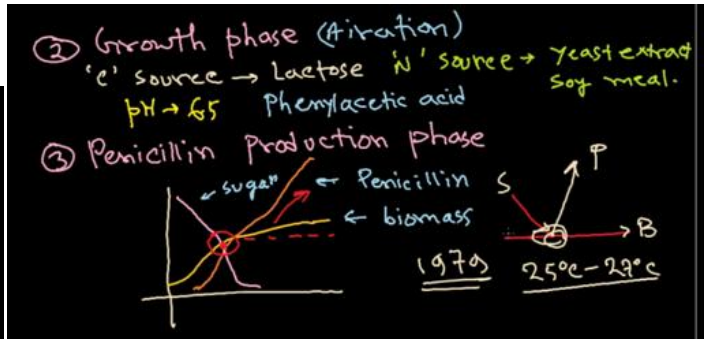


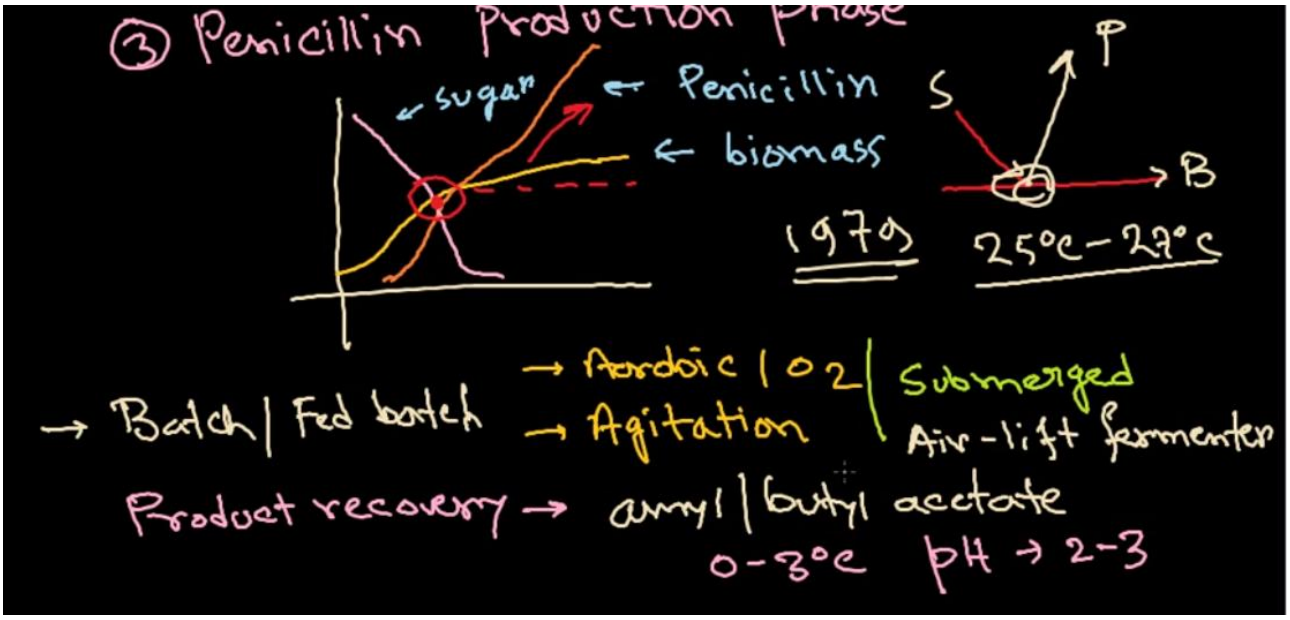




2.2.3 Industrial Prodution of penicillin







2.2.4 Devices

2.2.4.1 Rotary vacuum drum

2.3 Patent "Production of semisynthetic penicillins US 3912719 A"

ABSTRACT The old process for producing a synthetic penicillin, e.g. ampicillin or amoxicillin, which consisted of acylating solid -aminopenicillanic acid (6-APA) with an acid chloride (or chloride hydrochloride) after preparing the 6-APA by converting a silylated natural penicillin to an imino-chloride, as with PCl and thence to an imino-ether, as with methanol, and thence to 6-APA by hydrolysis followed by recovery of the solid 6-APA has been rendered more efficient and capable of being conducted in a single vessel by maintaining the imino-ether solution in the hydrolysis step at 50C. while adding a volume of water no greater than 10% of the volume of the imino-ether solution to produce a single phase containing -aminopenicillanic acid which is then acylated

with an acid chloride (or chloride hydrochloride) at about -40C. after the addition of a weak tertiary amine to produce the synthetic penicillin.

SUMMARY OF THE INVENTION In the process for producing a synthetic penicillin (e.g. amoxicillin or ampicillin) from a natural penicillin such as penicillin G or penicillin V by the consecutive steps of a. forming a solution in an anhydrous, unreactive organic solvent (preferably methylene chloride) of a silyl ester of said natural penicillin [preferably made by reaction of said natural penicillin with dichlorodimethylsilane (DDS) or hexamethyldisilazane HMDS) or trimethylchlorosilane (TMCS)] in the presence of a weal tertiary amine (preferably dimethylaniline),

b) adding at below 0C. (and preferably below 20C. and especially below 40C.) a halogenating agent (and preferably an acid halide and especially phosphorus pentachloride) to form a solution of the imino-halide,

c) mixing said solution at below -20C. (and preferably below 40C.) with alcohol (and preferably a lower alkanol and especially methanol) to form a solution of the imino-ether,

d) mixing said solution with water to produce 6- amino-penicillanic acid in a biphasic system,

e) isolating said 6-aminopenicillanic acid as a solid,

f) redissolving it in a solvent and g) adding thereto a carboxylic acid chloride (e.g.

D-( )-2-p-hydroxyphenylglycyl chloride hydrochloride or D-(-)-2-phenylglycyl chloride hydrochloride) as an acylating agent to produce said synthetic penicillin,

this invention provides the improvement which comprises maintaining the imino-ether solution in the hydrolys is step at 50C. while adding a volume of water no greater than 10% (and preferably no greater than 8%) of the volume of the imino-ether solution to produce a single phase containing 6-aminopenicillanic acid which is then, without intermediate isolation of the 6-aminopenicillanic acid, acylated at about 40C. after the addition of a weak tertiary amine (preferably N,N-dimethylaniline) to produce said synthetic penicillin.

In its more specific embodiments the present invention provides for the use of the process described above to produce ampicillin by the use of D-(-)-2- phenylglycyl chloride hydrochloride and amoxicillin by the use of D-(-)-2-p-hydroxyphenylglycyl chloride hydrochloride and epicillin by the use of D-(-)-2-amino- 2-(1,4-cyclohexadien-l-yl)acetyl chloride hydrochloride and cyclacillin by the use of l-aminocyclohexanecarboxyl chloride hydrochloride and methicillin by the use of 2,6-dimethoxybenzoyl chloride and nafcillin by the use of 2-ethoxy-1-naphthoyl chloride and oxacillin by the use of 5-methyl-3-phenyl-4-isoxazolecarbonyl chloride and cloxacillin by the use of 5- methyl-3-(2-chlorophenyl)-4-isoxazole-carbonyl chloride and dicloxacillin by the use of 5-methyl-3-(2,6-

dichlorophenyl)-4-isoxazole-carbonyl chloride and flu- 5 cloxacillin (floxacillin) by the use of 5-methyl-3-(2-chloro-6-fluorophenyl)-4-isoxazole-carbonyl chloride and indanyl carbenicillin by the use of S-indanyl phenylmalonyl chloride and 6-[D-a-(3-guanyl-l-ureido)- phenylacetamido]-penicillanic acid by the use of D-al0 (B-guanyl-l-ureido)phenylacetyl chloride hydrochloride and levopropylcillin by the use of (-)-2- phenoxybutyryl chloride and sulfocillin (sulbenicillin; sulfobenzylpenicillin) by the use of a-sulphophenylacetyl chloride and azidocillin by the use of D-(-)-al5 azidophenylacetyl chloride and 3,4-dichloro-a- SCHEME 1 0 CO2K 1 Potassium Penicillin V N.N-Dimcthylanilinc 0 (DMA) S c u ocn NH iCl l c u ocu iw CO H + Meoy SiMe methyl phosphates HCI The esterification of penicillin V potassium (1) in methylene chloride solution at 25 with dimethyldichlorosilane (DDS) in the presence of N,N- dimethylaniline gives rise to a mixture of monomer ester (2) and dimer ester (3) (Scheme 1). Low levels of DDS (0.60 moles/moles pen V) give predominantly dimer ester (3), whereas high levels of DDS (0.9-l.l moles/mole pen V) give rise to a mixture of both (2) and (3); monomer ester predominating. In either case, the esterifrcation is essentially quantitative. Long term stability studies indicate that the preferred technique for esterification is to add all of the DMA required for the cleavage (2.7-3.0 moles/mole pen V) to the suspension of pen V K salt in methylene chloride, prior to adding the DDS. This esterification mixture shows no tendency to undergo degradation after 16 hours at 25. An examination of esterification mixtures (0.94 moles DDS 0.22 moles DMA/mole pen V) after 16 hours showed approximately 9% degradation of the silyl ester to a compound tentatively assigned as the O-silylated amide, (8)

The treatment of the silylation mixture with phosphorous pentachloride (1.1-1.2 moles/mole Pen V) at 40 gives rise to the chloroimide (4). After 2 hours chlorination was quantitative and free from undesirable side reactions. No degradation was observed after 8 hours at -40.

The dropwise addition of precooled (60") anhydrous methanol to the chlorination mix (this order of addition is preferred), maintaining the temperature at 50, produces the imino ether hydrochloride free acid after l-2 hours reaction time at 50. The alcoholysis reactions of the chlorimide and the silyl ester are quantitative and also free from any undesirable side reactions; the latter reaction occurring within 10-15 minutes at 50.

The addition of 2.5-3% water by volume of methylyacetate. This reaction is nearly quantitative. In addition, there is no evidence to suggest that ,B-lactam breakage occurs during this step. Empirical data have shown that no loss of 6-APA occurs over 16 hours in this hydrolysis mix if it is stored that long.

The overall conversion of penicillin V to 6-APA in this process approaches 98-99%. Residual penicillin V assays of spent mother liquors are generally under 1%.

The resulting solution of 6-APA is treated with DMA at 50, followed by the addition of D-(-)-phenylglycyl chloride hydrochloride (PGl-l) at 40. After aqueous quench and workup via NSA/MILA, pure ampicillin trihydrate is produced in yields of 68-80% overall from penicillin V K salt.

Further laboratory investigations were then carried out by hydrolyzing methylation mix (prepared by adding chlorimide to methanol) with 6volume percent water at 45", followed by acylation at this temperature with

varying levels of DMA and PGH. Table 1 summarizes the effects of base and acid chloride on insolution yields of ampicillin.

lt appeared that the best conditions for acylation involved the use of 6-6.2 eq. of DMA and 1.1-1.3 eq. PGH (run numbers 9 and 10) at 45. These conditions gave rise to 69-72% of ampicillin in solution. Higher mole ratios of PGH (run numbers 4, 8, l2, l6) apparently resulted in over acylation of 6-APA (acylation of ampicillin), whereas lower levels of both DMA and PGH apparently resulted in incomplete acylation of the 6-APA (run numbers l-4).

A study of the effect of temperature on in solution yields of ampicillin was also carried out using the DMA/PGH levels described in Run No. 10 (Table 1). In these instances, methylation mix was prepared from known potency pen V K salt via esterification with DDS, chlorination with phosphorous pentachloride and by the addition of 25 eq. of methanol to the chlorimide. maintaining the addition temperature below 50. The single phase methylation mix was hydrolyzed at 50 with 2.6% water based on the volume of the methylation mix, and acylated at the temperatures described in Table ll.

TABLE I The Effect of DMA and PGH Levels on Ampicillin Yields in Solution TABLE I Contmued Run Moles of Moles of Calculated No. DMA added PGH added Ampi Free for for Acid in Soln.

Acylation Acylation A 2.0 ml. aliquot was taken from the acylation mix, stripped in vacuo, diluted to 20 20 mls. with pH 7.00 phosphate buffer and sent for bioassay. Yields are not corrected for input pen V potency.

"7r Ampiclllin in Solution (Bioassay meg/ml) (2O mls.) (Volume of Acylation mix) Yields are corrected for input pen V potency.

Somewhat higher yields were noted at temperatures above 50 (Run Nos. 2023). Interestingly, the rate of dissolution of the acid chloride was virtually instantaneous at l0, whereas it requires 20 minutes at 50. Bioassay data tend to indicate that better yields of ampicillin are obtained using the controlled addition of 25 ea. of methanol to chlorimide (compare bio yields in Table I with Table II). Thus, several isolation variations were carried out using this methylation technique, some of which are illustrated in Table 111.

TABLE III Isolation Conditions and Yields of Ampicillin Trihydrate* Chem Method Run Assay of Yield 7c of No. in meg/mg Theory in grns. Yld. lsoln.

24 853;856 98.7 4.17 70 l 25 810;8ll 93.8 15.8 76 l 26 8l7;8l2 94.1 5.4 77 2 27 1348;855 98.3 16.6 79 2 28 849;853 98.3 66.6 68 2 29 820 94.7 12.2 50 3" *Yields are not corrected for purity. "DMA removed by vacuum distillation at pH 7 (3.0N NaOH used for pH adjustment); NSA/MILA.

DMA removed by extraction (MIBK) at adjustment); NSA/MILA.

DMA removed by extraction (MIBK) at pH 7 (6N NH 0H used for pH adjustment) direct crystallization of ampicillin by pH adjustment.

' pH 7 (6N NH OH used for pH Workup in all cases consisted of aqueous quench of acylation mix at O-5. No emulsions were observed at this stage. The organic layer was removed and the aqueous was processed as follows:

Isolation method 1 involved adjustment of the rich aqueous with 3 N sodium hydroxide to pH 77.5. In addition to encountering an emulsion, a gummy solid precipitated during this step which was removed with difficulty via diatomaceous earth (Dicalite) treatment and filtration. The formation of this solid, however, was precluded by continuous pH adjustment at pH 7.5, but pH control was difficult. The two phase mix (DMA and aqueous) was concentrated at 50 in vacuo to complete DMA removal. Slow acidification with aqueous ,B-naphthalenesulfonic acid (NSA) gave ampicillin NSA salt. The conversion of the wet NSA cake to ampicillin trihydrate using MIBK-LA-l resin (MILA) gave yields up to 75% of good quality product.

Isolation method 2 involved adjustment of the rich aqueous with 6 N ammonium hydroxide to pH 77.5 in the presence of MIBK. An amorphous solid was found in addition to an emulsion, but was easily removed by filtration with added Dicalite. The MIBK layer containing DMA was removed and the clean aqueous processed via NSA/MILA to good quality ampicillin trihydrate.

Method 3 consisted of removal of the DMA by solvent extraction (MIBK) at pH 77.5 (6 N ammonium hydroxide used for pH adjustment), followed by direct crystallization of the ampicillin by pH adjustment. The yields were considerably lower (Table 3) using this technique.

Either of these three methods is capable of yielding good quality ampicillin trihydrate in reasonably good yields from penicillin V Method 2 has thus far processed most smoothly of the three methods.

The acylation to ampicillin was also investigated using other bases such as triethylamine, imidazole and pyridine. The yields respectively in each case (bioassay of acylation mix) under best conditions were 55% (6.5 eq. TEA, 1.4 eq. PGH), 27.2% (5 eq. imidazole, l.l eq. PGH) and 30% (2O eq. pyridine, l.l eq. PGH). These yields were all lower than those obtained using DMA.

Using the best conditions thus far obtained, an acylation of the resulting solution of 6-APA with D-(-)-2-(4-hydroxyphenyl)glycyl)chloride hydrochloride PHPGH) was examined at 40 using 6.2 eq. DMA/1.3 eq. PHPGH. Bioassay data indicated yields of amoxicillin in solution approaching average on three occas1ons.

The silyl esters of the process of the present invention are made, for example, by the use of such agents as are described in US. Pat. Nos. 3,499,909, 3,249,622, 3,654,266, 3,678,037, 3,741,959 and 3,694,437, e.g., trimethyl chlorosilane, hexamethyl disilazane, triethyl chlorosilane, methyl trichlorosilane, dimethyl dichlorosilane, triethyl bromosilane, tri-n-propyl chlorosilane, bromomethyl dimethyl chlorosilane, tri-n-butyl chlorosilane, methyl diethyl chlorosilane, dimethyl ethyl chlorosilane, phenyl dimethyl bromosilane, benzyl methyl ethyl chlorosilane, phenyl ethyl methyl chlorosilane, triphenylchlorosilane, triphenyl fluorosilane, tri-o-tolyl chlorosilane, tri-p-dimethylaminophenyl chlorosilane, N-ethyl triethylsilylamine, hexaethyl disilazane, triphenyl silylamine, tri-n-propyl silylamine, tetraethyl dimethyl disilazane, tetramethyl diethyl disilazane,

tetramethyl diphenyl disilazane, hexaphenyl disilazane, hexa-p-tolyl disilazane, etc. The same effect is produced by hexa-alkylcyclotrisilazanes, or octaalkylcyclotetrasilazanes. Other suitable silylating agents are silylamides and silylureides such as a trialkylsilylacetamide and a bis-trialkylsilylacetamide.

For optimum results, it is preferred to use high concentrations of the reactants. For example, in the formation of the silyl esters a 20 to 30%, preferably 25% by weight of the penicillin is suspended in an inert organic solvent and a base for the best results. The preferred base is N,N-dimethylaniline. Depending upon the specific starting material, the silane is employed preferably -in a slight excess i.e. 10 to 60%, above theoretical amounts. This enables the use of solvents which are not absolutely dry because trace amounts of water are removed therefrom by reacting with the excess silylating agent.

Examples of suitable alcohols for forming the imino ethers are primary and secondary alcohols having the vents such as methyl isobutyl ketone, dimethylformamide, ethyl acetate and acetonitrile.

Among these solvents, methylene chloride, chloro form, acetonitrile, and ethyl acetate are particularly useful. Since the halosilanes and silylated products are decomposed by moisture and other hydroxylic agents, solvents employed as reaction media must be substantially anhydrous and free from alcoholic impurities.

Useful weak tertiary bases include N,N- dimethylaniline, pyridine, any lutidine and quinoline; the term weak means those such amines having dissociation constants in the range of from 10' to 10*.

The halogenating agents include agents forming imide halides and, more specifically acid halides, particularly chlorides, which are derived from phosphorus,

sulfur, carbon or their oxygen acids, for example phosphorus oxychloride, phosphorus pentachloride, phosphorus trichloride, thionyl chloride, phosgene, oxalyl chloride.

general formula R OH in which R is selected f th The following examples are given in illustration of,

group consisting of (A) alkyl, having 1 to 12 carbon atoms, preferably at least 3 carbon atoms, such as methanol, ethanol, propanol, isopropanol, n-butanol, amylalcohol, decanol, etc.; (B) phenylalkyl having 1 to 7 alkyl atoms, such as benzylalcohol, 2-phenylethanol- 1, etc.; (C) cyloalkyl, such as cyclohexylalcohol, etc.; (D) hydroxyalkyl having 2 to 12 carbon atoms, preferably at least 3 carbon atoms, such as 1.6 hexanediol, etc.; (E) alkoxyalkyl having 3 to 12 carbon atoms, such as Z-methoxyethanol, butoxyethanol, etc.; (F) aryloxyalkyl having 2 to 7 carbon atoms in the aliphatic chain such as 2-pchlorophenoxyethanol, etc.; (G) aralkoxyalkyl, having 3 to 7 carbon atoms in the aliphatic chain, such as 2-(pmethoxybenzyloxy)-ethano1, etc.; (H) hydroxyalkoxyalkyl, having 4 to 7 carbon atoms, such as diglycol. Also, mixtures of these alcohols are suitable for forming the imino ethers.

For use as the anhydrous, unreactive organic solvent a wide range of anhydrous non-hydroxylic organic solvents are suitable, including hydrocarbons, such as benzene and toluene; chlorinated solvents such as

methylene chloride, chloroform, ethylene dichloride and chlorobenzene; ethers such as diethyl ether, diox- 2-isopropxyethanol, 2- 3O but not in limitation of, the present invention. All temperatures are in degrees Centigrade. 7Aminocephalosporanic acid is abbreviated as 7-ACA, methyl isobutyl ketone as MlBK and tetrahydrofuran as THF. Skellysolve B is a petroleum ether fraction of B.P. -68C. consisting essentially of n-hexane.

LA-l resin is a mixture of secondary amines wherein each secondary amine has the formula wherein each of R, R and R is a monovalent aliphatic hydrocarbon radical and wherein R, R and R contain in the aggregate from eleven to fourteen carbon atoms. This particular mixture of secondary amines, which is sometimes referred to - in these examples as Liquid Amine Mixture No. ll, is a clear amber liquid having the following physical characteristics: viscosity at 25C. of cpd., specific gravity at 20C. of 0.826; refractive index at 25C. of 1.4554; distillation range at 10 mm., up to 170C 0.5%, 170-220C. 3%, 220-230C.

ane and tetrahydrofuran; and other conventional sol 45 and above 230C. 6.5%.

DESCRIPTION OF THE PREFERRED EMBODIMENTS 1 MATERIALS Example I Step Compound WL. g. Volume. ml. Moles Eq.

A. Penicillin VK 1000 2.57 1.00

Methylene chloride 5000 N,N-Dimethylaniline 936 975 7.72 3.00 Dichlorodimethylsilane 366 342 2.83 2.20 B. Methylene chloride 5000 Phosphorous Pentachloride 643 3.09 1.20 C. Methanol 2064 2613 64.37 25.0 D. Water 362 362 20.0 7.83 E. N,N-Dimethylaniline 1934 2015 15.96 6.20

D-(')-phenylglycyl chloride Hydrochloride 750 3.35 1.30 F. Water 4000 MlBK 8000 6N Ammonium Hydroxide 4500 BNSA (NSA', Beta-naphthalene 3500 sulfonic acid) 15% MlLA 10900 Moles/mole penicillin VK salt. Based on 92% pure D-(-)-phcnylglycyl chloride hydrochloride. This refers to u 15% Weight/Volume solution of LA-l resin in methyl isobutyl ketone.

PROCEDURE All solvents should be dried, preferably with molecular sieves. Step A. Esterification 1. Potassium penicillin V 1000 g., 2.57 moles) is suspended in anhydrous methylene chloride (5000 ml.) with gentle stirring at 25 undeer a nitrogen atmosphere.

2. N,N-Dimethylaniline (975 mls., 7.72 moles) is added to the slurry over a minute period. No temperature rise was observed on a lab scale of 100 g. of K pen V.

3. Dichlorodimethylsilane (342 mls., 2.83 moles) is added over 15 to 20 minutes with gentle stirring at 25. An exothermic reaction ensues raising the temperature to 35-3 8 during the addition, resulting in the dissolution of the pen V K salt. The silylation mix is stirred for 45-60 minutes after the addition.

Step B. Chlorination 1. Methylene chloride (5000 ml.) is-added to the above clear yellow solution of silylation mix at 25 and the mixture is then cooled to 40 to 45.

2. Phosphorous pentachloride (643 g., 3.09 moles) is added in one portion with high speed agitation at 40 to 45. The temperatures rises to 35 to 38 and then falls to 40 to 45 over a -15 minute period. At this time nearly complete solution occurs and the mixture turns dark brown.

3. The chlorination mixture is stirred for 2 hours at 40 to 45.

Step C. Methylation 1. The above chlorination mix is cooled to 60 to 65.

2. Anhydrous methanol (2615 mls. 64.4 moles) precooled to 65 is added very slowly to the vigorously agitated chlorination mix such that the temperature is held between 55 and 50. After the addition of about 1100 mls., the mixture turns nearly colorless. The reaction is very exothermic and care should be taken not to exceed 50 during theearlier part of the addition of methanol.

3. Methylation is allowed to proceed at 50 to 52 for 2 hours.

Step D. Hydrolysis 1. Water at 25(362 mls., 20.1 moles, 2.6% V/V) is added over 5-10 minutes to the above light yellow solution at 50.

2. Single phase hydrolysis is allowed to proceed for 1 hour at 50.

Step E. Acylation 1. N,N-Dimethylaniline (2015 mls., 15.96 moles) is added to the hydrolysis mix over a -20 minute period-The temperature rises about 4 during this period, and the solution turns dark green. After about 1000 mls. are added, the mixture becomes a thick green slurry. 1

2. The slurry is warmed to 40 and solid D-(-)- phenylglycyl chloride hydrochloride (749.5 g., 3.35 moles) is added portionwise over 15-20 min. The reaction is slightly exothermic and the temperature rises to 35 and falls to 40 over a 10 min. period. Solution becomes complete during this period. The mixture is stirred at 40 for 45 minutes.

3. The mixture is warmed to 10 over a 30-45 min. period and 4000 mls. of water (25) is added over lO-15 min. with good agitation. The phases are separated and the methylene chloride layer is saved for solvent recovery.

4. The aqueous layer (pH 1.3) is layered with methyl isobutyl ketone (MIBK; 1000 mls.) and the pH is slowly adjusted to 7.5 7.7 over 10-15 min. with O-5C; 6N ammonium hydroxide (4000 ml.). The emulsion is treated with 100 g. of diatomaceous earth (Dicalite) and polish filtered and the cake washed with water (500 ml.) and MIBK (500 mls.).

5. The layers are separated and the aqueous layered with an equal volume of MIBK (about 2000 mls.).

6. With high speed agitation, the pH is slowly adjusted to 1.5-1.7 with B-naphthalenesulfonic acid (NSA) (2500-3000 mls.) over a 1 hour period at a rate of additon of NSA of 50 mls./min. When nucleation begins, the mixture is cooled to 0-5 over 1-2 hours.

7. The slurry is stirred at 0-5 for 2 hours, filtered and the cake washed with cold (0-5) water (2000 mls.) and 25 C. MIBK (2000 mls.).

8. The cake is sucked as dry as possible and slurried with high speed agitation in 15% of MILA (10;900 mls.) and water (1360 mls.) for 3 hours.

9. The ampicillin trihydrate is collected by filtration and displacement washed with cold.(0-5 C.) water (2000 mls.) and MIBK (2000 mls.) and oven dried at 45 for 18 hours. The yield of snow white trihydrate is .705-829 g. (68-80%); IR and NMR are consistant for structure. Biopotency indicates 97-99% purity. Chem. potency indicates about 97-99% purity.

EXAMPLE 2 Ampicillin Trihydrate Potassium penicillin V (100.0 g., 257,42 moles) was slurried in dry methylene chloride (500 ml.) under nitrogen, and N,N-dimethylaniline (97.48 ml., 93.58 g., 772.26 mmole, 3.0 eq.) was added in one portion at 25. Dimethyldichlorosilane (34.16 ml., 36.56 g.-, 283.16 mmole, 2.19 eq) was added over 1-2 min. at 25. The temperature rose to 35-37 during the addition and fellto 25-27 over 15-20 min. The mixture was stirred for a total of 30-45 min. and methylene chloride (500 ml.) was added. The solution wascooled to,40 to 45 and phosphorous pentachloride (64.33 g., 308.9 mmole, 1.2 eq.) was added in one portion at 40. The temperature rose to 35 and fell to 40 over 10-12 min. The chlorination was allowed to proceed for 2 hours at 40 to 45. The solution was cooled to 60 and precooled methanol (-60, 261.3 mls., 206.4 g., 6.45 moles, 25 eq.) was added dropwise very carefully maintaining the temperature below -50". The addition required about 20 min. Methylation was allowed to proceed for 2 hours at 50. Water at 25 (36.2 mls., 36.2 g., 2011 mmole, 7.81 eq., 2.6 V/V%) was added over 1 min. at 50 and single phase hydrolysis was allowed to proceed at -50" for 1 hour. N.N-' dimethylaniline (201.46 ml., 193.4 g., 6.2 eq.) was added slowly over 36 min. at 50. After the addition,

the mixture containing a green slurry was warmed to 40 over a 5-10 min. period. D-(-)-2-phenylglycyl chloride hydrochloride (assay purity, 74.95 g., 363.73 mmole, 1.3 eq.) was added in one portion at 40. Acylation was allowed to proceed at 40 for 40 minutes. The mixture was warmed to -10 and water (1000 ml.) was added over 5-10 minutes. The temper ature rose to about 5 C. during the addition. The layers.

were separated, and the aqueous was layered with methylene chloride (300 ml.) at 5. Dicalite g.) was added and the pH was adjusted to 7.5 with 6 N ammonium hydroxide (about 390 ml.) with high speed stirring maintaining the temperature at about 5. The resulting emulsion was filtered and the layers were separated. The aqueous was layered with an equal volume of methyl isobutyl ketone at 5l0. the pH was adjusted very slowly to 1.5 with 35% aqueous B-naphthalenesulfonic acid (NSA) solution (about 225 ml.) at a rate of about 2.0 ml./min. The solution was seeded at pH 3.5 and the slurry allowed to stir for 1.5 hours at about 10 and then cooled to 0-5. The slurry was held for 16 hours at 05 and the product collected by filtration and displacement washed with

water (05) followed by methyl isobutyl ketone (25). The cake was sucked as dry as possible and the slurry transferred to a tared beaker. A solution (MILA) of LA-l resin in methyl isobutyl ketone W/V) was added based on 200 mls./50 g. wet cake and water was added based on 25 mls./50 g. .wet cake. The slurry was stirred vigorously for 3 hours, filtered and washed with cold (05) water, methyl isobutyl ketone and oven dried at 45 for 18 hours giving 66.6 g. (68%) of snow white ampicillin trihydrate. Infrared and NMR spectra were completely consistent for structure: B-lactam potency was 856 mcg./mg. and the biopotency was 851 mcgJmg. indieating a purity of about 99%.

EXAMPLE 3 p-Hydroxyampicillin (Amoxicillin) Potassium penicillin V (25.0 g., 64.36 mmoles) was slurried in dry methylene chloride (100 mls.), followed by the addition of N,N-dimethylaniline (24.37 mls., 23.40 g., 193.08 mmoles) at 25 C. under nitrogen. Dimethyldichlorosilane (8.54 mls., 9.14 g., 70.79 mmoles) was added and the solution allowed to silylate for 1 hour. Methylene chloride (100 mls.) was added and the solution cooled to -40 C., and phosphorous pentachloride (16.1 g., 77.23 mmoles) was added in one portion. Chlorination was allowed to proceed for 1.5 hours at 40 C. The solution was cooled to -60 C. and pre-cooled methanol (60 C.; 65.3 mls., 51.6 g., 1609 mmoles) was added dropwise over a 15 minute period. During the addition of methanol, the temperature was not allowed to exceed 50 C., and methylation was allowed to proceed for 2 hours at 50 C. Water (2.6% V/V, 7.8 mls.) was added at 50 C. and hydrolysis allowed to proceed for 45 minutes at 50 C. N,N-Dimethylaniline (50.37 mls., 48.36 g., 398.92 mmoles) was added over a 5 minute period at 50 C. The solution was warmed to -40 C. and D-(-)-2-(4- hydroxyphenyl)glycyl chloride hydrochloride (90% pure; 20.64 g., 92.96 mmoles) was added at -40 C. and as soon as solution of the acid chloride was complete, a ml. aliquot was taken, stripped, dissolved in 20 mls. pH 7.0 buffer and sent for bioassay. Bioassay indicated 85% amoxicillin in solution. Two more runs were run under the same conditions and bioassay yields in solution were 82% and 89%. The average yield in solution was 85%.

EXAMPLE 4 Substitution in the procedure of Example 3 for the D()-2-(4-hydroxyphenyl)glycyl chloride hydrochloride of an equimolar weight of another acid chloride produces epicillin by the use of D-()-2-amino-2-amino-2-(1,4-cyclohexadien-l-yl)acetyl chloride hy drochloride and cyclacillin by the use of l-aminocyclohexanecarboxyl chloride hydrochloride and methicillin by the use of 2.6-dimethoxybenzoyl chloride and nafcillin by the use of 2-ethoxy-l-naphthoyl chloride and oxacillin by the sue of 5methyl-3-phenyl-4-isoxazole-carbonyl chloride and cloxacillin by the use of 5 -methyl-3-(2 ChlorophenyD-4-isoxazole-carbonyl chloride and dicloxacillin by the use of 5-methyl-3- (2',6-dichlorophenyl)-4-isoxazole-carbonyl chloride and flucloxacillin (floxacillin) by the use of 5-methyl-3- (2'-chloro-6'-fluorophenyl)-4-isoxazole-carbonyl chloride and indanyl carbenicillin by the use of S-indanyl phenylmalonyl chloride and 6-[D-B-(3-guanyl-1- ureido)-phenylacetamido]-penicillanic acid by the use of D-a-(3-guanyl-1-ureido)phenylacetyl chloride hydrochloride and levopropylcillin by the use of (-)-2- phenoxybutyryl chloride and sulfocillin (sulbenicillin; sulfobenzylpenicillin) by the use of asulphophenylacetyl chloride and azidocillin by the use of D-(-)-aazidophenylacetyl chloride and 3,4-dichloro-amethoxybenzylpenicillin by the use of 3,4-dichloro-amethoxyphenylacetyl chloride and 6-[D-m-chloro-phydroxyphenylacetamidolpenicillanic acid (U.S. Pat. No. 3,489,746) by the use of D-(-)-2-m-chloro-phydroxyphenylglycyl chloride hydrochloride and 6-[D- a-amino-(2-

thienyl)acetamido] penicillanic acid by the use of D-(-)-a-(2-thienyl)-glycyl chloride hydrochlo ride and 6-[D-a-amino-(3-thienyl)acetamido1peniciL lanic acid by the use of D-(-)-2(3-thienyl)glycyl chloride hydrochloride.

The amphoteric penicillins are isolated by the procedure of Example 2 and the others by conventional methods, e.g. extraction into alkaline water and backextraction at an acidic pH into a water-immiscible organic solvent from which, after drying the solution, they are precipitated in salt form as by the addition of sodium 2-ehtylhexanoate.

We claim:

1. In the process for producing a synthetic penicillin from a natural penicillin by the consecutive steps of a. forming a solution in an anhydrous, unreactive organic solvent of a silyl ester of said natural penicillin in the presence of a weak tertiary amine,

b. adding at below 0 C. a halogenating agent to form a solution of the imino-halide,

c. mixing said solution at below 20" C. with alcohol to form a solution of the imino-ether,

d. mixing said solution with water to produce 6- aminopenicillanic acid in a biphasic system,

c. isolating said 6-aminopenicillanic acid as a solid,

f. redissolving it in a solvent and g. adding thereto a carboxylic acid chloride as an acylating agent to produce said synthetic penicillin, the improvment which comprises maintaining the imino-ether solution in the hydrolysis step at 50C.

while adding a volume of water no greater than 10% of the volume of the iminoether solution to produce a single phase containing 6-aminopenicillanic acid which is then, without intermediate isolation of the 6-aminopenicillanic acid, acylated at about 40 C.

after the addition of a weak tertiary amine to produce said synthetic penicillin.

2. The process of claim 1 wherein the synthetic penicillin so produced is ampicillin and the acylating agent is D-()-2-phenylglycyl chloride hydrochloride.

3. The process of claim 1 wherein the synthetic penicillin so produced in amoxicillin and the acylating agent is D-(-)-2-p-hydroxyphenylglycyl chloride hydrochloride.

4. The process of claim 1 wherein the synthetic penicillin so produced is epicillin and the acylating agent is D-(-)-2-amino-2-(l ,4-cyclohexadien-l-yl)acetyl chloride hydrochloride.

5. The process of claim 1 wherein the synthetic penicillin so produced is cyclacillin and the acylating agent is 1aminocyclohexanecarboxyl chloride hydrochloride.

6. The process of claim 1 wherein the synthetic penicillin so produced is methicillin and the acylating agent is 2,6-dimethoxybenzoyl chloride.

7. The process of claim 1 wherein the synthetic penicillin so produced is nafcillin and the acylating agent is 2-ethoxy-l-naphthoyl chloride.

8. The process of claim 1 wherein the synthetic penicillin so produced is oxacillin and the acylating agent is 5-methyl-3phenyl4-isoxazole-carbonyl chloride.

9. The process of claim 1 wherein the synthetic penicillin so produced is cloxacillin and the acylating agent is 5-methyl-3-(2-chlorophenyl)-4-isoxazolecarbonyl chloride.

10. The process of claim 1 wherein the synthetic penicillin so produced is dicloxacillin and the acylating agent is 5-methyl-3-(2',6'-dichlorophenyl)-4- isoxazolecarbonyl chloride.

11. The process of claim 1 wherein the synthetic pen- I 5 ating agent is, S-indanyl phenylmalonyl chloride.

13. The process of claim 1 wherein the synthetic penicillin so produced is 6-[D-a-(3-guanyl-l-ureido)-phenylacetamido]-penicillanic acid and the acylating agent is D-a-(3-guanyl-1-ureido)phenylacetyl chloride hydrochloride.

14. The process of claim 1 wherein the synthetic penicillin so produced is levopropylcillin and the acylating agent is (-)-2-phenoxybutyryl chloride.

15. The process of claim 1 wherein the synthetic penicillin so produced is sulfocillin and the acylating agent is a-sulphophenylacetyl chloride.

16. The process of claim 1 wherein the synthetic penicillin so produced is azidocillin and the acylating agent is D-(-)-a-axidophenylacetyl chloride.

17. In the process for producing a synthetic penicillin from penicillin G or penicillin V by the consecutive steps of a. forming a solution in an anhydrous, unreactive organic solvent of a silyl ester of said penicillin in the presence of a weak tertiary amine,

b. adding at below 20 C. an acid halide to form a solution of the imino-halide,

c. mixing said solution at below -40 C, with a lower alkanol to form a solution of the imino-ether,

d. mixing said solution with water to produce 6- aminopenicillanic acid in a biphasic system,

e. isolating said 6-aminopenicillanic acid as a solid,

f. redissolving it in a solvent and g. adding thereto a carboxylic acid chloride as an acylating agent to produce said synthetic penicillin,

5 the improvement which comprises maintaining the I imino-ether solution in the hydrolysis step at 50 C. while adding a volume of water about 2.5 to 6% of the volume of the imino-ether solution to produce a single phase containing 6-aminopenicillanic acid 10 which is then, without intermediate isolating of the 6-aminopenicillanic acid, acylated at about 40 C. after the addition of a weak tertiary amine to produce said synthetic penicillin.

18. The process of claim 17 wherein the synthetic penicillin so produced is ampicillin and the acylating agent is D-(-)-2-phenylglycyl chloride hydrochloride.

19. The process of claim l7-wherein the synthetic penicillin so produced is amoxicillin and the acylating agent is D-(-)2-p-hydroxyphenylglycyl chloride hydro- 20 chloride. i 20. The process of claim 17 wherein the synthetic penicillin so produced is epicillin and the acylating agent is D-(-)-2-amino-2-(l ,4-cyclohexadien- 1 yl)acetyl chloride hydrochloride.

25 21. The process of claim 17 wherein the synthetic penicillin so produced is cyclacillin and theacylating agent is 1aminocyclohexanecarboxyl chloride hydrochloride.

22. The process of claim 17 wherein the synthetic penicillin so produced is methicillin and the acylating agent is 2,6-dimethoxybenzoyl chloride.

23. The process of claim 17 wherein the synthetic penicillin so produced is nafcillin and the acylating agent is 2-ethoXy-l-naphthoyl chloride.

24. The process of claim 17 wherein the synthetic penicillin so produced is oxacillin and the acylating agent is 5-methyl-3phenyl4-isoxazole-carbonyl chloride.

25. The process of claim 17 wherein the synthetic penicillin so produced is cloxacillin and the acylating agent is 5-methyl-3-(2-chlorophenyl)-4- isoxazolecarbonyl chloride. I

26. The process of claim 17 wherein the synthetic penicillin so produced is dicloxacillin and the acylating agent is 5-methyl-3-(2',6'-dichlorophenyl)-4- isoxazolecarbonyl chloride.

27. The process of claim 17 wherein the synthetic penicillin so produced is flucloxacillin and the acylating agent is 5-methyl-3-(2'-chloro-6-fluorophenyl)-4- isoxazolecarbonyl chloride.

28. The process of claim 17 wherein the synthetic penicillin so produced is indanyl carbenicillin and the acylating agent is S-indanyl phenylmalonyl chloride.

29. The process of claim 17 wherein the synthetic penicillin so produced is 6-[D-a-(3-guanyl-l-ureido)-phenylacetamido]penicillanic acid and the acylating agent is D-a-(3-guanyl-l-ureido)phenylacetyl chloride hydrochloride.

30. The process of claim 17 wherein the synthetic penicillin so produced is levopropylcillin and the acylating agent is (-)-2-phenoxybutyryl chloride.

31. The process of claim 17 wherein the synthetic penicillin so produced is sulfocillin and the acylating agent is a-sulphophenylacetyl chloride.

32. The process of claim 17 wherein the synthetic penicillin so produced is azidocillin and the acylating agent is D-(-)-a-azidophenylacetyl chloride.

l 33. in the process for producing a synthetic penicillin from penicillin G or penicillin \(by the l consecutive c. mixing said solution at below 40 C. with" a lower A alkanolto form a solution of the imino-ethe'r, d. mixing said solution with water to produce 6- aminopenicillanic acid in abiphasic system, 'e. isolating said 6-aminopenicillanic acid as'a solid, f. redissolving it in-a solvent and v i v adding thereto a carboxylic acid chloride as an acylating agent to produce said synthetic penicillin, the improvement which comprises maintaining the iminoether solution'in the, hydrolysis step at -5.0. C.

.while adding a volume of water about-2.5 to 6% of i 35. The process of claim 33wherein the synthetic penicillin so produced is amoxicillin and the acylating agent is D-(-)-2-p-hydroxyphenylglycyl chloride hydrochloride.

36. The process of claim 33 wherein the synthetic penicillin so produced is epicillin and the acylating agent is D-(-)-2-amino-2-(1,4-cyclohexadien-lyl)acetyl chloride hydrochloride.

37. The process of claim 33 wherein the synthetic penicillin so produced is cyclacillin and the acylating agent is l-aminocyclohexanecarboxyl chloride hydrochloride.

38. The process of claim 33 wherein the synthetic penicillin so produced is methicillin and the acylating agent is 2,6-dimethoxybenzoyl chloride.

39. The process of claim 33 wherein the synthetic penicillin so produced is nafcillin and the acylating agent is 2-ethoxy-l-naphthoyl chloride.

40. The process of claim 33 wherein the synthetic penicillin so produced is oxacillin and the acylating agent is 5-methyl-3-phenyl-4-isoxazole-carbonyl chloride.

41. The process of claim 33 wherein the synthetic penicillin so produced is cloxacillin and the acylating agent is 5-methyl-3-(2'-chlorophenyl)-4- isoxazolecarbonyl chloride.

42. The process of claim 33 wherein the synthetic penicillin so produced is dicloxacillin and the acylating agent is 5-methyl-3-(2',6'-dichlorophenyl)-4- isoxazolecarbonyl chloride.

43. The process of claim 33 wherein the synthetic penicillin so produced is flucloxacillin and the acylating agent is 5-methyl-3-(2'-chloro-6'-fluorophenyl)-4- jisoxazolecarbonyl chloride.

44. The process of claim 33 wherein the synthetic penicillin so produced is indanyl carbenicillin and the acylating agent is S-indanyl phenylmalonyl chloride.

45. The process of claim 33 wherein the synthetic penicillin so produced is 6-[D-a-(3-guanyl-1-ureido)- 1s ph e'nylace'ta mido] periic ill'anic acid arid th e acyl'ating agent is D- a-(3-guanyl-l-ureido)phenylacetyl chloride hydrochloride.

46. The process of claim 33 wherein 'thel synthetic p eni cil lin so prodiiced is levopropylcillin and the acylating agent is 2-phen oxybutyryl chloride.

47. The process of claim 33 wl'lereinthe synthetic ,pencillin so produced is sulfocillin and the acylating agentis a-sulphophenylace't'yl chloride.

" 48. The proces s of claim 33 wherein the synthetic penicillin so produced is azidocillin and the acylating agent is Dj-(-)-a-azidophenylacetyl chloride. i

49. In the process for producing a synthetic penicillin ,frorn penicillin V by the consecutive steps of a. forming a solution in anhydrous methylene chloride of a silyl ester of said penicillin V made by re- 1 action of said penicillin V, with dichlorodimethylsi lane or hexarnethyl-disilazane or trimethylchlorosilane in thepresence of dimethylaniline,

b. adding at below 40 C. phosphorus pentachloride to form a solution of the imino-halide,

c. mixing said solution at below -40 C. with methanol to form a solution of the imino-ether,

d. mixing said solution with water to produce 6- aminopenicillanic acid in a biphasic system,

e. isolating said 6-aminopenicillanic acid as a solid,

f. redissolving it in a solvent and g. adding thereto a carboxylic acid chloride as an acylating agent to produce said synthetic penicillin, the improvement which comprises maintaining the imino-ether solution in the hydrolysis step at C.

while adding a volume of water about 2.5 to 6% of the volume of the imino-ether solution to produce a single phase containing 6-aminopenicillanic acid which is then, without intermediate isolation of the 6-aminopenicillanic acid, acylated at about 40 C.

after the addition of dimethylaniline to produce said synthetic penicillin.

50. The process of claim 49 wherein the synthetic penicillin so produced is ampicillin and the acylating agent is D-(-)-2-phenylglycyl chloride hydrochloride.

51. The process of claim 49 wherein the synthetic penicillin so produced is amoxicillin and the acylating agent is D-(-)-2-p-hydroxyphenylglycyl chloride hydrochloride.

52. The process of claim 49 wherein the synthetic penicillin so produced is epicillin and the acylating agent is D-(-)-2-amino-2-(l,4-cyclohexadienl yl)acetyl chloride hydrochloride.

53. The process of claim 49 wherein the synthetic penicillin so produced is cyclacillin and the acylating agent is l-aminocyclohexanecarboxyl chloride hydrochloride.

54. The process of claim 49 wherein the synthetic penicillin so produced is methicillin and the acylating agent is 2,6-dimethoxybenzoyl chloride.

55. The process of claim 49 wherein the synthetic penicillin so produced is nafcillin and the acylating agent is 2-ethoxy-l-naphthoyl chloride.

56. The process of claim 49 wherein the synthetic penicillin so produced is oxacillin and the acylating agent is 5-methyl-3-phenyl-4-isoxazole-carbonyl chloride.

57. The process of claim 49 wherein the synthetic penicillin so produced is cloxacillin and the acylating agent is 5-methyl-3-(2'-chlorophenyl)-4- isoxazolecarbonyl chloride.

58. The process of claim 49 wherein the synthetic penicillin so produced is dicloxacillin and the acylating agent is -methyl-3-(2,6'-dichlorophenyl)-4- isoxazolecarbonyl chloride.

59. The process of claim 49 wherein the synthetic penicillin so produced is flucloxacillin and the acylating agent is 5-methyl-3-(2'-chloro-6-fluorophenyl)-4- isoxazolecarbonyl chloride.

60. The process of claim 49 wherein the synthetic penicillin so produced is indanyl carbenicillin and the acylating agent is S-indanyl phenylmalonyl chloride.

61. The process of claim 49 wherein the synthetic penicillin so produced is 6-[D-a-(3-guanyl-l-ureido)-phenylacetamido]-penicillanic acid and the acylating agent is D-a-(3-guanyl-l-ureido)phenylacetyl chloride hydrochloride.

62. The process of claim 49 wherein the synthetic penicillin so produced islevopropylcillin and the acylating agent is (-)-2-phenoxybutyryl chloride.

63. The process of claim 49 wherein the synthetic penicillin so produced is sulfocillin and the acylating agent is a-sulphophenylacetyl chloride.

64. The process of claim 49 wherein the synthetic penicillin so produced is azidocillin andthe acylating agent is D-(-)-a-azidophenylacetyl chloride.

65. The process of claim 49 wherein the synthetic penicillin so produced is 3,4-dichloro-a-methoxybenzyl penicillin and the acylating agent is 3,4-dichloro-amethoxyphenylacetyl chloride.

66. The process of claim 49 wherein the synthetic penicillin so produced is 6-[D-m-chlor - phydroxyphenylacetamido]penicillanic acid and the acylating agent is D-(-)-2-m-chloro-p-hydroxyphenylglycyl chloride hydrochloride.

67. The process of claim 49 wherein the synthetic penicillin so produced is 6-[D-a-amino-(2-thienyl)-acetamido1penicillanic acid-and the acylating agent is D-(-)-a-(2-thienyl)-glycyl chloride hydrochloride.

68. The process of claim 49 wherein the synthetic penicillin so produced is 6-[D-a-amino-(3-thienyl)-acetamido] penicillanic acid and the acylating agent is D-(-)-2-(3-thienyl)glycyl chloride hydrochloride.

2.4 Detailed Semi-synthetic Penicillin production steps and technologies[3]

2.4.1 General Process of Penicillin Production

15. Culture methods ◎ The fungus can be cultured in two methods ,namely Surface culture method Submerged culture method

16. Surface culture method ◎ In surface culture method ,the fungus is cultured on the surface of a liquid medium without agitation. ◎ After an appropriate incubation period ,the penicillin is extracted from the medium . ◎ This is an old method .

16. سطح ◎ ثقافة واساليب يمكن المثقفين فى طريقتين, وهى طريقة غمر سطح الثقافة الثقافة طريقة فطر. ◎ الثقافة طريقة السطحية الثقافة طريقة فطر هو المثقفين وذو سطح السائل متوسطة دون انفعال ◎ .بعد فترة حضانة مناسبة للنهار البنسلين يستخرج من المتوسطة ◎ .وهذا الاسلوب القديم.

[3] Fatima Antar

17. Submerged culture method ◎ In submerged culture method ,the fungus is grow in a liquid medium which is vigorously aerated and agitated. ◎ After an appropriate incubation period ,the penicillin is separated from the medium . ◎ Today penicillin is produced by the submerged method .

18. Production process medium fermentation centrifugation filtration Solvent extraction precipitation crystallization

FERMENTATION • It is done in a fed-batch mode as glucose must not be added in high amounts at the beginning of growth (which will result in low yield of penicillin production as excessive glucose inhibit penicillin production). • The fermentation conditions for the Penicillium mold, usually requires temperatures at 20-24°C while pH conditions are kept at 6.5 • The pressure in the bioreactor is much higher than the atmospheric pressure (1.02atm). This is to prevent contamination from occurring as it prevents external contaminants from entering. • It is necessary to mix the culture evenly throughout the culture medium. Fungal cells are able to handle rotation speed of around 200 rpm.

19. Media formulation ◎ Ph6.5 ◎ Temperature 20-24c ◎ Oxygen ◎ Nitrogen (corn steep liquor 8.5%) ◎ Glucose 1% (preferred for penicillium notatum) ◎ 80% ethanol ◎ Phenyl acetic acid ◎ Probenecid

20. ◎ Lactose 1% ◎ Calcium carbonate 1% ◎ Sodium hydrogen phosphate 0.4% ◎ Antifoaming agent : Vegetable oil

.17غمر ◎ الثقافة فى غمر طريقة طريقة, فان الثقافة هى تنمو فى السائل aerated الواسطة بقوة واثاروها ◎ .بعد فترة حضانة مناسبة للنهار البنسلين مفصولة عن متوسط ◎ .اليوم البنسلين الذى تنتجه المغمورة.

.18عملية الانتاج التخمر النابذة مذيب تصفية متوسط هطول الامطار بلورة 19

استخراج صياغة ◎ الوسائط ◎ ◎ Ph6.5 الحرارة 20-24ج ◎ الاكسجين والنتروجين (كورن الانحدار ◎ الخمر 8.5% الجلوكوز 1% (يفضل 80% notatum penicillium) ◎ ايثانول Probenecid ◎ ◎

استنتجت بان فرض مراقبة دولية على انهيدريد حمض 20 ◎ .اللاكتوز والغلوتين ◎ كربونات الكالسيوم 1% 1% فوسفات الصوديوم ◎ ◎ الهيدروجين 0.4% Antifoaming عميل: الزيوت النباتي

21. Heat sterilization ◎121 degree celcius at 30 psi (pounds per square inch). ◎For high temperature short time for sterilization is used to minimize the degradation of certain components of media.

22. FERMENTATION ◎ Usually done by fed-batch mode ◎ High amount of glucose result in low yield of Penicillin. ◎ Temperature : 20 to 24 c ◎ pH : 6.0 to 6.5 units ◎ Pressure : 1.02 atmosphere (higher than atmospheric pressure to prevent contamination)

23. Fermentation: • Sparging of air provided for providing sufficient oxygen required for cell viability. IMPELLER: •Rotor used to increase the pressure and flow of fluid. • Used to mix culture throughout the medium • Fungal cells are hardy • Hence handled at rotation speed around 200rpm FERMENTORS

24. SEED CULTURE: o First done in lab by adding penicillium spores to the liquid medium. o After growth , inoculated into the fermentor. o In some cases spores are directly inoculated into the fermentor. Spore: produced during stress condition

ϖFor الحرارة درجة مائوية التعقيم 30 121ϖ رطل لكل بوصة مربعة (رطل لكل بوصة مربعة). درجة حرارة عالية.

قصيرة التعقيم لتقليل تدهور عناصر معينة من وسائل الاعلام.

22.تخمير θ المعتاد عبر θ ضاق متقطع بقدر عال من الجلوكوز تؤدى الى تدنى غلة البنسلين. درجة الحرارة: 20 θ

24ج 6.0 :pH θ الى 6.5 وحدات الضغط : 1.02 جو) θ اعلى من الضغط الجوى لمنع تلوث) 23. تخمير

* :Sparging قدمت الجوية لتوفير ما يكفى من الاوكسجين اللازم خلية على البقاء. قابض المضخة: * دوار تستخدم لزيادة الضغط تدفق السائل. * استخدام مزيج الثقافة بين متوسطة * الخلايا الفطرية هاردى * ومن ثم معالجتها دوران حوالى 200 لفة فى الدقيقة FERMENTORS 24بذرة الثقافة o :اولا فى المعمل باضافة penicillium

25. FILTERATION: ◎ Rotary vaccum filter is used for large scale production. ◎ To remove biomass such as fungus, other impurities from the medium. ◎Phosphoric acid is added pH become 8.5 ◎This can leads to the loss of penicillin activity. ◎Thus pH is maintained at 6.0 to 6.5. ROTARY VACCUM FILTER

26. Addition of solvents : ◎ AMYL ACETATE or BUTYL ACETATE is added to dissolve penicillin in filtrate. ◎ Now, penicillin is present in the form of solution. ◎ Other solids are considered as wastes.

27. CENTRIFUGAL EXTRACTION: ◎ Tubular bowl or chamber bowl centrifuge is used. ◎ To separate solid waste from liquid component which contains the penicillin. ◎ Supernatent is transferred to downstream process.

28. EXTRACTION PENICILLIN + ACETATE SOLUTION 1.Phosphate buffer 2.Chloroform solution 3.Again phosphate buffer 4.Ether solution Mixed with

29. ◎ Penicillin is present in high concentration in ether solution ETHER SOLUTION CONTAINING PENICILLIN Mixed with SODIUM BICARBONATE Penicillin sodium salt BASKET CENTRIFUGATION ◎Solids are easily removed by basket centrifugation. ◎Penicillin salt is in stable powdered form at room temperature . Basket centrifuge

30. Fluid bed drying: ◎ To remove the moisture present in the penicillin salt. ◎ Hot gas is pumped from the base of the chamber. ◎ Powdered salt is contained in a vaccum chamber. ◎ Results in dried form of penicillin.

31. Storage: ◎Stored in containers in dried environment. ◎Then packaged into ◎Liquid penicillin ◎Penicillin in pills

32. process ◎ Medium (corn steep liquor lactose starter culture Yeast extract (penicillium) pHbuffers minerals) batch fermenter (10 times in 6 days to remove 30% culture add 30% fresh medium)

33. rotating filter filtrate fungal cells Dissolve in butyl acetate animal feed Potassium ions added to Precipitate salt of penicillin Wash, filter and dry 99.55% pure penicillin

25. ◎ FILTERATION: لازالة ◎ .الدوار يستخدم الكهرباء بهواء معكوس و يعمل عامل تصفية واسعة النطاق تصبح This◎ Phosphoric pH◎ الكتلة الحيوية مثل الفطريات وغيرها والشوائب من الوسيط. تتم اضافة حمض على 6.0 الى 6.5. الدوار 26 الكهرباء بهواء Thus pH◎ .8.5 يمكن ان يؤدى الى فقدان البنسلين النشاط خلات رصاص ثنائى او استبدل فيها شق بالميثايل ◎ AMYL :معكوس و يعمل عامل تصفية اضافة المذيبات الان البنسلين فى شكل الحل. المواد الصلبة ◎ .خلات رصاص ثنائى الى حل البنسلين فى تسلل فلسطينى بلباس تعتبر النفايات ◎ الاخرى.

27. فصل النفايات ◎ .استخراج: الوعاء او وعاء انبوبية قاعة ويستخدم جهاز الطرد المركزى ◎ اجهزة الطرد المركزى الصلبة الناتجة عن عنصر السائل الذى يحتوى على البنسلين. نقل Supernatent ◎ النهر.

28. استخراج البنسلين + حل 1.فوسفات خلات رصاص ثنائى المخزن المؤقت 2.الحل 3.جديد الكلوروفورم الفوسفات

البنسلين فى تركيزات عالية فى حل بالاثير الاثير الحل الذى يتضمن البنسلين ممزوجة بيكربونات الصوديوم ◎ .29 شكل المجفف مستقرة Penicillin◎ سلة النابذة ازالتها بسهولة سلة النابذة. الملح Solids◎ البنسلين لملح الصوديوم السائل: التجفيف لازالة الرطوبة الموجودة فى البنسلين الملح. ◎ عند درجة حرارة الغرفة. 30 الطرد سلة سرير المجفف ◎ فى الكهرباء بهواء معكوس و يعمل. نتائج ◎ الغازات الساخنة من قاعدة الحجرة. الملح المجفف ◎ ويضخ شكل البنسلين.

31. التخزين: ◎Stored فى حاويات فى المجفف .◎Liquid ◎Then ◎Penicillin فى حبوب البنسلين فى عملية ◎ 32 - الدفعة)) pHbuffers مستخلص الخميرة starter penicillium متوسطة (كورن ثقافة الخمر حاد اللاكتوز والغلوتين جهاز تخمير المعادن) (10 مرات خلال 6 ايام

الثقافة مستخلص الخميرة starter penicillium الذرة شديدة الانحدار الخمر اللاكتوز والغلوتين ◎) عملية متوسطة الدفعة) جهاز تخمير المعادن) (10 مرات خلال 6 ايام لازالة 30% الثقافة اضافة 30% جديدة) pHbuffers متوسطة) 33

– تتولى تصفية الخلايا الفطرية فى تسلل فلسطينى بلباس حل خلات رصاص ثنائى علف حيوانى استبدل فيها شق بالميثايل ايونات البوتاسيوم ان تعجل ملح البنسلين غسيل الملابس والتنظيف الجاف نسبة 99.55% فلتر محض البنسلين

2.4.2 Inoculum Development

- The preparation of a population of microorganisms from a dormant stock culture to an active state of growth that is suitable for inoculation inthe final production stage is called inoculum development. As a first step in inoculum development, inoculumis taken from a working stock culture to initiate growth in a suitable liquid medium.Bacterial vegetative cells and spores are suspended, usually, in sterile tap water, which is then added to thebroth. In case of nonsporulating fungi and actinomycetes the hyphae are fragmented and then transferredto the broth.Inoculum development is generally done using flask

cultures; flasks of 50 ml to 12 litres may be used andtheir number can be increased as per need. Where needed, small fermenters may be used.Inoculum development is Inoculum

اعداد السكان الكائنات المجهرية من ثقافة الاسهم النائمة الى حالة نشطة للنمو التى تناسب المرحلة النهائية - مخزونا من inoculum inoculumis التنمية. وكخطوة اولى فى التنمية inoculum للانتاج جاءت التطعيم يدعى الجراثيم البكتيرية الخلايا النباتى معلقة عادة فى جدل عقيم .medium الثقافة لبدء النمو بشكل مناسب السائل والفطريات الشعاعية مجزاة, ثم nonsporulating hyphae فى حالة الفطريات .thebroth ماء, قبل اضافته الى الثقافات; قوارير من 50 مل الى 12 لترا يمكن flask الطبخة.التنمية عموما باستخدام transferredto Inoculum يمكن زيادة العدد حسب الحاجة. عند الضرورة, يمكن استخدام اجهزة تخمير andtheir استخدامها التنمية Inoculum.الصغيرة

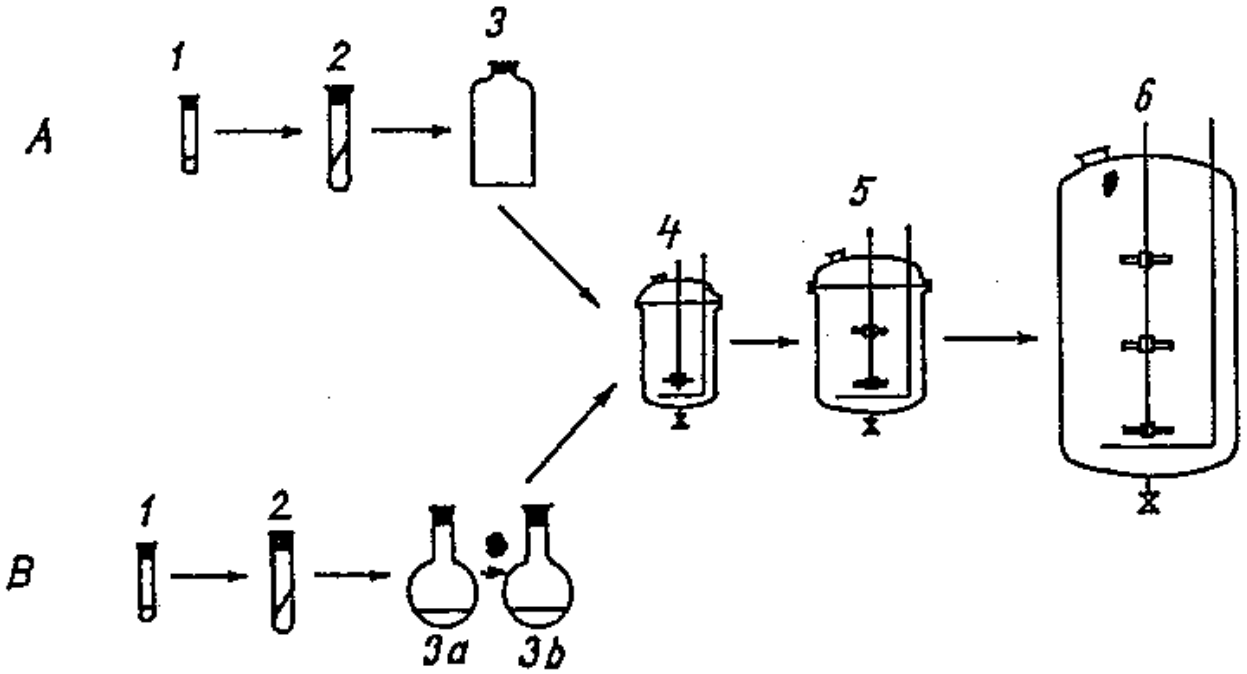
2.4.3 Solid StateFermentation

- In such fermentations,microbial growth and product formation occur at the surface of solid substrates. Examples of suchfermentations are mushroom cultivation, mold ripened cheeses, starter cultures, etc. More recently, thisapproach has been used for the production of extracellular enzymes, certain valuable chemicals, fungaltoxins, and fungal spores (used for biotransformation).Traditional substrates are several agricultural products, rice, wheat, maize, soybean, etc. The substrateprovides a rich and complex source of nutrients, which mayor may not need to be supplemented.Such substrates selectively support mycelial organisms, which can grow at high nutrient concentrations andproduce a variety of extracellular enzymes, e.g., a large number of filamentous fungi, and

الصلبة والتخميرات(مستنبتات الجراثيم المنتج فى سطح تشكيل قوى. ومن امثلة StateFermentation - الخ ومؤخرا استخدمت لانتاج thisapproach الثقافات starter الفطر وتربية العفن تحن والجبن suchfermentations معينة, والبوغات الفطرية fungaltoxins الانزيمات والمواد الكيميائية القيمة extracellular (biotransformation ركازات) التقليدى عدة والمنتجات الزراعية, والارز, والقمح, والذرة الصفراء وفول الصويا, الخ غنية ومعقدة من المغذيات, عمدة قد لا تحتاج الى اكمال ركازات.هذه الكائنات substrateprovides mycelial

مجموعة من الانزيمات, على سبيل extracellular andproduce انتقائية الدعم التى يمكن ان تنمو فى تركيزات عالية

المثال, عددا كبيرا من واختفاء قشريات القاع وظهور الفطريات,



2.4.4 Method of Penicillin Production in Submerged Culture on a Pilot-Plant Scale

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SUMMARY: This paper gives details of a 50 gal. fermentation vessel designed for investigating the formation of antibiotics (or other metabolic products) by micro- organisms grown in submerged culture. This vessel has been used for investigating the submerged culture production of penicillin by Penicillium chrysogenum X 1612 and Q176, and certain results relating to the size of the inoculum and the yields obtainable from these strains in synthetic and other media have been obtained. Culture fluids containing from 400 to 500 Oxford units penicillin/ml. have been obtained

طريقة انتاج البنسيلينيات لشركات من& المغمورة Pilot-Plant الثقافة على نطاق واسع

ا. ج. غوردون جرنفل ا. ب. ج. نولز شارليستون, ر. س. ا. ماكاليستر ومماركت ت. وايت معامل
الابحاث جون برو ويث ,.Ltd.,لندن

SUMNARY:هذه الورقة تفاصيل 50 جالون سفينة مصممة خصيصا بالتحقيق تخمير تشكيل المضادات الحيوية (او منتجات الايض) الكائنات الدقيقة تزرع فى غمر الثقافة. وقد استخدمت هذه السفينة التحقيق المغمورة الثقافة Penicillium انتاج مشتقات البنسلين ,chrysogenum X 1612 Q(176) وبعض النتائج المتصلة بحجم inoculumو مردود يمكن الحصول عليها من هذه السلالات المركبة وغيرها من وسائط الاعلام. الثقافة السوائل التى تحتوى على ما بين 400 الى 500 وحدات اكسفورد البنسلين/ملليلتر الحصول عليها.

with cultures of Q176 in a corn-steep liquor medium. A method of extracting penicillin from the broth has been worked out, based on solvent transfer, the method being applicable on virtually any scale of operation and involving only relatively simple equipment. It has the advantage of reducing the time of contact of penicillin with acid to such a degree that extraction at room temperature is possible, although extraction at still lower temperatures improves the yield. Using this method of extraction we have obtained calcium penicillin with a potency of 940 Oxford unitslmg., the overall recovery from the broth being of the order of 35-50%.

فى الذرة شديدة الانحدار الوسيطة. المشروبات الروحية طريقة استخراج البنسلين من الطبخة قد Q 176 مع ثقافات وضعت استنادا الى طريقة المذيبات ينطبق بالفعل على اى حجم العملية, التى تشمل معدات بسيطة نسبيا. لديه ميزة تقليل وقت اتصال البنسلين والاحماض لدرجة ان استخراج فى درجة حرارة الغرفة, وان كان لا يزال استخراج درجات الحرارة المنخفضة فى تحسين المحاصيل. باستخدام هذا الاسلوب فى استخراج حصلنا على امكانية الكالسيوم البنسلين unitslmg 50–35 من الطبخة التى الانعاش الشامل, اكسفورد%.

2.4.4.1 EXPERIMENTAL Methods and equipment

Analytical methods The course of each fementation was followed by periodic determinations of pH (electrometrically) ; sugar utilization (method of Schaffer & Hartman, 1920) ; ammonia content (micro-Kjedahl) ; and penicillin content (Grenfell et al. 1947). Other features could, of course, have been followed. Over a period of time, however, it was found that changes in the above constituents constituted the data of greatest significance and that from consideration of pH, sugar, and ammonia values it was normally possible to predict whether or not a fermentation was proceeding satisfactorily, and the time at which the culture fluid should be processed to obtain the best yield of penicillin. These two aspects are naturally of importance for production.

اساليب ومعدات تجريبية

السكر (استخدام (طريقة electrometrically pH) قرارات الدورى fementation الاساليب التحليلية كل واعقب المحتوى (الصغيرة) ; البنسلين المحتوى (جرنفل واخرون Kjedahl هارتمان, 1920) ; الامونيا Schaffer & 1947). ميزات اخرى يمكن بالتاكيد ان يتبع. على مدى فترة من الزمن, بيد انه تبين ان التغييرات فى مكونات والسكر الامونيا القيم عادة يمكن التنبؤ بمدى التخمر pH تشكل البيانات ذات الاهمية الكبرى وان من النظر تسير على نحو مرض, والوقت الذى يجب ان يكون سائل الثقافة المصنعة للحصول على افضل عائد البنسلين. وهذان الجانبان اهمية بالطبع للانتاج.

2.4.4.2 Culture media

Synthetic media No. 22A. This medium was developed for use in penicillin production by the Pennsylvania State University group of workers (un- published). The composition is: lactose B.P., 15 g.; glucose B.B., 5 g.; acetic acid (glacial), 4 g.; NH4N0,, 5 g.; KNO,, 3.5 g.; KH,P04, 2 g.; MgSO4,7&O, 0.5 g.; FeS0,,7H20, 0-2 g.; ZnS04,7H,0, 0.04 g.; CuS0,,5H20, 0.005 g.; phenylacetamide, 0.25 g.; water to 1 1. Corn-steep liquor medium. The composition of this medium is : corn-steep liquor (Stahley no. 14), 30 ml.; lactose B.P., 40 g.; CaCO,, 10 g.; phenylacet- amide, 0.25 g. ; water to 1 1. Antifoam (300 ml./200 1. medium) is added before sterilization,

وسائل الاعلام الاصطناعية رقم 22 الف هذه الوسيلة التى استحدثت لاستخدامها فى انتاج البنسيلينيات لشركات من جامعة ولاية بنسلفانيا مجموعة من العمال التابعة للامم المتحدة منشورة). تكوين: عدم تحمل اللاكتوز(B.P., 15 ز(; الجلوكوز باء باء 5 ;g انهيدريد الحمض الريبى النووى (4 g الجليدية ,NH4N0, و 5)ز ;(جرام, نظام الترفيه الاسرى(FeS0(7H20, 0-2 ز(04,7; لتحويل KNO, 3.5 g; KH,P04 2 g; MgSO4,7&O 0.5 المزيد من H,0, 0.04 غرام ;g phenylacetamide, 0.25 ;g CuS0,5H20, 0.005 ;g المياه 1. وزروع ومقام الانحدار الخمر متوسطة. تكوين هذه الوسيلة: الذرة Stahley الخمر حاد (رقم 14) 30 مل, عدم تحمل اللاكتوز باء –

الصفحة 40)ز(, و 10; كاتشو -g; phenylacet اميد, 0.25 غرام. ; المياه 1 300) Antifoam .ملل/200 1

– متوسط) قبل التعقيم

2.4.4.3 Submerged-culture

methods for P. chrysogenum 193 then connected by rubber tubing to the air filter tube of the aspirator and the latter clamped in an inverted position above the fermenter. The fermenter pressure was then lowered to 2 lb. by operating the air exit valve, the inocula- tion valve opened, and air at 30 lb. pressure passed from the branch air line into the aspirator, thus forcing the inoculum into the fermenter. On completion of this process the air passing into the aspirator was turned off, the inoculation nozzle valve closed, and the aspirator disconnected from the nozzle. The nozzle cap was then refitted and steam turned on at the nozzle steam line to resterilize the system from the valve seating upwards. The fermenter pressure was finally readjusted to 5-10 lb

غمرت الطرق ثقافة ص 193 ثم توصيل chrysogenum انابيب مطاطية على انبوبة فلتر الهواء من برابيش جهاز الشفط الاخيرة فى وضع معكوس فوق جهاز تخمير. ثم الضغط على اوعية التخمير 3,790 2 رطل تشغيل صمام الهواء inocula الخروج, الباب مفتوح صمام الهواء ضغط 30 رطل من فرع الخطوط الجوية فى برابيش جهاز الشفط, ويجبر inoculum فى اوعية التخمير. عند الانتهاء من هذه العملية فى الهواء يمر برابيش جهاز الشفط قد تم ايقافه, وتطعيم صمام اغلاق الفوهة, برابيش جهاز الشفط من الفتحة. غطاء الفوهة ثم اعيد تحديدها بخار تشغيل خط الفوهة resterilize حمام بخار صمام النظام للجلوس لاعلى. ان جهاز تخمير الضغط اخيرا للسماح 5–10 رطلا.

A previously sterilized aspirator assembly containing antifoam was then attached to the nozzle and additions of antifoam made when required by the same technique, except that the assembly was left attached to the fermenter throughout the run.

فوهة الاضافات التى antifoam تعقيم برابيش جهاز الشفط ثم ملحق antifoam وكانت الجمعية العامة تتضمن عندما تتطلب نفس الاسلوب, الا ان الجمعية كانت على جهاز تخمير خلال المرحلة

For centuries, the Irish peasants treated themselves with a miraculous preparation of which they had the secret. A few curious comrade scientists have investigated and discovered the miraculous active principle: penicillin ! Impure and small in quantity, certainly, but enough to cure wounds likely to become infected and end in gangrene .

ولقرون من الفلاحين معاملة الايرلندى انفسهم باعجوبة اعداد من السر. فضولى قليلة الرفيق العلماء التحقيق اكتشف مبدا النشطة المعجزة البنسلين! غير نقي وهؤلاء بكميات قليلة, بالتاكيد, لكن من المحتمل ان لعلاج الجروح عدد المصابين بفيروس نقص المناعة البشرى فى يديها وقدميها.

To make this "medicine", the Irish spread a piece of bread with butter and left it to rest for a fortnight in a warm and humid place.

ان تكون "الدواء" الايرلندى انتشار قطعة من الخبز مع الزبدة وتركت الباقين لمدة اسبوعين فى مكان دافئ ورطب.

فعلنا هذه التجربة مرة اخرى البنسلين انفسنا كشعب الايرلندية.

We did this experiment again to make penicillin ourselves, as the Irish did.

Beginning of the experiment: January 6, 2009

Beginning of the mold: January 18,

2.4.5 What is penicillin?

Penicillin is an antibiotic of the beta-lactam family that originated from the mold of a fungus: *Penicillium Notatum* . We now know that penicillin has the formula C9H11N2O4S --- R.

During mold: February 4, 2009

During mold: February 21, 2009

After a fortnight, we saw a greenish mold that is nothing other than penicillin. But we left it longer than expected because our growing medium should not be hot enough or wet enough. It is then sufficient to recover the mold and mix it with water to obtain the preparation of the Irish.

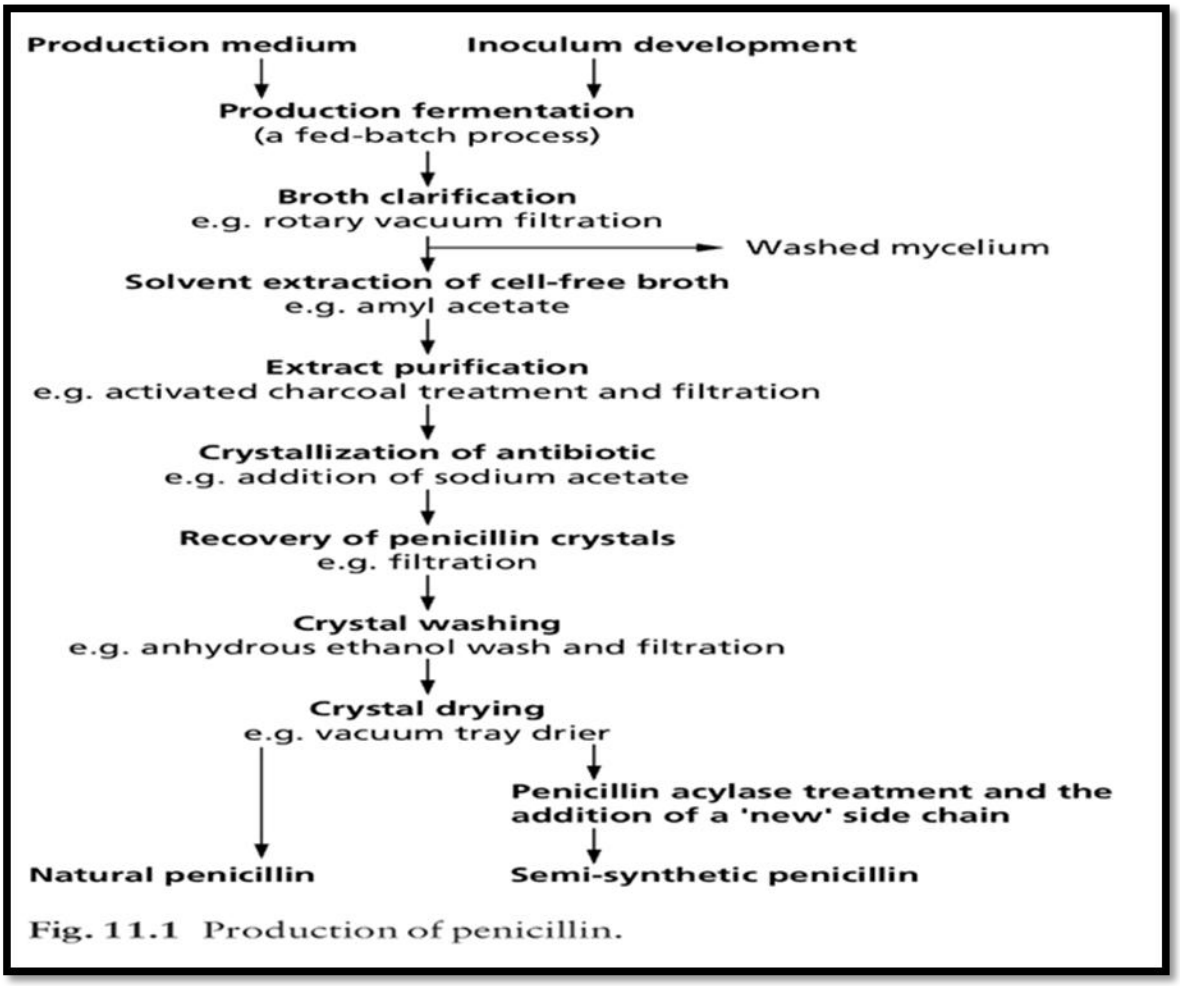


Fig. 11.1 Production of penicillin.

Penicillin production, including glucose, lactose, sucrose, ethanol and vegetable oils. About 65% of the carbon source is metabolized for cellular maintenance, 25% for

growth and 10% for penicillin production. In the past, a mixture of glucose and lactose was used, the former pro-ducing good growth, but poor penicillin yields, whereas the latter had the opposite effect. The mode of 'feeding' of a particular carbon source is vitally important, as it

can influence the production of this secondary metabo-lite (see Chapter 3, Secondary metabolism). Corn steep liquor is still used as a source of nitrogen, additional nutrients and side-chain precursors. Its acidic nature creates a requirement for calcium carbonate (1%, w/v)

and a phosphate buffer to neutralize the medium, there-by optimizing its pH for penicillin production. Ammo-nia, mineral salts and specific side-chain precursors, e.g. phenyl acetic acid or phenoxyacetic acid, may also be added. However, as some precursors are toxic,

they must be fed continuously at non-inhibitory concentrations.

Inoculum development is usually initiated by adding lyophilized spores to a small fermenter at at a concentration of ... spores/ml.

. Fungal mycelium may then be grown up through one or two further stages until there is sufficient to inoculate the production fermenter. Initially, there is a vegetative growth phase devoted to the development of biomass, which doubles every 6 h. This high growth rate is maintained for the first 2 days. To ensure an optimum yield of penicillin in the following production phase, the mycelium must develop as loose pellets, rather than compact forms. During the following production phase, the carbon source is fed at a low

rate and penicillin production increases. This continues for a further 6–8 days, provided that appropriate substrate feeds are maintained. Penicillin is excreted into the medium and is recoveredat the end of fermentation. Whole broth extraction may be performed, but can lead to downstream processing problems, as additional materials leach from the mycelium. Usually, penicillin recovery follows removal of mycelium using rotary vacuum filters, the efficiency of which may be affected by the culture mediacomposition, particularly its proteinaceous compo nents. Recovered mycelium is then washed to remove residual penicillin, prior to its use as animal feed or fertilizer. Antibiotic recovery is often by solvent extraction of the cell-free medium, which gives yields of up to 90%.

This involves reducing the pH of the filtered medium to 2.0–2.5 by addition of sulphuric or phosphoric acid, followed by a rapid two-stage continuous countercurrent extraction at 0–3°C using amyl acetate, butyl acetate or methyl isobutyl ketone. The low temperature is neces-

sary to reduce damage to penicillin due to the low pH. Alternatively, ion-pair extraction may be used at pH 5–7, in which range penicillin is stable. Any pigments

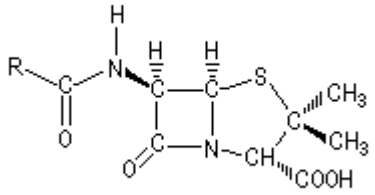
and trace impurities are removed by treating with activated charcoal. The penicillin is then retrieved from the solvent by addition of sodium or potassium acetate. This reduces the solubility of the penicillin and it precipitates as a sodium or potassium salt. Resultant

penicillin crystals are separated by rotary vacuum filtra- tion. Solvent is recovered from the separated liquor and any other materials used, such as the charcoal, which is

very important in terms of the overall economics of the process. Penicillin crystals are mixed with a volatile solvent, usually anhydrous ethanol, butanol or iso-propanol, to remove further impurities. The crystals are collected by filtration and air dried. At this stage the

penicillin is 99.5% pure. This product may be further processed to form a pharmaceutical grade product or is used in the production of semisynthetic penicillins.

2.4.6 But how do you make penicillin today?



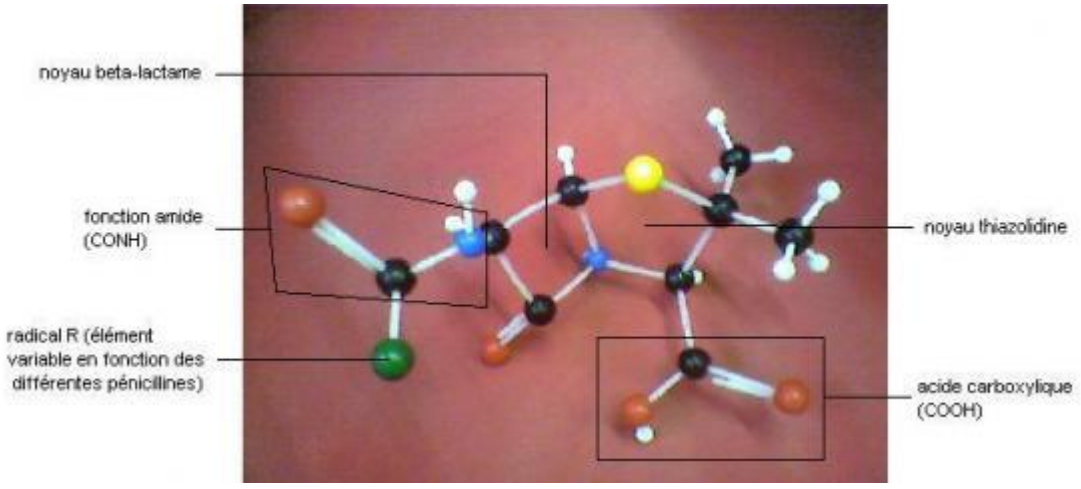
Lewis formula of penicillin

It is composed of two parts:

- Natural penicillin, or penicilloic acid, of formula C8H11N2O3S, corresponding to the fermentation of the fungus.

- The variable radical, of formula R, representing the different proteins that can be grafted synthetically to natural penicillin.

It can be decomposed into several subparts when it is in the form of penicillin: a thiazolidine ring acole at a beta-lactam ring, a carboxylic acid of formula COOH and an amide function of formula CONH.



B. The manufacture of yesterday

It all started on September 4, 1928 when Alexander Fleming, a Scottish doctor, accidentally discovered that a fungus named *Penicillium Notatum* could inhibit the growth of bacteria such as staphylococcus . He will call it "penicillin".

At that time the manufacture of penicillin is based on Fleming's original experience. This method of preparation of the first antibiotic could constitute the scheme of a universal manufacture. In fact, the manufacture of various antibiotics, modeled on that of penicillin, contains three main phases:

The preparation and preservation of the antibiotic-producing microorganism strain,

- His culture,

- The extraction of the antibiotic products of its metabolism.

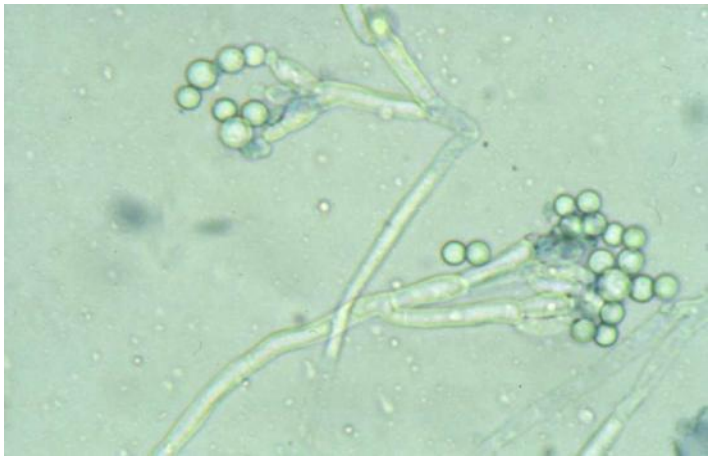
The strain consists of a microorganism, usually a fungus but sometimes a bacterium. It is most often a suitable variety with the best yield, obtained from the most diverse environments, suitably purified and mutated, and kept away from contamination.

اعداد وحفظ لانتاج مضادات حيوية ميكروب السلالة

–الثقافة

–استخراج المضادات الحيوية منتجات دولها الايض.

سلالة يتكون من ميكروب, عادة ما يكون فطر ولكن احيانا بالبكتريا . انه فى الغالب التشكيلة المناسبة العائد الافضل التى تم الحصول عليها من معظم بيئات متنوعة ملائما, مطهر والمتحولة, وابعدت عن التلوث.



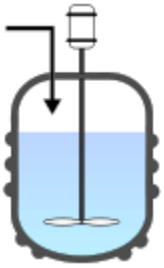
Penicillium Notatum Penicillium Notatum seen under a microscope

The microorganisms constituting the strain are then inoculated in a nutrient medium. The first methods used employed solid nutrient media distributed in thin layers in suitable containers, such as a box of

ruddy, maintained at a suitable temperature, about 20 ° C, in drying ovens. After a few days, an abundant fruiting of the mold is obtained, which is separated from the support medium. The latter, which contains the products of the *Penicillium* metabolism , is then treated for the extraction of the antibiotic.

من الكائنات المجهرية التى تشكل ضغطا على ثم تحصينهم المغذيات الوسيطة. اول الاساليب المستخدمة توزيع المواد الغذائية الصلبة الاعلام طبقات رقيقة فى اوعية مناسبة, مثل صندوق رودى , الحفاظ على درجة حرارة ملائمة على بعد حوالى 20 °C افران التجفيف, فى. وبعد بضعة ايام, وامل وفيرة العفن ويتم التى يفصلها عن دعم متوسط. وهذا النظام الذى يحتوى على منتجات الايض , Penicillium ثم تعامل لاستخراج المضادات الحيوية.

2.4.7 Fed-batch culture



Fed-batch culture is, in the broadest sense, defined as an operational technique in biotechnological processes where one or more nutrients (substrates) are fed (supplied) to the bioreactor during cultivation and in which the product(s) remain in the bioreactor until the end of the run.[1] An alternative description of the method is that of a culture in which "a base medium supports initial cell culture and a feed medium is added to prevent nutrient depletion".[2] It is also a type of semi-batch culture.

In some cases, all the nutrients are fed into the bioreactor. The advantage of the fed-batch culture is that one can control concentration of fed-substrate in the culture liquid at arbitrarily desired levels (in many cases, at low levels).

Generally speaking, fed-batch culture is superior to conventional batch culture when controlling concentrations of a nutrient (or nutrients) affect the yield or productivity of the desired metabolite.

وتعرف الثقافة الفدرالية ، بمعناها الواسع، بأنها تقنية تشغيلية في العمليات التكنولوجية الحيوية حيث يتم تغذية واحد أو أكثر من العناصر المغذية (الركيزة) بالمفاعل الحيوي أثناء الزراعة والتي يظل فيها المنتج (المنتجات) في المفاعل الحيوي حتى نهاية المدى. [1] وصف بديل لهذه الطريقة هو أن الثقافة التي يتم فيها إضافة "وسيط أساسي يدعم زراعة الخلايا الأولية ووسيلة تغذية لمنع استنزاف المغذيات". [2] وهو أيضا نوع من الثقافة شبه دفعة . في بعض الحالات، يتم تغذية جميع العناصر الغذائية في المفاعل الحيوي. وميزة ثقافة التغذية المتدفقة هي أنه يمكن للمرء أن يسيطر على تركيز الركيزة المغذية في سائل الثقافة عند مستويات مطلوبة عشوائيا (في كثير من الحالات، عند مستويات منخفضة.(

وبصفة عامة، تتغذى ثقافة التغذية المجمعة على ثقافة الدفعات التقليدية عندما تؤثر تركيزات المغذيات (أو المغذيات) على غلة أو إنتاجية المستقلب المطلوب.

2.4.8 Fermentation

Fermentation for penicillin is usually done in the fed-batch mode as glucose must not be added in high amounts at the beginning of growth which will result in low yield of penicillin production as excessive glucose inhibit penicillin production. In addition to that, penicillin is a secondary metabolite of the fungus, therefore, the fed-batch mode is ideal for such products as it allows the high production of penicillin. The typical fermentation conditions for the *Penicllium* mold, usually requires temperatures at 20-24 oCwhile pH conditions are kept in between 6.0 to 6.5. The pressure in the bioreactor is usually much higher than the atmospheric pressure(1.02atm) this is to prevent contamination from occurring as it prevents external contaminants from entering. Sparging of air bubbles is necessary to provide sufficient oxygen the viability of the fungus. Depending on the volume of medium, for 2 cubic metres of culture, the sparging rate should be about 2.5 cubic metres per minute. The impeller is necessary to mix the culture evenly throughout the culture medium, fungal cells are much hardy and they are able to handle rotation speed of around 200rpm.

2.4.9 Seed culture

Like any other scale up process, usually the seed culture is developed first in the lab by the addition of *Penicillium* spores into a liquid medium. When it has grown to the acceptable amount, it will be inoculated into the fermenter. In some cases,the spores are directly inoculated into the fermenter.

2.4.10 Removal of biomass

Filtration is necessary at this point of the bioprocess flow, as bioseparation is required to remove the biomass from the culture such as the fungus and other impurities away from the medium which contains the penicillin product. There are many types of filtration methods available today, however, the Rotary vacuum filter is commonly employed as it able to run in continuous mode in any large scale operations. Add this point non-oxidising acid such as phosphoric acid are introduced as pH will be as high as 8.5. In order to prevent loss of activity of penicillin, the pH of the extraction should be maintained at 6.0-6.5

2.4.11 Adding of solvent

In order to dissolve the penicillin present in the filtrate, organic solvents such as amyl acetate or butyl acetate are use as they dissolve penicillin much better than water at physiological pH. At this point, penicillin is present in the solution and any other solids will be considered as waste.

2.4.12 Materials

2.4.12.1 Amyl acetate

Amyl acetate (pentyl acetate) is an organic compound and an ester with the chemical formula CH3COO[CH2]4CH3 and the molecular weight 130.19 g/mol. It has a scent similar to bananas[3] and apples.[4] The compound is the condensation product of acetic acid and 1-pentanol. However, esters formed from other pentanol isomers (amyl alcohols), or mixtures of pentanols, are often referred to as amyl acetate.

Uses

It is used as a flavoring agent, as a paint and lacquer solvent, and in the preparation of penicillin.

It is an inactive ingredient in Liquid Bandages.[1]

OVERVIEW

Amyl acetate (A-mil AS-uh-tate) is a colorless liquid with a distinctive banana-like flavor and odor. Three major isomers of amyl acetate exist: normal (n-amyl), secondary (secamyl), and isoamyl (3-methyl-1-butyl) acetate. Isomers are two or more forms of a chemical compound with the same molecular formula, but different structural formulas and different chemical and physical properties. As an example, the boiling points of the three isomers of amyl acetate are 149.2°C (300.6°F), 142.0°C (287.6°F), and 140.0°C (284.0°F), respectively. Although the amyl acetates are probably best known as flavoring agents because of their distinctive banana-like flavor, they all have a number of interesting industrial applications also.

KEY FACTS

OTHER NAMES:

Pentyl acetate; acetic acid, amyl ester

FORMULA:

CH3COOC5H11

ELEMENTS:

Carbon, hydrogen, oxygen

COMPOUND TYPE:

Ester (organic)

STATE:

Liquid

MOLECULAR WEIGHT:

130.18 g/mol

MELTING POINT:

−70.8°C (−95.4°F)

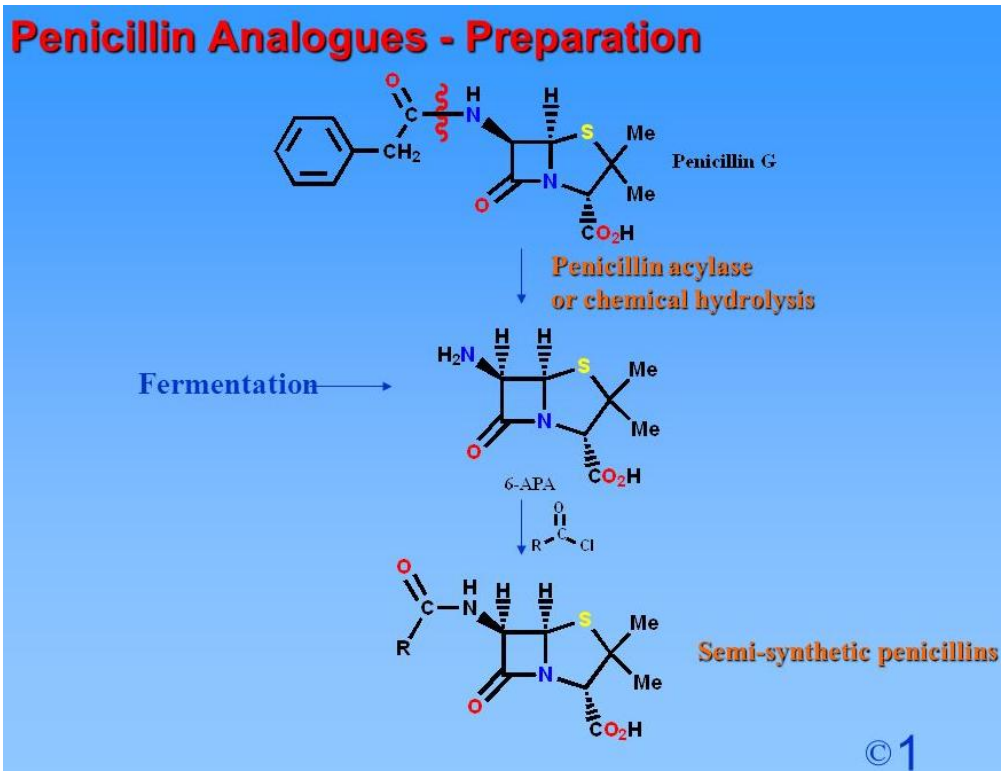
BOILING POINT:

149.2°C (300.6°F)

SOLUBILITY:

Slightly soluble in water; soluble in alcohol, ether, and most organic solvents

Amyl acetate Charcoal is the lightweight black carbon and ash residue produced by removing water and other volatile constituents from animal and vegetation substances. Charcoal is usually produced by slow pyrolysis — the heating of wood or other substances in the absence of oxygen (see char and biochar).



Commercial Production Of Penicillin

- Like all antibiotics, penicillin is a secondary metabolite, so is only produced in the stationary phase.

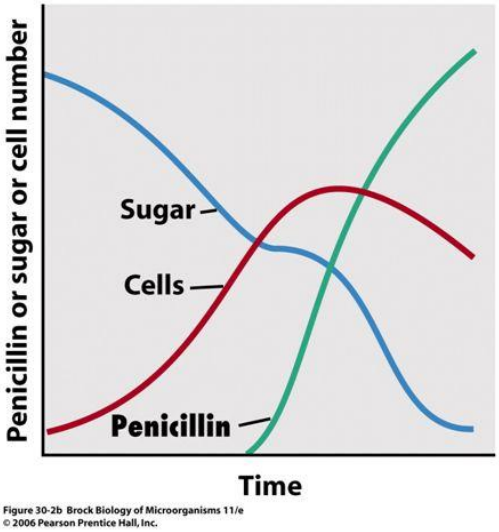


Figure 30-2b Brock Biology of Microorganisms 11/e
© 2006 Pearson Prentice Hall, Inc.

2.4.12.2 Charcoal

Charcoal is the lightweight black carbon and ash residue produced by removing water and other volatile constituents from animal and vegetation substances. Charcoal is usually produced by slow pyrolysis — the heating of wood or other substances in the absence of oxygen (see char and biochar).

Carbon source

Charcoal may be used as a source of carbon in chemical reactions. One example of this is the production of carbon disulphide through the reaction of sulfur vapors with hot charcoal. In that case the wood should be charred at high temperature to reduce the residual amounts of hydrogen and oxygen that lead to side reactions.

Purification and filtration

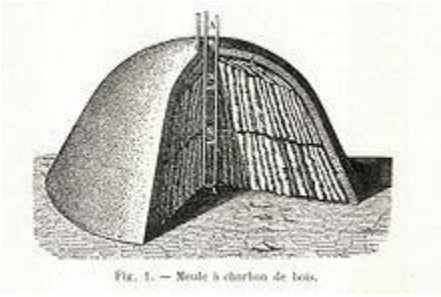
Activated carbon

Charcoal may be *activated* to increase its effectiveness as a filter. Activated charcoal readily adsorbs a wide range of organic compounds dissolved or suspended in gases and liquids. In certain industrial processes, such as the purification of sucrose from cane sugar, impurities cause an undesirable color, which can be removed with activated charcoal.

It is also used to absorb odors and toxins in gases, such as air. Charcoal filters are also used in some types of gas masks. The medical use of activated charcoal is mainly the absorption of poisons.[8] Activated charcoal is available without a prescription, so it is used for a variety of health-related applications. For example, it is often used to reduce discomfort and embarrassment due to excessive gas (flatulence) in the digestive tract.[9]

Animal charcoal or bone black is the carbonaceous residue obtained by the dry distillation of bones. It contains only about 10% carbon, the remainder being calcium and magnesium phosphates (80%) and other inorganic material originally present in the bones. It is generally manufactured from the residues obtained in the glue and gelatin industries. Its decolorizing power was applied in 1812 by Derosne to the clarification of the syrups obtained in sugar refining; but its use in this direction has now greatly diminished, owing to the introduction of more active and easily managed reagents. It is still used to some extent in laboratory practice. The decolorizing power is not permanent, becoming lost after using for some time; it may be revived, however, by washing and reheating. Wood charcoal also to some extent removes coloring material from solutions, but animal charcoal is generally more effective.

Medicine



Charcoal pile

Charcoal was consumed in the past as dietary supplement for gastric problems in the form of charcoal biscuits. Now it can be consumed in tablet, capsule or powder form, for digestive effects.[12] Research regarding its effectiveness is controversial.[13] To measure the mucociliary transport time the use was introduced by Passali in combination with saccharin.[14]

Red colobus monkeys in Africa have been observed eating charcoal for the purposes of self-medication. Their leafy diets contain high levels of cyanide, which may lead to indigestion. So they learned to consume charcoal, which absorbs the cyanide and relieves indigestion. This knowledge about supplementing their diet is transmitted from mother to infant.[15]

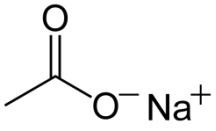
Degradation

The bacterium *Diplococcus* degrades charcoal, thereby raising charcoal's burning temperature.

2.4.12.3 Sodium acetate

Sodium acetate, CH3COONa, also abbreviated NaOAc,[8] also known as sodium ethanoate, is the sodium salt of acetic acid. This colorless deliquescent salt has a wide range of uses.

مركب كيميائي **أسيتات الصوديوم** أو **خلات الصوديوم** له الصيغة المجملة $C_2H_3NaO_2$ ، ويكتب عادة بالصيغة $CH_3COO^- Na^+ .3H_2O$ أو يمكن كتابتها بالشكل التالي ، $Na(CH_3COO) \cdot 3\ H_2O$.



-Applications

.Industrial

Sodium ethanoate is used in the textile industry to neutralize sulfuric acid waste streams and also as a photoresist while using aniline dyes. It is also a pickling agent in chrome tanning and helps to impede vulcanization of chloroprene in synthetic rubber production. In processing cotton for disposable cotton pads, sodium acetate is used to eliminate the buildup of static electricity.

Concrete longevity

Sodium ethanoate is used to mitigate water damage to concrete by acting as a concrete sealant, while also being environmentally benign and cheaper than the commonly used epoxy alternative for sealing concrete against water permeation.[9]

.Food

Sodium ethanoate may be added to food as a seasoning, sometimes in the form of sodium diacetate, a one-to-one complex of sodium acetate and acetic acid,[10] given the E-number E262. It is often used to give potato chips a salt and vinegar flavor.

.Buffer solution

As the conjugate base of acetic acid, a solution of sodium acetate and acetic acid can act as a buffer to keep a relatively constant pH level. This is useful especially in biochemical applications where reactions are pH-dependent in a mildly acidic range (pH 4-6).

- له العديد من التطبيقات المخبرية في الكيمياء التحليلية، على سبيل المثال في محاليل موقية (buffer) وذلك لضبط أس هيدروجيني الوسط.
- يستخدم بشكله اللامائي كمادة ساحبة للماء في الاصطناع العضوي.
- له تطبيق في الوسائد الحرارية ، والتي تحوي محلول فوق مشبع من هذا الملح والذي يمتاز بأن له القدرة على التبرد السريع لدرجة حرارة الغرفة دون أن يشكل بلورات.

بالضغط على قرص معدني في الوسادة تتشكل نواة تبلور مما يؤدي إلى تبلور المحلول بكامله. وبما أن عملية بلورة هذا الملح ناشرة للحرارة نحصل على الحرارة المطلوبة.

- يستخدم كمادة منظمة للحموضة في الإضافات الغذائية. E 262

-Reactions

Sodium acetate can be used to form an ester with an alkyl halide such as bromoethane:

$$CH3COONa + BrCH2CH3 \rightarrow CH3COOCH2CH3 + NaBr$$

Caesium salts catalyze this reaction.

-Nam

.IUPAC name

Sodium acetate

.Systematic IUPAC name

Sodium ethanoate

.Other names

Hot ice (Sodium acetate trihydrate)

-Properties

.Chemical formula

C2H3NaO2

.Molar mass	82.03 g·mol−1	Masse molaire2	82,0338 ± 0,0024 g/mol

C 29,28 %, H 3,69 %, Na 28,02 %, O 39,01 %,

136,08 g/mol (trihydrate)

.pKa	4,75 (pKb = 9.25)
.Density	1.528 g/cm3 (20 °C, anhydrous)

1.45 g/cm3 (20 °C, trihydrate)[2

Melting point	324 °C (anhydrous)	(615 °F;	597 K)
	58 °C (trihydrate)	(136 °F;	331 K)
Boiling point	881.4 °C	(1,618.5 °F;	1,154.5 K)

(anhydrous)		
122 °C	(252 °F;	395 K)
(trihydrate) decomposes		

2.4.12.4 Ethanol

Ethanol, also called alcohol, ethyl alcohol.

Properties	
Chemical formula	C_2H_6O
Molar mass	46.07 g·mol^{-1}
Appearance	Colorless liquid
Density	0.7893 g/cm^3 (at 20 °C)[2]
Melting point	−114.14 ± 0.03[2] °C (−173.45 ± 0.05 °F; 159.01 ± 0.03 K)
Boiling point	78.24 ± 0.09[2] °C (172.83 ± 0.16 °F; 351.39 ± 0.09 K)
Solubility in water	miscible
log *P*	−0.18
Vapor pressure	5.95 kPa (at 20 °C)
Acidity (pK_a)	15.9 (H_2O), 29.8 (DMSO)[3][4]
Magnetic susceptibility (χ)	−33.60·10^{-6} cm^3/mol
Refractive index (n_D)	1.3611[2]
Viscosity	1.2 mPa·s (at 20 °C), 1.074 mPa·s (at 25 °C)[5]
Dipole moment	1.69 D[6]

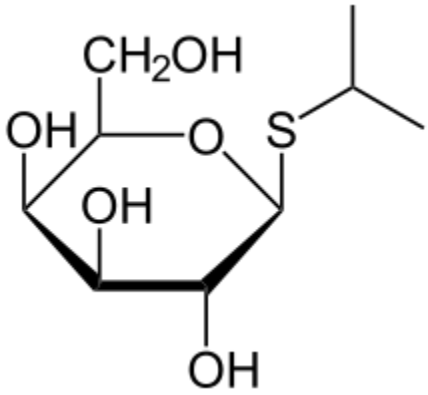
Physical properties


Ethanol burning with its spectrum depicted

Ethanol is a volatile, colorless liquid that has a slight odor. It burns with a smokeless blue flame that is not always visible in normal light. The physical properties of ethanol stem primarily from the presence of its hydroxyl group and the shortness of its carbon chain. Ethanol's hydroxyl group is able to participate in hydrogen bonding, rendering it more viscous and less volatile than less polar organic compounds of similar molecular weight, such as propane.

Ethanol is slightly more refractive than water, having a refractive index of 1.36242 (at λ=589.3 nm and 18.35 °C or 65.03 °F).[47] The triple point for ethanol is 150 K at a pressure of 4.3×10^{-4} Pa.[48]

2.4.12.5 Isopropyl β-D-1-thiogalactopyranoside (IPTG)

Isopropyl β-D-1-thiogalactopyranoside

Identifiers

CAS Number	• 367-93-1
3D model (JSmol)	• Interactive image
ChemSpider	• 571154
ECHA InfoCard	100.006.094
MeSH	Isopropyl+Thiogalactoside
PubChem CID	• 656894
InChI[show]	
SMILES[show]	
Properties	
Chemical formula	$C_9H_{18}O_5S$
Molar mass	238.30 g·mol^{-1}
Except where otherwise noted, data are given for materials in their standard state (at 25 °C [77 °F], 100 kPa).	
Infobox references	

Propriétés physiques	
T° fusion	105 °C[2]

Isopropyl β-D-1-thiogalactopyranoside (**IPTG**) is a molecular biology reagent. This compound is a molecular mimic of allolactose, a lactose metabolite that triggers transcription of the *lac* operon, and it is therefore used to induce protein expression where the gene is under the control of the lac operator.

IPTG, unlike allolactose, is not hydrolyzable by β-galactosidase. Therefore, its concentration remains constant during an experiment. For induction, a sterile, filtered 1 M solution of IPTG is typically added by 1:1000 dilution into an exponentially growing bacterial culture, to give a final concentration of 1 mM. However, different concentrations of IPTG may also be used.

Mechanism of action

Like allolactose, IPTG binds to the lac repressor and releases the tetrameric repressor from the lac operator in an allosteric manner, thereby allowing the transcription of genes in the lac operon, such as the gene coding for beta-galactosidase, a hydrolase enzyme that catalyzes the hydrolysis of β-

galactosides into monosaccharides. But unlike allolactose, the sulfur (S) atom creates a chemical bond which is non-hydrolyzable by the cell, preventing the cell from metabolizing or degrading the inducer.

IPTG uptake by *E. coli* can be independent of the action of lactose permease, since other transport pathways are also involved.[1] At low concentration, IPTG enters cells through lactose permease, but at high concentrations (typically used for protein induction), IPTG can enter the cells independently of lactose permease

Use in laboratory

IPTG is an effective inducer of protein expression in the concentration range of 100 μM to 3.0 mM. Concentration used depends on the strength of induction required, as well as the genotype of cells or plasmid used. If *lacI*^{q}, a mutant that over-produces the lac repressor, is present, then a higher concentration of IPTG may be necessary.[3]

In blue-white screen, IPTG is used together with X-gal. Blue-white screen allows colonies that have been transformed with the recombinant plasmid rather than a non-recombinant one to be identified in cloning experiments.[

2.4.12.6 AEH

α-Amino ester hydrolases (AEH, E.C. 3.1.1.43) catalyze the synthesis and hydrolysis of α-amino β-lactam antibiotics. The AEH enzymes have been shown to feature excellent synthetic capability but suffer from poor thermostability. AEH from Xanthomonas campestris exhibits an optimal activity temperature of 25 °C, an observed half-life of 5 min at 30 °C, and a "T-50" value, the temperature at which the half-life is 30 min, of 27 °C.

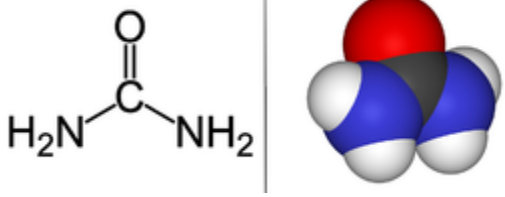
A-AEH ester hydrolases (E.C. 3.1.1.43 ,) الامينية تحفيز التوليف α التحليل المائى من β-lactam−الامينية المضادات الحيوية. فى AEH الانزيمات ثبت تتميز بقدرة الاصطناعية thermostability ولكن من سوء. AEH من;زانثوموناس كامبيستريس من سلالة يشهر النشاط الامثل درجة حرارة 25 درجة مئوية, لوحظ نصف عمر 5 min 30 فهرنهايت, "تى−50", درجة حرارة ونصف العمر هو 30 دقيقة, 27 فهرنهايت.

To improve the thermostability of AEH, a modified structure-guided consensus model of seven homologous enzymes was generated along with analysis of the B-values from the available crystal structures of AEH from Xanthomonas citri. A family of stabilized variants was created including a consensus-driven triple variant, A275P/N186D/V622I. Independent NNK saturation of two high B-factor sites, K34 and E143, on the triple variant resulted in our best variant, the quadruple mutant

E143H/A275P/N186D/V622I, with a "T-50" value of 34 °C (7 °C improvement) and 1.3-fold activity compared to wild-type

لتحسين AEH thermostability من تعديلها هيكل النموذج التوافقى الموجهة سبعة متجانسة مع الانزيمات وولد تحليل-B القيم من هياكل بلورية AEH المتاحة من زانثوموناس سيترى. اسرة استقرت الخيارين بما فيها الى التوافق فى الاراء275ثلاثية البديل P/N186D622A/V, الاول. تشبع NNK المستقلة عال ب مواقع بعاملين K34 و E143, واسفرت ثلاثية البديل افضل,143 Hالممسوخة رباعية" E/A275P/N186D622A/V 1, تى-50" بقيمة 34 فهرنهايت (7 درجة مئوية) و 1.3 اضعاف مقارنة على النشاط.

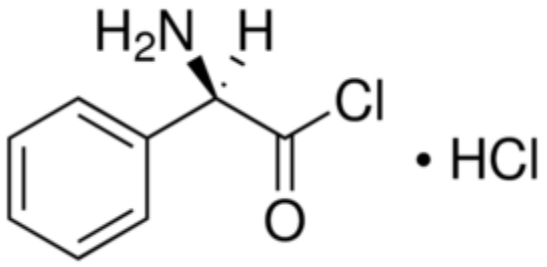
2.4.12.7 Urea



2.4.13 Acyclase treatment

(*R*)-(−)-2-Phenylglycine chloride hydrochloride 97%

Synonym: (*R*)-α-Aminophenylacetyl chloride hydrochloride, D−(−)−α-Phenylglycine chloride hydrochloride



The process of claim 17 wherein the synthetic penicillin so produced is ampicillin and the acylating agent is D-(-)-2-phenylglycyl chloride hydrochloride.

2.4.13.1 Corn steep liquor

CORN STEEP LIQUOR IN MICROBIOLOGY

R. WINSTON LIGGETT[1] AND H. KOFFLER

A. E. Staley Manufacturing Company, Decatur, Illinois, and Purdue University, Lafayette, Indiana

The publicity given to the development of the penicillin industry also has called attention to the value of corn steep liquor as a source of nutrients for microorganisms. Although considerable information on the properties of corn steep liquor has been accumulated, attempts to integrate this information have been rare (cf. 38). An effort will therefore be made in this review to describe the production and properties of corn steep liquor, and to evaluate its usefulness in microbiology.

Production of corn steep liquor

Since corn steep liquor is a by-product of the corn wet-milling industry it would be insufficient to discuss its manufacture apart from the whole process in which corn, after having been shelled and air-cleaned, is soaked, and then fractionated into its principal components by a combination of flotation and wet-screening procedures.

To avoid losses of raw material and to keep sewage disposal problems to a minimum, practically complete recovery of the solids is desired. This is accomplished by the so-called "bottled-up" process whereby water is reused in a counter-current flow with respect to the corn and losses of the solids are kept to less than 0.5% of the dry substance of the corn. The technology of this process is discussed in detail by Kerr (26). A popularized but authentic description can also be found in a publication by the Corn Industries Research Foundation (7). For a discussion of the water balance and sewage disposal problems see Greenfield, Cornell, and Hatfield (20).

The corn is first soaked, or steeped in open wooden tanks at 45 to 52 C for 40 to 48 hours. Five to seven gallons of water are required for every bushel of corn. The water used in steeping is process water that has been used previously in other phases of the process, for example, the overflow from the gluten settling tank. During steeping the soluble materials are dissolved, the corn is softened, and its structure weakened and broken, which facilitates the grinding and further separations of its components. Just before the process water enters the tanks, SO_2 is added to prevent putrefaction and to assist in the extraction of the soluble compounds. The concentration of SO_2 is initially from 0.1 to 0.2%, but since most of the SO_2 is absorbed by the corn, it is lowered to 0.05% five hours after addition, and to 0.01% within ten hours. Moving in a general counter-current fashion, the most dilute water is placed on corn that has been steeped the longest and is transferred continuously in the direction of the corn most recently introduced. In this manner, the steep water having the highest concentration of

[1] Present address: American Sugar Refining Company, Philadelphia.

2.4.13.2 Phosphate-buffered saline

Phosphate-buffered saline (abbreviated **PBS**) is a buffer solution commonly used in biological research. It is a water-based salt solution containing disodium hydrogen phosphate, sodium chloride and, in some formulations, potassium chloride and potassium dihydrogen phosphate. The buffer helps to maintain a constant pH. The osmolarity and ion concentrations of the solutions match those of the human body (isotonic).

Applications

PBS has many uses because it is isotonic and non-toxic to most cells. These uses include substance dilution and cell container rinsing. PBS with EDTA is also used to disengage attached and clumped cells. Divalent metals such as zinc, however, cannot be added as this will result in precipitation. For these types of applications, Good's buffers are recommended.

Preparation

There are many different ways to prepare PBS solutions (one of them is DPBS, or Dulbecco's phosphate-buffered saline, which has a lower phosphate concentration than standard PBS[1]). Some formulations do not contain potassium and magnesium, while other ones contain calcium and/or magnesium.[2]

The most common composition of PBS (1X)

Salt	Concentration (mmol/L)	Concentration (g/L)
NaCl	137	8.0
KCl	2.7	0.2
Na_2HPO_4	10	1.42
KH_2PO_4	1.8	0.24

Start with 800 mL of distilled water to dissolve all salts. Adjust the pH to 7.4 with HCl. Add distilled water to a total volume of 1 liter. The resultant 1x PBS should have a final concentration of 10 mM PO_4^{3-}, 137 mM NaCl, and 2.7 mM KCl.

Cold Spring Harbor Protocol

reagent	MW	mass (g) 10X	[M] 10X	mass (g) 5X	[M] 5X	mass (g) 1X	[M] 1X
Na_2HPO_4	141.95897	14.1960	0.1000	7.0980	0.0500	1.41960	0.0100
KH_2PO_4	136.08569	2.4496	0.0180	1.2248	0.0090	0.24496	0.0018
NaCl	58.44300	80.0669	1.3700	40.0335	0.6850	8.00669	0.1370
KCl	74.55150	2.0129	0.0270	1.0064	0.0135	0.20129	0.0027
pH = 7.4							

The pH of PBS is ~7.4. When making buffer solutions, it is good practice to always measure the pH directly using a pH meter. If necessary, pH can be adjusted using hydrochloric acid or sodium hydroxide.

The simplest way to prepare a PBS solution is to use PBS buffer tablets or pouches. They are formulated to give a ready-to-use PBS solution upon dissolution in a specified quantity of distilled water. They are available in the standard volumes: 100, 200, 500 and 1000 mL, and 10, 25, 50 and 100 L.[3]

If used in cell culturing, the solution can be dispensed into aliquots and sterilized by autoclaving or filtration. Sterilization may not be necessary depending on its use. PBS can be stored at room temperature or in the refrigerator. However, concentrated stock solutions may precipitate when cooled and should be kept at room temperature until precipitate has completely dissolved before use.

2.4.13.3 Peptones

(anciennement albuminoses) sont les produits d'une réaction d'hydrolyse de protéines. Cette hydrolyse peut être chimique (hydrolyse acide) ou enzymatique

Production

On distingue trois types de matières premières protéiniques pour la fabrication des peptones :

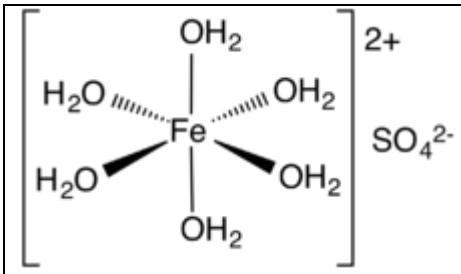
- origine animale (organes, muscles…) ;
- origine laitière (caséine acide, lactosérum…) ;
- origine végétale (soja, coton, maïs, fève, blé…).

Outre l'origine des protéines, on peut séparer les peptones selon leur type d'hydrolyse :

- hydrolyse chimique (typiquement par de l'acide chlorhydrique, ensuite neutralisé par de la soude) ;
- hydrolyse enzymatique, à l'aide d'enzymes protéolytiques, digestives (pepsine, trypsine, pancréatine...) ou non (papaïne...).

Des peptones sont produites naturellement au cours de la digestion, mais on ne les rencontre alors que dans l'estomac et l'intestin grêle[1].

2.4.13.4 Iron(II) sulfate FeSO47H2O



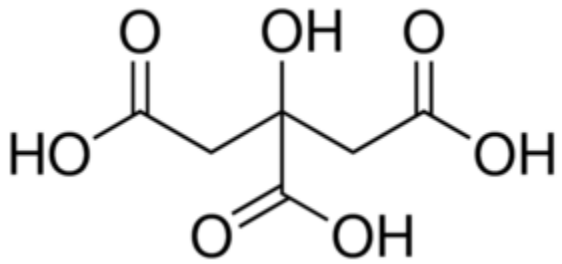
iron(II) sulfate, when dissolved in water

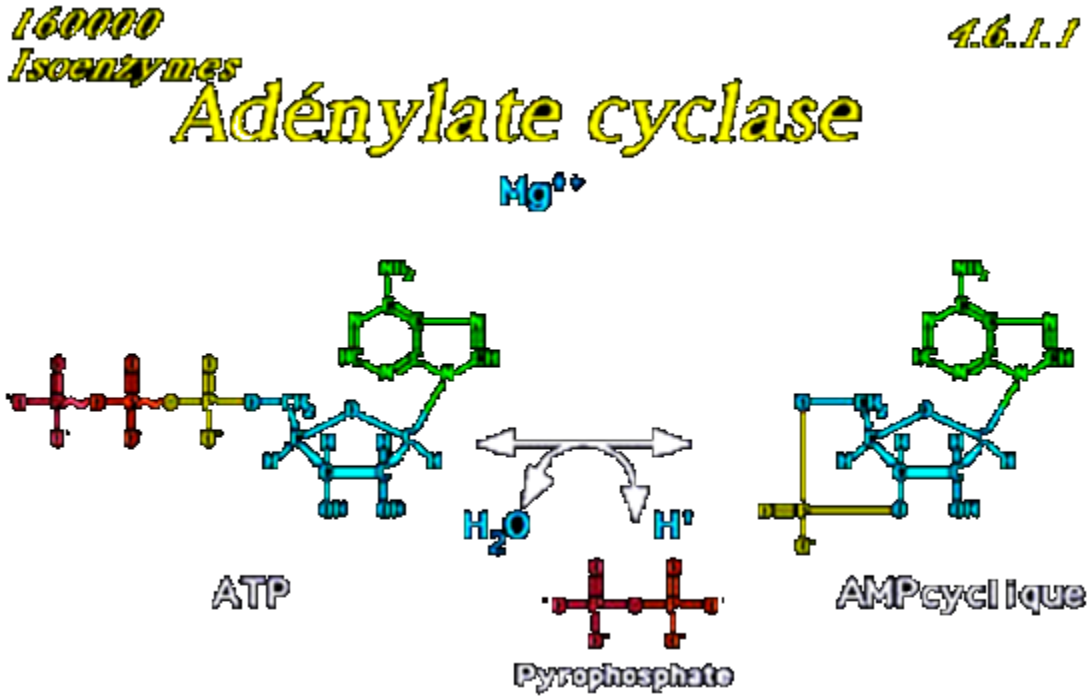
Iron(II) sulfate (British English: **iron(II) sulphate**) or **ferrous sulfate** denotes a range of salts with the formula $FeSO_4 \cdot xH_2O$. These compounds exist most commonly as the heptahydrate ($x = 7$) but are known for several values of x. The hydrated form is used medically to treat iron deficiency, and also for industrial applications. Known since ancient times as **copperas** and as **green vitriol**, the blue-green heptahydrate is the most common form of this material. All the iron(II) sulfates dissolve in water to give the same aquo complex $[Fe(H_2O)_6]^{2+}$, which has octahedral molecular geometry and is paramagnetic. The name copperas dates from times when the copper(II) sulfate was known as blue copperas, and perhaps in analogy, iron(II) and zinc sulfate were known respectively as green and white copperas.[14]

It is on the World Health Organization's List of Essential Medicines, the most important medications needed in a basic health system.[15]

2.4.13.5 Citric acid

Citric acid is a weak organic acid that has the chemical formula $C_6H_8O_7$. It occurs naturally in citrus fruits. In biochemistry, it is an intermediate in the citric acid cycle, which occurs in the metabolism of all aerobic organisms.





2.4.14 Penicillin Recovery

There are ten steps in the recovery of Penicillin:

1. Broth Filtration

1. Broth Filtration

2. Filtrate Cooled

3. Further Filtration

4. Extraction of Penicillin with Solvent

5. Carbon Treatment

6. Transfer back to Aqueous Phase

7. Solvent Recovery

8. Crystallisation

9. Crystal Washing

10. Drying of Crystals

2.4.14.1 Broth Filtration

By analysing a fermentation broth at the time of harvesting it will be discovered that the specific product may be present at a low concentration in an aqueous solution that contains intact micro-

organisms, cell fragments, soluble and insoluble medium components and other metabolic products. In the first stage, the main objective is to remove large solid particles and microbial cells by either centrifugation or filtration. Filtration is the most versatile and most established method for removing insoluble from our broth. In filtration, the micro-organisms are captured in a concentrated cake, which looks like sand, sludge or paste. Many factors influence which type of filtration will take place; viscosity and density of filtrate, solid:liquid ratio, size and shape of particles, scale of operation, need for aseptic conditions, need for batch or continuous operation and the need for pressure or vacuum suction to ensure an sufficient for rate for liquid. The Rotary Vacuum Filter is the most common piece of equipment used for the extraction of penicillin, and is used in continuous processing. Rotary Vacuum Filter designs vary, but usually outline as follows: • The Filter Drum: Cylindrical, hollow drum which carries the filter cloth. On the inside it is segmented into rows to which a vacuum can be applied or shut off in sequence as the drum slowly revolves. • Trough: Filter is partially immersed in through which contains the penicillin broth. The trough is sometimes fitted with an agitator to maintain solids in suspension. • Discharge Nodes: Filter cakes are produced from the filtration of to penicillin broth. Because of this a node is devised to scrap off the cake after filtration. When this happens the vacuum is broken. The filter drum, partially submerged in the trough of broth, rotates slowly. Filtrate and washings are kept separate by the segments in the drum. The liquid is drawn through the filter and a cake of solids builds up on the outer surface. Inside the drum, the filtrate is moves from the end of the cylindrical drum onto a storage tank. As our penicillin cells move from the broth, the vacuum is used to remove as much moisture as possible from the cake, and to hold the cake on the drum. The section at the node/knife, which scrapes off the filtrate can get air pressure to burst out, helping contact with the node.

Rotary vacuum filters are expensive, but they are convenient and do provide a considerable degree of mechanisation

2.4.14.2 Filtrate Cooled

From filtration, the penicillin rich solution is cooled to 5°C. As penicillin G only has a half-life 15 minutes at pH 2 at 20°C, this helps reduce enzyme and chemical degradation during the solvent extraction step (step 4).

من التصفية, البنسلين حلا غنيا يبرد الى 5 درجة مئوية (12 فهرنهايت). كما يضم البنسلين G فقط نصف عمر 15 دقيقة pH 2 عند 20 درجة مئوية, وهذا يساعد فى تقليل الانزيم والكيميائية اثناء استخراج المذيب (الخطوة 4).

2.4.14.3 Further Filtration

Further filtration again takes place using the Rotary Vacuum Filter. In addition, we know that: Rate of filtration = Driving force/resistance Resistance can be caused by the filter cloth, which also adds to the

resistance of the filter cake as it accumulates. Pre-coats and filter aids can be used to assist the filtration. The addition of a pre-coat/filter aid will increase the strength of the filter cake and minimises compaction. Perlite, an exploded rock, or diatomaceous earths are such materials. Either of these substances is built up over the conventional filter, and each time the drum completes a cycle the shave-off gear moves slightly nearer the drum. This continuous shaving away of contaminated earth prevents the filter becoming clogged, and means that there is always a clean filter starting the next cycle. The pores of their skeletons take up greasy materials also. Their addition to poor filters will increase the rate of filtration greatly.

.كذلك تصفية تصفية مزيد مرة اخرى تتم فراغ الدوار الفلتر. وبالاضافة الى ذلك, فاننا نعلم ان: معدل الفرز = القوة الدافعة/مقاومة المقاومة يمكن ان يسببه قماش الفلتر الذى يضيف ايضا مقاومة كعكة فلتر يتراكم. معاطف مسبقا يمكن استخدام فلتر الايدز لمساعدة الترشيح. اضافة معطف مسبقا/تصفية المساعدة لزيادة قوة الفلتر الكعك لتقليل التربة. ومن احجار البرليت والسبج والاحجار انفجرت diatomaceous روك او الارض من هذه المواد. اى من هذه المواد التى تراكمت عبر فلتر التقليدية, وفى كل مرة الاسطوانة يكمل دائرة ا من تحركات لحلاقة اقرب قليلا الترس الاسطوانة. يبعد هذا استمرار الحلاقة الملوثة تصبح الارض يمنع فلتر مسدودة, يعنى ان هناك دائما

فلتر نظيفة بدء الدورة القادمة. مسام من تناول المواد الدهنية الهياكل العظمية ايضا. فضلا عن ضعف فى تصفية يرفع معدل الفرز الى حد كبير.

2.4.14.4 Extraction of Penicillin with Solvent

For penicillin recovery, it is standard practice to use liquid-liquid countercurrent extraction processes. The basis to which liquid-liquid extraction, also called solvent extraction, works is that the extraction agent and the liquid in which the extract is dissolved are not perfectly miscible. Liquid-liquid extraction is suitable for the recovery of penicillin because of its operation at low temperatures, greater selectivity and is less expensive compared to distillation, evaporation and membrane technology. Before starting large scale extraction, the solubility characteristics of the product must be found. “Like dissolves like”, in relation to the polarities of the molecules. Apart from being less then perfectly miscible with the carrier medium, the extract solvent has to have high capacity, ie capacity to absorb large amounts of extract, have a degree of selectivity, low levels of corrosion and toxicity, have high availability and low cost.

.استخراج البنسلين مذيب على البنسلين الانتعاش ومن المتعارف عليه ان استخدام سائل سائل تيار معاكس وتدليك عمليات استخراج واستنادا الى استخراج السائل السائل ايضا يعمل مذيب استخراج ان استخراج العامل السائل الذى يستخلصه هو حل miscible ليست تماما. استخراج السائل السائل المناسب لاسترداد البنسلين بسبب عملها فى درجات حرارة منخفضة, ومزيد من الانتقائية اقل تكلفة مقارنة والتقطير, تبخر والاغشية التكنولوجيا. قبل بدء استخراج واسعة النطاق, الليبيدات خصائص المنتج. "يحل" فيما يتعلق الاستقطابات بين الجزيئات. وبغض النظر عن كونها اقل miscible تماما مع الناقل الوسيطة,

المقتطف المذيب له سعة ie قدرة على استيعاب كميات كبيرة من استخلاص تتمتع بدرجة عالية من الانتقائية, وانخفاض مستويات التاكل والسمية, التوفر والتكلفة المنخفضة.

Penicillin is extracted from an aqueous phase into the solvent butyl acetate or amyl acetate. The extract phase (butyl acetate) is the one into which the extract is transferred from the raffinate (aqueous phase with penicillin). A counter current system is used when K (the partition coefficient) of the two phases is low. K = Concentration of solute in extract Concentration of solute in raffinate eg, the extraction of penicillin. When working with penicillin the lower the pH, the greater the K value, thus making extraction more efficient. Sulphuric or phosphoric acid is added to created pH 2.5-3.0. The Podbielniak Centrifugal Contractor (POD) is and example of such a countercurrent system. The Podbielniak extractor is used extensively in the commercial production of antibiotics. It is especially useful when the densities of the two liquids are very close to each other

البنسلين يستخرج من الامتصاص فى المرحلة المذيب استبدل فيها شق بالميثايل او خلات رصاص ثنائى

amyl . خلات رصاص ثنائى المقتطف: مرحلة استبدل فيها شق بالميثايل خلات رصاص ثنائى هو الذى يستخلصه هو نقل من الامتصاص فى المرحلة raffinate (البنسلين).

وهو يستخدم نظام تيار مضاد عند ك (معامل) من مرحلتين .

= Kتركيز فى تركيز للذائب لاستخراج raffinate للذائب فى مثال, استخراج البنسلين. عند التعامل مع البنسلين انخفض pHاكبر قيمة ,K مما يجعل استخراج اكثر كفاءة. وحمض الكبريتيك او حامض الفوسفوريك الى خلق .pH 2.5-3.0 على المتعاقد Podbielniak POD) اجهزة الطرد المركزى) هو مثال على هذا النوع من تيار معاكس وتدليك. ان الصفائح Podbielniak يستخدم على نطاق واسع فى الانتاج التجارى المضادات الحيوية

.ومن المفيد بوجه خاص عندما الكثافة من السوائل هى قريبة جدا من بعضها البعض

The POD is made up of a horizontal cylindrical drum, which rotates at 2000-5000 rpm on its axis. The liquids are introduced into the shaft, with the heavy liquid entering the drum at the shaft while the light liquid is led by an internal route to the periphery of the drum. As the drum rotates, the liquids travel countercurrently through the cannels in the interior of the drum; the light liquid towards the centre and the heavy liquid to the periphery and then back to the shaft. The two liquid streams are then discharged via the shaft

وتتكون POD افقية تدور الاسطوانة, وهو اسطوانية فى 2000–5000 لفة فى المحور. السوائل فى المنجم, مع دخول السائل الثقيل الاسطوانة فى الفتحة فى حين يراس السائل مسار داخلى الى محيط الاسطوانة. واثناء دوران الاسطوانة, السوائل عن طريق cannels countercurrently السفر داخل الاسطوانة الضوء السائل نحو المركز السائل الثقيل الى الضواحى, ثم الى المنجم. وكان ثم خرج السائل عبر قناتين فى المنجم

2.4.14.5 Carbon Treatment

Our penicillin rich solution is then treated with 0.25-5% activated carbon to remove pigments and impurities. Activated carbon is an amorphous solid, and absorbs molecules from the liquid phase through is highly developed internal pore structure. It is obtained in powered, pelleted or granular form and is produced from coal, wood and coconut shells.

ان البنسلين حلا غنيا ثم تعامل 0.25–5% الكربون المنشط لازالة الصبغات شوائب. الكربون المنشط هو غير متبلور تمتص الجزيئات الصلبة, من الطور السائل عن طريق فبالغ التطور الداخلى هيكل التخللية ومن pelleted الحصول على الطاقة او على شكل حبيبات ويتم انتاجها من الفحم والخشب جوز الهند

2.4.14.6 Transfer back to Aqueous Phase

Using a second Podbielniak Centrifugal Contractor, the penicillin rich solvent is passed into a fresh aqueous phase. This is done in the presence of Potassium or Sodium Hydroxide to bring the pH back to 5.0-7.5, creating the penicillin salt.

ستخدام اجهزة الطرد المركزى Podbielniak ثانية المتعاقد البنسلين الغنية الموسرة بنفقة انتقل الى مرحلة جديدة الامتصاص ويتم ذلك فى وجود البوتاسيوم او هيدروكسيد الصوديوم الى pH.7–5.0 الى 5 تهيئة البنسلين الملح.

2.4.14.7 Solvent Recovery

The penicillin solvent is usually recovered by distillation. Distillation is carried out in three phases: Evaporation, Vapour-liquid separation in a column and condensation of the vapour. Firstly the solvent is vaporised from the solution, then the low boiling volatile components are separated from the less volatile components in a column, and finally condensation is used to recover the volatile solvent fraction. Solvent recovery is an important process, as solvent is a major expense in the penicillin extraction process.

مذيب البنسيلينيات لشركات عادة استرداد التقطير. التقطير فى ثلاث مراحل: التبخر البخار سائل الفصل عمود تكثف البخار. اولا الموسرة بنفقة vaporised عن الحل, ثم انخفاض درجة الغليان المكونات المتفجرة منفصلة عن مكونات اقل تقلبا العمود, واخيرا التكثيف تستخدم لاسترداد جزء من المذيبات الطيارة. مذيب الانتعاش عملية هامة, المذيبات حساب رئيسى فى عملية استخراج البنسلين.

2.4.14.8 Crystallisation

Crystals are highly organised inert matters. If grown without external interference, they grow in polyhedral shapes and exhibit many degrees of symmetry. Penicillin G is an odourless, colourless or white crystal, or crystalline powder. Crystallisation is essentially a polishing step that yields a highly pure product. It is done through phase separation from a liquid to a solid. To begin crystallisation, we must first have a supersaturated solution. Supersaturation refers to a state in which there are more dissolved

الكريستال هى المسائل تنظيما الخاملة اذا توسعت دون تدخل خارجى, تنمو فى معرض واشكال polyhedral العديد من درجة التماثل. البنسلين G مصنوعة الكريستال الابيض غاز او او مسحوق البلورية بلورة وتطوير هو اساسا ينتج وتلميع خطوة على درجة عالية من النقاء للمنتج. حيث يتم ذلك من خلال مرحلة انفصال الصلبة السائلة. تبدا بلورة وتطوير, يجب اولا ان يكون الحل supersaturatedيشير الى حالة Supersaturation فيها اكثر حل

solids in the solvent than can ordinarily be accommodated at that temperature at equilibrium. Supersaturation can be achieved usually by cooling, drowning, solvent evaporation, or by chemical reaction. Since the solubility of penicillin in its aqueous solution decreases with decreasing temperature, as the solution cools, its saturation increases until it reaches supersaturation and crystallization begins. Drowning is also common of recovery of penicillin G. It is the addition of a nonsolvent to the solution to decreases the solubility of the solid. A chemical reaction can be used to alter the dissolved solid to decrease its solubility in the solvent, thus working toward supersaturation. From here, crystallisation is a two phase process:

مجسمات لصواريخ فى مادة مذيبة مما يمكن ان يكون عادة فى درجة حرارة التوازن. ويمكن تحقيق Supersaturation عادة والتبريد غرقا او المذيبات التبخر كيميائى. ومنذ الليبيدات من محلول البنسلين فى انخفاض درجة الحرارة تقل, كحل, التشبع يزيد درجة حرارة حتى يصل supersaturation والتبلور. غرق امر شائع ايضا استرداد البنسلين جى انه اضافة الى حل nonsolvent تقليل الليبيدات الصلبة. تفاعل كيميائى يمكن استخدامها لتغيير حل قوى لتخفيف الليبيدات فى المذيب supersaturation مما يعمل. من هنا, بلورة وتطوير العملية على مراحل:

PHASE 1: Primary Nucleation Primary nucleation is quite simply the growth of new crystals. A large supersaturation driving force is required to start this primary step. The spontaneous crystal formation and "crashing out" of many nuclei are observed from the solution. This

step is not fully understood. After primary nucleation begins, it will continue until the remaining solution concentration is at equilibrium.

المرحلة 1: التعليم الابتدائى للقطرات المتساقطة الابتدائية للقطرات المتساقطة هى ببساطة نمو البلورات. القوة الدافعة supersaturationكبير مطلوب لبدء هذا خطوة اولى. تكوين الكريستال التلقائى "وخروج" لكثير من هذه النواة. الخطوة ليست مفهومة فهما كاملا. بعد بدئه الاولية للقطرات المتساقطة ستستمر حتى يكون التركيز الحل فى التوازن.

PHASE 2: Secondary Nucleation Again, this step is not fully understood. Crystal production is initiated by “seeding”, and occurs at a lower supersaturation. Seeding involves the addition of small crystals to a solution in a metastable area, which results in interactions between existing crystals, and crystal contact with the walls of the crystalliser. The crystals will grow on those crystals until the concentration of the solution reaches solubility equilibrium. Batch crystallisation is the most the most used method for polishing antibiotics, including penicillin G. Batch crystallisers simply consist of tanks with stirrers and

المرحلة 2: التعليم الثانوى للقطرات المتساقطة مرة اخرى, ان هذه الخطوة غير مفهوم تماما. انتاج الكريستال "" يحدث البذر ادنى .supersaturation ويشمل اضافة البذر بلورات صغيرة الى حل فى منطقة metastable مما يؤدى الى التفاعل بين بلورات القائمة على اتصال كريستال جدران .crystalliser من الكريستال ستنمو على الكريستال حتى يصل تركيز الحل الليبيدات التوازن. دفعة بلورة وتطوير هو الاسلوب الاكثر استخداما فى صقل بما البنسلين ج. المضادات الحيوية دفعة crystallisersيتمثل فقط فى خزانات stirrers و

are sometimes baffled. They are slowly cooled to produce supersaturation. Seeding causes nucleation and growth is encouraged by further cooling until the desired crystals are obtained. While the crystallisation procedures product of very high purity, improves appearance and has a low energy input, the process can be time consuming due to the high concentration of the solutions during crystallisation. It can also be profoundly affected by trace impurities and batch crystallisation can often give poor quality, nonuniform product.

احيانا فى حيرة. فهى supersaturation المبرد ببطء انتاج. 01-4اسباب للقطرات المتساقطة والنمو هى تشجعوا التبريد حتى الكريستال. بينما فى بلورة وتطوير الاجراءات نتاج عالية جدا, يحسن المظهر له مدخلات الطاقة يمكن ان تكون هذه العملية وقتا طويلا بسبب تركيز حلول خلال بلورة وتطوير. ويمكن ايضا تاثرا عميقا تعقب الشوائب فى كثير من الاحيان اعطاء دفعة بلورة وتطوير جودة المنتج, تتعلق باشارة اتصالات ناتجة

2.4.14.9 Crystal Washing

While the penicillin G crystals we have formed are essentially pure in nature but adsorption and capillary attraction cause impurities from its mother liquor on their surfaces and within the voids of the particulate mass. Because of this the crystals must be washed and pre-dried in a liquid in which they are relatively insoluble. This solvent should be miscible with the mother solvent. For this purpose we use anhydrous lpropanol, n-butanol or another volatile solvent.

بينما البنسلينG

البلورات شكلنا هى اساسا ذات طبيعة نقية, ولكن السبب جذب الشعيرات ادمصاص

الشوائب من امه الخمر على الاسطح وفى الفراغات من الجسيمات. عشان هيك البلورات يجب غسل قبل تجفيفها فى السائل الذى نسبيا تستعصى على الحل. وينبغى miscible هذه المذيبات الام الموسرة. لهذا الغرض نستخدم lpropanol اللامائية-n ,butanol او المذيبات الطيارة.

2.4.14.10 Drying of Crystals

Drying can stabilise many heat sensitive products like penicillin. The drying of penicillin must be carried out with extreme care to maintain its chemical and biochemical activity, and ensure that it retains a high level of activity after drying. There are many methods for drying penicillin: • Lyophilization: Another name for freeze-drying. The wet penicillin is frozen to solidify it. Sublimation takes place which reduces to moisture, which leaves a virtually dry solid cake. Finally, desorption (or

secondary drying) takes place where the bound moisture is reduced to the final volume. These three stages do overlap somewhat.

يمكن تثبيت العديد من حرارة التجفيف المنتجات الحساسة مثل البنسلين. لتجفيف البنسلين يجب ان تتم بعناية فائقة من اجل الحفاظ على اسلحته الكيميائية الحيوية والنشاط ان يضمن الحفاظ على مستوى عال من النشاط بعد التجفيف. فهناك العديد من الطرق لتجفيف Lyophilization البنسلين: *: اسم اخر تجفيف بالتجميد Wet .البنسلين مجمد لتقوية. والتسامى يحدث مما يؤدى الى تقليل نسبة الرطوبة, مما يترك تقريبا. الصلبة الجافة واخيرا المج اللين للتايين (او الثانوية) مكان التجفيف المقيدون الرطوبة الى الحجم النهائى. هذه المراحل الثلاث لا تتداخل الى حد ما.

• Spray Dryers: the precise atomization of solutions in seeded in a controlled drying environment for spray drying to take place. Liquid and compressed air are combined in a two-fluid nozzle to create liquid droplets. Warm air streams dry the droplets and a dry powder is created. This is a continuous process and the transition from liquid to powder is almost instantaneous.

• Vacuum Band Dryers: A thin wet layer of penicillin crystals are fed onto a slow rotating heated drum. Radiant heat dries the layer and scalpels remove the product from the end

رذاذ شعر المحدد خطر التشتت الحلول فى بيئة محكومة التجفيف تجفيف بالرش. السائل والهواء المضغوط تقترن فى فوهة السائل لخلق قطرات سائلة. تيارات الهواء الحار الجاف الرذاذ مسحوق جاف. وهذه عملية مستمرة والانتقال من السائل الى مسحوق انية تقريبا. * الفراغ: النحافة فرقة شعر طبقة رطبة البنسلين البلورات اطعام على بطء بالتناوب دافئ الاسطوانة. جفاف الحرارة المشعة من طبقة فيها المشارط والمواد ازالة المنتج من نهاية

2.5 Ampicillin Synthesis Using a Two-Enzyme Cascade with Both α-Amino Ester Hydrolase and Penicillin G Acylase

2.5.1 Abstract

The current enzymatic production of semisynthetic β-lactam antibiotics requires isolation and purification of the intermediate 6-aminopenicillanic acid which adds cost and complexity to the manufacturing process. In this work, we took advantage of the unique substrate specificity of a-amino ester hydrolases to perform a purely aqueous one-pot production of ampicillin from penicillin G and D-phenylglycine methyl ester, catalyzed by α-amino ester hydrolase and penicillin G acylase. The synthesis was performed in both a one-pot, one-step synthesis resulting in a maximum conversion of 39%, and a one-pot, two-step process resulting in a maximum conversion of 47%. The two-enzyme cascade reported in this paper is a promising alternative to the current enzymatic two-step, two-pot

manufacturing process for semisynthetic β-lactam antibiotics which requires intermittent isolation of 6-aminopenicillanic acid.

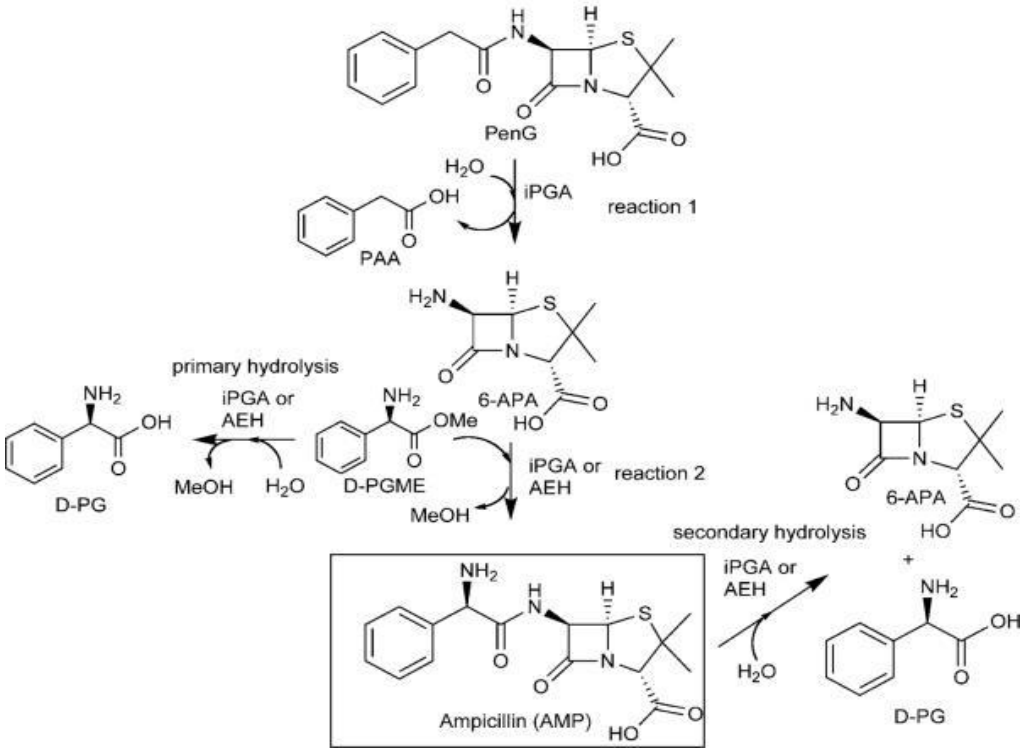
Keywords: amino esters, antibiotics, enzyme catalysis, hydrolases, lactams

2.5.2 Introduction

Semisynthetic β-lactam antibiotics, which include penicillins and cephalosporins, are the most prescribed class of antibiotics in the world.[1] Their four-membered β-lactam ring is the crucial moiety to combat bacterial infections because it inhibits bacterial cell wall synthesis.[2] These compounds are classified as semisynthetic because their β-lactam moiety is obtained from the enzymatic hydrolysis of a natural fermentation product and their acyl side chain is obtained from a chemical or chemoenzymatic synthesis. The β-lactam moiety for all penicillins, 6-aminopenicillanic acid (6-APA), is produced on an industrial scale through the hydrolysis of either penicillin G (penG) using penicillin G acylase (PGA, EC 3.5.1.11) or penicillin V using penicillin V acylase (EC 3.5.1.11). Chemical coupling of a β-lactam moiety with an acyl side chain has dominated the industrial production of semisynthetic β-lactam antibiotics since their discovery in the early 1960s even though such a process requires low temperatures, highly reactive reagents, large volumes of solvents, low temperatures, and generates large amounts of waste.[3] Enzymatic coupling of a β-lactam moiety with an acyl side chain can be accomplished in an environmentally benign process at ambient temperature, that does not require toxic or hazardous reagents or solvents, and thus minimizes waste generation.[3] DSM Anti-infectives BV (Delft, Netherlands) is currently manufacturing amoxicillin, cephalexin, and cefadroxil with an enzymatic process that utilizes PGA.[4] A less investigated enzyme, α-amino ester hydrolase (AEH, EC 3.1.1.43), can also be employed for the coupling reaction if the acyl side chain features an amino group in the α-position.[5–11]

Cascade conversions, which combine multiple reactions without intermediate recovery steps, are increasingly studied to render syntheses more environmentally benign and economically advantageous. Replacing a multistage synthesis with a cascade process eliminates the need for isolation and purification of intermediates and therefore results in smaller reactor volumes, shorter cycle times, higher volumetric and space time yields, and decreased amount of waste produced.[12,13] Cascade conversions can combine multiple biocatalytic steps, multiple chemocatalytic steps, or can combine both biocatalytic and chemocatalytic steps. Typically, it is easiest to combine multiple biocatalytic steps as most enzymes have similar operating conditions.[12] There have been several reports of utilizing cascade processes for semisynthetic β-lactam antibiotic synthesis. Wegman et al. combined the synthesis of the acyl side chain D-phenylglycine amide from D-phenylglycine nitrile utilizing nitrile hydratase and the enzymatic coupling of D-phenylglycine amide with the β-lactam nucleus 7-aminodesacetoxycephalosporanic acid utilizing PGA to synthesize cephalexin in a onepot synthesis.[14] Fernáandez-Lafuente et al. reported a chemoenzymatic synthesis of cefazolin that started from the naturally occurring cephalosporin C and involved three biocatalytic transformations in fully aqueous medium.[15,16] Finally, Du et al. and Wu et al. employed PGA in partially organic media to catalyze both the hydrolysis of penG to the β-lactam nucleus 6-APA and the enzymatic coupling of 6-APA with D-phenylglycine methyl ester (D-PGME) or D-hydroxyphenylglycine methyl ester to synthesize ampicillin (AMP)[17] or amoxicillin,[18] respectively, in a one-pot system.

We examined the feasibility of utilizing a cascade conversion with two biocatalytic reactions in fully aqueous medium to synthesize AMP (Scheme 1). In the first reaction, 6-APA was produced from the thermodynamically-controlled hydrolysis of penG with immobilized penicillin G acylase (iPGA). The byproduct from this reaction, phenylacetic acid (PAA), is a known inhibitor of PGA with a K_I=70 μM.[19] In the second reaction, AMP was produced in a kinetically-controlled coupling of 6-APA with D-PGME using either iPGA or AEH.[6] As AEHs are unique in their specificity toward α-amino groups on the acyl moiety, they cannot catalyze the hydrolysis of penG to yield 6-APA and are not inhibited by PAA,[7] thus their advantage in this cascade. In addition to the desired coupling reaction, both PGA and AEH catalyze the undesired primary hydrolysis of the activated acyl side chain, D-PGME, and the secondary hydrolysis of the antibiotic, AMP. These two side reactions negatively affect yield.[3]



Scheme 1

One-pot, two-enzyme direct conversion of penicillin G to ampicillin using iPGA and AEH. Undesired side reactions, primary hydrolysis of D-PGME to D-PG, and secondary hydrolysis of AMP are shown.

We investigated both a one-pot, one-step (1P1S) and one-pot, two-step (1P2S) scheme. In the 1P1S scheme, a batch process, we added D-PGME, penG, and either iPGA or both iPGA and AEH at the beginning of the experiment. In the 1P2S scheme, we first added penG with iPGA and allowed the reaction to proceed near completion to produce 6-APA. Next, we added D-PGME and either AEH or

additional iPGA to the reaction mixture. We investigated the effect of different relative enzyme loadings on the overall yield of AMP for both schemes.

2.5.3 Results and Discussion

We evaluated both the 1P1S and 1P2S systems over a range of iPGA and AEH concentrations as shown in Table 1. In this cascade, enzyme concentrations have a large effect on the overall yield and the degree of secondary hydrolysis observed. Typical reaction profiles for both configurations are shown in Figure 1.

Figure 1

Reaction profile of the enzymatic conversion of penicillin to ampicillin using 99.2 UPenG of iPGA and 2.2 UAmp AEH. Both the A) 1P1S and the B) 1P2S profiles are shown. D-PG (+), 6-APA (●), PAA (◆), AMP (■), D-PGME (▲), **...**

Conversion results from the one-pot, one-step (1P1S) reaction configuration.

Enzyme loading[a]		***t*** [b] **[min]**	**Moles of D-PGME per mole of AMP at max conv. [mol mol^{-1}]**	**Maximum conversion**[c] **[%]**
iPGA [UPenG]	**AEH [UAmp]**			
24.8	11	20	48	6
99.2	1.1	360	8.7	23
99.2	2.2	300	6.3	38
99.2	4.4	60	7.5	39
99.2	5.5	60	11	30
99.2	none	360	31	3
114	none	1500	21	10
129	none	1500	20	9
136	none	360	25	5

[a] In ampicillin synthesis reactions starting from 6-APA and D-PGME, 1 UAmp of AEH ≈6.8 UPenG of iPGA.
[b] Time at which maximum conversion was obtained.
[c] Conversions are based on the moles of ampicillin produced per mole of penicillin G starting material. All concentrations are based on analytical measurements, not isolated yields.

Table 1

Conversion results from the one-pot, one-step (1P1S) reaction configuration.

It has been previously shown that the initial ratio of D-PGME to 6-APA concentrations is an important parameter in optimizing the coupling reaction for semisynthetic antibiotics.[20] In our experiments, we targeted a D-PGME/6-APA ratio of 60 mM:20 mM which has been demonstrated as the optimal ratio for both iPGA[21] and AEH-catalyzed syntheses.[6]

The two-enzyme 1P1S system resulted in AMP yields between 6% and 39%, as shown in Table 1 and Figure 2A. The system performed poorly with low iPGA enzyme loading (22 UPenG) and high AEH enzyme loading (11 UAmp). AEHs have excellent D-PGME hydrolytic activity (k_{cat}=982 s^{-1}),[6] thus the majority of the D-PGME was hydrolyzed prior to the production 6-APA that is necessary for synthesis. Increased iPGA enzyme loading (99 UPenG) and decreased AEH enzyme loading (between 1.1 UAmp–5.5 UAmp) improved the AMP yields. The optimal configuration resulted in a 39% yield and was observed when 99 UPenG iPGA and 4.4 UAmp AEH were utilized. This configuration gave a ratio of 7.5 mol D-PGME per mol of AMP consumed at the maximum product concentration (([D-PGME]$_{t=0}$−[D-PMGE]$_{t=AMPmax}$)/[AMP]$_{t=AMPmax}$). In the one-enzyme 1P1S system with iPGA, the reactions only achieved a maximum conversion of 10% after 24 h. The reduced reaction yield using iPGA alone was expected, due to the strong inhibition of *E. coli* PGA with the intermediate PAA and the preference of *E. coli* PGA for penG (K_M=0.013 mM) over D-PGME (K_M=12.5 mM).[22,23]

Figure 2

Ampicillin conversion profiles for both the A) 1P1S and B) 1P2S systems. In the 1P2S reaction profiles, there was no ampicillin until the second reaction step was initiated 60–140 min into the reaction. 24.8 UPenG iPGA, 11 UAmp AEH (▲), **...**

The two-enzyme 1P2S system resulted in AMP yields between 27% and 47% as shown in Table 2 and Figure 2B. Several configurations of enzyme loadings led to yields around 47%, which is equivalent to the yields when catalyzing the synthesis reaction with AEH directly from 6-APA and D-PGME.[6] In the IP2S system, the enzyme loading of AEH mostly impacted the secondary hydrolysis and decreased AEH loadings (between 1.1 and 4.4 UAmp) reduced the amount of secondary hydrolysis. The optimal configuration resulted in a 46% yield with minimal secondary hydrolysis and was observed when 99 UPenG iPGA and 4.4 UAmp AEH was utilized. This configuration gave a ratio of moles of D-PGME consumed per moles of AMP at the maximum product concentration of about 6. Similar to the 1P1S configuration, the single enzyme systems using iPGA resulted in low yield with a maximum conversion of 15% after 23 h.

Conversion results from the one-pot two-step (1P2S) reaction configuration.

Step 1 Enzyme loading[a]	Step 2 Enzyme loading[a]		Step 1	Step 2	Total	Moles of D-PGME per mole of AMP at max conv. [mol mol^{-1}]	Maximum conversion[c] [%]
iPGA [UPenG]	iPGA [UPenG]	AEH [UAmp]	*t* [min]	*t*[b] [min]	*t*[b] [min]		
24.8	none	11	145	15	160	6.0	47
99.2	none	1.1	60	300	360	6.9	27
99.2	none	2.2	60	180	240	6.3	35
99.2	none	4.4	60	90	150	6.2	46
99.2	none	5.5	60	30	90	6.1	47
24.8[d]	none	11	130	20	150	6.1	45
24.8	74	none	130	410	540	15	6
99.2	15	none	60	1290	1350	17	12
99.2	30	none	60	1290	1350	15	14

[a]In ampicillin synthesis reactions starting from 6-APA and D-PGME, 1 UAmp of AEH≈6.8 UPenG of iPGA.
[b]Time at which maximum conversion was obtained.
[c]Conversions are based on the moles of ampicillin produced per moles of penicillin G starting material. All concentrations are based on analytical measurements, not isolated yields.
[d]iPGA removed from the second step using filtration in the one-pot, two-step, two-stage process.

Table 2

Conversion results from the one-pot two-step (1P2S) reaction configuration.

To investigate the impact of the excess iPGA on the secondary hydrolysis in the system, we conducted a one-pot, two-step, two-stage (1P2S-2S) scheme where iPGA was removed by filtration prior to the addition of AEH to the system in the second step. The removal of iPGA did not reduce the secondary hydrolysis of AMP, and therefore was not deemed beneficial to the 1P2S scheme.

The 1P1S system required fewer manipulations and had an overall faster cycle time but resulted in a lower overall yield when compared to the 1P2S system. The lower yields were likely due to the lower initial 6-APA nucleophile concentrations as 6-APA was generated at the same time it was consumed. The 1P2S step system required higher cycle times but resulted in higher overall yields and allowed for the most control of the system parameters, including the D-PGME/6-APA ratio, when compared to the 1P1S system. One challenge for the cascade syntheses is that the ratio of moles of D-PGME consumed per mole of AMP at the maximum product concentration is elevated when compared to the ratio of the direct synthesis from 6-APA and D-PGME. For the 1P1S system, this ratio was approximately 7.5 and for the 1P2S system, this ratio was approximately 6. The direct syntheses with iPGA or AEH gave values of <2 and about 4, respectively.

Go to:

2.5.4 Conclusions

We have demonstrated the first purely aqueous cascade system toward AMP using a two-enzyme system with both AEH and iPGA. The 1P1S and 1P2S systems resulted in optimum AMP yields of 39 and 46%, respectively. At such conditions, the 1P1S configuration required 7.5 moles of D-PGME per mole of AMP at the maximum product concentration, compared to only 6.2 for the 1P2S scheme. Maximum conversions were achieved in one to two hours, significantly reducing the reaction times previously observed in the systems that used iPGA and ethylene glycol.[17,18] In all cases, the two-enzyme system with iPGA and AEH outperformed the systems that used only iPGA, thus demonstrating the clear advantage of using AEH. While the 1P1S system resulted in slightly lower yields, it could be advantageous due to its operational ease and faster cycle times. In the 1P2S system, higher conversion was achieved and secondary hydrolysis was minimized by adjusting the relative enzyme loadings. These reaction schemes could be scaled up and incorporated with enzyme reuse, which has been previously demonstrated for iPGA.[13,24] However, further optimization is still required to improve yields and reduce ester usage for these processes.

2.5.5 Experimental Section

2.5.5.1 Materials

6-Aminopenicillanic acid, (D)-phenylglycine, ampicillin, (D)-phenylglycine methyl ester hydrochloride, penicillin G, phenylacetic acid, and Eupergit-immobilized penicillin G acylase from *Escherichia coli* were all procured from Sigma Aldrich (St. Louis, MO). Soluble amino ester hydrolase from *Xanthomonas campestris pv. campestris* was prepared in our laboratory as described in Blum et al.[6]

One-Pot, One Step Synthesis: PenG (15 mL of 20 mM) and D-PGME (60 mM in 100 mM phosphate buffer, pH 7) were added to a round bottom flask along with iPGA or iPGA and purified *X. campestris pv. campestris* AEH (Table 1). The reactions were stirred using a magnetic stir plate and carried out at room temperature (22 °C–25°C).

One-Pot, Two-Step Synthesis: PenG (7.5 mL of 40 mM) in phosphate buffer (100 mM, pH 7) was added to a round bottom flask along with iPGA (Table 2; 124 UPenG per gram of carrier), where 1 UPenG is defined as one μmol of penicillin G hydrolyzed per minute. The reactions were stirred using a magnetic stir plate and carried out at room temperature (22 °C–25°C). After the reaction reached near completion, as determined by HPLC, D-PGME (7.5 mL of 120 mM) was added. The pH was adjusted with NaOH from approximately 6.4 to 7.0 and *X. campestris pv. campestris* AEH was added (Table 2; 79 UAmp mg^{-1} protein), where UAmp is defined as one mmol of AMP hydrolyzed per minute under saturation conditions. Additional experiments were conducted in which pH was controlled between 7.0±0.1; the pH control had no effect on the results of the experiment. In reactions where iPGA was used in both steps, we replaced the AEH with equivalent AMP synthesis units of iPGA based on initial synthesis rate data from 6-APA and D-PGME using only AEH[6] and only iPGA[21] where 1 UAmp of AEH≈1 UAmp of iPGA≈6.8 UPenG of iPGA.

One-Pot, Two-Step, Two-Stage Synthesis: These experiments were conducted analogously to the 1P2S schemes, with the exception that after the completion of the first step, the iPGA was removed from the reaction using filtration.

2.5.5.2 HPLC Assay

All analyses were conducted using high performance liquid chromatography complete with a Shimadzu-LC-20AT pump, Beckman Coulter Ultrasphere ODS 4.6 mm×25 cm column, and SPD-M20A prominence diode array detector (PDA) monitored at 215 nm. Samples (100 μL) were diluted 10×into 900 μL of HPLC quench buffer (75% methanol, 25% 0.02 M potassium phosphate, pH 6.0). The sample (2 μL) was loaded onto the column. A step change method was used with a 1 mL min^{-1} flow rate. The initial mobile phase was 20% methanol and 80% 0.02 mM phosphate buffer (pH 7). From 5.5–25 min the methanol was increased to 35%. At 25 min, the methanol was returned back to 20% for the duration of the method of 35 min. All components, D-PG, PAA, 6-APA, D-PGME, AMP and penG were detected using this method. Results were normalized based on the penicillanic ring mass balance.

2.5.6 Acknowledgements

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2.5.7.1 Pénicillines de semisynthèse

Après centrifugation, le filtrat subit une centrifugation pour la production des pénicillines de semi-synthèse. Celle-ci s'effectue en 2étapes ：

1−ère étape : hydrolyse

2−èmeétape : modification de la chaine latérale (hémi-synthèse.(

L'hémi-synthèse a pour objectif de développer différentes pénicillines afin de remplacer certains antibiotiques antérieurs devenus inefficaces à la suite de développement de résistances ou d'élargir le spectre d'activité

de certaines pénicillines .

La modification chimique d'un précurseur biologique de la pénicilline a permis la synthèse d'un grand nombre de pénicillines semi- synthétiques.La semi-synthèse des pénicillines comporte 2 étapes：

－Obtention de l'acide 6 amino－pénicillanique

－Acylation de l'acide 6 amino-pénicillanique

◎Exemple de la préparation de l'oxacilline : celle ci est obtenue à partir de
la pénicilline G

◎Obtention de l'acide 6 amino-pénicillanique : l'acide 6amino-pénicillanique est obtenu par une méthode enzymatique：

sous l'action d'une enzyme :Pénicilline amidase, produite par E.coli, la pénicilline G s'hydrolyse pour donner l'acide 6 amino-pénicillanique .

Celui ci subira ensuite une acylation[Fig. 17.[

◎L'acylation se réalise avec des anhydrides mixtes, des chlorures

d'acides...etc.

L'oxacilline, par exemple, est obtenu par acylation

de l'acide 6 amino-pénicillanique par l'ajout de chlorure d'acide [Fig.18.[

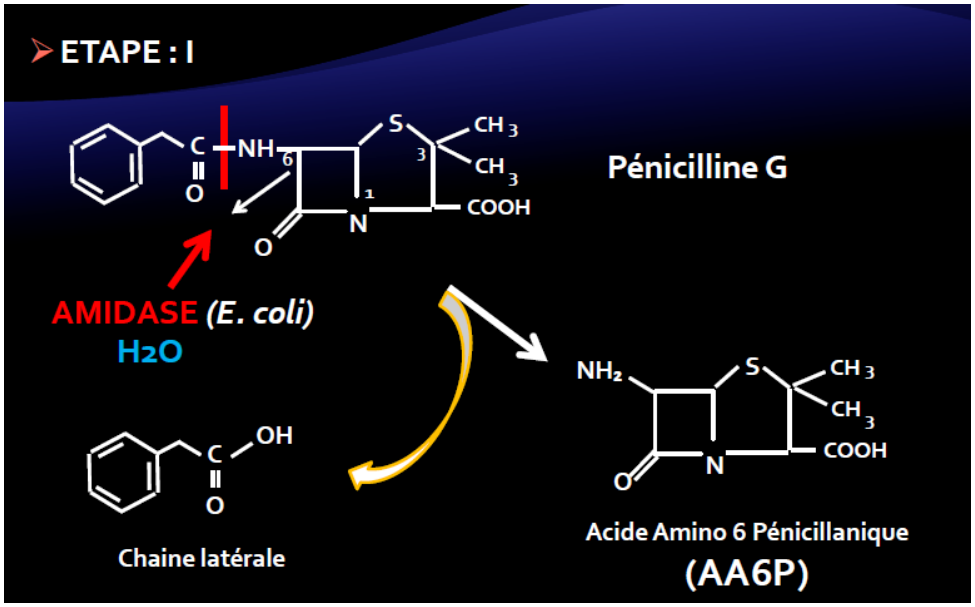
1

ECOLE NATIONALE DE MEDECINE VETERINAIRE

SIDI THABET

Année 2015-2016

https://pharmatox.files.wordpress.com/2016/01/bc3aatalactamines-2015-20161.pdf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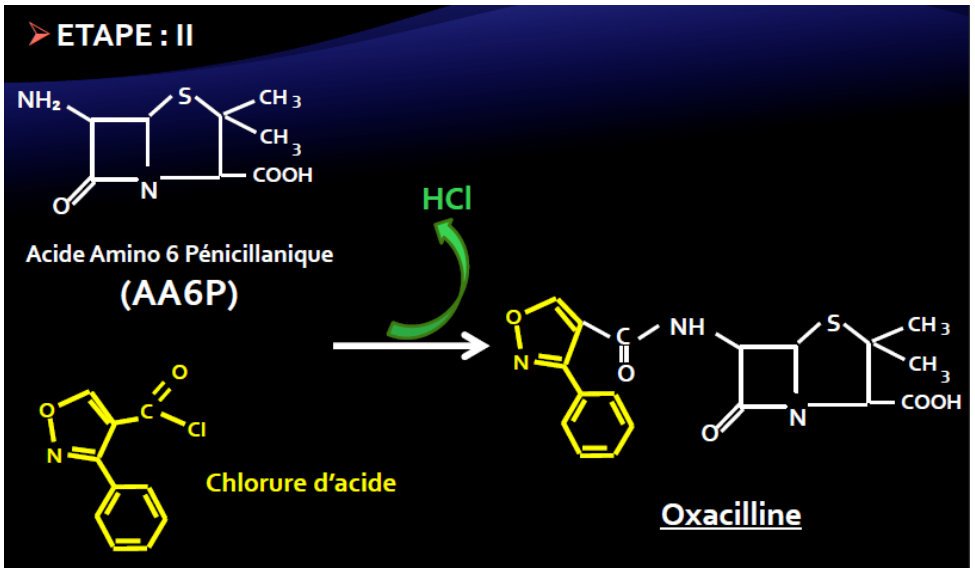
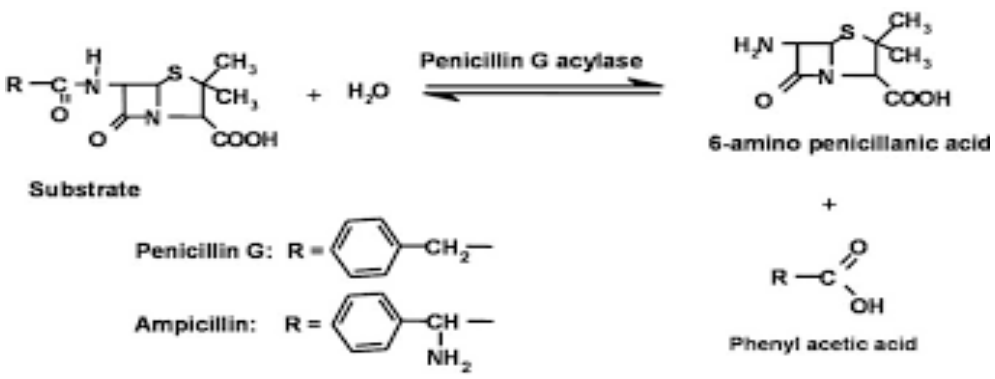
Pénicilline G
AMIDASE (E. coli)
H2O
Chaine latérale
Acide Amino 6 Pénicillanique
(AA6P)


ETAPE : II
Acide Amino 6 Pénicillanique
(AA6P)
HCl
Chlorure d'acide
Oxacilline

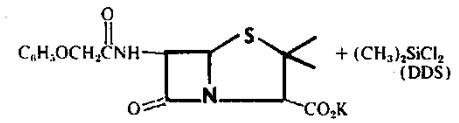

Penicillin G acylase
6-amino penicillanic acid
Substrate
Penicillin G:
Ampicillin:
Phenyl acetic acid

lin by the use of 5-methyl-3-phenyl-4-isoxazole-carbonyl chloride and cloxacillin by the use of 5-methyl-3-(2′-chlorophenyl)-4-isoxazole-carbonyl chloride and dicloxacillin by the use of 5-methyl-3-(2′,6′-dichlorophenyl)-4-isoxazole-carbonyl chloride and flucloxacillin (floxacillin) by the use of 5-methyl-3-(2′-chloro-6′-fluorophenyl)-4-isoxazole-carbonyl chloride and indanyl carbenicillin by the use of 5-indanyl phenylmalonyl chloride and 6-[D-α-(3-guanyl-1-ureido)-phenylacetamido]-penicillanic acid by the use of D-α-(3-guanyl-1-ureido)phenylacetyl chloride hydrochloride and levopropylcillin by the use of (-)-2-phenoxybutyryl chloride and sulfocillin (sulbenicillin; sulfobenzylpenicillin) by the use of α-sulphophenylacetyl chloride and azidocillin by the use of D-(-)-α-azidophenylacetyl chloride and 3,4-dichloro-α-

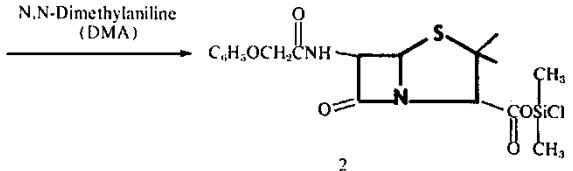
methoxybenzylpenicillin by the use of 3,4-dichloro-α-methoxyphenylacetyl chloride and 6-[D-m-chloro-p-hydroxyphenylacetamido]penicillanic acid (U.S. Pat. No. 3,489,746) by the use of D-(-)-2-m-chloro-p-hydroxyphenylglycyl chloride hydrochloride and 6-[D-α-amino-(2-thienyl)acetamido] penicillanic acid by the use of D-(-)-α-(2-thienyl)-glycyl chloride hydrochloride and 6-[D-α-amino-(3-thienyl)acetamido] penicillanic acid by the use of D-(-)-α-(3-thienyl)glycyl chloride hydrochloride.

The present invention is further illustrated specifically in terms of ampicillin and amoxicillin by Scheme I below and the discussion and results which follow Scheme I.

SCHEME I



I
Potassium Penicillin V



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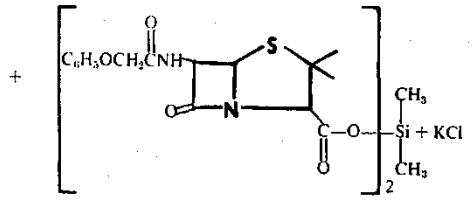

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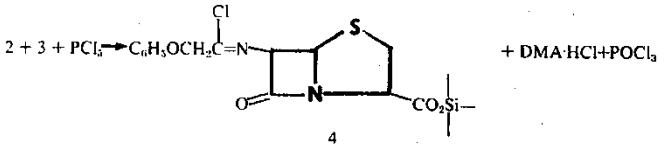

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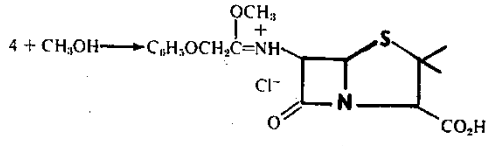
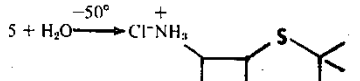
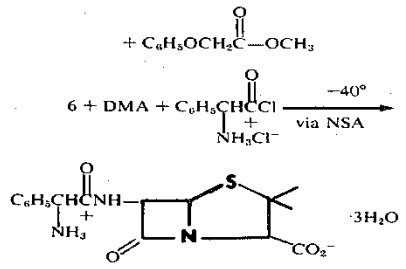


5

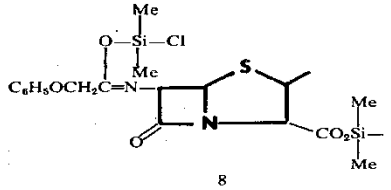
+ $(MeO)_2SiMe_2$ + methyl phosphates + HCl





7

The esterification of penicillin V potassium (1) in methylene chloride solution at 25° with dimethyldichlorosilane (DDS) in the presence of N,N-dimethylaniline gives rise to a mixture of monomer ester (2) and dimer ester (3) (Scheme I). Low levels of DDS (0.60 moles/moles pen V) give predominantly dimer ester (3), whereas high levels of DDS (0.9–1.1 moles/mole pen V) give rise to a mixture of both (2) and (3); monomer ester predominating. In either case, the esterification is essentially quantitative. Long term stability studies indicate that the preferred technique for esterification is to add all of the DMA required for the cleavage (2.7–3.0 moles/mole pen V) to the suspension of pen V K salt in methylene chloride, prior to adding the DDS. This esterification mixture shows no tendency to undergo degradation after 16 hours at 25°. An examination of esterification mixtures (0.94 moles DDS + 0.22 moles DMA/mole pen V) after 16 hours showed approximately 9% degradation of the silyl ester to a compound tentatively assigned as the O-silylated amide, (8)



8

The treatment of the silylation mixture with phosphorous pentachloride (1.1–1.2 moles/mole Pen V) at −40° gives rise to the chloroimide (4). After 2 hours chlorination was quantitative and free from undesirable side reactions. No degradation was observed after 8 hours at −40°.

The dropwise addition of precooled (−60°) anhydrous methanol to the chlorination mix (this order of addition is preferred), maintaining the temperature at −50°, produces the imino ether hydrochloride free acid (5) after 1–2 hours reaction time at −50°. The alcoholysis reactions of the chlorimide and the silyl ester are quantitative and also free from any undesirable side reactions; the latter reaction occurring within 10–15 minutes at −50°.

The addition of 2.5–3% water by volume of methylation mix at −50° rapidly (e.g. within 5 minutes) cleaves the imino ether to 6-APA and methy phenoxyacetate. This reaction is nearly quantitative. In addition, there is no evidence to suggest that β-lactam breakage occurs during this step. Empirical data have shown that no loss of 6-APA occurs over 16 hours in this hydrolysis mix if it is stored that long.

The overall conversion of penicillin V to 6-APA in this process approaches 98–99%. Residual penicillin V assays of spent mother liquors are generally under 1%.

The resulting solution of 6-APA is treated with DMA at −50°, followed by the addition of D-(-)-phenylglycyl chloride hydrochloride (PGH) at −40°. After aqueous quench and workup via NSA/MILA, pure ampicillin trihydrate is produced in yields of 68–80% overall from penicillin V K salt.

Further laboratory investigations were then carried out by hydrolyzing methylation mix (prepared by adding chlorimide to methanol) with 6volume percent water at −45°, followed by acylation at this temperature with varying levels of DMA and PGH. Table I summarizes the effects of base and acid chloride on in-solution yields of ampicillin.

It appeared that the best conditions for acylation involved the use of 6–6.2 eq. of DMA and 1.1–1.3 eq. PGH (run numbers 9 and 10) at −45°. These conditions gave rise to 69–72% of ampicillin in solution. Higher mole ratios of PGH (run numbers 4, 8, 12, 16) apparently resulted in over acylation of 6-APA (acylation of ampicillin), whereas lower levels of both DMA and PGH apparently resulted in incomplete acylation of the 6-APA (run numbers 1–4).

A study of the effect of temperature on in solution yields of ampicillin was also carried out using the DMA/PGH levels described in Run No. 10 (Table 1). In these instances, methylation mix was prepared from known potency pen V K salt via esterification with DDS, chlorination with phosphorous pentachloride and by the addition of 25 eq. of methanol to the chlorimide. maintaining the addition temperature below −50°. The single phase methylation mix was hydrolyzed at −50° with 2.6% water based on the volume of the methylation mix, and acylated at the temperatures described in Table II.

TABLE I

The Effect of DMA and PGH Levels on Ampicillin Yields in Solution

TABLE I—Continued

Run No.	Moles of DMA added for Acylation	Moles of PGH added for Acylation	Calculated[1] % Ampi Free Acid in Soln.[2]
1	4.0	1.1	25.4
2	4.2	1.3	21.9
3	4.4	1.5	26.0
4	4.6	1.7	14.7
5	5.0	1.1	38.7
6	5.2	1.3	40.1
7	5.4	1.5	50.0
8	5.6	1.7	40.2
9	6.0	1.1	69.6
10	6.2	1.3	71.6
11	6.4	1.5	67.2
12	6.6	1.7	54.7
13	7.0	1.1	59.4
14	7.2	1.3	63.2
15	7.4	1.5	66.0
16	7.6	1.7	61.1
17	8.0	1.1	61.8
18	8.2	1.3	65.6

[1]A 2.0 ml. aliquot was taken from the acylation mix, stripped in vacuo, diluted to 20 mls. with pH 7.00 phosphate buffer and sent for bioassay. Yields are not corrected for input pen V potency.

[2]% Ampicillin in Solution =

$$\frac{\text{(Bioassay mcg/ml) (20 mls.) (Volume of Acylation mix)}}{\text{(2 mls.) (1000 mcg/mg) (1000 mg/gm) (Theoretical Yld in gms)}} \times 100$$

TABLE II

The Effect of Temperature on Ampicillin Yields in Solution[1]

Run No.	Moles of DMA for Acylation	Moles of PGH for Acylation	Acylation Temperature	% Ampi in Soln.
19	6.2	1.3	−50° C.	81.0
20	6.2	1.3	−40° C.	88.9
21	6.2	1.3	−30° C.	85.5
22	6.2	1.3	−20° C.	85.5
23	6.2	1.3	−10° C.	87.5

[1]Yields are corrected for input pen V potency.

Somewhat higher yields were noted at temperatures above −50° (Run Nos. 20–23). Interestingly, the rate of dissolution of the acid chloride was virtually instantaneous at −10°, whereas it requires 20 minutes at −50°.

Bioassay data tend to indicate that better yields of ampicillin are obtained using the controlled addition of 25 ea. of methanol to chlorimide (compare bio yields in Table I with Table II). Thus, several isolation variations were carried out using this methylation technique, some of which are illustrated in Table III.

TABLE III

Isolation Conditions and Yields of Ampicillin Trihydrate*

Run No.	Chem Assay in mcg/mg	% of Theory	Yield in gms.	% Yld.	Method of Isoln.
24	853;856	98.7	4.17	70	1[a]
25	810;811	93.8	15.8	76	1
26	817;812	94.1	5.4	77	2[b]
27	848;855	98.3	16.6	79	2
28	849;853	98.3	66.6	68	2
29	820	94.7	12.2	50	3[c]

*Yields are not corrected for purity.

[a]DMA removed by vacuum distillation at pH 7 (3.0N NaOH used for pH adjustment); NSA/MILA.

Workup in all cases consisted of aqueous quench of acylation mix at 0–5°. No emulsions were observed at this stage. The organic layer was removed and the aqueous was processed as follows:

Isolation method 1 involved adjustment of the rich aqueous with 3 N sodium hydroxide to pH 7–7.5. In addition to encountering an emulsion, a gummy solid precipitated during this step which was removed with difficulty via diatomaceous earth ("Dicalite") treatment and filtration. The formation of this solid, however, was precluded by continuous pH adjustment at pH 7.5, but pH control was difficult. The two phase mix (DMA and aqueous) was concentrated at 50° in vacuo to complete DMA removal. Slow acidification with aqueous β-naphthalenesulfonic acid (NSA) gave ampicillin NSA salt. The conversion of the wet NSA cake to ampicillin trihydrate using MIBK-LA-1 resin (MILA) gave yields up to 70–75% of good quality product.

Isolation method 2 involved adjustment of the rich aqueous with 6 N ammonium hydroxide to pH 7–7.5 in the presence of MIBK. An amorphous solid was found in addition to an emulsion, but was easily removed by filtration with added "Dicalite". The MIBK layer containing DMA was removed and the clean aqueous processed via NSA/MILA to good quality ampicillin trihydrate.

Method 3 consisted of removal of the DMA by solvent extraction (MIBK) at pH 7–7.5 (6 N ammonium hydroxide used for pH adjustment), followed by direct crystallization of the ampicillin by pH adjustment. The yields were considerably lower (Table 3) using this technique.

Either of these three methods is capable of yielding good quality ampicillin trihydrate in reasonably good yields from penicillin V Method 2 has thus far processed most smoothly of the three methods.

The acylation to ampicillin was also investigated using other bases such as triethylamine, imidazole and pyridine. The yields respectively in each case (bioassay of acylation mix) under best conditions were 55% (6.5 eq. TEA, 1.4 eq. PGH), 27.2% (5 eq. imidazole, 1.1 eq. PGH) and 30% (20 eq. pyridine, 1.1 eq. PGH). These yields were all lower than those obtained using DMA.

Using the best conditions thus far obtained, an acylation of the resulting solution of 6-APA with D-(-)-2-(4-hydroxyphenyl)glycyl)chloride hydrochloride PHPGH) was examined at −40° using 6.2 eq. DMA/1.3 eq. PHPGH. Bioassay data indicated yields of amoxicillin in solution approaching 85% average on three occasions.

The silyl esters of the process of the present invention are made, for example, by the use of such agents as are described in U.S. Pat. Nos. 3,499,909, 3,249,622, **3,654,266, 3,678,037, 3,741,959** and 3,694,437, e.g., trimethyl chlorosilane, hexamethyl disilazane, triethyl chlorosilane, methyl trichlorosilane, dimethyl dichlorosilane, triethyl bromosilane, tri-n-propyl chlorosilane, bromomethyl dimethyl chlorosilane, tri-n-butyl chlorosilane, methyl diethyl chlorosilane, dimethyl ethyl chlorosilane, phenyl dimethyl bromosilane, benzyl methyl ethyl chlorosilane, phenyl ethyl methyl chlorosilane, triphenylchlorosilane, triphenyl fluorosilane, tri-o-tolyl chlorosilane, tri-p-dimethylaminophenyl chlorosilane, N-ethyl triethylsilylamine, hexaethyl

Volume Par Cm3 	hauteur	rayon
Amylacetate=5849.14	34	7.4
AEH=SODIUM ACETAT=PENICILLIN=CHAORECOL TREATMENT=crystal dring=Ethanol=40212.38L	50.5	16
Acyclase treatement=ampicillin=56297.34	70	16

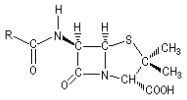
Cm3=ml

Cm3=10^{-3}l

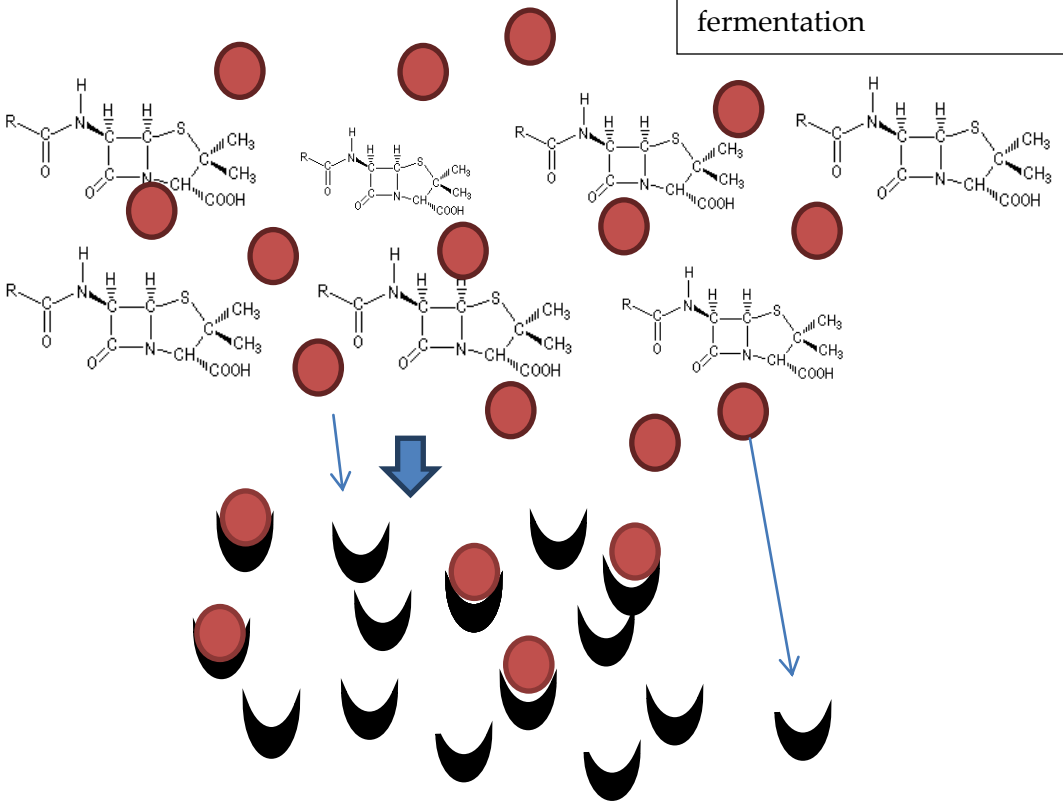
V=3.14*r²*h

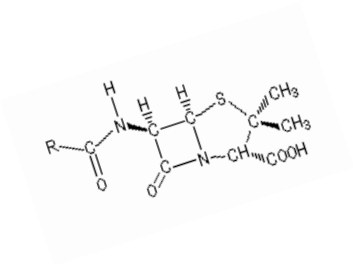
:produit indesirable

: Charcoal



:penicillin produit par fermentation



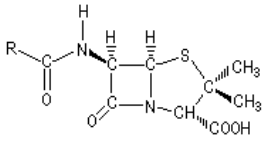


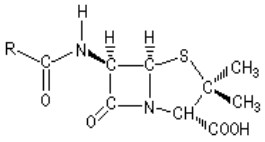
Sodium acetate

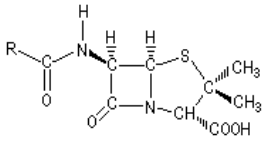
Filtration

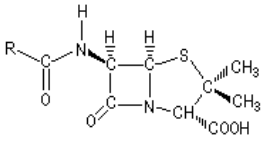
Ethaol

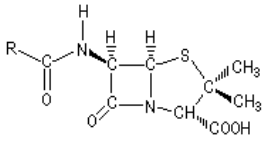
filtration

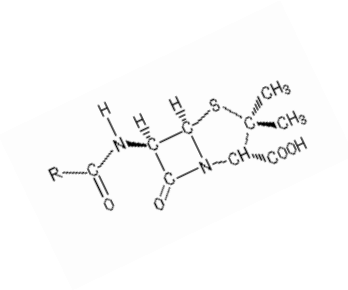






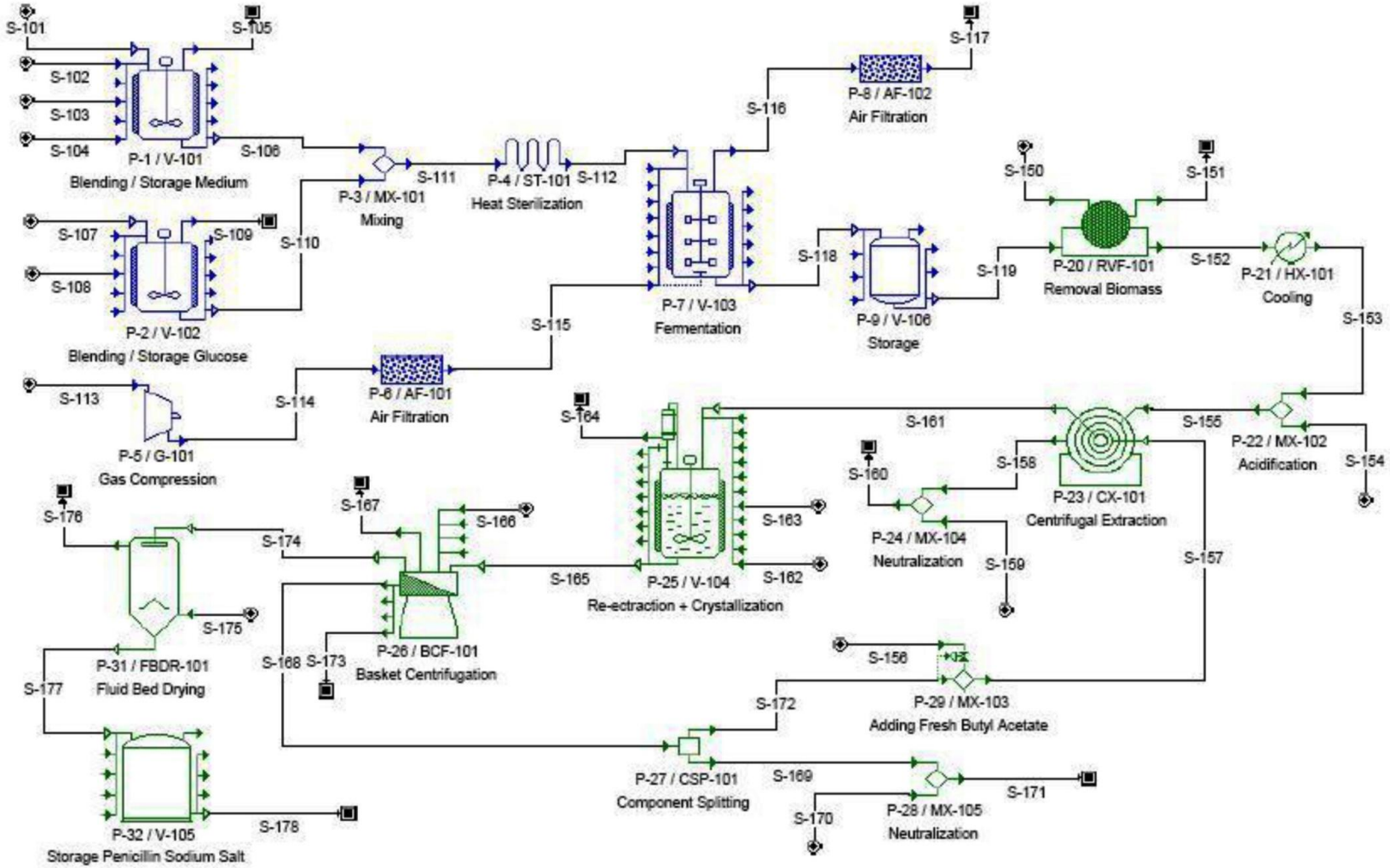






2.6 Large Scale Penicillin Production

2.6.1 Process Flow Diagram



As in any bioprocess facility, there has to be an upstream and downstream process, the upstream processes in this case are refering to processes before input to the fermenter, while the downstream processes refers to the processes that are done to purify the output of the fermenter until it reaches to the desired product.

2.6.2 Medium for Penicillium

Medium preparation is necessary in bioprocesses which as it generally involve the use of microorganism to achieve their products. In the case of the Penicillium fungus, the medium usually contain its carbon source which is found in corn steep liquor and glucose. Medium also consist of salts such as Magnesium sulphate, Potassium phosphate and Sodium nitrates. They provide the essential ions required for the fungus metabolic activity.

Corn_steep_liquor.jpg

Corn steep syrup

2.6.3 Heat sterilisation

Medium is sterilse at high heat and high pressure usually through a holding tube or sterilse together with the fermenter. The pressurized steam is use usually and the medium is heated to 121°C at 30psi or twice of atmospheric pressure. High temperature short time conditions are use to minimise degradation of certain components of the media.

Sterilisation machine

2.6.4 Fermentation

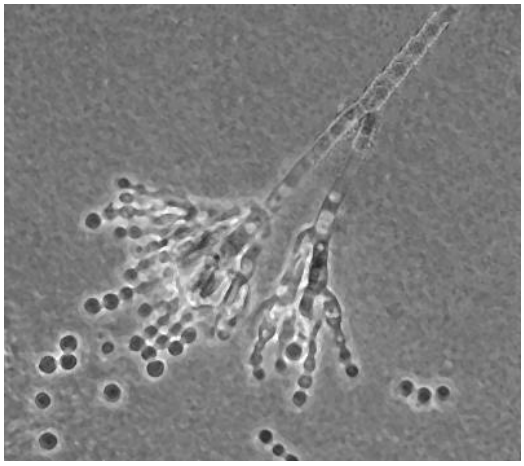
Fermentation for penicillin is usually done in the fed-batch mode as glucose must not be added in high amounts at the beginning of growth which will result in low yield of penicillin production as excessive glucose inhibit penicillin production. In addition to that, penicillin is a secondary metabolite of the fungus, therefore, the fed-batch mode is ideal for such products as it allows the high production of penicillin. The typical fermentation conditions for the *Penicllium* mold, usually requires temperatures at 20-24 °C while pH

conditions are kept in between 6.0 to 6.5. The pressure in the bioreactor is usually much higher than the atmospheric pressure(1.02atm) this is to prevent contamination from occurring as it prevents external contaminants from entering. Sparging of air bubbles is necessary to provide sufficient oxygen the viability of the fungus. Depending on the volume of medium, for 2 cubic metres of culture, the sparging rate should be about 2.5 cubic metres per minute. The impeller is necessary to mix the culture evenly throughout the culture medium, fungal cells are much hardy and they are able to handle rotation speed of around 200rpm.

Fermenters.jpg

2.6.5 Seed culture

Like any other scale up process, usually the seed culture is developed first in the lab by the addition of *Penicillium* spores into a liquid medium. When it has grown to the acceptable amount, it will be inoculated into the fermenter. In some cases,the spores are directly inoculated into the fermenter.



The Penicillium fungus

2.6.6 Removal of biomass

Filtration is necessary at this point of the bioprocess flow, as bioseparation is required to remove the biomass from the culture such as the fungus and other impurities away from the medium which contains the penicillin product. There are many types of filtration methods available today, however, the Rotary vacuum filter is commonly employed as it able to run in continuous mode in any large scale operations. Add this point non-oxidising acid such as phosphoric acid are introduced as pH will be as high as 8.5. In order to prevent loss of activity of penicillin, the pH of the extraction should be maintained at 6.0-6.5.

Rotary_vacuum_filter.jpg

2.6.7 Adding of solvent

In order to dissolve the penicillin present in the filtrate, organic solvents such as amyl acetate or butyl acetate are use as they dissolve penicillin much better than water at physiological pH. At this point, penicillin is present in the solution and any other solids will be considered as waste.

solvent.jpg

Amyl Acetate as Solvent

2.6.8 Centrifugal extraction

Centrifugation is done to separate the solid waste from the liquid component which contains the penicillin. Usually a tubular bowl or chamber bowl centrifuge is use at this point.The supernatant will then be transferred further in the downstream process to continue with extraction.

disk_centrifuge.jpg

Disk centrifuge- One of the most common type of centrifuge for large scale production

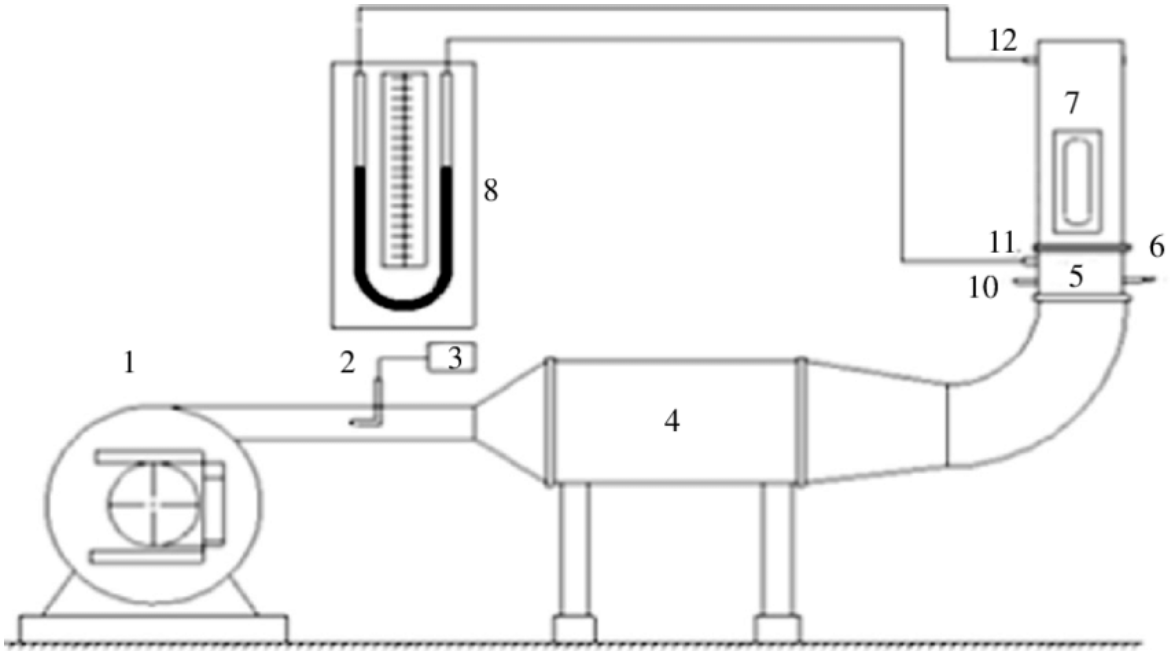
2.6.9 (Batch) Extraction

Penicillin dissolve in the solvent will now undergo a series of extraction process to obtain better purity of the penicillin product. The acetate solution is first mixed with a phosphate buffer, followed by a chloroform solution, and mixed again with a phosphate buffer and finally in an ether solution. Penicillin is present in high concentration in the ether solution and it will be mixed with a solution of sodium bicarbonate to obtain the penicillin-sodium salt, which allow penicillin to be stored in a stable powder form at room temperature. The penicillin-sodium salt is obtained from the liquid material by basket centrifugation, in which solids are easily removed.

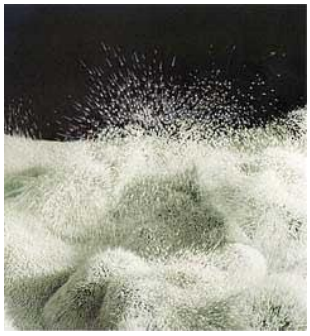
	
Batch extraction unit	Basket Centrifuge- Extremely using in the removal of solids in this case Penicillin salt

2.6.10 Fluid bed drying

Drying is necessary to remove any remaining moisture present in the powdered penicillin salt. In fluid bed drying, hot gas is pump in from the base of the chamber containing the powdered salt inside a vacuum chamber. Moisture is then remove in this manner and this result in a much drier form of penicillin.



Schematic diagram of the fluidized bed dryer: (1) blower, (2) pitot tube, (3) differential pressure transmitter, (4) electrical heater, (5) plenum chamber, (6) distributor, (7) drying chamber, (8) differential manometer, (9) humidity measurement sensor, (10) temperature control sensor, and (11, 12) pressure tap



spray_powder.jpg

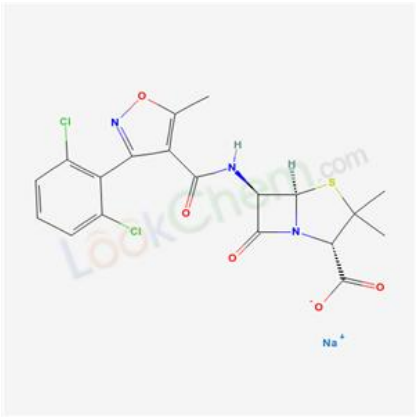
Powdered penicillin being blowned by hot air

2.6.11 Storage

Penicillin salt is stored in containers and kept in a dried environment. It will then be polished and package into various types of products such as liquid penicillin or penicillin in pills. Dosage of the particular penicillin is determined by clinical trials that are done on this drug.

Penicilin_sodium.jpg

The White Penicillin-Sodium salt



Chemical Structure of the Penicillin Sodium Salt

Chemical Structure of the Penicillin Sodium Salt

http://slideplayer.com/slide/10446753/"EXTRACTION & PURIFICATION of PENICILLIN

2.7 العفن الذي أنقذ العالم و طريقة صنع البنسلين

لأحد, 27 يناير 2013

- قطعة من الخبز أو قشر الحمضيات
- دورق مخروطي 750 مل
- وسيط (انظر للخطوة الرابعة)
- 1 لتر مخبار مدرج Éprouvette graduée
- عدد من زجاجات الحليب النظيفة ..

خطوات العمل :

1- لتحضير البنسلينيوم : تُعرّض قطعة من الخبز أو قشر الحمضيات لبيئة تكون درجة حرارتها 70 درجة فهرنهايت (25 درجة مئوية) و ينبغي أن يكون العفن أزرق أو أخضر .

2- لتعقيم الأدوات : ضع الدورق في فرن عند درجة حرارة 315 درجة فهرنهايت (157.2 درجة مئوية) على مدار الساعة ، أو تعقيم الأدوات في قدر الضغط لمدة لا تقل عن 15 دقيقة ، اغسل زجاجات الحليب جيداً .

3- ملء الدورق المخروطي : تقطع قطع الخبز أو قشر الحمضيات لقطع صغيرة و يملأ بها الدورق و نضعها بعد ذلك في الظلام عند درجة حرارة 70 درجة فهرنهايت (21.1 درجة مئوية) لمدة 5 أيام (فترة الاحتضان) ، بعد فترة الحضانة يمكن الإحتفاظ بالدورق في الثلاجة لمدة لا تزيد عن 10-14 يوم .

4- لتحضير الوسيط : أذب المكونات التالية حسب الترتيب المسرود في 500 مل من ماء الصنبور البارد

44,0 جرام لاكتوز أحادي الهيدرات , 25.0 جرام نشا الذره ,3,0 جرام نيتريت الصوديوم , 0.25 جرام كبريتات المغنيسيوم , 0.50 جرام فوسفات البوتاسيوم الأحادي , 2.75 جرام جلوكوز أحادي الهيدريد , 0.044 جرام كبريتات الزنك , 0.044 جرام كبريتات المنجنيز . ثم أضف أخيراً ماء الصنبور البارد لعمل لتر واحد . إستخدم حمض الهيدروكلوريك لضبط ال ph بين 5.0 و 5.5 .

5- ملء الزجاجات بمادة الوسيط : نملأ زجاجات الحليب بهذه الوسائط ، نستخدم عادةً كمية تكفي بحيث عندما نضع زجاجة بجانبها لا يصل هذا الوسيط إلى المكونات .

6- إضافة أبواغ البنسلين (العفن) : أولاً نقوم بتعقيم زجاجات الوسيط في قدر الضغط أو في الفرن كما فعلنا في الدورق المخروطي و عندما تبرد الزجاجات نضع بها ملعقة من أبواغ (عفن) الخبز أو قشر الحمضيات .

7- إحتضان الزجاجة : تترك الزجاجات للراحة بدون عائق في الجانبين عند درجة حرارة 70 درجة فهرنهايت (21.1 درجة مئوية) لمدة 7 أيام ، إذا تكوّن البنسلين سيكون الجزء السائل في الوسيط بعد هذه الفترة (الحضانة) ، و أخيراً تصفية الوسيط و تبريده على الفور ، إذا كان يجب استخدامه يستخدم في أقرب وقت ممكن و إن كان ينبغي تجنب ذلك .

لا ينبغي للبنسلين المتكون من هذه التجربة أن يستخدم إلا إذا كان لغرض البقاء أو استخدامات أخرى

فمن الممكن للعفن السام أن ينمو جنباً إلى جنب مع البنسلين ، حتى لو كنت تعرف مالذي تقوم به فمن الممكن لمتبطات نمو العفن وقف نمو أبواغ البنسلين .

2 http://chemi101.blogspot.com/2013/01/blog-post.html

2.8 Sabouraud Agar

Agar Sabouraud agar in a Petri dish with a colony of Trichophyton rubrum var. rodhaini.

Sabouraud's agar (which is named after Raymond Sabouraud) is an isolation medium for Fungi (molds and yeasts).

It was created by, and is named after, Raymond Sabouraud in 1892. Later adjusted by Chester W. Emmons when the pH was brought closer to the neutral range and the dextrose concentration lowered to support the growth of other fungi. The pH of 5.6 of the traditional sabouraud agar inhibits bacterial growth.

(Dermatophyte_test_medium&action)

2.9 Uses of Ethyl acetate

Ethyl acetate is used in the following areas:

- solvent to remove nail polish (called solvent);
- solvent for dangerous glues to "sniff" because it causes a feeling of intoxication that can damage the brain;
- solvent for nitrocellulose;
- produce to decaffeinate coffee beans and tea leaves;
- solvent for chromatography mixed with a non-polar solvent such as hexane;
- solvent for extractions (antibiotics);

2.9.1 Synthesis of Ethylacetate

Ethyl acetate is synthesized by the Fischer esterification process, resulting from a reaction between acetic acid and ethanol. An acid, such as sulfuric acid, catalyzes the reaction. $CH_3CH_2OH + CH_3COOH \rightarrow CH_3COOCH_2CH_3 + H_2O$.

2.10 Revelation of efficacite to penicillin

2.10.1 LBmedium

The aim of the culture to tested the penicillin soluble

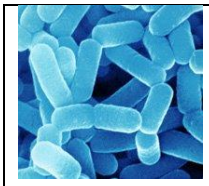
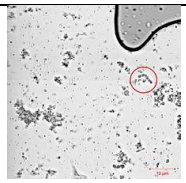
Preparation of medium

they are called the two main bacteria of yogurt *Lactobacillus bulgaricus* and *Streptococcus thermophilus.*

Pour les articles homonymes, voir LB.

LB culture medium in a bottle and in a culture dish. The LB culture medium (For lysogeny broth or incorrectly Luria-Bertani medium) is a nutrient culture medium, initially used for bacterial culture1. It was first developed by Bertani, who named it lysogeny broth (lysogenic broth) in its first publication2. LB media have become an industry standard for culturing Escherichia coli since the 1950s. They have been used extensively in molecular microbiology for the preparation of DNA plasmids and recombinant proteins. It remains to this day, one of the most used environments for the maintenance and culture of recombinant lines of Escherichia coli. There are various compositions of LB. Although they are different, they usually share some of the common components they have to support the growth of species in culture. • Peptides and peptones of casein• Vitamins (Vitamin B included) • trace elements (eg nitrogen, sulfur, magnesium) • Minerals

2.11 Bactéris of yaourt

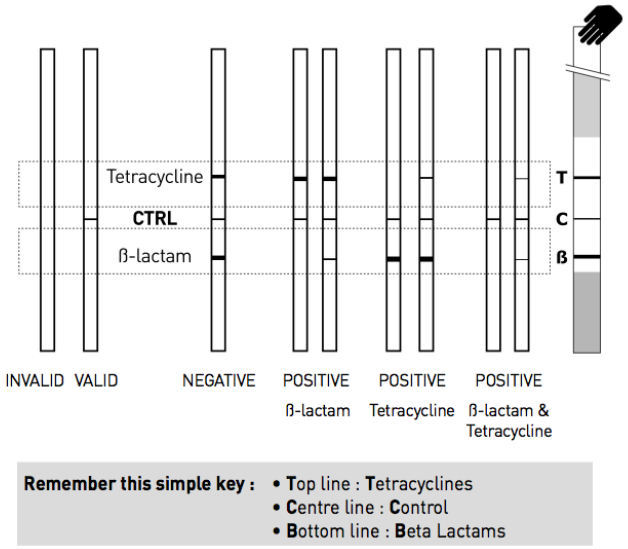
	
Lactobacillus delbrueckii subsp. Bulgaricus is a microorganism of the genus Lactobacillus. It is a gram positive bacillus. His discovery was due to the Bulgarian student of medicine Stamen Grigoroff (in) in 19051, and named in 1919, Thermobacterium bulgaricum, by the Danish Orla Sigurd Jensen (da) (1870-1949). From 1971 to 1983, its name was Lactobacillus bulgaricus, renamed by Morrison Rogosa and Danish Poul Arne Hansen (1902-1972). Caractéristiques: - gram + - anaérobie - catalase – - oxydase –	The thermophilic streptococcus(or Streptococcus thermophilus1,2) is a thermophilic food bacterium (growth optimum at 43 ° C), present only in the fermentation of milk, where it is used in particular in association with the bacterium Lactobacillus delbrueckii subsp. bulgaricus for making yoghurt. • as a cocci (rounded shell), 0.7-1 µm, forming strings or pairs • with positive Gram stain • its optimum growth temperature is between 37 ° C and 60 ° C, depending on the strain. Does not grow at 15 ° C but all strains grow at 45 ° C and most at 50 ° C • strict homofermentative bacterium (producing L-lactate), microaerophilic • non-pathogenic its cultivation requires B vitamins and some amino acids.

In Gram-positive bacteria, the different β-lactams reach the transpeptidases through the already formed or in-process peptidoglycan wall. In contrast, in Gram-negative bacteria, they only reach these enzymes after penetration through the pores of the outer membrane

2.11.1 Twin sensor

The test requires the use of two components. The first component is a microwell containing predetermined amounts of receptors and antibodies bound to gold particles. The second is a gauge composed of a set of membranes with specific capture lines.

For a valid test, the red control line should be visible after the second incubation. Both ether are the specific test lines placed on both sides of the control line. The line of β-lactam antibiotics [penicillins and cephalosporins] is located under the "control" whereas the tetracycline-related line is located above. When the reagent from the microwell is resuspended with a milk sample, the two receptors will bind the corresponding analytes if they are present during the first 3 minutes of incubation at 40 ° C. Then, when the dipstick is immersed in the milk, the liquid begins to run vertically on the gauge and passes through the catchment areas.

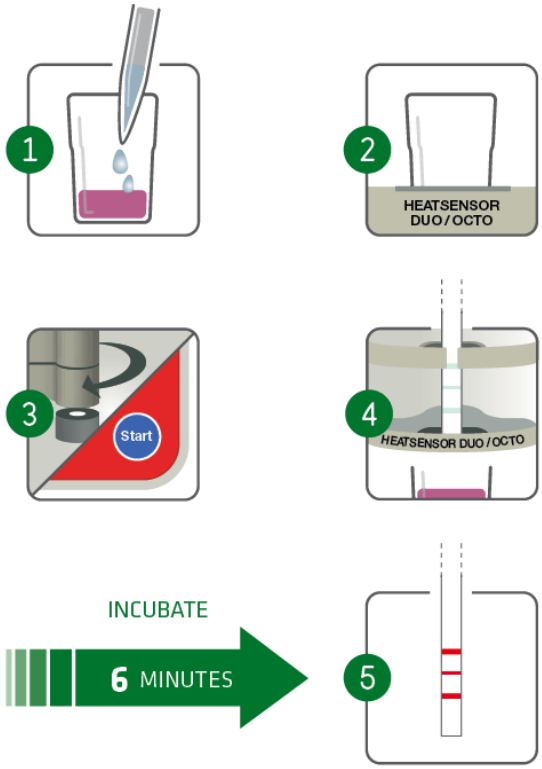


When the sample is free of antibiotics, color development occurs at the specific capture lines, indicating the absence of targeted analytes in the milk sample. On the contrary, the presence of antibiotics in the sample will not cause the appearance of the colored signal at the specific capture lines.

Beta-lactams and tetracycline antibiotics are the antibiotics most commonly used in the treatment of bacterial infections in dairy cattle. A specific indication for administering both types of antibiotics is infectious mastitis. These drugs are also administered to animals in foods for the promotion of growth and for collective prophylaxis.

The monitoring of beta-lactams and tetracyclines in milk is important because of the hypersensitivity of certain individuals to these antibiotics and the emergence of bacterial strains resistant to antibiotics. In addition, the overall residual level of antibiotics could alter the efficiency of industrial processing from raw milk to the preparation of cheese or other fermented dairy products.

Maximum Residue Limits (MRLs) have been specified for food products and milk to control the levels of these antibiotics reaching the consumer. The kit is available in a version specific to the European Union Maximum Residue Limits (KIT020).



5 http://www.intermed.be/fr/produits-professionels/laboratoire-diagnostiques/produits-laitiers/twinsensor.html

2.11.2 Analysis of Penicillin purity: ELISA Kit

www.abnova.com:

Catalog Number KA3305
96 assays
Version: 03
Intended for research use only
KA3305 3 / 9

During routine testing of milk samples for antibiotics, in more than 90% of the positive cases, betalactam preparations or penicillins are detected. The method of choice for the determination of penicillin contamination in food has always been a microbiological assay. These procedures allow however no quantitative determination and no identification of the antibiotic drug, which is achieved by a sensitive ELISA test kit or immunoaffinity columns together with HPLC. Principle of the Assay

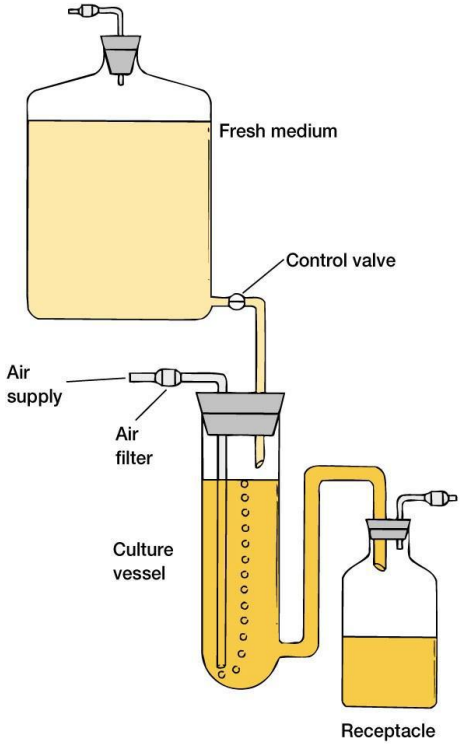
The Penicillin ELISA Kit is based on the principle of the enzyme linked immunosorbent assay.

A penicillin conjugate is bound on the surface of a microtiter plate. A penicillin conjugate is bound on the surface of a microtiter plate. Penicillin containing samples or standards and an antibody directed against penicillin are given into the wells of the microtiter plate. Immobilized and free penicillin compete for the antibody binding sites. After one hour incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A peroxidase conjugate directed against the penicillin antibody is given into the wells and after another hour incubation, the plate is washed again. Then a substrate solution is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured photometrically at 450 nm. The concentration of penicillin is indirectly proportional to the color intensity of the test sample.

2.12 Le chémostat

- Rythme d'introduction du milieu stérile = rythme d'élimination du milieu.

- Un élément nutritif essentiel est fournit en quantités limitées (i.e. un acide aminé



Mold culture Penicillium chrysogenum in liquid Sabouraud medium, with gentle agitation.

It is noted that, unlike bacteria that develop in a liquid medium without forming colonies mildew by clouding the medium, molds form spherical structures (due to their centrifugal growth from a spore) and that the medium remains perfectly limpid (a disorder of the environment thus translating a microbial contamination)

3_image: http://droguet-sebastien.e-monsite.com/medias/images/penicillium-sabouraud-liquide-1-.jpg?fx=r_1200_800

Reference: http://droguet-sebastien.e-monsite.com/pages/activites-technologiques-terminale-2014-2015/at03-etude-des-mycetes.html

2.13 Principles of pO2 Measurement with the Clark Electrode

The Clark Oxygen Electrode

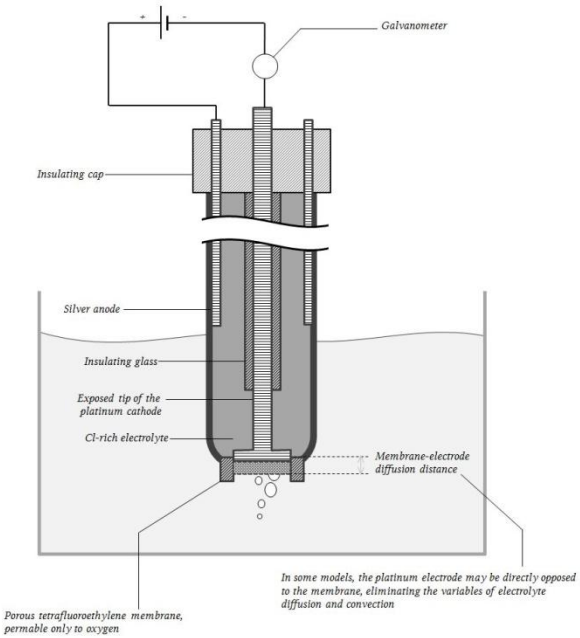
The principles of amperometric oxygen measurement are discussed at some length in the chapter on the platinum oxygen cathode.

In brief:

- A silver anode and platinum cathode are suspended in an electrolyte.
- Oxygen is dissolved in the electrolyte.
- A voltage of known magnitude (about 700 mV) is applied to the electrodes.
- Oxygen is reduced at the cathode and silver is oxidised at the anode.
- The resulting current increases as the voltage increases.
- The current reaches a plateau when the rate of reaction is determined by the diffusion of oxygen rather than the voltage.
- This plateau correlates to the oxygen tension in the electrolyte.

The major difference between this electrode and the earlier oxygen cathode is the addition of an oxygen-permeable membrane. Something resembling the original patent application diagram can be found here.

Its butchered representation can be found below.



Reference

derangedphysiology.com/main/core-topics-intensive-care/arterial-blood-gas-interpretation/Chapter 2.0.5/principles-po2-measurement-clark-electrode

2.14 Uses of Ethyl acetate

Ethyl acetate is used in the following areas:

- solvent to remove nail polish (called solvent);
- solvent for dangerous glues to "sniff" because it causes a feeling of intoxication that can damage the brain;
- solvent for nitrocellulose;
- produce to decaffeinate coffee beans and tea leaves;
- solvent for chromatography mixed with a non-polar solvent such as hexane;
- solvent for extractions (antibiotics);

Summary

Ethyl acetate is synthesized by the Fischer esterification process, resulting from a reaction between acetic acid and ethanol. An acid, such as sulfuric acid, catalyzes the reaction. $CH_3CH_2OH + CH_3COOH \rightarrow CH_3COOCH_2CH_3 + H_2O$.

Since this reaction is reversible and produces a chemical equilibrium, the yield is low unless the water is removed. In the laboratory, ethyl acetate can be separated from water using the Dean-Stark process.

2.15 Synthesis of ethyl acetate

synthesis of ethyl acetate.html https://www.youtube.com/watch?v=cFxZ0NircIk

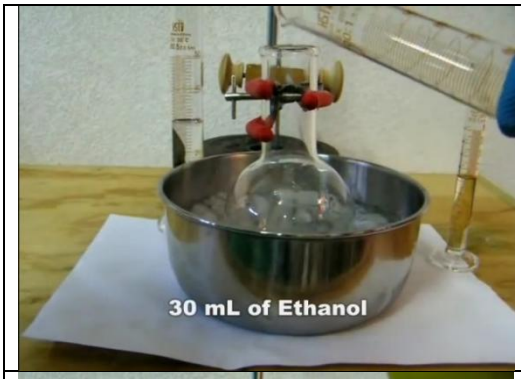
30ml acetic acid

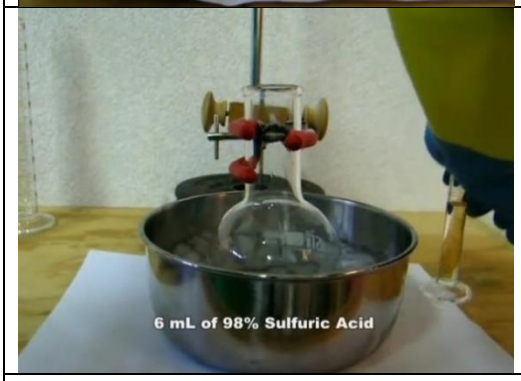
30ml ethanol

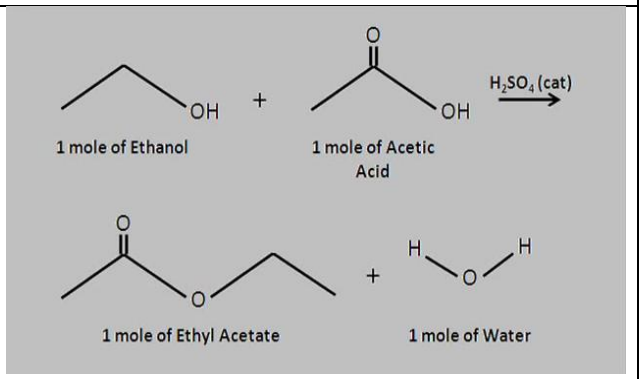
6ml sulfuric acid

3g Na2co3+15ml H2O

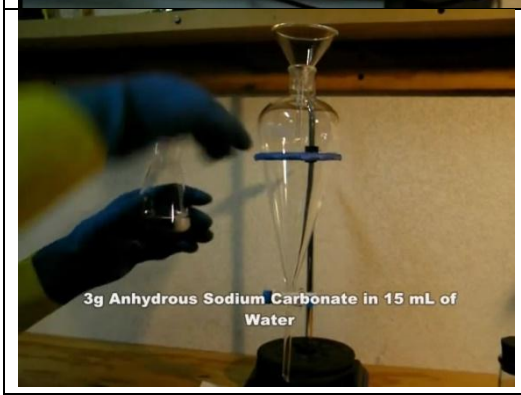
3g CaCL2+15ml H2O

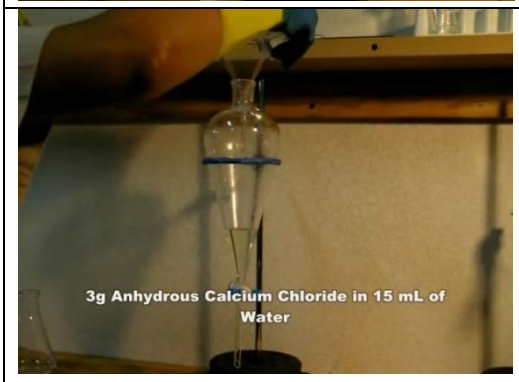







تم النسخ إلى الحافظة


3g Anhydrous Sodium Carbonate in 15 mL of Water


3g Anhydrous Calcium Chloride in 15 mL of Water

2.16 Dean Stark's device

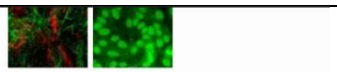
The device is generally used for azeotropic distillation. For example, to remove water produced by a reaction involving toluene. A heteroazeotropic mixture of toluene and water evaporates from the flask, but only toluene returns (being of lower density) since it floats above the water which accumulates in the "burette".

For example in the case of the esterification of butanol with acetic acid catalyzed by sulfuric acid. The vapors contain 63% ester, 24% water and 8% alcohol; after condensation, the organic phase which returns to the medium contains 86% of ester, 11% of alcohol and 2% of water while the aqueous phase consists of 97% pure water)

2.17العمل مع المجهر (working with microscope)

OBJECTIVE POWER	4X, 10X, 40X, 100X
OBJECTIVE SIZE	N/A
EYEPIECE POWER	10X, 25X
EYEPIECE SIZE	N/A
EYEPIECE FOCUSABILITY	N/A
CAMERA	N/A
CAMERA PIXEL	N/A
CAMERA SENSOR	N/A
REDUCTION LENS	N/A
STAGE	N/A
MICROSCOPE STAND	No
LIGHTING CONFIGURATION	Transmitted

PRODUCT CODE	5555268
MICROSCOPE TYPE	Compound
SPECIALIZED	EPI-Fluorescenc
APPLICATION	Clinic, Veterinary, Laboratory
MAGNIFYING TYPE	Multi-Power
MAGNIFICATION POWER	40X to 2500X
OPTICS	Achromatic
FIELD VIEW	N/A
HEAD TYPE	Trinocular
OBJECTIVE POWER	4X, 10X, 40X, 100X



$1,619.99

IN STOCK

- Epi-fluorescence illumination with blue and green exciting light filters
- Transmitted brightfield illumination
- Trinocular compensation free viewing head, easy to mount digital camera to catch ordinary and high contrast fluorescent images
- 4 high quality fluorescence objectives: FLUOR 4x, 10x, 40x(S), 100x(S,Oil)
- Large stain-resistant double layer stage can hold two slides in parallel

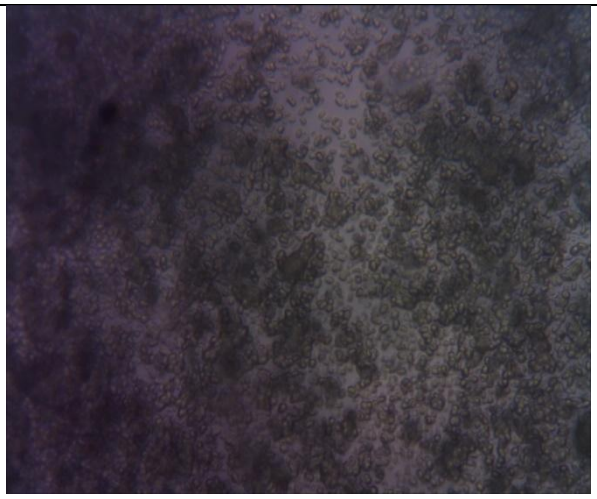
Qty: 1 ADD TO CART

Add to Wishlist | Add to Compare

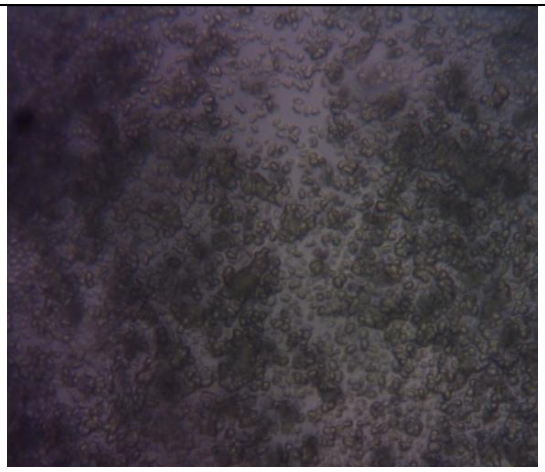
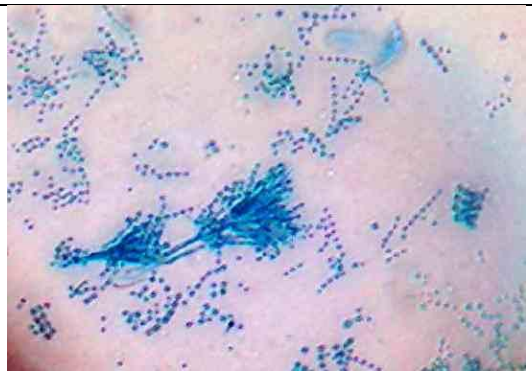
نوع المكثف	N / A
بصريات مكثفة	N / A
بارلو لينس	N / A
قزحية	N / A
مزود الطاقة	N / A
التعليم	N / A
اسم العلامة التجارية	OMAX
اللون	N / A

نوع خفيف	الهالوجين ، بخار الزئبق
قوة الضوء	N / A
شكل خفيف	N / A
عدد من المصابيح الكهربائية	2
المرحلة DIV	N / A
مكثف	N / A
نوع المكثف	N / A
بصريات مكثفة	N / A
بارلو لينس	N / A
قزحية	N / A
مزود الطاقة	N / A
التعليم	N / A

Your search - **microscope fluorescent (YJ-1006285)** - did not match any



Techniques	Aspect microscopique (G×40)	Caractères microscopiques

Adhesive tape A small piece of tape is applied by the sticky face on the colony then deposited on a slide. Then observation under immersion microscope: the goal (× 40) then to (× 100) (Joffin, 2013)		Spores
Lactophenol blue cotton A fragment of the colony is removed with the help of a platinum loop and deposited on aslide in a dye drop afterwards cover with a coverslip-object that makes the preparation crushed (Chabasse et al., 2002		-Conidiophores isolés -Pénicilles constitués de phialides branchés directement à l'extrémité du conidiophore

Since this reaction is reversible and produces a chemical equilibrium, the yield is low unless the water is removed. In the laboratory, ethyl acetate can be separated from water using the Dean-Stark process.

2.18 Preparation of medium saboureu

2.18.1 Experimental protocol

	g		ml		g	ml	
	15		1000				250
	20		1000				
	10		1000				
glucose	2		100	eau	0.5	25	5
peptone	1		100		0.25	25	2.5
agar	1.5		100		0.375	25	

Materials:

- Becher 100ml, Stirrer
- Erlenmeyer, Petri dish, Libra

- Glucose, Microbiological medium agar, Tryptone yeast extract, KH2PO4, MgCl2, CaCl2, distilled water, autoclave, charcoel treatment, acetic acid, ethanol, amino acid (instead of peptone)
- Microwave or Bunsen burner and penicillium orange (green spot)
  Incubator

Procedure:

- The glassworks are washed with tap water and then with distilled water and sterilized the glassworks by the autoclave
- 0.5 g of glucose, 0.25 g of tryptone, 0.4 g of agar and 0.25 g of KH 2 PO 4, 0.25 g of MgCl 2 are weighed into the Erlenmeyer flask using a pipette and 0.25 ml of CaCl 2 are measured.
- The test solution is filled with 25 ml of water and poured into the Erlenmeyer flask.
- We put the Erlenmeyer on the magnetic stirrer at 100 °C until two minutes left to cool a little
- Pour the mixture into the semi-covered dough box until the solidified solid (gel)
  We put some spore of the green spot on the gel obtained we semi cover and put it in the incubator 48 h, we read
- After 48h and reading the box the penicillium and ready to grow in a liquid medium

Feasibility Study and Business Plan

3 Feasibility study

3.1 احتياجات السوق في الدول العربية وتركي

3.1.1 Situation in Egypt

1 أسماء أمين

2017-12-18 11:15:17

طباعة

البنسلين – أرشيفية

قال محمود فؤاد رئيس المركز المصرى للحق فى الدواء إن أزمة البنسلين انتهت بنسبة 95% منذ حوالى 48 ساعة.

وأوضح محمود فؤاد خلال مداخلة هاتفية ببرنامج "8 الصبح"، المذاع على فضائيةdmc ، أن هذه الأزمة لها أسباب محددة بأن مصر تستهلك من 6 إلى 8 ملايين عبوة بنسلين سنويا، مضيفا أن هناك شركة عامة مسؤولة عن استيراد حوالى 80% من الاستهلاك السنوى، وبدورها تورده للحكومة.

وأضاف فؤاد أن رئيس مجلس إدارة أحد الشركات قبل أن يترك عمله بالشركة أنشأ جمعية عمومية مصغرة للشركة ومجلس إدارة مقررين الاستغناء عن حوالى 45 منتجا من بينها البنسلين، ووافق مجلس الإدارة، على أن تتولى شركة خاصة جديدة هذه المهمة، إلا أنهم اكتشفوا فى النهاية أن رئيس مجلس الإدارة تنازل لنفسه من الباطن.

قالت الدكتورة رشا زيادة، رئيس الإدارة المركزية لشئون الصيادلة بوزارة الصحة والسكان، إن وزارة الصحة والسكان لديها مخزون استراتيجي من المواد الخام المصنعة لعقار «البنسلين» يكفي لإنتاج 2 مليون عبوة من البنسلين.

وأوضحت لـ«الدستور»، أن الاستهلاك الطبيعي للبنسلين يبلغ 300 ألف حقنة شهريًا على مستوى المحافظات.

وأكدت أن وزارة الصحة والسكان، وفرت 3 ملايين و800 ألف عبوة بنسلين طويل المفعول، ما بين مستورد ومحلي منذ ديسمبر الماضي، حيث قامت بتوفير 800 ألف عبوة بنسلين مستورد، وتم توزيع 400 ألف منها على شركات التوزيع، والاحتفاظ بـ400 ألف كمخزون استراتيجي بالوزارة، مشيرة إلى أن وزارة الصحة سوف تستورد مليون عبوة أخرى من المستورد خلال شهر فبراير المقبل.

وأضافت أنه تم توزيع الإنتاج المحلي الذي بلغ مليونا و200 ألف عبوة، وتم تخزين مثلها كمخزون استراتيجي، لافتةً إلى أنه تم توزيع البنسلين على الصيدليات دون كوتة محددة، مما ساعد على توافره في أى صيدلية.

وحذرت «زيادة» من تلاعب بعض الصيدليات في سعر العقار، أو إيهام المرضى بعدم وجوده، مناشدة المواطنين سرعة الاتصال بإدارة التفتيش الصيدلي في هذه الحالة؛ لاتخاذ الإجراءات اللازمة ضد هذه الصيدليات.

كانت وزارة الصحة والسكان قد شهدت نقصا حادا في حقن «البنسلين» طويل المفعول، خلال العام الماضي

هذة الحقن تشتهر بأسم البنسلين طويل (ممتد) المفعول. Long Acting Penicillin

توجد فى تركيز واحد فقط و هو مليون و 200 ألف وحدة دولية (1,200,000. (U

أشهر أستعمالاتها هى للوقاية من مرض الحمى الروماتيزمية.

الجرعة المعتادة هى مرة واحدة فقط فى الشهر .

كتبت ـ أسماء سرور وهدير الحضرى

نشر فى : الأربعاء 13 ديسمبر 2017 - 8:51 م | آخر تحديث : الأربعاء 13 ديسمبر 2017 - 8:51 م

-الصيادلة: احتياجات السوق 200 إلى 400 ألف عبوة شهريًا.. وقانون الهيئة العامة للدواء يمهد لحل الأزمة

قال المتحدث الرسمى لوزارة الصحة خالد مجاهد، إن 4 شركات محلية ستكون مسئولة عن إنتاج البنسلين خلال الفترة المقبلة، لتقليل الاعتماد على الاستيراد، وضمان عدم تكرار أزمة نقص الحقن، التى حدثت خلال الفترة الماضية، مشيرا إلى توفر رصيد استراتيجى يكفى لمدة 5 أشهر بنهاية ديسمبر الحالى.

https://www.shorouknews.com/news/view.aspx?cdate=13122017&id=5d982b45-ef04-42df-a526-b122c49eaeb1

11يناير 2018 4:52 م

تصاعدت أزمة نقص البنسلين والأنسولين المستورد بدمياط، وسط صرخات الأهالى بتوفير البنسلين والأنسولين لإنقاذ أبنائهم وإنقاذ المرضي خاصة من هم بحاجة إلى البنسلين وسرعة حل الأزمة وتنفيذ وعوده بتوفير الأدوية، عقب زيادة سعرها. وبيعها بالسوق السوداء بأضعاف ثمنها الأصلي.

وتأتى أزمة نقص البنسلين لتصدر المشهد فبعد إن كان متوافرا بالأسواق بسعر 9 جنيهات للزجاجة وصل سعره إلى 150 جنيها بدعوى أنه ناقص بالسوق وأنه مستورد.

تقول أمانى السيد إنها تعانى في الحصول على الأنسولين المستورد من مستشفيات التأمين الصحى وترفض الحصول على الأنسولين المصري لأنه غير فعال ويسبب اضطرابات في نسبة السكر في الدم ولهذا تلجأ إلى شرائه من الصيدليات بسعر 45 جنيها للعبوة الواحدة.

وتابع أحمد العزب موظف: ابنى الوحيد بيضيع منى، كل ده عشان مش عارف أجيب علاجه بحس أن قلبى بيتقطع، ومش عارف أعمل له حاجة، لفيت على جميع صيدليات المحافظة من أجل الحصول على علبة بنسلين وفي الآخر لقيت علبة واحدة ب150 جنيها وحجة الصيدلى أنها ناقصة بالسوق وأنها مستوردة ومش موجودة فاضطريت اشتريها وكمان 15 يوما هحتاج واحدة تانية وهدور تانى.

يقول الدكتور إيهاب قطارية وكيل نقابة الصيادلة بدمياط ورئيس لجنة الصيدليات، إن أزمة نقص البنسلين على مستوى الجمهورية ومتفاقمة رغم تصريحات وزير الصحة بأنه لا توجد أزمة وتم ضخ كميات كبيرة من البنسلين في الأسواق، فالبنسلين غير موجود بالمرة بالصيدليات باستثناء بعض الصيدليات التى توجد لديها علبة أو أكثر ويتم بيعها بالسوق السوداء بفارق سعر أزيد وبالنسبة للمستشفيات الحكومية فهناك شروط وتعقيدات للمرضي للحصول على البنسلين منها يجب إحضار صورة ميلاد الطفل أو الرقم القومى للمريض إضافة إلى الوقوف في طوابير للحصول على البنسلين ومن الممكن أنه بعد تلك المشقة لا يحصل عليه وداخل الأزمة ظهر مافيا السوق السوداء، ومحتكر الأدوية من أصحاب الصيدليات ممن خزنوا كميات من البنسلين، ليطرحوه فى السوق السوداء، وهو أمر اعتاد عليه معدومى الضمير من أصحاب الصيدليات في أزمات نقص الأدوية.

الصحة: «نوفارتس» توقفت عن إنتاج البنسلين وتوريده لمصر

"وهو خبر بتاريخ اليوم الموافق الخميس 18 يناير 2018 01:22 مساءً.

الصحة: «نوفارتس» توقفت عن إنتاج البنسلين وتوريده لمصر العرب نيوز ينشر لكم جديد الاخبار – ونبدء مع اهم الاخبار الصحة: «نوفارتس» توقفت عن إنتاج البنسلين وتوريده لمصر – العرب نيوز – الصحة: «نوفارتس» توقفت عن إنتاج البنسلين وتوريده لمصر .حيث ننشر لكم متابعينا في كل بقاع الوطن العربي جديد الاخبار اليوم عبر موقعنا العرب نيوز ونبدء مع الخبر الابرز، (العرب نيوز _ طريقك لمعرفة الحقيقة) – اخبر الدكتور مصطفى السيد مدير إدارة التفتيش الصيدلى بوزارة الصحة والسكان، إن هناك نوعين من البنسلين في السوق المصري.

وأوضح في تصريح خاص للدستور أن النوع الأول من إنتاج شركة نوفارتس العالمية، ولكنها أوقفت إنتاجه وتوزيعه لأنهم لا يحتاجون له لعدم وجود مرضي بالحمى الرماتزميه في دول أوروبا، موضحا أن مرضي الحمى الروماتزمية يتواجدون بأعداد كبيرة في دول العالم الثالث والهند، أما النوع الثاني وهو المنتج الصيني ويوزع لمنطقة الشرق الأوسط وفي مصر وهذا النوع هو المتواجد الآن في مصر.

والمح إلى أن أسباب تخبط شركات قطاع الأعمال التي كانت تنتج فيما مضي وحدوث الأزمة هى أن المرضي المصرين لديهم "عقدة الخواجة"، ويميلون لشراء الدواء المستورد عن الدواء المحلي، فأصبحت الشركات المحلية التي كانت تنتج بكميات كبيرة تواجه خسارة كبيرة وخفضت الإنتاج لعدم الإقبال عليه في السوق المصري.

واضاف إن المرضى يسألون الآن عن الدواء المستورد الذين كانوا يأخذونه، مؤكدا أنه خلال أسبوع سوف تنتهي الأزمة نهائيًا.

وأوضح أن لا توجد أزمة الآن فى البنسلين حيث أنه متوفر فى كافة الصيدليات الخاصة والحكومية.

3.1.2 Situation in Sudan

وقف استيراد الأدوية يهدد السودان بأزمة جديدة

الخرطوم . عاصم إسماعيل

31أغسطس 2017

أخبار مرتبطة

105%ارتفاع أسعار الأدوية بالسودان... وشكاوى المواطنين تتزايد

أزمة الدولار تهدّد صناعة الأدوية في السودان

اختفاء البنسلين من صيدليات مصر وبيعه في الأسواق بـ 25 ضعف سعره

أدوية مفقودة في تونس

وحذرت السلطات السودانية قبل أيام من التحايل على القرار الرئاسي الذي حظر استيراد الأدوية التي تُنتج محليا، فيما بدأت وزارة المالية والبنك المركزي في تنفيذ القرار، مع الالتزام باستيراد الأدوية المزمنة وفق سعر الدولار الرسمي.

وبدد وزير الصحة، بحر إدريس، في تصريحات صحافية قبل أيام، مخاوف المهنيين من شح الأدوية، قائلا إن الدولة تدعم الدواء بنحو 120 مليون دولار سنويا، فضلا عن توفير الدولار اللازم لاستيراد الأدوية بالسعر الرسمي.

وافتتح الرئيس السوداني عمر البشير، في إبريل/نيسان الماضي، أكبر مخزن دواء في العاصمة الخرطوم، بسعة تخزين 46 ألف متر مكعب، وبمواصفات ومعايير تستجيب لأسس التخزين الجيد، والتخلص من العمل اليدوي عبر استخدام التكنولوجيا والرافعات الحديثة لنقل الأدوية.

3.1.3 مقالات:واقع صناعة الدواء في العالم العربي - الخليج نموذجًا

19 فبراير 2017

صناعة الدواء في الخليج العربي

تولي دول مجلس التعاون الخليجي الرعاية الصحية اهتمامًا بالغًا لتطوير الخدمات الصحية لسكانها ويظهر ذلك من خلال حجم ما تقدمه الميزانية للقطاع الصحي حيث بلغت نحو 21.5 مليار دولار في العام 2011 وبلغت فاتورة قطاع التأمين الصحي في دول المجلس في العام 2010 أكثر من 13 مليار دولار.

كما حفزت الحكومات القطاع الخاص على الاستثمار في الصناعات الدوائية من خلال تقديم القروض والإعفاءات والحوافز وبادرت بإقامة شركات بالمشاركة مع القطاع الخاص للصناعات الدوائية والمستلزمات الطبية، إذ لا تزال دول الخليج تستورد احتياجاتها من الأدوية بنسبة كبيرة تقارب الـ95% مقارنة مع ما تنتجه محليًا، لذا تتوفر فرص كبيرة للمستثمرين لتغطية هذا النقص الهائل في الطلب.

mid.gif

وارتفعت عدد مصانع الأدوية في دول مجلس التعاون من 18 مصنعًا في العام 1995 باستثمارات قدرها 174.4 مليون دولار إلى 55 مصنعًا في عام 2004 باستثمارات بلغت 793.1 مليون دولار، وحافظت السعودية على صدارة دول المجلس في عدد المصانع، بواقع 27 مصنعًا، وعدد المصانع في الإمارات وصل لـ16 مصنعًا، حيث ارتفع من 14 مصنعًا عام 2014 إلى 16 في 2015 يعمل لإنتاج أكثر من 1000 صنف دوائي مبتكر ومثيل .

ولا تزال السعودية تمثل الوزن الأكبر بين دول المجلس في صناعات الدواء، ففي دارسة أشارت أن الصناعات الدوائية السعودية تمثل 80% من إجمالي السوق الخليجية وتتخطى حاجز الـ13 مليار ﷼ سنويًا كما أنها تحقق نموًا سنويًا بلغ 12%، وتظهر الدارسة أن المصانع السعودية تغطي 20% فقط من حاجة السوق المحلية الدوائية ويذهب الباقي للتصدير.

وبلغت قيمة سوق الأدوية في دول مجلس التعاون الخليجي 10.1 مليار دولار في عام 2014، وتأتي السعودية على رأس دول المجلس بحجم سوق أدوية يبلغ 6.3 مليارات دولار، وتحتل الإمارات المرتبة الثانية حيث قدرت قيمة السوق الدوائية بـ2.4 مليار دولار ومن المتوقع أن تصل إلى 3.7 مليار دولار بحلول عام 2020، وجاءت الكويت في المرتبة الثالثة، كما تصل قيمة الأدوية المستوردة في دول الخليج العربية إلى نحو 9.5 مليارات دولار سنويًا، بنسبة تصل إلى 90% من حجم الاستهلاك المحلي.

ورغم حجم هذه الأرقام يؤكد خبراء أن صناعة الدواء في السعودية خصوصًا والخليج عمومًا بحاجة إلى مزيد من الاستثمارات في هذه الصناعة الواعدة، فالسعودية مثلاً استوردت في العام 2014 نحو 96.28 ألف طن من الأدوية بلغت قيمتها نحو 20.6 مليار ﷼ في حين أنها صدرت 54.2 ألف طن من الأدوية المصنعة محليًا بلغت قيمتها 2.21 مليار ﷼ في نفس العام.

3.1.4 الصومال

طائرة إغاثية ثالثة تحمل مساعدات طبية تصل للصومال ونؤكد لكم باننا نسعى دائما لامدادكم بكل ماهو جديد وحصري والان ندخل في التفاصيل

الرياض – عبدالله السعيد – إنفاذاً لتوجيهات خادم الحرمين الشريفين الملك سلمان بن عبدالعزيز آل سعود وسمو ولي عهده الأمين –حفظهما الله– حيال تقديم المساعدات الطبيه للحكومة الصومالية، وصلت الاثنين إلى مقديشو طائرة القوات الجوية الملكية السعودية تحمل على متنها مساعدات طبية يرافقها فريق من مركز الملك سلمان للإغاثة والأعمال الإنسانية.

وكان في استقبال الطائرة في العاصمه مقديشو عدد من المسؤولين الصوماليين، وسيستكمل فريق المركز خطته لتسليم المساعدات وتوزيعها بالتنسيق مع الجهات ذات العلاقه، ويأتي هذا الدعم استمراراً لما تقدمة المملكة من مساعدات للأشقاء في جمهورية الصومال، بما يخدم المواطن الصومالي في كافة الاحتياجات وبما يتوافق مع المعايير الدولية.

الجدير بالذكر أن مركز الملك سلمان للإغاثة قدم العديد من المشاريع الإغاثية والإنسانية للأشقاء في الصومال.

3.1.5 الاحتلال يحارب صناعة الدواء في غزة

غزة . يوسف أبو وطفة

23أغسطس 2015

أخبار مرتبطة

مصانع غزة تترقب المواد الخام وحرية التصدير

أزمة الدولار تهدّد صناعة الأدوية في السودان

النظام الصحي بغزة مهدّد بالانهيار التام.. رواتب مقطوعة وقمامة ومخلفات أدوية

105%ارتفاع أسعار الأدوية بالسودان... وشكاوى المواطنين تتزايد

عام تفاقم الأزمات ... 2017 الأسوأ اقتصاديا على غزة

غزة تحتاج إلى أطباء

لم يعد مصنع "الشرق الأوسط" للأدوية في غزة المحاصرة، قادراً على العمل بكامل طاقته الإنتاجية، نتيجة المنع الإسرائيلي المتكرر لدخول المواد الكيمائية، التي تدخل في صناعة الأدوية والمستحضرات الطبية، والتي تقوم الشركة بإنتاجها منذ تأسيسها أواخر العام 1999، بسبب الذرائع الأمنية الإسرائيلية.

وتعاني الشركات والمؤسسات العاملة في مجال الصناعات الدوائية وتوريد المستلزمات الطبية في القطاع، من الممارسات الإسرائيلية، المتمثلة في منع دخول المواد الأولية المكونة للأدوية، وإرجاع المعدات وعدم السماح لها بالمرور عبر معبر كرم أبو سالم التجاري الذي يربط القطاع بالأراضي المحتلة عام 1948، وهو ما يكبد هذه المؤسسات خسائره مالية باهظة.

ويقول المدير العام لمصنع "الشرق الأوسط" للأدوية، الطبيب مروان الأسطل، لـ "العربي الجديد"، إنّ الناتج المحلي للمصنع يغطي ما نسبته 15% من حاجة السوق المحلي في غزة، بين مضادات حيوية وكبسولات وكريمات علاجية خاصة، حيث بلغ عدد الأصناف الإجمالية التي ينتجها نحو 90 صنفاً.

3.1.6 مصانع غزة تترقب المواد الخام وحرية التصدير

غزة ـ يوسف أبو وطفة

20أكتوبر 2017

ينتظر أصحاب المصانع والمنشآت الإنتاجية في قطاع غزة، المحاصر إسرائيلياً للعام الحادي عشر على التوالي، انعكاسا إيجابيا لتسلّم حكومة الوفاق الوطني مهامها، عبر رفع الحصار والتخفيف من الإجراءات المفروضة على حركة البضائع ودخول المواد الخام.

ويبدو انتظار أصحاب هذه المنشآت مشروعًا، في ظل الخسائر المالية الكبيرة التي تكبدوها، طيلة السنوات الماضية، بفعل الحصار وتلاحق الحروب التي شنها الاحتلال على القطاع وطاولت العديد من هذه المنشآت التي كانت مصدر رزق لآلاف العاملين.

وتعتبر المصانع العاملة في القطاع مصدرا من مصادر تشغيل الأيدي العاملة، في ظل ارتفاع معدلات البطالة لأكثر من 44%، بينهم نحو 60% من فئة الشباب، في الوقت الذي وصل فيه اعتماد الأسر الغزية على المعونات الإغاثية لأكثر من 80.%

وبحسب تقديرات اللجنة الشعبية لكسر الحصار (منظمة غير حكومية)، فإن متوسط دخل الفرد اليومي في القطاع الذي يقطنه أكثر من مليوني مواطن غزي، وصل إلى نحو دولار أميركي فقط، في حين ارتفع عدد العاطلين عن العمل إلى نحو ربع مليون لا يجدون فرص عمل.

ويقول رئيس اللجنة الحكومية لكسر الحصار عن غزة، علاء البطة، لـ "العربي الجديد"، إن إنجاز المصالحة الفلسطينية يشكل بداية حقيقية لإنهاء الحصار، والعمل على تخفيف الإجراءات المفروضة على القطاع، طيلة السنوات الماضية، وسينعكس إيجابًا على حياة الفلسطينيين وعلى الاقتصاد الغزي.

ويوضح أن نحو 4 آلاف منشأة ومصنع تعمل في القطاع، يعمل فيها عشرات الآلاف من العمال، تنتظر وقف هذه الإجراءات التي اتخذت بفعل الحصار، طيلة السنوات الماضية، وأدت إلى توقف بعض المصانع وتعطل أخرى بشكل شبه كلي، وخفض إنتاجية العدد الأكبر من تلك المصانع التي ظلت تعمل.

ويلفت البطة إلى أنه، ووفقًا لآخر إحصائية موجودة، فإن نحو 90% من المصانع توقفت بشكل كلي وشكل شبه كلي، خلال الفترة الماضية، بفعل نقص المواد الخام ومنع إدخال الاحتلال لها عبر وضعها على قوائم السلع ذات الاستخدام المزدوج، ما تسبب في تسريح أعداد كبيرة من العاملين بها.

3.1.7 مختصون: 22 نوعاً من الأدوية قاتلة في السعودية

مختصون: 22 نوعاً من الأدوية قاتلة في السعودية في حال لم تصرف بوصفة طبية معتمدة وهذه الأدوية تقود إلى مضاعفات خطيرة بنسبة 30 بالمئة إضافة إلى التسمم والإصابة بالأمراض كالسرطانات وأمراض الكبد وتشوه الأجنة

...وأكد أن حقنة البنسلين الواحدة أو أي مضاد حيوي آخر، يمكن أن يتسبب في الوفاة، إذا تم حقنه وريدياً أو في العضلة، وإذا كان لدى المريض حساسية، يتم عمل اختبار الحساسية قبل القيام بحقن المضادات الحيوية، كون الأقراص، والكبسولات، والأشربة تؤدي إلى أعراض خطرة في حال وجود حساسية لدى المريض.

الشرقية خالية من حقن البنسلين

عصام أبو الفتوح: الشرقية خالية من حقن البنسلين

عصام أبو الفتوح: شرق خالية من البنسلين حقن المملكة أخبار حقن البنسلين، عصام أبو الفتوح: الشرقية خالية من حقن البنسلين ننشر لكم زوارنا الجدد أخبار اليوم من خلال موقعنا الإخباري والبدء مع أهم الأخبار عصام أبو الفتوح: الشرقية خالية من حقن البنسلين.

أخبار المملكة الدكتور عصام أبو الفتوح، الشرقية، على اختفاء حقن البنسلين طويل المفعول، والذي يسبب أزمة حقيقية؛ بسبب الأمراض المزمنة، والمخدرات، وأنه يحتاج إلى عدد كبير من المواطنين.

وفي مكالمة هاتفية مع البرنامج، "ما بعد ذلك"، طالبت علا شوشة، وهي هيئة الإذاعة الساتلية لتس، بزيادة سعر البنسلين حتى تتمكن شركات الإنتاج من توفير الكميات المطلوبة في السوق. وأوضح أن الشركات لم تعد قادرة على إنتاج البنسلين، وبسبب تحرير سعر الصرف، هناك نقص في الإنتاج، وتوقفت المصانع عن إنتاج كميات كافية لتغطية احتياجات السوق.

شكرا لكم على متابعتنا ونحن نعدكم دائما لتقديم كل ما هو أفضل .. ونقل الأخبار من جميع مصادر الأخبار وتسهيل قراءتها. لا ننسى عمل إك لصفحتنا في الفيسبوك ومتابعة آخر الأخبار على تويتر. مع تحيات موقع عائلة المملكة.

المصدر: الصباح العربي

لأربعاء 17 يناير 2018 | اسطنبول 12° C

تركيا بوست المصدر:الأناضول

تستعد السعودية لاستخدام ‘‘نظام تتبع الأدوية’’ الذي طورته وزارة الصحة التركية، ويعد الأول من نوعه في العالم.

وقال مسؤولون في الوزارة، لمراسلنا، إن النظام الذي نفذته مؤسسة الأدوية والأجهزة الطبية التركية، التابعة لوزارة الصحة، تمكن من تخطي منافسيه من أوروبا وأمريكا، في تقييمات فنية صعبة، ضمن المشروع الذي أطلقته السعودية.

ووقع الجانبان التركي والسعودي اتفاقية بهذا الخصوص، في 21 أغسطس/آب الحالي؛ حيث سيتم العمل على مدار عام لتطوير نظام تتبع الأدوية السعودي، SAUDI DTTSومع دخوله حيز الخدمة، سيكون بوسع النظام رصد سوق الأدوية بالمملكة بنسبة 100.%

وزار مسؤولو أكثر من 20 دولة بينها السعودية وكوريا الجنوبية وكازخستان وقرغيزيا، مؤسسة الأدوية والأجهزة الطبية التركية، للاطلاع عن كثب على نظام تتبع الأدوية المطبق في تركيا، والذي يتيح مراقبة الأدوية التي تدخل السوق في عموم البلاد، سواء المنتجة محليا أو المستوردة.

وبهذه الطريقة يتم الحيلولة دون بيع الأدوية المزورة أو المهربة، والمنتهية صلاحيتها.

وبفضل أكواد رموز الاستجابة السريعة، الموجودة على علب الأدوية، يمكن تعقبها منذ دخولها السوق وحتى وصولها إلى المستهلك.

ومع إضافة آخر تحديث للنظام ‘‘أين دوائي؟’’ الذي يمكن تحميله على الهواتف الذكية، الموجه لمرضى سرطان الثدي بالمرحلة الأولى، بات بوسع المرضى رؤية أين يتواجد الدواء المطلوب، في أي أقرب صيدلية .

3.1.8 الدليل الوطني للأدوية المسجّلة في لبنان

Penicilline

لتصنيف العلاجي	الاسم	أساسي / جنيسي	التركيبة العلمية	العيار	الشكل الصيدلاني	سعر المبيع من العموم
J01CE01	PENICILLINE G PANPHARMA	G	Benzylpenicillin (sodium) - 1,000,000IU	1,000,000IU	Injectable powder for solution	33,606 L.L
J01CE01	PENICILLINE G PANPHARMA	G	Benzylpenicillin (sodium) - 5.000.000IU	5MUI	Injectable powder for solution	72,396 L.L
J01CE01	PENICILLINE G SOD. INJ.	G	Benzylpenicillin (sodium) - 1,000,000IU	1,000,000IU	Injectable powder for solution	64,045 L.L
J01CE01	PENICILLINE G SODIQUE	G	Benzylpenicillin (sodium) - 5.000.000IU	5MIO	Injectable powder for solution	129,703

Ampicilline

صنيف العلاجي	الاسم	أساسي / جنيسي	التركيبة العلمية	العيار	الشكل الصيدلاني	سعر المبيع من العموم
J01CA01	AMPICILLINE INJ.	G	Ampicillin (sodium) - 500mg	500mg	Injectable powder for solution	49,050 L.L
J01CA01	AMPICILLINE INJ.	G	Ampicillin (sodium) - 1g	1g	Injectable powder for solution	98,100 L.L

Amoxicilline

لتصنيف العلاجي	الاسم	أساسي / جنيسي	التركيبة العلمية	العيار	الشكل الصيدلاني	سعر المبيع من العموم
J01CA04	AMOXICILLIN	G	Amoxicillin (trihydrate) - 500mg	500mg	Capsule	85,726 L.L

التصنيف العلاجي	الاسم	أساسي / جنيسي	التركيبة العلمية	العيار	الشكل الصيدلاني	سعر المبيع من العموم
J01CR02	AMOXICILLINA/ ACIDO CLAVULANICO	G	Amoxicillin (sodium) - 1g, Clavulanic Acid (potassium) - 200mg	1.2g	Injectable powder for solution	395,532 L.L
J01CA04	AMOXICILLINE INJ.	G	Amoxicillin (sodium) - 500mg	500mg	Injectable powder for solution	65,867 L.L
J01CA04	AMOXICILLINE INJ.	G	Amoxicillin (sodium) - 1g	1g	Injectable powder for solution	100,902 L.L
J01CA04	AMOXICILLINE INJ.	G	Amoxicillin (sodium) - 1g	1g	Injectable powder for solution	21,338 L.L
J01CR02	AMOXICILLINE/ACIDE CLAVULANIQUE PANPHARMA	G	Amoxicillin (sodium) - 1g, Clavulanic Acid (potassium) - 200mg	1.2g	Injectable dry powder	34,923 L.L
J01CR02	AMOXICILLINE/ACIDE CLAVULANIQUE PANPHARMA	G	Amoxicillin (sodium) - 1g, Clavulanic Acid (potassium) - 200mg	1.2g	Injectable dry powder	82,937 L.L

نوال الأشقر – لبنان 24

تشهد أسعار سوق الأدوية في لبنان انخفاضاً ملحوظاً تخطى في بعض الأنواع نسبة الخمسين في المئة. فما سبب هذا الإنخفاض، وهل هو مرحلي؟

نقابة الصيادلة: الأسعار إلى مزيد من الإنخفاض

نقابة الصيادلة أكدت لـ"لبنان 24" أن أكثرية الأدوية انخفضت أسعارها بنسبة 25 %، وبعضها انخفض أكثر من 50 %. المستشار الإعلامي لنقيب الصيادلة جو سلّوم أعاد سبب تدني الأسعار إلى عاملين: "أولاً أعادت وزارة الصحة جدولة أسعار

الأدوية وعمدت إلى مقارنة أسعارها في بلد المنشأ، وخفضت السعر على هذا الأساس. ثانياً تقلب أسعار العملات الأجنبية وانخفاض أسعارها في بلد المنشأ نتيجة انخفاض صرف سعر اليورو.''

هذا التدني في أسعار الأدوية خلق ارتياحاً لدى المواطنين، إلتقينا عينة منهم في صيدلية ''محيو''، سارع أفرادها إلى إجراء مقارنة بين المبلغ الذي كانوا يتكبدونه شهرياً لشراء أدويتهم وما أصبح عليه اليوم. أحدهم رد معلقاً: ''اتساءل عن سبب هذا الإنخفاض اليوم، هل مافيا الدواء إلى تقهقر، أمّا أنه انخفاض مرحلي؟''

إلاّ أنّ هذا الإرتياح الشعبي يقابله امتعاض من قبل الصيادلة، كما أوضحوا لنا في صيدلية محيو أن ''التخفيض كانت مفاعيله سلبية علينا كصيادلة، ونتج عنه تقلص في مداخيلنا بسبب تدني الأسعار وبقاء الجعالة على حالها، ولاسيما أن وزارة الصحة ومنذ ثلاث سنوات خفّضت الجعالة، وجعلتها مقطوعة بنسبة 46 $ فقط لكل دواء سعره فوق 300 $ حتى لو وصل سعره إلى مليون أو أكثر، كل دواء سعره فوق 100 $ انخفضت الجعالة من 22 % الى 20,7 % ، وفوق الـ 300 دولار لا تتعدى نسبة الربح 18 %. وكان يجب أن يلزموا الوكيل بنسب معينة من الربح.''

وهذا نموذج مقارنة عن أسعار بعض الأدوية التي انخفضت بشكل كبير، كما كانت عليه وكما هي اليوم:

Cipralex –من 64000 إلى 25000.

Augmentin 1 g –من 26526 إلى 12080.

Plavix –من 129707 إلى 71052.

Diamicron –من 24813 إلى 9183.

Seroquel xr 300-من 359841 إلى 151305.

على أي حال سوق الدواء ليس سوى حلقة من منظومة صحية متكاملة يشوبها الكثير من الثغرات، ومحاربة الفساد الصحي فيها يحتاج إلى عشرات الوصفات السياسية قبل الطبية، تكون فيها "الوصفة الموحدة" أو الإرداة السياسية الجامعة برفع الغطاء عن "مافيا القطاع الصحي" بداية طريق الإصلاح.

3.2 Antibiotics technologies and global markets

بلغت قيمة سوق المضادات الحيوية النظامية العالمية 39.6 مليار دولار في عام 2013، ومن المتوقع أن تصل إلى 41.2 مليار دولار بحلول عام 2018 بمعدل نمو سنوي مركب يبلغ 0.8٪.

-تتوقع أبحاث بنك البحرين والكويت أن يزداد سوق الولايات المتحدة من 15.8 مليار دولار في عام 2013 إلى 16.4 مليار دولار في 2018، بمعدل نمو سنوي مركب قدره 0.7٪ في الفترة من 2013 إلى 2018.

- سيزداد السوق الأوروبي للمضادات الحيوية بمعدل نمو سنوي مركب قدره 0.5٪ من 9.8 مليار دولار في عام 2013 إلى 10.1 مليار دولار بحلول عام 2018.

https://www.prnewswire.com/news-releases/antibiotics-technologies-and-global-markets-262708941.html

3.2.1 Annual Output of 10,000 Tons of Penicillin Industrial Salt Project of Songyuan City (China)

2013/04/07 Source: Jilin Daily

Market Prospects

Anti-microbial infection drugs sales in today's global pharmaceutical market possesses the second place in a large class of drugs. Production and sales of penicillin is the largest in the world. After sixty years of development in the course of penicillin, especially in the 80s, and 90s, due to its efficacy, small side effects, low price, it is highly clinical welcomed. And it has become one of the first clinical drug choices of antibiotics. Globally, the world's antibiotic market experienced a middle term growth period in the 70s, the rapid growth period in the 80s, and maturity in the 90s, it has entered a new century of transition. The 1998 statistics show that annual world sale of anti-infective market is 40 billion U.S. dollars, accounting for about 10% of therapeutic drug market; the antibiotic is 25-26 billion U.S. dollars, 62-65% of the anti-infective drug, the largest share in the anti-infective market of the world.

Meanwhile, according to the relevant information over the years, annual growth rate of antibiotics is 8% on average. Also in this class of antibiotic drugs, sale of penicillin is 3.317 billion U.S. dollars, accounting for 13% of total sales of antibiotics.

With the different levels of development of global semi-synthetic penicillin and increase of market sales at the same time, it also brings about increase of 6-APA, 7-ADCA and other intermediates with penicillin as the raw material. In 1985, global production of 6-APA was 4200 tons, in 1990, 7000 tons, and from 1997 to the present, global production of 6-APA has been over 12000 tons. Annual growth rate is 15%. And the cephalosporins antibiotics with 7-ADCA and 7ACA as intermediates has dominated 31% antibiotics share

of the world; from the future development trend of this kind of drug, the sales turnover of ceftriaxone, thienamycin sub-methylamine, ceftizoxime, cefuroxime axetil, cephalosporins, imatinib could reach 300 million U.S. dollars. Therefore, it will bring about increase of demand for 7-ADCA etc. intermediates during a given period. However, 7-ADCA is major derivative of penicillin G industrial salt, which shows the large development space of penicillin.

In addition, from dynamics of large penicillin companies, DSM Company, which accounts for 30% raw material market share of penicillin in the world, plans to establish a new joint venture company with Chemferm to further expand the scale of production of penicillin and its derivatives. This new plant to be built in the Netherlands plans to process 6-APA into 7-ADCA by enzymatic processing in order to reduce the production costs of 7-ADCA, improve efficiency and eliminate pollution. Bivchemie is another penicillin producer in the world, and the penicillin produced by this company occupies 10% of market share in the world. Aimed at the condition that the profitability of pharmaceutical raw material is low, the company said, it will not give up penicillin production, and look forward to the start of penicillin market and prices rise. Penicillin production in China in this year is expected to reach 34,000 tons, of which 20% are directly used as injection, 50% as the cephalosporin in producing intermediates products, and 30% as export to earn foreign currency.

China is a superpower in penicillin industrial salt export, and in 2003, exports accounted for 60% of global market share.

Penicillin of China is mainly focused in Huayao, Huaxing, Harbin Pharmaceutical, Shiyao and Lukang Pharmaceutical five enterprises, accounting for 90% of total output of penicillin.

http://english.jl.gov.cn/Investment/Opportunities/Industry/MedicineandBiotechnology/201304/t20130407_1439841.html

Scale of Project Construction

Construction scale is 10,000 tons of penicillin industrial salt.

Table 1 Product Plan

Name	Standard	Packaging	Reference discount kg / billion
Penicillin sodium (days powder)	CP2000 BP98	4 kg / Tin 5 billion / Tin	0.624
Penicillin potassium (sterile powder)	CP2000 CP2000 CP2000 BP98	4 kg / Tin 5 billion / Tin 3.15 kg / Tin 5 billion / Tin	0.652
Pharmaceutical intermediate penicillin potassium (penicillin industrial powder)	Industry standards VSP25	40 billion / barrel	0.652
Penicillin V potassium (oral powder)	CP2000 BP2000	40 billion / barrel	
Oxytetracycline (base)	BP2002/VSP26 Ministry of Health standards	25 kg / barrel 25 kg / bag	

3.3 Penicillin products on market (China, ..)[4]

3.3.1 Penicillin streptomycin injection For veterinary use only

US $2-2.4 / Box **5000 Boxes** (Min. Order) Dosage Form: **Injection,oral liquid** Animal Type: **Cattle,Fowl,Pets,Cattle, Fowl** Function: **Antibacterial Drugs** Appearance: **Colourless, Transparent** Place of Origin: **Hebei,China (Mainland)** Brand Name: **Depond**	 Hebei Depond Animal Health Care Science And Technology Co., Ltd., China (Mainland)

[4] from www.alibaba.com

3.3.2 Penicillin

US $0.1-10 / Milliliter **32 Milliliters** (Min. Order) Dosage Form: **Powder** Animal Type: **Cattle,Fowl,Horse,Pets,Pig,Sheep** Function: **Antibacterial Drugs** Appearance: **White Powder** Place of Origin: **Tianjin,China (Mainland)** Brand Name: **Zoohance**	 Tianjin Glory Technology Co., Ltd., China (Mainland)

3.3.3 Animal antibodies & penicillin powder/ penicillin price

US $16-20 / Kilogram **1 Kilogram** (Min. Order) Dosage Form: **Powder** Animal Type: **Aquatic Animals,Cattle,Fowl,Horse,Pets,Pig,Sheep** Function: **Antibacterial Drugs** Type: **Animal Health Products** Appearance: **White crystalline powder** Place of Origin: **Henan,China (Mainland)**	 Zhengzhou Zhenhua Pharmaceutical Technology Service Co., Ltd., China (Mainland) Trade Assurance

3.3.4 Antibacterial Veterinary Prouducts Penicillin 30% Injection for cattle

US $0.1-1 / Piece **1 Piece** (Min. Order) Dosage Form: **Injection** Animal Type: **Cattle,Horse,Sheep,Dog** Function: **Antibacterial Drugs** Appearance: **an oil solution of fine particles suspended.** Place of Origin: **Hebei,China (Mainland)** Brand Name: **TIANYUAN**	 Hebei Tianyuan Pharmaceutical Co., Ltd., China (Mainland)

3.3.5 Penicillin G injection300.000iu

US $0.5-3 / Unit **50 Cartons** (Min. Order) Dosage Form: **Injection** Animal Type: **Cattle,Horse,Sheep** Function: **Antibacterial Drugs** Place of Origin: **Jiangxi,China (Mainland)** Brand Name: **Bolai** Model Number: **100ml**	 Jiangxi Bolai Pharmacy Co., Ltd., China (Mainland)

3.3.6 Veterinary products Procaine penicillin

US $0.39-1.09 / Box **1 Box** (Min. Order) Dosage Form: **Injection** Animal Type: **Cattle,Horse,Other Special Breeding Animals,Pets,Pig,Sheep** Function: **Antiviral** Appearance: **liquid** Place of Origin: **Hebei,China (Mainland)** Brand Name: **Jiuding**	 Shijiazhuang Jiuding Animal Pharmaceutical Co., Ltd., China (Mainland)

3.3.7 Cefalexin Medicine Grade/alternative to penicillin in patients with penicillin hypersensitivity

US $20-50 / Ton **1 Ton** (Min. Order) Dosage Form: **Aerosol** Animal Type: **Aquatic Animals** Function: **Antibacterial Drugs** Place of Origin: **Shandong,China (Mainland)** Brand Name: **WNN** Model Number: **WNN**	 Weifang Union Biochemistry Co., Ltd., China (Mainland)

3.3.8 Benzyl Penicillin+Penicillin powder for injection

US $0.1-1.5 / Box **2000 Boxes** (Min. Order) Dosage Form: **Injection,Powder** Animal Type: **Cattle,Fowl,Horse,Pig,Sheep** Function: **Antibacterial Drugs** Place of Origin: **Hebei,China (Mainland)** Brand Name: **GRDR** Model Number: **Vet - Medicine**	 Hebei Guangren Pharmaceutical Technology Co., Ltd., China (Mainland) Trade Assurance, Transaction Level: **7** Transactions(6 months), 1,000+

3.3.9 Procaine penicillin G & Dihydrostreptomycin sulphate 20:20 injectable suspension

US $2.4-2.8 / Box **10000 Boxes** (Min. Order) Dosage Form: **Injection** Animal Type: **Aquatic Animals,Cattle,Fowl,Horse,Pets,Pig,Sheep,camel dog** Function: **Antibacterial Drugs** Place of Origin: **Hebei,China (Mainland)** Brand Name: **ZDHF** Model Number: **20:20,20:25**	 Shijiazhuang ZDHF Stock-Raising Co., Ltd. China (Mainland) Trade Assurance Transaction Level: **1** Transaction(6 months), 100+

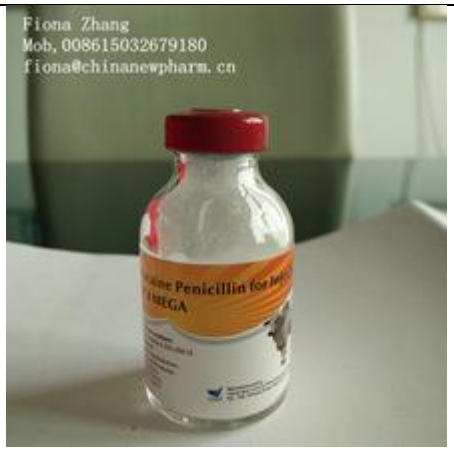
3.3.10 High Quality Veterinary USP Penicillin G 54-35-3

US $30-60 / Kilogram **1 Kilogram** (Min. Order) Dosage Form: **Powder** Animal Type: **Aquatic Animals,Cattle,Fowl,Horse,Other Special Breeding Animals,Pets,Pig,Sheep** Function: **Antibacterial Drugs** Appearance: **White or almost white crystalline powder** Place of Origin: **Hubei,China (Mainland)** Model Number: **Top grade**	 , Wuhan Vanz Pharm Inc., China (Mainland) Trade Assurance Transaction Level: **39** Transactions(6 months), 10,000+

3.3.11 PENICILLIN G PROCAINE NON-STERILE(ORAL GRADE)

Function: **Antibacterial Drugs** Place of Origin: **Shanghai,China (Mainland)** Model Number: **USP**	 HUA YUN INTERNATIONAL (SINGAPORE)PTE LTD , Singapore

3.3.12 veterinary products streptomycin sulfate+procaine penicillin+benzyl penicillin powder for livestock

US $0.01-1 / Box **20000 Boxes** (Min. Order) Dosage Form: **Injection,Powder** Animal Type: **Cattle** Function: **Antibacterial Drugs** Place of Origin: **Hebei,China (Mainland)** Brand Name: **getion or your brand** Hebei New Century Pharmaceutical Co., Ltd., China (Mainland)	

3.3.13 Penicillin ,Penicillin industrial salt

200 Kilograms (Min. Order) Dosage Form: **Powder** Animal Type: **Cattle,Fowl,Horse,Pig,Sheep** Function: **Antibiotic** Place of Origin: **CN**	

Brand Name: **puertai** Model Number: **6008**	Zhengzhou MCT International Co., Ltd., China (Mainland)

3.3.14 Procaine Penicillin 20%+Dihydrostreptomycin Sulfate 25% Injection for animal use

5000 Pieces (Min. Order) Dosage Form: **Injection** Animal Type: **Cattle,Horse,Sheep** Function: **Antibacterial Drugs** Place of Origin: **Hebei,China (Mainland)** Brand Name: **VEYONG** Model Number: **20%+25% 20%+20%**	 Hebei Veyong Animal Pharmaceutical Co., Ltd., China (Mainland)

3.3.15 APA Penstrep 20 S | High quality Veterinary Medicine| Dog Medicine with Penicillin (Vietnam)

US $0.5-1.5 / Unit **200 Units** (Min. Order) Dosage Form: **Suspension** Animal Type: **Pets** Function: **Antibacterial Drugs** Type: **Antibiotic** Place of Origin: **Ho Chi Minh City,Vietnam** Brand Name: **APA**	 APA UNITED NANO TECHNOLOGY CO., LTD , Vietnam

3.3.16 GMP, Dihydrostreptomycin sulphate + Procaine penicillin G suspension injection for veterinary medicine/cattle/poultry < ASIFAC> (Vietnam)

US $0.01-0.05 / Unit **2000 Units** (Min. Order) Dosage Form: **Injection,Suspension** Animal Type: **Cattle,Horse,Pig,Sheep** Function: **Antibacterial Drugs** Type: **Antibiotic** Appearance: **White suspension** Place of Origin: **Dong Nai,Vietnam** BRANCH OF THINH A VETERINARY MEDICINE TRADING AND MANUFACTURING JOINT STOCK COMPANY , Vietnam	

3.3.17 Benzyl Penicillin for Injection

US $0.1-0.2 / Box **10000 Boxes** (Min. Order) Place of Origin: **CN** Brand Name: **ZMC** Zhejiang Medicines & Health Products I/E Co., Ltd. China (Mainland)	

3.3.18 Benzyl penicillin potassium Injection for Veterinary

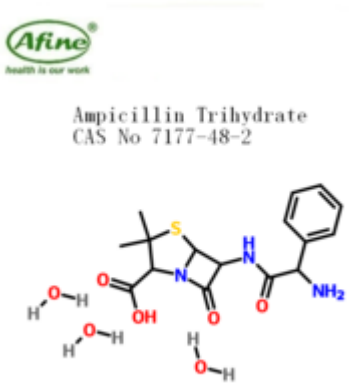
10 Kilograms (Min. Order) Dosage Form: **Injection** Animal Type: **Cattle,Pig** Function: **Antibacterial Drugs** Place of Origin: **Zhejiang,China (Mainland)** Model Number: **veterinary** white crystal powder: **off-white crystal powder** Hangzhou Union Biotechnology Co., Ltd. China (Mainland) Trade Assurance Transaction Level: **2** Transactions(6 months), 20,000+	

3.3.19 Top quality acid-resisting penicillin antibiotics Oxacillin Sodium Sterile

US $10-300 / Kilogram **1 Kilogram** (Min. Order) Dosage Form: **Injection,Powder,Tablet** Animal Type: **Cattle,Fowl,Horse,Pets,Pig,Sheep** Function: **Antibacterial Drugs** Appearance: **White or almost white powder.** Place of Origin: **CN** Brand Name: **TOP-PHARMCHEM** Shaanxi TOP Pharm Chemical Co., Ltd. China (Mainland)	

3.4 Ampicillin products on market (China, ..)

3.4.1 Ampicillin Trihydrate CAS 7177-48-2

US $30-50 / Kilogram **25 Kilograms** (Min. Order) MF: **C16H19N3O4S.3(H2O)** Other Names: **Ampicillin Trihydrate** Purity: **99%min** Type: **Antibiotic and Antimicrobial Agents** Grade Standard: **Medicine Grade** Usage: **Animal Pharmaceuticals** Afine Chemicals Limited China (Mainland) Trade Assurance	

3.4.2 Pharmaceutical raw materials ampicillin trihydrate from GMP manufacturer, CAS7177-48-2

US $1-50 / Kilogram **25 Kilograms** (Min. Order) MF: **C17H20N4O6** Purity: **96-102.0%** Type: **Antibiotic and Antimicrobial Agents** Grade Standard: **Medicine Grade,Tech Grade** Usage: **Animal Pharmaceuticals** Appearance: **White or almost white, crystalline powder**	 Beijing Infoark Co., Ltd. , China (Mainland)

3.4.3 high purity and free sample ampicillin

US $3-10 / Kilogram **1 Kilogram** (Min. Order) MF: **C16H19N3O4S3H2O** Other Names: **amfipen** Other name: **amfipen** Purity: **99%min** Type: **Antibiotic and Antimicrobial Agents,Antipyretic Analgesics and NSAIDs,Auxiliaries and Other Medicinal Chemicals,ampicillin**	 Daily Hi Industry (Shanghai) Co., Ltd.

Grade Standard: **Food Grade,Medicine Grade,ampicillin**	China (Mainland)

3.4.4 Ampicillin trihydrate CAS /NO:7177-48-2

US $1-1000 / Kilogram **1 Kilogram** (Min. Order) MF: **C16H19N3NaO4S** Other Names: **Ampicillin** Purity: **99%min** Type: **Urinary System Agents** Grade Standard: **Medicine Grade** Usage: **Animal Pharmaceuticals**	 Xi'an Sgonek Biological Technology Co., Ltd., China (Mainland) Trade Assurance , Transaction Level: **33** Transactions(6 months), 10,000+

3.4.5 GMP APPROVED HIGH PURITY LOW PRICE AMPICILLIN TRIHYDRATE

US $23.0-24.0 / Kilograms **200 Kilograms** (Min. Order) MF: **C16H19N3O4S.3H2O** Other Names: **AMPICILLINE** Purity: **99%min** Type: **Antibiotic and Antimicrobial Agents,Antibiotic and Antimicrobial Ag** Grade Standard: **Medicine Grade, Medicine Grade** Usage: **Animal Pharmaceuticals**	 Landy Enterprise Limited , China (Mainland) Trade Assurance

3.4.6 Veterinary hormones antibiotic compacted powder Ampicillin trihydrate

US $15-105 / Kilogram

1 Kilogram (Min. Order)

MF: **C16H19N3NaO4S**

Other name: **Ampicillin trihydrate compacted**

Other Names: **Ampicillin trihydrate**

Purity: **99%**

Type: Anesthetic Agents,Anti-Allergic Agents,Antibiotic and Antimicrobial Agents,Antidote,Antineoplastic Agents,Antiparasitic Agents,Antipyretic Analgesics and NSAIDs,Auxiliaries and Other Medicinal Chemicals, Blood System Agents, Cardiovascular Agents,Central Nervous System Agents,Disinfectant and Preservatives,Electrolyte

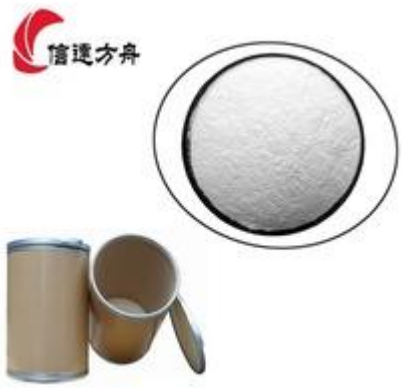
Balance and Dialysis Agents,Endocrine System Agents,Gastrointestinal Agents,Immune Function Agents,Respiratory System Agents,Urinary System Agents,Vitamins, Amino Acids and Coenzymes

Grade Standard: **Cosmetic Grade,Feed Grade,Food Grade,Medicine Grade,Tech Grade**

3.4.7 Pharmaceutical raw material Ampicillin/cas no 69-53-4

US $2-10 / Kilogram **1 Kilogram** (Min. Order) MF: **C16H19N3O4S3H2O** Other Names: **amfipen** Purity: **99%-101%** Type: **Antibiotic and Antimicrobial Agents,Antipyretic Analgesics and NSAIDs,Auxiliaries and Other Medicinal Chemicals,Ampicillin** Grade Standard: **Cosmetic Grade,Food Grade,Medicine Grade,Ampicillin** Usage: **Animal Pharmaceuticals**	Daily Hi Industry (Shanghai) Co., Ltd. China (Mainland)

3.4.8 Pharmaceutical raw material Ampicillin Trihydrate powder,Ampicillin compacted

US $20-50 / Kilogram **25 Kilograms** (Min. Order) MF: **C16H19N3O4S** Purity: **99%** Type: **Antibiotic and Antimicrobial Agents** Grade Standard: **Feed Grade,Medicine Grade** Usage: **Animal Pharmaceuticals,Antibacterial Drugs** Appearance:: **white or a kind of white crystal powder**	 Beijing Infoark Co., Ltd. , China (Mainland)

3.4.9 High quality competitive price 69-53-4 Ampicillin in bulk supply

US $1-1000 / Kilogram **1 Kilogram** (Min. Order) MF: **C16H19N3NaO4S** Other Names: **Ampicillin** Purity: **99%min** Type: **Urinary System Agents** Grade Standard: **Medicine Grade** Usage: **Animal Pharmaceuticals**	 Xi'an Sgonek Biological Technology Co., Ltd. China (Mainland) Trade Assurance Transaction Level: **33** Transactions(6 months), 10,000+

3.4.10 PHARMACEUTICAL GRADE 99% AMPICILLIN MANUFACTURER

US $23.0-24.0 / Kilograms **200 Kilograms** (Min. Order) MF: **C16H19N3O4S.3H2O** Other Names: **AMPICILLINE** Purity: **99%min** Type: **Antibiotic and Antimicrobial Agents** Grade Standard: **Medicine Grade, Medicine Grade** Usage: **Animal Pharmaceuticals** Landy Enterprise Limited , China (Mainland) Trade Assurance	

3.4.11 High Quality Ampicillin CAS:7177-48-2 Ampicillin Trihydrate Powder

US $20-40 / Kilogram **1 Kilogram** (Min. Order) MF: **C16H19N3O4S.3H2O, C16H19N** Other Names: **AMPICILLINE** Purity: **99%min** Type: **Antibiotic and Antimicrobial Agents,API ampicillin trihydrate veterinary antibiotic ampicillin powder** Grade Standard: **Feed Grade,Medicine Grade,Tech**	

Grade Usage: **Animal Pharmaceuticals**	Zhengzhou Panpan Chemical Co., Ltd. China (Mainland) Trade Assurance

3.4.12 factory supply High quality pharmaceutical Grade Ampicillin//69-53-4

US $1-1000 / Kilogram **1 Kilogram** (Min. Order) MF: **C16H19N3NaO4S** Other Names: **Ampicillin** Purity: **99%min** Type: **Urinary System Agents** Grade Standard: **Medicine Grade** Usage: **Animal Pharmaceuticals**	 Xi'an Sgonek Biological Technology Co., Ltd. China (Mainland) Trade Assurance Transaction Level: **33** Transactions(6 months), 10,000+

3.4.13 Pharmaceutical Material 99% Ampicillin Compacted

US $23.0-24.0 / Kilograms **200 Kilograms** (Min. Order) MF: **C16H19N3O4S.3H2O** Other Names: **AMPICILLINE** Purity: **99%min** Type: **Antibiotic and Antimicrobial Agents,Antibiotic and Antimicrobial Ag** Grade Standard: **Medicine Grade, Medicine Grade** Usage: **Animal Pharmaceuticals** Landy Enterprise Limited, China (Mainland) Trade Assurance	

3.4.14 China API Medicine grade ampicillin animals compacted powder

US $20-40 / Kilogram **1 Kilogram** (Min. Order) MF: **C16H19N3O4S.3H2O, C16H19N** Other Names: **AMPICILLINE** Purity: **99%min** Type: **Antibiotic and Antimicrobial Agents,China API Medicine grade ampicillin animals compacted powder** Grade Standard: **Feed Grade,Medicine Grade,Tech Grade** Usage: **Animal Pharmaceuticals**	 Zhengzhou Panpan Chemical Co., Ltd., China (Mainland) Trade Assurance

3.4.15 Pharmaceutical raw material Ampicillin

US $2-10 / Kilogram **1 Kilogram** (Min. Order) MF: **C16H19N3O4S3H2O** Other Names: **amfipen** Other name: **amfipen** Purity: **99%min** Type: **Antibiotic and Antimicrobial Agents,Auxiliaries and Other Medicinal Chemicals,ampicillin** Grade Standard: **Food Grade,Medicine Grade,ampicillin**	 Daily Hi Industry (Shanghai) Co., Ltd. China (Mainland)

3.4.16 SG supply Ampicillin powder CAS:69-53-4

US $20-500 / Kilogram **5 Kilograms** (Min. Order) MF: **C16H19N3O4S** Other Names: **Ampicillin** Purity: **99%min** Type: **Ampicillin** Grade Standard: **Medicine Grade** Color: **White**	 Xi'an Sgonek Biological Technology Co., Ltd., China (Mainland) Trade Assurance, Transaction Level: **33** Transactions(6 months), 10,000+

3.4.17 HIGH QUALITY PHARMACEUTICAL MATERIAL AMPICILLIN POWDER

US $23.0-24.0 / Kilograms **200 Kilograms** (Min. Order) MF: **C16H19N3O4S.3H2O** Other Names: **AMPICILLINE** Purity: **99%min** Type: **Antibiotic and Antimicrobial Agents** Grade Standard: **Medicine Grade** Usage: **Animal Pharmaceuticals**	 Landy Enterprise Limited , China (Mainland) Trade Assurance

3.4.18 China API ampicillin trihydrate veterinary antibiotic ampicillin powder

US $20-40 / Kilogram **1 Kilogram** (Min. Order) MF: **C16H19N3O4S.3H2O, C16H19N** Other Names: **AMPICILLINE** Purity: **99%min** Type: **Antibiotic and Antimicrobial Agents,API ampicillin trihydrate veterinary antibiotic ampicillin powder** Grade Standard: **Feed Grade,Medicine Grade,Tech Grade** Usage: **Animal Pharmaceuticals**	 Zhengzhou Panpan Chemical Co., Ltd., China (Mainland) Trade Assurance

3.4.19 FACTORY SUPPLY LOW PRICE COMPACTED AMPICILLIN GRANULES

US $23.0-24.0 / Kilograms **200 Kilograms** (Min. Order) MF: **C16H19N3O4S.3H2O** Other Names: **AMPICILLINE** Purity: **99%min** Type: **Antibiotic and Antimicrobial Agents** Grade Standard: **Medicine Grade, Medicine Grade** Usage: **Animal Pharmaceuticals**	 Landy Enterprise Limited, China (Mainland) Trade Assurance

3.4.20 High quality ampicillin trihydrate drug ampicillin compacted powder

US $20-40 / Kilogram **1 Kilogram** (Min. Order) MF: **C16H19N3O4S.3H2O, C16H19N** Other Names: **AMPICILLINE** Purity: **99%min** Type: **Antibiotic and Antimicrobial Agents,High quality ampicillin trihydrate drug ampicillin compacted powder** Grade Standard: **Feed Grade,Medicine Grade,Tech Grade** Usage: **Animal Pharmaceuticals**	 Zhengzhou Panpan Chemical Co., Ltd., China (Mainland) Trade Assurance

3.4.21 Wholesale 99% Purity CAS No 69-52-3 Ampicillin sodium (Georgia)

0.1 Kilograms (Min. Order) MF: **C16H18N3NaO4S** Other Names: **Reasonable** Purity: **99%min** Type: **Auxiliaries and Other Medicinal Chemicals, fitness** Grade Standard: **Food Grade,Medicine Grade** Appearance: **White Powder** Synprotech LLC , Georgia	

3.4.22 Ampicillin 5%, Colistin 20MUI/100g WSP, antibiotics (Vietnam)

US $0.01 / Pieces **1000 Pieces** (Min. Order) Dosage Form: **Powder** Animal Type: **Cattle,Fowl,Pig** Function: **Antibacterial Drugs** Place of Origin: **Hanoi,Vietnam** Model Number: **FIVEVET** Brand Name: **Five-Ampicon** CENTRAL VETERINARY MEDICINE JOINT STOCK COMPANY NO.5 , Vietnam	

3.4.23 Hydroxyl ampicillin/penicillin CAS:61336-70-7

US $1-100 / Kilogram **0.01 Kilograms** (Min. Order) MF: **C16H19N3O5S** Other Names: **Hydroxyl ampicillin penicillin** Purity: **99%** Type: **Anesthetic Agents,Anti-Allergic Agents,Antibiotic and Antimicrobial Agents,Antidote,Antineoplastic Agents,Antiparasitic Agents,Antipyretic Analgesics and NSAIDs,Auxiliaries and Other Medicinal Chemicals,Blood System Agents,Cardiovascular Agents,Central Nervous System Agents,Disinfectant and Preservatives,Electrolyte Balance and Dialysis Agents,Endocrine System Agents,Gastrointestinal Agents,Immune Function Agents,Respiratory System Agents,Urinary System Agents,Vitamins, Amino Acids and Coenzymes**	Grade Standard: **Cosmetic Grade,Feed Grade,Food Grade,Medicine Grade** Usage: **Animal Pharmaceuticals**  Hefei Joye Import & Export Co., Ltd. China (Mainland) Trade Assurance

3.5 Amoxicillin products on market (Products of China, ..)

3.5.1 Pet Medicine Antibiotics Amoxicilin Powder 10% for Cat chicken poultry amoxycillin poultry

US $1-20 / Kilogram **10 Kilograms** (Min. Order) Dosage Form: **Powder** Animal Type: **Aquatic Animals,Cattle,Fowl,Horse,Other Special Breeding Animals,Pets,Pig,Sheep** Function: **Antibacterial Drugs** Place of Origin: **CN;HUB,Hubei, China (Mainland)** Brand Name: **Longxiang**	 Hubei Longxiang Pharmaceutical Tech Co., Ltd. , China (Mainland)

3.5.2 Farming medicine Antibiotics Amoxicilin Powder 10% antibiotics for chickens

US $1-20 / Kilogram **10 Kilograms** (Min. Order) Dosage Form: **Powder** Animal Type: **Aquatic Animals,Cattle,Fowl,Horse,Other Special Breeding Animals,Pets,Pig,Sheep** Function: **Antibacterial Drugs** Place of Origin: **CN;HUB,Hubei, China (Mainland)**	

Brand Name: **Longxiang** Model Number: **10%**	Hubei Longxiang Pharmaceutical Tech Co., Ltd. China (Mainland)

3.5.3 USP BP EP CP Amoxicilin,amoxicilina powder

US $10-100 / Kilogram **25 Kilograms** (Min. Order) MF: **C16H19N3O5S.3H2O** Other Names: **Almodan** Purity: **more than 99%** Type: **Antibiotic and Antimicrobial Agents** Grade Standard: **Medicine Grade** Usage: **Animal Pharmaceuticals** Shanghai Ruizheng Chemical Technology Co., Ltd., China (Mainland) Transaction Level: **25** Transactions(6 months), 130,000+	

3.5.4 Pharmaceutical grade medicine Amoxicilin,amoxicilina powder

US $10-100 / Kilogram **1 Kilogram** (Min. Order) MF: **C16H19N3O5S.3H2O** Other Names: **Almodan** Purity: **99%** Type: **Antibiotic and Antimicrobial Agents** Grade Standard: **Medicine Grade,Tech Grade** Usage: **Animal Pharmaceuticals** Xi'an Geekee Biotech Co., Ltd. , China (Mainland) Trade Assurance Transaction Level: **42** Transactions(6 months), 10,000+	

3.5.5 Amoxicillin raw material Amoxicilin

US $20-26 / Kilogram **10 Kilograms** (Min. Order) MF: **C16H25N3O8S** Other Names: **Amoxicillin trihydrate** Purity: **99%** Type: **Antibiotic and Antimicrobial Agents,Respiratory System Agents** Grade Standard: **Feed Grade,Medicine Grade,Tech Grade** Usage: **Animal Pharmaceuticals**	 Hebei Weierli Animal Pharmaceutical Group Co., Ltd. China (Mainland) Trade Assurance

3.5.6 jiangying,wuxi factory supply amoxicilin capsules/amoxicilina powder/amoxiciline

200000 Pieces (Min. Order) MF: **C16H19N3O5S.3H2O** Purity: **99%** Type: **Antibiotic and Antimicrobial Agents** color: **white** Place of Origin: **CN** CAS No.: **26787-78-0** SJ (Jiangsu) Pharmaceutical Co., Ltd. China (Mainland) Trade Assurance Transaction Level: **4** Transactions(6 months), 2,000+	

3.5.7 Amoxicilin Trihydrate Compact CAS 26787-78-0

US $60-100 / Kilogram **100 Grams** (Min. Order) MF: **C16H19N3O5S** Other Names: **amoxicillintrhydrate** Purity: **95%up** Type: **Anti infection** Grade Standard: **Medicine Grade** Sulphated ash: **0.1%** Sinoway Industrial Co., Ltd.	

China (Mainland) Trade Assurance	

3.6 صناعة الأردن (Jordan)

اسم المشروع	**مصنع البنسلين**
الموقع	عمان – الاردن
صاحب العمل	شركة الحكمة
المساحة المبنية	8000م^2
نطاق المشروع	تصميم خطوط الإنتاج وتشمل تصميم جميع التخصصات والخدمات لجميع منطقة التصنيع داخل مصنع الإنتاج

P. O. BOX 142 882 Amman - Um uthina - Saed Bin Abi waqas st

info@ucs-jo.com

00962 (6) 5529476

00962 (6) 5522476

http://ucs-jo.com/ar/index.php/project/item/53-apm-sterile-production-lines

Les précurseurs de β-lactame de toutes les pénicillines et céphalosporines sont produits par fermentation dans des fermenteurs jusqu'à 1000 m3. La concentration des produits dans le milieu à la fin de la fermentation qui prend entre cinq et sept jours, est jusqu'à 100 g / L de pénicilline et 20 g / L de céphalosporine C.

3.6.1 ادوية المضاد الحيوي تبا تحقيق يفجر مفاجأة : الصيدليات تجني أرباح بمئات الملايين و شركات عملاقة تسيطر على الدواء بزيادة الاسعار 600%

تحقيق يفجر مفاجأة : الصيدليات تجني أرباح بمئات الملايين و شركات عملاقة تسيطر على الدواء بزيادة الاسعار 600%

27-12-2017 03:49 ع للحكومة بنفس الاسم التجاري بـ 282.18 دينار تباع في احدى سلسلات الصيدليات بـ 506.33 اي بفارق 224 دينارا اي بنسبة 100% فيما تباع احد انواع الادوية المخصصة لعلاج العيون ب4.4 قروش للحكومة بينما تباع في الصيدليات بـ 245 قرشا اي بنسبة 55 ضعف ما يباع للحكومة.

و تبين ان هناك ادوية مضادات حيوية تباع الحبة الواحدة ب 2.6 قرش بينما تباع في الصيدليات بـ 28.6 قرش اي اكثر من عشرة اضعاف ونوع اخر تباع الحبة الواحدة بـ 1.6 قرش بينما تباع في الصيدليات الخاصة بـ52 قرشا اي 32 ضعف السعر الحقيقي.

أسعار دواء أوجمنتين أقراص تركيز 1 جرام و 625 مجم

سعر دواء اوجمنتين اقراص مضاد حيوي 1 جم 2018

سعر دواء اوجمنتين اقراص مضاد حيوي 1 جم 2018

سعر دواء أوجمنتين أقراص مضاد حيوي 1 جم في مصر 2018:

89.75 جنيه مصري

سعر دواء أوجمنتين أقراص 1 جم في السعودية 2018 :

97.9ريال سعودي

سعر أقراص أوجمنتين تركيز 625 مجم في مصر 2018:

52.5 جنيه مصري

سعر اقراص دواء دواء اوجمنتين 625 مجم مضاد حيوي 2018

سعر اقراص دواء اوجمنتين 625 مجم مضاد حيوي 2018

سعر أقراص أوجمنتين 625 مجم في المملكة العربية السعودية 2018:

79.75 ريال سعودي

سعر دواء اوجمنتين 375 أقراص مضاد حيوي في مصر 2017

36جنيه مصري

سعر اقراص اوجمنتين مضاد حيوي 375 مجم في السعودية

45.95ريال سعودي

موضوع كامل عن سعر دواء اوجمنتين شرب 156 و 312 و 457 و 600 مجم بكل التركيزات و البدائل بسعر أرخص اضغط هنا

بدائل أوجمنتين أقراص مثيل أقراص أوجمنتين سعر أقل سعر أرخص بنفس التركيبة و المادة الفعالة و الاستخدام :

كيورام أقراص 1 جم : سعر دواء كيورام أقراص في مصر 76 جنيه مصري.

ايموكسكلاف 1 جم أقراص : سعر ايموكسكلاف 1 جم في مصر 50.25 جنيه مصري.

هاي بيوتيك أقراص 1 جم : سعر هاي بيوتيك 1 جم في مصر 75 جنيه مصري.

ميجاموكس 1 جم أقراص : سعر ميجاموكس 1 جم في مصر 75 جنيه مصري.

أموكلاوين 1 جم أقراص سعر اموكلاوين 1 جم في مصر 34.5 جنيه مصري.

سعر ومواصفات ميجاموكس – مضاد حيوى واسع المجال – 1 جم 14 قرص

أفضل سعر لـ ميجاموكس – مضاد حيوى واسع المجال – 1 جم 14 قرص من دوايا فى مصر هو 50 ج.م.

طرق الدفع المتاحة هدفع عند الاستلام

تكلفة التوصيل هى 5 ج.م.

تباع المنتجات المماثلة لـ ميجاموكس – مضاد حيوى واسع المجال – 1 جم 14 قرص فى دوايا, سيف مع اسعار تبدأ من 40 ج.م.

أول ظهور لهذا المنتج كان فى إبريل 17, 2014

من بين المنتجات المماثلة لـ ميجاموكس – مضاد حيوى واسع المجال – 1 جم 14 قرص أرخص سعر هو 40 ج.م. من دوايا

3.7 انتاج فرنسي والاستهلاك في فرنسا (France)

3.7.1 AMOXICILLINE BIOGARAN 500 mg, gélule

- **AMOXICILLINE BIOGARAN 500 mg, 12 gélules** *P,* **Prix :** 2,55€ **Taux de remboursement :** 65%
- **Médicament princeps :** CLAMOXYL 500 mg, 12 gélules, **Prix** : 2,55€

3.7.2 Evolution des consommations d'antibiotiques en France entre 2000 et 2015

L'Agence nationale de sécurité du médicament et des produits de santé (ANSM) analyse chaque année les données relatives à la consommation des antibiotiques en France. Les résultats présentés dans la nouvelle édition de son rapport montrent notamment que la consommation des antibiotiques repart à la hausse depuis 2010, et que la France reste parmi les pays européens où celle-ci est la plus élevée. Ce niveau élevé est très préoccupant car une utilisation non maîtrisée des antibiotiques est responsable du développement des résistances bactériennes. De surcroît, l'éventail des solutions de recours que constituent les antibiotiques dits « de réserve » s'appauvrit en raison de la diminution du nombre de substances antibiotiques disponibles et d'une innovation thérapeutique trop modeste.

La consommation d'antibiotiques a globalement diminué de 11,4 % entre 2000 et 2015, mais elle est en hausse de 5,4 % depuis 2010.

Plusieurs points doivent être soulignés :

La consommation d'antibiotiques en ville représente 93 % de la consommation totale.

Elle se caractérise par :

Un usage important des pénicillines et notamment de l'association amoxicilline-acide clavulanique, qui est particulièrement génératrice de résistances ;

Une diminution de l'usage des quinolones, ce qui constitue un point positif ;

Des durées de prescription très variables, avec une moyenne se situant à 9,2 jours ;

Des disparités de consommation importantes entre plusieurs régions françaises.

À l'hôpital, la consommation d'antibiotiques représente 7 % de la consommation totale. Elle a peu évolué au cours de ces dernières années et se caractérise par :

Une stabilisation de la consommation des céphalosporines de 3ème et 4ème générations ;

Une diminution de la consommation de la colistine injectable, substance active qui exige un suivi spécifique en raison du développement de souches bactériennes multi-résistantes.

En revanche, d'autres évolutions demeurent défavorables, comme la progression de l'usage des carbapénèmes.

En Europe, aucun changement majeur n'a été observé depuis 2000 dans la cartographie des consommations.

En ville, la France se situe en 2015 au 4ème rang et son niveau de consommation reste très supérieur à la moyenne européenne. A l'hôpital, cependant, la consommation française se rapproche de la moyenne européenne

Le travail d'analyse effectué par l'ANSM a pour but de contribuer au meilleur usage des antibiotiques.

L'objectif poursuivi ne doit pas seulement être quantitatif et aboutir à ce que la consommation française rejoigne la moyenne européenne. Une évolution qualitative de la consommation doit également être recherchée. Les prescriptions inadaptées, inutiles ou trop longues doivent être évitées. Le bon usage demeure ainsi plus que jamais une priorité.

3.7.3 AMOXICILLINE ACIDE CLAVULANIQUE 500 mg/62,5 mg

Médicament	Notice	Prix	Remboursement
AMOXICILLINE/ACIDE CLAVULANIQUE SANDOZ 500 mg/62,5 mg ADULTES, 24 comprimés pelliculés *P*	Notice	7,48€	65%
AMOXICILLINE/ACIDE CLAVULANIQUE SANDOZ 500 mg/62,5 mg ADULTES, 16 comprimés pelliculés *P*	Notice	6,15€	65%

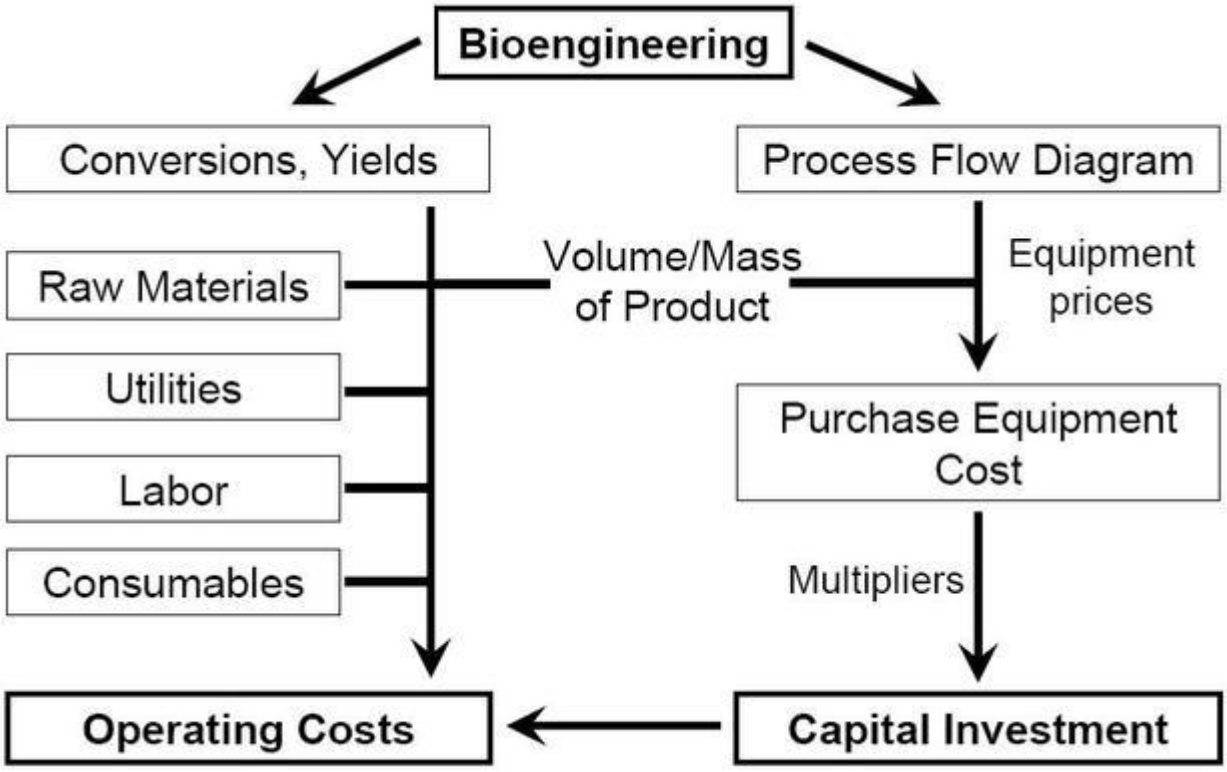
3.8

3.9 تكلفة الانتاج (Production costs) لمصنع ينتج 200 طون بنسلين سنويا

The cost of production of penicillin

وتبلغ التكلفة المقدرة لإنشاء مصنع للبنسلين 625 طنا في السنة ما بين 50 و 52 مليون دولار أمريكي تقريبا

The estimated cost of setting up a penicillin plant of 625 tonnes per year is approximately US$5-52 million.



وكما هو مبين في الرسم البياني أعلاه، فإن التكلفة المقدرة تأتي من عنصرين رئيسيين .وتشمل هذه :

1. تكاليف االستثمارات الرأسمالية

2. تكاليف الإنتاج

1. تكاليف االستثمارات الرأسمالية

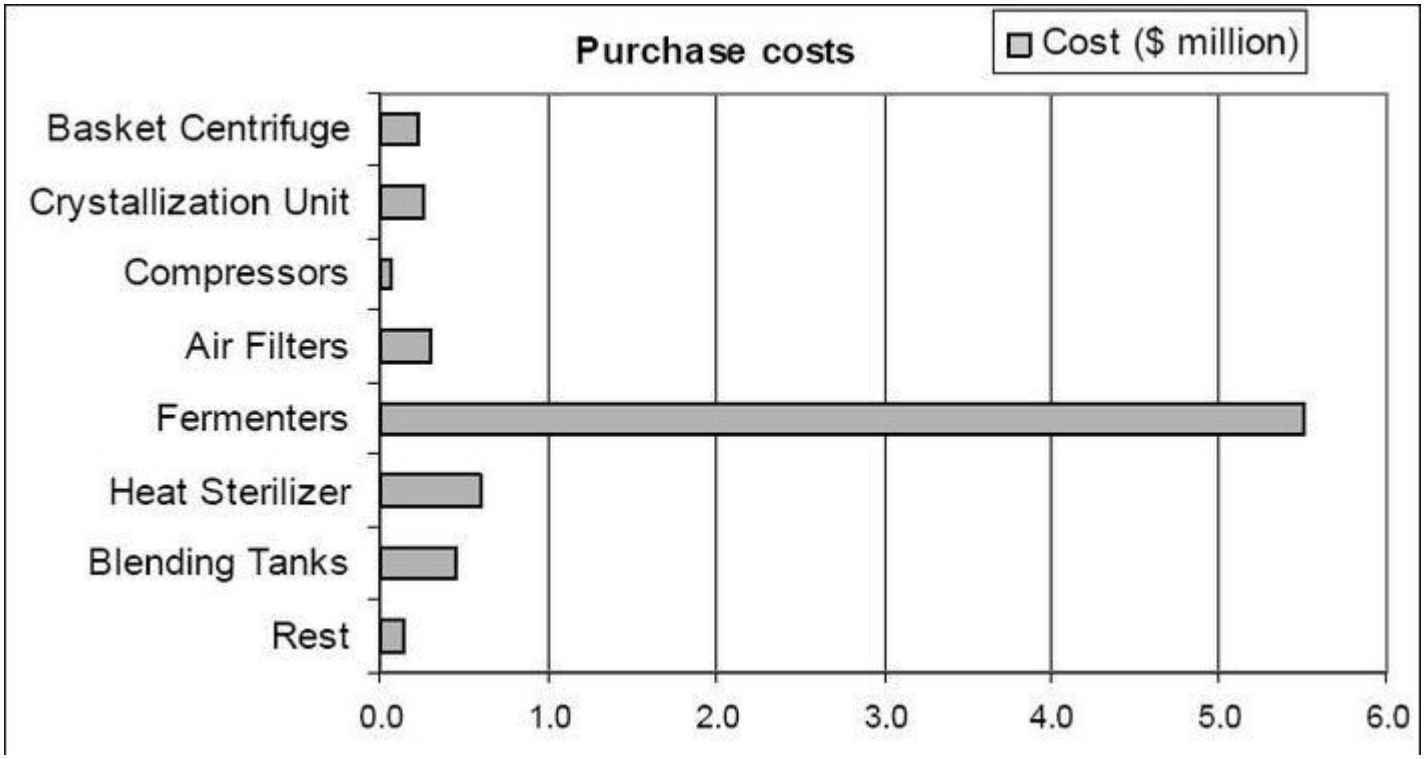
ويشمل ذلك تكاليف البناء والتشييد وتكاليف المعدات .الجدول أدناه هو تقدير تقريبي لتكاليف استثمار رأس المال، حيث تم فصل المكونات إلى تكاليف مباشرة وغير مباشرة .

Description	Range %	Ave	Used	of	Costs	
I. Direct Costs (DC)				FCI		
A. Equipment plus				FCI		
1. Purchased equipment (**PEC**)				FCI	$300,000	
2. Installation, insulation, painting	20-150	50	50%	PEC	$150,000	
3. Instrumentation & control, installed	20-60	35	35%	PEC	$105,000	
4. Piping, installed	30-60	40	40%	PEC	$120,000	
5. Electrical, installed	10-20	15	15%	PEC	$45,000	
B. Buildings including services	10-200	45	45%	PEC	$135,000	
C. Service facilities	20-100	50	50%	PEC	$150,000	
D. Yard Improvement	5-20	15		PEC		
Total Direct Costs (TDC)					**$1,005,000**	
II. Indirect costs (IDC)						
A. Engineering & supervision	20-30	25	25%	TDC	$0	
B. Legal expenses	1-3	2	2%	FCI	$0	H19*F15
C. Construction & contractor's fee	35-50	40	40%	FCI	$402,000	H19*F16
D. Contingency	7-15	10	10%	FCI	$0	H19*F17
Total Indirect Costs					**$402,000**	
III. Fixed Capital Investment (**FCI**)=DC+IDC	0.4-1				**$2,093,751**	(H12+H14)/(1-sum(f15:f17))
IV. Working Capital (WC)	10-20	15	15%	TCI	$369,485	
V. Total Capital Investment (**TCI**)=**FCI+WC**					**$2,463,236**	H19/(1-F20)

Source: Peters, et al., Plant Design and Economics for Chemical Engineers (2003)
Harrison, et al., Bioseparations Science and Engineering (2003)

تكاليف المعدات

ويتوقف ذلك على حجم النبات المستمد من حجم وعدد المخمرات والمبلغ السنوي للمنتجات التي يتم إنتاجها .ويوضح الرسم البياني التالي تكلفة شراء المعدات المقدرة لإنشاء مصنع للبنسلين .



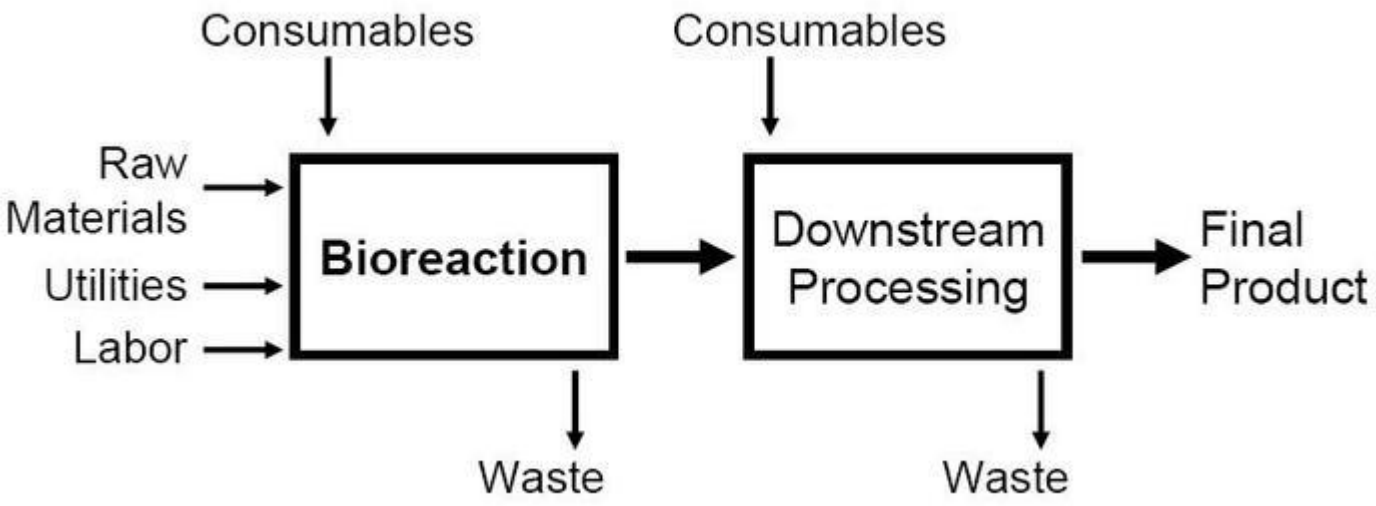
.2تكاليف الإنتاج

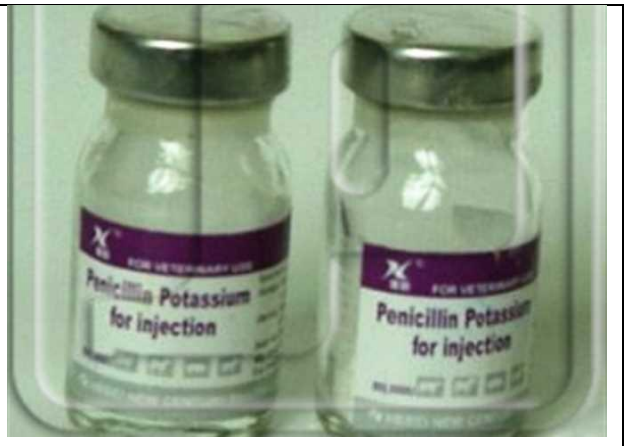
تكلفة الإنتاج الإجمالية المقدرة تشمل أيضا تكلفة التشغيل .

Description	Range %	Used	of	
Fixed Capital Inv (FCI)				2,500,000
Total Capital Inv (TCI)	110-120	115%	FCI	2,875,000
I. Manufacturing cost				
A. Direct Production Costs	66		TPC	
1. Raw materials	10-80		TPC	500,000
2. Operating labor	20-50		TPC	1,000,000
3. Direct supervisory labor	10-20	15%	Op Labor	150,000
4. Utilities	1-30	15%	TPC	701,937
5. Maintenance and Repair	2-10	6%	FCI	150,000
6. Operating supplies	10-20	15%	Mainten.	22,500
7. Lab/QCont/QAssurance	10-20	15%	Op Labor	22,500
8. Patents and royalty	0-6	3%	TPC	140,387
9. Waste Disposal	1-20	1%	TPC	46,796
B. Fixed Charges	10-20		FCI	
1. Depreciation	Depends	10%	FCI	250,000
2. Local taxes	1-4	2.5%	FCI	62,500
3. Insurance	0.4-1	0.7%	FCI	17,500
4. Rent	8-12		Value	
5. Financing	0-10	6%	TCI	172,500
C. Plant overhead	50-70	60%	Labor+Maint	780,000
Total Manufacturing Cost				**4,016,620**
II. General Expense				
A. Administrative costs	20	15%	Labor+Maint	195,000
B. Distribution & selling	2-20	5%	TPC	233,979
C. R&D	2-5	5%	TPC	233,979
III. Total Product Cost (TPC) =TMC+Gen Exp				**4,679,577** G29/(1-E2
Factor depending on TPC		29%		
Term not depending on TPC				3,322,500
Source: Peters, et al., Plant Design and Economics for Chemical Engineers (2003)				
Harrison, et al., Bioseparations Science and Engineering (2003)				

تكاليف التشغيل

تتضمن تكلفة التشغيل التكلفة الازمة للمواد الخام والمواد االستهالكية والنفايات واستهالك الطاقة وتكلفة العمالة واالستهالك .





1 تكاليف المواد الخام

•مقدار تكلفة التكلفة x

•التسعير يعتمد اعتمادا كبيرا على المصدر والحجم

2 المواد الاستهلاكية

العوامل :

)ط) المبلغ لكل دفعة

'2'استبدال التردد / ساعات التشغيل

'3'السعر

•المواد الاستهلاكية الرئيسية

4 استهلاك الطاقة

•استهلاك الطاقة النموذجية :

)ط) عملية التدفئة والتبريد

'2'التبخر / التقطير

'3'تهوية المفاعل الحيوي، والتحريض

'4'الطرد المركزي، وتعطيل الخلايا، وما إلى ذلك .

•تكاليف المرافق

(1)الكهرباء: 4.5 سنت / كيلوواط ساعة

'2'البخار: 4.40 دولار للطن

'3'مياه التبريد: 8 سنتات / م 3

5 تكلفة العمالة

•مقدار العمل :

)ط) محسوبة من الطلب على كل خطوة من خطوات العملية

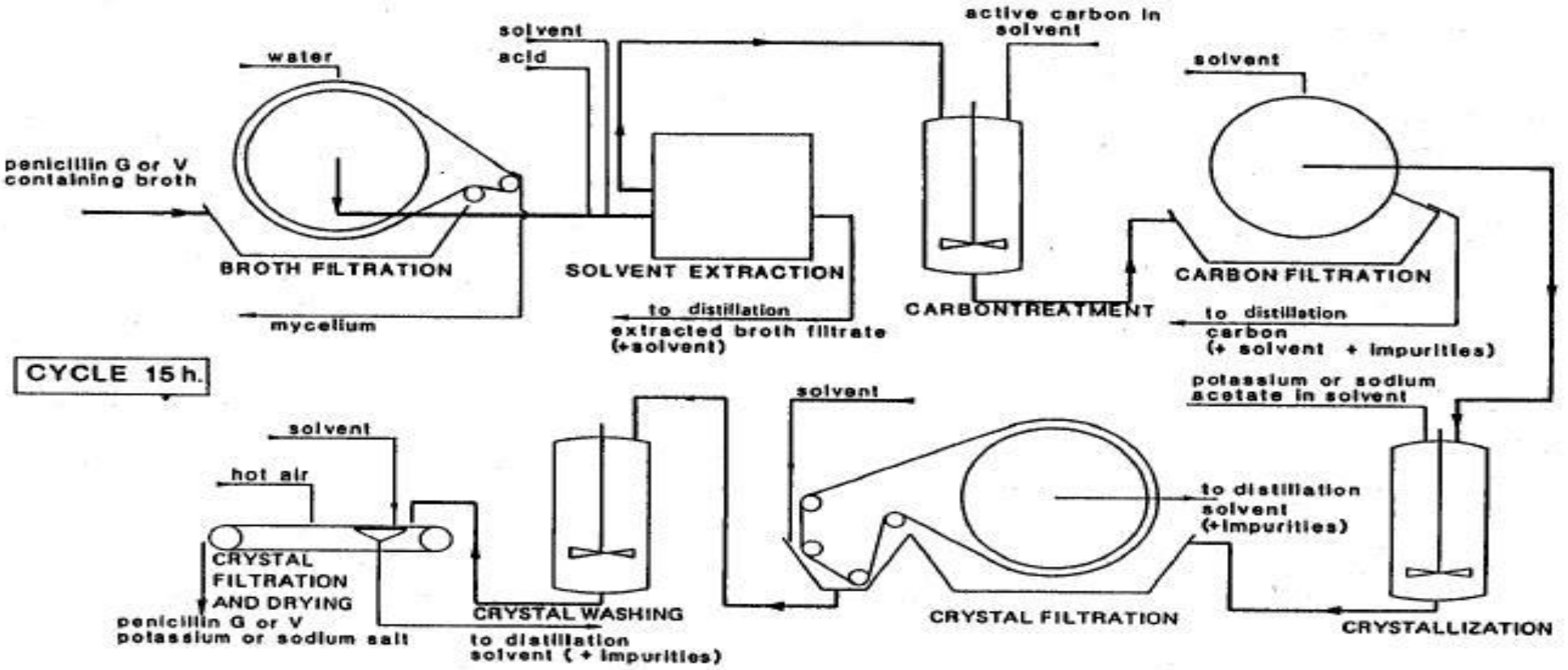
'2'يحدد عدد الأشخاص لكل نوبة / عدد التحولات

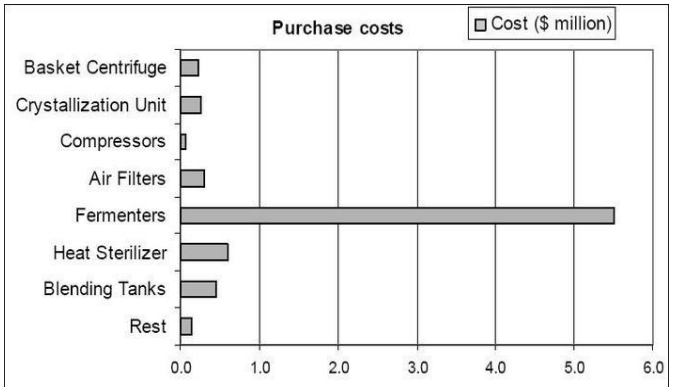
•التكلفة كل ساعة

)ط) القيمة المتوسطة للشركة الداخلية

(1)راتنجات الامتزاز / اللوني)إي) الأغشية (فيلتيراتيونس، غسيل الكلى، ديافيلتراتيون، ه (	'2الأدب، مثل العمالة الماهرة: 34 دولارا في الساعة
3 النفايات أنواع النفايات وتكاليفها *)ط) النفايات الصلبة •غير خطرة: 35 دولار للطن •الخطرة: $ 145 / طن '2النفايات السائلة / مياه الصرف: 0.5 دولار / م 3 '3الانبعاثات: تعتمد التكلفة على التركيب	**6 الاستهلاك** •تكلفة االستهالك = "رد" التكلفة االستثمارية •فترة الاستهلاك Life≈ وقت المشروع: 3–10 سنوات •طريقة االستهالك :)ط) خط مستقيم (نفس دولار سنويا ('2انخفاض الرصيد

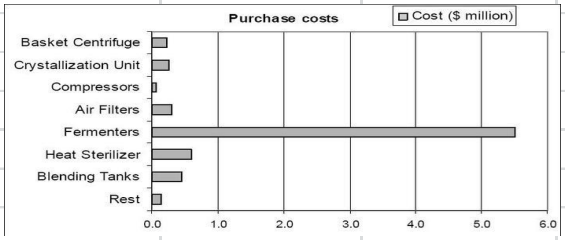
https://penicillin.wikispaces.com/Estimated++cost

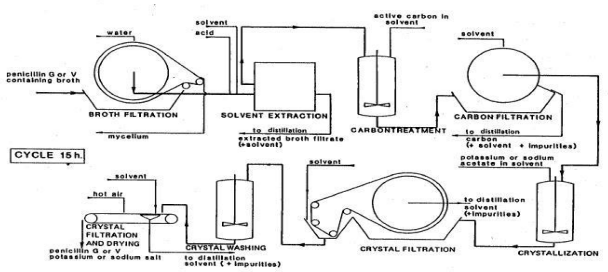

water
solvent
acid
active carbon in solvent
solvent
penicillin G or V containing broth
BROTH FILTRATION
SOLVENT EXTRACTION
CARBONTREATMENT
CARBON FILTRATION
mycelium
to distillation
extracted broth filtrate (+solvent)
to distillation
carbon (+ solvent + impurities)
CYCLE 15 h.
potassium or sodium acetate in solvent
solvent
hot air
solvent
to distillation solvent (+impurities)
CRYSTAL FILTRATION AND DRYING
penicillin G or V potassium or sodium salt
CRYSTAL WASHING
to distillation solvent (+ impurities)
CRYSTAL FILTRATION
CRYSTALLIZATION


Purchase costs
Cost ($ million)
Basket Centrifuge
Crystallization Unit
Compressors
Air Filters
Fermenters
Heat Sterilizer
Blending Tanks
Rest
0.0
1.0
2.0
3.0
4.0
5.0
6.0

MEGBI-APP010218

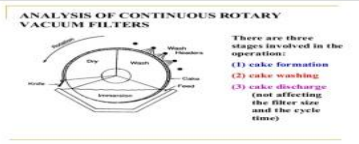
Penicillin Recovery

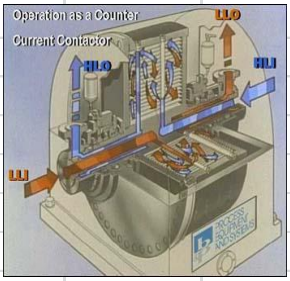




Material Costs

SYSTEM	#	Piece Priece	Total
valve	18	$60	$1.08
CNC LAB			
Rotary Vacuum Filter	5	$15,000	$75,000





$2,300-23,000/ Piece

Corn_steep_liquor

1 ton

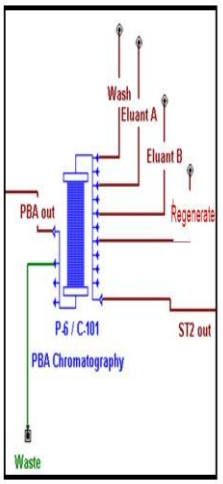
US $ 499-599 / Ton

Min. Order: **20** **$570** **$570**

solvent amyl acetate Kg $6

heat_sterilization 1 **$3,400** **$3,400**

Fermenters 1000 L		40	**$5,000**	**$200,000**	
					
Crystallization Unit		1	**$3,000**	**$3,000**	
					
Mixer+ blending tank		**1**	**$2,268**	**$2,268**	
US $2,268-12,368 / Piece					https://www.made-in-china.cor
					
Air Filter		2	**$8,000**	$16,000	
$7					
Total			**$37,304**	$300,239.08	

PH metre	1	**$170**	**$170**	
$ 159.5-200				
control system	1	**$350**	**$350**	
temperature sensor	1	**$100**	**$100**	
$5-300				
	1	**$272.09**	**$272.09**	
PO2 metre				
Lutron PO2-250 Oxygen Meter				
i-zone.in/index.php?route=common/home				
Homogenizer	1	**$1,000.00**	**$1,000.00**	
ali baba.com				
PBA chromatography colunm	3	**$302.00**	**$906.00**	GENERAL DESCRIPTION : PBA CHROMATOGRAPHY
colunm volume=344.10L				
PBA chromatography colunm	2	**$310.00**	**$620.00**	4 different PBA Chromatography Column; 1)Column Loading (Load) 2)Column Washing (Wash) 3)Column Elution (Elute) 4)Column Regeneration (Regenerate) 
colunm volume=358.19L.				
PBA chromatography colunm	1	**$268.00**	**$268.00**	
colunm volume=276.87L				
PBA chromatography colunm	1	**$265.00**	**$265.00**	
colunm volume=271.76L				

blinding tank			1	**$250.00**	**$250.00**			
vessel volume=20349.40L								
Stirred reactor			1	**$365.00**	**$365.00**			
vessel volume=198.34L								
$520-3000								
Stirred reactor			1	**$570.00**	**$570.00**			
vessel volume=5107.53L								
Diafilter			1	**$62.00**	**$62.00**			
membrane Area=24.11 m2								
Diafilter			1	**$45.00**	**$45.00**			
membrane Area=13.99 m2								
stirred reactor			1	**$624.00**	**$624.00**			
vessel volume=9841.19L								
total				**$5,158**	**$6,072**			

total plan Direct cost								
direct costs								
Equipment purchase cost			$30,480,444					
installation			$150,000					
process piping			$120,000					
Instrumentation			$105,000					
Electrical			$45,000					
Buildings			$135,000					
Auxiliary facilities			$150,000					
yard improvement								
toyal			$31,035,444					
			indirect costs					
			Engineering	$20,554,000				
			construction	$402,000				
			legal expenses					
			contingency					
Cost of workers								
labor type	$/h		Annual Amoun	Annual cost($)				
operator		69	117,606	8,114,800				

Description	Range %	Ave	Used	of	Costs	
I. Direct Costs (DC)				FCI		
A. Equipment plus				FCI		
1. Purchased equipment (**PEC**)				FCI	$300,000	
2. Installation, insulation, painting	20-150	50	50%	PEC	$150,000	
3. Instrumentation & control, installed	20-60	35	35%	PEC	$105,000	
4. Piping, installed	30-60	40	40%	PEC	$120,000	
5. Electrical, installed	10-20	15	15%	PEC	$45,000	
B. Buildings including services	10-200	45	45%	PEC	$135,000	
C. Service facilities	20-100	50	50%	PEC	$150,000	
D. Yard Improvement	5-20	15		PEC		
Total Direct Costs (TDC)					$1,005,000	
II. Indirect costs (IDC)						
A. Engineering & supervision	20-30	25	25%	TDC	$0	
B. Legal expenses	1-3	2	2%	FCI	$0	H19*F15
C. Construction & contractor's fee	35-50	40	40%	FCI	$402,000	H19*F16
D. Contingency	7-15	10	10%	FCI	$0	H19*F17
Total Indirect Costs					$402,000	
III. Fixed Capital Investment (**FCI**)=DC+IDC	0.4-1				$2,093,751	(H12+H14)/(1-sum(f15:f17))
IV. Working Capital (WC)	10-20	15	15%	TCI	$369,485	
V. Total Capital Investment (**TCI**)=**FCI**+**WC**					$2,463,236	H19/(1-F20)

Source: Peters, et al., Plant Design and Economics for Chemical Engineers (2003)
Harrison, et al., Bioseparations Science and Engineering (2003)

Materials Cost

Glucose	$5-60	1 kg	$60
salts			$1
water			$0
H3PO4			$1
NaOH			0,500
WF1			$0
Ammonia			0,700
Air			0,000
EDTA			$19
Tris base			$6
triton-x-100			$2
Mr ETOH			$3
Urea			$2
CNBr			$11
Formic acid			$2
guanidine HCl			$2
Na2O6S4			0,600
Sodium sulfite			0,400
sodium chloride			$1
Enzymes			$500,000
Acetonitrile			$3
Ammonium Acetat			$15
Zinc chloride			$12
total			$500,139

Description	Range %	Used	of	
Fixed Capital Inv (FCI)				2,500,000
Total Capital Inv (TCI)	110-120	115%	FCI	2,875,000
I. Manufacturing cost				
A. Direct Production Costs	66		TPC	
1. Raw materials	10-80		TPC	500,000
2. Operating labor	20-50		TPC	1,000,000
3. Direct supervisory labor	10-20	15%	Op Labor	150,000
4. Utilities	1-30	15%	TPC	701,937
5. Maintenance and Repair	2-10	6%	FCI	150,000
6. Operating supplies	10-20	15%	Mainten.	22,500
7. Lab/QCont/QAssurance	10-20	15%	Op Labor	22,500
8. Patents and royalty	0-6	3%	TPC	140,387
9. Waste Disposal	1-20	1%	TPC	46,796
B. Fixed Charges	10-20		FCI	
1. Depreciation	Depends	10%	FCI	250,000
2. Local taxes	1-4	2.5%	FCI	62,500
3. Insurance	0.4-1	0.7%	FCI	17,500
4. Rent	8-12		Value	
5. Financing	0-10	6%	TCI	172,500
C. Plant overhead	50-70	60%	Labor+Maint	780,000
Total Manufacturing Cost				**4,016,620**
II. General Expense				
A. Administrative costs	20	15%	Labor+Maint	195,000
B. Distribution & selling	2-20	5%	TPC	233,979
C. R&D	2-5	5%	TPC	233,979
III. Total Product Cost (TPC) =TMC+Gen Exp				**4,679,577** G29/(1-E2
Factor depending on TPC		29%		
Term not depending on TPC				3,322,500

Source: Peters, et al., Plant Design and Economics for Chemical Engineers (2003)
Harrison, et al., Bioseparations Science and Engineering (2003)

$30,174,133

Laborers	#	
	9	150000$
	1	16500$

تصليحات
150000$

البيع كلفة
1000000$

المصنع انشاء كلفة
المكان+المعدات+ تركيبا
$641,211

سنويا الربح
$1,000,000
$655,000

المدخول =

سنويا التشغيل كلفة
كهرباء+ عمال+تصليحات
$345,000

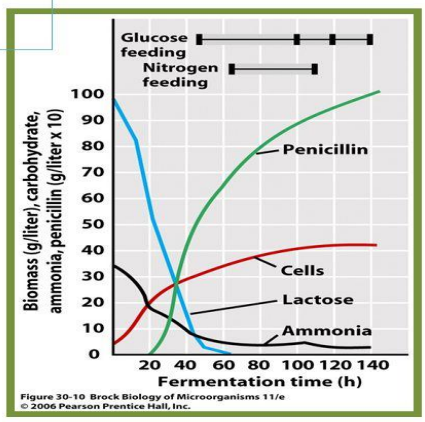
الانتاج كلفة مجموع
$986,211

Amortisation	years
$655,000	1
$1,310,000	2
$1,965,000	3

MEGBI-APP010218

Penicillin Recovery

The graph shows the kinetics of the penicillin fermentation with *Penicillium chrysogenum*.



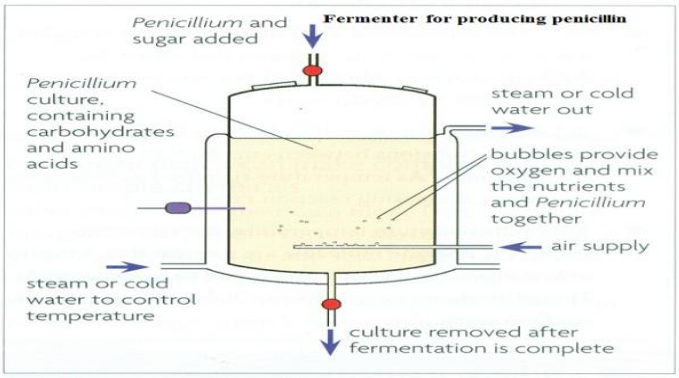
Enzyme processes in the production of β-Lactam antibiotics

Penicillins and chephalosporins belong to the class of β-lactam antibiotics that are formed from the common precursor tripeptide isopenicillin N. The β-lactam structure is formed by ring-closure reactions between Cys and Val, where (S)-Val is isomerized to (R)-Val. The β-lactam precursors of all penicillins and cephalosporins are produced by fermentation in fermentors of up 1000 m3.

The concentration of the products in the medium on completion of fe Les précurseurs de β-lactame de toutes les pénicillines et céphalosporines sont produits par fermentation dans des fermenteurs jusqu'à 1000 m3.

La concentration des produits dans le milieu à la fin de la fermentation qui prend entre cinq et sept jours, est jusqu'à 100 g / L de pénicilline et 20 g / L de céphalosporine C.

http://slideplayer.com/slide/4214453/ Methods of industrial production



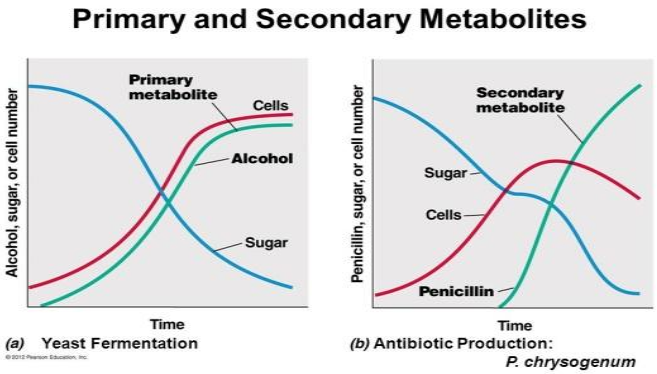
1m3=1000L

g/1000=Kg

1ton=1000Kg

lb الباوند الواحد = 0,4536 كيلوغرام

fermenteur m3	penicillin g/l	
1000	100	
1,000,000 L	100,000,000 g	
1,000L	100,000 g	100Kg



Clicker Question:

rendement maximum de production de penicilline disponible lorsque la concentration de lactosse et cell de liqueure de cornsteep dans le milieu de base ont été ajustée à 60 Kg /m3
et 30kg/m3 respectivement

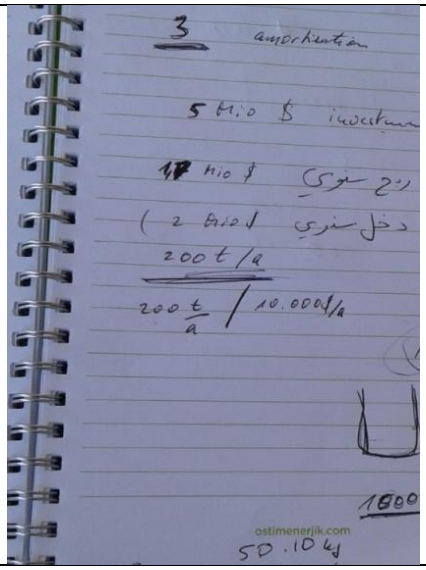
https://translate.googleusercontent.com/translate_c?depth=1&hl=fr&prev=search&rurl=translate.google.com&sl=en&sp=nmt4&u=https://healtheappointments.com/cdn-cgi/l/email-protection&xid=17259,15700021,15700105,15700124,15700149,15700168,15700173,15700201&usg=ALkJrhgdgWS-zdzncshWKpiJLN8lQ8wQ6Q#0a63646c654a626f6b667e626f6b7a7a6563647e676f647e7924696567

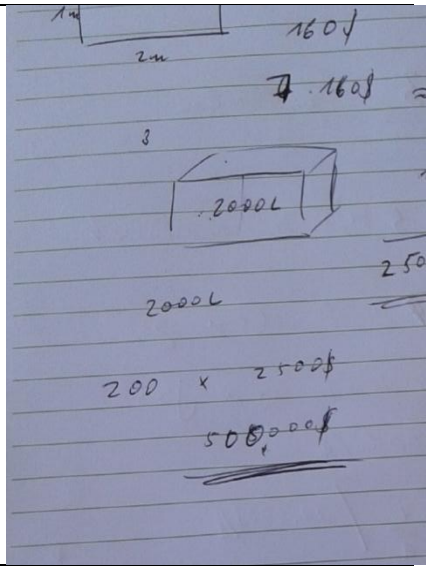
			Kg /m3	
lactose			60	
liqueure de cornsteep			30	
		#	Volume	$
		1	1000L	1250
		40	40000L	50000
	200 tons /ans			
			penicilline	cost$
			1Kg	5
			1000Kg	5000
			200 tons	1000000

Fermentation medium In addition to physical parameters like pH, agitation and aeration rate, air saturation, temperature, dissolved CO2 and foaming, medium composition is a very important factor strongly influencing fermentation processes, often being object of extensive process development and optimization studies. Common fermentation media for L-lysine production contain various carbon and nitrogen sources, inorganic ions and trace elements (Fe++, Mn++), amino acids, vitamins (biotin, thiamine-HCl, Nicothinamide) and numerous complex organic compounds. An overexpression of genes is also achieved by optimizing the composition of the media and the culture technique in addition to physiological and genetic parameters .
CARBON SOURCE Mutants of Corynebacterium and related microorganisms enable the inexpensive production of amino acids from cheap renewable carbon sources by direct fermentation. Various carbohydrates are utilized individually or as a mixture for the production of L-lysine such as glucose, fructose, sucrose, molasses (sucrose, glucose, fructose etc.), maltose, blackstrap molasses, starch hydrolyzate (glucose, oligosaccharides), lactose, maltose, starch and starch hydrolysates, cellulose, cellulose hydrolysate, organic acids such as acetic acid, propionic acid, benzoic acid, formic acid, malic acid, citric acid and fumaric acid, alcohols such as ethanol, propanol, inositol and glycerol and certainly hydrocarbons, oils and fats such as soy bean oil, sunflower oil, groundnut oil and coconut oil as well as fatty acids such as e.g. palmitic acid, stearic acid and linoleic acid.
42 NITROGEN SOURCE Various sources of nitrogen are utilized individually or as mixtures for the commercial and pilot scale production of L-lysine, including inorganic compounds such as gaseous and aqueous ammonia, ammonium salts of inorganic or organic acids such as ammonium sulfate, ammonium nitrate, ammonium phosphate, ammonium chloride, ammonium acetate and ammonium carbonate. Alternatively, natural nitrogen containing organic materials like soybean-hydrolyzate, soyprotein HCl-hydrolyzate (total nitrogen of about 7%), soybean meal, soybean cake hydrolysate, corn steep liquor, casein hydrolysate, yeast extract, meat extract, malt extract, urea, peptones and amino acids may also be utilized.
43 INFLUENCE OF OXYGEN L-lysine fermentation is an aerobic process demanding large amounts of oxygen and strongly influenced by the air saturation in bioreactor. Lactic acid is formed as a byproduct under anaerobic conditions, which is reconsumed after the establishment of aerobic conditions.
44 pH The pH is a very important factor strongly influencing microbial fermentations. Basic compounds such as sodium hydroxide, potassium hydroxide, ammonium hydroxide, calcium carbonate, urea, ammonia and gaseous ammonia, or inorganic acid compounds such as phosphoric or sulfuric acid and organic acids are utilized

	Rotary Vacuum Filter							
www.911metallurgist.com/blog/rotary-drum-filter								
				kg		lb (ponde)		
				0.4536		1		
				90.72		200		
			907.2 L	← 907.2		2000		
			5	Rotory vacuum				

the capacity of a vacuum rotory drum filter varies from about 200 to 2,000 Lb.of dry concentrate per square foot of filtering surface per 24 hours according to the nature of the materiel and the amount of water that it contains.For the puproses of rough calculation it is usual to assume a capacity of 1000lb .Per square foot, although this figure is generally exceeded under conditions the moisture cake normally ranges from 8 to 12%.



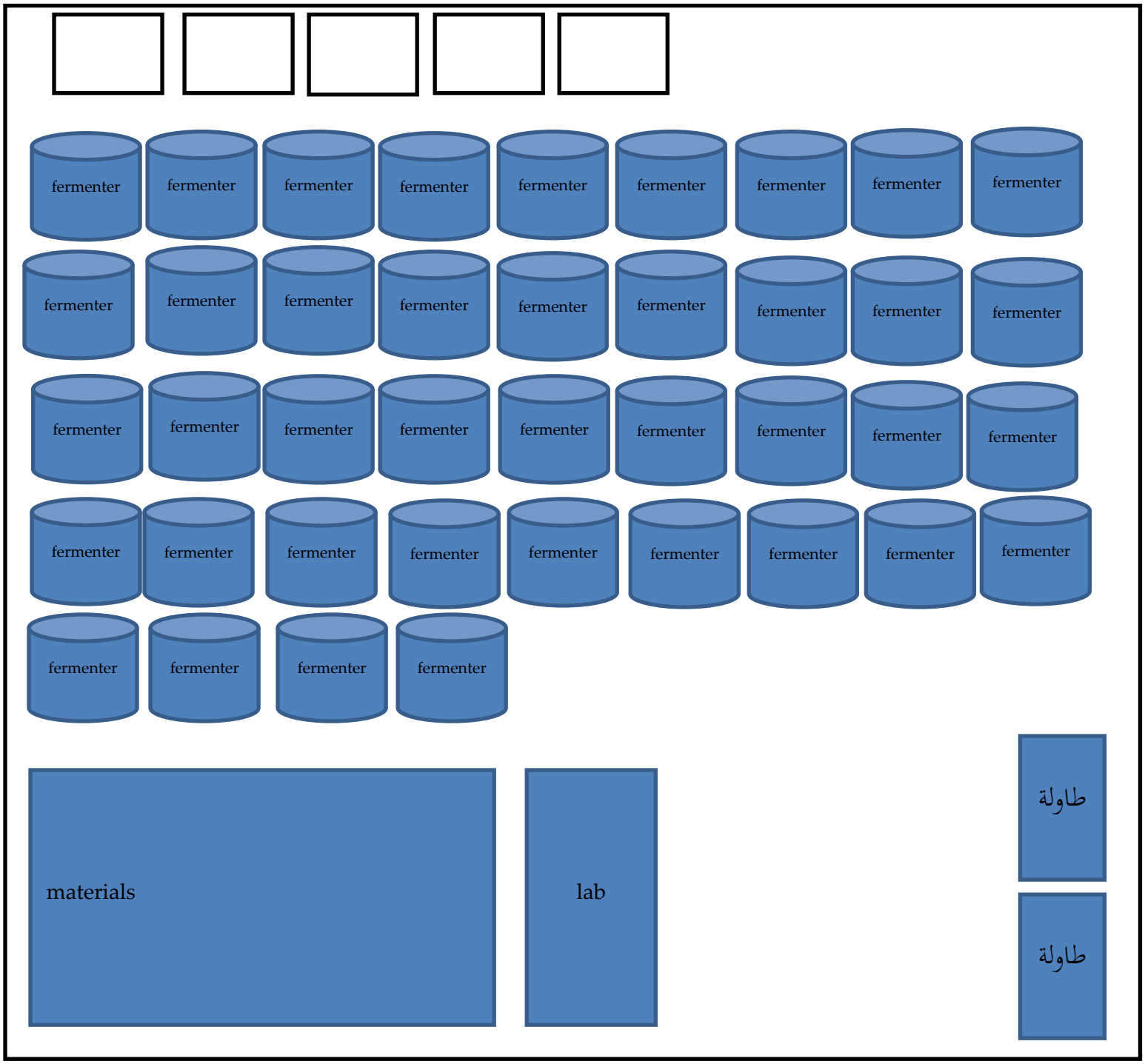


3.9.1 تلخيص (Summary)

سنويا الانتاج	المنشئة كلفة	
200 t	العاملة اليد كلفة	$248,400
	المواد كلفة+الالات	$806,451.82
	total	$1,054,852

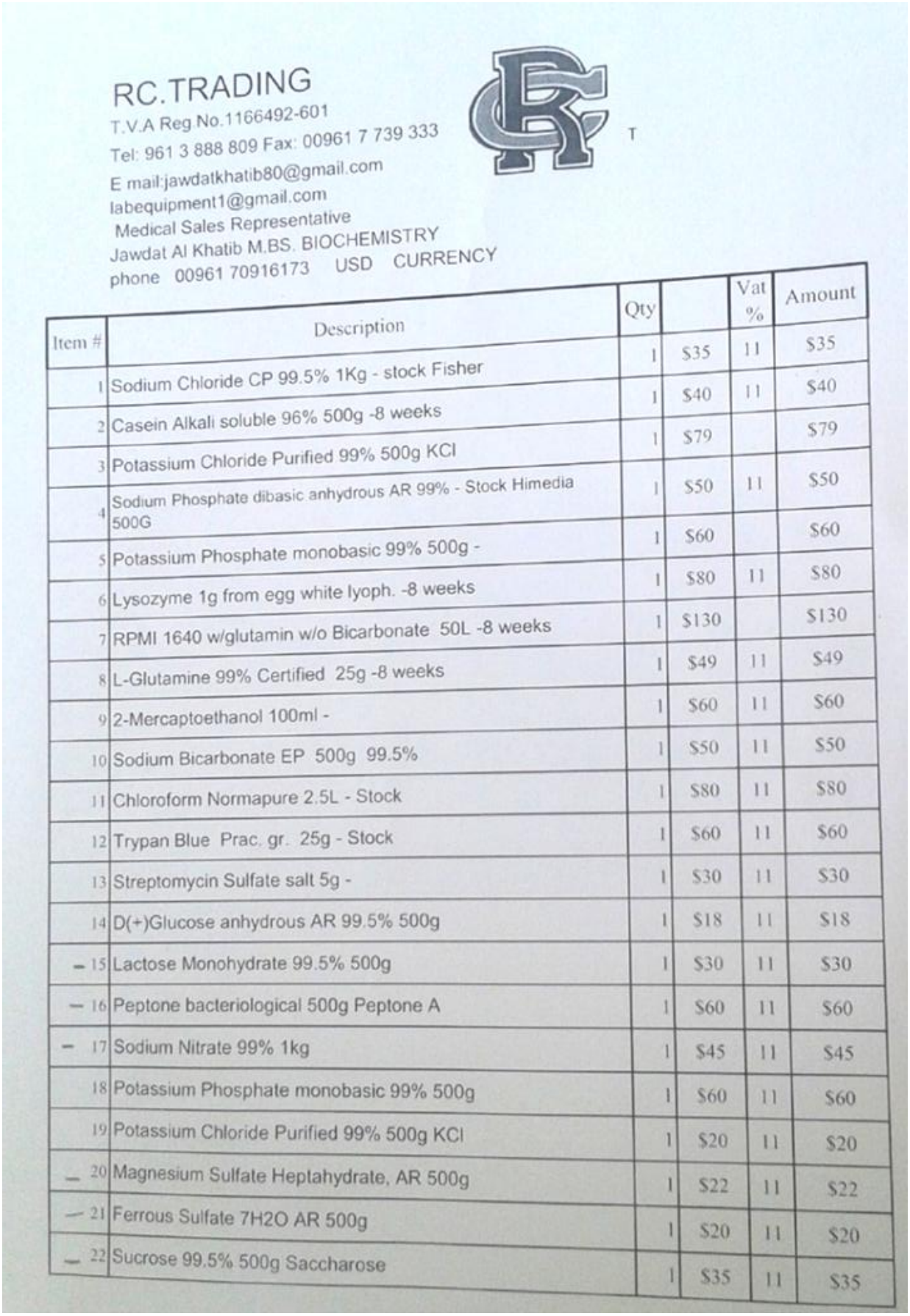
المحطة لتشغيل السنوية الكلفة	
صيانة	150,000
عمال	$248,400
كهرباء	$45,000
total	$443,400

3.9.2 المصنع (production site) (150qm)



3.9.3 Consumables and Materials

3.9.3.1 Offer from Jawdat AlKatibe



RC.TRADING
T.V.A Reg.No.1166492-601
Tel: 961 3 888 809 Fax: 00961 7 739 333
E mail:jawdatkhatib80@gmail.com
labequipment1@gmail.com
Medical Sales Representative
Jawdat Al Khatib M.BS. BIOCHEMISTRY
phone 00961 70916173 USD CURRENCY

Item #	Description	Qty		Vat %	Amount
1	Sodium Chloride CP 99.5% 1Kg - stock Fisher	1	$35	11	$35
2	Casein Alkali soluble 96% 500g -8 weeks	1	$40	11	$40
3	Potassium Chloride Purified 99% 500g KCl	1	$79		$79
4	Sodium Phosphate dibasic anhydrous AR 99% - Stock Himedia 500G	1	$50	11	$50
5	Potassium Phosphate monobasic 99% 500g -	1	$60		$60
6	Lysozyme 1g from egg white lyoph. -8 weeks	1	$80	11	$80
7	RPMI 1640 w/glutamin w/o Bicarbonate 50L -8 weeks	1	$130		$130
8	L-Glutamine 99% Certified 25g -8 weeks	1	$49	11	$49
9	2-Mercaptoethanol 100ml -	1	$60	11	$60
10	Sodium Bicarbonate EP 500g 99.5%	1	$50	11	$50
11	Chloroform Normapure 2.5L - Stock	1	$80	11	$80
12	Trypan Blue Prac. gr. 25g - Stock	1	$60	11	$60
13	Streptomycin Sulfate salt 5g -	1	$30	11	$30
14	D(+)Glucose anhydrous AR 99.5% 500g	1	$18	11	$18
15	Lactose Monohydrate 99.5% 500g	1	$30	11	$30
16	Peptone bacteriological 500g Peptone A	1	$60	11	$60
17	Sodium Nitrate 99% 1kg	1	$45	11	$45
18	Potassium Phosphate monobasic 99% 500g	1	$60	11	$60
19	Potassium Chloride Purified 99% 500g KCl	1	$20	11	$20
20	Magnesium Sulfate Heptahydrate, AR 500g	1	$22	11	$22
21	Ferrous Sulfate 7H2O AR 500g	1	$20	11	$20
22	Sucrose 99.5% 500g Saccharose	1	$35	11	$35

23	Zinc Sulfate 7H2O 99% Purified 500g	1	$20	11	$20
24	Copper II Sulfate 5H2O EP 500g	1	$25	11	$25
25	Protose BE (Beef extract powder) 500g	1	$120	11	$120
26	Ammonium Persulfate EP 98% 500g	1	$20	11	$20
27	Parafilm 4"x38 meter 125Ft	1	$38	11	$38
28	Ethyl acetate AR 2.5L	1	$60	11	$60
29	Phosphate Buffer Saline PH 7.2 100g PBS	1	$50	11	$50
30	Chloroform Normapure 2.5L	1	$80	11	$80
31	Cotton Blue Lactophenol 100ml	1	$50	11	$50

Offer from Bourhan Kabbara

Ampicillin Pilot Plans			
ID			cost$
Glucose		500g	20
Lactose		500g	24
Peptone		500g	56
NaNo3		500g	32
Na2HPO4		500g	25
MgSO47H2O		500g	18
FeSO47H2O		500g	20
Sucrose		500g	18
ZnSO47H2O		500g	20
CuSO45H2O		500g	18
(NH4)2SO4		500g	30
Sodium acetate		500g	22
Ethyl acetate		2.5 L	60
Sodium acetate		500g	22
Chloroform		2.5L	75
Lacto phenol cotton blue stain		100ml	46
Titriplex		250g	25
total			531
K2HPO4 (dibasic)			
yest extract			
CaCO3			
Corn steep liquor			
Beef extract			
Na2SO4			

4 Business Plan from Jan 2017

بسم الله الرحمن الرحيم

Business Plan 2017-2023 for project "Manufactoring and operation of a semi-synthetic penicillin production plant"

Initial Document: Ras Nhache, 12.1.2017, Last update: 21 Januar 2017 (initially for Themar Tarablus)

صناعة منشآت لإنتاج أدوية

Manufactoring of medical biotechnological production plants

للاتصال \Contact

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عنوان المؤسسة:

بناية ميناء بلازا الطابق الأول (دوار الميناء، فوق محمصة زينة)، طريق ميناء – بيروت

أوقات الاستقبال: الاثنين – الخميس الساعة 8–12

الجمعة: الساعة 8–11

Content

5 Company profile

5.1.1 رؤيتنا (Vision)

To supply Middle East Countries with home produced medicine and giving the youth in the region an opportunity of work.

5.1.2 رسالتنا (Mission)

To supply North Lebanon pharmacies and several countries of the Middle East market with penicillin, ampicillin and other semi-synthetic penicillins in the next years.

5.1.3 الاهداف (Goals)

The goal is to install in Jan-June 2017 an ampicillin pilot plant and in the second half of the year 2017 work for marketing in North Lebanon.

6 Organisation and Management

6.1 Management

Director: Eng. Samir Mourad

CV_SamirMourad13012017.pdf

6.2 Shareholders

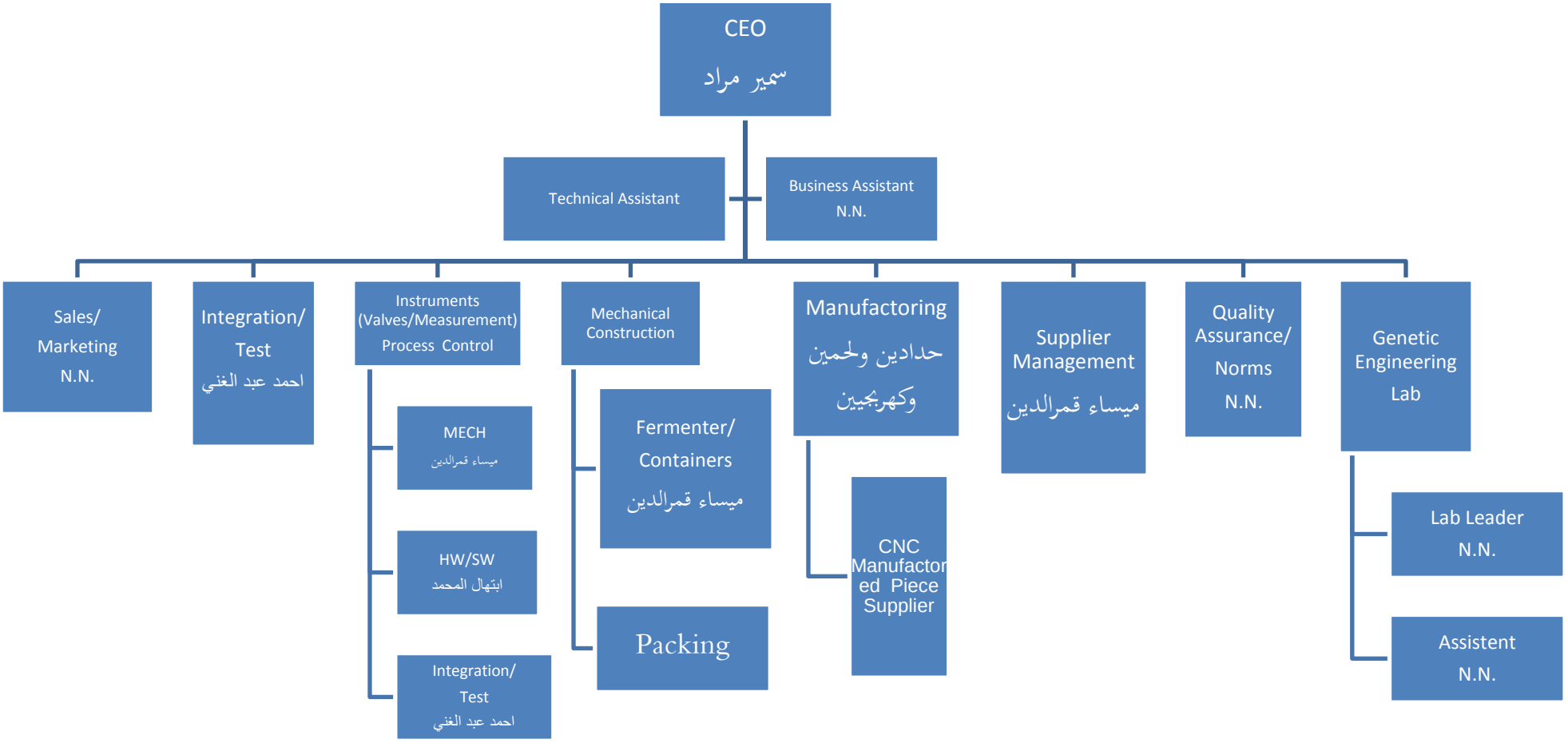
Actual investors list (Investment sum: 1.2 Mio EUR)

AECENAR (Public Research Institution)	140,000 EUR
Naser Al-Araimi	1,200 EUR
Samir Mourad	1,058,700 EUR
~~David Yildiz~~	~~100 EUR~~[5]

[5] 28.07.2020: Investment already given back

6.3 Organizational chart (current and future)

LG Biotech Organigram for manufactoring semi-synthetic penicillin production plants



6.4 Employees

6.4.1 Number of employees

		Number of employees	Sum
Administration	Technical Assistant Business Assistant	1 1	2
Marketing/Sales	Sales/Marketing	2	2

Development	• Integration/Test
	• Instruments (Valves/Measurement)
	• Process Control
	  • MECH
	  • HW/SW
	  • Integration/Test
	• Mechanical Construction
	  • Fermenter/Containers
	  • Packing
	• Quality Assurance/Norms
		2
		2
		
		1
		1
		2
		
		1
		1
		1
			11

Manufactoring	• Supplier Management • Manufactoring • CNC Manufactored Piece Supplier	1 2	3
Genetic Engineering Lab	Lab Leader Assistent	1 1	2
	Total number of employees		20

Actually (Jan 2017) 3-5 part time.

6.4.2 CVs summary

tbd

6.4.3 Management team gaps (which positions are missing in the company)

See above in Organigram (N.N. positions):

1. Business Assistent

2. Marketing/Sales

7 Market analysis

7.1.1 Potential Customers

All microbiology laboratories (including clinic labs) & all pharmacies in the region

7.1.1.1 Potential Customers in North Lebanon

Name	Contact	Actual need	Remarks	Required antibiotics supply, Return of Invest Range

...

tbd (to be done) as trainee task

7.1.1.2 Others

To be identified later on

7.1.2 Market needs, trends and growth

tbd (to be done) as trainee task

7.1.2.1 Competetors

	Acillin (from online phamacy)	LG Biotech (our company)
Price for on ampicillin unit (1 pill)	About 0.20$	0.15$
quality		same chemical analysis result as competitors

7.2 Distribution channels

Actually in North Lebanon. Contact bureau in Tripoli/North Lebanon.

7.3 Suppliers

Steel suppliers: Sabalbal (Tripoli), ...

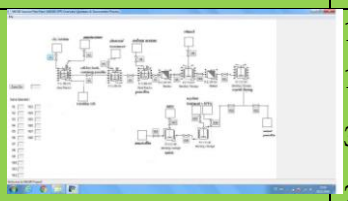
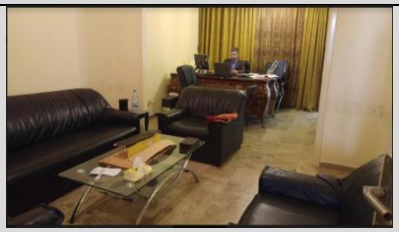
Instruments: Jamal&Chaban (Tripoli), ...

Manufactoring of parts with CNC: Riyaco (Beddawi), ...

Biomedical, biotechnology material: numlab (Beirut)

8 Funding request: 200,000 $

8.1 Costs of Building the amoxillin production pilot plant, marketing and begin of production (May 2017-May 2019)

<table>
<tr><td colspan="2"> 
</td><td>200,000 $ MEGBI Genetic Engineering Lab (Installation 2009-2011)
30,000$ lab assistant for 2 years
  </td></tr>
<tr><td colspan="2">Semi-synthetic penicillin production pilot plant</td><td></td></tr>
<tr><td>Pilot-plant mechanics</td><td>Process-control system for pilot plant</td><td></td></tr>
<tr><td>  </td><td> 
  </td><td>150,000 $ mechanics, PCS (2012-16)
3500 $ automatic valves, pipes
2000 $ integration
5000$ process control system
30,000$ optimizing plant (temp./Sterilization in process)
60,000$ production personal (2 persons) for 2 years
5000$ material for 2 years</td></tr>
<tr><td>Qualification process</td><td></td><td>In 2017: 15,000$</td></tr>
<tr><td>Administration, Marketing</td><td></td><td></td></tr>
<tr><td>  </td><td></td><td>24,000 $ Administration for 2 years
24,000 $ Marketing for 2 years</td></tr>
<tr><td>Facility: flat with 5 rooms
2 rooms Genetic Engineering Lab
1 room production plant</td><td></td><td>20,000 $ Renting for 2 years (April 2017- April 2019)</td></tr>
</table>

2 rooms administration/sales		
	Total cost	**568,500 $** **(218,500 $ still open)**

8.1.1.1 Estimated Return of Invest (ROI)

10$ selling price for 500mg × 60 pills ampicillin

competetor: 30$

Market: Lebanon/Jordan/Turkey and other Arab Countries
About 500 customers x 10 packages/month x 10 $/package x 12 months =

1,2 Mio. $ per year

8.1.1.2 Estimated win

Investment 1.2 $, ROI - operation cost: 6 Mio.$ -> win is about 500% in 3 years.

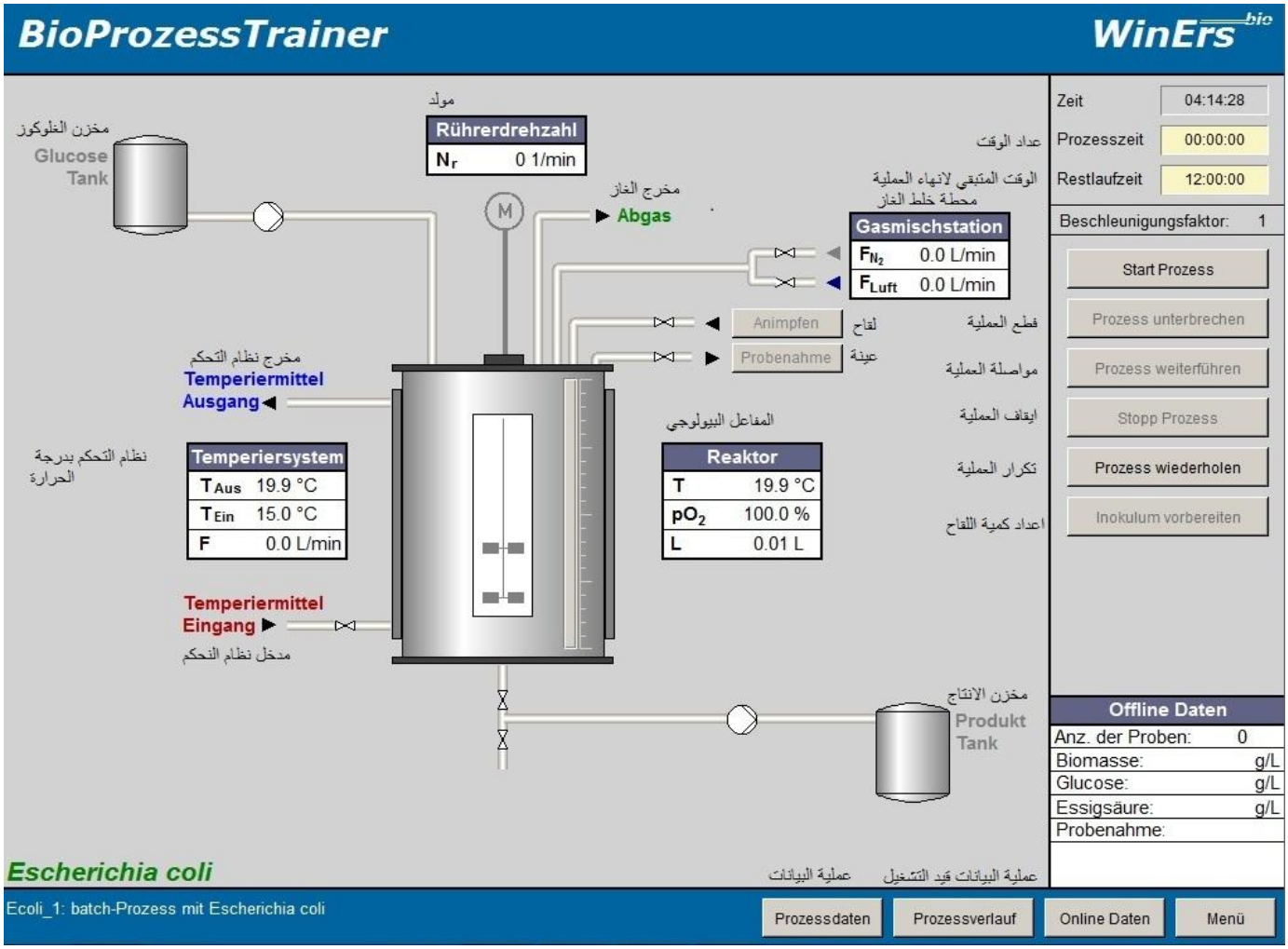
8.1.1.3 Milestones

		Milestone	Funding need
2017	كانون الثاني	Control Valve Testrig finished	
	شباط	Mounting automatic valves for pilot plant	
	آدار		
	نيسان	Lab/Bureau/Production Plant in Flat in Ras Nhache	50k$
	أيار	Process Control System for pilot plant	
	حزيران	Process Control System for pilot plant	
	تموز	Integration Test	
	آب	Packing unit	
	أيلول	Packing unit	
	تشرين الاول	Qualification	50k$
	تشرين الثاني	Qualification	
	كانون الاول		
2018		Start of Production	100$

9 تشغيل البيورياكتور (bioreactor) - عمليات تفصيلية داخل البيورياكتور

9.1 كيفية عمل المفاعل البيولوجي (bioreactor)[6]

الصورة في الاسفل مأخوذة من برنامج حاسوبي من [Hass, Pörtner 2011] و اضيفت اليها الترجمة العربية



للمصطلحات. بهاذا البرنامج يمكننا ان نرى ديناميك النظام على مدار الوقت اذا تم تشغيله والتدخل فيه. بالتالي سنقوم بمحاكاة (simulation) تجربة (experiment) مع إ. كولي.

بالتالي نقوم بتشغيل مثالي للبيورياكتور

ويمكننا ان نفعل ذلك حقيقياً او بحاكات (simulation) بإستخدام برنامج BioProcessTrainer

[6] معظم مضمون هذه الفقرة من [Hass, Pörtner 2011]

أولاً : نقوم بادخال 10 ليتر من المستنبت (medium) الكلوكوز الى المفاعل .

ثانياً: نشغل المحرك الكهربائي (Ruehrer) بسرعته الأولية 50 دورة / دقيقة .

(ثالثاً : نفتح محطة دمج الغاز (Gasmischstation) ونثبت سرعة دخول الغاز الى المفاعل البيولوجي على 10 ليتر/دقيقة.)

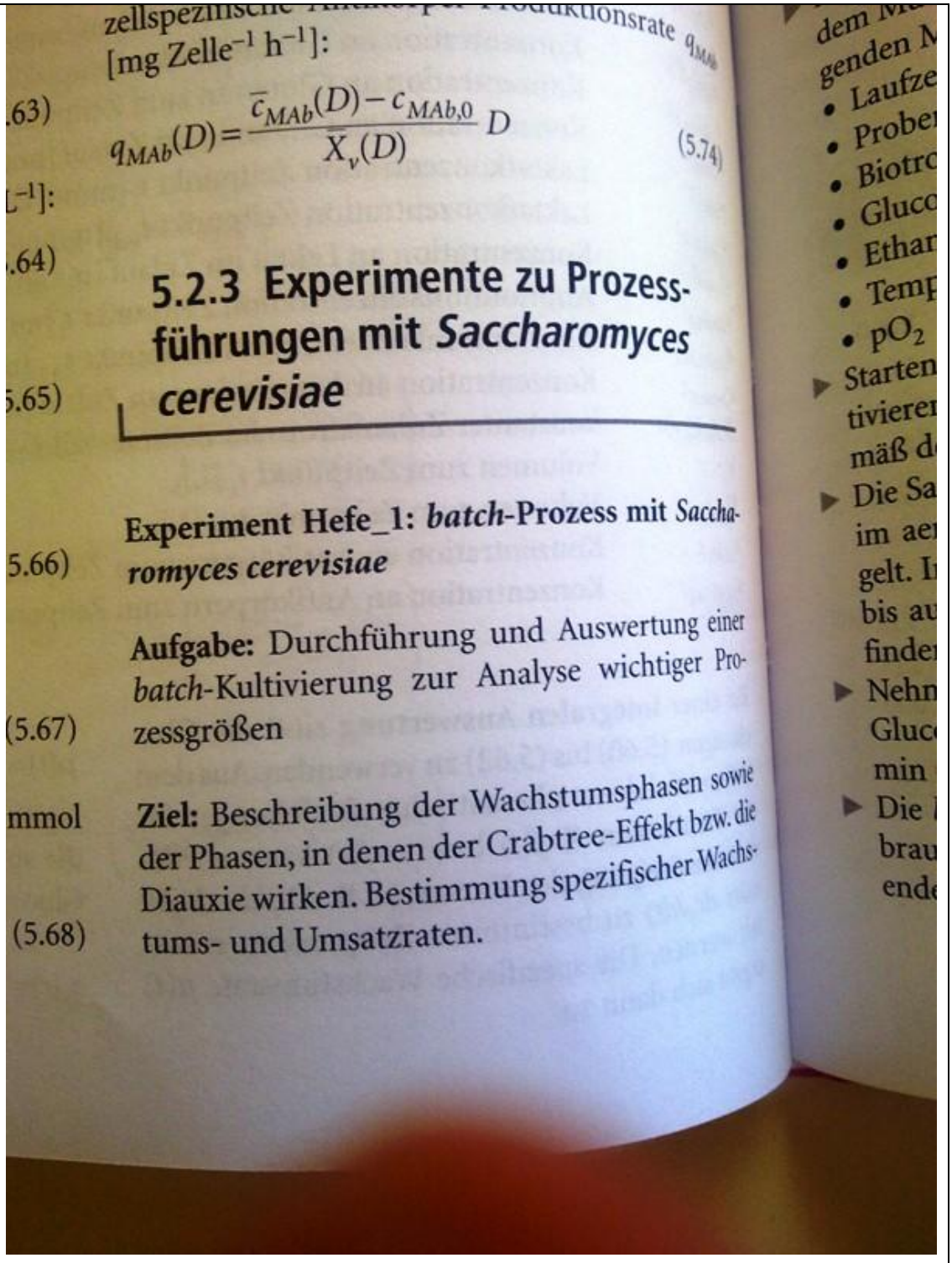
رابعاً : نشغل نظام التحكم بدرجة الحرارة (Temperiersystem) وهو عبارة عن أنبوب يلتف حول المفاعل و يدخل فيه السائل من الطرف الأسفل و يخرج من الطرف الأعلى , و يكون السائل بارداً أو ساخناً بحسب درجة حرارة المفاعل . الهدف من هذا النظام ابقاء درجة الحرارة في المفاعل ثابتة .

خامساً : نحدد كمية البكتيريا (اذا استخدمنا البنامج BioProcessTrainer: بالضغط على Inokulum)) (vorbereitenثم ندخلها الى المفاعل البيولوجي (اذا استخدمنا البنامج BioProcessTrainer: بالضغط على (animpfen)) على أن تدخل الكمية خلال 20 ثانية .

سادساً: نأخذ عينة من المفاعل (اذا استخدمنا البنامج BioProcessTrainer: من خلال الضغط على(Probenahme). و في البرنامج على الفور تظهر كمية كل مكون من مكونات العينة في جدول المعلومات تسمى (offline daten) و هي على الشكل التالي biomasse,glucose , essigsauere)).

- الغاز الذي يدخل الى المفاعل يخرج منه عبر المخرج المسمى الAbgas.
- (اذا استخدمنا البنامج BioProcessTrainer: عندما نضغط على (online daten) خلال العملية يظهر جدول يبرز المتغيرات الحالية لكل من : معدل الحرارة المستنبت (T), معدل الحجم المستنبت (V), pH , pO2, كمية الأوكسيجين و ثاني أوكسيد الكربون (O2,CO2)).
- (اذا استخدمنا البنامج BioProcessTrainer: عندما نضغط على (Prozessverlauf) نحصل على رسم بياني لكل من : درجة الحرارة , سرعة المحرك , الحجم الاجمالي للمفاعل , ال pH و pO2, في اللحظة التي نضغط بها , هذه المعطيات نحصل عليها من خلال مجسات موصولة الى المفاعل.)
- (اذا استخدمنا البنامج BioProcessTrainer: عندما نضغط على (Prozessdaten) نحصل على رسم بياني لنفس المعطيات السابقة و لكن لفترة من الوقت و ليس للللحظة التي نضغط بها.)

9.2/اختبار مع خميرة (S.cerevisiae) مع حل ملي ببرنامج BioProcessTrainer



zellspezifische [illegible] Produktionsrate q_{MAb}

[mg Zelle^{-1} h^{-1}]:

$$q_{MAb}(D) = \frac{\bar{c}_{MAb}(D) - c_{MAb,0}}{\bar{X}_v(D)} D \qquad (5.74)$$

5.2.3 Experimente zu Prozessführungen mit *Saccharomyces cerevisiae*

Experiment Hefe_1: *batch*-Prozess mit *Saccharomyces cerevisiae*

Aufgabe: Durchführung und Auswertung einer *batch*-Kultivierung zur Analyse wichtiger Prozessgrößen

Ziel: Beschreibung der Wachstumsphasen sowie der Phasen, in denen der Crabtree-Effekt bzw. die Diauxie wirken. Bestimmung spezifischer Wachstums- und Umsatzraten.

Grundlagen: Kap. 3.4 und Kap. 4.1
Auswertungsgleichungen: Kap. 5.2.2.2

Einstellungen am BioProzessTrainer:

- Wählen Sie aus dem Hauptmenü das Experiment **Hefe_1**. Hierdurch wird der **BioProzessTrainer** initialisiert. Nach der Initialisierung befinden sich $V_R = 10$ L einer auf 35 °C temperierten und mit Sauerstoff gesättigten ($pO_2 = 100\,\%$) Mediumslösung im Reaktor. Die Anfangskonzentrationen für Glucose und Ethanol betragen:
  - Glucose: 10 g L^{-1}
  - Ethanol: 0 g L^{-1}

Die Konzentration an Biomasse X_R nach dem Animpfen soll bei 4 g L^{-1} liegen.

- Berechnen Sie die erforderliche Biomassekonzentration X_I im Inokulum (Volumen Inokulum $V_I = 200$ mL)

$$X_I = X_R \frac{V_R + V_I}{V_I} \tag{5.75}$$

Vorgehensweise:

- Bereiten Sie ein Datenblatt gemäß beiliegendem Muster (siehe Beispiellösung) für die folgenden Messgrößen vor:
  - Laufzeit t
  - Probenvolumen (hier 10 mL)
  - Biotrockenmassekonzentration
  - Glucosekonzentration
  - Ethanolkonzentration
  - Temperatur
  - pO_2
- Starten Sie das Experiment **Hefe_1** durch Aktivieren des Start-Buttons (aerob/anaerob) gemäß den Hinweisen auf der DVD.
- Die Sauerstoffkonzentration im Medium wird im aeroben Fall bei 60 % Luftsättigung geregelt. Im anaeroben Fall wird die Luftsättigung bis auf 0 % absinken. Hinweise zur Regelung finden sich in Kap. 6.
- Nehmen Sie Proben (zu Biotrockenmasse, Glucose und Ethanol) im Abstand von ca. 30 min (Prozesszeit).
- Die *batch*-Kultur ist mit dem kompletten Verbrauch an Substraten (Glucose, Ethanol) beendet.
- Tragen Sie die Daten für die Messgrößen in die vorbereitete Tabelle gemäß Musterlösung ein.
- Wiederholen Sie das Experiment unter anaeroben Bedingungen. Zur Wiederholung des Teil-Experiments drücken Sie den Wiederholungs-Button entsprechend den Hinweisen auf der DVD.
- Zum Beenden des Experiments **Hefe_1** drücken Sie den Ende-Button entsprechend den Hinweisen auf der DVD.

Auswertung:

- Stellen Sie aus den Rohdaten die Verläufe von Biotrockenmasse, Glucose- und Ethanolkonzentration als Funktion der Zeit dar.
- Unterteilen Sie den Verlauf in die exponentielle Phase (aerobes Wachstum mit Crabtree-Effekt bei Glucoseüberschuss und Ethanolbildung, Diauxie bei niedrigen Glucosekonzentrationen und Ethanolverbrauch).
- Berechnen Sie in den jeweiligen Zeitintervallen zwischen zwei Probenahmen die im Folgenden aufgeführten Größen und stellen Sie diese ebenfalls als Funktion der Zeit dar.
  - spezifische Wachstumsrate μ
  - Verdopplungszeit t_D
  - spezifische Substrataufnahmerate für Glucose q_{Glc}
  - spezifische Substrataufnahmerate für Glucose q_{Glc} und Ethanol q_{Eth} (bei Glucoselimitierung)
  - spezifische Ethanolbildungsrate $q_{p,Eth}$ bei Glucoseüberschuss
  - Ausbeutekoeffizient Biotrockenmasse/Glucose $Y_{X_{TC}/Glc}$ unter den verschiedenen Prozesszuständen (vgl. Kap. 3.4)
  - Ausbeutekoeffizient Biotrockenmasse/Ethanol $Y_{X_{TC}/Eth}$ unter den verschiedenen Prozesszuständen (vgl. Kap. 3.4)

  (Vorbereitung für Experimente in Kap. 5.3)
- Vergleichen Sie dabei die differentielle und die integrale Methode zur Bestimmung der genannten Kenngrößen.

Beispiellösung zu HEFE_1

Experiment Nr	HEFE_1	aerobe Prozessführung
Name	HEFE_1	
Organismus	*Saccharomyces cerevisiae*	

Startvolumen	10,0 L
Anfangskonzentration Glucose	10,0 g/L
Anfangskonzentration Ethanol	0,0 g/L
Animpfkonzentration	4,0 g/L
Temperatur	35,0 °C
Sauerstoffgehalt	60,0 %

Probe #	Laufzeit t [min]	Laufzeit t [h]	Biotrockenmasse X [g/L]	Glucose G [g/L]	Ethanol E [g/L]	Temperatur T [°C]	pH pH [–]	pO_2 pO_2 [%]
1	5,00	0,08	4,1	9,4	0,2	34,9	6,9	60,0
2	30,00	0,50	4,7	7,2	0,8	35,0	6,7	59,9
3	60,00	1,00	5,4	4,1	1,7	35,0	6,4	60,0
4	90,00	1,50	6,3	0,9	2,4	35,0	6,1	62,1
5	120,00	2,00	6,9	0,0	2,2	35,0	6,0	60,0
6	150,00	2,50	7,0	0,0	1,7	35,0	6,0	60,0
7	180,00	3,00	7,1	0,0	1,3	35,0	6,0	60,0
8	210,00	3,50	7,2	0,0	0,9	35,0	6,0	60,1
9	240,00	4,00	7,3	0,0	0,6	35,0	6,0	60,1
10	270,00	4,50	7,4	0,0	0,3	35,0	6,0	60,2
11	300,00	5,00	7,4	0,0	0,1	35,0	6,0	59,9
12	330,00	5,50	7,4	0,0	0,1	35,0	6,0	60,0
13	360,00	6,00	7,4	0,0	0,0	32,0	6,0	72,0

HEFE_1: Auswertung der Kenndaten der aeroben Kultivierung nach der differentiellen Methode

Laufzeit t [h]	Intervall-mitte t [h]	Vol. vor Probe V_i [L]	Vol. nach Probe V_{i+1} [L]	μ [1/h]	t_d [h]	q_{Glc} [1/h]	q_{Eth} [1/h]	$Y_{XTG/Glc}$ [–]	$Y_{XTG/Eth}$ [–]
0,08		10	9,99						
0,50	0,29	9,99	9,98	0,327	2,118	1,200	0,327	0,273	–1,000
1,00	0,75	9,98	9,97	0,277	2,500	1,228	0,356	0,226	–0,778
1,50	1,25	9,97	9,96	0,308	2,253	1,094	0,239	0,281	–1,286
2,00	1,75	9,96	9,95	0,182	3,812	0,273	–0,061	0,667	3,000
2,50	2,25	9,95	9,94	0,029	24,087	0,000	–0,144	–	0,200
3,00	2,75	9,94	9,93	0,028	24,433	0,000	–0,113	–	0,250
3,50	3,25	9,93	9,92	0,028	24,780	0,000	–0,112	–	0,250
4,00	3,75	9,92	9,91	0,028	25,127	0,000	–0,083	–	0,333
4,50	4,25	9,91	9,90	0,027	25,473	0,000	–0,082	–	0,333
5,00	4,75	9,9	9,89	0,000	–	0,000	–0,054	–	0,000
5,50	5,25	9,89	9,88	0,000	–	0,000	0,000	–	–
6,00	5,75	9,88	9,87	0,000	–	0,000	–0,027	–	0,000

HEFE_1: Auswertung der Kenndaten der aeroben Kultivierung nach der integralen Methode

Laufzeit t [h]	Intervall-mitte t [h]	Vol. vor Probe [L]	Vol. nach Probe [L]	μ [1/h]	t_d [h]	q_{Glc} [1/h]	q_{Eth} [1/h]	$Y_{XTG/Glc}$ [–]	$Y_{XTG/Eth}$ [–]
0,08	–	10	9,99	0,521	1,331	2,312	0,799	0,225	–0,799
0,50	0,29	9,99	9,98	0,367	1,889	1,459	0,426	0,251	–0,426
1,00	0,75	9,98	9,97	0,250	2,767	0,855	0,172	0,293	–0,172
1,50	1,25	9,97	9,96	0,172	4,024	0,487	0,027	0,354	–0,027
2,00	1,75	9,96	9,95	0,116	5,979	0,253	–0,056	0,459	0,056
2,50	2,25	9,95	9,94	0,074	9,384	0,103	–0,099	0,715	0,099
3,00	2,75	9,94	9,93	0,042	16,436	0,012	–0,116	3,417	0,116
3,50	3,25	9,93	9,92	0,019	36,439	–0,036	–0,113	–0,532	0,113
4,00	3,75	9,92	9,91	0,004	185,548	–0,051	–0,096	–0,074	0,096
4,50	4,25	9,91	9,9	–0,004	–191,610	–0,040	–0,070	0,091	0,070
5,00	4,75	9,9	9,89	–0,003	–274,205	–0,009	–0,038	0,296	0,038
5,50	5,25	9,89	9,88	0,008	90,707	0,036	–0,006	0,209	0,006
6,00	5,75	9,88	9,87	0,027	25,421	0,088	0,022	0,309	–0,022

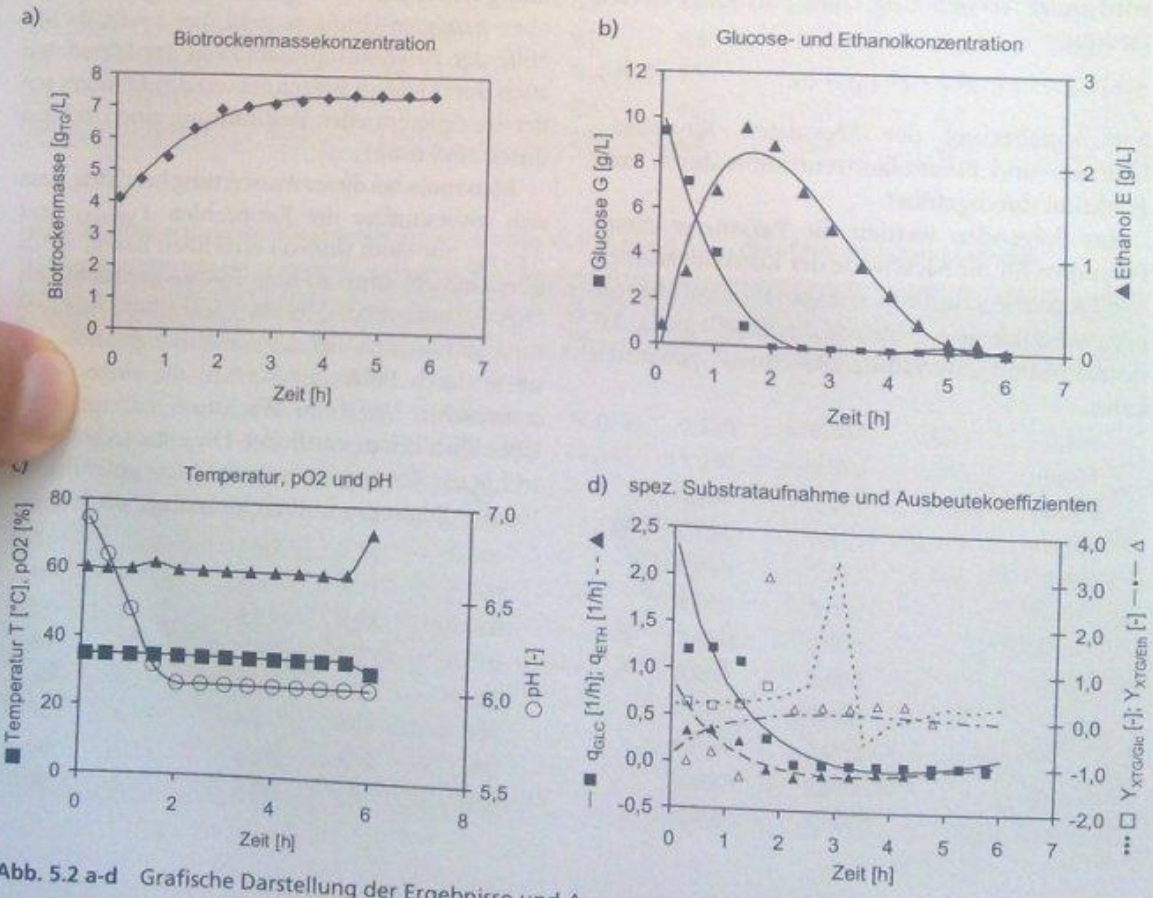


Abb. 5.2 a-d Grafische Darstellung der Ergebnisse und Auswertungen für die aerobe Kultivierung HEFE_1

In den grafischen Darstellungen der Ergebnisse und Auswertungen wurden die Kenngrößen sowohl mit der differentiellen Methode, als auch mit der integralen Methode berechnet. Differentiell erhaltene Größen sind in den Grafiken durch diskrete Symbole gekennzeichnet. Integral berechnete Größen sind in den Grafiken durch Linien verbunden (Abb. 5.2 d).

Anhand der Grafiken kann man erkennen, dass eine exponentielle Wachstumsphase für maximal 2,0 Stunden, also ungefähr bis zum Abbau der Glucose anhält. Ferner sieht man, dass der Crabtree-Effekt – also die Erzeugung von Ethanol unter aeroben Bedingungen – über den ganzen Konzentrationsbereich der Glucose zu beobachten ist. Ein Abbau von Ethanol ist erst zu beobachten, wenn die Glucose vollständig abgebaut ist.

Die integrale Berechnung von Kennzahlen wird unter Verwendung eines Polynoms vierter Ordnung

$$z(t) = a \cdot t^4 + b \cdot t^3 + c \cdot t^2 + d \cdot t + e \qquad (5.76)$$

zur Annäherung der Messdaten Biomasse-, Glucose- und Ethanolkonzentration durch eine Funktion durchgeführt.

Im Folgenden werden die Parameter dieses Polynoms für die Messwerte der Konzentrationen von Biomasse sowie Glucose und Ethanol angegeben, wie man sie z. B. durch die Tabellenkalkulationsfunktion „Trendlinie berechnen“ gewinnen kann.

	X	Glc	Eth
a	0,0015	0,0152	–0,0119
b	0,0143	–0,3716	0,2406
c	–0,3990	3,0074	–1,5830
d	2,1490	–9,7415	3,4557
e	3,8253	10,6680	–0,2621
R^2	0,9902	0,9836	0,9668

Die entsprechenden Ableitungen ergeben sich aus einem Polynom dritter Ordnung

$$\frac{dz(t)}{dt} = 4a \cdot t^3 + 3b \cdot t^2 + 2c \cdot t + d \qquad (5.77)$$

In der Abb. 5.2 d erkennt man deutlich den ausgleichenden Charakter der unterlegten Polynome. Die Kenngrößen aus der integralen Bestimmung weisen eine geringere Streuung auf (in der oben gezeigten Grafik täuscht dies etwas, da mit Hilfe der Polynomrepräsentation der Messdaten auch dort Größen berechnet werden können, wo bei der differentiellen Bestimmung eine Division durch Null droht).

Man muss bei dieser Auswertung beachten, dass sich insbesondere die Kennzahlen $Y_{XTG/Glc}$ und $Y_{XTG/Eth}$ nur dann sinnvoll errechnen lassen, wenn überhaupt Wachstum von Biomasse stattfindet. Dies ist insbesondere bei der integralen Auswertung zu beachten. Bei der Annäherung der Messwerte durch Polynome können die eingesetzten statistischen Verfahren Wachstum anzeigen, wo tatsächlich keines stattfindet. Die entsprechend errechneten Kennzahlen müssen daher unter diesen Randbedingungen kritisch hinterfragt werden.

Experiment Nr.	HEFE_1	anaerobe Prozessführung
Name	HEFE_1	
Organismus	*Saccharomyces cerevisiae*	
Startvolumen	10,0	L
Anfangskonzentration Glucose	10,0	g/L
Anfangskonzentration Ethanol	0,0	g/L
Animpfkonzentration	4,0	g/L
Temperatur	35,0	°C
Sauerstoffgehalt	0,0	%

Probe #	Laufzeit t [min]	Laufzeit t [h]	Biotrockenmasse X [g/L]	Glucose G [g/L]	Ethanol E [g/L]	Temperatur T [°C]	pH pH [–]	pO_2 pO_2 [%]
1	0,00	0,00	4,0	9,7	0,1	35,0	7,0	–0,4
2	30,00	0,50	4,1	7,8	1,0	35,0	6,8	–0,4
3	60,00	1,00	4,2	5,6	2,1	35,0	6,6	–0,4
4	90,00	1,50	4,2	3,2	3,3	35,0	6,3	–0,4
5	120,00	2,00	4,3	1,0	4,3	35,0	6,1	–0,5
6	150,00	2,50	4,3	0,0	4,8	35,0	6,0	–0,2
7	180,00	3,00	4,3	0,0	4,8	35,0	6,0	–0,2
8	210,00	3,50	4,3	0,0	4,8	35,0	6,0	–0,2
9	240,00	4,00	4,3	0,0	4,8	35,0	6,0	–0,2
10	270,00	4,50	4,3	0,0	4,8	35,0	6,0	–0,2
11	300,00	5,00	4,3	0,0	4,8	35,0	6,0	–0,2
12	330,00	5,50	4,3	0,0	4,8	35,0	6,0	–0,2
13	360,00	6,00	4,3	0,0	4,8	35,0	6,0	–0,2

HEFE_1: Auswertung der Kenndaten der anaeroben Kultivierung nach der differentiellen Methode

Laufzeit t [h]	Intervall-mitte t [h]	Vol. vor Probe V_i [L]	Vol. nach Probe V_{i+1} [L]	μ [1/h]	t_d [h]	q_{Glc} [1/h]	q_{Eth} [1/h]	$Y_{XTG/Glc}$ [–]	$Y_{XTG/Eth}$ [–]
0,00		10,00	9,99						
0,50	0,25	9,99	9,98	0,049	14,036	0,938	0,444	0,053	–0,111
1,00	0,75	9,98	9,97	0,048	14,383	1,060	0,530	0,045	–0,091
1,50	1,25	9,97	9,96	0,000	–	1,143	0,571	0,000	0,000
2,00	1,75	9,96	9,95	0,047	14,729	1,035	0,471	0,045	–0,100
2,50	2,25	9,95	9,94	0,000	–	0,465	0,233	0,000	0,000
3,00	2,75	9,94	9,93	0,000	–	0,000	0,000	–	–
3,50	3,25	9,93	9,92	0,000	–	0,000	0,000	–	–
4,00	3,75	9,92	9,91	0,000	–	0,000	0,000	–	–
4,50	4,25	9,91	9,9	0,000	–	0,000	0,000	–	–
5,00	4,75	9,9	9,89	0,000	–	0,000	0,000	–	–
5,50	5,25	9,89	9,88	0,000	–	0,000	0,000	–	–
6,00	5,75	9,88	9,87	0,000	–	0,000	0,000	–	–

HEFE_1: Auswertung der Kenndaten der anaeroben Kultivierung nach der integralen Methode

Laufzeit t [h]	Intervall-mitte t [h]	Vol. vor Probe [L]	Vol. nach Probe [L]	μ [1/h]	t_d [h]	q_{Glc} [1/h]	q_{Eth} [1/h]	$Y_{XTG/Glc}$ [–]	$Y_{XTG/Eth}$ [–]
0,00		10	9,99	0,061	11,378	1,224	0,603	0,050	–0,603
0,50	0,25	9,99	9,98	0,044	15,584	1,175	0,574	0,038	–0,574
1,00	0,75	9,98	9,97	0,031	22,081	1,045	0,508	0,030	–0,508
1,50	1,25	9,97	9,96	0,021	32,938	0,860	0,416	0,024	–0,416
2,00	1,75	9,96	9,95	0,013	53,275	0,642	0,310	0,020	–0,310
2,50	2,25	9,95	9,94	0,007	99,306	0,414	0,199	0,017	–0,199
3,00	2,75	9,94	9,93	0,003	255,019	0,197	0,095	0,014	–0,095
3,50	3,25	9,93	9,92	0,000	17060,531	0,014	0,007	0,003	–0,007
4,00	3,75	9,92	9,91	–0,001	–573,953	–0,112	–0,053	0,011	0,053
4,50	4,25	9,91	9,9	–0,001	–593,558	–0,157	–0,073	0,007	0,073
5,00	4,75	9,9	9,89	0,000	29816,419	–0,096	–0,043	0,000	0,043
5,50	5,25	9,89	9,88	0,002	311,562	0,095	0,049	0,023	–0,049
6,00	5,75	9,88	9,87	0,005	131,084	0,439	0,215	0,012	–0,215

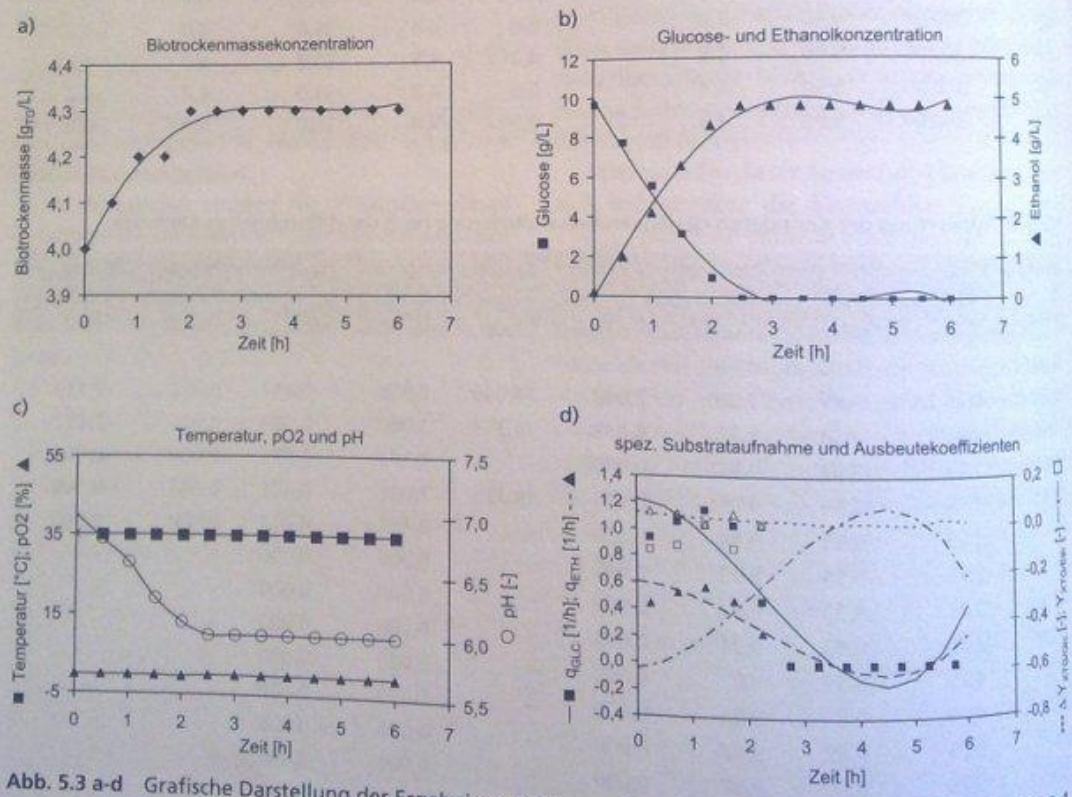
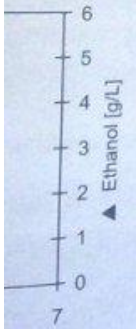
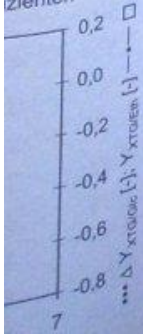


Abb. 5.3 a-d Grafische Darstellung der Ergebnisse und Auswertungen für die anaerobe Kultivierung HEFE_1

Modellbildung

$Y_{XTG/Eth}$ [–]
−0,603
−0,574
−0,508
−0,416
−0,310
−0,199
−0,095
−0,007
0,053
0,073
0,043
−0,049
−0,215





5.2 Experimente zum Wachstumsverhalten der Beispielorganismen

Die Berechnung der Kenngrößen der anaeroben Hefekultivierung erfolgt mit den gleichen Methoden wie die Berechnung der Kenngrößen der aeroben Kultivierung. Damit die Auswertungen der aeroben (Abb. 5.2) und der anaeroben Kultivierung (Abb. 5.3) einfacher zu vergleichen sind, ist die Zeitachse beibehalten worden. Es ist aber zu beachten, dass die Auswertung der Kenngrößen nur bis zum vollständigen Verbrauch der Glucose sinnvoll ist. Weil im anaeroben Betrieb dann kein Wachstum mehr erfolgt, ergeben Berechnungen von Kennzahlen, die die Wachstumsrate beinhalten keinen Sinn.

Im Vergleich der beiden Kultivierungen fällt auf, dass die Wachstumsphase bei der anaeroben Kultivierung kürzer ist als bei der aeroben Kultivierung. Die Hefezellen haben keine Möglichkeit, Ethanol zur Energiegewinnung umzusetzen. Dies zeigt sich deutlich im Ausbeutekoeffizienten Biotrockenmasse/Glucose. Dieser ist bei der anaeroben Prozessführung deutlich geringer.

Die Erwartung für den Ausbeutekoeffizienten Biotrockenmasse/Ethanol ist für die anaerobe Prozessführung Null, da kein Abbau von Ethanol zur Energiegewinnung stattfinden kann.

Experiment Hefe_2: *batch*-Prozess mit *Saccharomyces cerevisiae* bei verschiedenen anfänglichen Biomas… …bstratkonzentrationen sowie bei ver… …mperaturen

Aufgabe: Du… …Ausw… …ng von *batch*-Kultivier… …gs-konzentrationen … verschiedenen Tem…

Ziel: Ermittlung opti… zur Erzielung einer h… tion

Grundl… …4 und… Auswer… …gen:

Führen … …rim… riment … …wobei …

Überlegen Sie sich hierzu eine … Sie mit möglichst wenigen Experim… wertbaren Aussagen kommen kön… torieller Versuchsplan [Soravia and…

Auswertung:
Bereiten Sie die Daten wie in Exper… auf und werten Sie diese entsprech…

- Welche Startzellkonzentration w… optimal ansehen?
- Welche optimalen Anfangsko… für Glucose würden Sie nach Au… Ergebnisse vorschlagen (Begrün…
- Vergleichen Sie diese Werte mit … ten.

Führen Sie ein Experiment mit den … wählten Bedingungen durch und üb… ob Ihre Prognosen zutreffen.

Experiment Hefe_3: *batch*-Prozess… *romyces cerevisiae;* Bestimmung d… und des respiratorischen Quotient… rend einer Kultivierung.

Aufgabe: Durchführung einer einf… Kultivierung mit geregeltem pO_2-… mung des k_La-Wertes in Abhängig… Rührerdrehzahl mit den *onlin*… Sauerstoffkonzentration im Abgas … löstsauerstoffkonzentration c_{O_2}.

Bestimmung des Verlaufs des res… Quotienten (*RQ*) während einer *b*… rung mit Hilfe der Abgasanalytik.

Ziel: Verfolgen des k_La-Wertes aus… …ößen während einer Kultivierun… …Zusammenhangs zwischen k_La-… …ße Rührerdrehzahl.

…en des *RQ* während einer… *Saccharomyces cerevisiae*

…p. 2.3

…hungen: Kap. 2.3

9.3 /اختبار دفع العملية مع ال E.coli

المهمة : اجراء و تقييم تكبير البكتيريا اذا لقح المستنبت مرة واحدة في البداية بE.coli (batch cultivation).

الهدف : وصف لمرحلة النمو، وتحديد سرعة النمو.

الاعدادات على برنامج التدريب BioProcessTrainer:

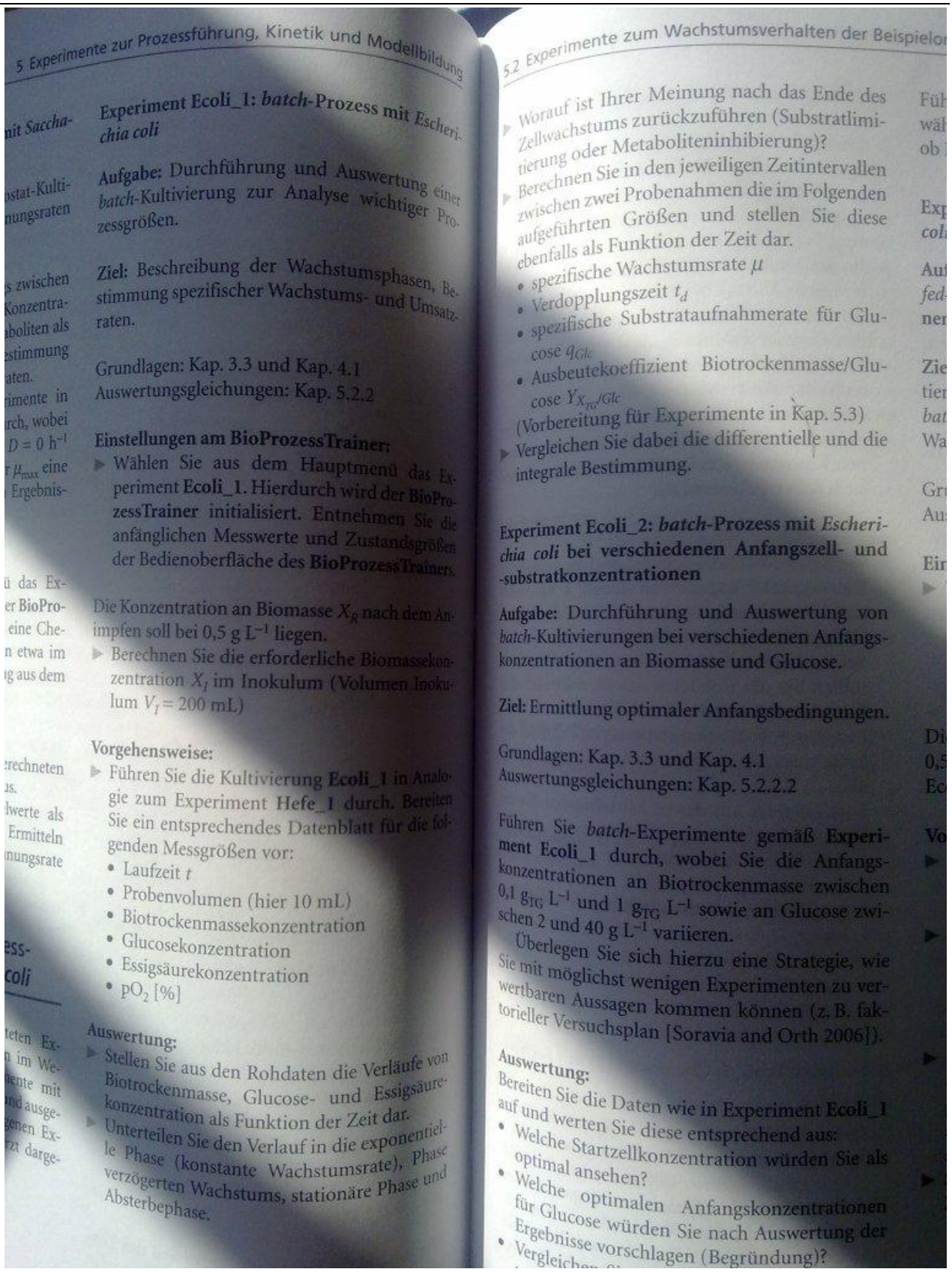
–> نختار من القائمة الرئيسية الاختبار (Ecoli_1) . و هكذا يتم استهلال (initialization) الBioProzessTrainer. خذ القيم (values) الابتدائية من شاشة التحكم لBioProzessTrainer

كثافة البيوماس (biomass) بعد التلقيح يجب ان تكون تقريباً 0.5g في الليتر.

–> احسب كثافة البيوماس (biomass) اللازمة في حجم اللقاح اذا هو يساوي 200 ml .

طريقة العمل:

عُد ورقة معطيات (data sheet) بعد قياس المتغيرات ...(انظر في الاسفل باللغة الالمانية)



5 Experimente zur Prozessführung, Kinetik und Modellbildung

Experiment Ecoli_1: *batch*-Prozess mit *Escherichia coli*

Aufgabe: Durchführung und Auswertung einer *batch*-Kultivierung zur Analyse wichtiger Prozessgrößen.

Ziel: Beschreibung der Wachstumsphasen, Bestimmung spezifischer Wachstums- und Umsatzraten.

Grundlagen: Kap. 3.3 und Kap. 4.1
Auswertungsgleichungen: Kap. 5.2.2

Einstellungen am BioProzessTrainer:

▶ Wählen Sie aus dem Hauptmenü das Experiment **Ecoli_1**. Hierdurch wird der **BioProzessTrainer** initialisiert. Entnehmen Sie die anfänglichen Messwerte und Zustandsgrößen der Bedienoberfläche des **BioProzessTrainers**.

Die Konzentration an Biomasse X_R nach dem Animpfen soll bei 0,5 g L^{-1} liegen.

▶ Berechnen Sie die erforderliche Biomassekonzentration X_I im Inokulum (Volumen Inokulum $V_I = 200$ mL)

Vorgehensweise:

▶ Führen Sie die Kultivierung **Ecoli_1** in Analogie zum Experiment **Hefe_1** durch. Bereiten Sie ein entsprechendes Datenblatt für die folgenden Messgrößen vor:

- Laufzeit t
- Probenvolumen (hier 10 mL)
- Biotrockenmassekonzentration
- Glucosekonzentration
- Essigsäurekonzentration
- pO_2 [%]

Auswertung:

▶ Stellen Sie aus den Rohdaten die Verläufe von Biotrockenmasse, Glucose- und Essigsäurekonzentration als Funktion der Zeit dar.

▶ Unterteilen Sie den Verlauf in die exponentielle Phase (konstante Wachstumsrate), Phase verzögerten Wachstums, stationäre Phase und Absterbephase.

5.2 Experimente zum Wachstumsverhalten der Beispielor

▶ Worauf ist Ihrer Meinung nach das Ende des Zellwachstums zurückzuführen (Substratlimitierung oder Metaboliteninhibierung)?

▶ Berechnen Sie in den jeweiligen Zeitintervallen zwischen zwei Probenahmen die im Folgenden aufgeführten Größen und stellen Sie diese ebenfalls als Funktion der Zeit dar.

- spezifische Wachstumsrate μ
- Verdopplungszeit t_d
- spezifische Substrataufnahmerate für Glucose q_{Glc}
- Ausbeutekoeffizient Biotrockenmasse/Glucose $Y_{X_{TG}/Glc}$

(Vorbereitung für Experimente in Kap. 5.3)

▶ Vergleichen Sie dabei die differentielle und die integrale Bestimmung.

Experiment Ecoli_2: *batch*-Prozess mit *Escherichia coli* bei verschiedenen Anfangszell- und -substratkonzentrationen

Aufgabe: Durchführung und Auswertung von *batch*-Kultivierungen bei verschiedenen Anfangskonzentrationen an Biomasse und Glucose.

Ziel: Ermittlung optimaler Anfangsbedingungen.

Grundlagen: Kap. 3.3 und Kap. 4.1
Auswertungsgleichungen: Kap. 5.2.2.2

Führen Sie *batch*-Experimente gemäß **Experiment Ecoli_1** durch, wobei Sie die Anfangskonzentrationen an Biotrockenmasse zwischen 0,1 g_{TG} L^{-1} und 1 g_{TG} L^{-1} sowie an Glucose zwischen 2 und 40 g L^{-1} variieren.

Überlegen Sie sich hierzu eine Strategie, wie Sie mit möglichst wenigen Experimenten zu verwertbaren Aussagen kommen können (z. B. faktorieller Versuchsplan [Soravia and Orth 2006]).

Auswertung:

Bereiten Sie die Daten wie in Experiment **Ecoli_1** auf und werten Sie diese entsprechend aus:

- Welche Startzellkonzentration würden Sie als optimal ansehen?
- Welche optimalen Anfangskonzentrationen für Glucose würden Sie nach Auswertung der Ergebnisse vorschlagen (Begründung)?
- Vergleichen

9.4 Principles of pO2 Measurement with the Clark Electrode

The Clark Oxygen Electrode

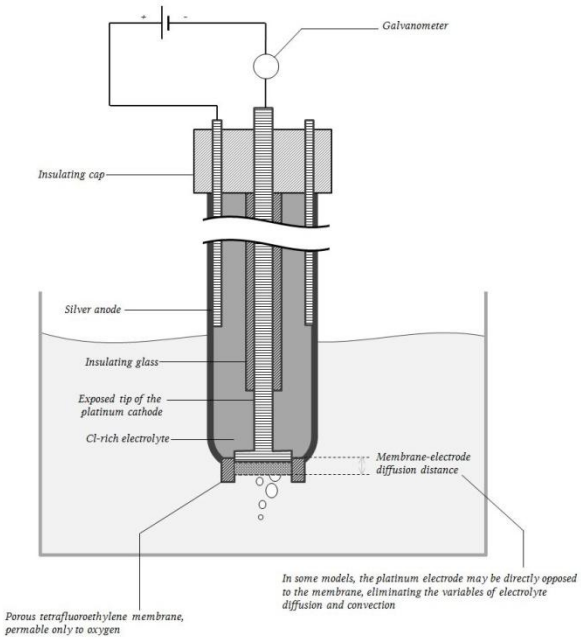
The principles of amperometric oxygen measurement are discussed at some length in the chapter on the platinum oxygen cathode.

In brief:

- A silver anode and platinum cathode are suspended in an electrolyte.
- Oxygen is dissolved in the electrolyte.
- A voltage of known magnitude (about 700 mV) is applied to the electrodes.
- Oxygen is reduced at the cathode and silver is oxidised at the anode.
- The resulting current increases as the voltage increases.
- The current reaches a plateau when the rate of reaction is determined by the diffusion of oxygen rather than the voltage.
- This plateau correlates to the oxygen tension in the electrolyte.

The major difference between this electrode and the earlier oxygen cathode is the addition of an oxygen-permeable membrane. Something resembling the original patent application diagram can be found here.

Its butchered representation can be found below.



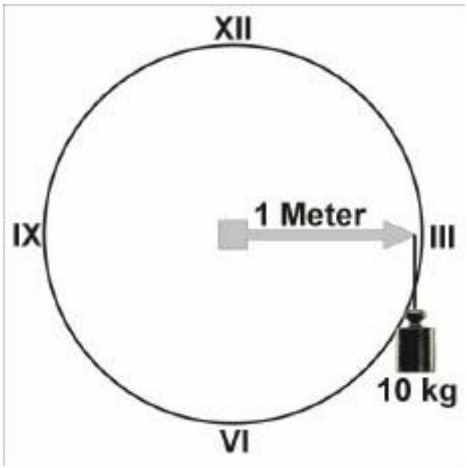
Reference

derangedphysiology.com/main/core-topics-intensive-care/arterial-blood-gas-interpretation/Chapter 2.0.5/principles-po2-measurement-clark-electrode

10 Automation System Basics

10.1 Torque at Stepper Motors and Servos

Wenn man an den Zeiger einer Turmuhr in der Stellung auf 3 Uhr ein Gewicht von 10 kg hängt, wirkt auf die Achse ein Drehmoment von 100 Nm (also 10000 Ncm). Ein Getriebemotor mit 100 Ncm könnte beispielsweise bei einem Hebel von 1 cm (an der Achse) noch 10 kg heben.



10.1.1 Product Example (from www.cnclablb.com)

	Metal Gear Servo TowerPro MG995 Servo - 9kg Price : 8$ Serial number : ACT0005

Description:

Modulation: Digital

Torque: 4.8V: 130.54 oz-in (**9.40 kg-cm**) 6.0V: 152.76 oz-in (11.00 kg-cm)

Speed: 4.8V: 0.20 sec/60° 6.0V: 0.16 sec/60°

Weight: 1.94 oz (55.0 g)

Dimensions:Length:1.60 in (40.7 mm)

Width:0.78 in (19.7 mm)

Height:1.69 in (42.9 mm)

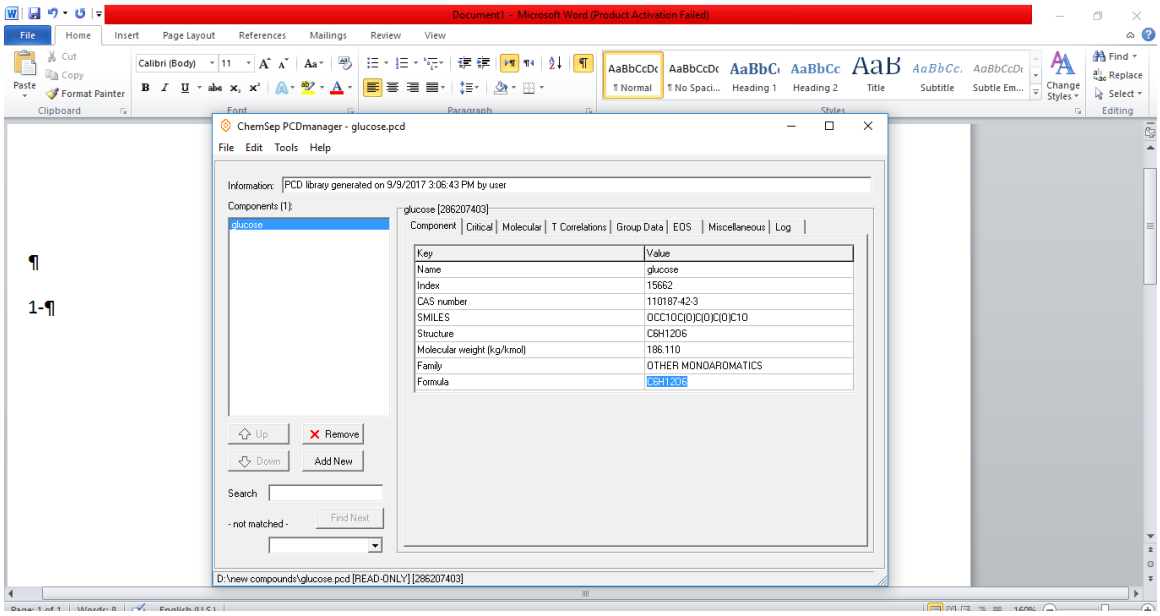
11 Chemical Process Simulation

11.1 Chemical Process Simulation with COCO[7]

11.1.1 How to add new compounds with COCO

❖ Steps:

1- Open PCD manager



2- Press Add New

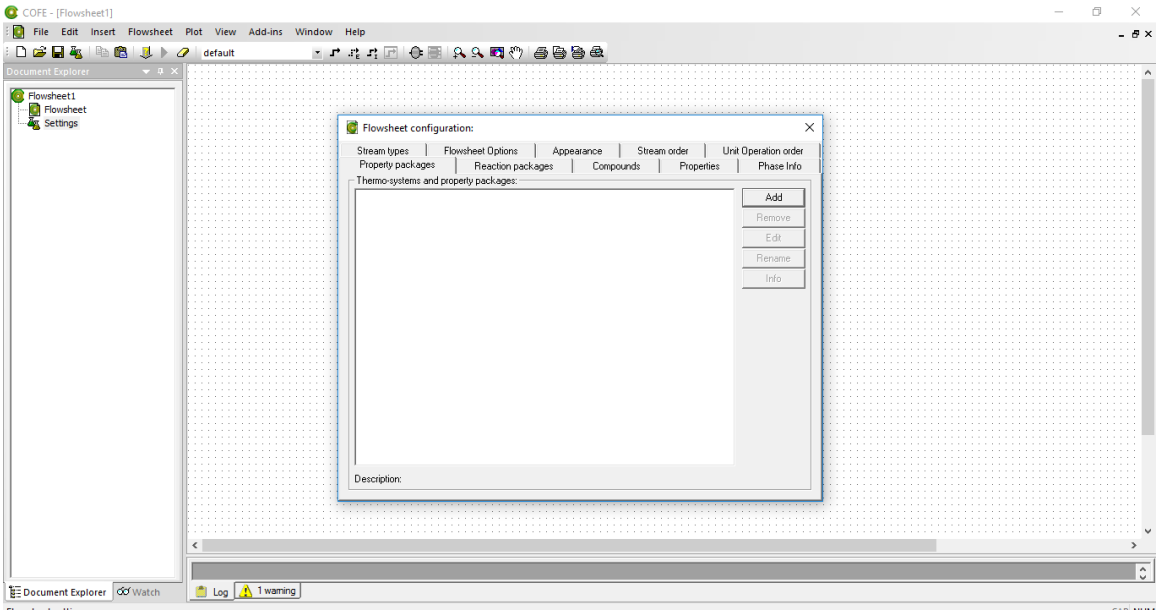
3- Enter compound's information

4- Save as in a file in local disk D

5- Open coco program

6- Press sittings(left) then press new

[7] Razan Kalawoun


COFE - [Flowsheet1]
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Flowsheet Options
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Reaction packages
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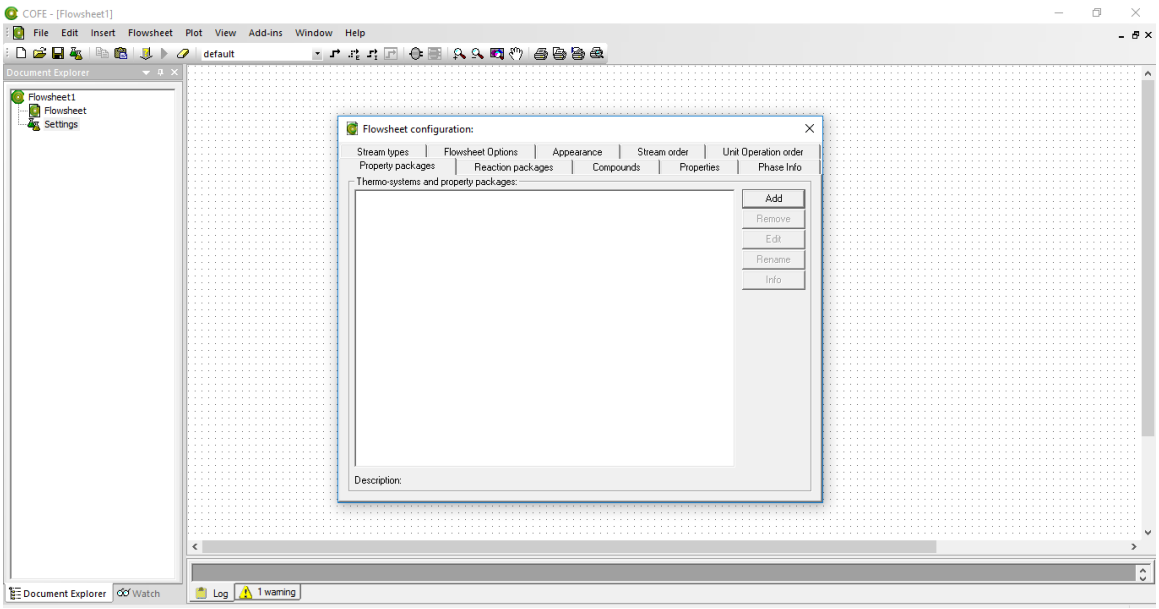
Info
Description:
Document Explorer
Watch
Log
1 warning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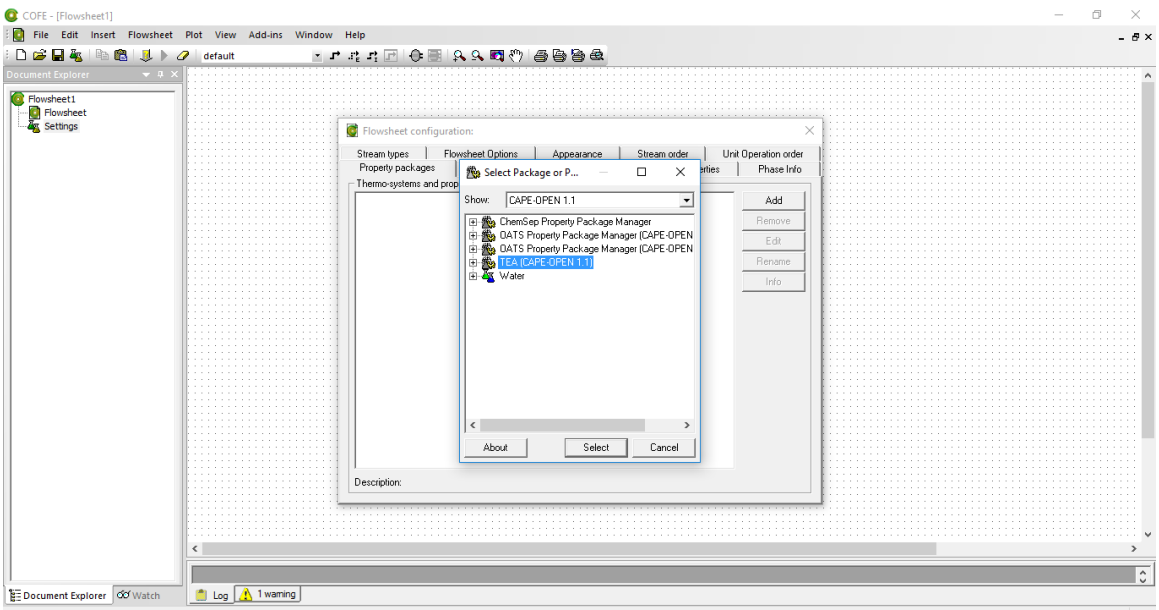
7-Select tea


COFE - [Flowsheet1]
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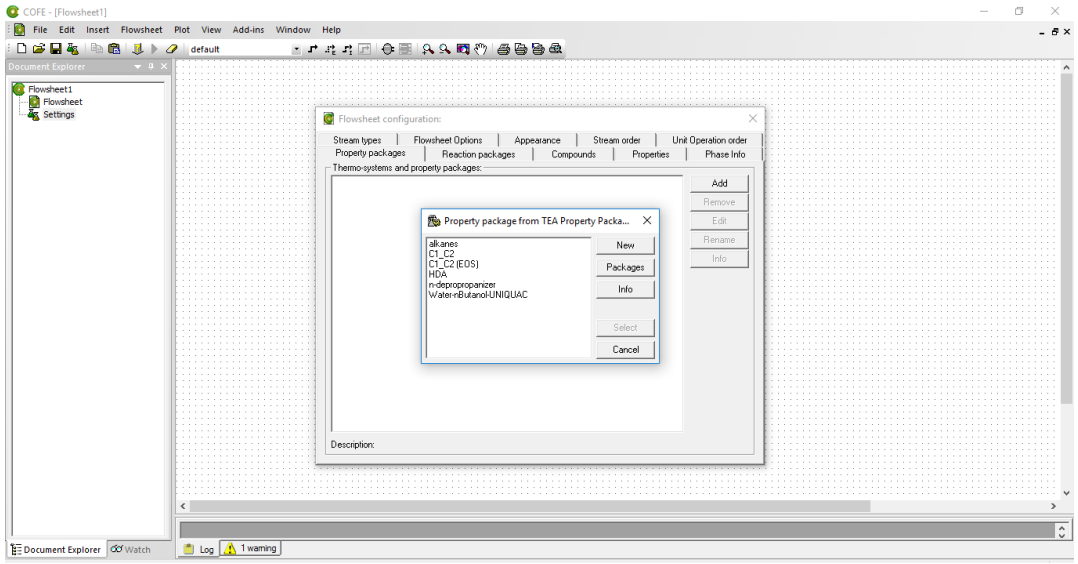
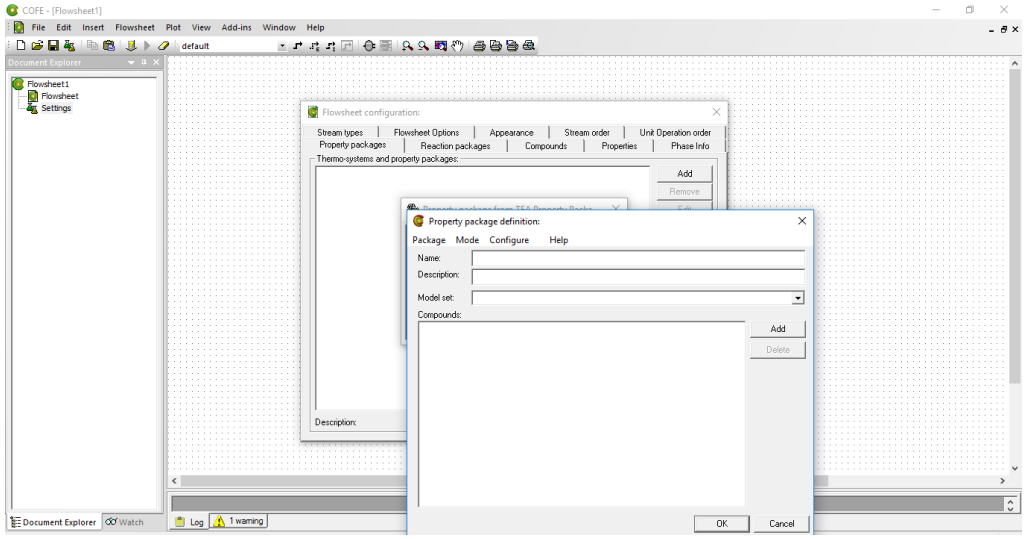
Flowsheet configuration:
Stream types
Flowsheet Options
Appearance
Stream order
Unit Operation order
Property packages
Reaction packages
Compounds
Properties
Phase Info
Thermo-systems and property packages:
Select Package or P...
Show:
CAPE-OPEN 1.1
ChemSep Property Package Manager
OATS Property Package Manager (CAPE-OPEN
OATS Property Package Manager (CAPE-OPEN
TEA (CAPE-OPEN 1.1)
Water
About
Select
Cancel
Add
Remove
Edit
Rename
Info
Description:
Document Explorer
Watch
Log
1 warning

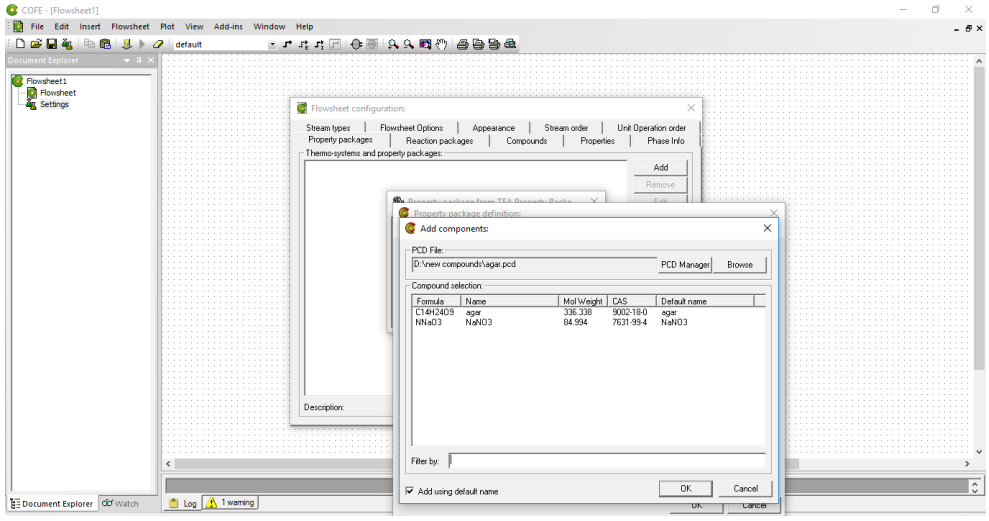
8-Then press new



9-Press add



10-press browse (right)



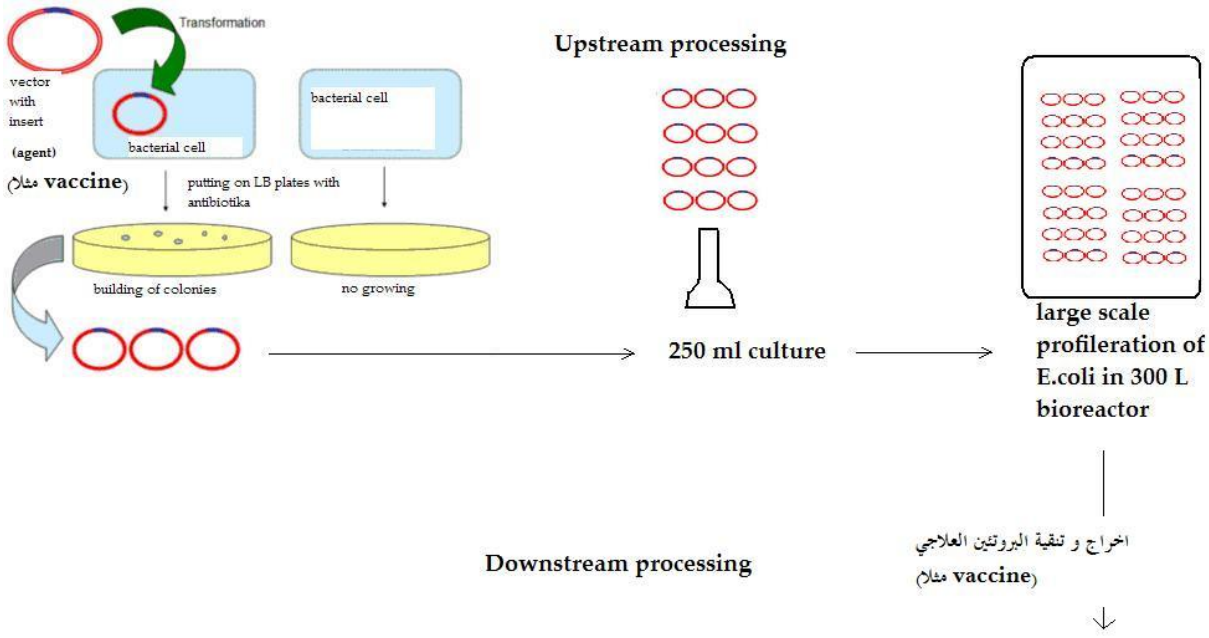
11-choose the compound you add it

12 تصميم و تصنيع 300L بيوريكتور (bioreactor/fermenter)

12.1 مواصفة (Specification)

12.1.1 نظرة عامة

نريد ان نبني 300L بيوريكتور (bioreactor/fermenter) لتصنيع البروتيئينات (proteins) العيلاجية في الجرسومة E.coli.



12.1.2 متطلبات (Requirements)

[Req1] It has to be a 300 L fermenter

[Req2] The tube is 1 m high and with has a radius of 318 mm

[Req3] Two steel sheets 316 1mx2m have to be used

[Req4] Sensors: 1. pH sonde, 2. temperature, 3. oxygen, each is 12 mm x 120 mm 4. filling level (dt. Füllstandsmesser)

[Req5] Motor: ca. 1-8 U/sec., d.h. 60 – 480 U/min.

[Req6] NaOH, HCl inlet, each is controlled with a valve (not a motor). That means it has to be above (or on higher level as) the fermenter tube.

[Req7] One oxygen inlet

[Req8] LB media inlet, recombinant culture inlet

[Req9] For temperature control: heat exchanger

12.2 التصمیم (design)

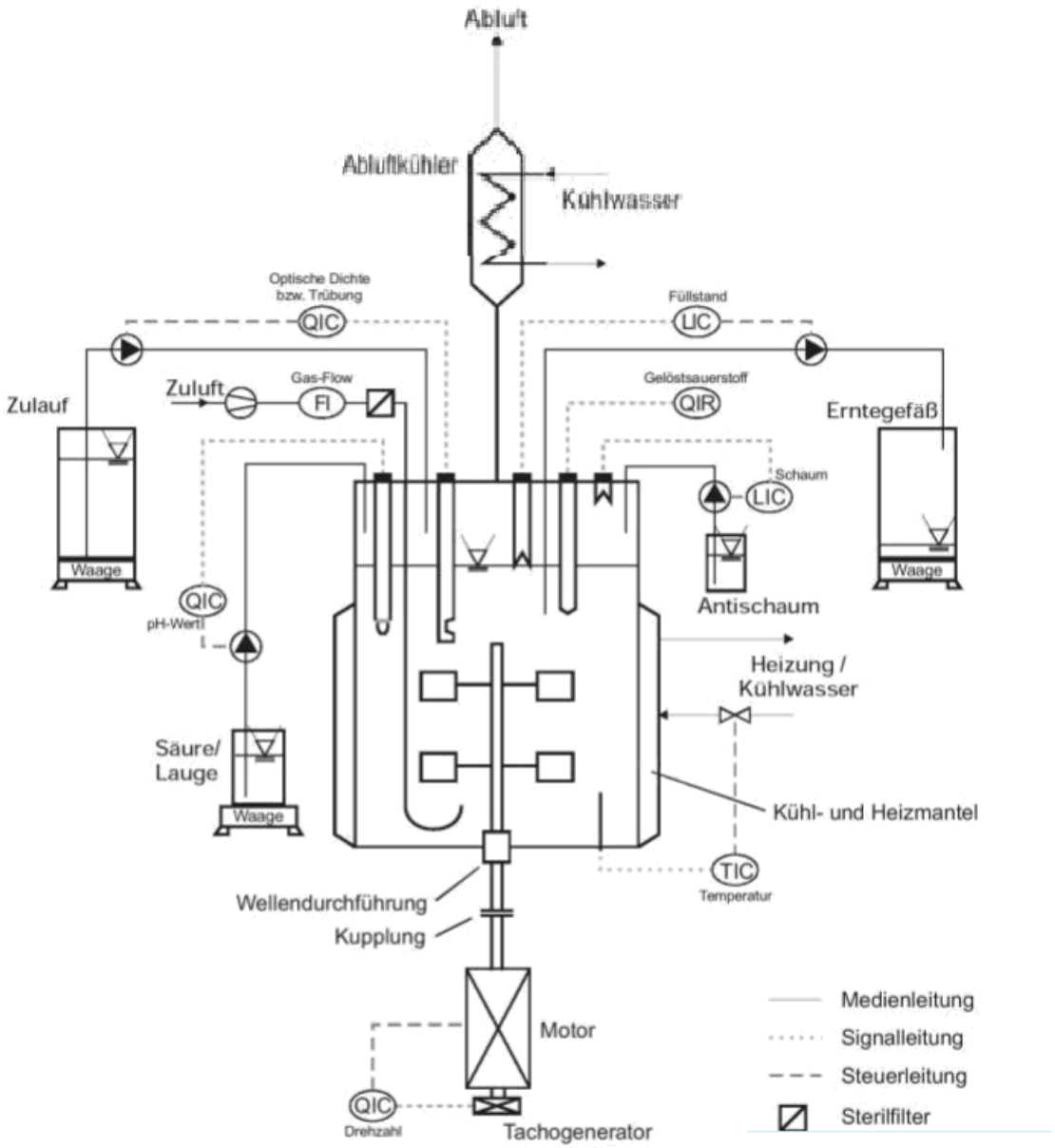
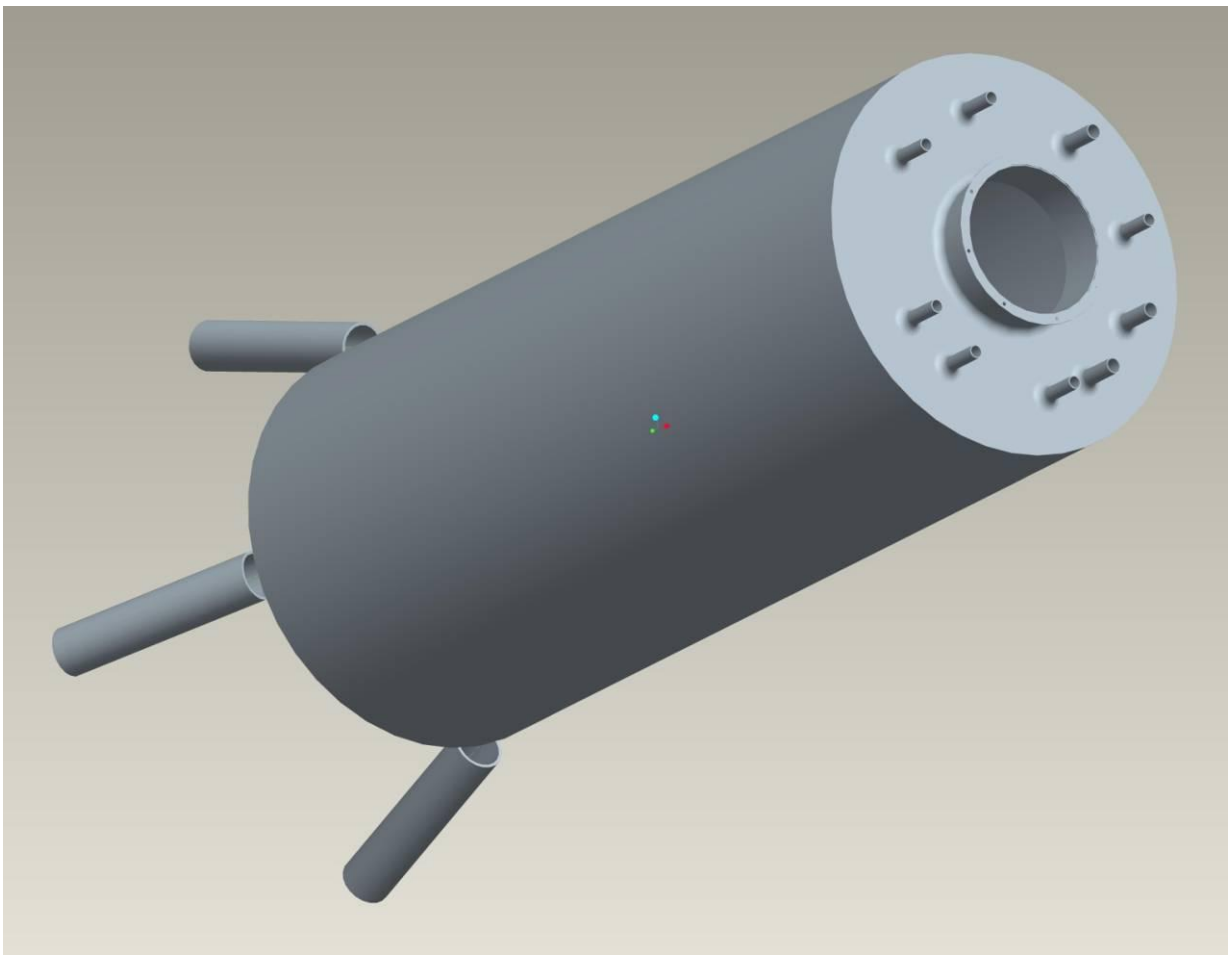


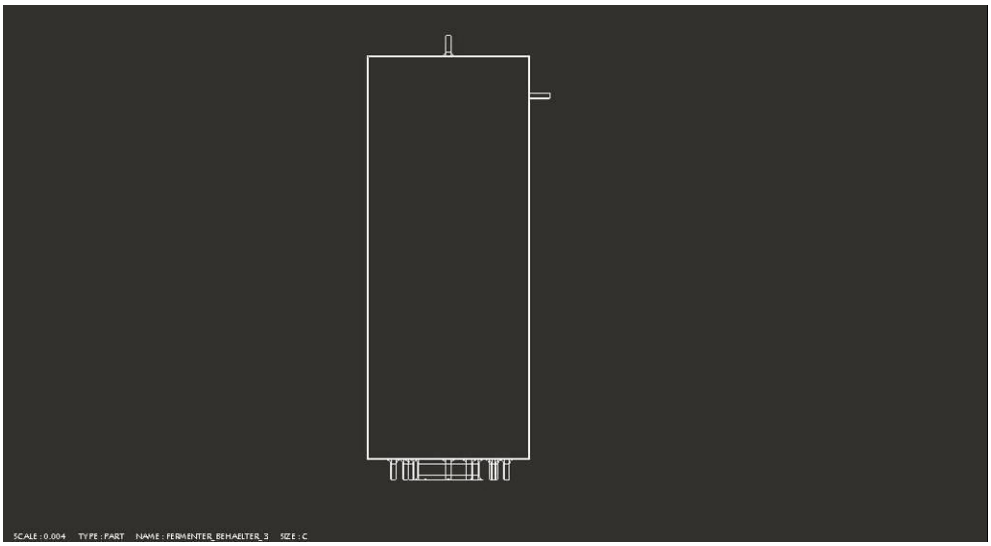
Figure: Instrumentation

12.3 Construction

الخزان(1)

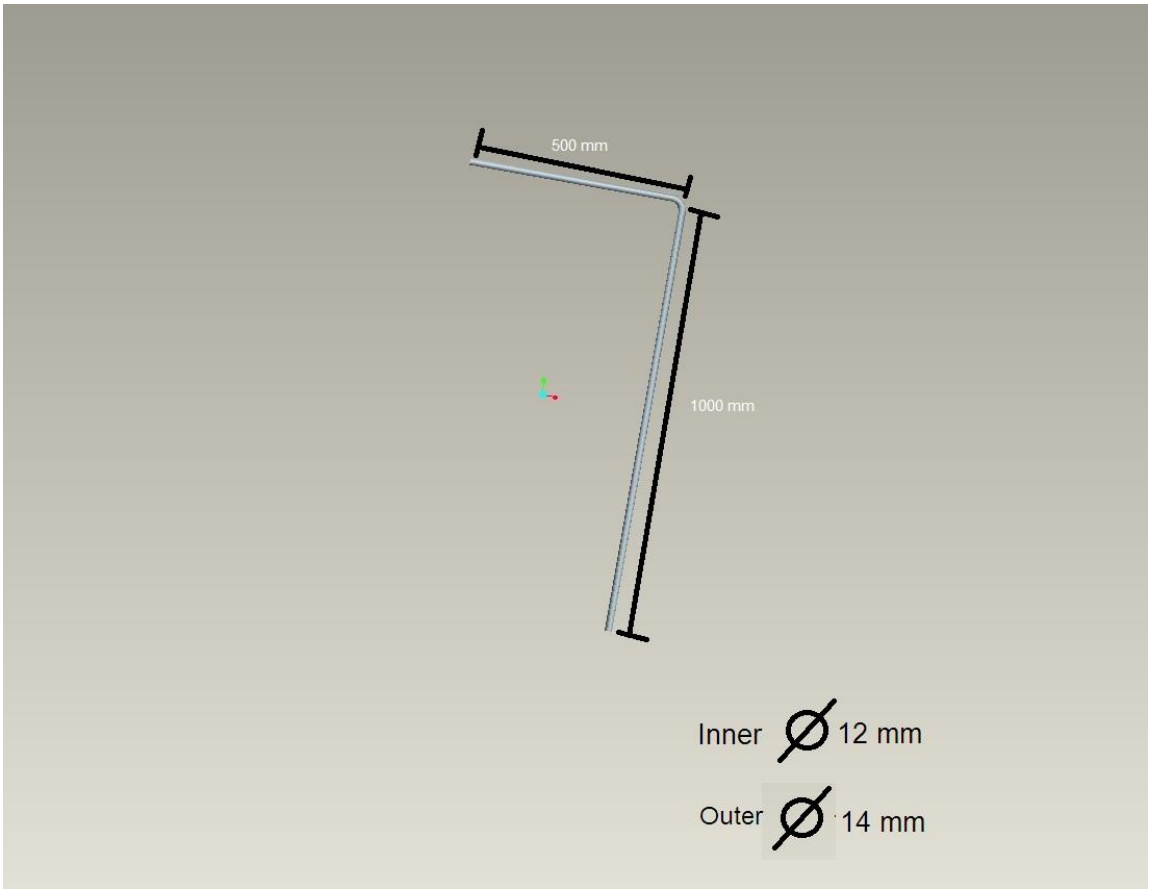


الخزان هو القطعة الأساسية للبيوريكتور



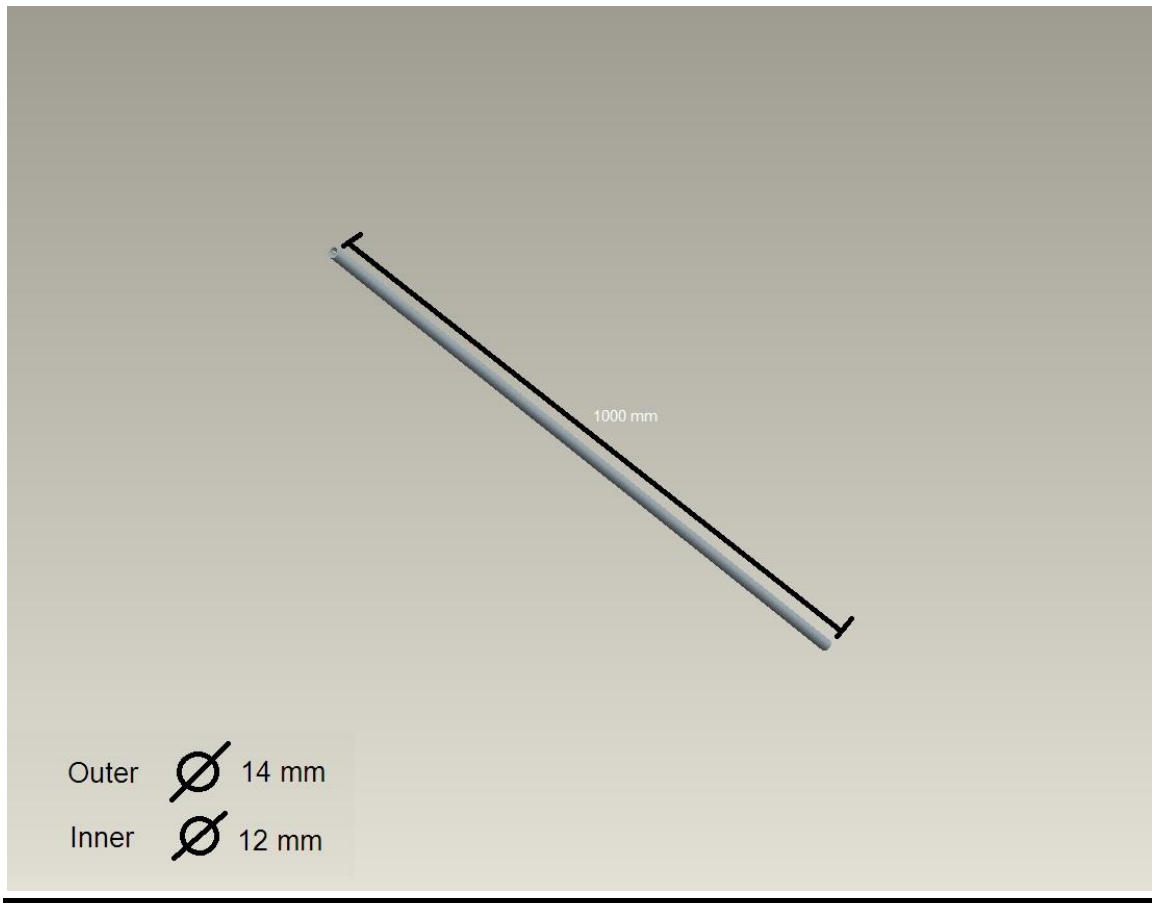
الرسمة التكنيكية للخزان

(.2)مدخل السائل القلوي(base)



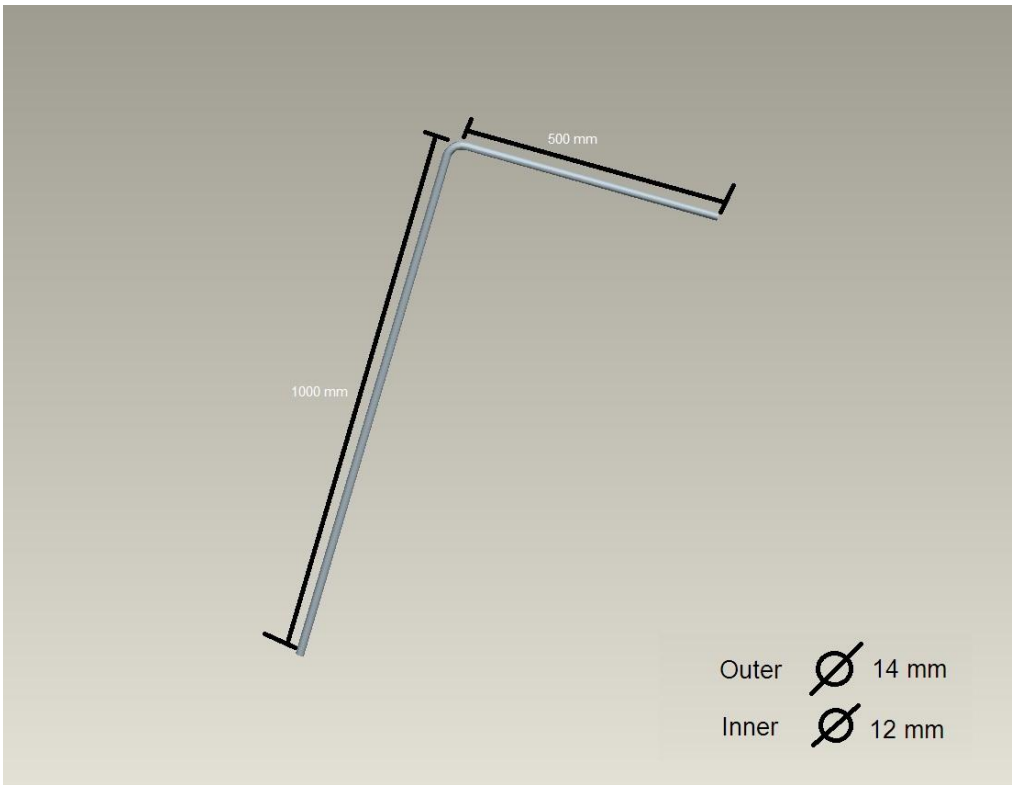
بهذا الأنبوب يتم ادخال سائل قلوي

(3.)مجرخ الغاز



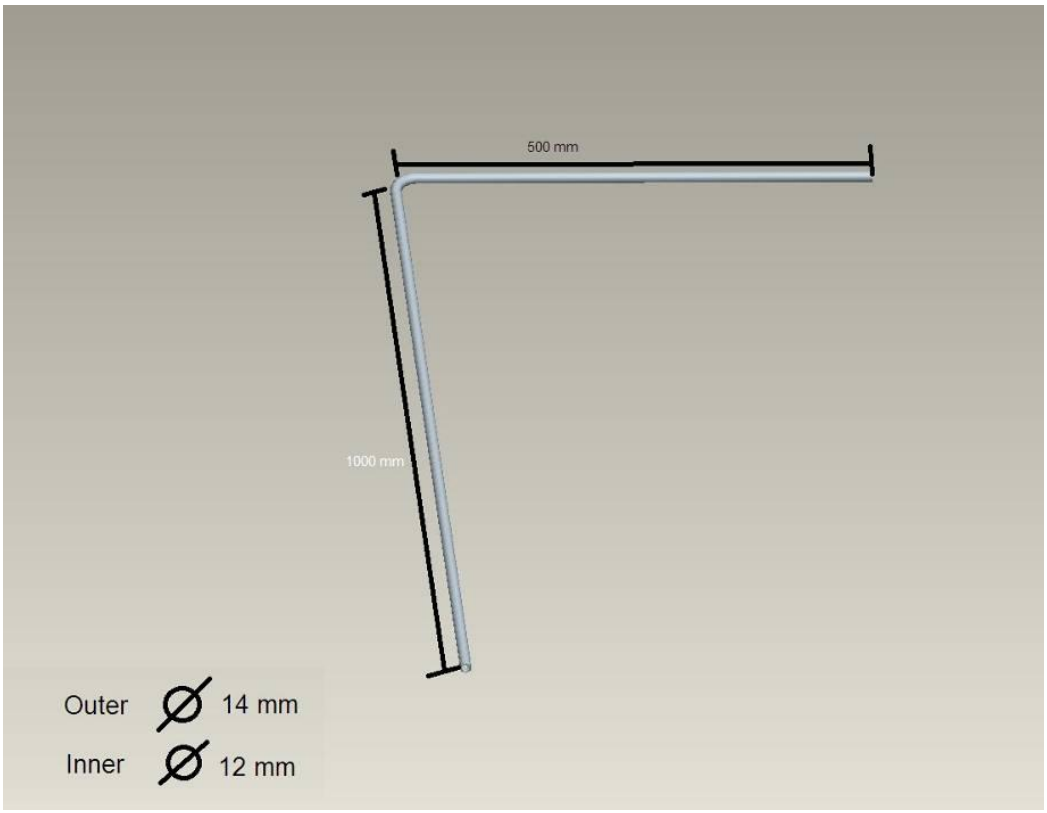
هذا الأنبوب يخرج الغازات المستهلكة

(4.) مدخل اللقاح



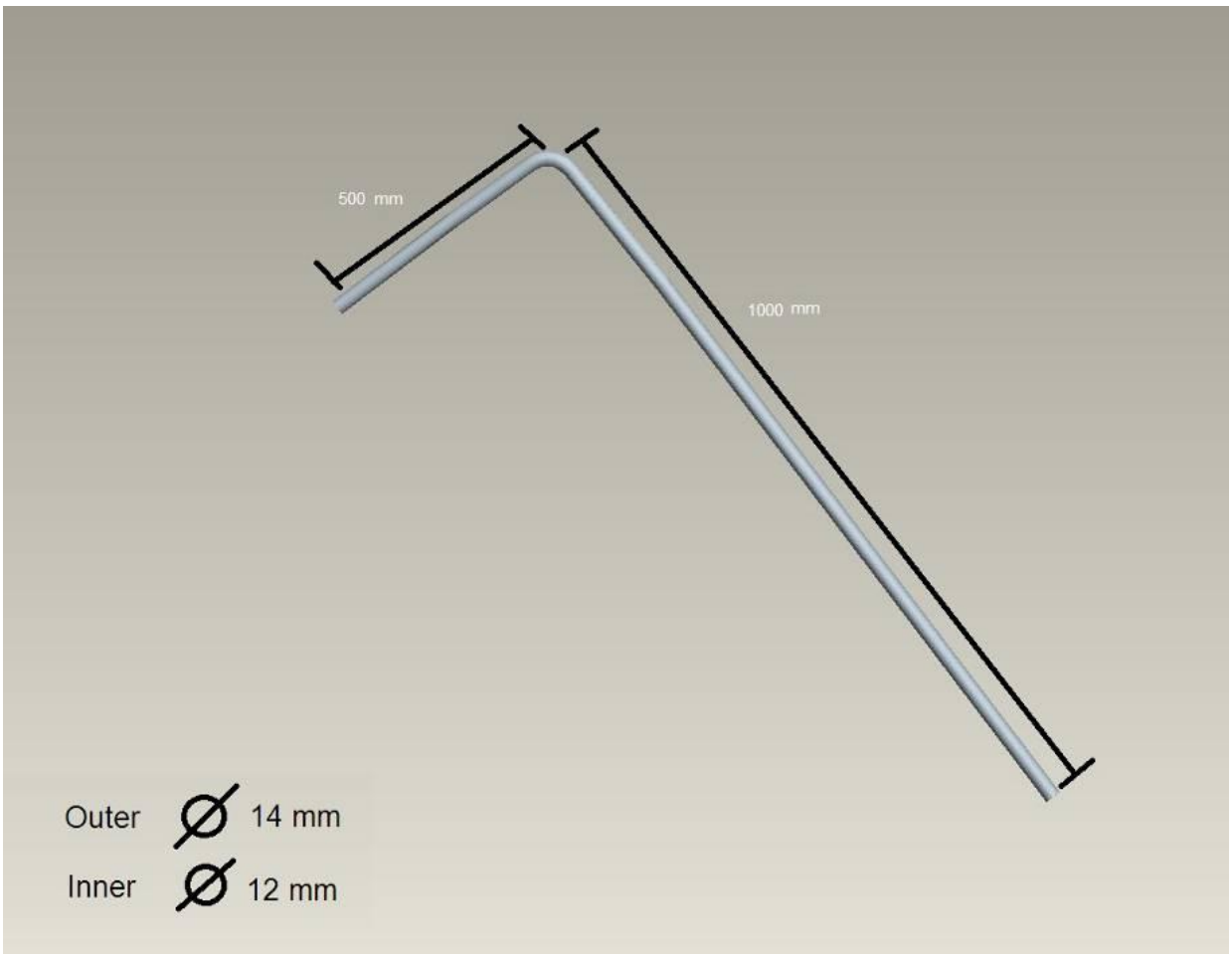
هذا الأنبوب يدخل لقاحا في الخزان

(مخرج الانتاج (5)

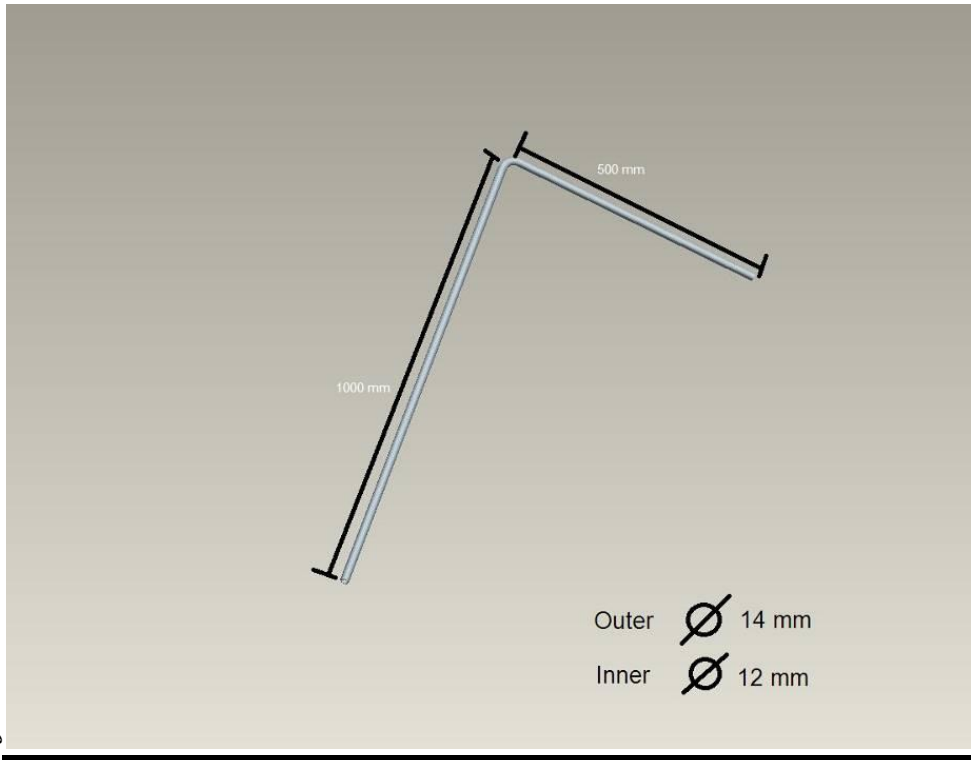


هذا الأنبوب يخرج الانتاج النهائي

(.6)مدخل الغذاء



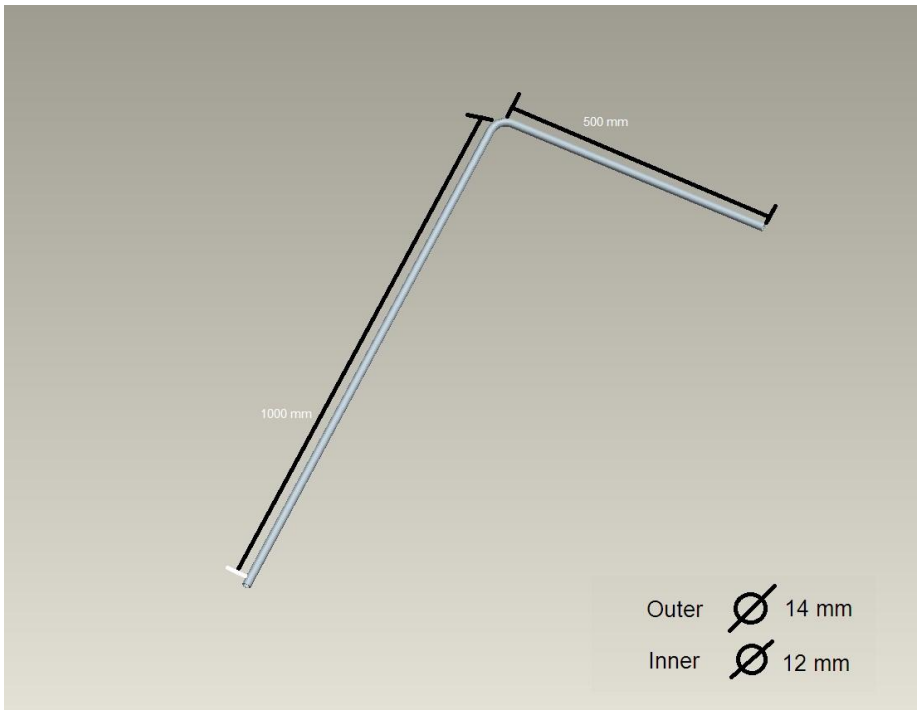
هذا الأنبوب يدخل غذاء للبكتاريا



مخرج العينات (7.) هذا الأنبوب

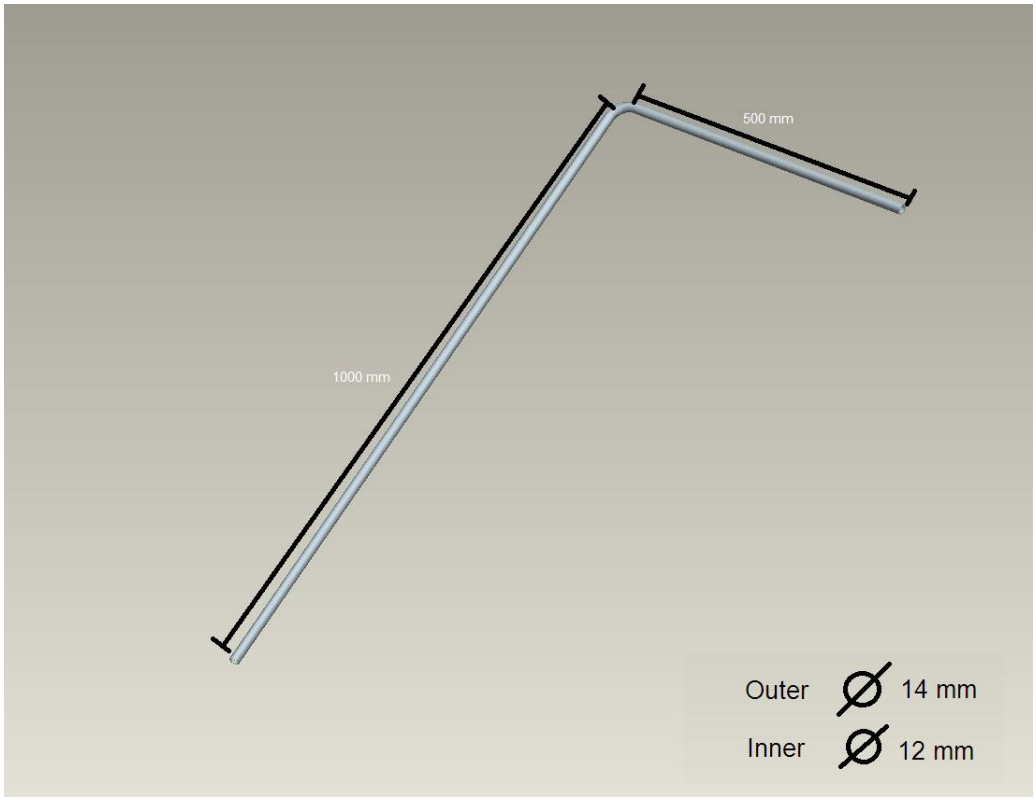
يخرج عينات التي تفحص في المختبر

(8.) مدخل السائل الحمضي



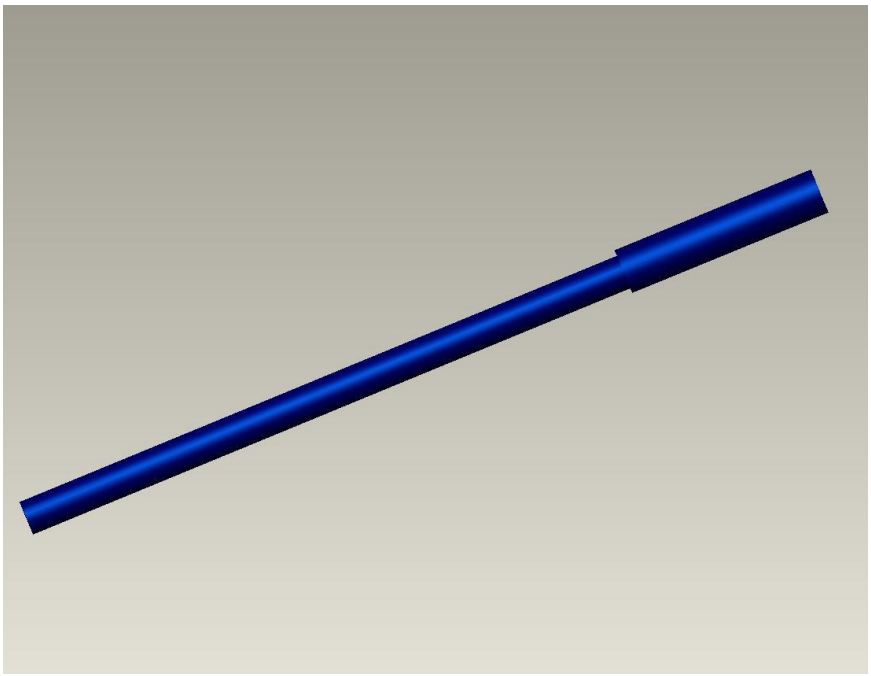
هذا الأنبوب يدخل سائل حمضي في الخزان

(9.)مدخل الأكسيجين



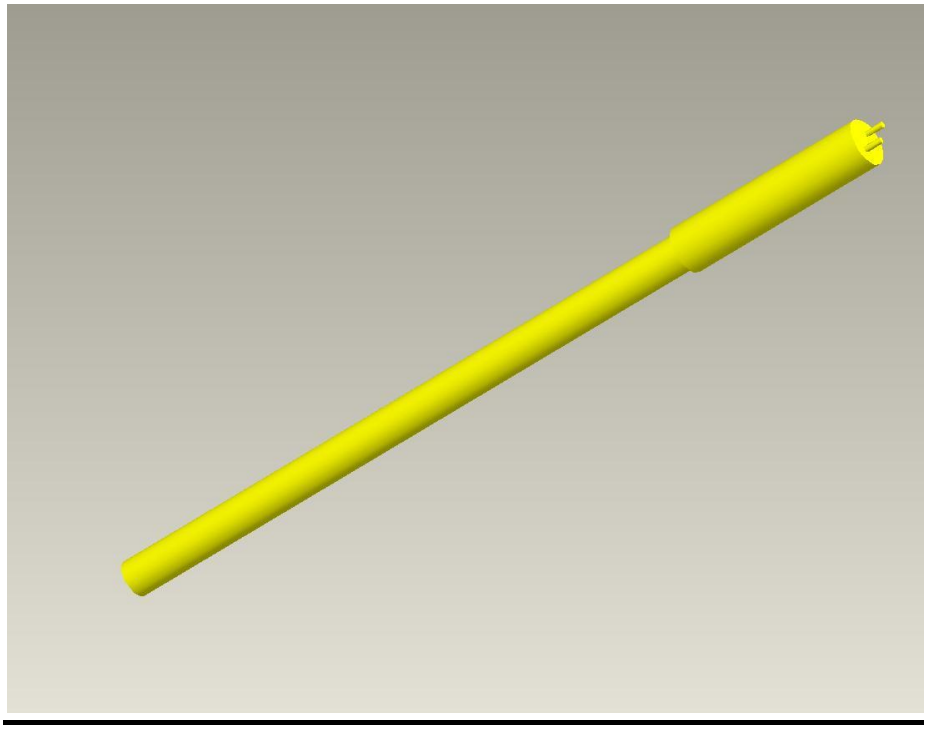
العمليات في الخزان هي بحاجة الى أكسيجين الذي يدخل عبر هذا الأنبوب

(10.)محسس الحرارة



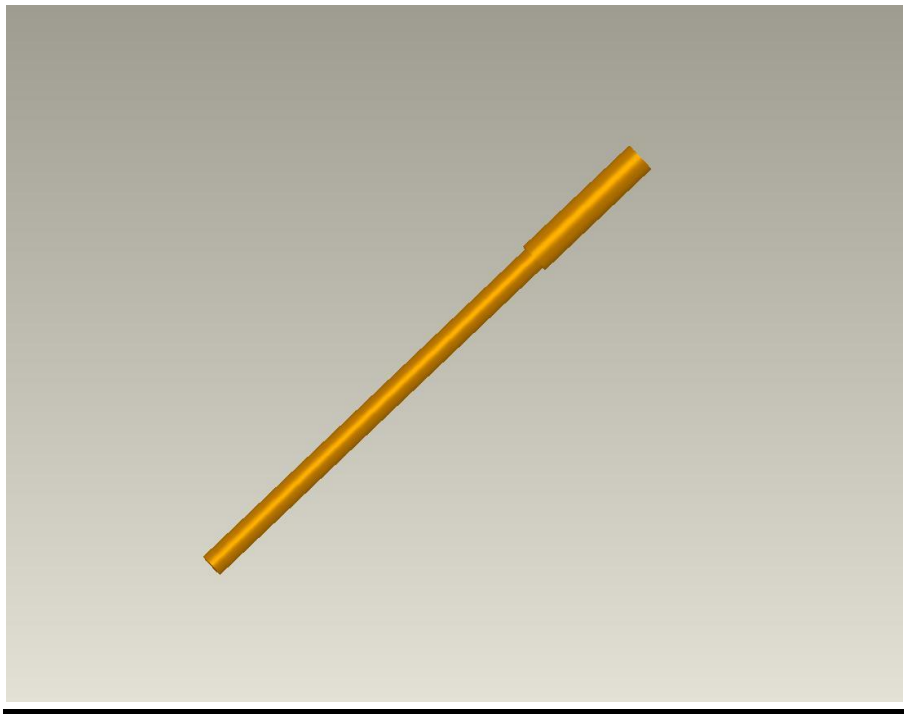
محسس الحرارة يقيس درجة الحرارة داخل الخزان

(.11)محسس الأكسيجين



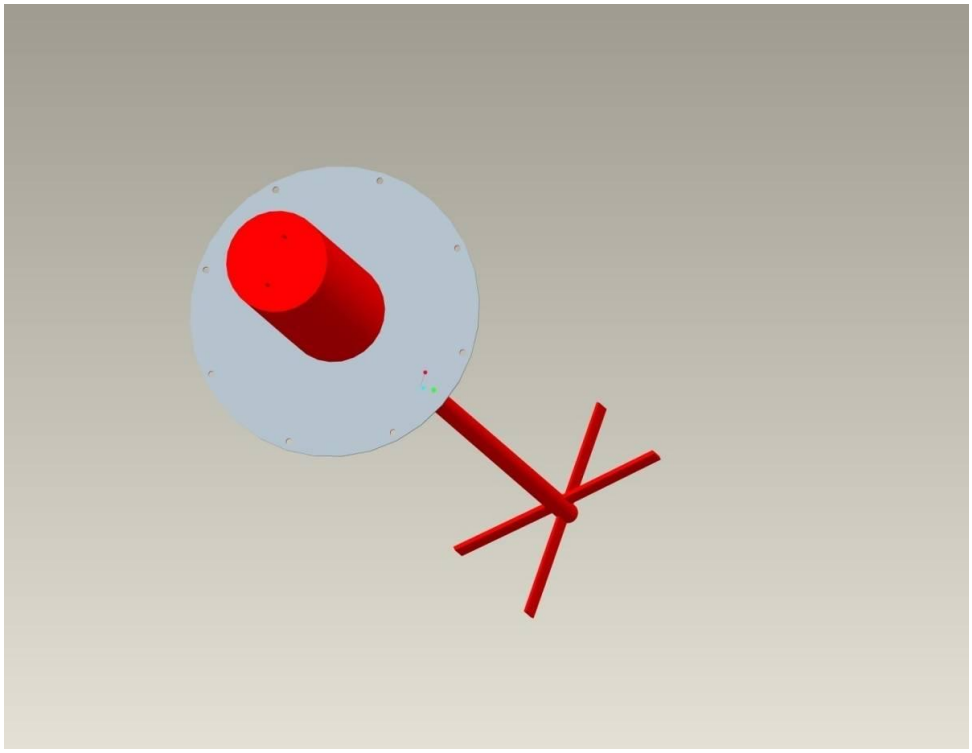
محسس الأكسيجين يقيس كمية الأكسيجين داخل الخزان

(.12)محسس الرقم الهيدروجيني (pH value)

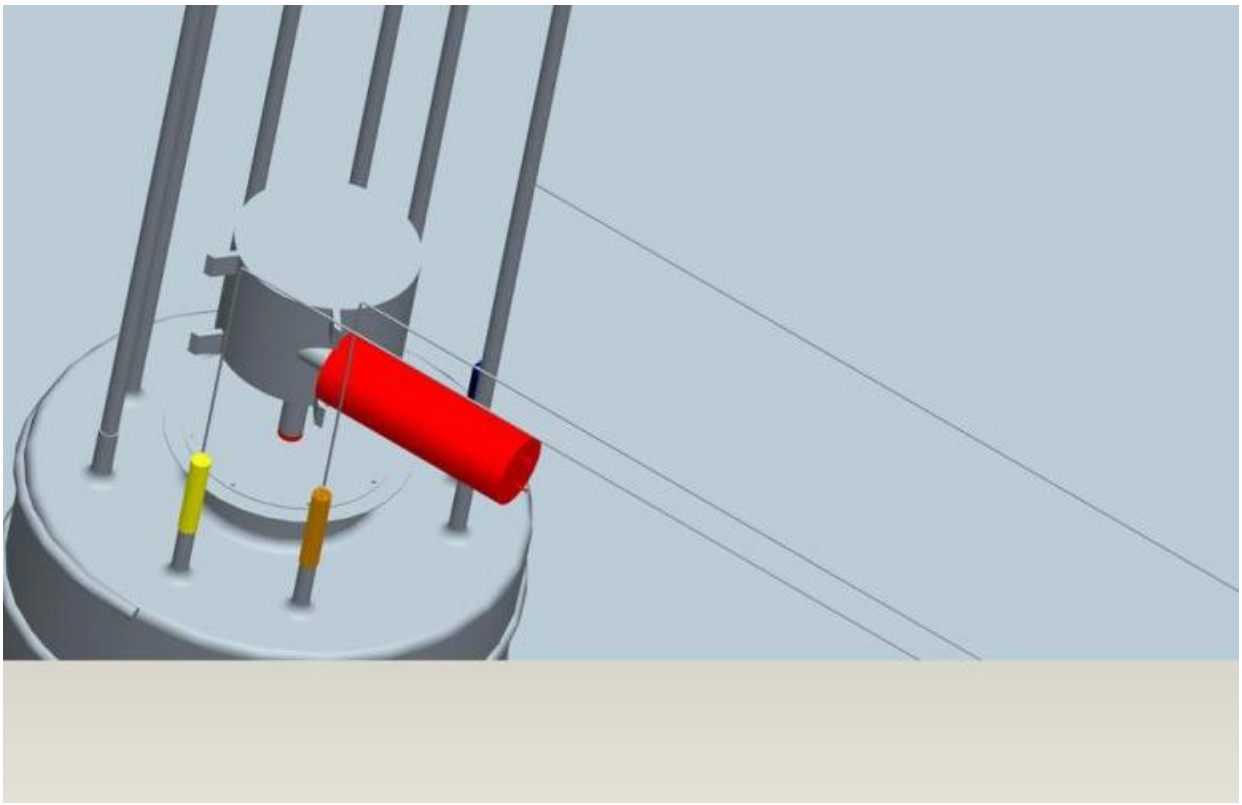


محسس الرقم الهيدروجيني يقيس الرقم الهيدروجيني للسائل داخل الخزان

(13.)المحرك

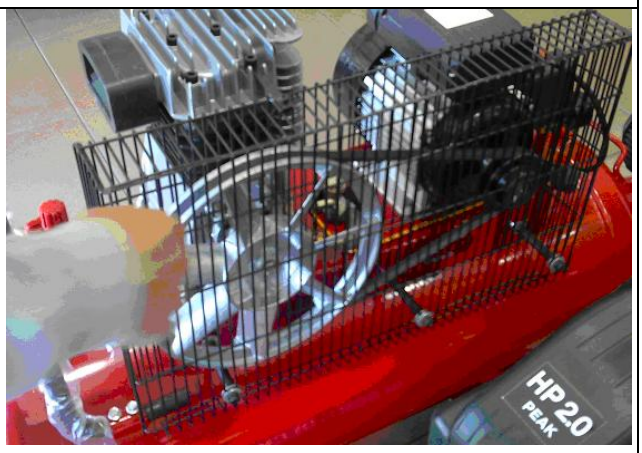


المحرك يحرك السائل داخل الخزان



Zur Anpassung der Drehgeschwindigkeit (Motor hat 1400 rpm) wird ein Getriebe eingesetzt.

Vom Schrottplatz hat das obige Getriebe 53 USD gekostet. Als Getriebeöl muss unbedingt 90iger Öl eingesetzt werden (0,5-1 Liter, 1Liter kostet 6 USD).

Das Getriebe links kostet 300 USD, das Getriebe oben rechts 280 USD. Händler: Seitenstrasse links vor Jamal&Chaban (wenn man aus der Innenstadt kommt), Tripoli, Libanon	Alternativ dazu kann eine Umsetzung auf die folgende Art geschehen:
	

Zur **Abdichtung** des Rührerrohres gegen die obere Reaktorwand wird eine einfache Gleitringdichtung verwendet.

Fig.: Gleitringdichtung

Schnitt durch eine drehrichtungsunabhängige, einfachwirkende Gleitringdichtung.

1) Gewindestift

2) O-Ring (Sekundärdichtung)

3) Spannstift als Verdrehsicherung für den Gleitring (4)

4) Gleitring

5) Gegenring

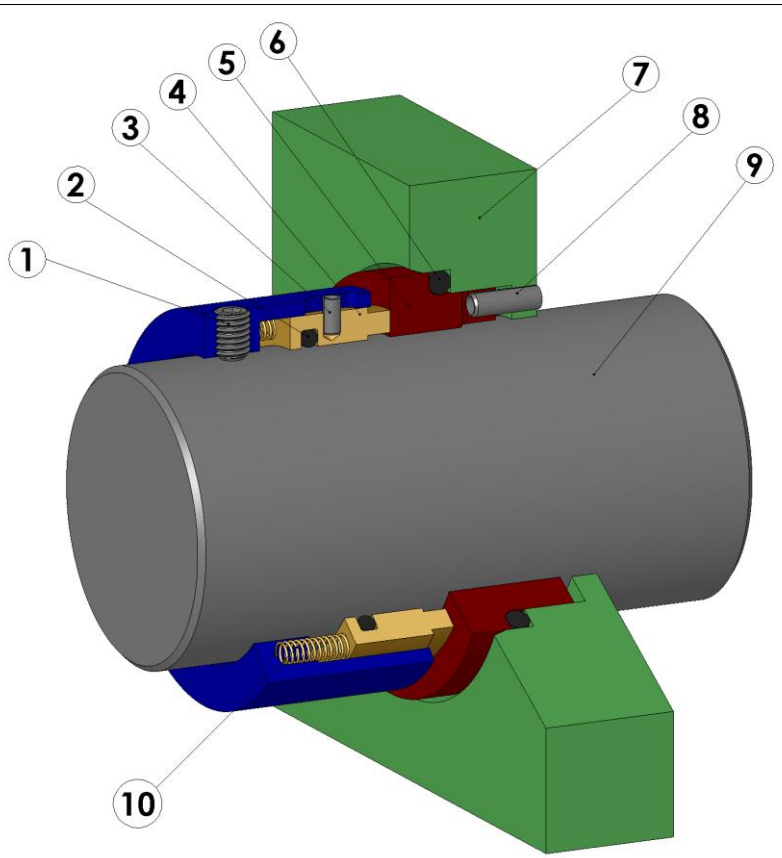
6) O-Ring (Sekundärdichtung)

7) Gehäusewand (nur angedeutet)

8) Spannstift als Verdrehsicherung für den Gegenring (5)

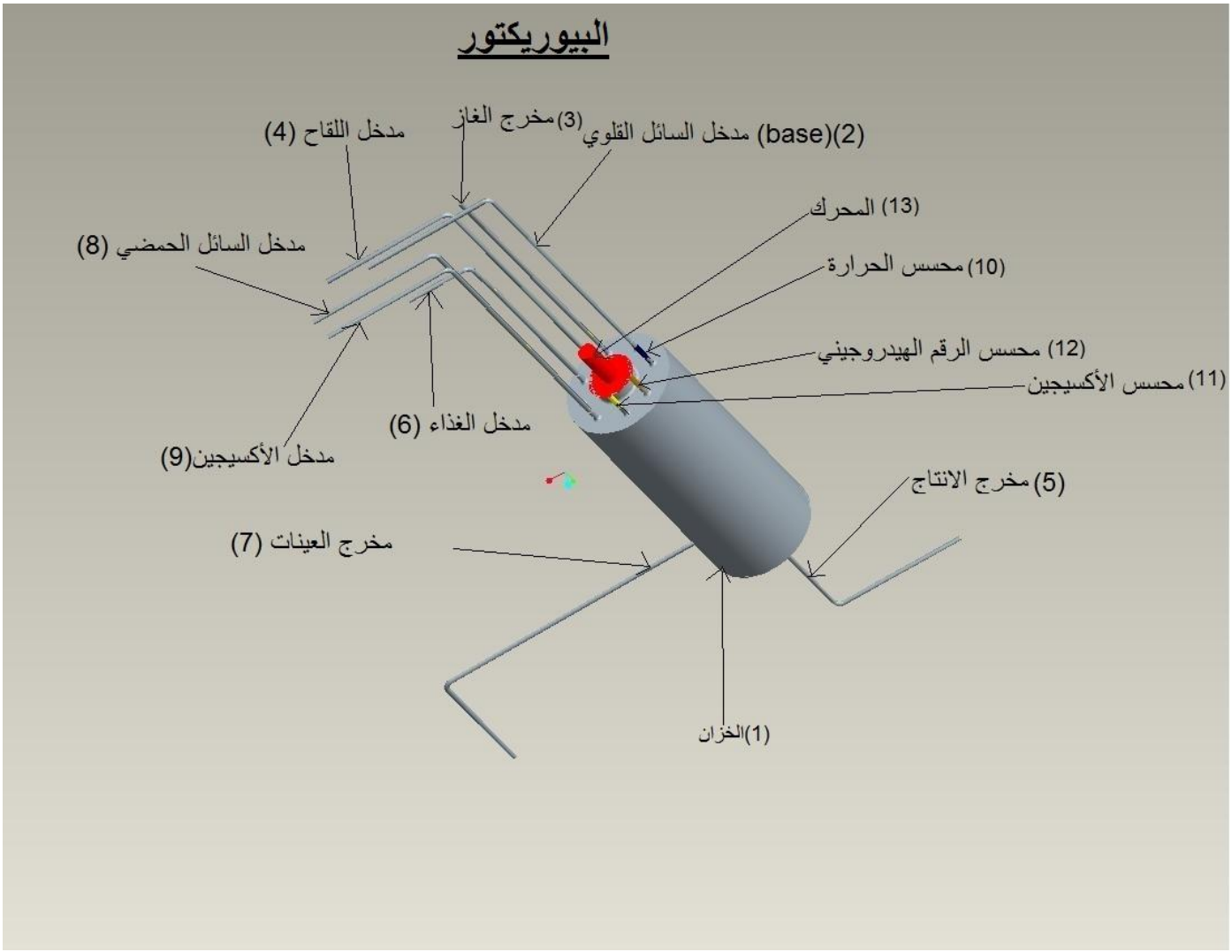
9) Welle/Achse

10) Federn

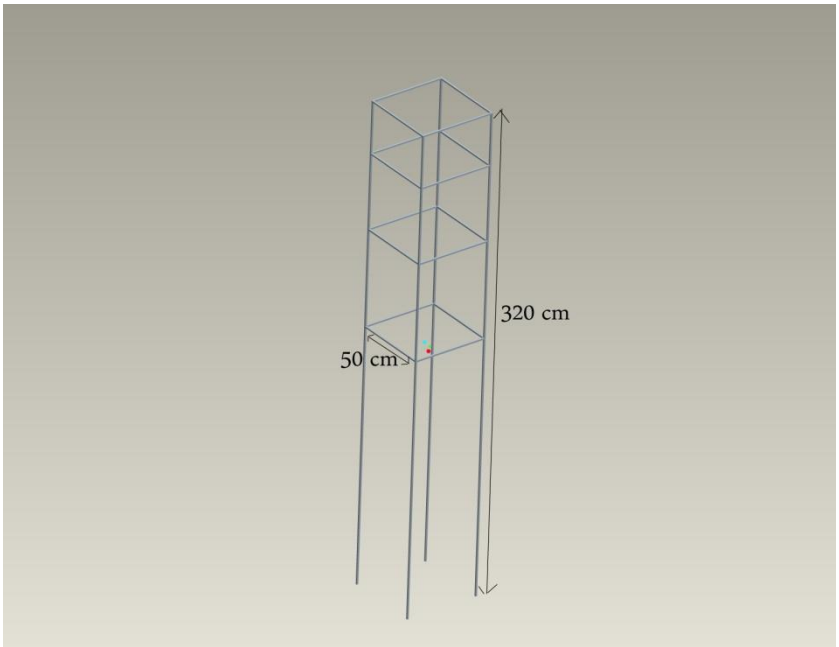


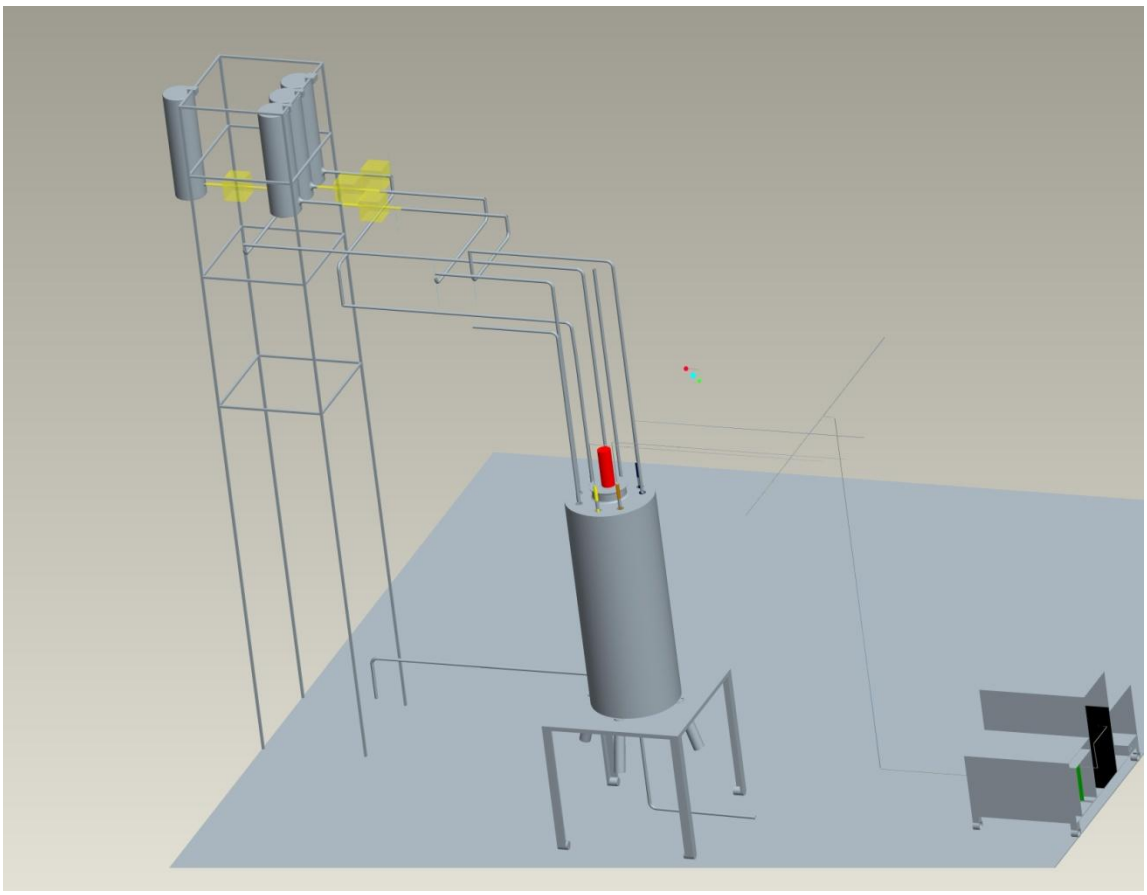
Gleitringdichtungen oder auch sogenannte dynamische Dichtungen übernehmen die Abdichtung rotierender Wellen gegenüber einer Wand, z. B. eines Maschinengehäuses. Hauptkomponenten sind zwei aufeinander gleitende Bauteile, der befederte Gleitring (im oberen Bild Position a) und ein Gegenring. Einer der beiden Ringe sitzt starr im stationären Gehäuse (Stator) (im oberen Bild Position d), der andere ist mithilfe von Verdrehsicherungsstiften auf der rotierenden Welle befestigt (Rotor). Die Flächen zwischen diesen beiden Teilen sind - abhängig von der Art der Gleitringdichtung – zumeist plan und bestehen in der Regel aus Kohlenstoff-Graphitwerkstoffen, Metall, Keramik, Kunststoff oder kunstharzgebundenem Kohlenstoff.

The mixing can also be done by a magnetic stirrer if the vessel from staniless steel:

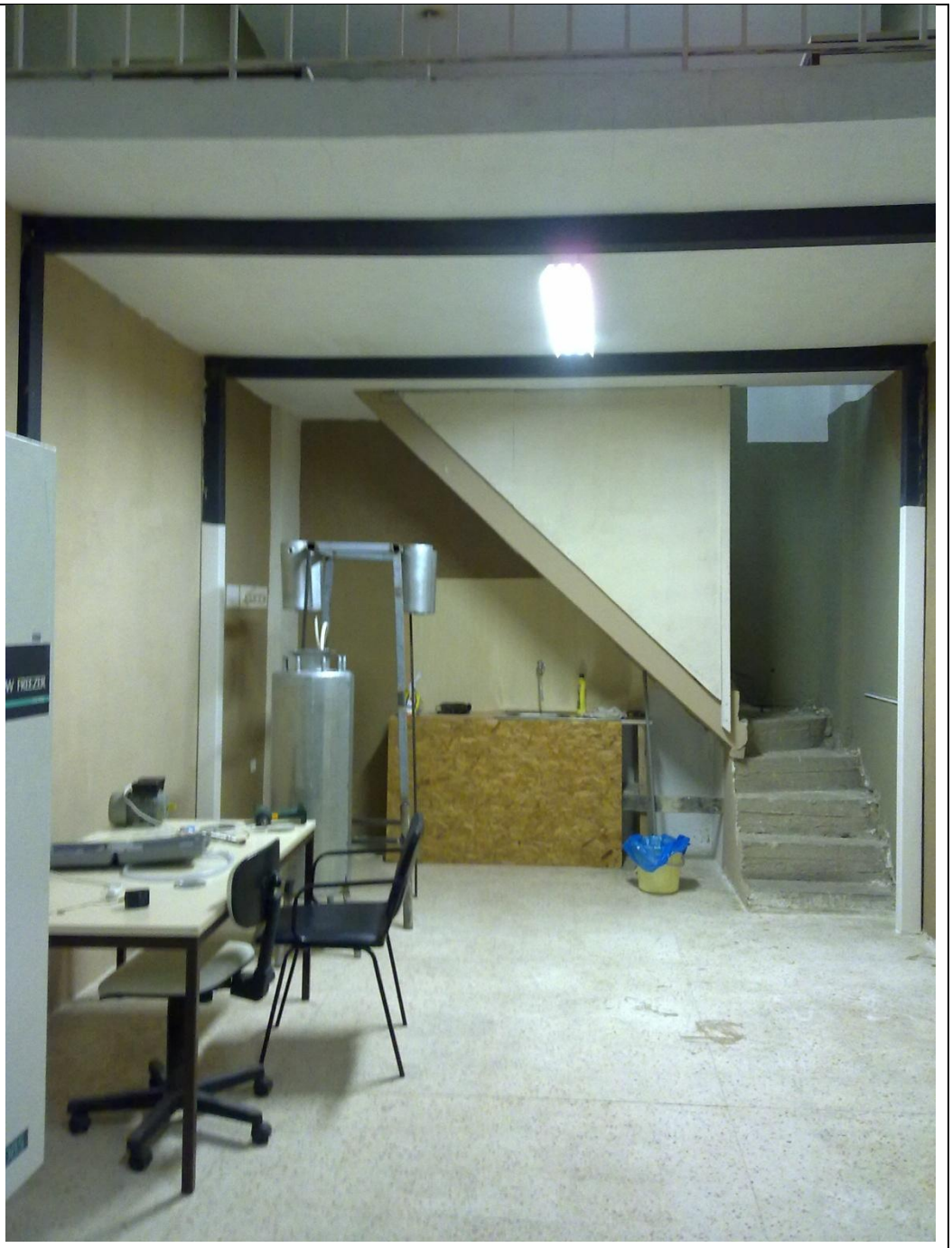


12.3.1 تركيب (System Integration)





12.4 تصنيع البيورياكتور (Manufacting of bioreactor)



لان المحرك يدور 1450 مرة في الدقة ونحتاج 200 مرة في دقيقة نحتاج وسيلة لتخفيض سرعة الدوران.

وذلك سيحقق ان شاء الله مثل الصورة في الاسفل

12.5 تکالیف

material piece list for initial inner bioreactor

piece	Length (m)	number	cost per meter or piece	Cost of pieces
fermenter box		1	$500	$500
motor for fermenter		1	$60	$60
Rohr	1,13	1	$40	$45
	0,73	1	$40	$29
	0,90	4	$40	$144
	0,65	2	$40	$52
	0,51	2	$40	$41
Stange	3,00	4	$15	$180
	0,50	8	$15	$60
Valve		5	$15	$75
			Costs (without sensors)	**$1.186,20**

13 التحكم للبيورياكتور (bioreactor automation)

13.1 مواصفات (Specification)

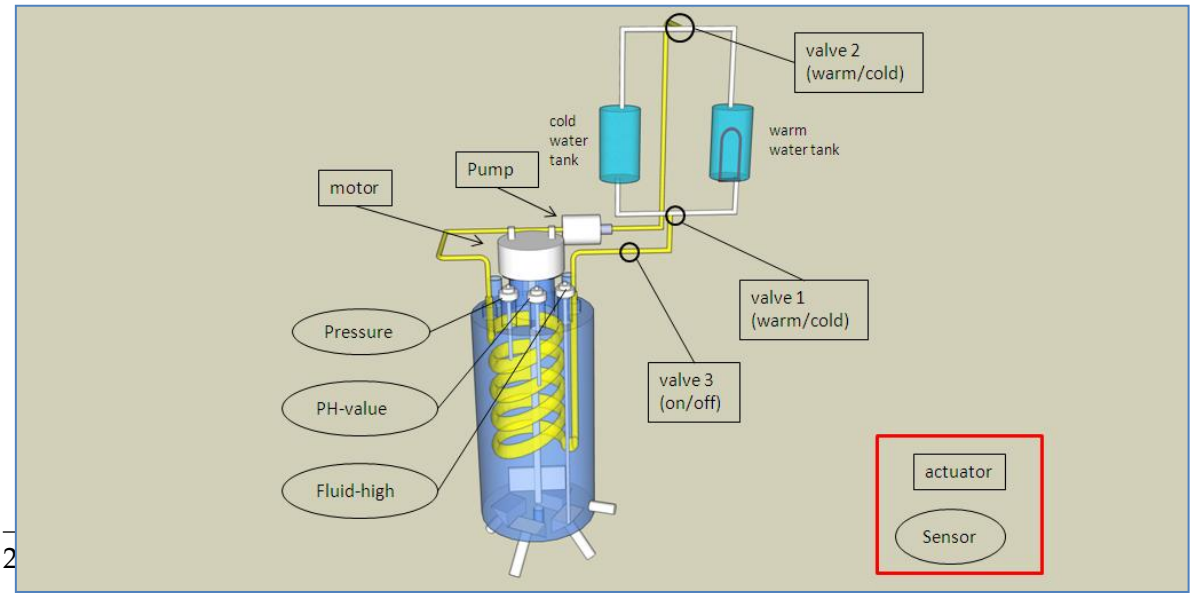
13.1.1 اجهزة الاحساس (sensors)

مقياس الحرارة داخل البيورياكتور		
مقياس الPH		
مقياس العلوّ		

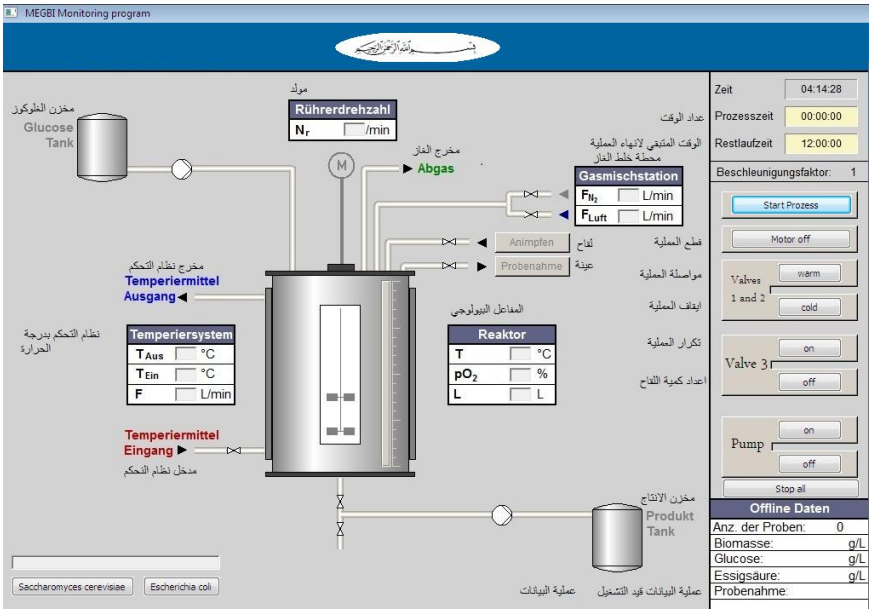
13.1.2 محركات (actuators)

		المولّد (Motor)
		Electronic valve 1
		Electronic valve 2
		Electronic valve 3
		الترومبة (Pump)

System Design *13.2*



شاشة التحكم


MEGBI Monitoring program
مولد
Rührerdrehzahl
Nr /min
مخزن الغلوكوز
Glucose Tank
مخرج الغاز
Abgas
عداد الوقت
الوقت المتبقي لانتهاء العملية
محطة خلط الغاز
Gasmischstation
FH2 L/min
FLuft L/min
Animpfen
لقاح
Probenahme
عينة
قطع العملية
مواصلة العملية
ايقاف العملية
تكرار العملية
اعداد كمية اللقاح
مخرج نظام التحكم
Temperiermittel Ausgang
نظام التحكم بدرجة الحرارة
Temperiersystem
TAus °C
TEin °C
F L/min
المفاعل البيولوجي
Reaktor
T °C
pO2 %
L L
Temperiermittel Eingang
مدخل نظام التحكم
مخزن الانتاج
Produkt Tank
Saccharomyces cerevisiae
Escherichia coli
عملية البيانات
عملية البيانات قيد التشغيل
Zeit 04:14:28
Prozesszeit 00:00:00
Restlaufzeit 12:00:00
Beschleunigungsfaktor: 1
Start Prozess
Motor off
Valves 1 and 2
warm
cold
Valve 3
on
off
Pump
on
off
Stop all
Offline Daten
Anz. der Proben: 0
Biomasse: g/L
Glucose: g/L
Essigsäure: g/L
Probenahme:

Implementation *13.3*

13.3.1 السفتوير (Software)

260613_MEGBI.py

```
#-------------------------------------------------------------------------------
------------
# Name:        MEGBI monitoring software
# Purpose:
#
# Author:      abdurrahman
#
# Created:     09/05/2013
# Copyright:   (c) aecenar 2013
# Licence:     <AECENAR>
#-------------------------------------------------------------------------------
------------

##!/usr/bin/env python
## -*- coding: iso-8859-15 -*-

import wx
import random
import sys

import time


#import ctypes
from ctypes import *

import thread


#**********************                    panel frame
**********************
#*******************************************************************************
************


wx.SetDefaultPyEncoding("iso-8859-15")
BACKGROUND_IMAGENAME = "reactor-h-bild.bmp"
##"hintergrundbild.jpg"


class MyBackgroundPanel(wx.Panel):

    def __init__(self, parent):
        wx.Panel.__init__(self, parent)
        self.bmp = wx.Bitmap(BACKGROUND_IMAGENAME)
        self.SetSize(self.bmp.GetSize())
        self.Bind(wx.EVT_PAINT, self.on_paint)


    def on_paint(self, event = None):
        dc = wx.BufferedPaintDC(self, self.bmp)


class MyFrame(wx.Frame):

    def __init__(self, parent = None, title = "MEGBI Monitoring program"):
```

1/7

260613_MEGBI.py

```
        self.testUSB                = True
        self.dll                    = None
        self.USBAdr0                = 0
        self.USBAdr1                = 1
        self.USBAdr2                = 2
        self.USBOpened              = False
        self.counterUSBBoards       = 3

        wx.Frame.__init__(self, parent, -1, title)

        panel = MyBackgroundPanel(self)

        LABELSTYLE = wx.BORDER_SUNKEN | wx.ST_NO_AUTORESIZE |
wx.ALIGN_CENTER_HORIZONTAL

        #Start Prozess
        self.prozess_Start_Flow_Read = wx.Button(panel, -1, "          Start Prozess
       ", pos=(855,202))
        self.Bind(wx.EVT_BUTTON, self.OpenMotorANDStartRead,
self.prozess_Start_Flow_Read)

        #Stop of Motor
        self.button_Stop_Read_motor = wx.Button(panel, -1, "             Motor off
        ", pos=(855,242))
        self.Bind(wx.EVT_BUTTON, self.StopMotor, self.button_Stop_Read_motor)



        #Valve 1 and 2
        self.valve_1_and_2_warm = wx.Button(panel, -1, "warm", pos=(910,282))
        self.Bind(wx.EVT_BUTTON, self.Valve1AND2Warm, self.valve_1_and_2_warm)
        self.valve_1_and_2_cold = wx.Button(panel, -1, "cold", pos=(910,322))
        self.Bind(wx.EVT_BUTTON, self.Valve1AND2Cold, self.valve_1_and_2_cold)


        #Valve 3
        self.valve_3_on = wx.Button(panel, -1, "on", pos=(910,370))
        self.Bind(wx.EVT_BUTTON, self.Valve3on, self.valve_3_on)
        self.valve_3_off = wx.Button(panel, -1, "off", pos=(910,410))
        self.Bind(wx.EVT_BUTTON, self.Valve3off, self.valve_3_off)



        #Pump
        self.pump_on = wx.Button(panel, -1, "on", pos=(910,466))
        self.Bind(wx.EVT_BUTTON, self.PumpOn, self.pump_on)
        self.pump_off = wx.Button(panel, -1, "off", pos=(910,506))
        self.Bind(wx.EVT_BUTTON, self.PumpOff, self.pump_off)

        #Stop all
        self.stopp_all = wx.Button(panel, -1, "                  Stop all
    ", pos=(845,535))
        self.Bind(wx.EVT_BUTTON, self.StopAll, self.stopp_all)
```

260613_MEGBI.py

```
        #modeselect
        self.mode_1 = wx.Button(panel, -1, "Saccharomyces cerevisiae", pos=(10,650))
        self.Bind(wx.EVT_BUTTON, self.Mode1, self.mode_1)
        self.mode_2 = wx.Button(panel, -1, "Escherichia coli", pos=(165,650))
        self.Bind(wx.EVT_BUTTON, self.Mode2, self.mode_2)


# lable


        # Reaktor
        self.temperature_reaktor = wx.StaticText(
            panel, size = (26, -1), pos = (595, 380), style = LABELSTYLE
        )
        self.p_h_reaktor = wx.StaticText(
            panel, size = (26, -1), pos = (595, 405), style = LABELSTYLE
        )
        self.fluid_high_reaktor = wx.StaticText(
            panel, size = (26, -1), pos = (595, 428), style = LABELSTYLE
        )


        # Ruehrerzahl
        self.n_r_ruehrerzahl = wx.StaticText(
            panel, size = (26, -1), pos = (400, 117), style = LABELSTYLE
        )



        #Gasmischstation
        self.f_n_two_gasmischstation = wx.StaticText(
            panel, size = (26, -1), pos = (722, 194), style = LABELSTYLE
        )
        self.f_air_gasmischstation = wx.StaticText(
            panel, size = (26, -1), pos = (722, 219), style = LABELSTYLE
        )



        #Temperiersystem
        self.t_aus_temperiersystem = wx.StaticText(
            panel, size = (26, -1), pos = (202, 380), style = LABELSTYLE
        )
        self.t_ein_temperiersystem = wx.StaticText(
            panel, size = (26, -1), pos = (202, 405), style = LABELSTYLE
        )
        self.f_temperiersystem = wx.StaticText(
            panel, size = (26, -1), pos = (202, 428), style = LABELSTYLE
        )



        # Mode
        self.mode = wx.StaticText(
            panel, size = (245, -1), pos = (10, 625), style = LABELSTYLE
```

```
        )



        # Layout
        self.Fit()

    def on_timer(self, event = None):  #new_value =
str(windll.K8061.ReadAnalogChannel(1,1))



    # Reaktor
        new_value = str(self.dll.ReadAnalogChannel(1,1))
        self.temperature_reaktor.SetLabel(new_value)
        self.temperature_reaktor.Refresh()
        new_value = str(self.dll.ReadAnalogChannel(1,2))
        self.p_h_reaktor.SetLabel(new_value)
        self.p_h_reaktor.Refresh()
        new_value = str(self.dll.ReadAnalogChannel(1,3))
        self.fluid_high_reaktor.SetLabel(new_value)
        self.fluid_high_reaktor.Refresh()



    # Ruehrerzahl
        new_value = str(self.dll.ReadAnalogChannel(1,4))
        self.n_r_ruehrerzahl.SetLabel(new_value)
        self.n_r_ruehrerzahl.Refresh()


    #Gasmischstation
        new_value = str(self.dll.ReadAnalogChannel(1,5))
        #self.f_n_two_gasmischstation.SetLabel(new_value)
        self.f_n_two_gasmischstation.Refresh()
        new_value = str(self.dll.ReadAnalogChannel(1,6))
        #self.f_air_gasmischstation.SetLabel(new_value)
        self.f_air_gasmischstation.Refresh()


    #Temperiersystem
        new_value = str(self.dll.ReadAnalogChannel(1,7))
        #self.t_aus_temperiersystem.SetLabel(new_value)
        self.t_aus_temperiersystem.Refresh()
        new_value = str(self.dll.ReadAnalogChannel(1,8))
        #self.t_ein_temperiersystem.SetLabel(new_value)
        self.t_ein_temperiersystem.Refresh()
        new_value = str(random.randint(20, 25))
        #self.f_temperiersystem.SetLabel(new_value)
        self.f_temperiersystem.Refresh()
```

260613_MEGBI.py

```
#**********************                Run USB System
**********************
#*******************************************************************************
************
    def OpenUSBBoardThread(self):
        self.dll = windll.K8061
        i = self.counterUSBBoards
        for doit in range(0,i+1):
            try:
                self.dll.OpenDevice()
                self.USBOpened = True
# debug info
                print 'USB Board is now connected!'
#end debug info
            except:
                txt = 'Please Check USB Board connection'
                print txt
                return


#**********************                    STOP Motor
********************
#*******************************************************************************
************

    def StopMotor(self, event):
        self.dll.ClearDigitalChannel(1,1)
        print 'Digital Channel Cleared, motor turn off'



#**********************                    START Prozess
********************
#*******************************************************************************
************


    def OpenMotorANDStartRead(self, event):

        wx.MessageBox("Do you want to open motor and start monitoring?", "start
monitoring", wx.OK|wx.ICON_INFORMATION)


# open the USB board

        self.OpenUSBBoardThread()

        time.sleep(0.5)

        self.dll.SetDigitalChannel(1,1)

        self.timer = wx.Timer()
        self.timer.Bind(wx.EVT_TIMER, self.on_timer)
        self.timer.Start(1000)
```

260613_MEGBI.py

```
#**********************                        Valve 1 and 2
*******************
#*****************************************************************************************
***********

    def Valve1AND2Warm(self, event):
        self.dll.SetDigitalChannel(1,2)
        print 'Valve 1 and 2: warm'


    def Valve1AND2Cold(self, event):
        self.dll.ClearDigitalChannel(1,2)
        print 'Valve 1 and 2: cold'


#**********************                        Valve 3
*******************
#*****************************************************************************************
***********

    def Valve3on(self, event):
        self.dll.SetDigitalChannel(1,3)
        print 'Valve 3: on'


    def Valve3off(self, event):
        self.dll.ClearDigitalChannel(1,3)
        print 'Valve 3: off'




#**********************                        Pump
*******************
#*****************************************************************************************
***********

    def PumpOn(self, event):
        self.dll.SetDigitalChannel(1,4)
        print 'Pump: on'


    def PumpOff(self, event):
        self.dll.ClearDigitalChannel(1,4)
        print 'Pump: off'




#**********************                        Stopp all
*******************
```

260613_MEGBI.py

```
#*****************************************************************************************
************

    def StopAll(self, event):
        self.dll.ClearDigitalChannel(1,1)
        self.dll.ClearDigitalChannel(1,2)
        self.dll.ClearDigitalChannel(1,3)
        self.dll.ClearDigitalChannel(1,4)
        print 'All stopped'



#**********************                    Mode
*******************
#*****************************************************************************************
************


    def Mode1(self, event):
        self.mode.SetLabel("Saccharomyces cerevisiae")
        print 'Saccharomyces cerevisiae'



    def Mode2(self, event):
        self.mode.SetLabel("Escherichia coli")
        print 'Escherichia coli'


#**********************                main definition and loop
*******************
#*****************************************************************************************
************

def main():
    """Testing"""
    app = wx.PySimpleApp()
    f = MyFrame()
    f.Center()
    f.Show()
    app.MainLoop()

if __name__ == "__main__":
    main()
```

7/7

13.3.2 الهاردوير (Hardware)

- K8061 Test & Diagnosis Utility (Rev. V1.1)
- USB cable
- Boards for sensors und actuators

13.3.3 Example for input/outputs of K8061 for the bioreactor system

#	Unit	Type	Symbol	Input/ Output	Port/Pin	Signal	part/range	Remark
1	Emergency switch (Software)	switch	N	I	PH0	Digital	0-1	Bioreactor control OFF
1	rotor	switch	C	O	PJ0-3	Digital	0-1	Turning ON/OFF
1	Pump for heating water	220 V pump with relais	M	O	PA0-1-2	Digital	Stufen 0,1,2,3	Pump control
1	Speed counter with photocell	Hitachi	S_G	I	PT7	digital (impulses)	50 Hz	Drehzahlzähler für Generator
2	Level meter	Farnell	L	I	PA3-4	digital	0-100%	Bioreactor tank
1	Control valve	Danfoss	V	O	PB3-4	digital/analog	1-2/3-1	…
1	Control valve	Danfoss	V	O	PB3-4	digital/analog	1-2/3-1	…
…	…	…	…	…	…	…	…	…
5	Temperature sensor	Pt100 TFK01	T	O	PAD00-PAD04	analog (4-20 mA)	-200 - +600°C	
3	Pressure sensor	Mano-meter	p	I	PAD08-PAD10	analog (4-20 mA)	0-180 bar	
3	Mass flow	Danfoss	dm/dt	I	PAD11-PAD13	analog (4-20 mA)		

Table 6.1: Specification for actuators and sensors

Remark:
In sensors for the output signal is 4-20 mA standard, ie the lowest measured value corresponds to 4 mA, 20 mA corresponds to the top and what is below 4 mA for line fault detection. Actuators at the output signal must be amplified.

13.3.4 Extended USB interface Board K8061

The Velleman K8061 module has 33 Ein-/Outputs and is connected via a USB port on the PC. The connection is galvanically-optically isolated, so that damage to the PC is not possible.

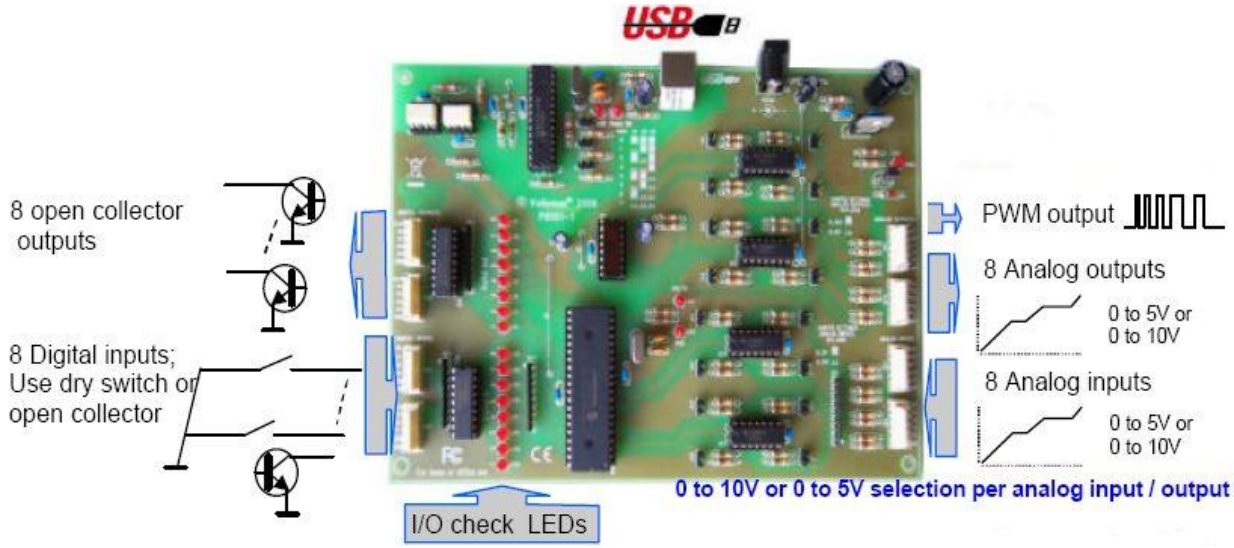


Abb. 8.1-2: I/O-Karte K8061

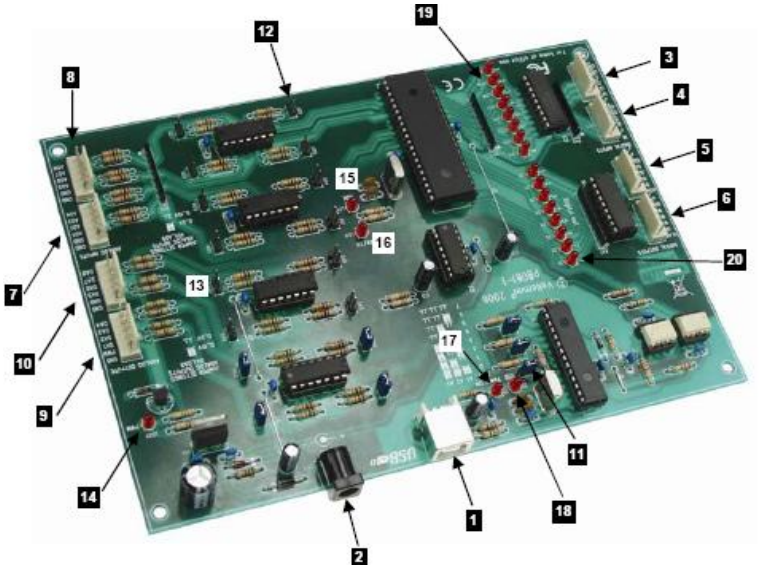
Characteristics:

- 8 analog Inputs with 10 bit-Auflösung: 0…5 V oder 10 VDC / 20 kΩ
- 8 analog Outputs with 8 bit-Auflösung: 0…5 V oder 10 VDC / 47 Ω
- 8 digital Inputs: „Open Collector"-Kompatibel (Anschluss an GND=0) with integrated LED display
- 8 digitale „Open Collector"-Outputs (max. 50 V/100 mA) with integrated LED display
- One 10 bit PWM-Ausgang: 0 bis 100% „Open Collector"-Ausgang (max 100 mA / 40 V) with integrated LED display
- General response time: 4ms per command
- USB Port: 2.0

Specifications:

- Power consumption from USB port: about 60 mA
- can be connected to the PC up to 8 cards
- Powered by PS1205 adapter: 12Vdc / 300 mA
- PWM frequency: 15.6 KHz
- Standard time: 48 ms (Microchip and K8061D.DLL drivers)
- Enhanced Execution time: 21 ms (K8061_C.DLL V1.0 for RE applications use)
- PCB Dimensions: 195 x 142 x 20mm (2.7 "x 5.6" x 0.8 ").

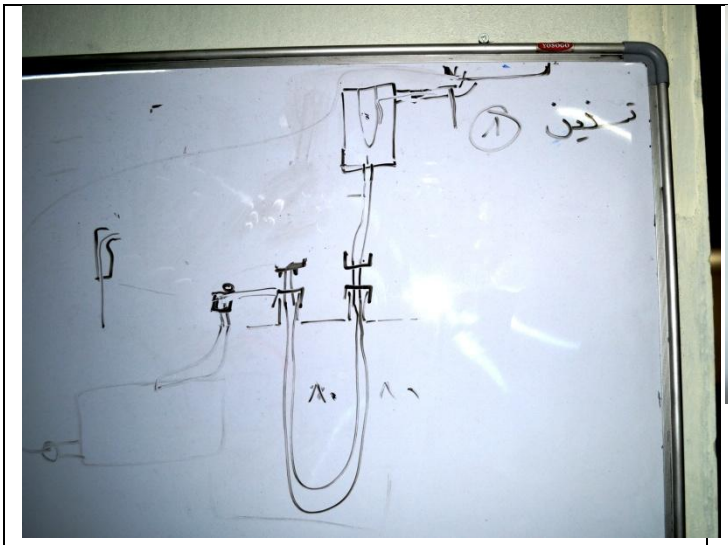
Connections of the K8061:



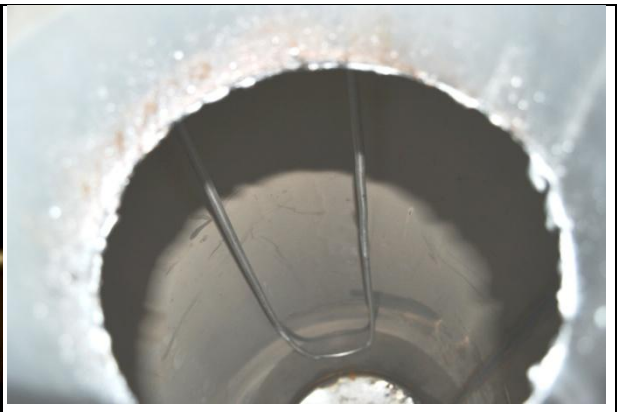
- 1: K8061-PC USB port
- 2: power supply (12VDC non-stabilized, at least 300mA)
- 3 and 4: Digital inputs 1-4/5-8: External "LOW" activate (with GND).
- 5 and 6: Digital Outputs 1-4/5-8: "open collector" outputs
- 7 and 8: Analog inputs 1-4/5-8: With their help, you can digitize read an analog voltage applied to it via the PC.

These inputs require a stable DC voltage (0-5V or 0-10V).

14 Bioreactor Temperature System Integration



Zeichnung

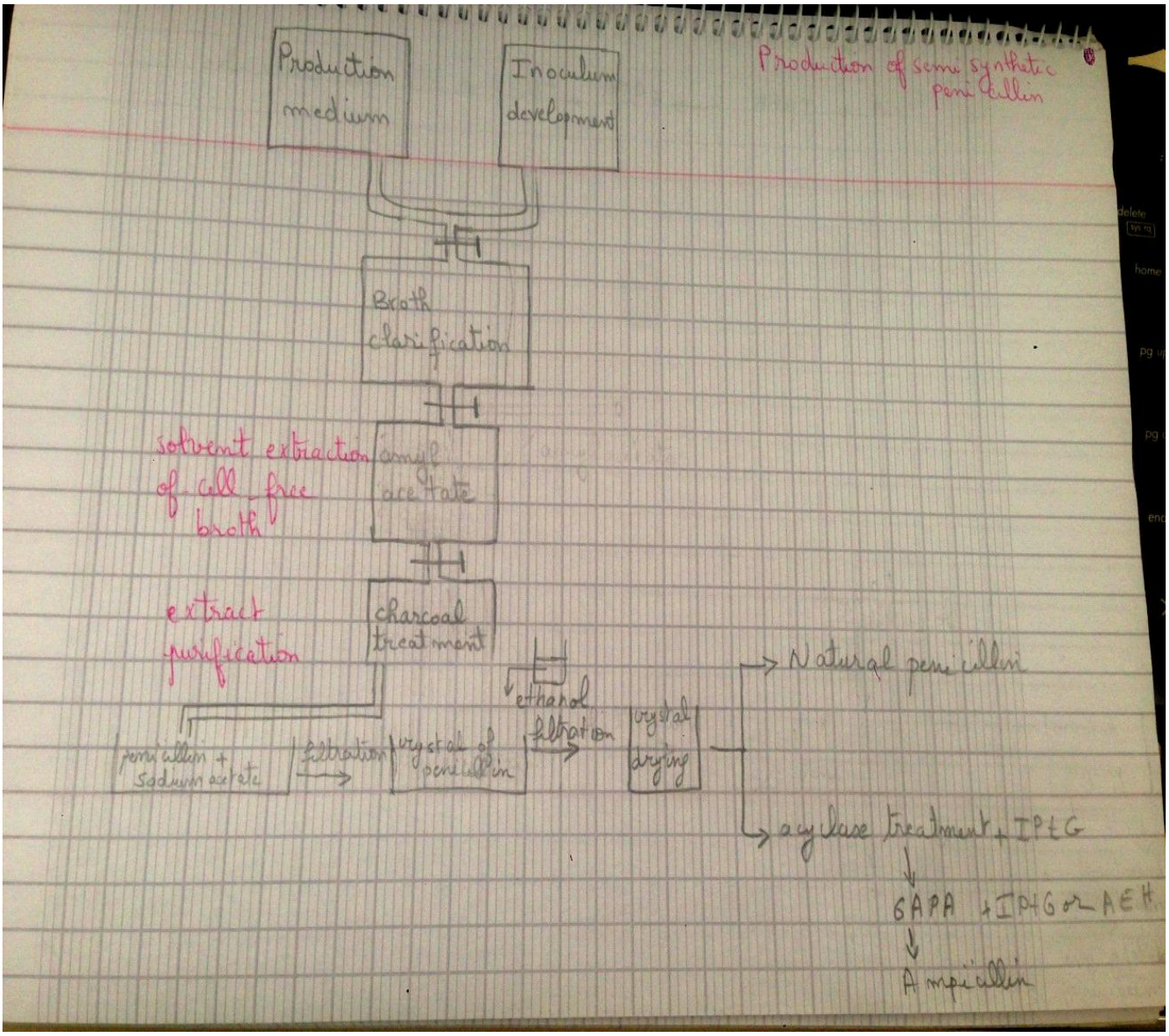


Temperiersystem innen

Bioreactor_mitTemperiersystem

15 Concept for MEGBI-APP Plant Design[8]

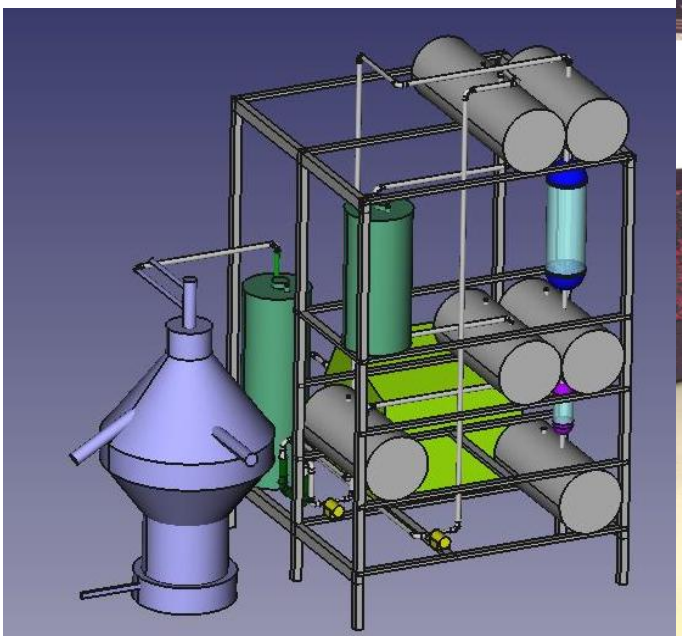
15.1 Flow diagram



15.2 Mechanical structure

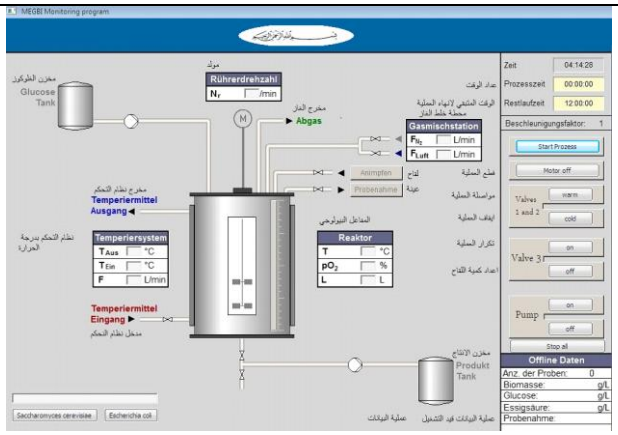
The concept is to install a simplified semi-synthetic penicillin production line based on the already existing mechanical structure (see picture below on the right).

[8] from [MEGBI-APP 2016]

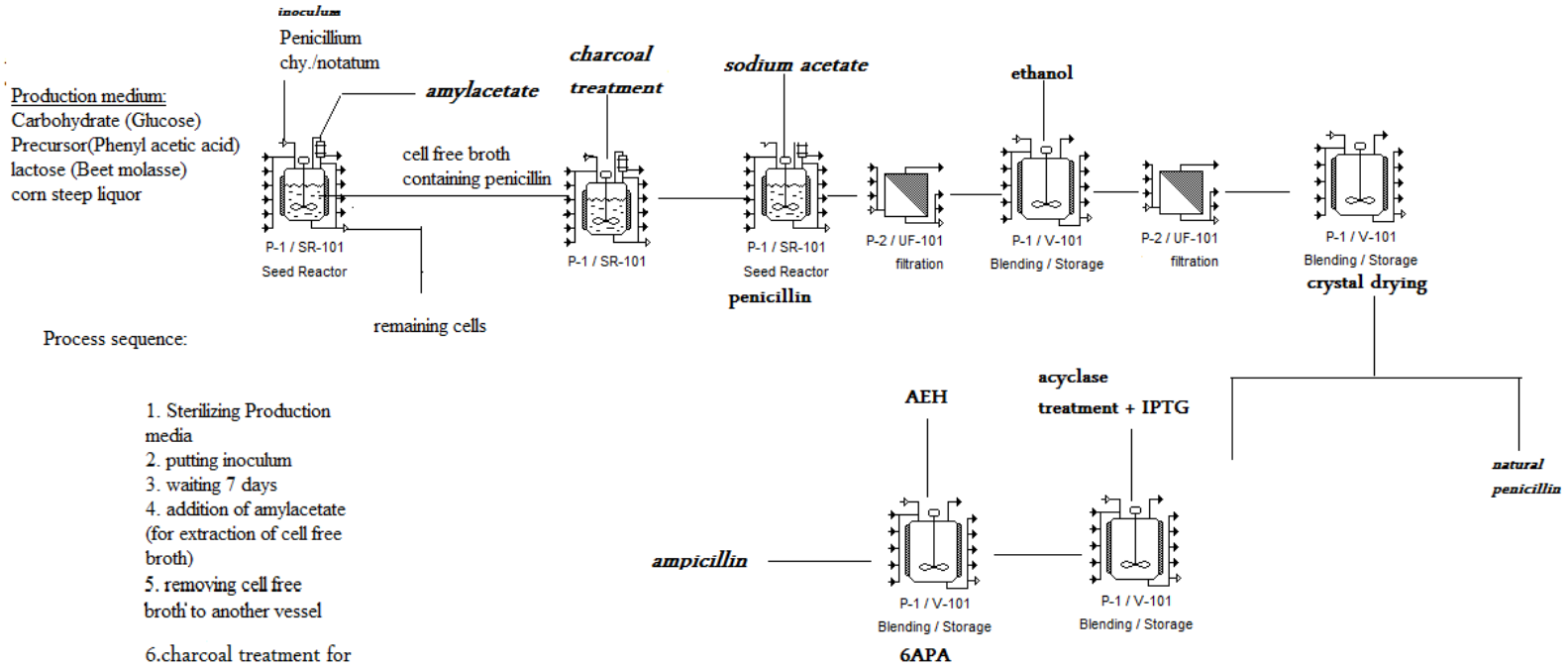


15.3 Automation System

The automation system shall have a C++/phython user interface and a Simatic S7 interface to the sensors/actuators.

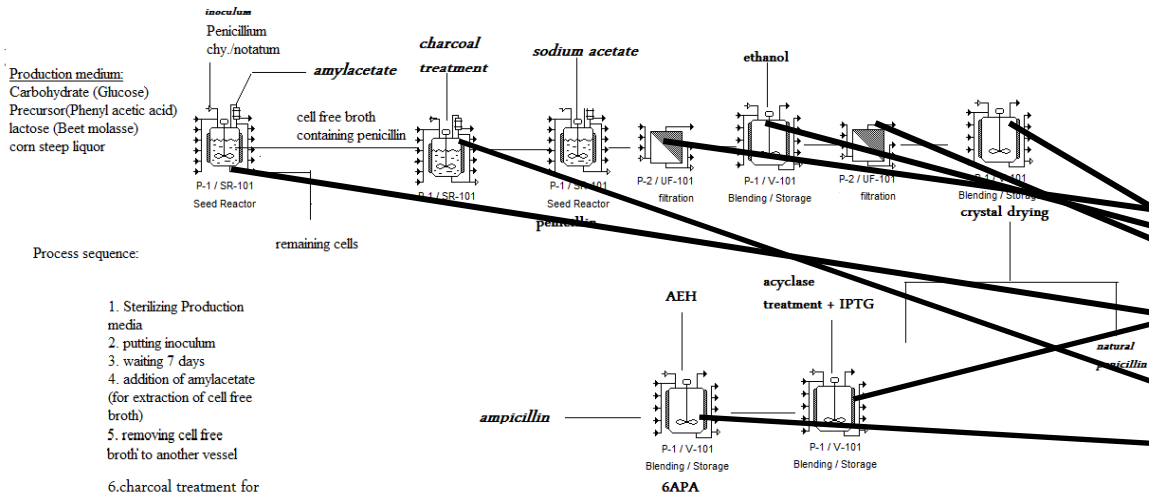


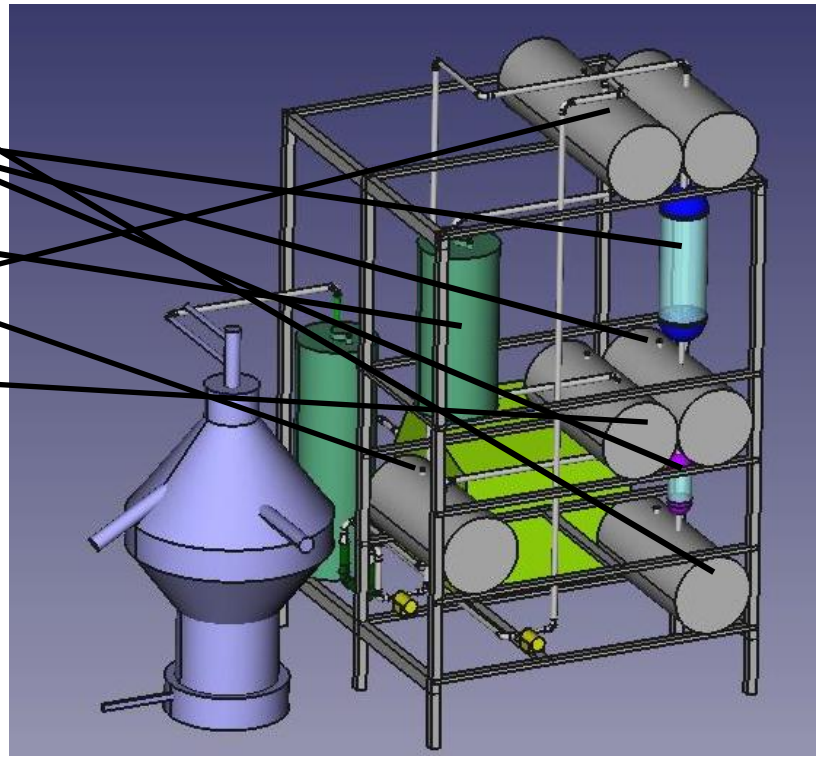
16 System Design (Ampicillin Pilot Production Plant Design)

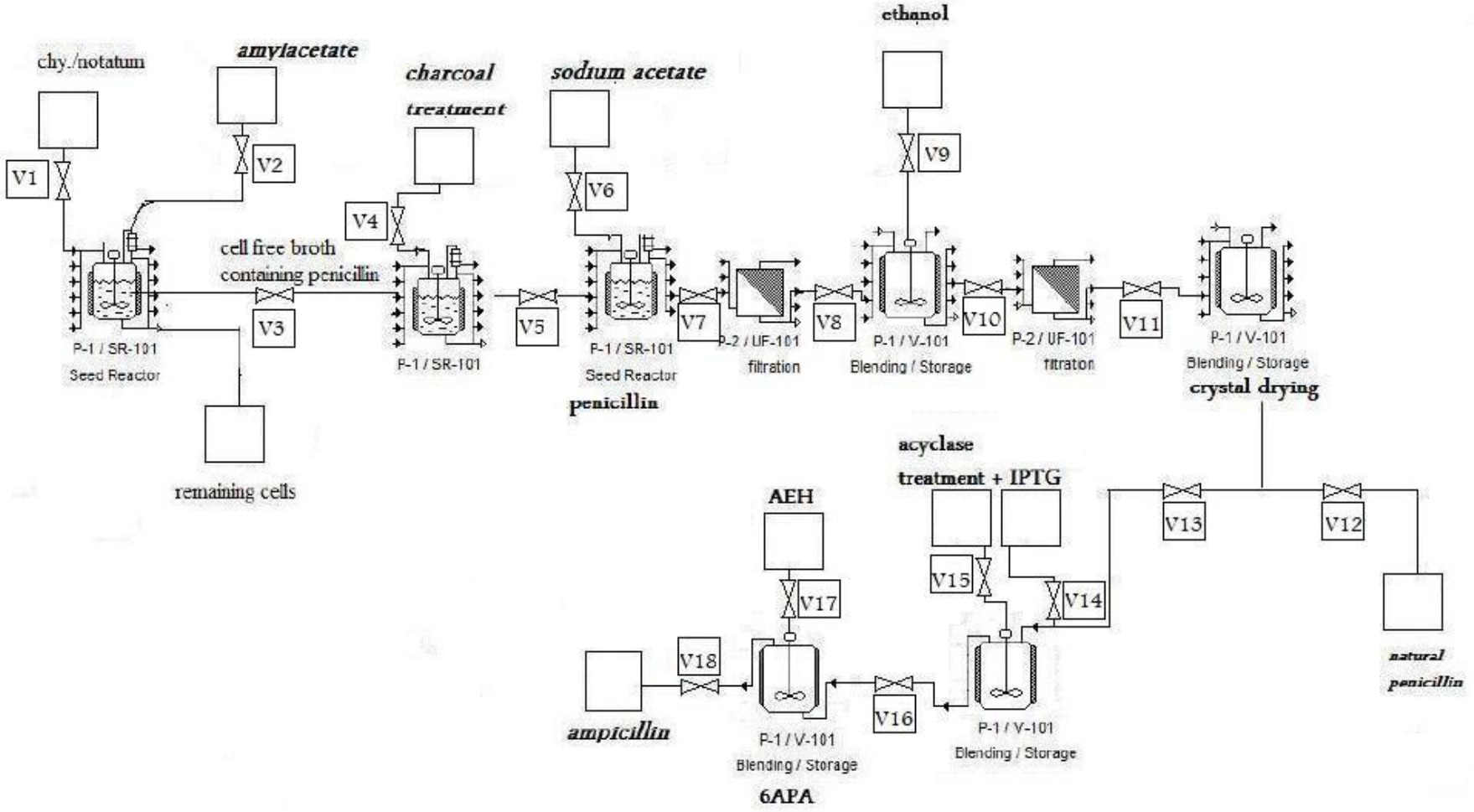


Process sequence:

1. Sterilizing Production media
2. putting inoculum
3. waiting 7 days
4. addition of amylacetate (for extraction of cell free broth)
5. removing cell free broth to another vessel
6. charcoal treatment for purification
7. sodium acetate for crystallization
8. filtation
9. addition ethanol
10. filtation
11. acyclase treatment+ IPTG for removing side chaine
12. addition AEH for ampicillin production


inoculum
Penicillium
chy./notatum
amylacetate
charcoal
treatment
sodium acetate
ethanol
Production medium:
Carbohydrate (Glucose)
Precursor(Phenyl acetic acid)
lactose (Beet molasse)
corn steep liquor
cell free broth
containing penicillin
P-1 / SR-101
Seed Reactor
P-2 / UF-101
filtration
P-1 / V-101
Blending / Storage
crystal drying
remaining cells
Process sequence:
1. Sterilizing Production media
2. putting inoculum
3. waiting 7 days
4. addition of amylacetate (for extraction of cell free broth)
5. removing cell free broth to another vessel
6.charcoal treatment for purification
7.sodium acetate for crystallization
8.filtation
9.addition ethanol
10.filtation
11.acyclase treatment+ IPTG for removing side chaine
12.addition AEH for ampicillin production
AEH
acyclase
treatment + IPTG
ampicillin
6APA
natural
penicillin




chy./notatum
amylacetate
charcoal
treatment
sodium acetate
ethanol
V1
V2
V3
V4
V5
V6
V7
V8
V9
V10
V11
cell free broth
containing penicillin
P-1 / SR-101
Seed Reactor
P-1 / SR-101
P-1 / SR-101
Seed Reactor
penicillin
P-2 / UF-101
filtration
P-1 / V-101
Blending / Storage
P-2 / UF-101
filtration
P-1 / V-101
Blending / Storage
crystal drying
remaining cells
acyclase
treatment + IPTG
AEH
V12
V13
V14
V15
V16
V17
V18
natural
penicillin
ampicillin
P-1 / V-101
Blending / Storage
P-1 / V-101
Blending / Storage
6APA

16.1.1 chrystal drying

easily but, instead, tends to block the filter. From the viewpoint of drying, it would be best that the crystals be large, within the range of about 1 mm or above. After the filtration stage, the amount of mother liquor in the crystals is low. The large crystal size also improves purity because the same thickness of the attached mother liquor on the surface, which contains impurities, results in a lower level of impurities in large crystals. If the mother liquor remains on the surface of the crystals, it solidifies, with the impurities that it contains, on the surface of the crystal. It should be mentioned here that crystal sizes above approximately 1 mm tend to be harmful. For crystals larger than 1 mm, it may be difficult to maintain the steady state in a continuous process, due to the decreased overall crystal surface required for releasing supersaturation. Furthermore, large crystals may break in the centrifuge.

The aim of the earlier discussion was to explain how crystals of a desired size could be produced. Furthermore, the CSD should be as narrow as possible for easy drying. In principle, the drying of crystals can be carried out in the same way as that of any particulate material. However, there are some cases when the crystalline structure itself poses problems in drying. We will briefly discuss these cases.

Most crystals are so soft that the corners of the crystalline particles tend to get rounded if collisions occur between the crystals during drying and, as a result, the quality of the product suffers. In addition, dust may be a problem. For example, a traditional rotary dryer is not suitable for most crystals. Surprisingly, both fluid-bed dryers and pneumatic dryers are relatively gentle, perhaps, because of the shorter residence time.

Then, there is the problem of crystal water. These are often salt hydrates, i.e., inorganic crystals with different numbers of water molecules attached to each molecule of the basic molecule. Drying may remove crystal water, which leads to quality problems in the product. Furthermore, crystallization at high temperatures may cause the agglomeration and solidification of the product during storage.

16.1.2 Filtration of sodium acetate and after adding of ethanol

...

16.1.3 Package 1: vessels for storing and mixing

Placing of storages based on flowdiagram

Costs: 1500$

16.1.4 Package 2: Chromatographic Columns

Costs: 1400$

16.1.5 Package 3: Pumps & Valves

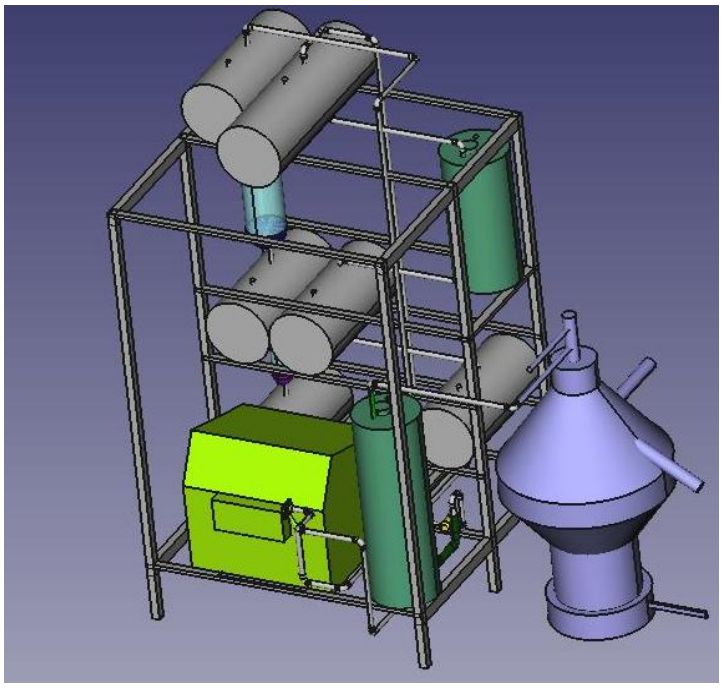
Costs: 3500$

16.1.6 Package 4: Piping

Costs: 1500$

16.2 Manufactored 24.12.-30.12.2015 (based on minimal system)

16.2.1 Design

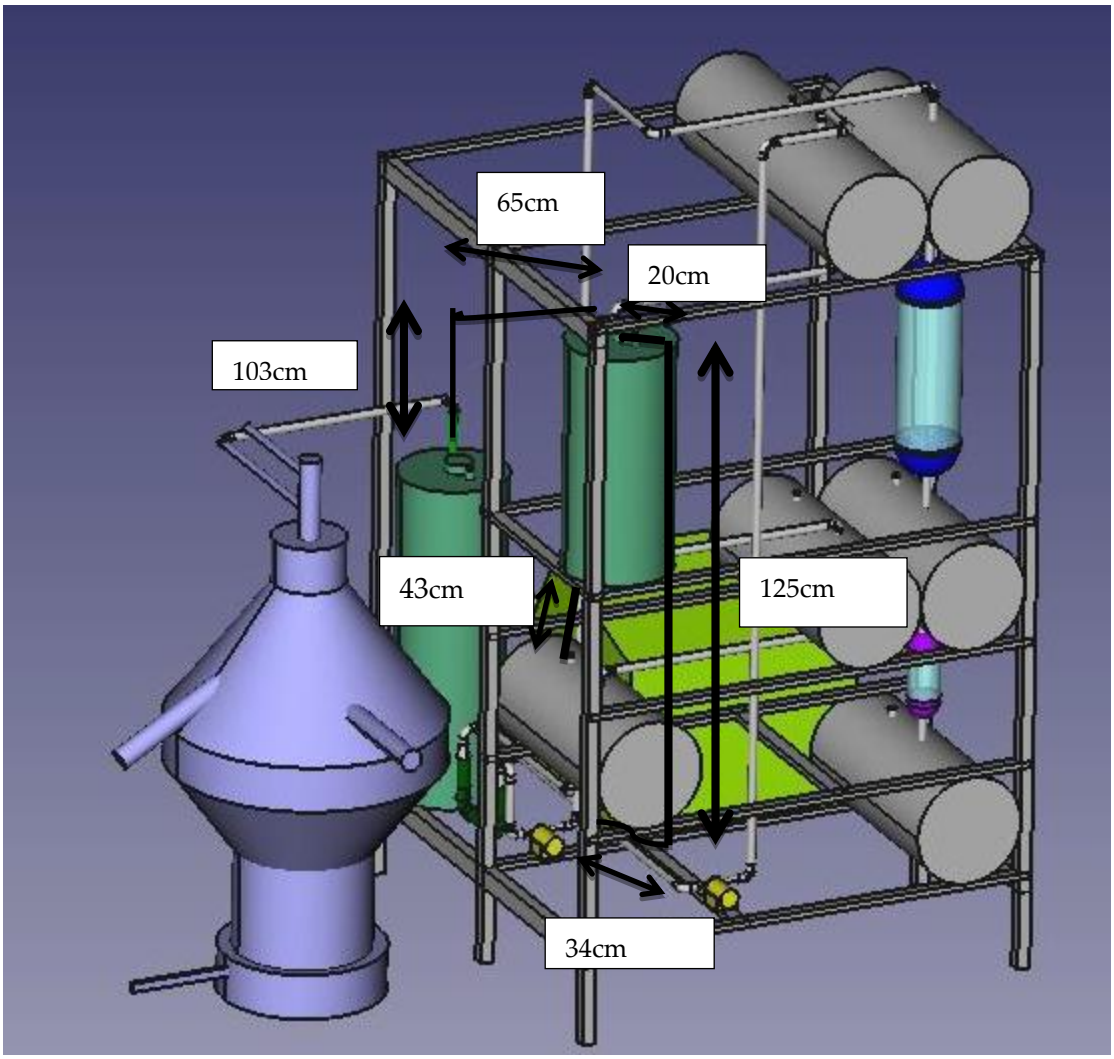


16.3 Simpliyfied System

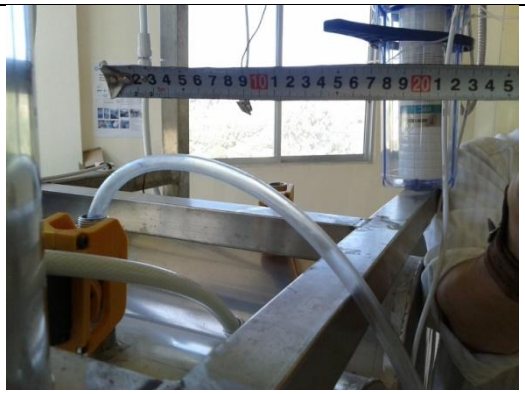
17 Installing Heat Sterilization Unit for the MEGBI-APP test rig

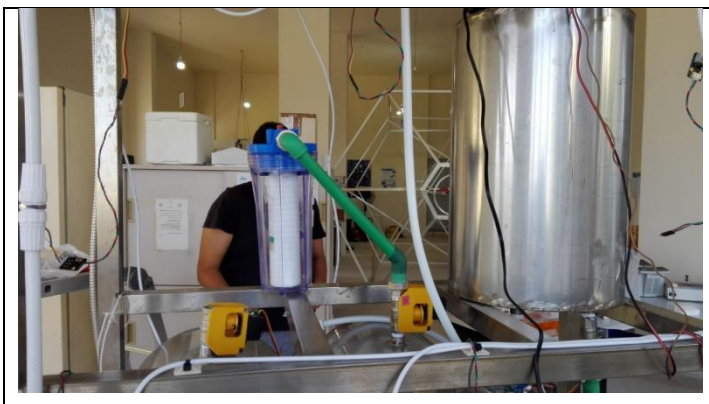
We will put an azone system to heat the water inside the bioreactor and control the 2-barrel test.

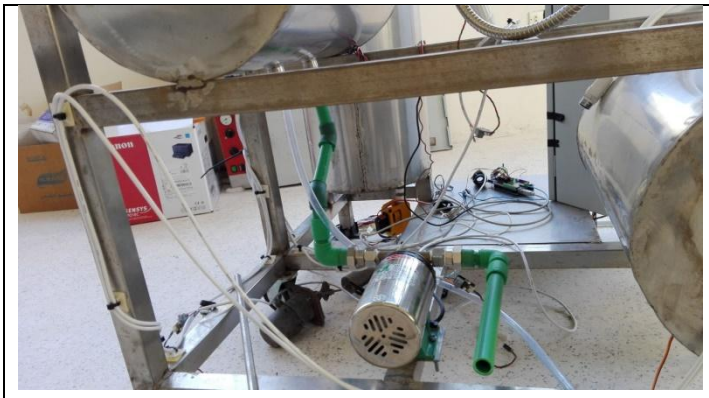
A system of tubes connects all the bioreactors use in our manipa to become sterilize the hot water vapor haudes so at the end when opening valve



New additions to the painting in black





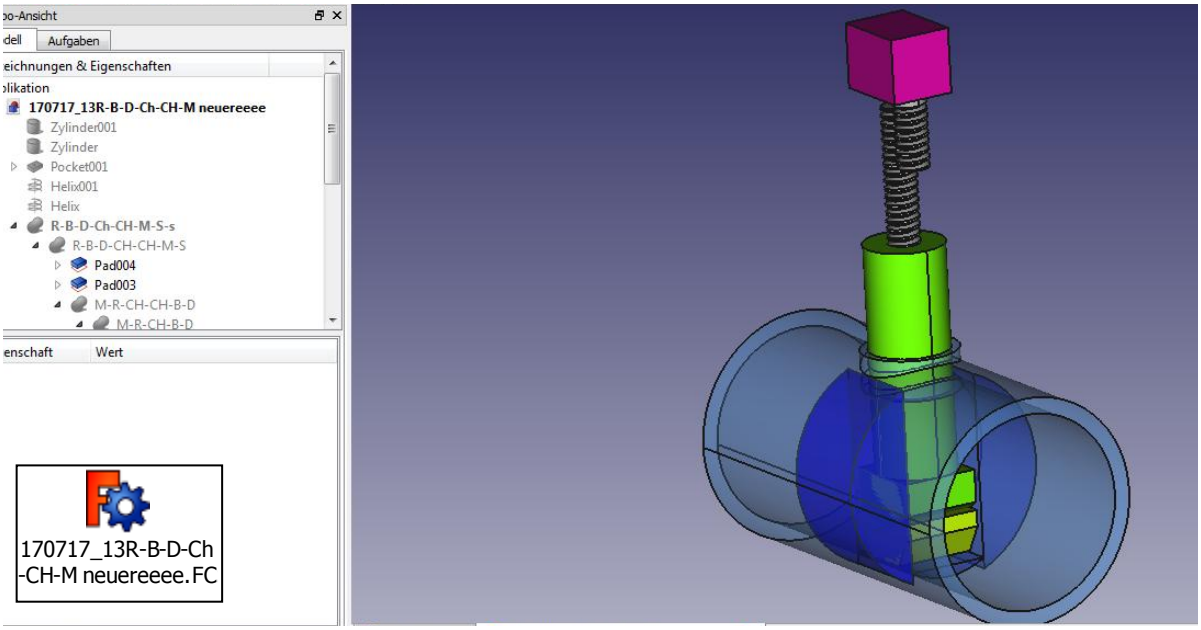



Boiler for steam generation for autoclaving

18 MEGBI-APP Process Control System 2017

18.1 Automatic Valves: Conception

18.1.1 Preliminary Design of Automatic Control Valve



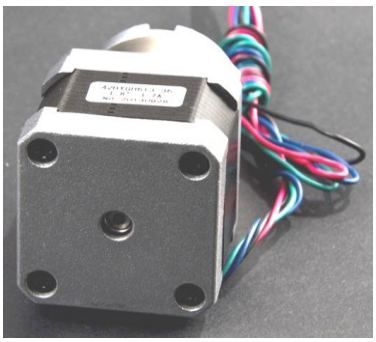
18.1.2 Alternative 1: DC Motor for automatic valves

from www.cnclablb.com: **Metal DC Geared Motor - 12V 50RPM 9kg.cm rated torque,** Price : 15.95$, Serial number : ACT0022

Description: This is a metal DC geared motor, 100% pure copper coils, high-density molecular layer, 100:1 metal reducer, small size, large torque. The maximum torque could arrive 50 kg.cm, stable and durable!

Specification: Rated voltage: 12 V, Gear reduction ratio: 100:1, D output shaft diameter: 6 mm, No-load speed: 50 RPM @ 12 v, No-load current: 0.17 A, Rated speed: 45 RPM @ 12 v, Current rating: 0.68 A, Rated torque: 9 kg.cm, Locked-rotor torque: 50 kg.cm, Locked-rotor current: 2.19 A, Power: 5W, Weight: 210 g, **Shipping List:** Metal DC Geared Motor - 12V 50RPM 50kg.cm x1

18.1.3 Alternative 2: Stepper Motor



From www.cnclablb.com

from www.cnclablb.com: Bipolar Stepper Motor with Planet Gear Box (18kg.cm), Price : 40$, Serial number : ACT0017, **!!!needs additional drive!!!**

18.1.4 Alternative3: Servo

18.1.4.1 Low Cost Servo

	from www.cnclablb.com: Metal Gear Servo TowerPro MG995 Servo - 9kg, Price : 8$ Serial number : ACT0005 **Description:** Modulation: Digital, Torque: 4.8V: 130.54 oz-in (**9.40 kg-cm**) 6.0V: 152.76 oz-in (11.00 kg-cm) Speed: 4.8V: 0.20 sec/60° 6.0V: 0.16 sec/60°, Weight: 1.94 oz (55.0 g), Dimensions:Length:1.60 in (40.7 mm), Width:0.78 in (19.7 mm), Height:1.69 in (42.9 mm)

18.1.4.2 High cost Servo

	DF15MG Tilt/Pan Kit, Price : 47.5$, Mark : DFRobot, Serial number : FIT0046 This is a 2DOF Pan and Tilt Kit assembly for horizontal surface mount. It equipped with a DF15MG servo which offers 15 kg high-torque

18.2 Actual Motorized Valve Implementation

18.2.1 Hardware and Electronics

18.2.1.1 Adopted Motor: Low Cost Servo (Alternative 3 (Low Cost Variante)

The adopted motor is the TowerPro MG995 DC Servo Motor with the following specs:

- Modulation: Digital
- Torque: 4.8V: 9.40 kg-cm 6.0V: 11.00 kg-cm
- Speed: 4.8V: 0.20 sec/60° 6.0V: 0.16 sec/60°
- Weight: 1.94 oz (55.0 g)
- Dimensions:Length:1.60 in (40.7 mm)
- Width:0.78 in (19.7 mm)
- Height:1.69 in (42.9 mm)
- LINK – CNC LAB Shop

Figure 18-1 – TowerPro MG995

The adopted motor provides the required torque to turn the ball valve.

A set of 18 servos are used with a control unit shown in 6.2.2 to allow opening and closing of 18 ball valves.

18.2.1.2 Motor Controller and Interfaces

To accommodate 18 servo motors and ensure best response the Arduino Mega 2560 was chosen for the following reasons:

- Enough PPM capable IO count to control the servos. The Arduino Mega 2560 allows control of 48 Servo motors while most of other Arduino boards allow control of only 12 servos max.
- Availability of an IO shield that makes powering and connecting all the servos much more convenient and much less time consuming.

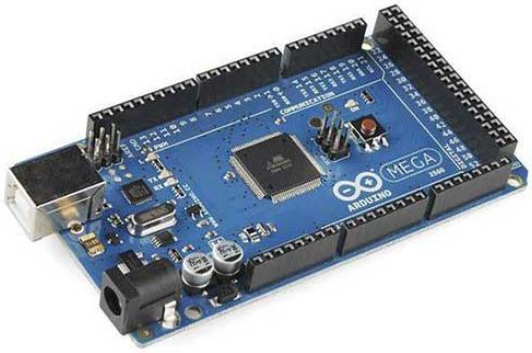


Figure 18-2 – Arduino Mega - LINK

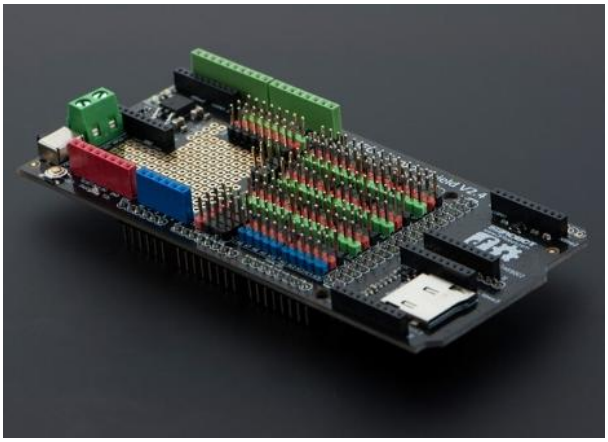


Figure 18-3 – Mega Sensor Shield - LINK

Interfacing between MEGBI python GUI and the servos can be accomplished in two ways:

a. Via Digital input signals on the Arduino Shield.

b. Via Communication through the Arduino USB port.

Digital interface mode and communication mode can be used at the same time if necessary.

The following IO map illustrates the IO allocation for the servos and the digital inputs on the Arduino Shield:

VAVLE ID	COMMAND PIN (ARDUINO INPUT)	SERVO PIN (ARDUINO OUTPUT)
1	DIO 33	DIO 14
2	DIO 34	DIO 15
3	DIO 35	DIO 16
4	DIO 36	DIO 17
5	DIO 37	DIO 18
6	DIO 38	DIO 19
7	DIO 39	DIO 20
8	DIO 40	DIO 21
9	DIO 41	DIO 22
10	DIO 42	DIO 23
11	DIO 43	DIO 24
12	DIO 44	DIO 25
13	DIO 45	DIO 26
14	DIO 46	DIO 27
15	DIO 47	DIO 28
16	DIO 48	DIO 29
17	DIO 49	DIO 30
18	DIO 50	DIO 31

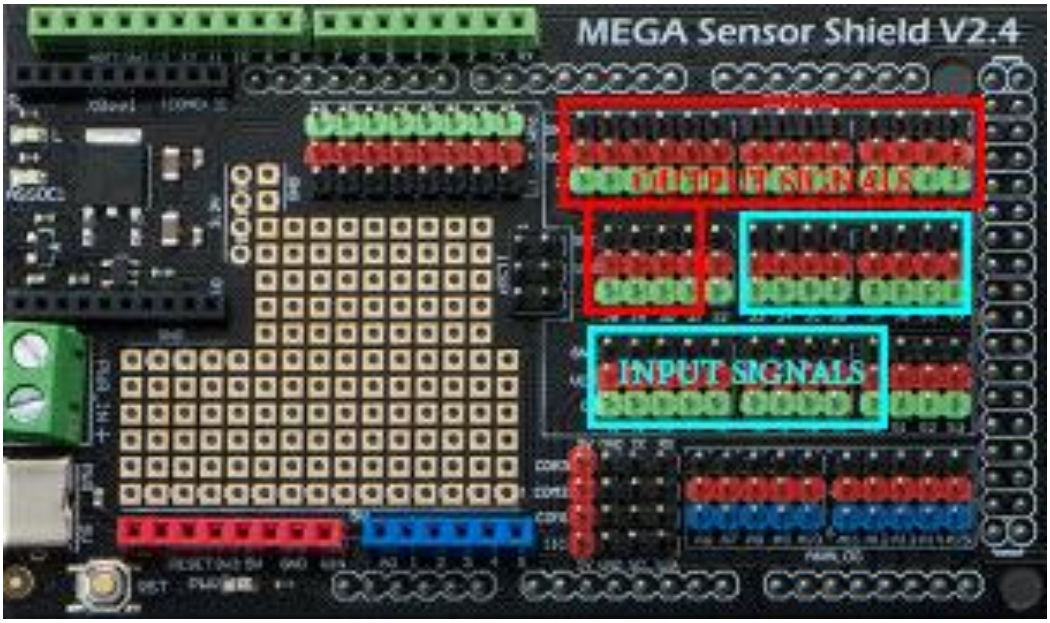


Figure 18-4 – Inputs and Outputs Allocation

The digital input mode of control allows closing and openning the valves by set or clearing the corresponding DIO respectively.

On the other hand, controlling the valves via USB communication with Arduino is implemented in an example Python code using a couple of Python classes discussed in more details in part 6.2.2.

18.2.1.3 Power Management

One of the reasons of choosing Arduino Mega IO shield was powering the motors as mentioned earlier, as 18 Servo motors can consume a hefty amount of power.

Each servo motor can consume up to 1.2 Amps at 5V at certain moments when closing or openning the valves. Thus in terms of power management the following mesures were taken:

- The IO shield allows powering the servos from a separate power connector (Green screw terminal in Fig6-4) thus isolating the limited Arduino regulator from motors consumption and ensuring microcontroller chip performance and functionality.
- Within the Arduino Firmware, precautions were taken so that the servos are only consuming power while opening or closing and for a limited time beyond that. After the time delay of a motor's activity the motor is powered down to cut its consumption to almost zero Amps.

Having mentioned the above points, selecting the motors power supply is highly related to the number of motors that are expected to be active simultaneously. For example, if the automatic mode of the plant requires that 6 motors have to be active at a certain moment; and active means is currently in the process of opening or closing; then the power supply should be a 5 VDC with at least 6 x 1.2A = 7.2 Amps.

The arduino board itself can be powered either by a USB cable connected to PC or by any standard wall adapter with voltage between 7.4V and 12V.

18.2.2 Firmware and Software

18.2.2.1 Arduino Firmware

The Arduino controller is loaded with a firmware featuring the following: • Control of 18 Servo motors with preset positions for closed and opened valve. • Digital Input control for all 18 valves. • Communication protocol class for two way communication with Python GUI on PC. • Power management for all motors. The firmware was developed by CNC LAB. The code is developed with maintenance and scalability in mind.	 ArduinoSourceCode.zip

CommandMessanger.h

```
/*
CmdMessenger - library that provides command based messaging
Permission is hereby granted, free of charge, to any person obtaining
a copy of this software and associated documentation files (the
"Software"), to deal in the Software without restriction, including
without limitation the rights to use, copy, modify, merge, publish,
distribute, sublicense, and/or sell copies of the Software, and to
permit persons to whom the Software is furnished to do so, subject to
the following conditions:
The above copyright notice and this permission notice shall be
included in all copies or substantial portions of the Software.
THE SOFTWARE IS PROVIDED "AS IS", WITHOUT WARRANTY OF ANY KIND,
```

```
EXPRESS OR IMPLIED, INCLUDING BUT NOT LIMITED TO THE WARRANTIES OF
MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE AND
NONINFRINGEMENT. IN NO EVENT SHALL THE AUTHORS OR COPYRIGHT HOLDERS BE
LIABLE FOR ANY CLAIM, DAMAGES OR OTHER LIABILITY, WHETHER IN AN ACTION
OF CONTRACT, TORT OR OTHERWISE, ARISING FROM, OUT OF OR IN CONNECTION
WITH THE SOFTWARE OR THE USE OR OTHER DEALINGS IN THE SOFTWARE.
*/
#ifndef CmdMessenger_h
#define CmdMessenger_h
#include <inttypes.h>
#if ARDUINO >= 100
#include <Arduino.h>
#else
#include <WProgram.h>
#endif
//#include "Stream.h"
extern "C"
{
// callback functions always follow the signature: void cmd(void);
typedef void(*messengerCallbackFunction)(void);
}
#define MAXCALLBACKS 50 // The maximum number of commands (default: 50)
#define MESSENGERBUFFERSIZE 64 // The length of the commandbuffer (default: 64)
#define MAXSTREAMBUFFERSIZE 512 // The length of the streambuffer (default: 64)
#define DEFAULT_TIMEOUT 5000 // Time out on unanswered messages. (default: 5s)
// Message States
enum
{
kProccesingMessage,// Message is being received, not reached command separator
kEndOfMessage,// Message is fully received, reached command separator
kProcessingArguments,// Message is received, arguments are being read parsed
};
#define white_space(c) ((c) == ' ' || (c) == '\t')
#define valid_digit(c) ((c) >= '0' && (c) <= '9')

class CmdMessenger
{
private:
// **** Private variables ***
bool startCommand;// Indicates if sending of a command is underway
uint8_t lastCommandId;// ID of last received command
uint8_t bufferIndex;// Index where to write data in buffer
uint8_t bufferLength;// Is set to MESSENGERBUFFERSIZE
uint8_t bufferLastIndex;// The last index of the buffer
char ArglastChar;// Bookkeeping of argument escape char
char CmdlastChar;// Bookkeeping of command escape char
bool pauseProcessing;// pauses processing of new commands, during sending
bool print_newlines;// Indicates if \r\n should be added after send command
char commandBuffer[MESSENGERBUFFERSIZE];// Buffer that holds the data
char streamBuffer[MAXSTREAMBUFFERSIZE];// Buffer that holds the data
uint8_t messageState;// Current state of message processing
bool dumped;// Indicates if last argument has been externally read
bool ArgOk;// Indicated if last fetched argument could be read
char *current;// Pointer to current buffer position
char *last;// Pointer to previous buffer position
char prevChar;// Previous char (needed for unescaping)
Stream *comms;// Serial data stream
char command_separator;// Character indicating end of command (default: ';')
char field_separator;// Character indicating end of argument (default: ',')
char escape_character;// Character indicating escaping of special chars
messengerCallbackFunction default_callback;// default callback function
messengerCallbackFunction callbackList[MAXCALLBACKS];// list of attached callback
functions
// **** Initialize ****
void init(Stream &comms,const char fld_separator,const char cmd_separator,const char
esc_character);
void reset();
// **** Command processing ****
```

```
inline uint8_t processLine(char serialChar) __attribute__((always_inline));
inline void handleMessage() __attribute__((always_inline));
inline bool blockedTillReply(unsigned int timeout = DEFAULT_TIMEOUT, byte ackCmdId = 1)
__attribute__((always_inline));
inline bool checkForAck(byte AckCommand) __attribute__((always_inline));
// **** Command sending ****
/**
* Print variable of type T binary in binary format
*/
template < class T >
void writeBin(const T & value)
{
const byte *bytePointer = (const byte *)(const void *)&value;
for (unsigned int i = 0; i < sizeof(value); i++)
{
printEsc(*bytePointer);
bytePointer++;
}
}
// **** Command receiving ****
int findNext(char *str, char delim);
/**
* Read a variable of any type in binary format
*/
template < class T >
T readBin(char *str)
{
T value;
unescape(str);
byte *bytePointer = (byte *)(const void *)&value;
for (unsigned int i = 0; i < sizeof(value); i++)
{
*bytePointer = str[i];
bytePointer++;
}
return value;
}
template < class T >
T empty()
{
T value;
byte *bytePointer = (byte *)(const void *)&value;
for (unsigned int i = 0; i < sizeof(value); i++)
{
*bytePointer = '\0';
bytePointer++;
}
return value;
}
// **** Escaping tools ****
char *split_r(char *str, const char delim, char **nextp);
bool isEscaped(char *currChar, const char escapeChar, char *lastChar);
void printEsc(char *str);
void printEsc(char str);
public:
// ****** Public functions ******
// **** Initialization ****
CmdMessenger(Stream & comms, const char fld_separator = ',',
const char cmd_separator = ';',
const char esc_character = '/');
void printLfCr(bool addNewLine = true);
void attach(messengerCallbackFunction newFunction);
void attach(byte msgId, messengerCallbackFunction newFunction);
// **** Command processing ****
void feedinSerialData();
bool next();
```

```
bool available();
bool isArgOk();
uint8_t commandID();
// **** Command sending ****
/**
* Send a command with a single argument of any type
* Note that the argument is sent as string
*/
template < class T >
bool sendCmd(byte cmdId, T arg, bool reqAc = false, byte ackCmdId = 1,
unsigned int timeout = DEFAULT_TIMEOUT)
{
if (!startCommand) {
sendCmdStart(cmdId);
sendCmdArg(arg);
return sendCmdEnd(reqAc, ackCmdId, timeout);
}
return false;
}
/**
* Send a command with a single argument of any type
* Note that the argument is sent in binary format
*/
template < class T >
bool sendBinCmd(byte cmdId, T arg, bool reqAc = false, byte ackCmdId = 1,
unsigned int timeout = DEFAULT_TIMEOUT)
{
if (!startCommand) {
sendCmdStart(cmdId);
sendCmdBinArg(arg);
return sendCmdEnd(reqAc, ackCmdId, timeout);
}
return false;
}
bool sendCmd(byte cmdId);
bool sendCmd(byte cmdId, bool reqAc, byte ackCmdId);
// **** Command sending with multiple arguments ****
void sendCmdStart(byte cmdId);
void sendCmdEscArg(char *arg);
void sendCmdfArg(char *fmt, ...);
bool sendCmdEnd(bool reqAc = false, byte ackCmdId = 1, unsigned int timeout =
DEFAULT_TIMEOUT);
/**
* Send a single argument as string
* Note that this will only succeed if a sendCmdStart has been issued first
*/
template < class T > void sendCmdArg(T arg)
{
if (startCommand) {
comms->print(field_separator);
comms->print(arg);
}
}
/**
* Send a single argument as string with custom accuracy
* Note that this will only succeed if a sendCmdStart has been issued first
*/
template < class T > void sendCmdArg(T arg, unsigned int n)
{
if (startCommand) {
comms->print(field_separator);
comms->print(arg, n);
}
}
/**
* Send double argument in scientific format.
* This will overcome the boundary of normal d sending which is limited to abs(f) <=
```

```
MAXLONG
*/
void sendCmdSciArg(double arg, unsigned int n = 6);
/**
* Send a single argument in binary format
* Note that this will only succeed if a sendCmdStart has been issued first
*/
template < class T > void sendCmdBinArg(T arg)
{
if (startCommand) {
comms->print(field_separator);
writeBin(arg);
}
}
// **** Command receiving ****
bool readBoolArg();
int16_t readInt16Arg();
int32_t readInt32Arg();
char readCharArg();
float readFloatArg();
double readDoubleArg();
char *readStringArg();
void copyStringArg(char *string, uint8_t size);
uint8_t compareStringArg(char *string);
/**
* Read an argument of any type in binary format
*/
template < class T > T readBinArg()
{
if (next()) {
dumped = true;
return readBin < T >(current);
}
else {
return empty < T >();
}
}
// **** Escaping tools ****
void unescape(char *fromChar);
void printSci(double f, unsigned int digits);
};
#endif
```

CommandMsg.cpp

```
/*
CmdMessenger - library that provides command based messaging
Permission is hereby granted, free of charge, to any person obtaining
a copy of this software and associated documentation files (the
"Software"), to deal in the Software without restriction, including
without limitation the rights to use, copy, modify, merge, publish,
distribute, sublicense, and/or sell copies of the Software, and to
permit persons to whom the Software is furnished to do so, subject to
the following conditions:
The above copyright notice and this permission notice shall be
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THE SOFTWARE IS PROVIDED "AS IS", WITHOUT WARRANTY OF ANY KIND,
EXPRESS OR IMPLIED, INCLUDING BUT NOT LIMITED TO THE WARRANTIES OF
MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE AND
NONINFRINGEMENT. IN NO EVENT SHALL THE AUTHORS OR COPYRIGHT HOLDERS BE
LIABLE FOR ANY CLAIM, DAMAGES OR OTHER LIABILITY, WHETHER IN AN ACTION
OF CONTRACT, TORT OR OTHERWISE, ARISING FROM, OUT OF OR IN CONNECTION
WITH THE SOFTWARE OR THE USE OR OTHER DEALINGS IN THE SOFTWARE.
Initial Messenger Library - Thomas Ouellet Fredericks.
CmdMessenger Version 1 - Neil Dudman.
CmdMessenger Version 2 - Dreamcat4.
CmdMessenger Version 3 - Thijs Elenbaas.
3.6 - Fixes
```

```
- Better compatibility between platforms
- Unit tests
3.5 - Fixes, speed improvements for Teensy
3.4 - Internal update
3.3 - Fixed warnings
- Some code optimization
3.2 - Small fixes and sending long argument support
3.1 - Added examples
3.0 - Bugfixes on 2.2
- Wait for acknowlegde
- Sending of common type arguments (float, int, char)
- Multi-argument commands
- Escaping of special characters
- Sending of binary data of any type (uses escaping)
*/
extern "C" {
#include <stdlib.h>
#include <stdarg.h>
}
#include <stdio.h>
#include "CmdMessenger.h"
#define _CMDMESSENGER_VERSION 3_6 // software version of this library
// **** Initialization ****
/**
* CmdMessenger constructor
*/

CmdMessenger::CmdMessenger(Stream &ccomms, const char fld_separator, const char
cmd_separator, const char esc_character)
{
init(ccomms, fld_separator, cmd_separator, esc_character);
}
/**
* Enables printing newline after a sent command
*/
void CmdMessenger::init(Stream &ccomms, const char fld_separator, const char cmd_separator,
const char esc_character)
{
default_callback = NULL;
comms = &ccomms;
print_newlines = false;
field_separator = fld_separator;
command_separator = cmd_separator;
escape_character = esc_character;
bufferLength = MESSENGERBUFFERSIZE;
bufferLastIndex = MESSENGERBUFFERSIZE - 1;
reset();
default_callback = NULL;
for (int i = 0; i < MAXCALLBACKS; i++)
callbackList[i] = NULL;
pauseProcessing = false;
}
/**
* Resets the command buffer and message state
*/
void CmdMessenger::reset()
{
bufferIndex = 0;
current = NULL;
last = NULL;
dumped = true;
}
/**
* Enables printing newline after a sent command
*/
void CmdMessenger::printLfCr(bool addNewLine)
{
print_newlines = addNewLine;
```

```
}
/**
* Attaches an default function for commands that are not explicitly attached
*/
void CmdMessenger::attach(messengerCallbackFunction newFunction)
{
default_callback = newFunction;
}
/**
* Attaches a function to a command ID
*/
void CmdMessenger::attach(byte msgId, messengerCallbackFunction newFunction)
{
if (msgId >= 0 && msgId < MAXCALLBACKS)
callbackList[msgId] = newFunction;
}
// **** Command processing ****
/**
* Feeds serial data in CmdMessenger
*/
void CmdMessenger::feedinSerialData()
{
while (!pauseProcessing && comms->available())
{
// The Stream class has a readBytes() function that reads many bytes at once. On
Teensy 2.0 and 3.0, readBytes() is optimized.
// Benchmarks about the incredible difference it makes:
http://www.pjrc.com/teensy/benchmark_usb_serial_receive.html
size_t bytesAvailable = min(comms->available(), MAXSTREAMBUFFERSIZE);
comms->readBytes(streamBuffer, bytesAvailable);
// Process the bytes in the stream buffer, and handles dispatches callbacks, if
commands are received
for (size_t byteNo = 0; byteNo < bytesAvailable; byteNo++)
{
int messageState = processLine(streamBuffer[byteNo]);
// If waiting for acknowledge command
if (messageState == kEndOfMessage)
{
handleMessage();
}
}
}
}
/**
* Processes bytes and determines message state
*/
uint8_t CmdMessenger::processLine(char serialChar)
{
messageState = kProccesingMessage;
//char serialChar = (char)serialByte;
bool escaped = isEscaped(&serialChar, escape_character, &CmdlastChar);
if ((serialChar == command_separator) && !escaped) {
commandBuffer[bufferIndex] = 0;
if (bufferIndex > 0) {
messageState = kEndOfMessage;
current = commandBuffer;
CmdlastChar = '\0';
}
reset();
}
else {
commandBuffer[bufferIndex] = serialChar;
bufferIndex++;
if (bufferIndex >= bufferLastIndex) reset();
}
return messageState;
}
```

```
/**
* Dispatches attached callbacks based on command
*/
void CmdMessenger::handleMessage()
{
lastCommandId = readInt16Arg();
// if command attached, we will call it
if (lastCommandId >= 0 && lastCommandId < MAXCALLBACKS && ArgOk &&
callbackList[lastCommandId] != NULL)
(*callbackList[lastCommandId])();
else // If command not attached, call default callback (if attached)
if (default_callback != NULL) (*default_callback)();
}
/**
* Waits for reply from sender or timeout before continuing
*/
bool CmdMessenger::blockedTillReply(unsigned int timeout, byte ackCmdId)
{
unsigned long time = millis();
unsigned long start = time;
bool receivedAck = false;
while ((time - start) < timeout && !receivedAck) {
time = millis();
receivedAck = checkForAck(ackCmdId);
}
return receivedAck;
}
/**
* Loops as long data is available to determine if acknowledge has come in
*/
bool CmdMessenger::checkForAck(byte ackCommand)
{
while (comms->available()) {
//Processes a byte and determines if an acknowlegde has come in
int messageState = processLine(comms->read());
if (messageState == kEndOfMessage) {
int id = readInt16Arg();
if (ackCommand == id && ArgOk) {
return true;
}
else {
return false;
}
}
return false;
}
return false;

}
/**
* Gets next argument. Returns true if an argument is available
*/
bool CmdMessenger::next()
{
char *temppointer = NULL;
// Currently, cmd messenger only supports 1 char for the field seperator
switch (messageState) {
case kProccesingMessage:
return false;
case kEndOfMessage:
temppointer = commandBuffer;
messageState = kProcessingArguments;
default:
if (dumped)
current = split_r(temppointer, field_separator, &last);
if (current != NULL) {
dumped = true;
```

```
return true;
}
}
return false;
}
/**
* Returns if an argument is available. Alias for next()
*/
bool CmdMessenger::available()
{
return next();
}
/**
* Returns if the latest argument is well formed.
*/
bool CmdMessenger::isArgOk()
{
return ArgOk;
}
/**
* Returns the commandID of the current command
*/
uint8_t CmdMessenger::commandID()
{
return lastCommandId;
}
// **** Command sending ****
/**
* Send start of command. This makes it easy to send multiple arguments per command
*/
void CmdMessenger::sendCmdStart(byte cmdId)
{
if (!startCommand) {
startCommand = true;
pauseProcessing = true;
comms->print(cmdId);
}
}
/**
* Send an escaped command argument
*/
void CmdMessenger::sendCmdEscArg(char* arg)
{
if (startCommand) {
comms->print(field_separator);
printEsc(arg);
}
}
/**
* Send formatted argument.
* Note that floating points are not supported and resulting string is limited to 128 chars
*/
void CmdMessenger::sendCmdfArg(char *fmt, ...)
{
const int maxMessageSize = 128;
if (startCommand) {
char msg[maxMessageSize];
va_list args;
va_start(args, fmt);
vsnprintf(msg, maxMessageSize, fmt, args);
va_end(args);
comms->print(field_separator);
comms->print(msg);
}
}
/**
* Send double argument in scientific format.
* This will overcome the boundary of normal float sending which is limited to abs(f) <=
```

```
MAXLONG
*/
void CmdMessenger::sendCmdSciArg(double arg, unsigned int n)
{
if (startCommand)
{
comms->print(field_separator);
printSci(arg, n);
}
}
/**
* Send end of command
*/
bool CmdMessenger::sendCmdEnd(bool reqAc, byte ackCmdId, unsigned int timeout)
{
bool ackReply = false;

if (startCommand) {
comms->print(command_separator);
if (print_newlines)
comms->println(); // should append BOTH \r\n
if (reqAc) {
ackReply = blockedTillReply(timeout, ackCmdId);
}
}
pauseProcessing = false;
startCommand = false;
return ackReply;
}
/**
* Send a command without arguments, with acknowledge
*/
bool CmdMessenger::sendCmd(byte cmdId, bool reqAc, byte ackCmdId)
{
if (!startCommand) {
sendCmdStart(cmdId);
return sendCmdEnd(reqAc, ackCmdId, DEFAULT_TIMEOUT);
}
return false;
}
/**
* Send a command without arguments, without acknowledge
*/
bool CmdMessenger::sendCmd(byte cmdId)
{
if (!startCommand) {
sendCmdStart(cmdId);
return sendCmdEnd(false, 1, DEFAULT_TIMEOUT);
}
return false;
}
// **** Command receiving ****
/**
* Find next argument in command
*/
int CmdMessenger::findNext(char *str, char delim)
{
int pos = 0;
bool escaped = false;
bool EOL = false;
ArglastChar = '\0';
while (true) {
escaped = isEscaped(str, escape_character, &ArglastChar);
EOL = (*str == '\0' && !escaped);
if (EOL) {
return pos;
}
```

```
if (*str == field_separator && !escaped) {
return pos;
}
else {
str++;
pos++;
}
}
return pos;
}
/**
* Read the next argument as int
*/
int16_t CmdMessenger::readInt16Arg()
{
if (next()) {
dumped = true;
ArgOk = true;
return atoi(current);
}
ArgOk = false;
return 0;
}
/**
* Read the next argument as int
*/
int32_t CmdMessenger::readInt32Arg()
{
if (next()) {
dumped = true;
ArgOk = true;
return atol(current);
}
ArgOk = false;
return 0L;
}
/**
* Read the next argument as bool
*/
bool CmdMessenger::readBoolArg()
{
return (readInt16Arg() != 0) ? true : false;
}
/**
* Read the next argument as char
*/
char CmdMessenger::readCharArg()
{
if (next()) {
dumped = true;
ArgOk = true;
return current[0];
}
ArgOk = false;
return 0;
}
/**
* Read the next argument as float
*/
float CmdMessenger::readFloatArg()
{
if (next()) {
dumped = true;
ArgOk = true;
//return atof(current);
return strtod(current, NULL);
```

```
}
ArgOk = false;
return 0;
}
/**
* Read the next argument as double
*/
double CmdMessenger::readDoubleArg()
{
if (next()) {
dumped = true;
ArgOk = true;
return strtod(current, NULL);
}
ArgOk = false;
return 0;
}
/**
* Read next argument as string.
* Note that the String is valid until the current command is replaced
*/
char* CmdMessenger::readStringArg()
{
if (next()) {
dumped = true;
ArgOk = true;
return current;
}
ArgOk = false;
return '\0';
}
/**
* Return next argument as a new string
* Note that this is useful if the string needs to be persisted
*/
void CmdMessenger::copyStringArg(char *string, uint8_t size)
{
if (next()) {
dumped = true;
ArgOk = true;
strlcpy(string, current, size);
}
else {
ArgOk = false;
if (size) string[0] = '\0';
}
}
/**
* Compare the next argument with a string
*/
uint8_t CmdMessenger::compareStringArg(char *string)
{
if (next()) {
if (strcmp(string, current) == 0) {
dumped = true;
ArgOk = true;
return 1;
}
else {
ArgOk = false;
return 0;
}
}
return 0;
}
// **** Escaping tools ****
```

```
/**
* Unescapes a string
* Note that this is done inline
*/
void CmdMessenger::unescape(char *fromChar)
{
// Move unescaped characters right
char *toChar = fromChar;
while (*fromChar != '\0') {
if (*fromChar == escape_character) {
fromChar++;
}
*toChar++ = *fromChar++;
}
// Pad string with \0 if string was shortened
for (; toChar < fromChar; toChar++) {
*toChar = '\0';
}
}
/**
* Split string in different tokens, based on delimiter
* Note that this is basically strtok_r, but with support for an escape character
*/
char* CmdMessenger::split_r(char *str, const char delim, char **nextp)
{
char *ret;
// if input null, this is not the first call, use the nextp pointer instead
if (str == NULL) {
str = *nextp;
}
// Strip leading delimiters
while (findNext(str, delim) == 0 && *str) {
str++;
}
// If this is a \0 char, return null
if (*str == '\0') {
return NULL;
}
// Set start of return pointer to this position
ret = str;
// Find next delimiter
str += findNext(str, delim);
// and exchange this for a a \0 char. This will terminate the char
if (*str) {
*str++ = '\0';
}
// Set the next pointer to this char
*nextp = str;
// return current pointer
return ret;
}
/**
* Indicates if the current character is escaped
*/
bool CmdMessenger::isEscaped(char *currChar, const char escapeChar, char *lastChar)
{
bool escaped;
escaped = (*lastChar == escapeChar);
*lastChar = *currChar;
// special case: the escape char has been escaped:
if (*lastChar == escape_character && escaped) {
*lastChar = '\0';
}
return escaped;
}
/**
* Escape and print a string
```

```
*/
void CmdMessenger::printEsc(char *str)
{
while (*str != '\0') {
printEsc(*str++);
}
}
/**
* Escape and print a character
*/
void CmdMessenger::printEsc(char str)
{
if (str == field_separator || str == command_separator || str == escape_character || str
== '\0') {
comms->print(escape_character);
}
comms->print(str);
}
/**
* Print float and double in scientific format
*/
void CmdMessenger::printSci(double f, unsigned int digits)
{
// handle sign
if (f < 0.0)
{
Serial.print('-');
f = -f;
}
// handle infinite values
if (isinf(f))
{
Serial.print("INF");
return;
}
// handle Not a Number
if (isnan(f))
{
Serial.print("NaN");
return;
}
// max digits
if (digits > 6) digits = 6;
long multiplier = pow(10, digits); // fix int => long
int exponent;
if (abs(f) < 10.0) {
exponent = 0;
}
else {
exponent = int(log10(f));
}
float g = f / pow(10, exponent);
if ((g < 1.0) && (g != 0.0))
{
g *= 10;
exponent--;
}
long whole = long(g); // single digit
long part = long((g - whole)*multiplier + 0.5); // # digits
// Check for rounding above .99:
if (part == 100) {
whole++;
part = 0;
}
char format[16];
sprintf(format, "%%ld.%%0%dldE%%+d", digits);
char output[16];
```

```
sprintf(output, format, whole, part, exponent);
comms->print(output);
}
```

ValvesControl.ino

```
#include <Servo.h>
#include "CmdMessenger.h"
//#include <MemoryFree.h>
//To control a valve use one of the following option:
// 1- Set the corresponding Valve Input Signal
// Input Pin: 33 -> 50
// Valve index: 0 -> 18
// 2- Send a serial command with the following syntax:
// v<x><y> where
// x is a character {0-9,:,;,<,?,@,A} corresponding the valve index {0-17} respectively
// y is a character {0,1} corresponding to OPEN and CLOSE respectively
//The servos are to be connected as follows:
// Output Pin: 14 -> 31
// Valve index: 0 -> 18
#define OPEN 138
#define CLOSE 35
enum {
cmd_connect,
rep_connected,
cmd_open_valve,
cmd_close_valve,
rep_valve_state,
rep_error,
};
const int BAUD_RATE = 9600;
CmdMessenger c = CmdMessenger(Serial,',',';','/');
/*
char servoCharV[18] ={'0', '1', '2', '3', '4',
'5', '6', '7', '8', '9',
':', ';', '<', '=', '>',
'?', '@', 'A'};
*/
int signalsPins[18] ={33, 34, 35, 36, 37,
38, 39, 40, 41, 42,
43, 44, 45, 46, 47,
48, 49, 50};
int servosPins[18] = {14, 15, 16, 17, 18,
19, 20, 21, 22, 23,
24, 25, 26, 27, 28,
29, 30, 31};
bool virtualSignals[18];
bool preVirtualSignals[18];
bool prevInputSignals[18];
bool servoStates[18];
bool prevServoStates[18];
Servo servos[18];
long servosTimers[18];
long detachInterval = 3000;
bool anyAttached = false;
void setup()
{
pinMode(13,OUTPUT);
Serial.begin(BAUD_RATE);
attach_callbacks();
//Serial.println("Initializing Valves");
InitValves();
digitalWrite(13,HIGH);
//Serial.println("Initialization Complete");
}
//long fmpm = 0;
```

```
void loop()
{
c.feedinSerialData();
//StateMachine();
UpdateValves();
/*if(millis() - fmpm >= 1000)
{
Serial.println(freeMemory());
fmpm = millis();
}*/
}
/* callback */
void on_connect(void)
{
c.sendCmd(rep_connected,"OK");
}
/* callback */
void on_open_valve(void)
{
int value1 = c.readBinArg<int>();
if(value1 >= 0 && value1 < 18)
{
virtualSignals[value1] = true;
c.sendCmdStart (rep_valve_state);
c.sendCmdBinArg<int16_t>((int16_t)value1);
c.sendCmdBinArg<int16_t>((int16_t)1);
c.sendCmdEnd ();
//c.sendBinCmd(rep_valve_state,value1,1);
}
else
c.sendBinCmd(rep_error,"Invalid Valve Index");
}
/* callback */
void on_close_valve(void)
{
int value1 = c.readBinArg<int>();
if(value1 >= 0 && value1 < 18)
{
virtualSignals[value1] = false;
c.sendCmdStart (rep_valve_state);
c.sendCmdBinArg<int16_t>((int16_t)value1);
c.sendCmdBinArg<int16_t>((int16_t)0);

c.sendCmdEnd ();
//c.sendBinCmd(rep_valve_state,value1,0);
}
else
c.sendBinCmd(rep_error,"Invalid Valve Index");
}
/* callback */
void on_unknown_command(void)
{
c.sendCmd(rep_error,"Unknown Command");
}
/* Attach callbacks for CmdMessenger commands */
void attach_callbacks(void)
{
c.attach(cmd_connect,on_connect);
c.attach(cmd_open_valve,on_open_valve);
c.attach(cmd_close_valve,on_close_valve);
c.attach(on_unknown_command);
}
/*int machineState = 0;
int rxVIdx = -1;
void StateMachine()
{
char c;
if(Serial.available())
```

```
{
c = Serial.read();
//Serial.print("Rx: ");
//Serial.print(char(c));
//Serial.print(" - S ");
//Serial.print(machineState);
switch(machineState)
{
case 0:
if(c == 'v') machineState++;
else if(c == 'C') Serial.println("OK");
break;
case 1:
rxVIdx = int(c) - 0x30;
//Serial.print(" - IDX ");
//Serial.print(rxVIdx);
machineState++;
break;
case 2:
if(rxVIdx >= 0 && rxVIdx < 18)
{
if(c == '1') virtualSignals[rxVIdx] = true;
else if(c == '0') virtualSignals[rxVIdx] = false;
//Serial.print(" - OC ");
//Serial.print(virtualSignals[rxVIdx]? "Open":"Close");
}
machineState = 0;
rxVIdx = -1;
break;

default:
machineState = 0;
rxVIdx = -1;
break;
}
//Serial.print(" - NS ");
//Serial.println(machineState);
}
}*/
void UpdateValves()
{
for(int i = 0; i < 18; i++)
{
bool doMove = true;
bool state = !digitalRead(signalsPins[i]);
//Serial.print(!state? "1":"0");
//Serial.print("-");
if(state != prevInputSignals[i])
{
prevInputSignals[i] = state;
}
else if((preVirtualSignals[i] != virtualSignals[i]))
{
state = virtualSignals[i];
preVirtualSignals[i] = virtualSignals[i];
}
else
doMove = false;
//state = state || virtualSignals[i];
if(doMove)
ControlValve(i, state? OPEN:CLOSE, false);
//Serial.print(servoStates[i]? "1":"0");
//Serial.print("-");
}
//Serial.println();
DetachServos();
}
void InitValves()
{
bool ledState = false;
```

```
for(int i = 0; i < 18; i++)
{
pinMode(signalsPins[i], INPUT_PULLUP);
virtualSignals[i] = false;
preVirtualSignals[i] = false;
servoStates[i] = false;
prevServoStates[i] = false;
ControlValve(i, CLOSE, true);
//delay(1000);
delay(100);
DetachServos();
prevInputSignals[i] = !digitalRead(signalsPins[i]);
digitalWrite(13,ledState);
ledState = !ledState;
}
DetachServos();
}
void OpenValve(int idx)
{
ControlValve(idx, OPEN, false);
}
void CloseValve(int idx)
{
ControlValve(idx, CLOSE, false);
}
void ControlValve(int idx, int state, bool force)
{
servoStates[idx] = (state == OPEN);
virtualSignals[idx] = servoStates[idx];
if((servoStates[idx] != prevServoStates[idx]) || force)
{
//Serial.print((state == OPEN)? "Open":"Close");
//Serial.print(" Servo "); Serial.println(idx);
prevServoStates[idx] = servoStates[idx];
if(!servos[idx].attached())
servos[idx].attach(servosPins[idx]);
servos[idx].write(state);
servosTimers[idx] = millis();
anyAttached = true;
}
}
void DetachServos()
{
if(!anyAttached) return;
bool ledState = false;
bool tempAnyAttach = false;
for(int i = 0; i < 18; i++)
{
bool isat = servos[i].attached();
//Serial.print(isat? "1":"0"); Serial.print("-");
if(isat)
{
if(millis() - servosTimers[i] >= detachInterval)
servos[i].detach();
else
tempAnyAttach = true;
}
digitalWrite(13,ledState);
ledState = !ledState;
}
//Serial.println();
anyAttached = tempAnyAttach;
}
```

18.2.2.2 Python Software

__pycache__	29.07.2017 ...	Dateiordner		
__init__.py	06.02.2017 ...	PY-Datei	1 KB	
arduino.py	29.07.2017 ...	PY-Datei	7 KB	
PyCmdMessenger.py	29.07.2017 ...	PY-Datei	23 KB	
pyValveControl.py	31.07.2017 ...	PY-Datei	2 KB	

pycache:

arduino.cpython-36.pyc	29.07.2017 ...	PYC-Datei	5 KB
PyCmdMessenger.cpython-36.pyc	29.07.2017 ...	PYC-Datei	17 KB

PythonCode.zip

Two Python classes are available to allow two communication with Arduino:

- "arduino.py" Class defines and Arduino object with all the communication hardware settings and buffers encapsulated to send and receive general binary data. [Ref [6]] (Harms)
- "PyCmdMessenger.py" Class encapsulates a communication protocol that allows developer to define custom commands and replies and the class instance can manage and parse all communication with Arduino. . [Ref [6]] (Harms)

An additional Python code file is also included:

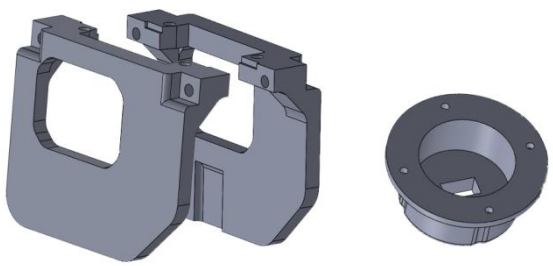
"pyValveControl.py" This code illustrates how to use the above mentioned classes to define the requires commands and replies that are compatible with the Arduino firmware and shows how to control the valves using the USB communication mode. [Developed by CNC LAB]

18.2.3 Retrofit,3D Models and 3D Prints

Retrofitting the ball valve with Servo motor was achieved by designing a functional mechanism that ensures the following:

- Fixating the Motor body to the Valve body to prevent motor body from rotating.
- Coupling the motor shaft with the valve shaft while improving or at least not hindering the motor torque.
- Minimize the scale factor of the mechanism.

The following design was modeled, 3D printed and tested during 3 iterations. Tweaked and optimized with each iteration.



ServoBraket.STL

ServoHorn.STL

Figure 18-5 – Retrofit 3D Model

The 3D model was printed and test as shown in the following pictures:

Figure 18-6 - Out Of Printer

Figure 18-7 - Valve Assembly

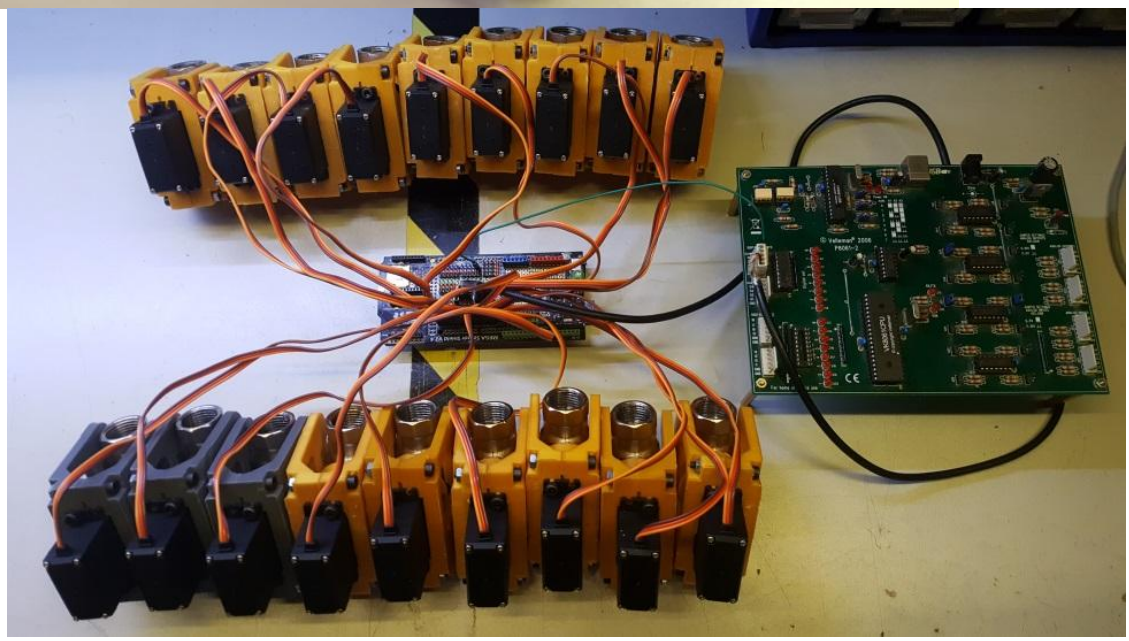
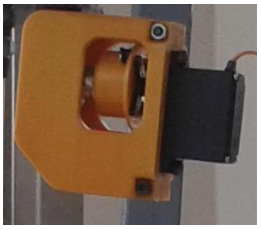


Figure 18-8 - Complete System

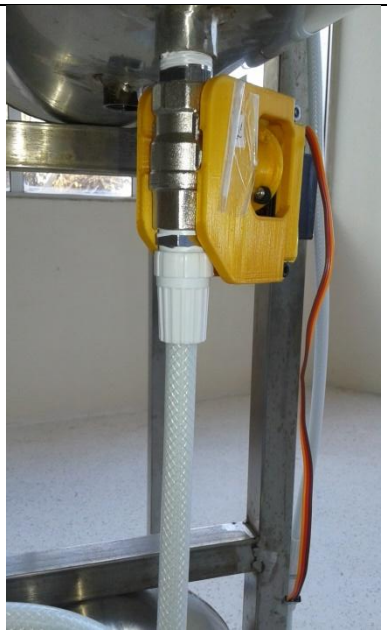
18.3 Integration

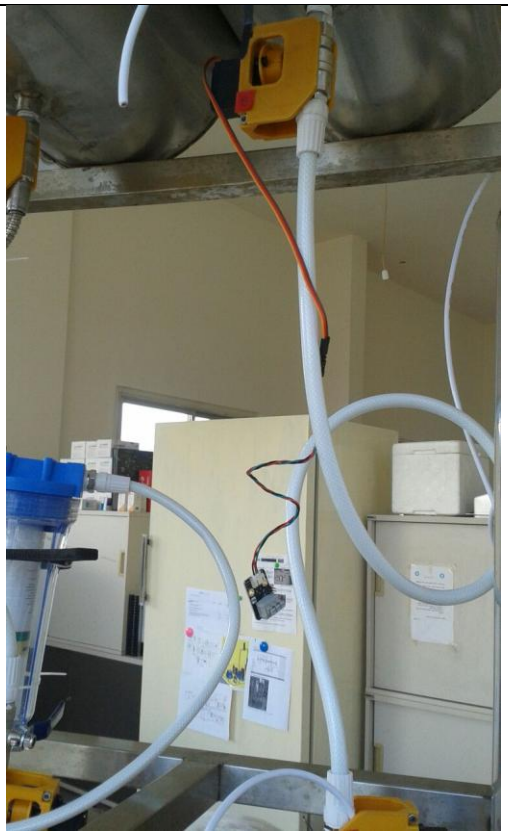
18.3.1 Costs

	#	Cost/#	Total
 Valve	18	$60	$1.080
	36	$2	$72
	36	$1	$36

Stand 29.10.17: Noch offen zur Beendigung des Teststandes: Anschlüsse für 18 Valves

18.3.2 Piping

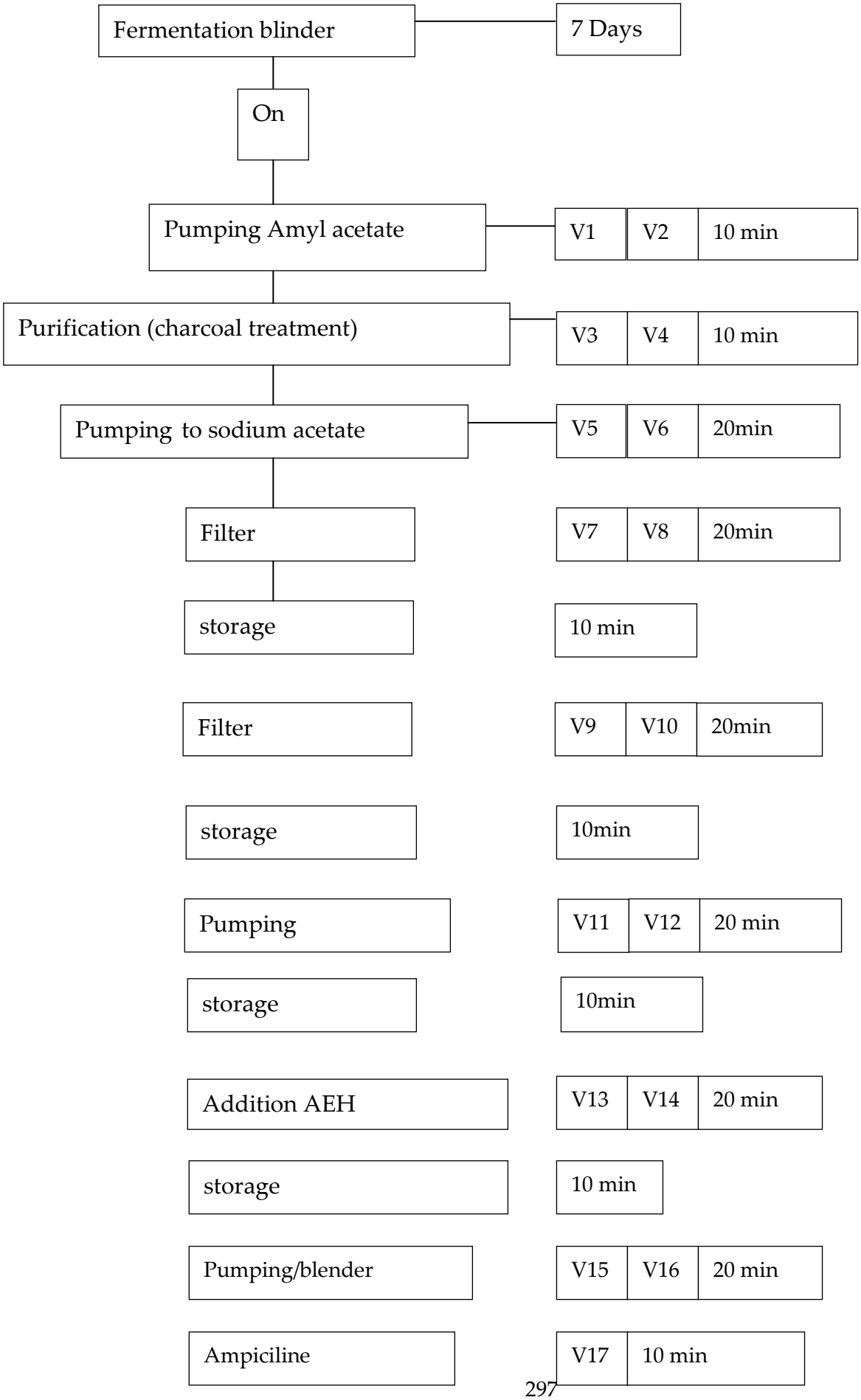




18.3.3 Connecting to automation system

Possibility: Portating GUI to Raspberry

18.4 Process Control Algorithm (from [MEGBI-APP 2018]



18.5 Installation issues concerning automation system (MEGI-APP)

Date		task	المنفذ
30-8-2018	من 8 لل1	تشغيل ال mixerعلى الكهرباء	عبد الرحمن او محمد
	ساعتان	قص حديدة الmixer لتلائم ال bioreacteur penicilline	
29-8-2018	نص نهار	تركيب فلتر الهواء	عبد الله
2-9-2018		تركيب ال الحنفيات و valves التحقق من ال5n 4n 3n 2n valves الautomatic	فاطمة عبد الرحمن
2-9-2018		تسكير الbioreacteur الفتحات	فاطمة

19 Suppliers

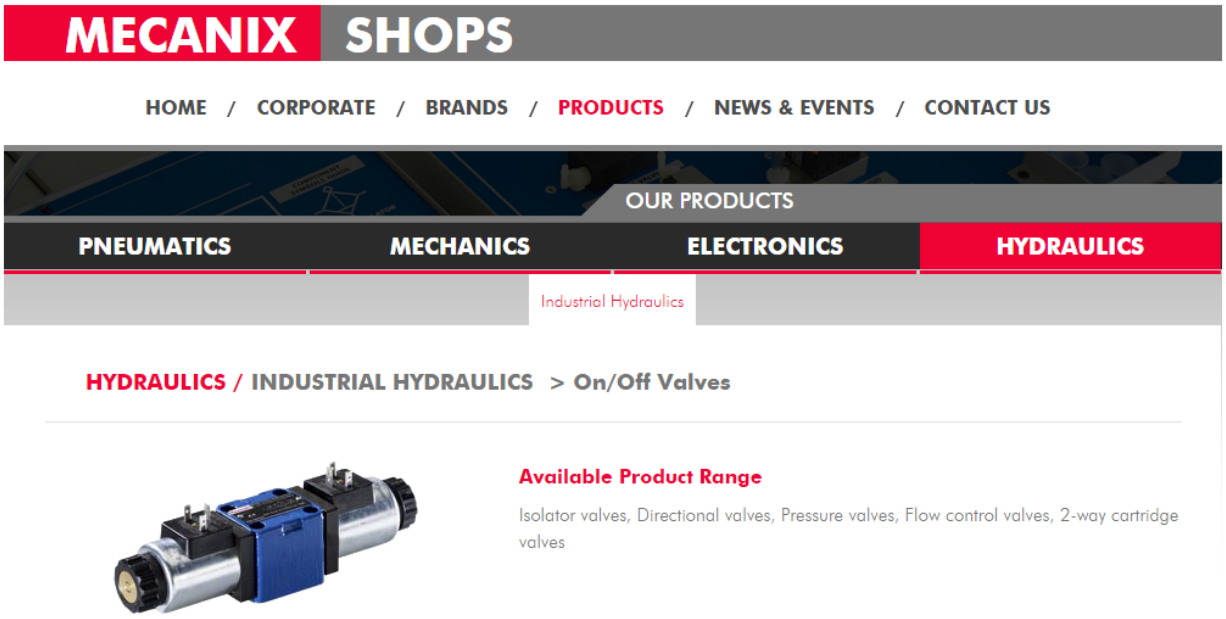
19.1 Mechanical Parts (Valves, Sensors)

Sin El Fil, Horch Tabet
P. : +961-1-486701/2 - +961-1-490754/5
M. : +961-3-783778 / +961-3-763678
F. : +961-1-490929

| +961-76-500880

Mail : P.O. Box 55384 Beirut, Lebanon
Email : sales@mecanixshops.com

19.1.1 Valves



19.1.2 Temperature Sensors

ELECTRONICS / SENSORS > THERMOCOUPLE

Available Product Range

Din Head, Air Probe, MGO, Ceramic, SS316

Product Description:

PT100 EASY-UP

Diameter 6mm, three-wires cable

19.1.3 Pressure Sensors

https://mecanixshops.com/products/Electronics/Sensors/Pressure-Sensor

Sensors | Controllers | Power Units | Weighing Systems | Process Automation | Motion & Drives | Switches | Cables

ELECTRONICS / SENSORS > Pressure Sensor

Available Product Range

Vacuum, Flush, Differential, Atex

Product Description:

TPFADA Series flush diaphragm pressure transmitters are based on bonded strain gauge on stainless steel technology.

Thanks to the strong flush diaphragm made with 17-4 PH stainless steel, **TPFADA is particularly suitable for pressure measurement where the media is with high viscosity (thick fluids, oils, rubber, pulps, chemical products, etc.) and the traditional transducers with internal measuring chamber cannot be used.**

The high thickness of the diapragm makes the product very reliable and suitable for heavy industrial application. Internal state of the art electronics allows a wide range of current and voltage signal outputs, as well as the innovative "Digital Autozero & Span" function is able to perform an easy and quick automatic zero adjustment after the installation, simply with the touch of a magnetic pen, supplied as standard.

Main features:

- Ranges: from 0...10 to 0...1000 bar
- Output signal 4...20mA 2-wires / 0.1...5.1Vdc / 0.1...10.1Vdc / 0...5Vdc / 0...10Vdc / 1...5Vdc / 1...6Vdc / 1...10Vdc
- Protection rating: IP65/IP67
- Wetted parts: 17-4PH Stainless Steel
- Flush fitting stainless steel measuring diaphragm
- Digital Autozero & Span function

19.1.4 Flow Meters

https://mecanixshops.com/products/Electronics/Process-Automation/Flow-Meter

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OUR PRODUCTS

PNEUMATICS | MECHANICS | ELECTRONICS

Sensors | Controllers | Power Units | Weighing Systems | Process Automation | Motion & Drives | Switch

ELECTRONICS / PROCESS AUTOMATION > Flow Meter

Available Product Range

Magnetic, Ultrasonic, Rotary, Mass

19.1.5 Visualization Software

s://mecanixshops.com/products/Electronics/Process-Automation/Process-visualization-software

OUR PRODUCTS

PNEUMATICS | MECHANICS | ELECTRONICS | HYDRAULICS

Sensors | Controllers | Power Units | Weighing Systems | Process Automation | Motion & Drives | Switches | Cables

ELECTRONICS / PROCESS AUTOMATION > Process Visualization Software

Available Product Range

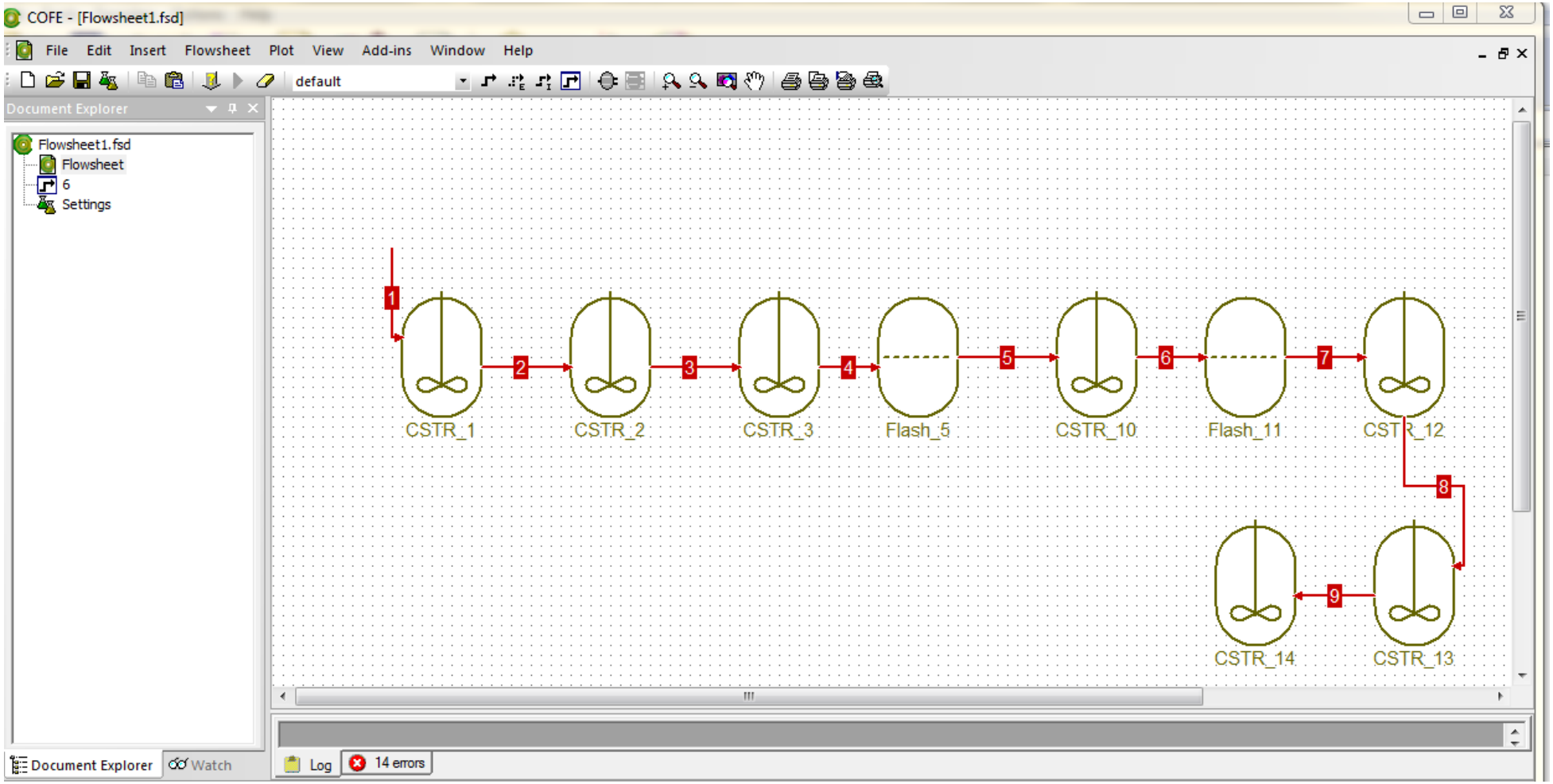
-Tank configuration-Transmitter configuration-Tankpark visualization-Displaying of measured values-Displaying of limit values-Trend monitoring-Data logging-Database handling-Archiving-Other log functions(alarm)-Remote connection (LAN or Internet)

Product Description:

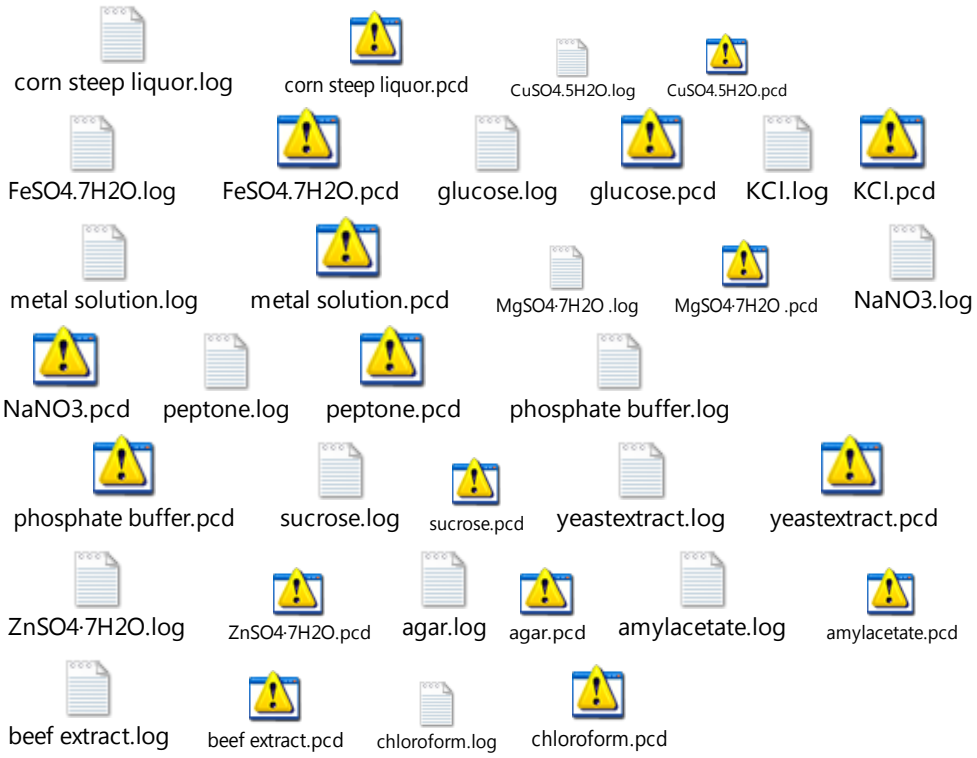
- -Tank configuration
- -Transmitter configuration
- -Tankpark visualization
- -Displaying of measured values
- -Displaying of limit values
- -Trend monitoring
- -Data logging
- -Database handling
- -Archiving
- -Other log functions (alarms)
- -Remote connection (LAN or Internet)

20 Chemical Process Simulation of MEGBI-APP

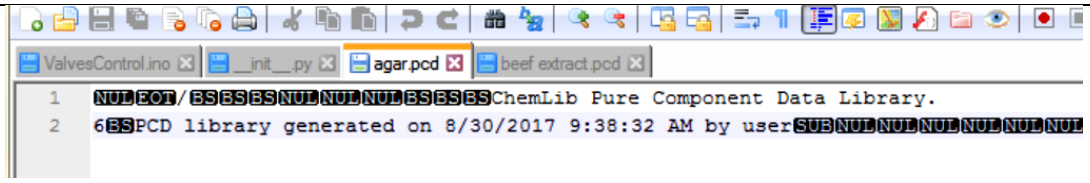
20.1 Flowsheet



20.2 Compounds (pcd files)

all	 pcd-Files.zip
Agar Chloroform Corn steep liquor CuSO4 . 5H2O FeSO4 . 7H2O KCl MgSO4 . 7H2O NaNO3 Peptone Phosphate buffer Sucrose Yeast extract ZnSO4 . 7H2O	 corn steep liquor.log, corn steep liquor.pcd, CuSO4.5H2O.log, CuSO4.5H2O.pcd, FeSO4.7H2O.log, FeSO4.7H2O.pcd, glucose.log, glucose.pcd, KCl.log, KCl.pcd, metal solution.log, metal solution.pcd, MgSO4·7H2O .log, MgSO4·7H2O .pcd, NaNO3.log, NaNO3.pcd, peptone.log, peptone.pcd, phosphate buffer.log, phosphate buffer.pcd, sucrose.log, sucrose.pcd, yeastextract.log, yeastextract.pcd, ZnSO4·7H2O.log, ZnSO4·7H2O.pcd, agar.log, agar.pcd, amylacetate.log, amylacetate.pcd, beef extract.log, beef extract.pcd, chloroform.log, chloroform.pcd

20.2.1 Example files

agar.log	 agar.log	Added component 13097 agar agar; LIX=13097; CAS number[70]; old=; new=9002-18-0; chk=126179476; on 8/30/2017 9:45:08 AM by user agar; CAS=9002-18-0; Molecular weight[13]; old=*; new=336.337; chk=146441160; on 8/30/2017 9:51:53 AM by user agar; CAS=9002-18-0; Structure[3]; old=; new=C14H24O9; chk=177770139; on 8/30/2017 10:08:35 AM by user agar; CAS=9002-18-0; Molecular weight[13]; old=336.337; new=336.3382; chk=180295062; on 8/30/2017 10:08:39 AM by user agar; CAS=9002-18-0; Family[4]; old=0; new=73; chk=185080742; *->other polyfunctional organics on 8/30/2017 10:28:49 AM by user Added component 19599 NaNO3 NaNO3; LIX=19599; CAS number[70]; old=; new=7631-99-4; chk=133951884; on 8/30/2017 10:41:34 AM by user NaNO3; CAS=7631-99-4; Molecular weight[13]; old=*; new= 84.99; chk=180189686; on 8/30/2017 10:42:11 AM by user NaNO3; CAS=7631-99-4; Structure[3]; old=; new=NaNO3; chk=205267212; on 8/30/2017 11:04:42 AM by user NaNO3; CAS=7631-99-4; Structure[3]; old=NaNO3; new=NNaO3; chk=205267193; on 8/30/2017 11:05:16 AM by user NaNO3; CAS=7631-99-4; Molecular weight[13]; old=84.99; new=84.99467; chk=195302116; on 8/30/2017 11:05:18 AM by user NaNO3; CAS=7631-99-4; Molecular weight[13]; old=84.99467; new=84.994; chk=206263521; on 8/30/2017 11:05:34 AM by user NaNO3; CAS=7631-99-4; Family[4]; old=0; new=80; chk=211514386; *->sodium salts on 8/30/2017 11:08:35 AM by user
Agar.pcd	 agar.pcd	 ValvesControl.ino, __init__.py, agar.pcd, beef extract.pcd 1 NUL EOT / BS BS BS NUL NUL NUL BS BS BS ChemLib Pure Component Data Library. 2 6 BS PCD library generated on 8/30/2017 9:38:32 AM by user SUB NUL NUL NUL NUL NUL NUL

Control System of Antibiotics Production Pilot Plant (MEGBI-APP)

Version 2020

Developers & Operation Manual

Author: Eng. Abdullah Q.

Last update: 30.06.2030

Content

System Overview

HMI

PLC

Electrical system

Periphery: Actuators & Sensors

Electric Mixer Temp sensor1 (Thermocouple k) Actuator Valve1 Actuator Valve2 Pump Temp sensor 2 (Thermocouple k) Pressure sensor Resistor of Heater Solenoid valve

21 Hardware and Development Environment

21.1 Human Machine Interface (DOP107-BV)

A human machine interface (HMI) is a platform which permits interaction between users and automation equipment.

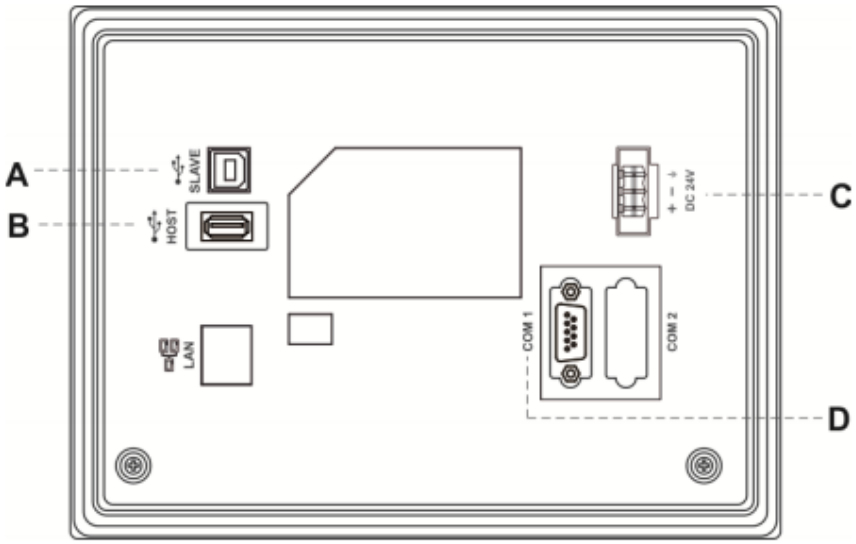
The HMI adopt the latest Cortex-A8 / Dual Core high-speed processor and 65,536 color LCD screen with high brightness and contrast. In addition, they are equipped with the HMI programming software DOPSoft 4.0 and built-in Lua editor for easy programming as well as alarm / history log / user authority functions for highly efficient management.

21.1.1 Specifications

Model		DOP-107BV
Display	Panel type	7" TFT LCD (65535 colors)
	Resolution	800 x 480 pixels
	Backlight	LED backlight (half-life under room temperature 25°C > 20,000 hours)*1
	Display range	154.08 mm * 85.92 mm
	Brightness	400 cd / m² (Typ.)
CPU		ARM Cortex-A8 (800 MHz)
Flash ROM		256 Mbytes
RAM		256 Mbytes
Touchscreen		4-wire resistive touchscreen > 1,000,000 operated
Buzzer		Multi-tone frequency (2 K – 4 KHz) / 80 dB
Network interface		N/A
USB		1 USB Slave Ver 2.0; 1 USB Host Ver 2.0
SD		N/A
Serial communication port	COM1	RS-232 (supporting flow control) / RS-485*2
	COM2	RS-422 / RS-485*2
	COM3	N/A
Auxiliary function key		N/A
Calendar		Built-in
Cooling method		Natural cooling
Approvals		CE / UL (please use shielding network cable and magnetic ring with the filter of 300 ohm / 100 MHz)
Panel waterproof level		IP65 / NEMA4 / UL TYPE 4X (indoor use only)
Operation voltage*2		DC +24V (-15% to +15%) (please use an isolated power supply) Supplied by Class 2 or SELV circuit (isolated from MAINS by double insulation)
Leakage current		500 V_{AC} for 1 minute (between DC24V terminal and FG terminal)
Power consumption*2		8.6 W (Max)*3
Backup battery		3V lithium battery CR2032 × 1
Backup battery life		About 3 years or more at 25°C (subject to operation temperature and condition)
Operation temperature		0°C to 50°C (32°F to 122°F)
Storage temperature		-20°C to +60°C (-4°F to 140°F)
Operating environment		10% - 90% RH [0°C - 40°C], 10% - 55% RH [41°C - 50°C]; pollution degree: 2
Vibration resistance		Conforms to IEC61131-2: continuous vibration 5 Hz - 8.3 Hz with amplitude 3.5 mm; 8.3 Hz - 150 Hz with amplitude 1G
Shock resistance		Conforms to IEC60068-2-27: 11 ms, 15 G Peak, in X, Y, Z directions each for 6 times
Dimension (W) x (H) x (D) mm		215 x 161 x 35.5
Mounting dimension (W) x (H) mm		196.9 x 142.9
Weight		Approx. 700 g

21.1.2 Descripton

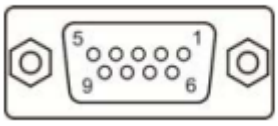
DOP-107BV (rear view)



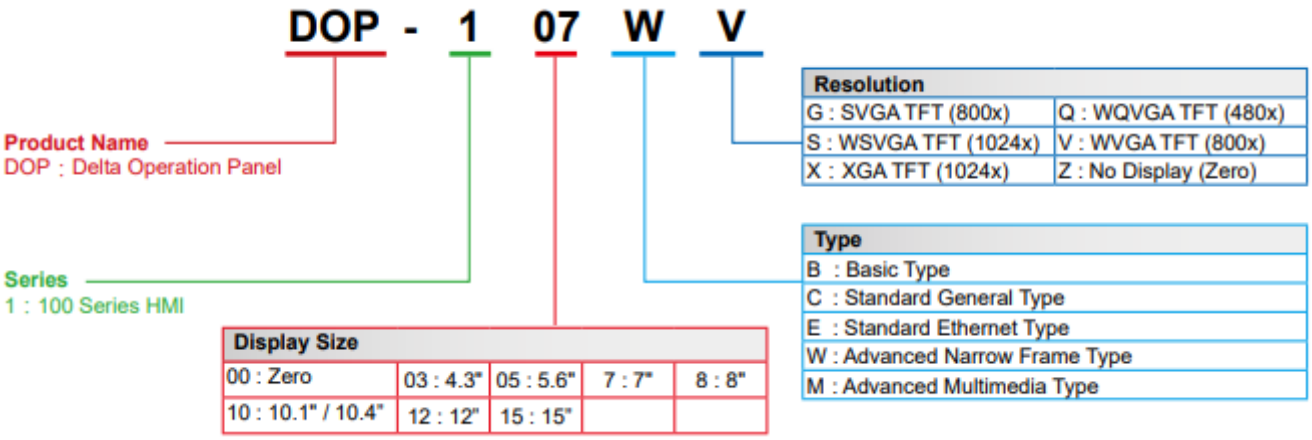
A	USB Slave	B	USB Host
C	Power input terminal (24 AWG wire min.)	D	COM1

21.1.3 Communication port pin assignment

DOP-107BV COM1

COM Port	Pin	MODE1		MODE2		MODE3	
		COM1	COM2	COM1	COM2	COM1	COM2
		RS-232	RS-485	RS-485	RS-485	RS-232	RS-422
	1	-	-	D+	-	-	TXD+
	2	RXD	-	-	-	RXD	-
	3	TXD	-	-	-	TXD	-
	4	-	D+	-	D+	-	RXD+
	5	GND		GND		GND	
	6	-	-	D-	-	-	TXD-
	7	RTS	-	-	-	RTS	-
	8	CTS	-	-	-	CTS	-
	9	-	D-	-	D-	-	RXD-

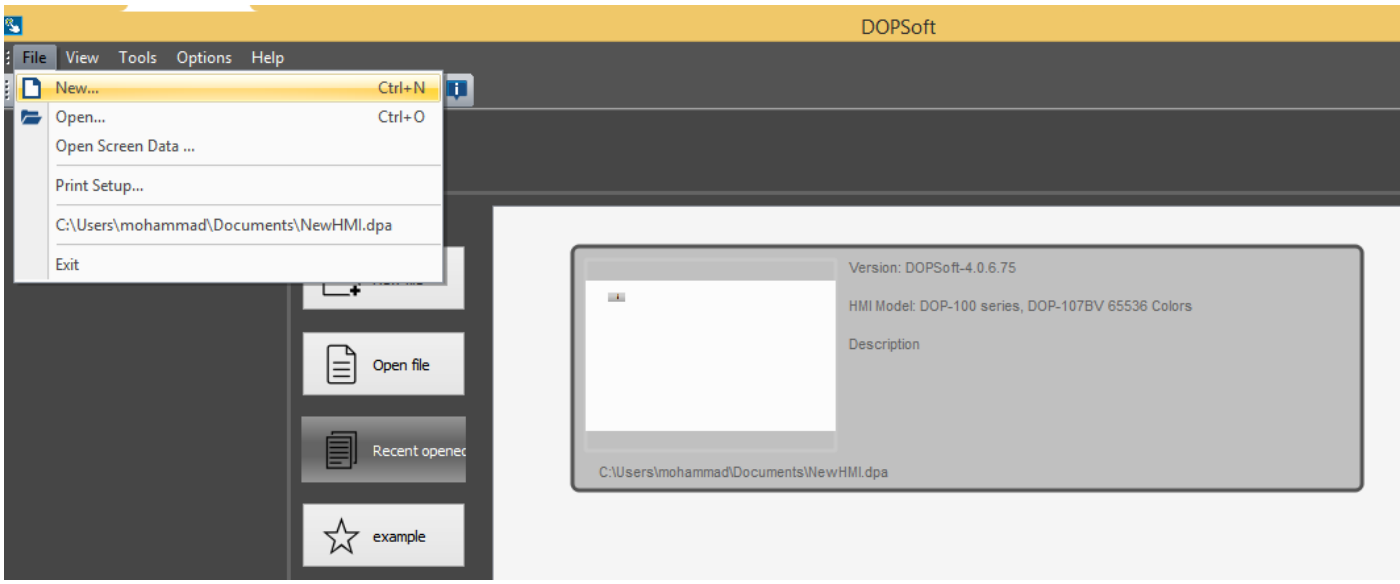
21.1.4 Model Description



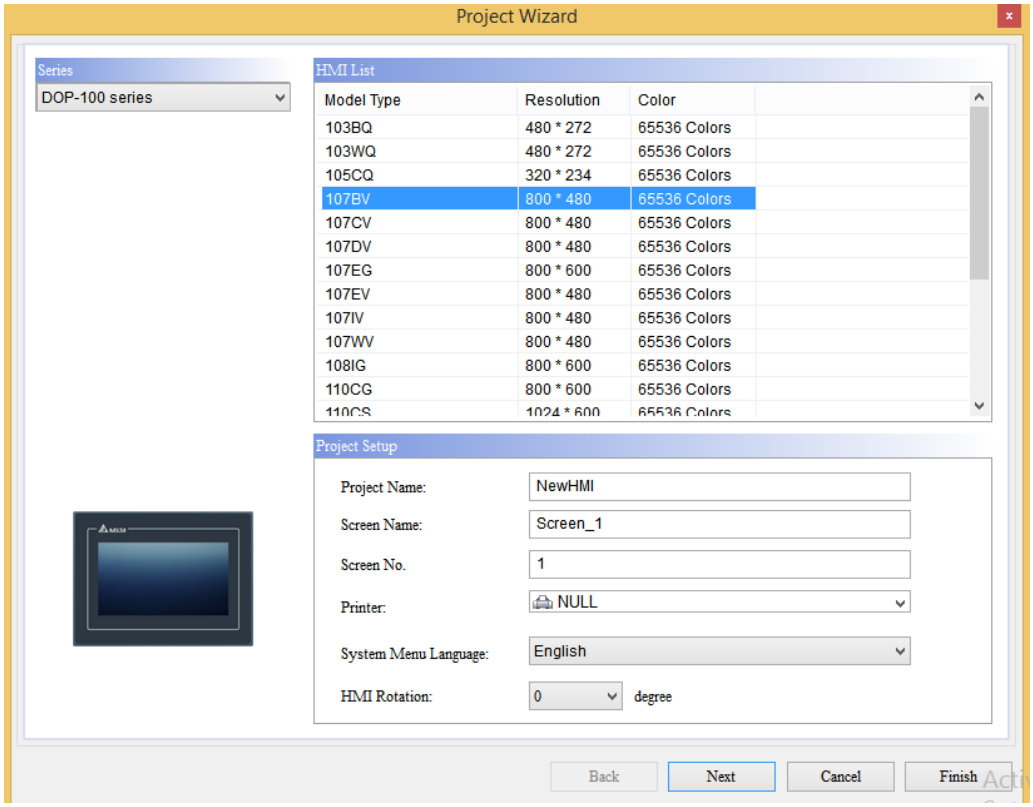
21.1.5 Software DOPSoft 4.0 for HMI programming

21.1.5.1 Create a Project

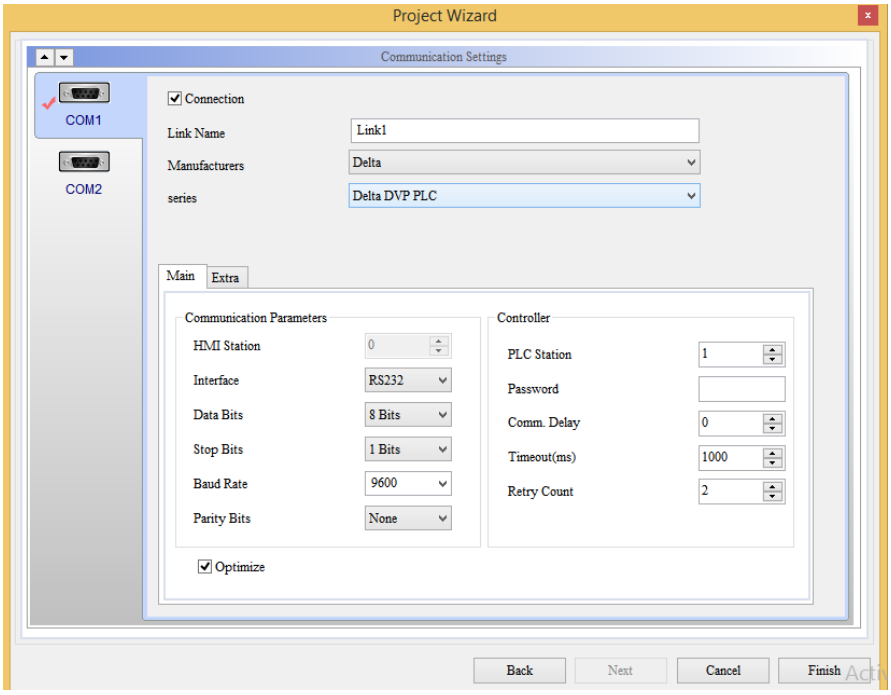
- We click on «File-New »

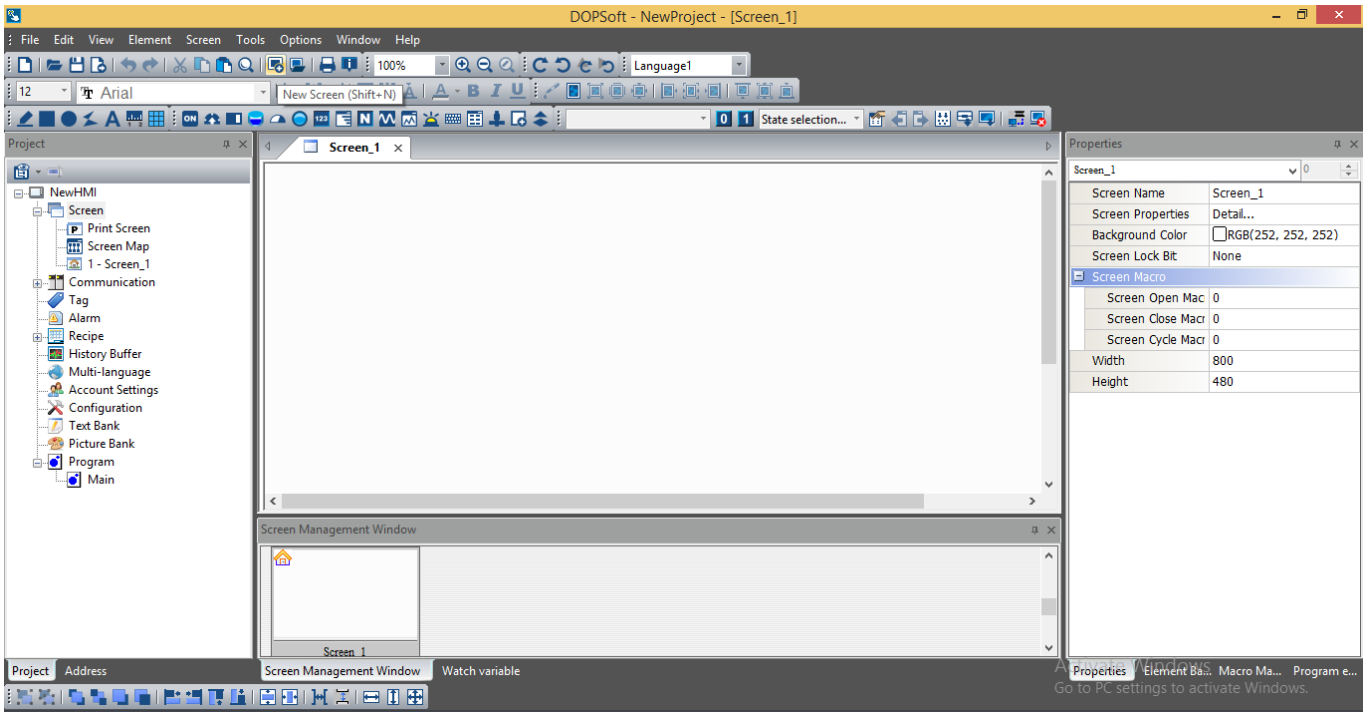


- We choose the HMI product «107BV »
- We put a name in the «Project Name»
- We click on «Next »



- **We choose the following :**
  Port of communication « COM1 »
  Manufacturers : « Delta »
  Series : « Delta DVP PLC »
  Address of PLC Station : « 1 »
  Interface : « RS232»
  We choose the “communication parameters” that correspond to the PLC
- We click on «Finish »

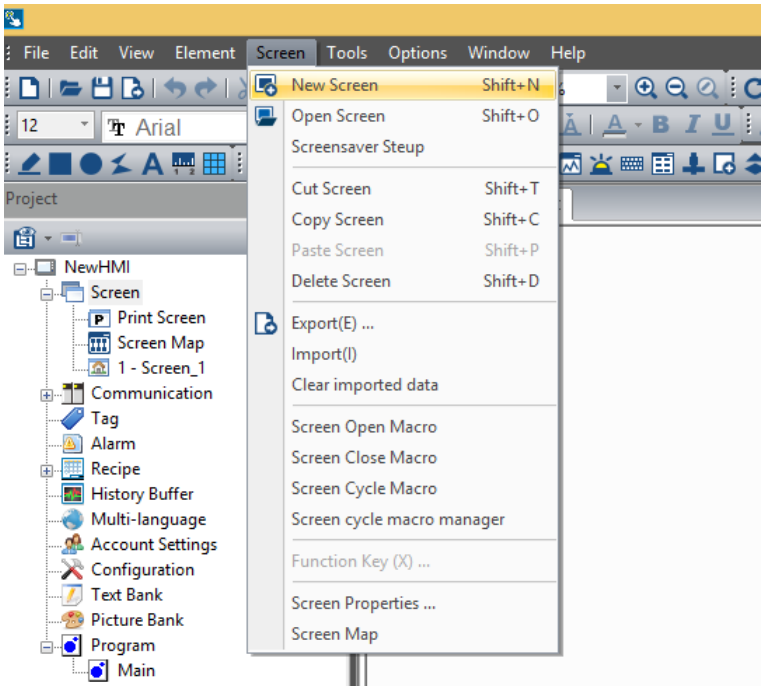




21.1.5.2 Design a project

a. <u>Add pages</u>

To add pages, we click on « Screen-New Screen » or « Shift+N »

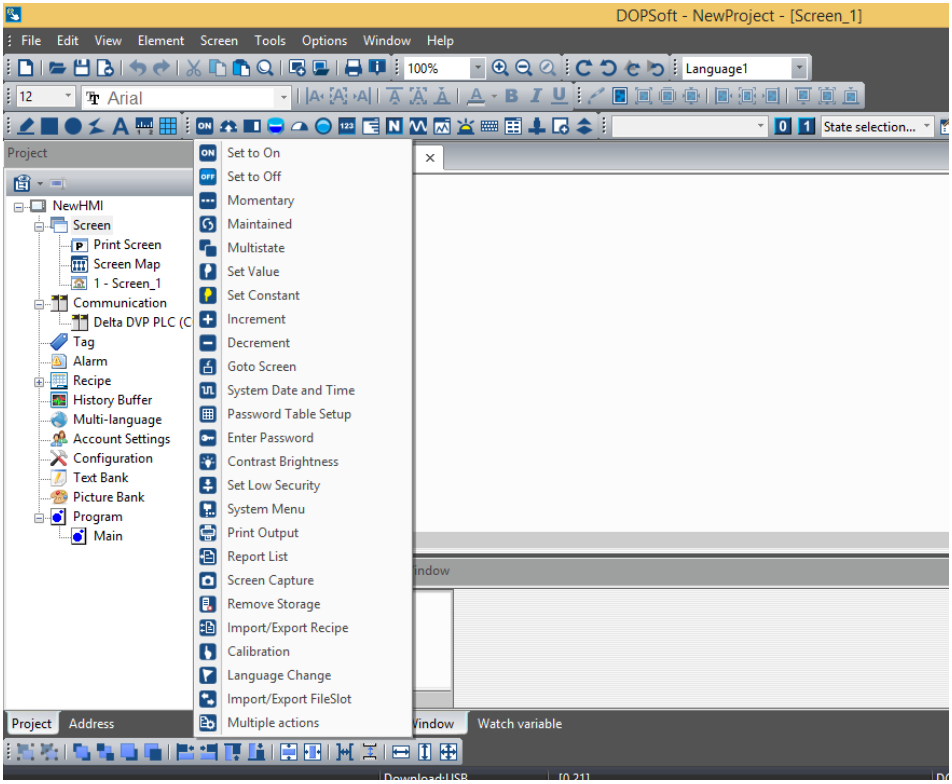


b. **<u>Button (Write - Bit)</u>**

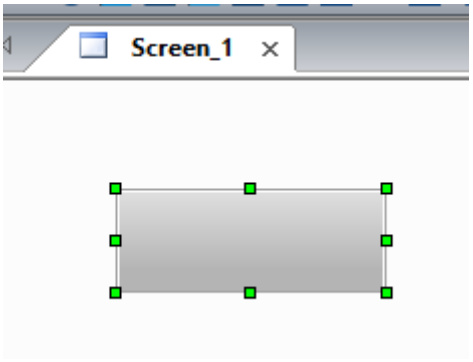
Set to On (ON Only)

Set to Off (OFF Only)

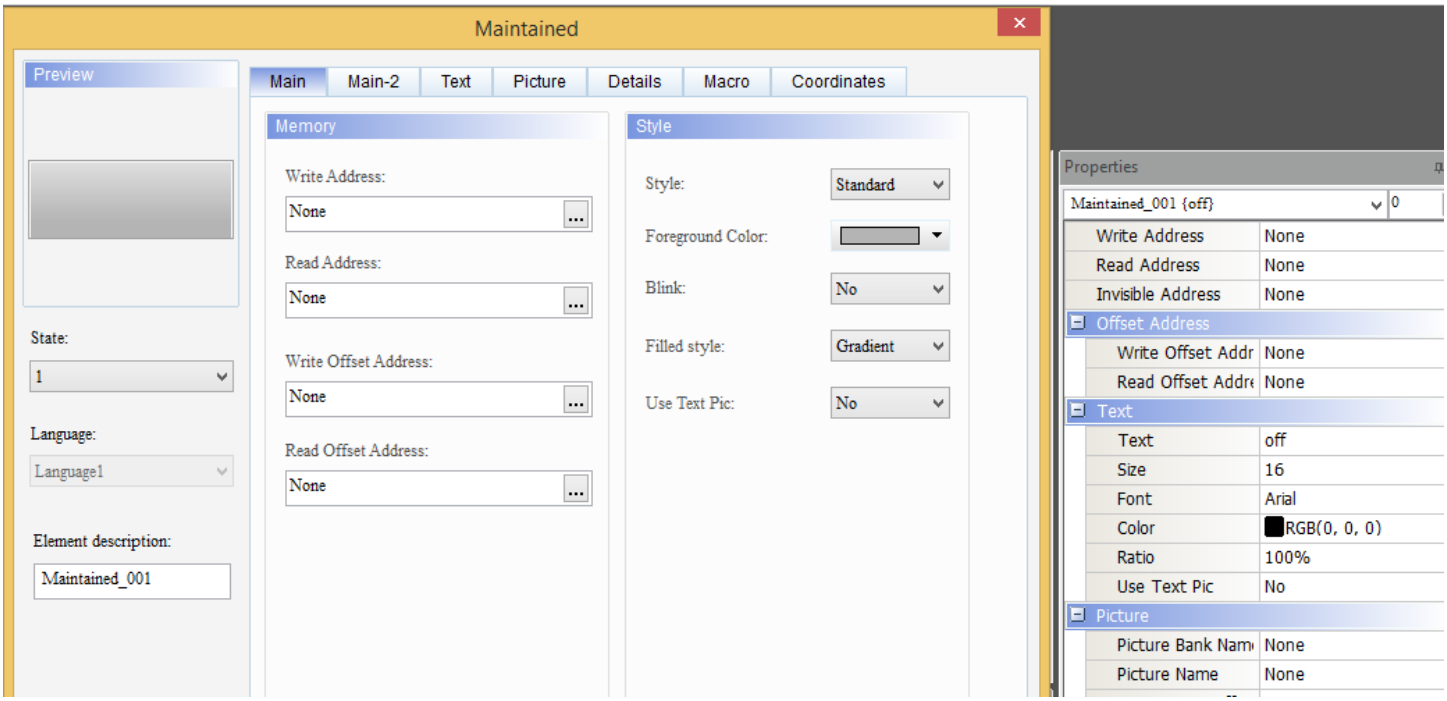
Maintained (OFF & ON)



Button

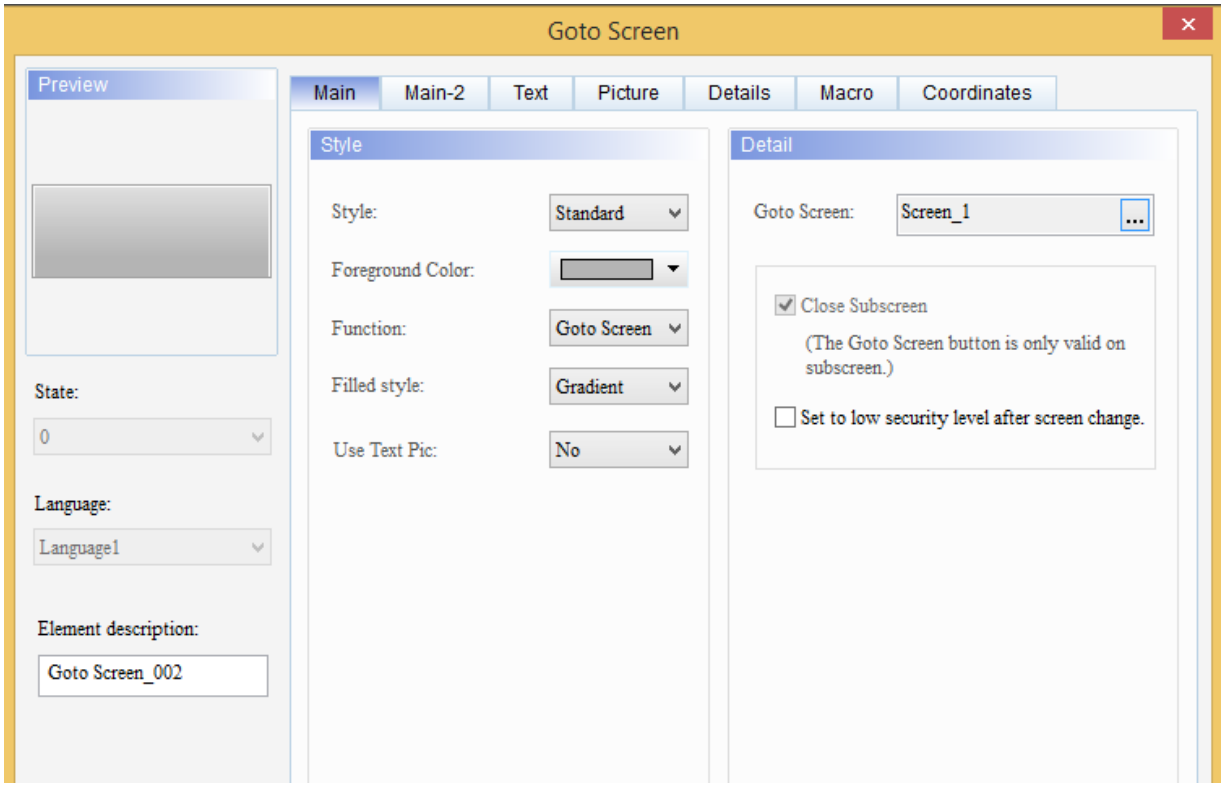


- We press the button to display the properties
- We enter the address of bit device for PLC in the «Proporties - Write address »

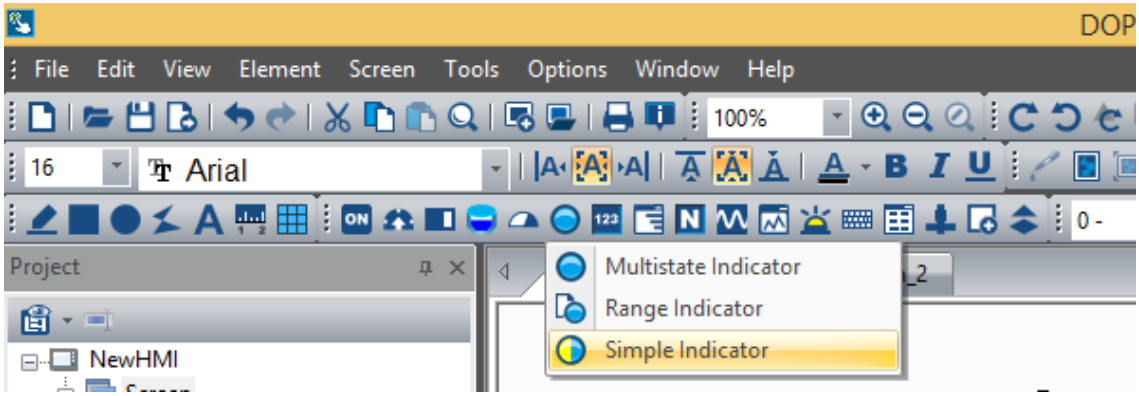


c. <u>Button - Goto Screen (Go to another page)</u>

We choose the name of the page we want to navigate to in the « Proporties - Goto screen »


Goto Screen
Preview
Main
Main-2
Text
Picture
Details
Macro
Coordinates
Style
Detail
Style:
Standard
Foreground Color:
Function:
Goto Screen
Filled style:
Gradient
Use Text Pic:
No
Goto Screen:
Screen_1
Close Subscreen
(The Goto Screen button is only valid on subscreen.)
Set to low security level after screen change.
State:
0
Language:
Language1
Element description:
Goto Screen_002

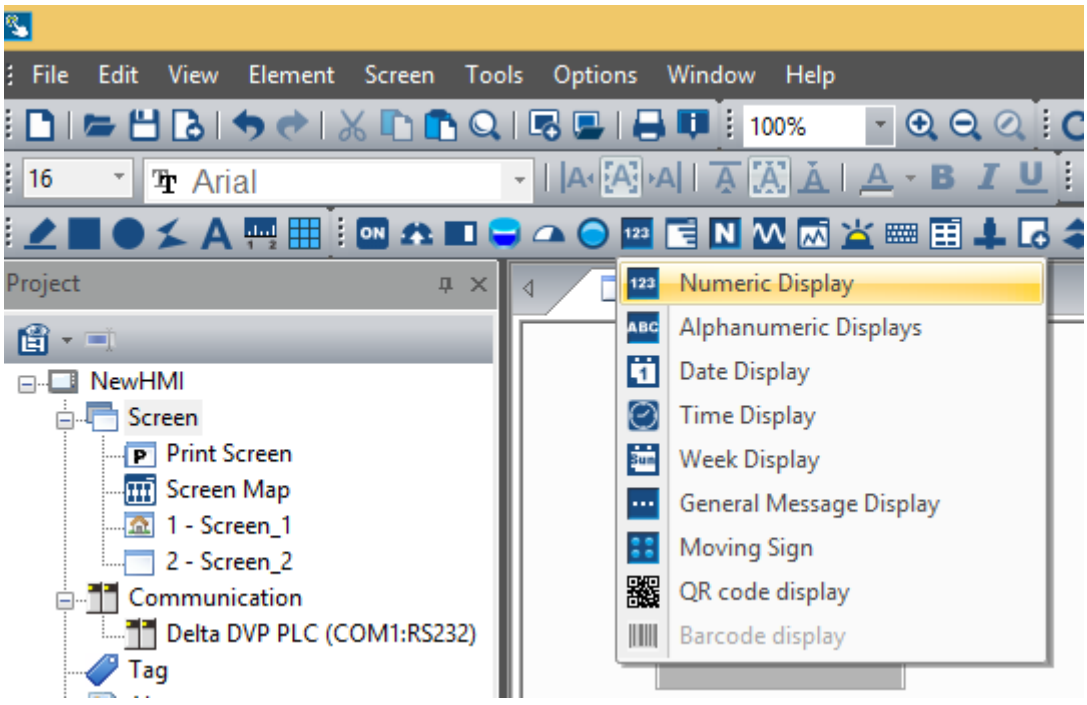
d. Indicator (Read-Bit)

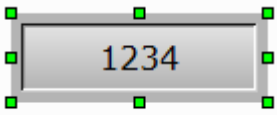


Simple Indicator

We enter the address of bit device for PLC in « Proporties - Read address »

e. Numeric Display (Read-Word)





Numeric Display

We enter the address of Word device for PLC in the « Proporties - Read address »

21.2 DELTA PLC (DVP20SX211R)

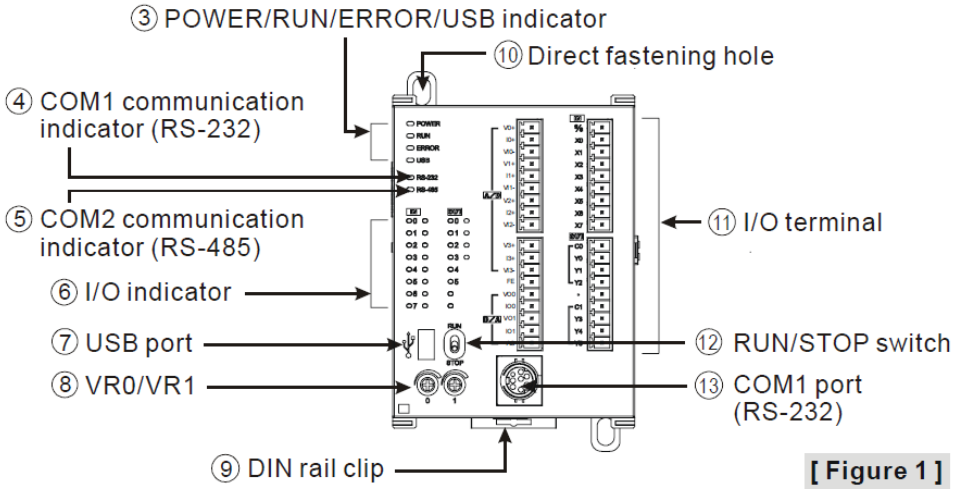
DELTA PLC - DVP20SX211R

21.2.1 Specifications

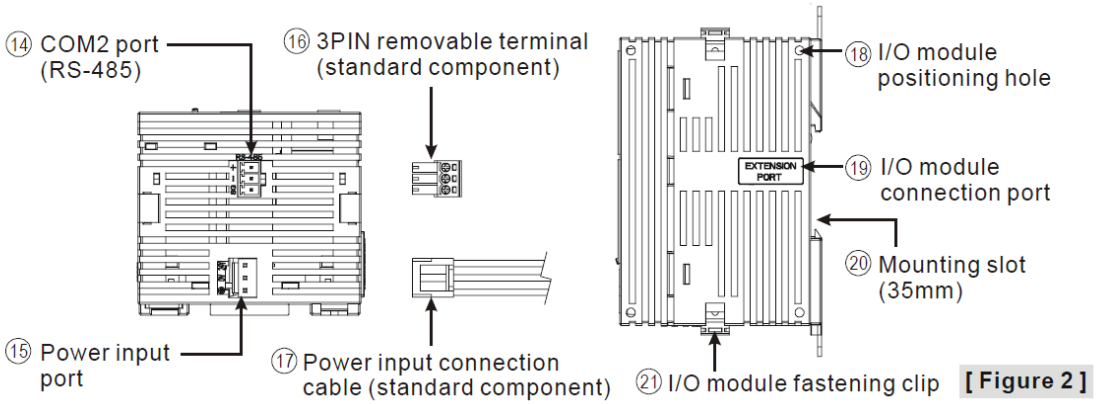
_ Program capacity: 16k steps/Data register: 10k words
_ Higher execution speed compared to the competition: LD: 0.35μs, MOV: 3.4μs
_ Built-in mini USB, RS-232 and RS-485 ports (Master/Slave) Supports standard MODBUS ASCII/RTU protocol and PLC Link function
_ Supports real time clocl for version 2.0 and above (no battery required) It operates for at least one week after power off.
_Built-in 4 analog inputs / 2 analog outputs / 8 Digital Inputs & 6 Digital Outputs (Relay)
_ Supports DVP-S series left-side and right-side modules
_Power supply voltage : 24V DC

Built-in Analog I/O			
Analog Input		**Analog Output**	
Channels	4	**Channels**	2
Resolution	12-bit	**Resolution**	12-bit
Spec.	-20~20 mA or -10~10 V or 4~20 mA	**Spec.**	0~20 mA or -10 V~10 V or 4~20 mA

21.2.2 Product Profile



[Figure 1]



[Figure 2]

21.2.3 Point Specifications

21.2.3.1 Input point Specifications

Items \ Spec.		Input Point		
		24VDC (-15% ~ 20%) single common port input		
Input No.		X0, X2	X1, X3	X4 ~ X7
Input type		DC (SINK or SOURCE)		
Input Current (± 10%)		24VDC, 5mA		
Input impedance		4.7K Ohm		
Action level	Off→On	> 15VDC		
	On→Off	< 5VDC		
Response time	Off→On	< 2.5μs	< 10μs	< 20us
	On→Off	< 5μs	< 20μs	< 50us
Filter time		Adjustable within 0 ~ 20ms by D1020 (Default: 10ms)		

21.2.3.2 Output point Specifications

Items \ Spec.		Output Point
		Relay
Output No.		Y0 ~ Y5
Max. frequency		1Hz
Working voltage		250VAC, < 30VDC
Max. load	Resistive	1.5A/1 point (5A/COM)
	Inductive	#2
	Lamp	20WDC/100WAC
Response time	Off→On	Approx. 10 ms
	On→Off	

21.2.3.3 Analog input & Analog output Specifications

Items	Analog Input (A/D)			Analog Output (D/A)		
	Voltage	Current		Voltage	Current	
Analog I/O range	±10V	±20mA	4 ~ 20mA#1	±10V	0 ~ 20mA	4 ~ 20mA#1
Digital conversion range	±2,000	±2,000	0 ~ +2,000	±2,000	0 ~ +4,000	0 ~ +4,000
Resolution #2	12-bit					

21.2.3.4 Point Wiring

V0+	S/S
I0+	X0
VI0-	X1
V1+	X2
I1+	X3
VI1-	X4
V2+	X5
I2+	X6
VI2-	X7

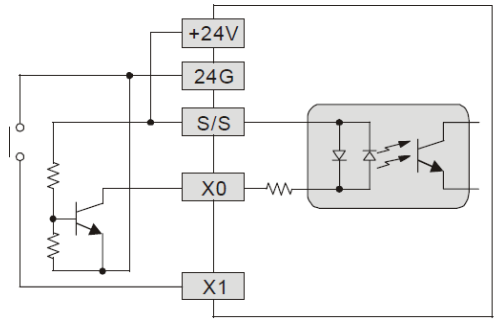
V3+	C0
I3+	Y0
VI3-	Y1
FE	Y2
VO0	●
IO0	C1
VO1	Y3
IO1	Y4
AG	Y5

21.2.3.5 Input Point Wiring

There are 2 types of DC inputs, SINK and SOURCE. (See the example below. For detailed point configuration, please refer to the specification of each model.)

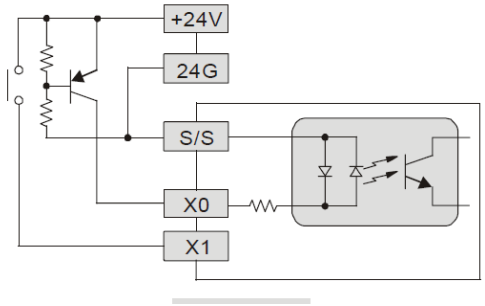
- DC Signal IN – SINK mode

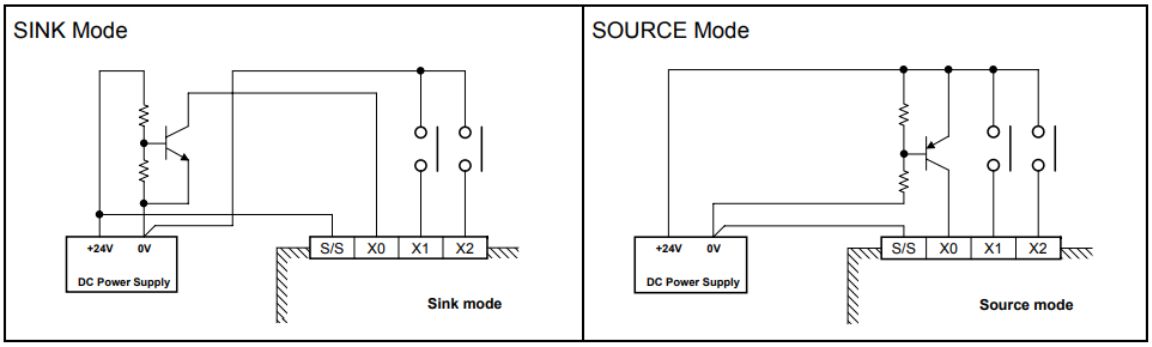
Input point loop equivalent circuit



- DC Signal IN – SOURCE mode

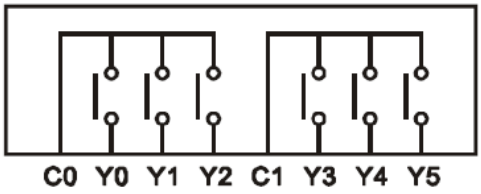
Input point loop equivalent circuit



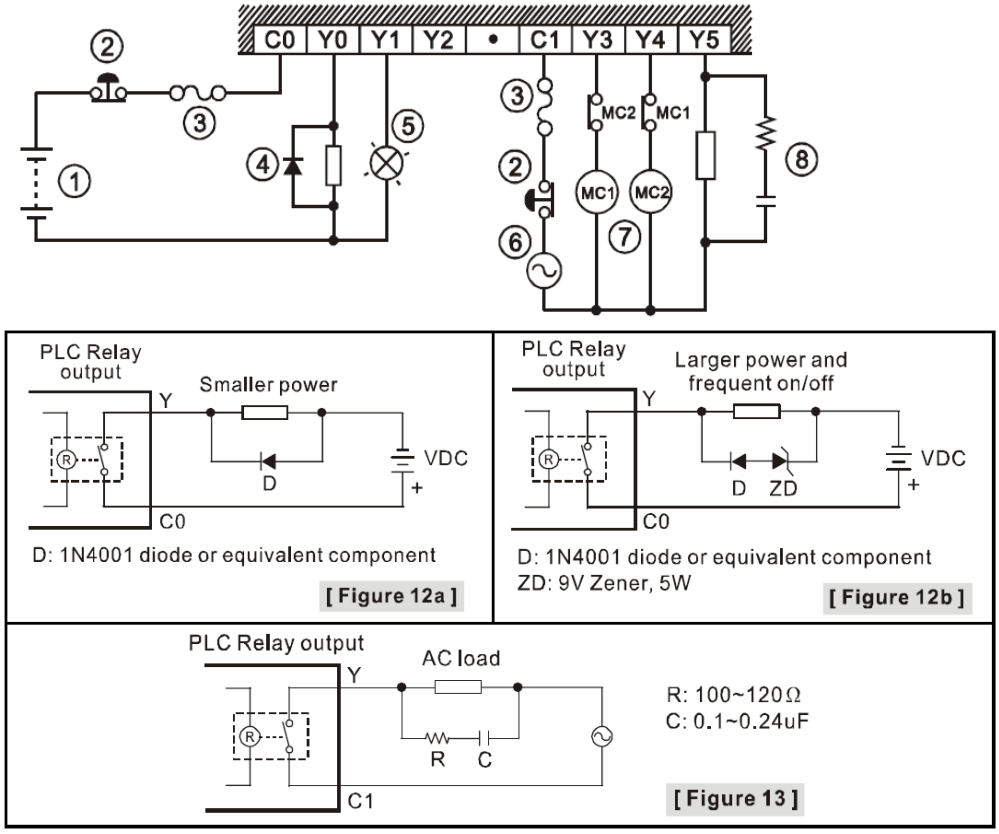


21.2.4 Output Point Wiring

Output terminals, Y0, Y1, and Y2, of relay models use C0 common port; Y3, Y4, and Y5 use C1 common port; as shown in the Figure . When output points are enabled, their corresponding indicators on the front panel will be on.

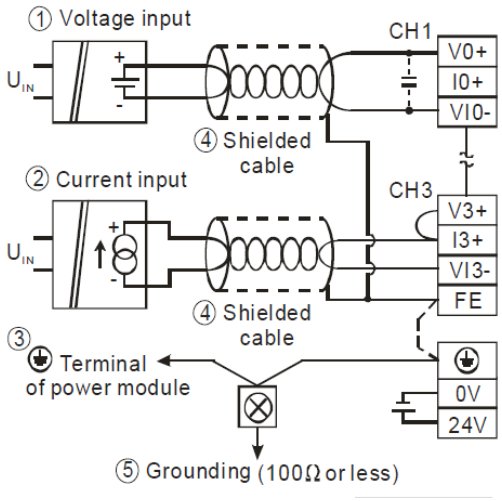


Relay (R) output circuit wiring

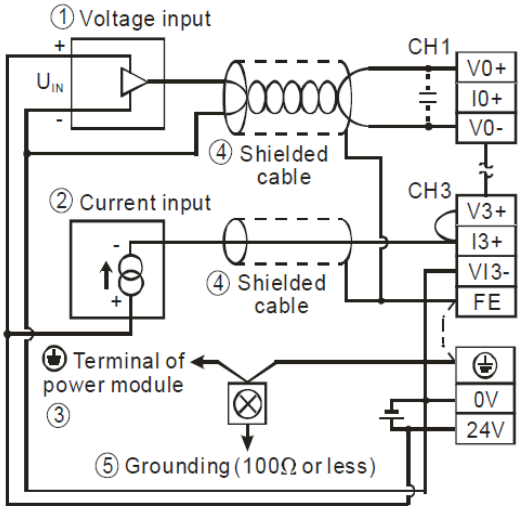


21.2.5Analog input A/D & Analog output D/A External Wiring

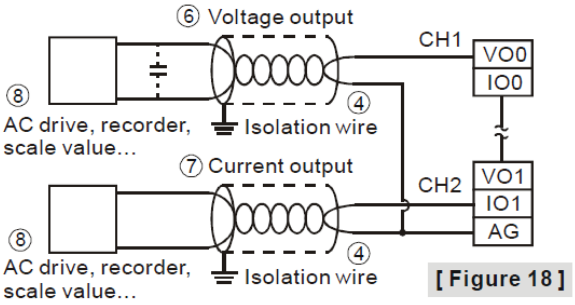
- A/D: Active



- A/D: Passive



• D/A



[Figure 18]

21.2.6 DVP20SX2 Memory Map

Specifications					
Control Method				Stored program, cyclic scan system	
I/O Processing Method				Batch processing method (when END instruction is executed)	
Execution Speed				LD instructions – 0.54μs, MOV instructions – 3.4μs	
Program language				Instruction List + Ladder + SFC	
Program Capacity				15872 steps	
Bit Contacts	X	External inputs		X0~X377, octal number system, 256 points max.	Total 480+32 I/O(*4)
	Y	External outputs		Y0~Y377, octal number system, 256 points max.	
	M	Auxiliary relay	General	M0~M511, 512 points, (*1) M768~M999, 232 points, (*1) M2000~M2047, 48 points, (*1)	Total 4096 points
			Latched	M512~M767, 256 points, (*2) M2048~M4095, 2048 points, (*2)	
			Special	M1000~M1999, 1000 points, some are latched	
	T	Timer	100ms (M1028=ON, T64~T126: 10ms)	T0~T126, 127 points, (*1) T128~T183, 56 points, (*1)	Total 256 points
				T184~T199 for Subroutines, 16 points (*1)	
				T250~T255(accumulative), 6 points (*1)	
			10ms (M1038=ON, T200~T245: 1ms)	T200~T239, 40 points, (*1)	
				T240~T245(accumulative), 6 points, (*1)	
			1ms	T127, 1 points, (*1) T246~T249(accumulative), 4 points, (*1)	

	C	Counter	16-bit count up		C0~C111, 112 points, (*1) C128~C199, 72 points, (*1) C112~C127, 16 points, (*2)	Total 233 points
			32-bit count up/down		C200~C223, 24 points, (*1) C224~C232, 9 points, (*2)	
			32bit high-speed count up/down	Soft-ware	C235~C242, 1 phase 1 input, 8 points, (*2) C233~C234, 2 phase 2 input, 2 points, (*2)	Total 22 points
				Hard-ware	C243~C244, 1 phase 1 input, 2 points, (*2) C245~C250, 1 phase 2 input, 6 points, (*2) C251~C254 2 phase 2 input, 4 points, (*2)	
	S	Step point	Initial step point		S0~S9, 10 points, (*2)	Total 1024 points
			Zero point return		S10~S19, 10 points (use with IST instruction), (*2)	
			Latched		S20~S127, 108 points, (*2)	
			General		S128~S911, 784 points, (*1)	
			Alarm		S912~S1023, 112 points, (*2)	

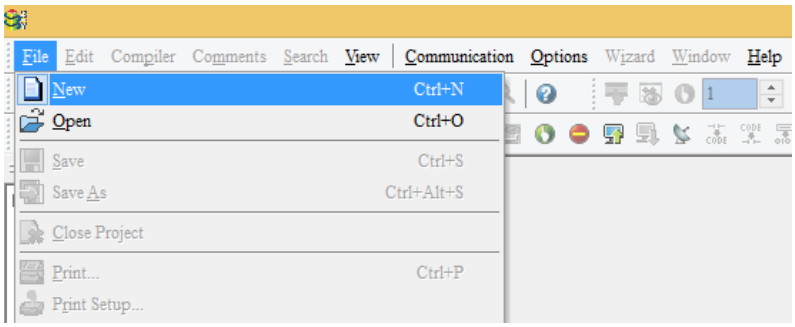
Specifications					
Word Register	T	Current value		T0~T255, 256 words	
	C	Current value		C0~C199, 16-bit counter, 200 words	
				C200~C254, 32-bit counter, 55 words	
	D	Data register	General	D0~D407, 408 words, (*1) D600~D999, 400 words, (*1) D3920~D9799, 5880 words, (*1)	Total 10000 points
			Latched	D408~D599, 192 words, (*2) D2000~D3919, 1920 words, (*2)	
			Special	D1000~D1999, 1000 words, some are latched	
			Righ-side special module	D9900~D9999, 100 words (*1) (*6)	
			Left-side special module	D9800~D9899, 100 words (*1) (*7)	
			Index	E0~E7, F0~F7, 16 words, (*1)	
Pointer	N	Master control loop		N0~N7, 8 points	
	P	Pointer		P0~P255, 256 points	
	I	Interrupt Service	External interrupt	I000/I001(X0), I100/I101(X1), I200/I201(X2), I300/I301(X3), I400/I401(X4), I500/I501(X5), I600/I601(X6), I700/I701(X7), 8 points (01: rising-edge trigger ⌐, 00: falling-edge trigger ¬)	
			Timer interrupt	I602~I699, I702~I799, 2 points (Timer resolution = 1ms) I805~I899, 1 point (Timer resolution = 0.1ms) (Supported by V2.00 and above)	
			High-speed counter interrupt	I010, I020, I030, I040, I050, I060, I070, I080, 8 points	
			Communication interrupt	I140(COM1), I150(COM2), I160(COM3), 3 points, (*3)	

Constant	K	Decimal	K-32,768 ~ K32,767 (16-bit operation), K-2,147,483,648 ~ K2,147,483,647 (32-bit operation)
	H	Hexadecimal	H0000 ~ HFFFF (16-bit operation), H00000000 ~HFFFFFFFF (32-bit operation)
Serial Ports		SA2	COM1: built-in RS-232 ((Master/Slave) COM2: built-in RS-485 (Master/Slave) COM3: built-in RS-485 (Master/Slave) COM1 is typically the programming port.
		SX2	COM1: built-in RS-232 ((Master/Slave) COM2: built-in RS-485 (Master/Slave) COM3: built-in USB (Slave) COM1 is typically the programming port.
Real Time Clock			Year, Month, Day, Week, Hours, Minutes, Seconds
Special I/O Modules			Right side: Up to 8 I/O modules can be connected Left side: Up to 8 high-speed I/O module can be connected
File Register (*5)			K0~K4999, 5000 points (*2)

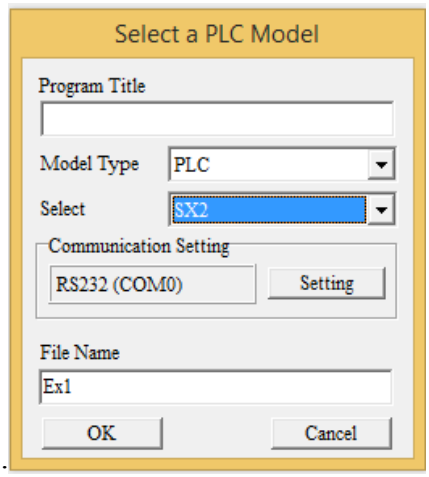
21.2.7 Software "WPL Soft" for PLC programming

21.2.7.1 Create a Project

- We click on «File-New »

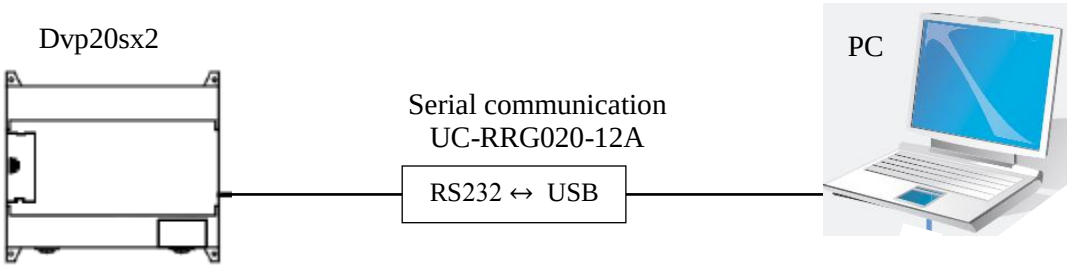


- We choose the PLC product «SX2 »
- We put a name in the «File Name»
- We click on «OK »

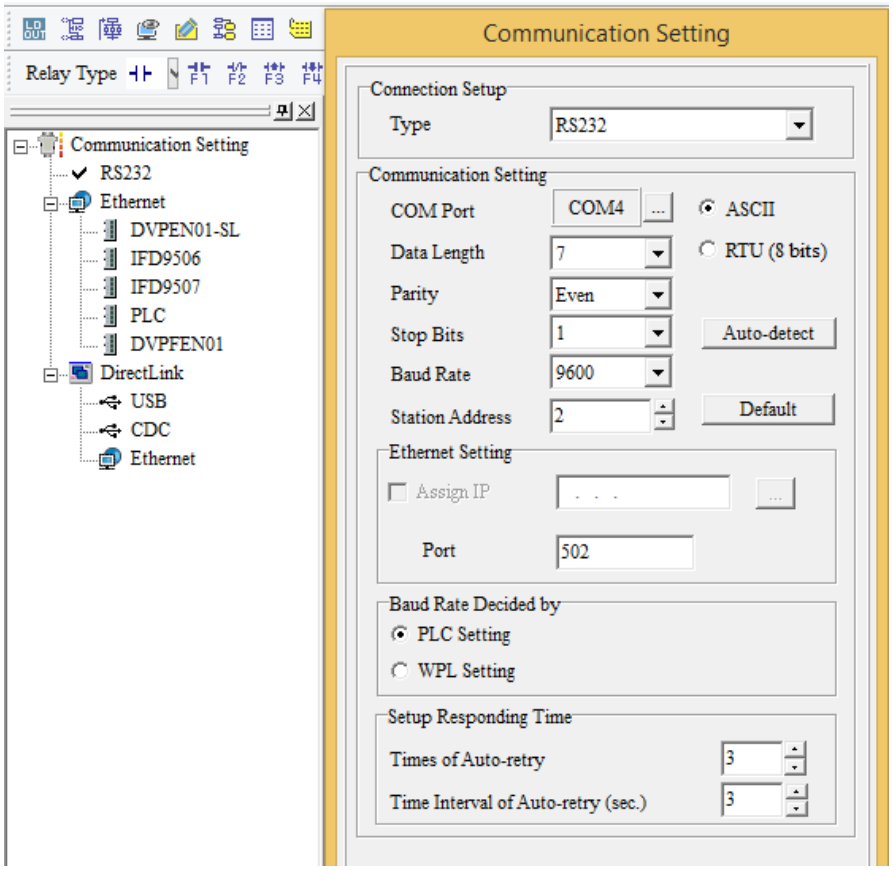


21.2.7.2 The necessary steps to download the program on the PLC

We Use Programming cable (UC-PRG020-12A) connecting a computer and a PLC.

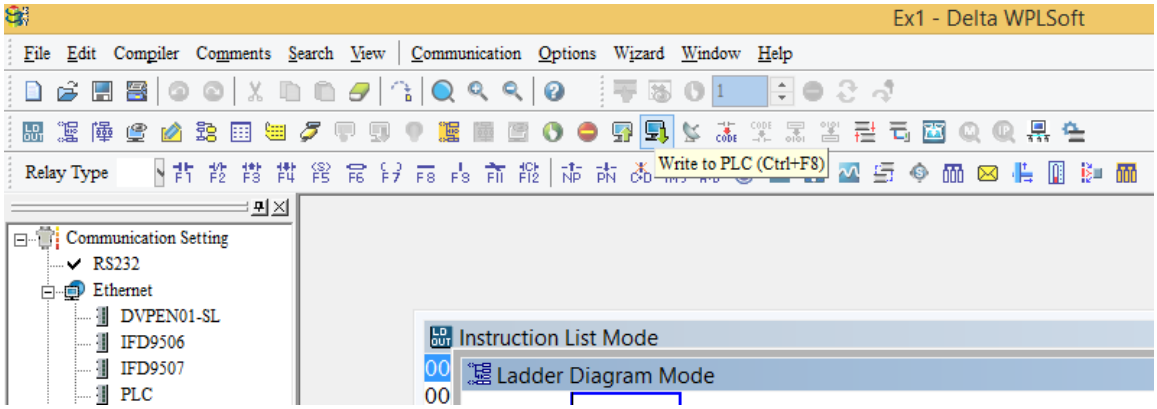


- We click on “Communication setting- RS232 “ to check the port (COM).
- we put The PLC address in Station address



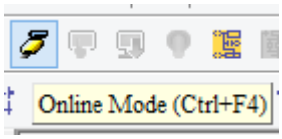
21.2.7.3 Downloading a PLC program

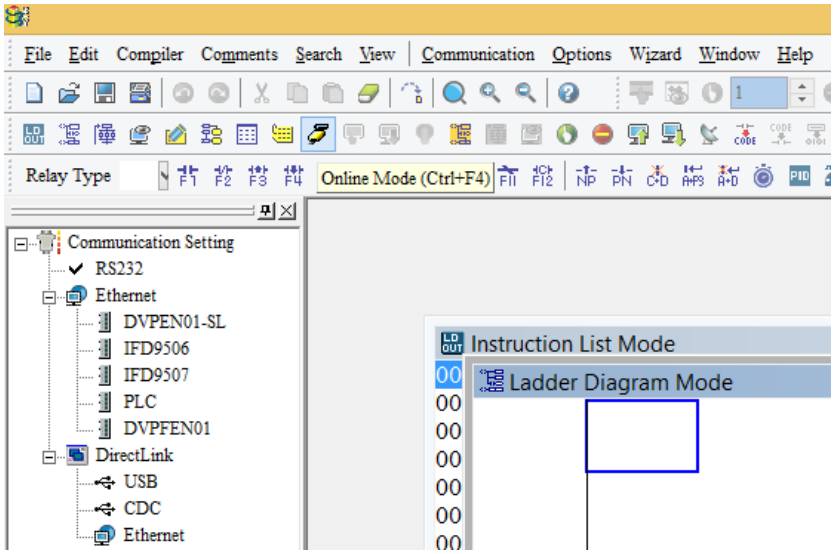
To download the program, we click on the following form :



21.2.7.4 Monitoring a Program

To monitor the program's work in the PLC, we click on the following form:



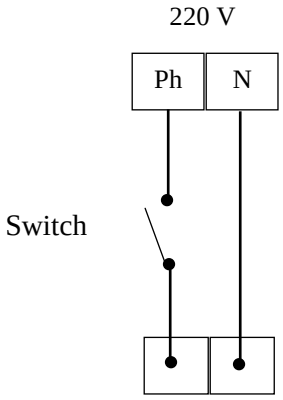


22 Connecting the sensors & actuators

22.1 Control Panel

22.2 Pump 1

Power circuit between the Switch & the Pump 1

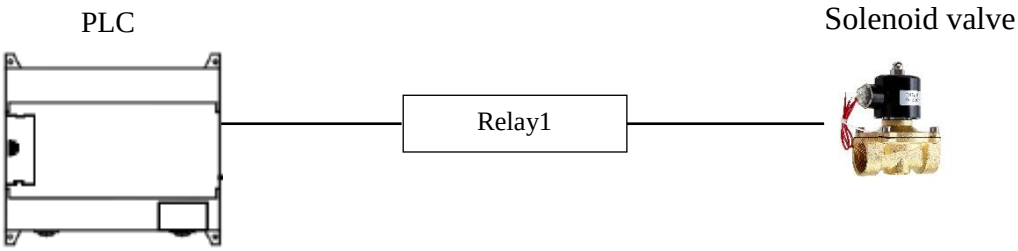


Switch of Pump 1

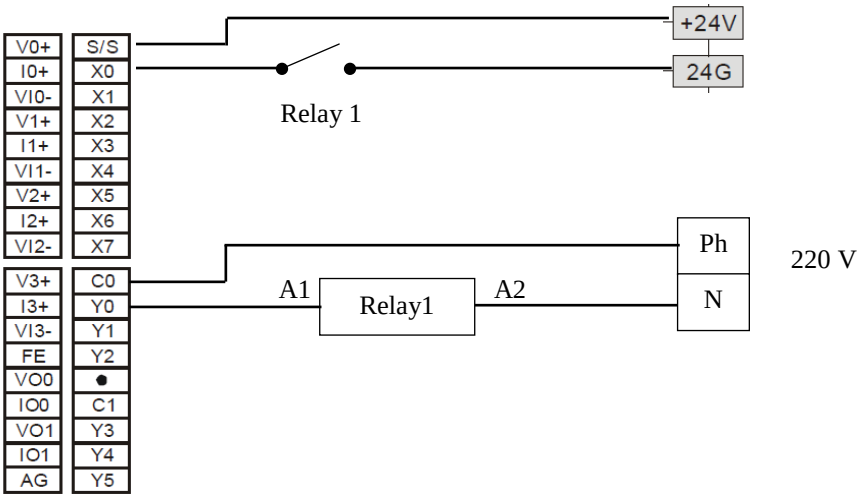
22.3 Solenoid valve

Voltage: AC220V
Fluid Temperature: 0~200℃

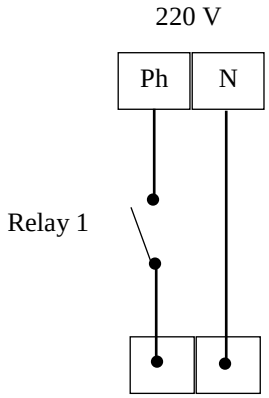
Connecting between the PLC & the Solenoid valve



Control circuit between the PLC & the relay 1



Power circuit between relay 1 & solenoid valve

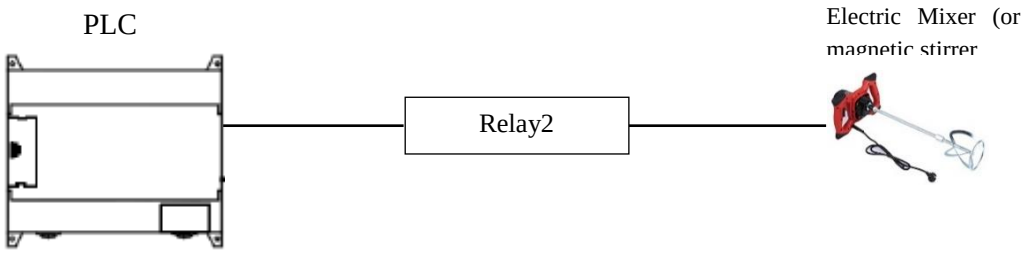


Solenoid valve

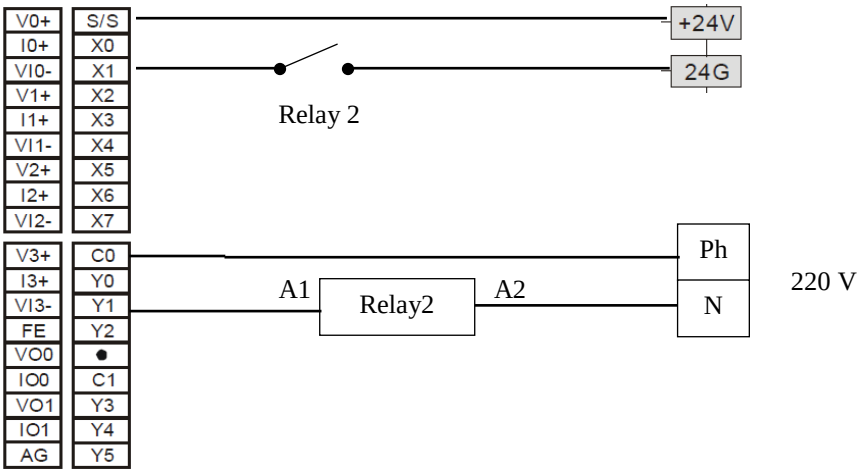
22.4 Electric Mixer / Stirrer

Voltage: AC220V

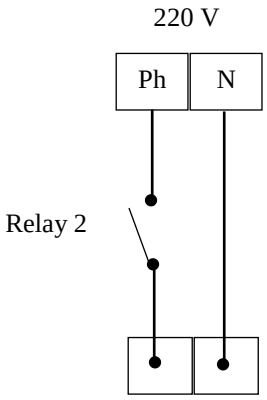
Connecting between the PLC & the electric Mixer (or magnetic stirrer)



Control circuit between the PLC & the relay 2



Power circuit between relay 2 & Electric Mixer

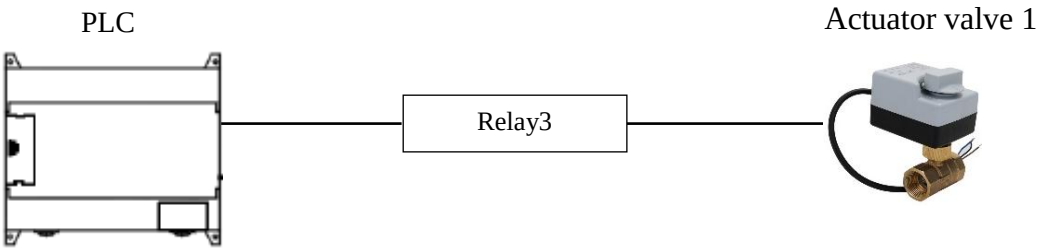


Electric Mixer

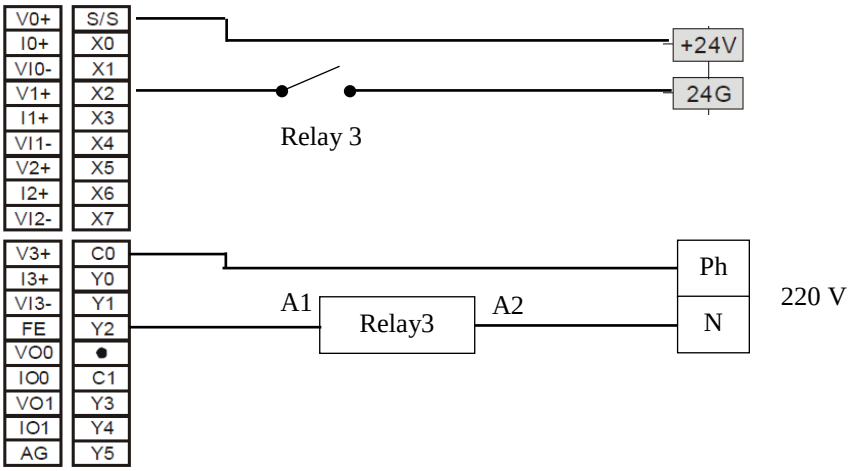
22.5 Electric Actuator Valve 1

Voltage: AC220V

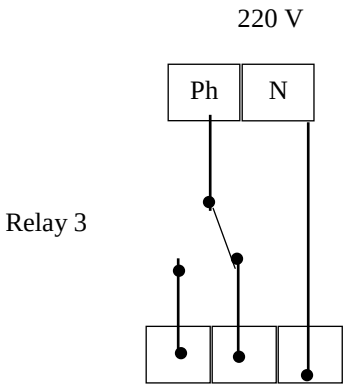
Connection between the PLC & the Actuator Valve 1



Control circuit between the PLC & the relay 3



Power circuit between relay 3 & Actuator valve 1

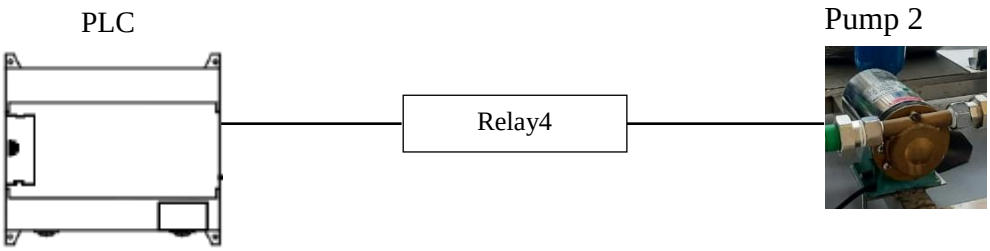


Actuator valve 1

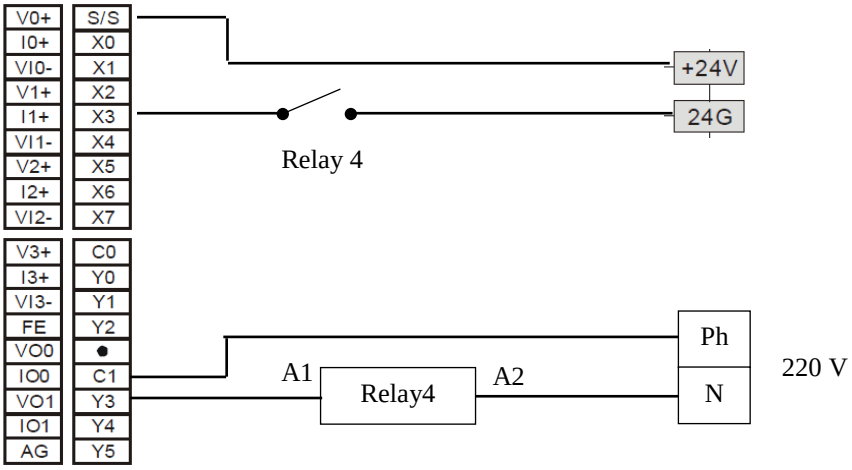
22.6 Pump 2

Voltage: AC220V

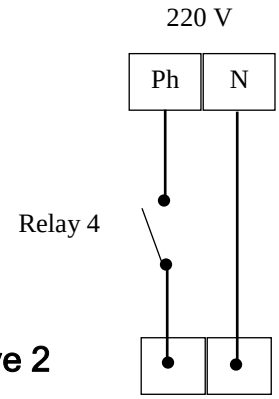
Connecting between the PLC & the Pump 2



Control circuit between the PLC & the relay 4



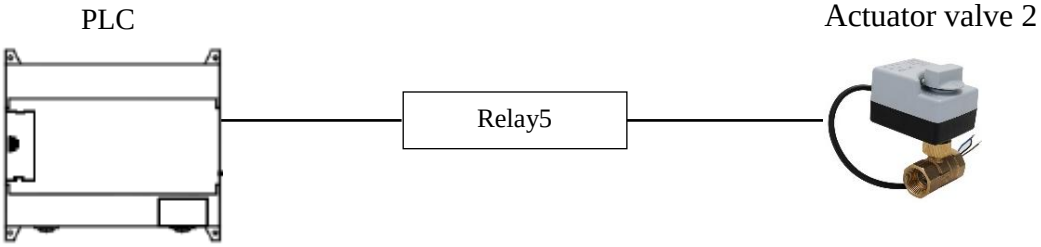
Power circuit between relay 4 & the Pump 2



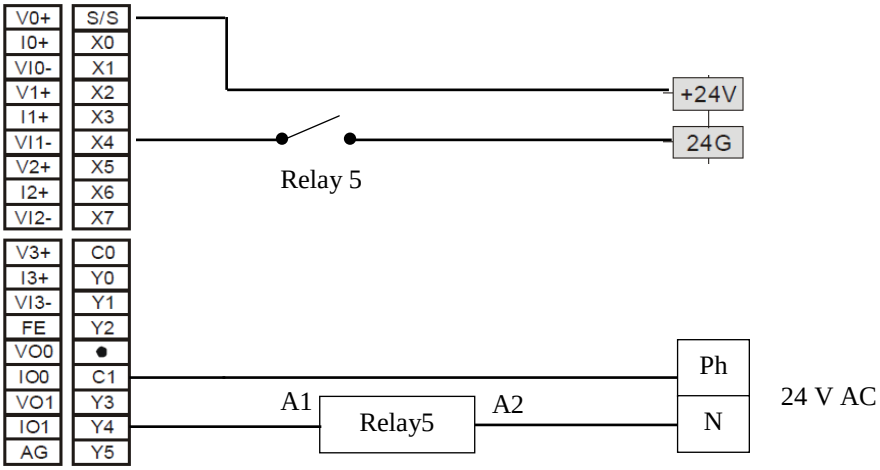
22.7 Electric Actuator Valve 2

Voltage: AC 24V

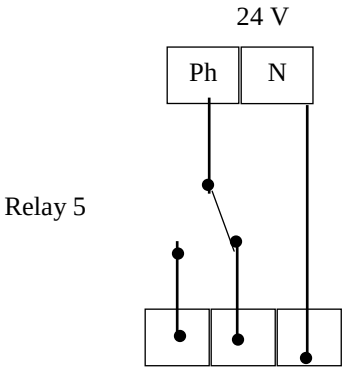
Connecting between the PLC & the Actuator Valve 2



Control circuit between the PLC & the relay 5



Power circuit between relay 5 & Actuator valve 2

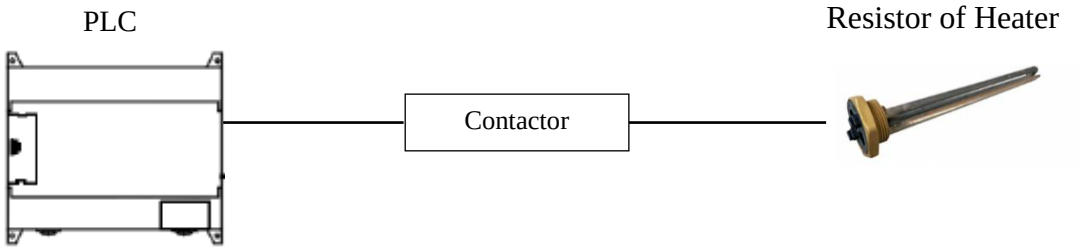


Actuator valve 2

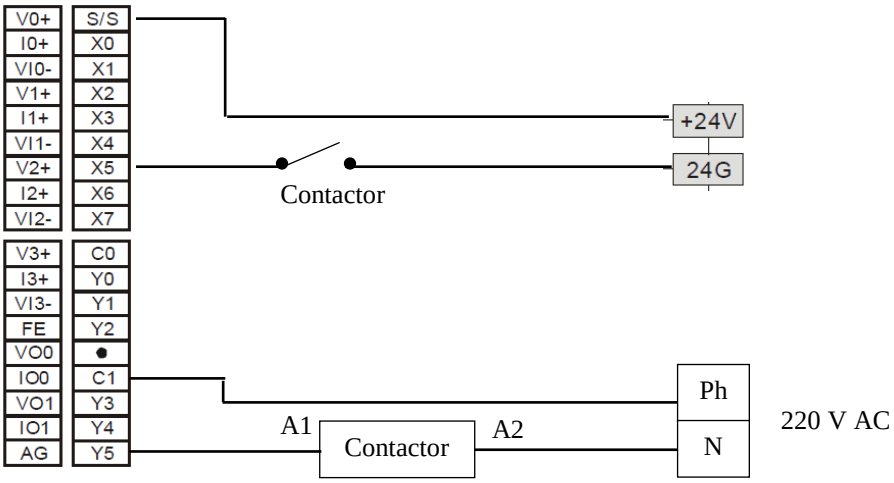
22.8 Resistor of Heater

Voltage: AC 220V

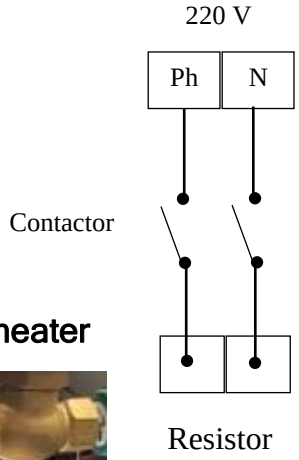
Connecting between the PLC & the Resistor of Heater



Control circuit between the PLC & the Contactor

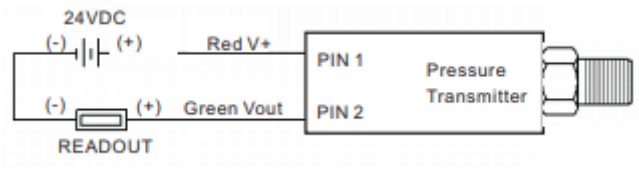


Power circuit between Contactor & the Resistor of heater

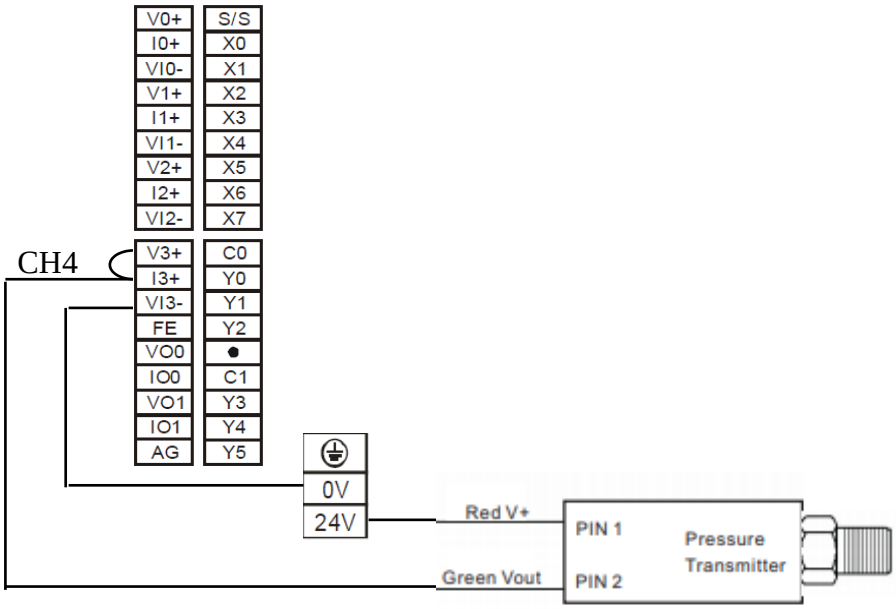


22.9 Pressure sensor of heater

MODEL : GPT220
Range : 0-16bar
Output : 4-20 mA
Power : 12- 36V
Temperature : 220^0 C

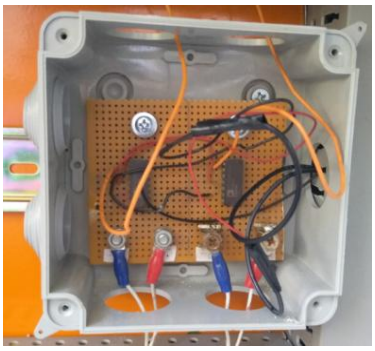


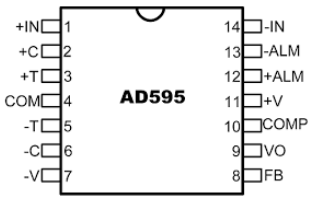
Connecting between the PLC & the Pressure sensor



22.10 Temperature sensor of penicillin fermenter tank

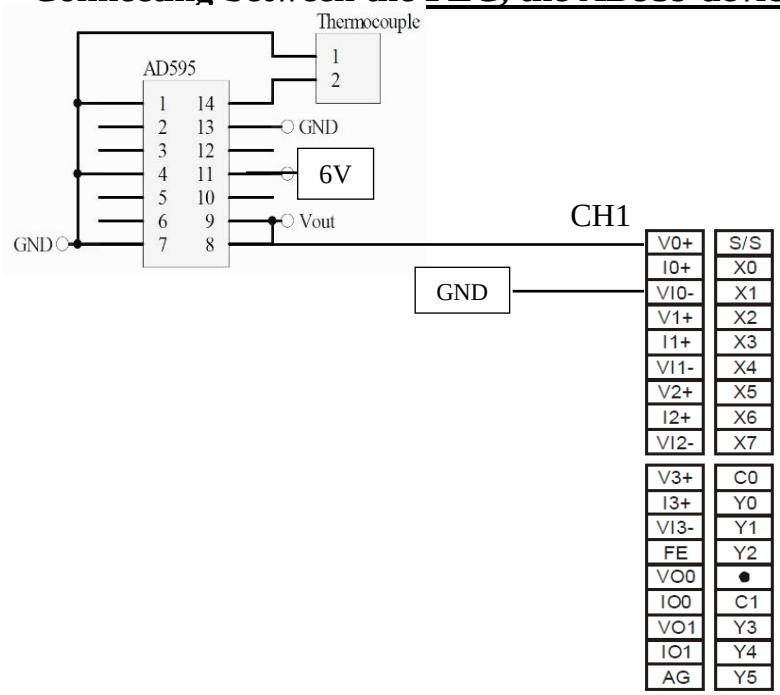
Temperature sensor (K-Thermocouple)





AD959 device

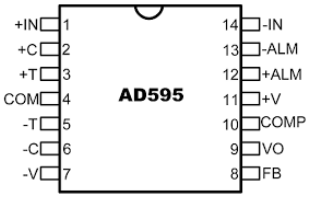
Connecting between the PLC, the AD959 device & the Temperature sensor

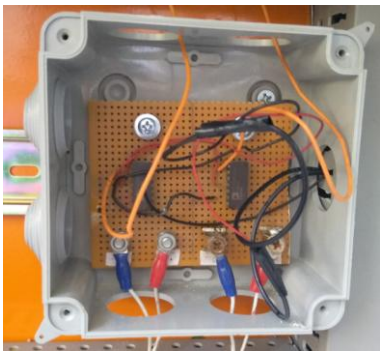


22.11 Temperature sensor of Heater tank

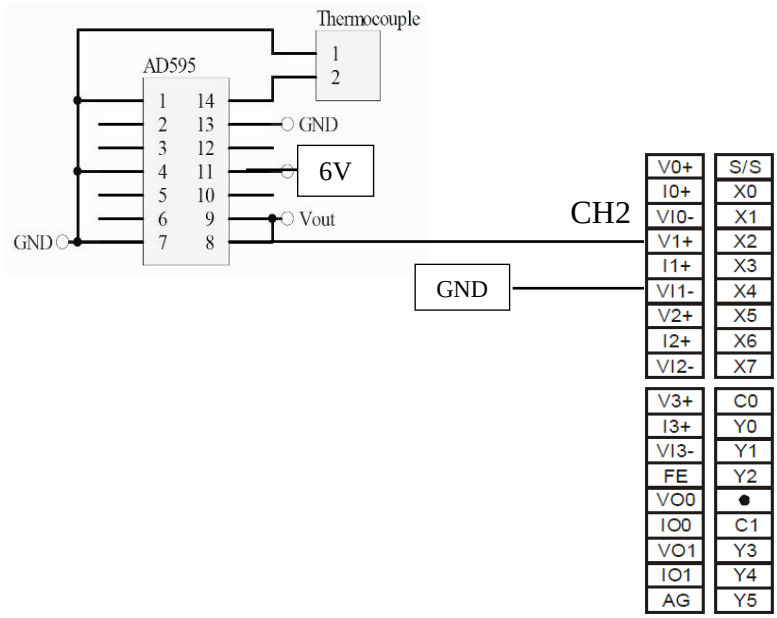
Temperature sensor (K-Thermocouple)

AD959 device





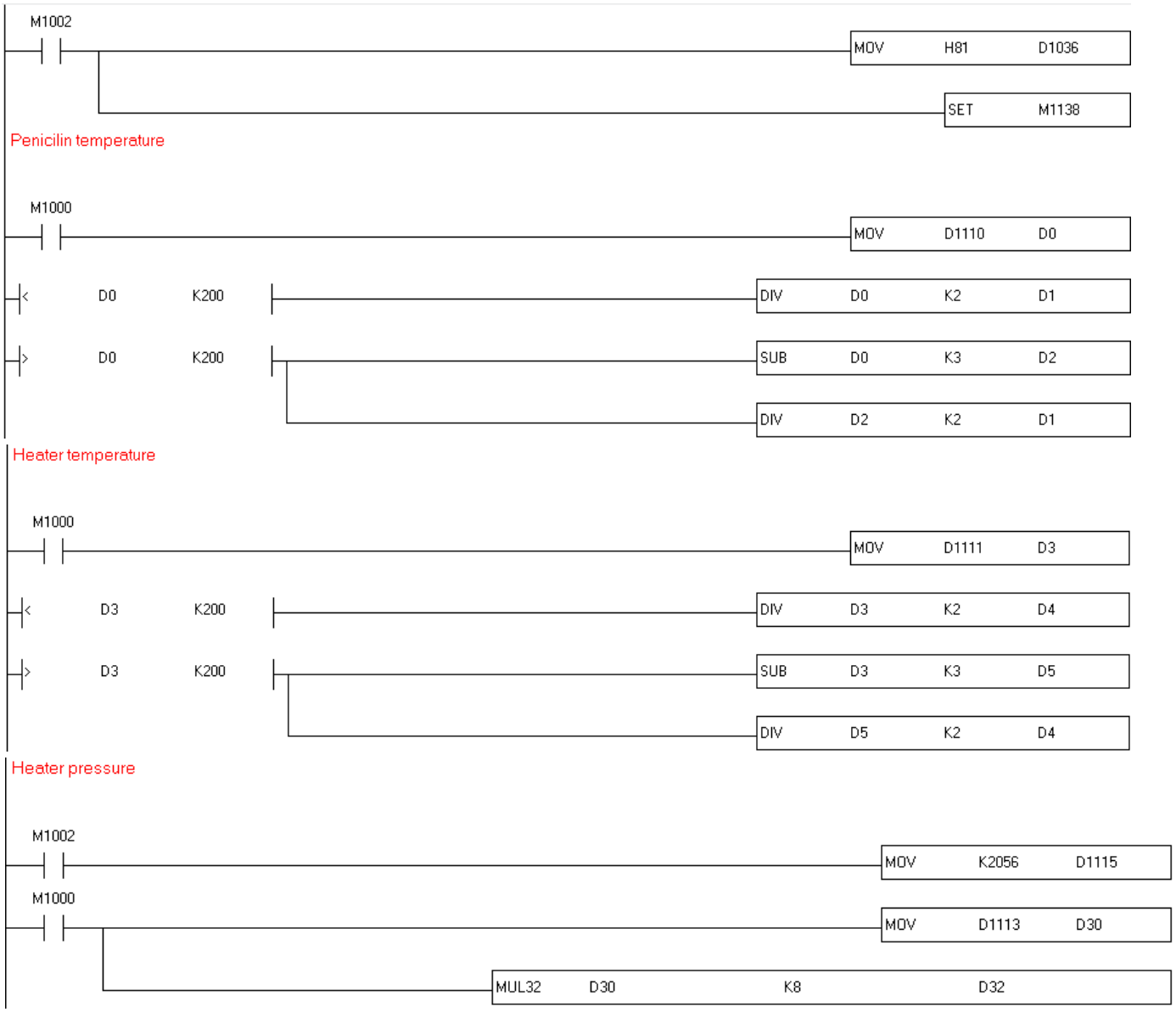
Connecting between the PLC, the AD959 device & the Temperature sensor (K-Thermocouple)

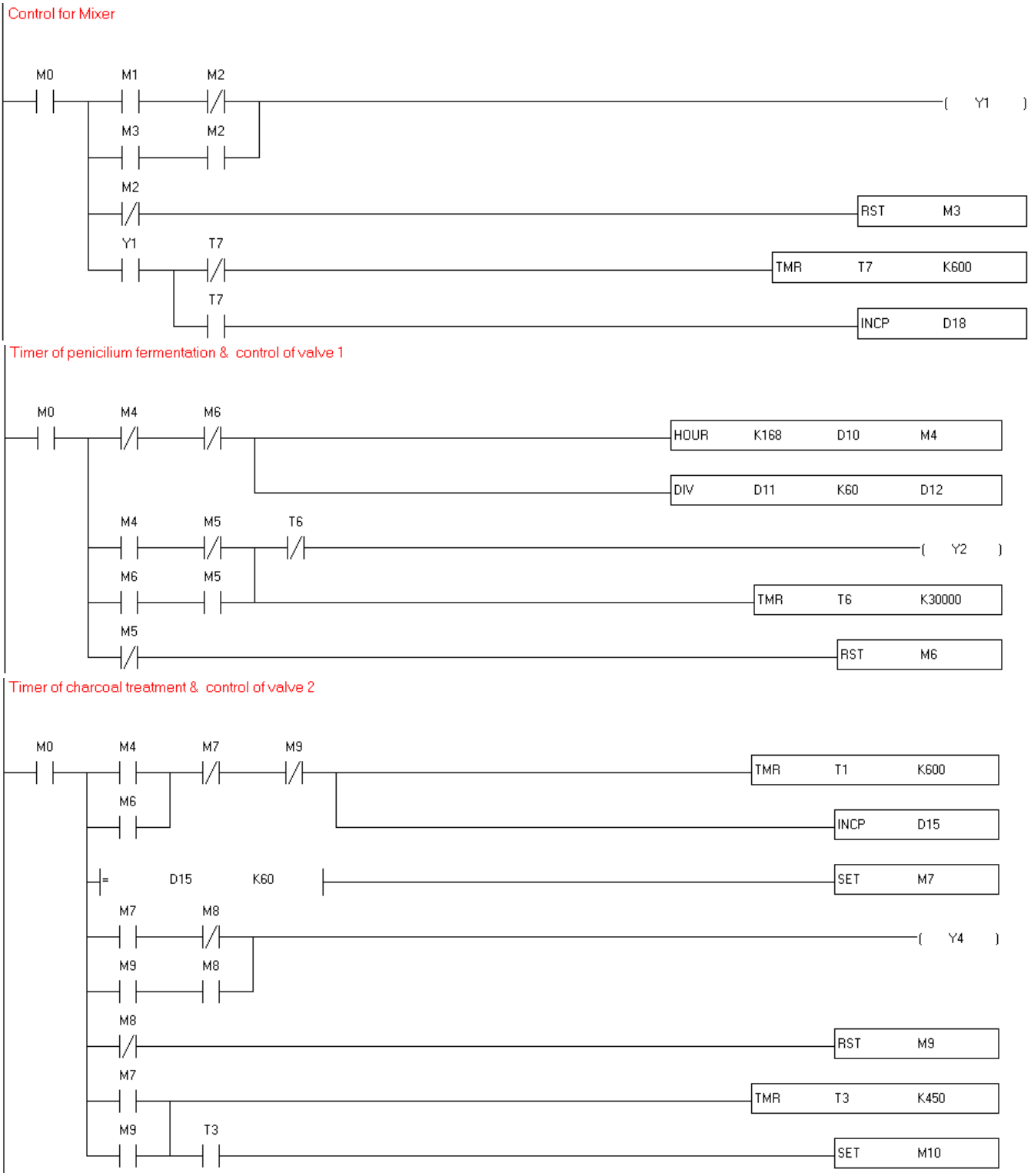


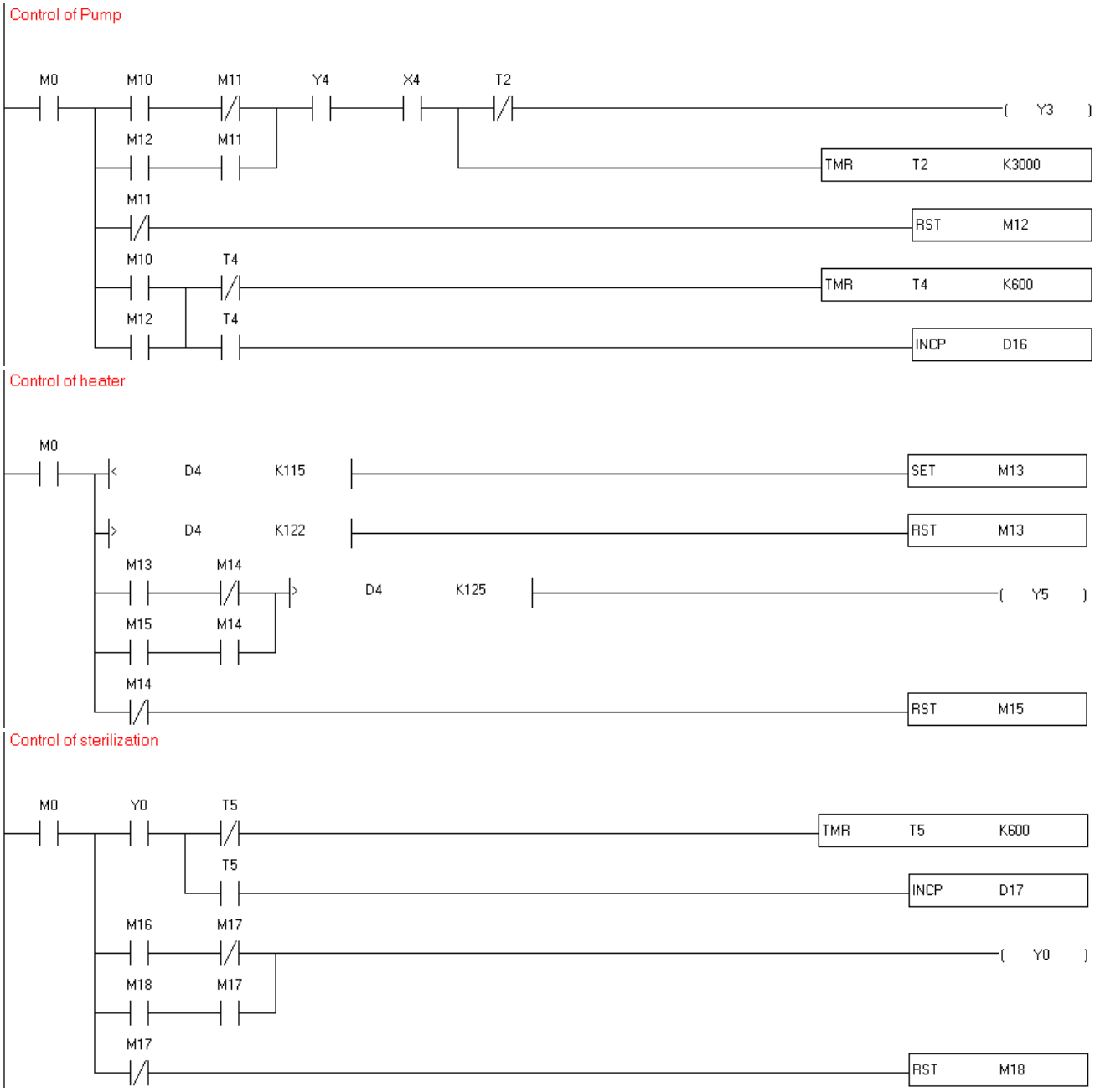
23 Control system for PLC & HMI

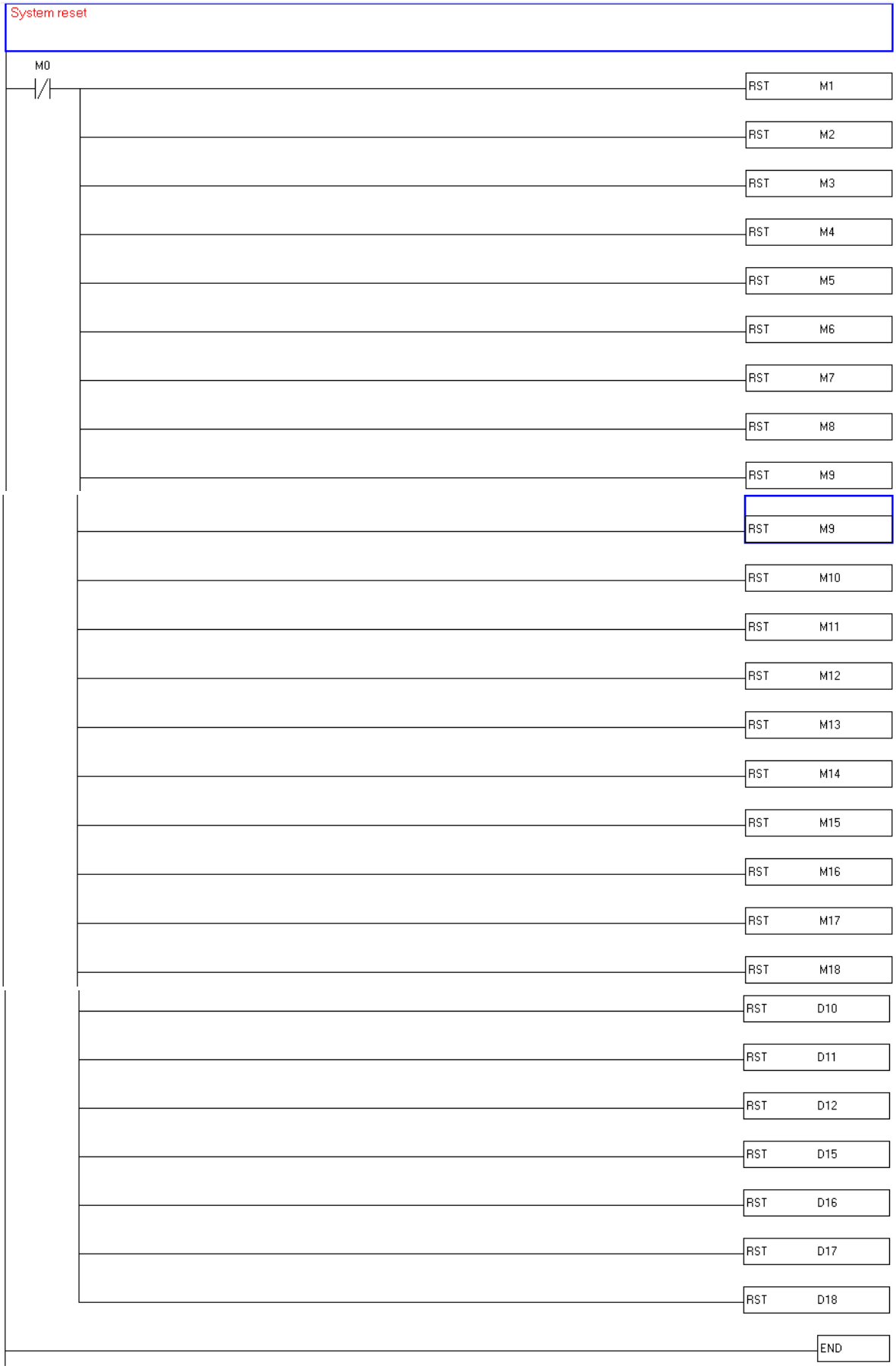
23.1 Programme of PLC

MEGBI-APP-Control System.dvp	Program code (please click and open in any editor, e.g. notepad++)




Control for Mixer
M0
M1
M2
Y1
M3
M2
M2
RST M3
Y1
T7
TMR T7 K600
T7
INCP D18
Timer of penicilium fermentation & control of valve 1
M0
M4
M6
HOUR K168 D10 M4
DIV D11 K60 D12
M4
M5
T6
Y2
M6
M5
TMR T6 K30000
M5
RST M6
Timer of charcoal treatment & control of valve 2
M0
M4
M7
M9
TMR T1 K600
M6
INCP D15
= D15 K60
SET M7
M7
M8
Y4
M9
M8
M8
RST M9
M7
TMR T3 K450
M9
T3
SET M10


Control of Pump
M0
M10
M11
Y4
X4
T2
Y3
M12
M11
TMR T2 K3000
M11
RST M12
M10
T4
TMR T4 K600
M12
T4
INCP D16
Control of heater
M0
D4 K115
SET M13
D4 K122
RST M13
M13
M14
D4 K125
Y5
M15
M14
M14
RST M15
Control of sterilization
M0
Y0
T5
TMR T5 K600
T5
INCP D17
M16
M17
Y0
M18
M17
M17
RST M18


System reset
M0
RST M1
RST M2
RST M3
RST M4
RST M5
RST M6
RST M7
RST M8
RST M9
RST M9
RST M10
RST M11
RST M12
RST M13
RST M14
RST M15
RST M16
RST M17
RST M18
RST D10
RST D11
RST D12
RST D15
RST D16
RST D17
RST D18
END

23.2 HMI Program

23.2.1 Auto mode

Press “Start”

- Start Timer 1 of tank 1, Mixer ON
- Delay 168 hours (7 days)
- If Timer 1= 168 hours, Open Valve 1
- Start Timer 2 of tank 2
- If Timer 2 = 1 hour, Open Valve 2
- Pump 2 ON for 5 min after Valve 2 is open

23.2.2 Manuel mode (interactive)

Press “Start”

Fermentation pen cilium :

a) Mixer :
- Press “Manual”
- for OFF Press “Manual OFF”
- for ON press “Manal ON”

b) Valve :
- Press “Manual”
- for Open Press “Manual Open”
- for Close press “Manal Close ”

Charcoal treatment :

a) Valve :
- Press “Manual”
- for Open Press “Manual Open”
- for Close press “Manal Close ”

b) Pump : (if valve 2 Close, Pump not working)
- Press “Manual”
- for OFF Press “Manual OFF”
- for ON press “Manal ON”

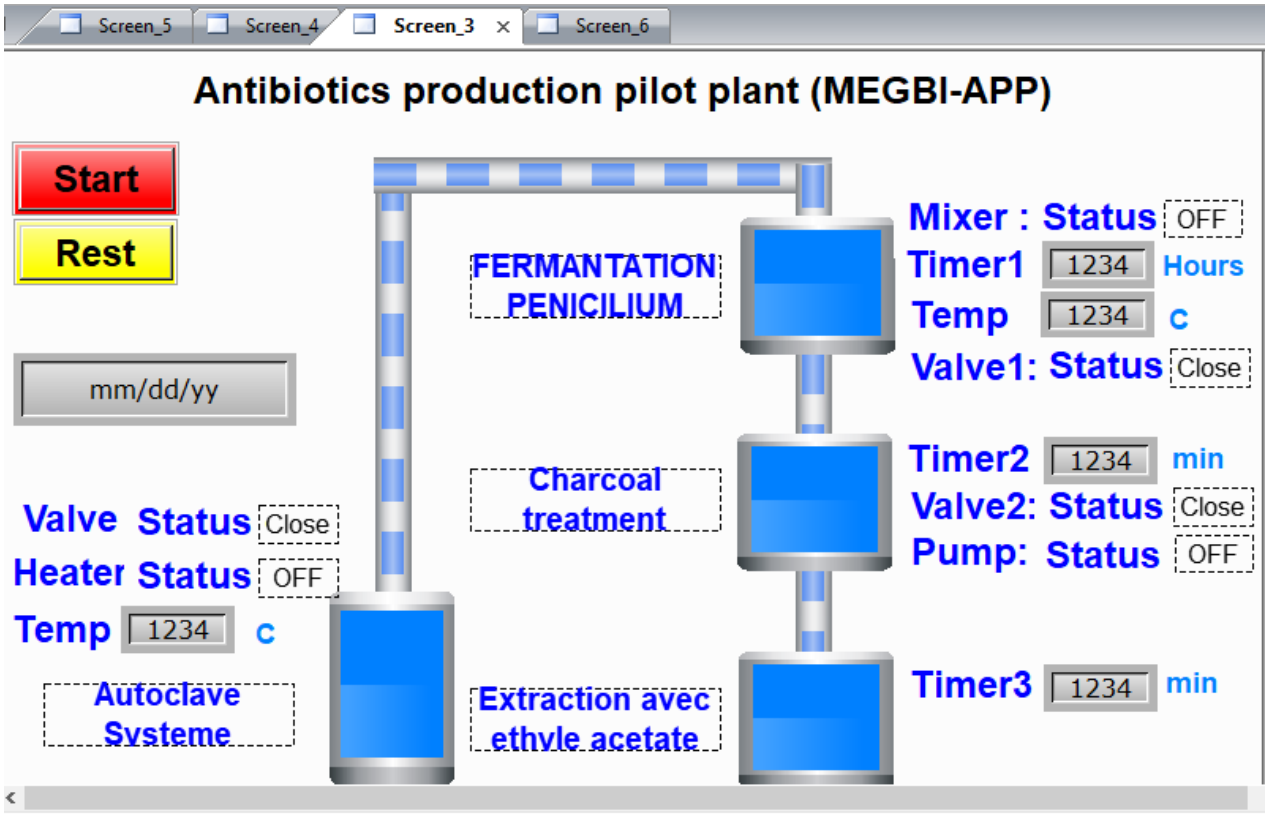
Autoclave system:

a) Heater :
- Press “Manual”
- for OFF Press “Manual OFF”
- for ON press “Manal ON” (if Temperature > 122^0 C Heater OFF)

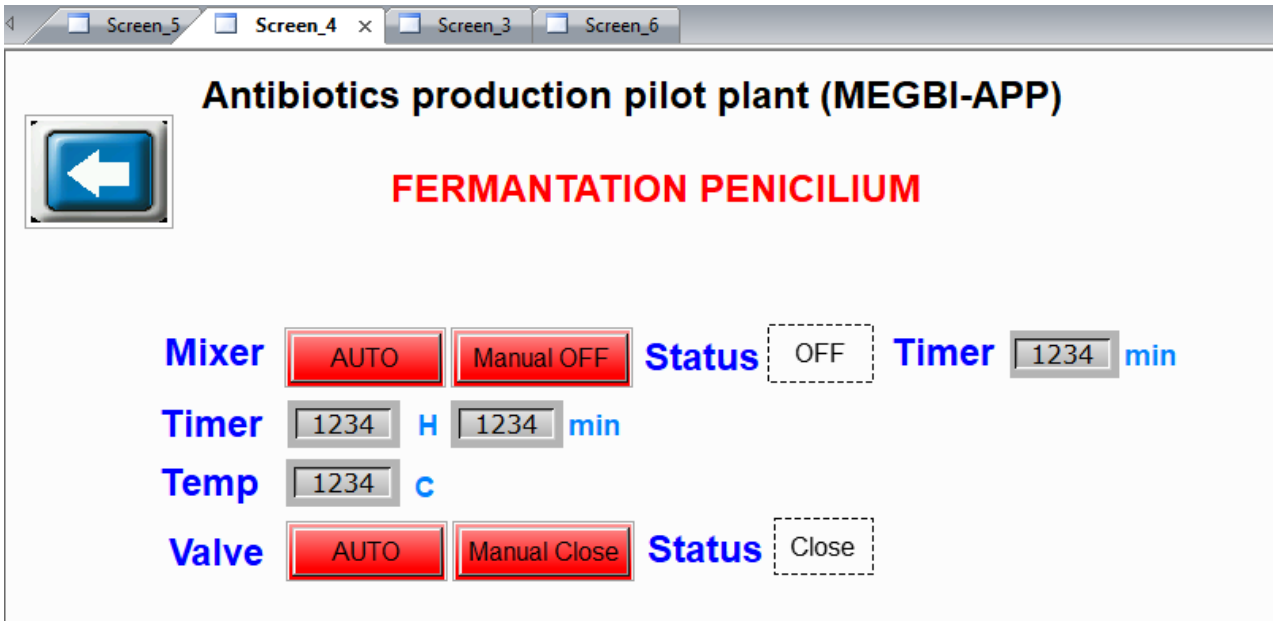
b) Solenoid valve
- Press “Manual”
- for Open Press “Manual Open”
- for Close press “Manual Close”

23.2.3 HMI pages

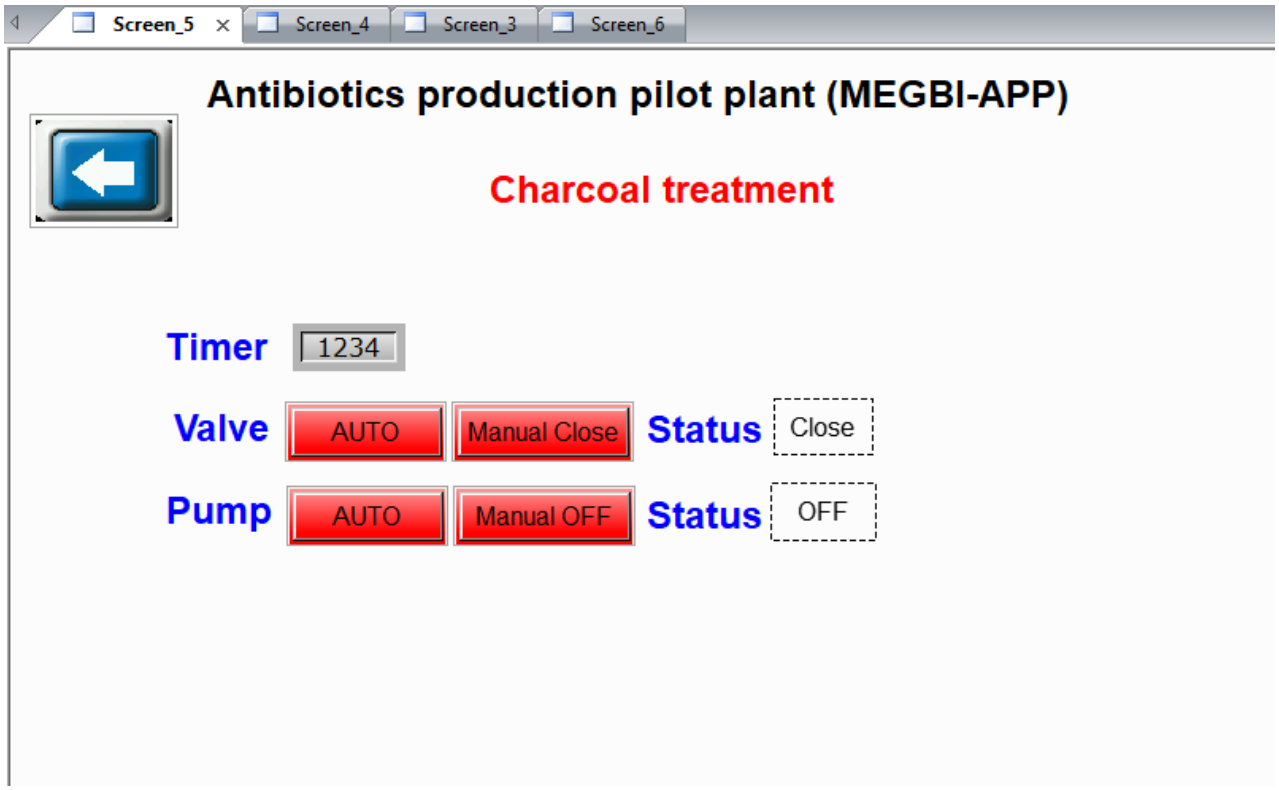
23.2.3.1 Main page



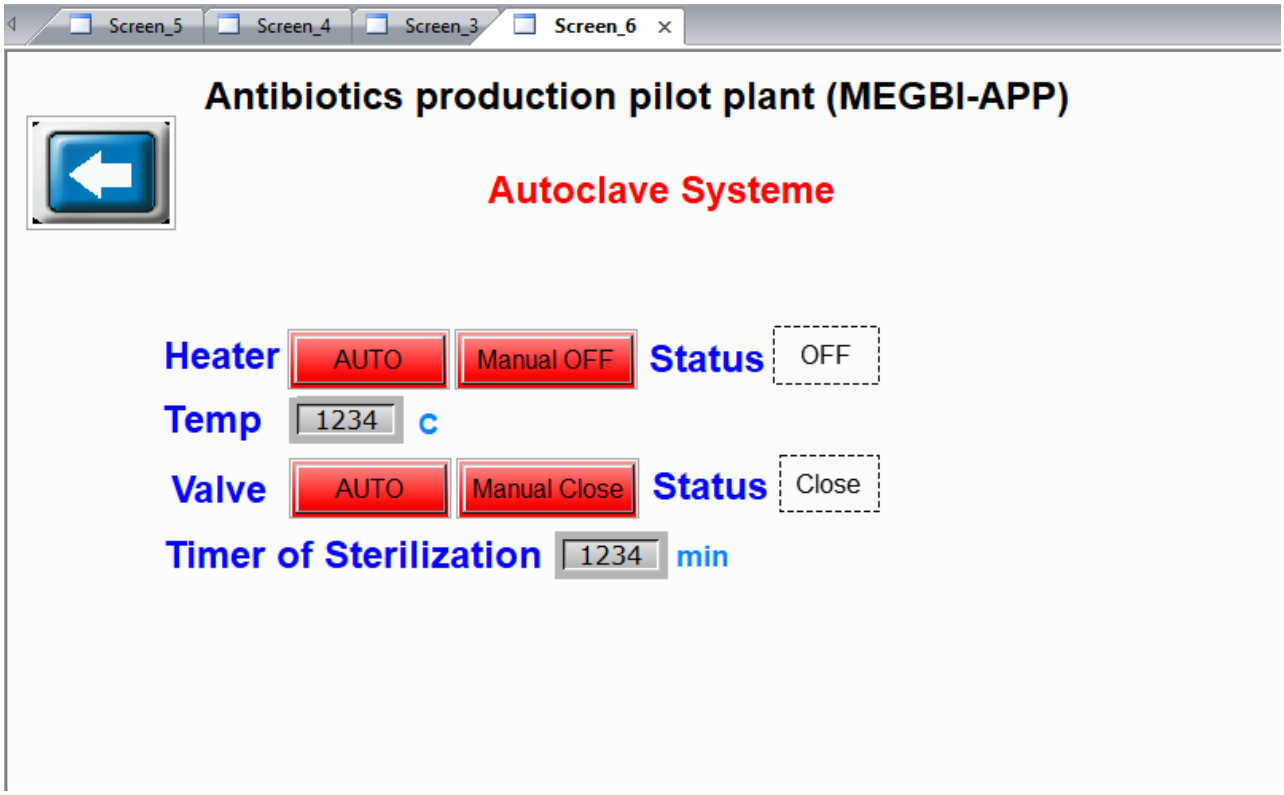
23.2.3.2 Fermentation pencilium page



23.2.3.3 Charcoal treatment page



23.2.3.4 Autoclave system page

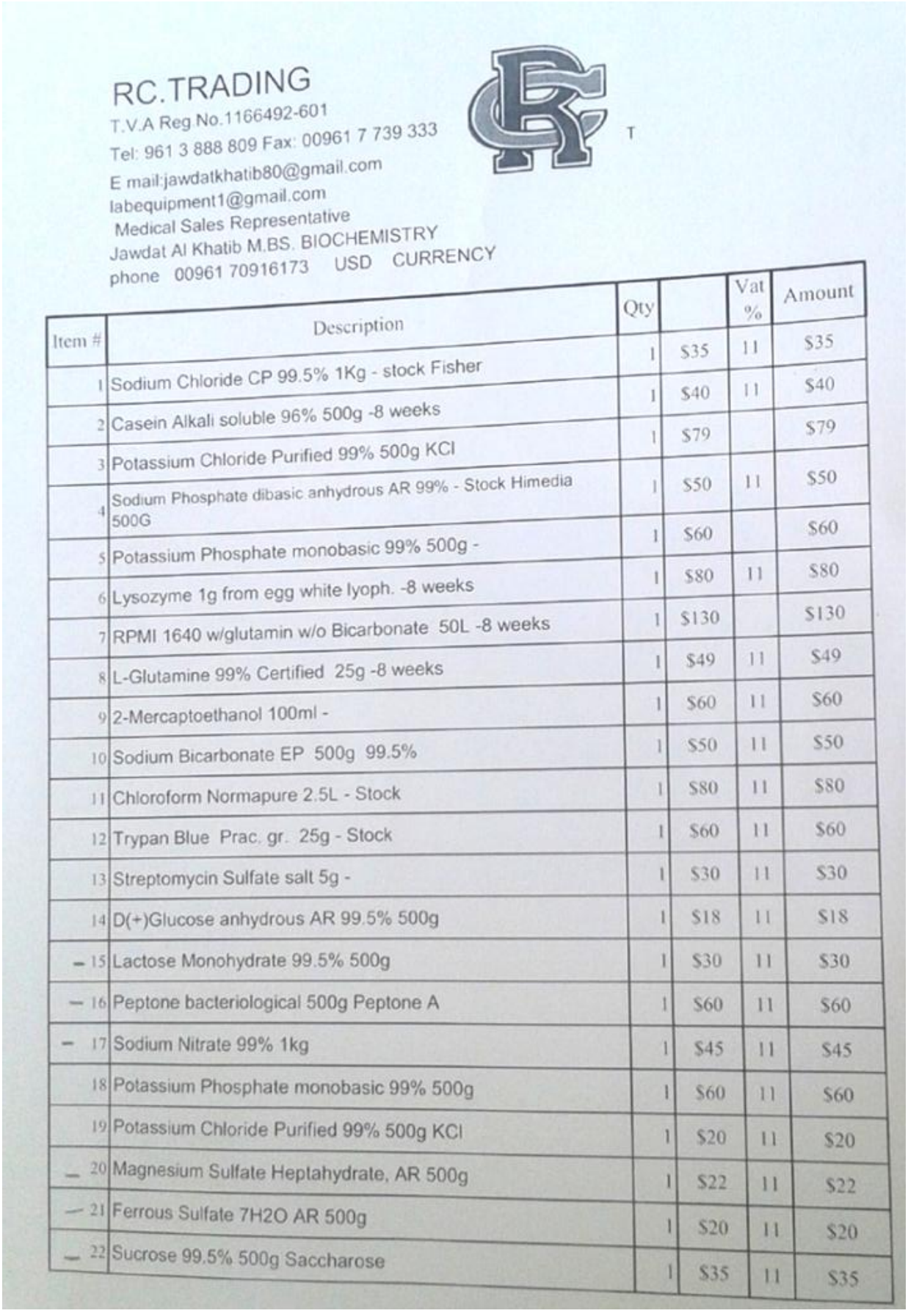


Operators Manual

24 Materials for MEGBI-APP

24.1.1 Consumables and Materials

24.1.1.1 Offer from Jawdat AlKatibe



RC.TRADING
T.V.A Reg No.1166492-601
Tel: 961 3 888 809 Fax: 00961 7 739 333
E mail:jawdatkhatib80@gmail.com
labequipment1@gmail.com
Medical Sales Representative
Jawdat Al Khatib M.BS. BIOCHEMISTRY
phone 00961 70916173 USD CURRENCY

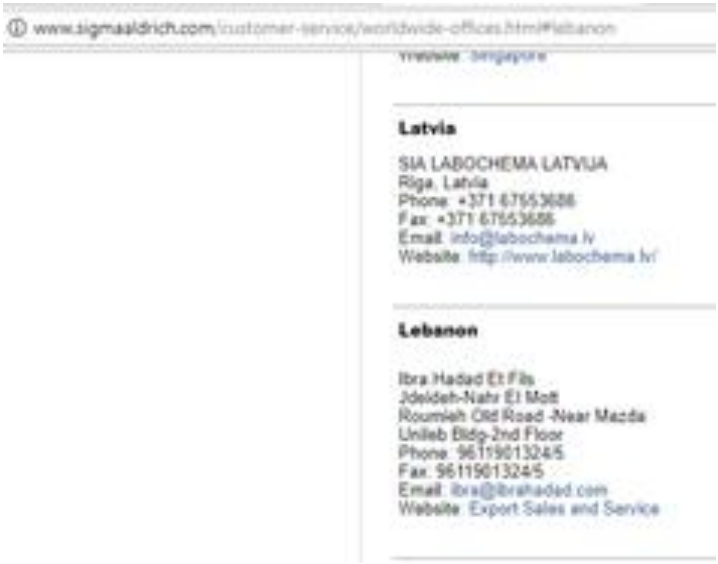
Item #	Description	Qty		Vat %	Amount
1	Sodium Chloride CP 99.5% 1Kg - stock Fisher	1	$35	11	$35
2	Casein Alkali soluble 96% 500g -8 weeks	1	$40	11	$40
3	Potassium Chloride Purified 99% 500g KCl	1	$79		$79
4	Sodium Phosphate dibasic anhydrous AR 99% - Stock Himedia 500G	1	$50	11	$50
5	Potassium Phosphate monobasic 99% 500g -	1	$60		$60
6	Lysozyme 1g from egg white lyoph. -8 weeks	1	$80	11	$80
7	RPMI 1640 w/glutamin w/o Bicarbonate 50L -8 weeks	1	$130		$130
8	L-Glutamine 99% Certified 25g -8 weeks	1	$49	11	$49
9	2-Mercaptoethanol 100ml -	1	$60	11	$60
10	Sodium Bicarbonate EP 500g 99.5%	1	$50	11	$50
11	Chloroform Normapure 2.5L - Stock	1	$80	11	$80
12	Trypan Blue Prac. gr. 25g - Stock	1	$60	11	$60
13	Streptomycin Sulfate salt 5g -	1	$30	11	$30
14	D(+)Glucose anhydrous AR 99.5% 500g	1	$18	11	$18
15	Lactose Monohydrate 99.5% 500g	1	$30	11	$30
16	Peptone bacteriological 500g Peptone A	1	$60	11	$60
17	Sodium Nitrate 99% 1kg	1	$45	11	$45
18	Potassium Phosphate monobasic 99% 500g	1	$60	11	$60
19	Potassium Chloride Purified 99% 500g KCl	1	$20	11	$20
20	Magnesium Sulfate Heptahydrate, AR 500g	1	$22	11	$22
21	Ferrous Sulfate 7H2O AR 500g	1	$20	11	$20
22	Sucrose 99.5% 500g Saccharose	1	$35	11	$35

23	Zinc Sulfate 7H2O 99% Purified 500g	1	$20	11	$20
24	Copper II Sulfate 5H2O EP 500g	1	$25	11	$25
25	Protose BE (Beef extract powder) 500g	1	$120	11	$120
26	Ammonium Persulfate EP 98% 500g	1	$20	11	$20
27	Parafilm 4"x38 meter 125Ft	1	$38	11	$38
28	Ethyl acetate AR 2.5L	1	$60	11	$60
29	Phosphate Buffer Saline PH 7.2 100g PBS	1	$50	11	$50
30	Chloroform Normapure 2.5L	1	$80	11	$80
31	Cotton Blue Lactophenol 100ml	1	$50	11	$50

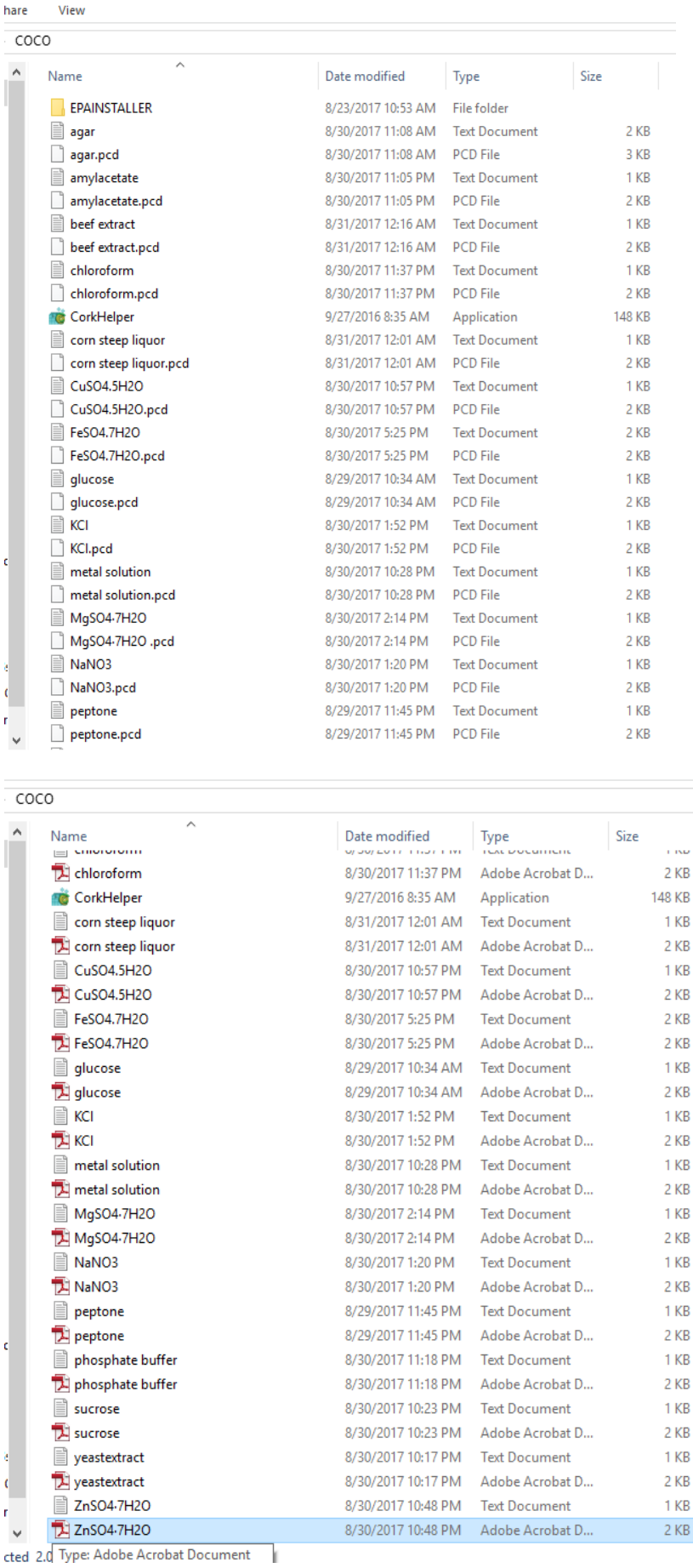
Offer from Bourhan Kabbara

Ampicillin Pilot Plans			
ID			cost$
Glucose		500g	20
Lactose		500g	24
Peptone		500g	56
NaNo3		500g	32
Na2HPO4		500g	25
MgSO47H2O		500g	18
FeSO47H2O		500g	20
Sucrose		500g	18
ZnSO47H2O		500g	20
CuSO45H2O		500g	18
(NH4)2SO4		500g	30
Sodium acetate		500g	22
Ethyl acetate		2.5 L	60
Sodium acetate		500g	22
Chloroform		2.5L	75
Lacto phenol cotton blue stain		100ml	46
Titriplex		250g	25
total			531
K2HPO4 (dibasic)			
yest extract			
CaCO3			
Corn steep liquor			
Beef extract			
Na2SO4			

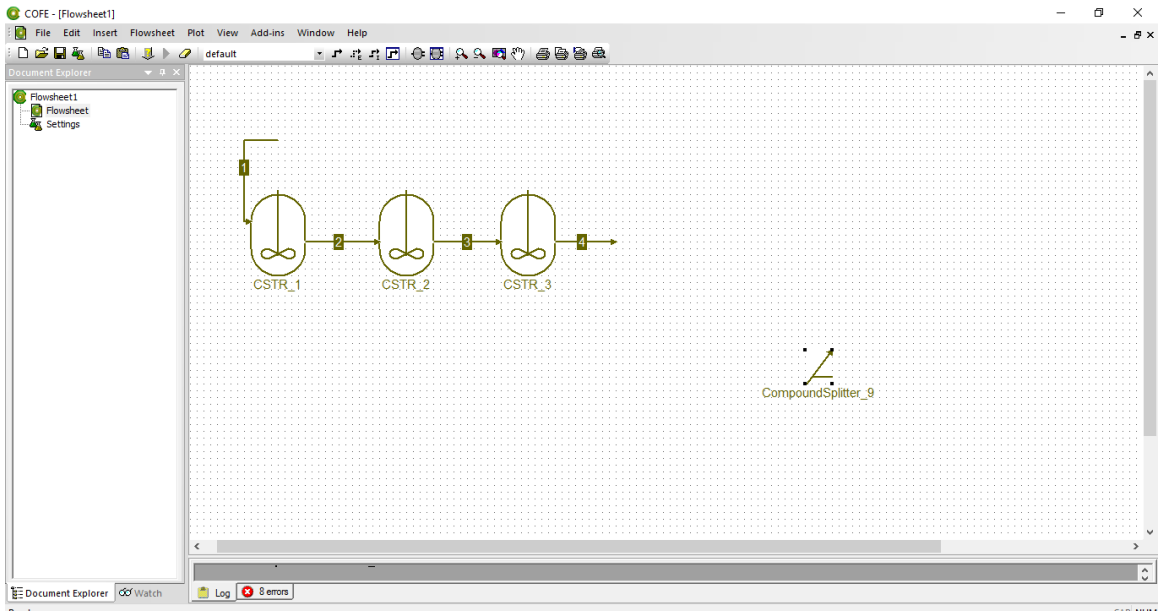
24.2 Chemicals from Sigma Aldrich



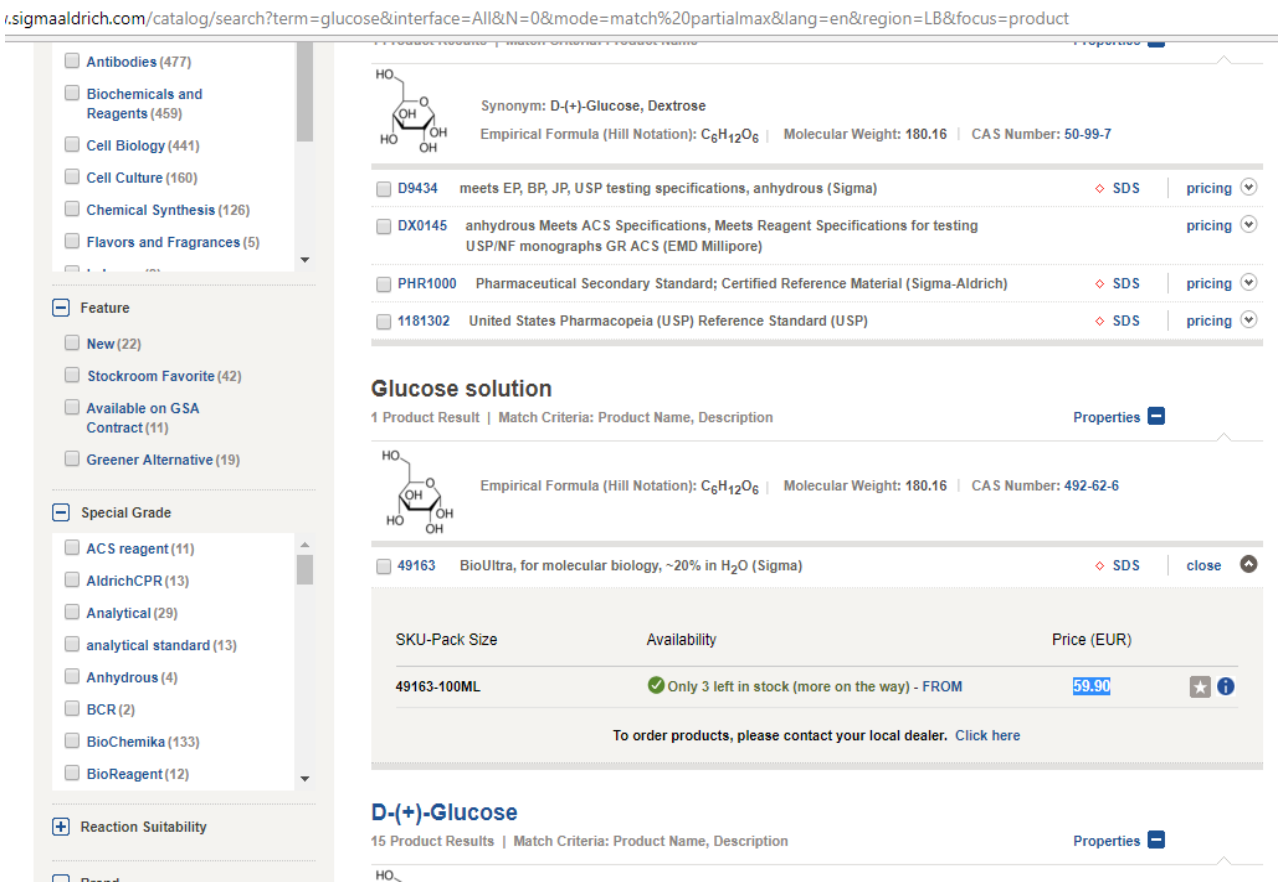
24.2.1 new compounds on coco



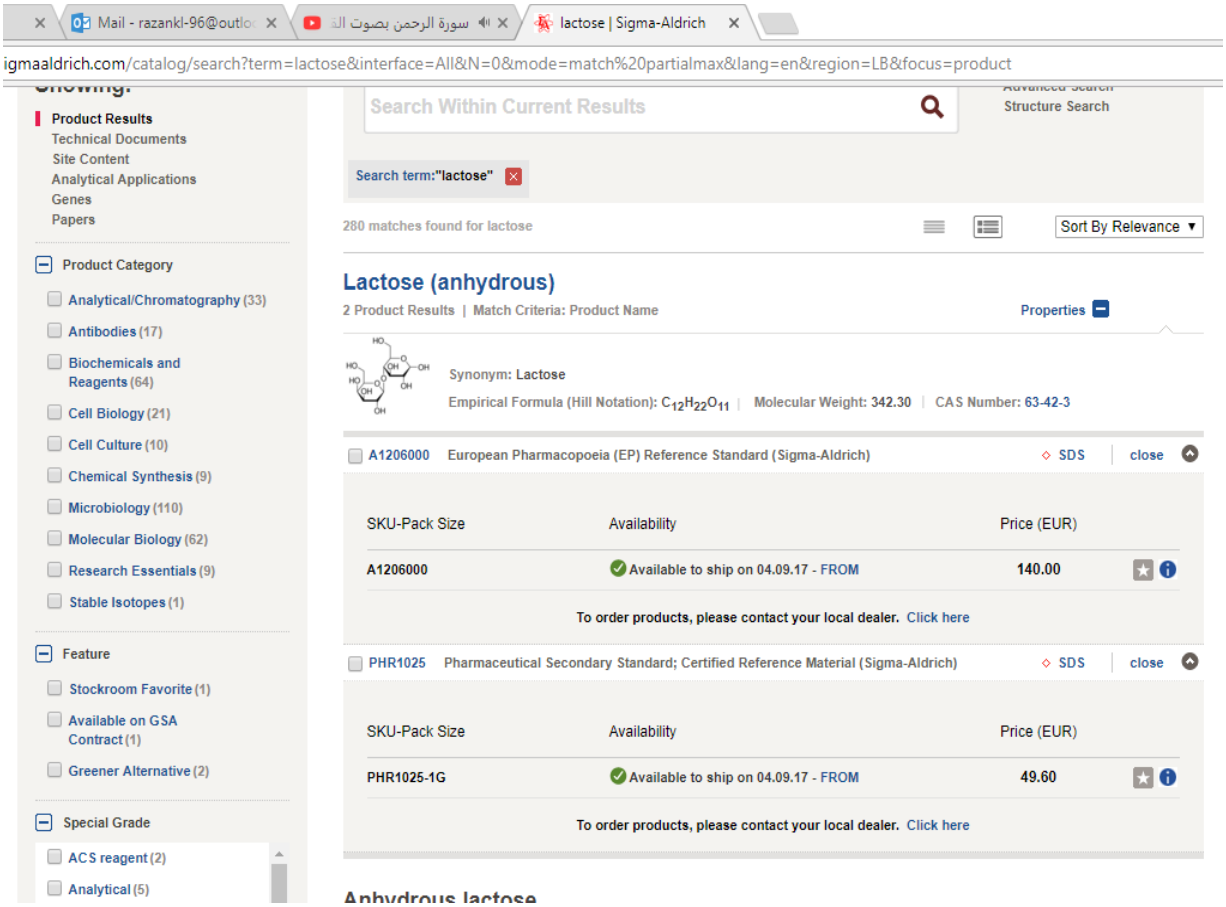
Problem: I can't use these compounds in coco (cofe 64)



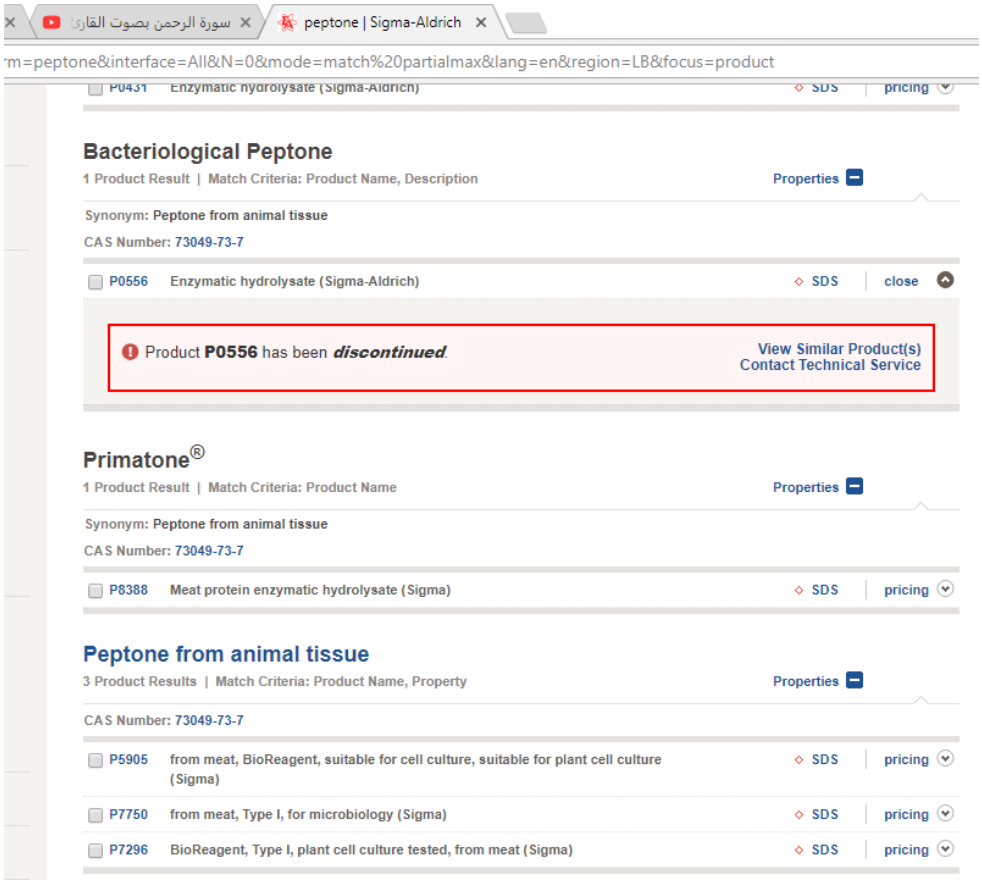
24.2.2 Glucose:



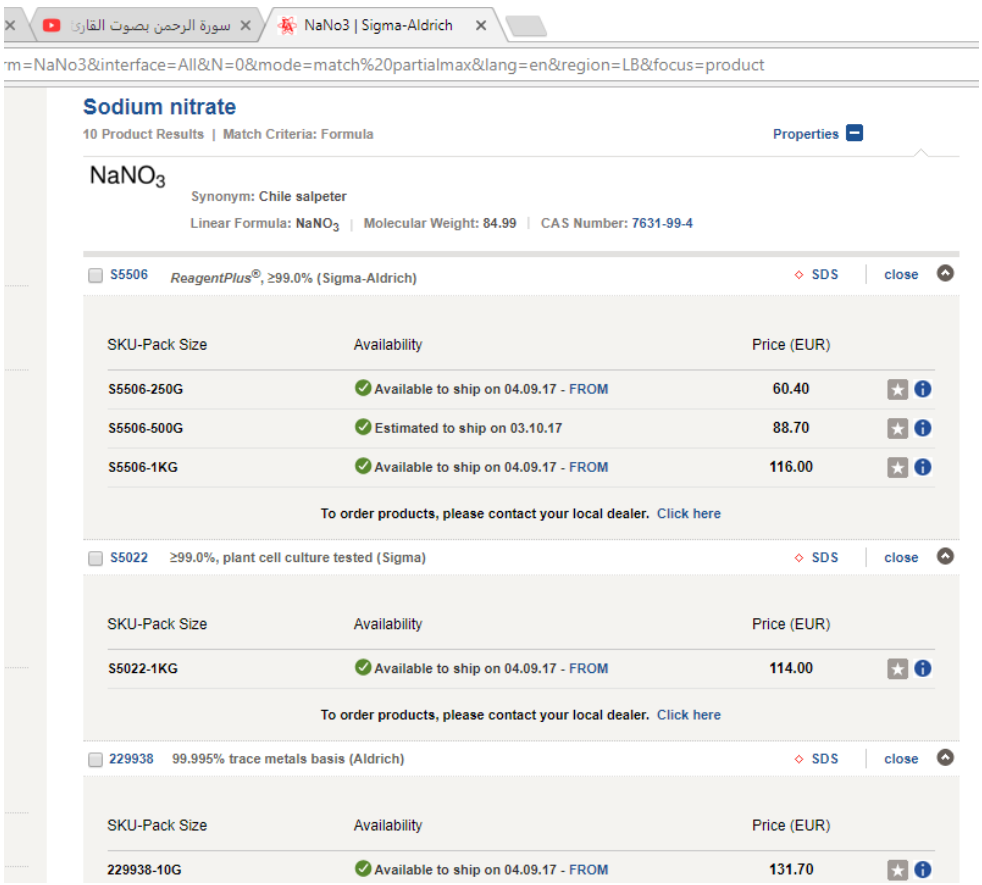
24.2.3 Lactose:

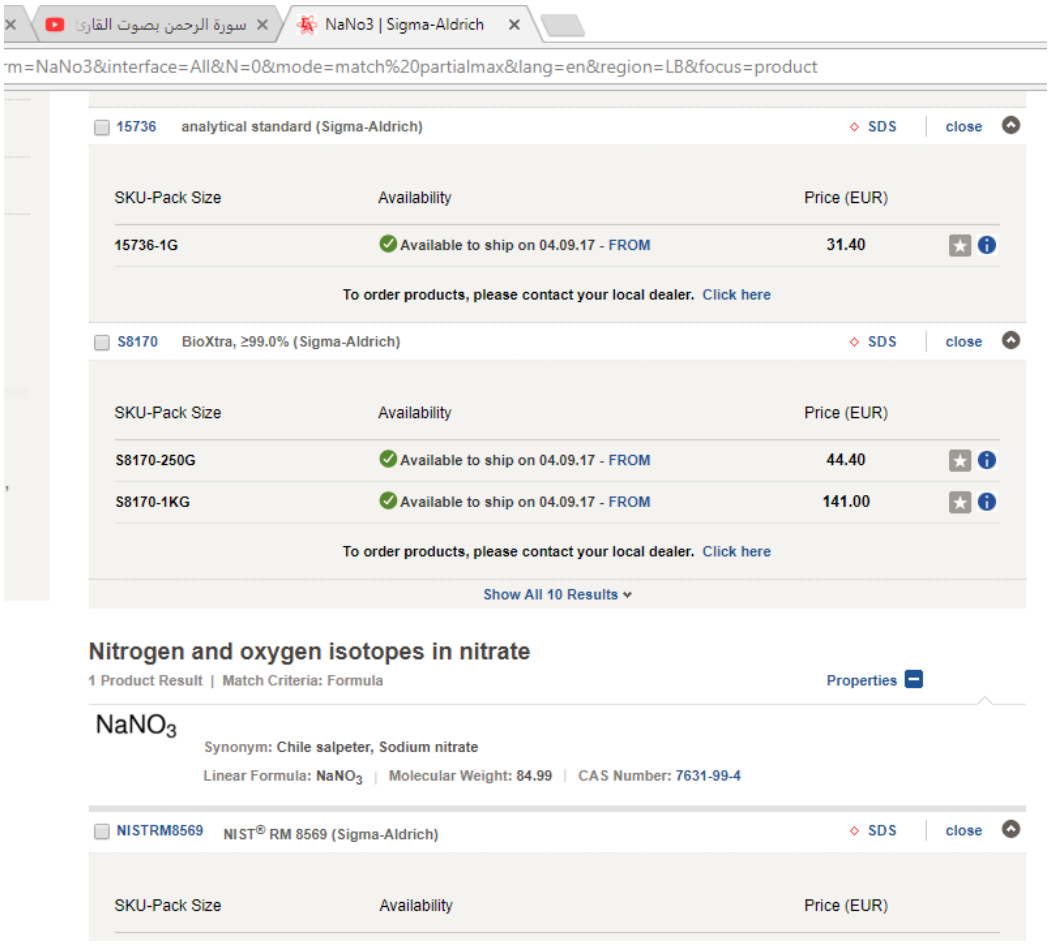


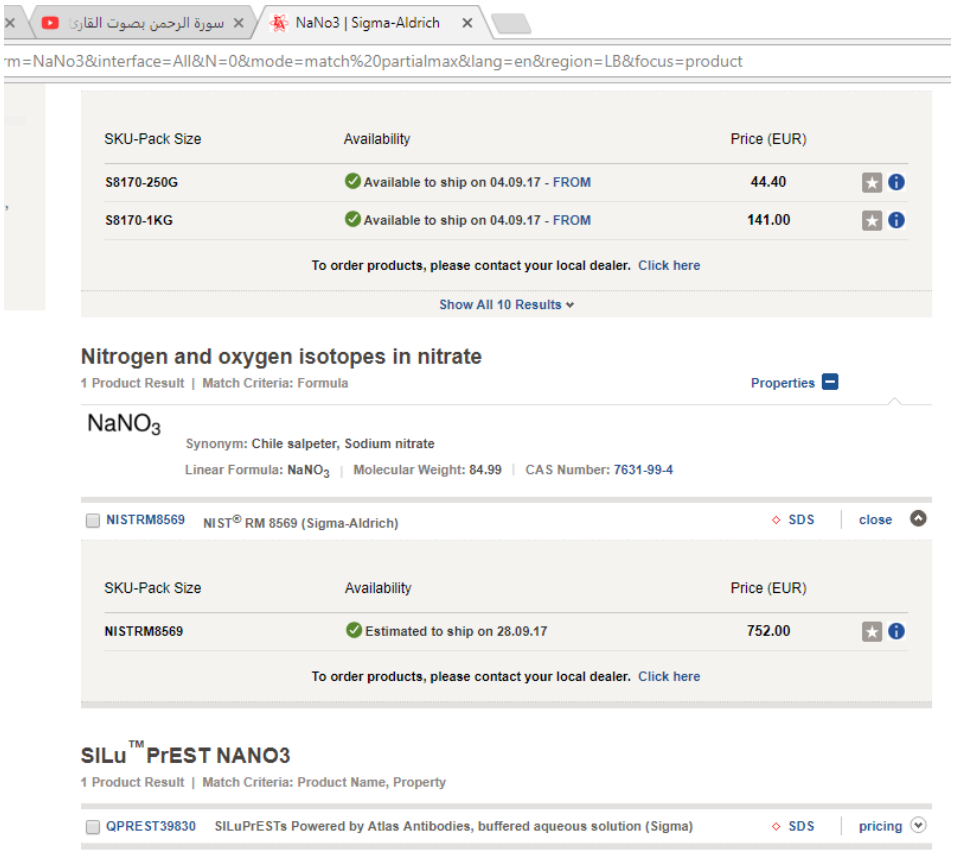
24.2.4 Peptone:



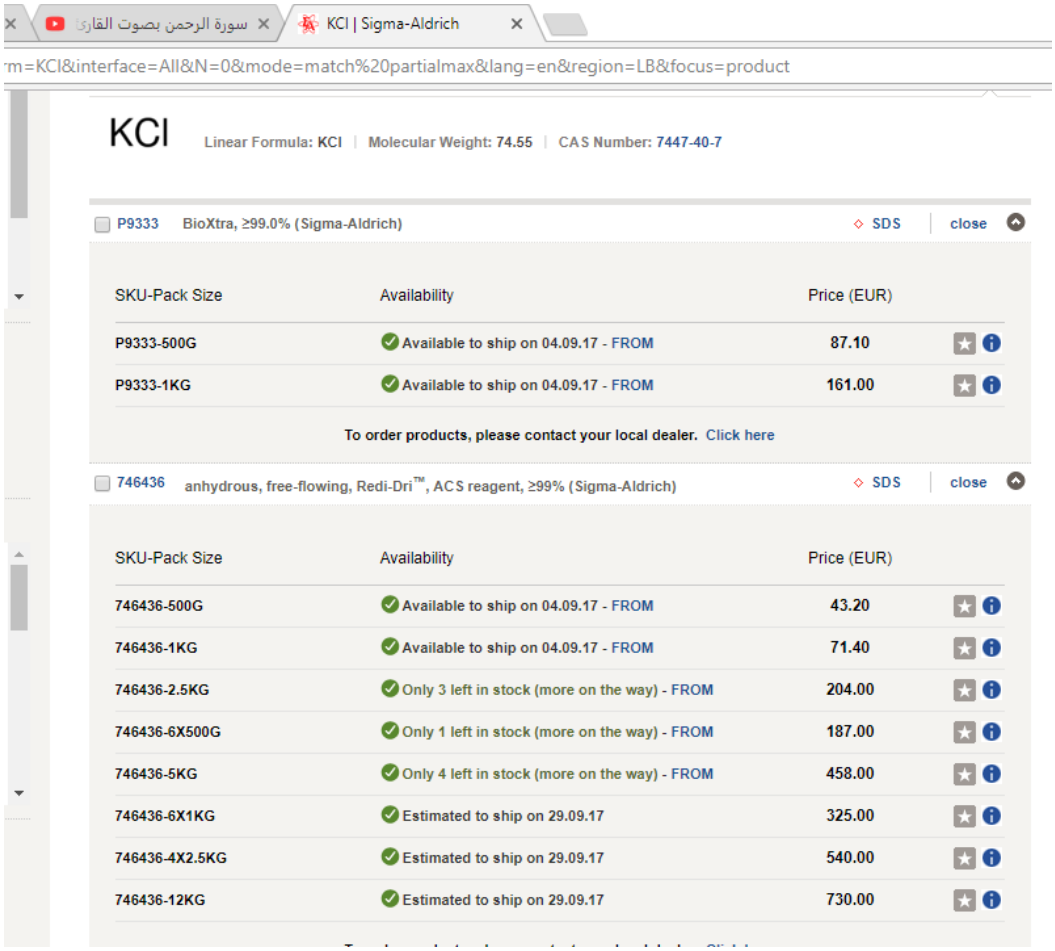
24.2.5 NaNO3:

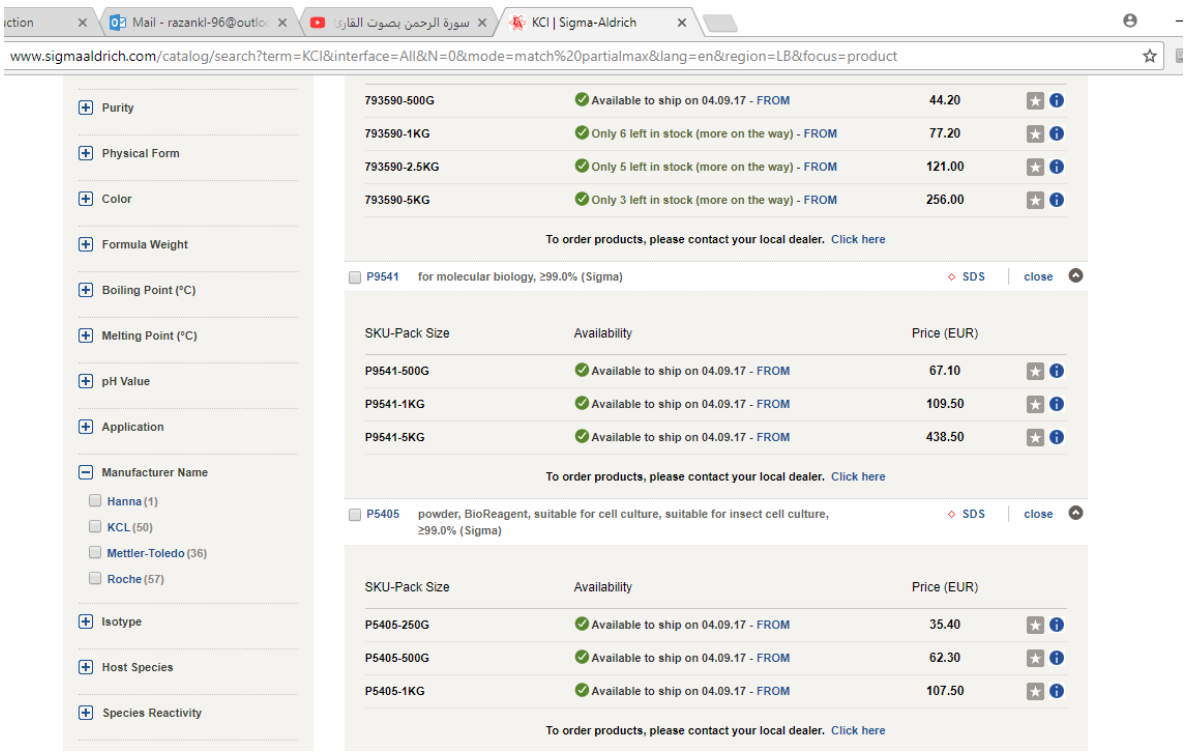



سورة الرحمن بصوت القارئ
NaNo3 | Sigma-Aldrich
m=NaNo3&interface=All&N=0&mode=match%20partialmax&lang=en&region=LB&focus=product
15736 analytical standard (Sigma-Aldrich)
SDS
close
SKU-Pack Size
Availability
Price (EUR)
15736-1G
Available to ship on 04.09.17 - FROM
31.40
To order products, please contact your local dealer. Click here
S8170 BioXtra, ≥99.0% (Sigma-Aldrich)
SDS
close
SKU-Pack Size
Availability
Price (EUR)
S8170-250G
Available to ship on 04.09.17 - FROM
44.40
S8170-1KG
Available to ship on 04.09.17 - FROM
141.00
To order products, please contact your local dealer. Click here
Show All 10 Results
Nitrogen and oxygen isotopes in nitrate
1 Product Result | Match Criteria: Formula
Properties
NaNO3
Synonym: Chile salpeter, Sodium nitrate
Linear Formula: NaNO3 | Molecular Weight: 84.99 | CAS Number: 7631-99-4
NISTRM8569 NIST® RM 8569 (Sigma-Aldrich)
SDS
close
SKU-Pack Size
Availability
Price (EUR)

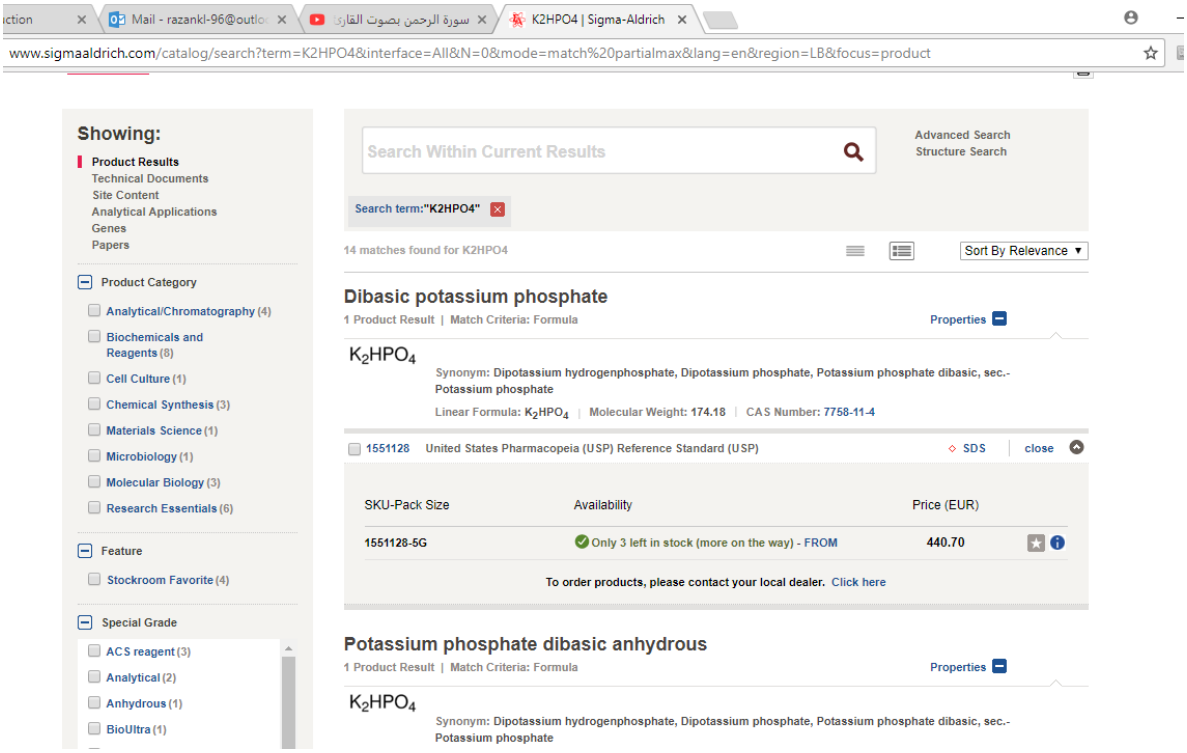

سورة الرحمن بصوت القارئ
NaNo3 | Sigma-Aldrich
m=NaNo3&interface=All&N=0&mode=match%20partialmax&lang=en&region=LB&focus=product
SKU-Pack Size
Availability
Price (EUR)
S8170-250G
Available to ship on 04.09.17 - FROM
44.40
S8170-1KG
Available to ship on 04.09.17 - FROM
141.00
To order products, please contact your local dealer. Click here
Show All 10 Results
Nitrogen and oxygen isotopes in nitrate
1 Product Result | Match Criteria: Formula
Properties
NaNO3
Synonym: Chile salpeter, Sodium nitrate
Linear Formula: NaNO3 | Molecular Weight: 84.99 | CAS Number: 7631-99-4
NISTRM8569 NIST® RM 8569 (Sigma-Aldrich)
SDS
close
SKU-Pack Size
Availability
Price (EUR)
NISTRM8569
Estimated to ship on 28.09.17
752.00
To order products, please contact your local dealer. Click here
SILu™ PrEST NANO3
1 Product Result | Match Criteria: Product Name, Property
QPREST39830 SILuPrESTs Powered by Atlas Antibodies, buffered aqueous solution (Sigma)
SDS
pricing

24.2.6 KCl


سورة الرحمن بصوت القارئ
KCl | Sigma-Aldrich
m=KCl&interface=All&N=0&mode=match%20partialmax&lang=en&region=LB&focus=product
KCl
Linear Formula: KCl | Molecular Weight: 74.55 | CAS Number: 7447-40-7
P9333 BioXtra, ≥99.0% (Sigma-Aldrich)
SDS
close
SKU-Pack Size
Availability
Price (EUR)
P9333-500G
Available to ship on 04.09.17 - FROM
87.10
P9333-1KG
Available to ship on 04.09.17 - FROM
161.00
To order products, please contact your local dealer. Click here
746436 anhydrous, free-flowing, Redi-Dri™, ACS reagent, ≥99% (Sigma-Aldrich)
SDS
close
SKU-Pack Size
Availability
Price (EUR)
746436-500G
Available to ship on 04.09.17 - FROM
43.20
746436-1KG
Available to ship on 04.09.17 - FROM
71.40
746436-2.5KG
Only 3 left in stock (more on the way) - FROM
204.00
746436-6X500G
Only 1 left in stock (more on the way) - FROM
187.00
746436-5KG
Only 4 left in stock (more on the way) - FROM
458.00
746436-6X1KG
Estimated to ship on 29.09.17
325.00
746436-4X2.5KG
Estimated to ship on 29.09.17
540.00
746436-12KG
Estimated to ship on 29.09.17
730.00


Mail - razankl-96@outlo
سورة الرحمن بصوت القارئ
KCl | Sigma-Aldrich
www.sigmaaldrich.com/catalog/search?term=KCl&interface=All&N=0&mode=match%20partialmax&lang=en&region=LB&focus=product
Purity
Physical Form
Color
Formula Weight
Boiling Point (°C)
Melting Point (°C)
pH Value
Application
Manufacturer Name
Hanna (1)
KCL (50)
Mettler-Toledo (36)
Roche (57)
Isotype
Host Species
Species Reactivity
793590-500G
Available to ship on 04.09.17 - FROM
44.20
793590-1KG
Only 6 left in stock (more on the way) - FROM
77.20
793590-2.5KG
Only 5 left in stock (more on the way) - FROM
121.00
793590-5KG
Only 3 left in stock (more on the way) - FROM
256.00
To order products, please contact your local dealer. Click here
P9541 for molecular biology, ≥99.0% (Sigma)
SDS
close
SKU-Pack Size
Availability
Price (EUR)
P9541-500G
Available to ship on 04.09.17 - FROM
67.10
P9541-1KG
Available to ship on 04.09.17 - FROM
109.50
P9541-5KG
Available to ship on 04.09.17 - FROM
438.50
To order products, please contact your local dealer. Click here
P5405 powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99.0% (Sigma)
SDS
close
SKU-Pack Size
Availability
Price (EUR)
P5405-250G
Available to ship on 04.09.17 - FROM
35.40
P5405-500G
Available to ship on 04.09.17 - FROM
62.30
P5405-1KG
Available to ship on 04.09.17 - FROM
107.50
To order products, please contact your local dealer. Click here

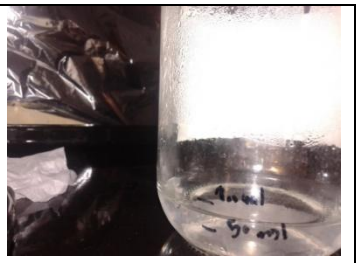
24.2.7 K2HPO4



24.3 Preparation of ethyl acetate

Synthesis: Ethyl acetate is synthesized by the Fischer esterification process, resulting from a reaction between acetic acid and ethanol. An acid, such as sulfuric acid, catalyzes the reaction. CH3CH2OH + CH3COOH → CH3COOCH2CH3 + H2O.

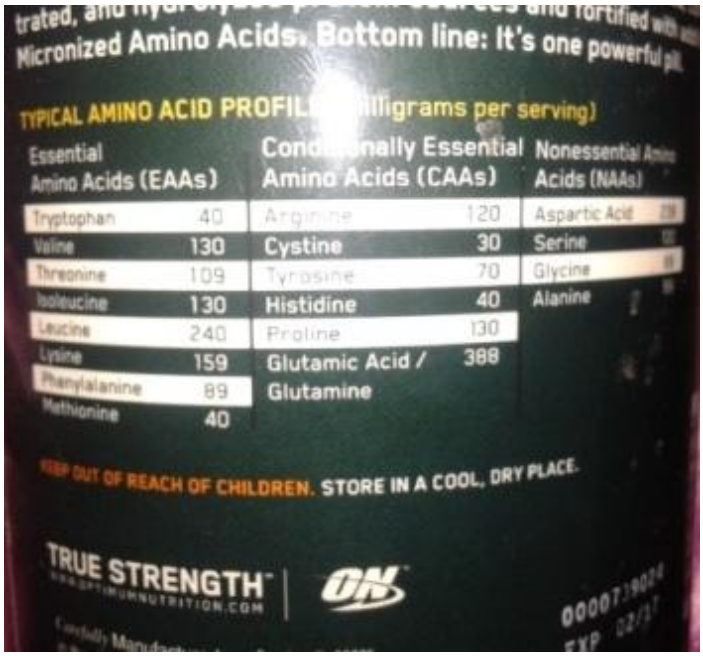
Since this reaction is reversible and produces a chemical equilibrium, the yield is low unless the water is removed. In the laboratory, ethyl acetate can be separated from water using the Dean-Stark process 200 ml vinegar is placed in an Erlenmeyer flask and heated to boiling to evaporate the water after cooling. 100 ml of ethanol are placed in an Erlenmeyer flask and the slowly cooled reaction is added.

	 Warming of vinegar	 Ethyl acetate

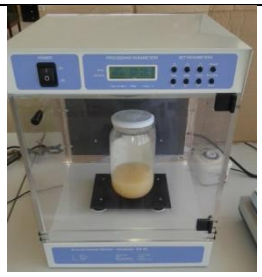
To increase the yield, the technique of ...

25 Experimental Laboratory scale production of penicillin

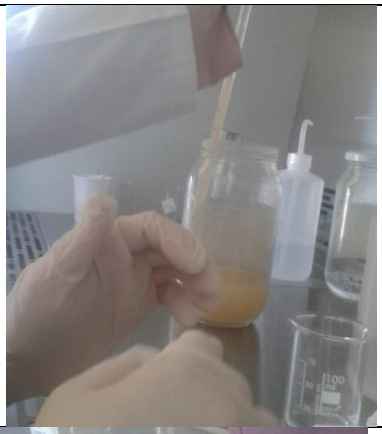
25.1 Experiment 1: Synthese of penicillin by Amino acids

<table>
<tr>
<td> 

Material:

amino+ Table sugar, milk poudre (lactose), eau</td>
<td> 
Incubation of liquid medium in the incubator at 26 0c + chaker</td>
</tr>
<tr>
<td> 
</td>
<td>After fermentation of content

Amino sugar and table salt, lactose (lacto milk) water and qlq penicillium spore</td>
</tr>
</table>

take 10ml 	It is also pu 
Take 1( epared)	It is also 
 incubation with charcoal treatment (0.43g)	Then we filter we put the contents in a tube (10ml) we incubate it is desired that the contents contain soluble penicillin
	Tube content of liquid it is desired that the contents contain soluble penicilli

25.1.1 Culture of bacteria of yogurt bacteria+ penicillin

The aim of the culture to tested the penicillin soluble
Preparation of medium
they are called the two main bacteria of yogurt *Lactobacillus bulgaricus* and *Streptococcus thermophilus.*
How long does it take for them?
About 20 minutes. Do you imagine how many twins this will give
it if we all divide 3x per hour!

Materials

petri dish, yoghurt, Nacl, tripton yeast extract agar, distilled water, magnetic stirrer, Bunsen burner, wooden cord, handle, flame.

Protocol : work under high The glassworks are washed with tap water and then with distilled water and sterilized the glassworks by the autoclave

Water in a 250 ml beaker and put the tube of tryptone to melt the contents

After adding 10 ml of water in the tube after homogenization is poured into the Erlenmeyer flask.

We put the Erlenmeyer on the magnetic stirrer at 100 ○ C until two minutes left to cool a little

Pour the mixture into the semi-covered dough box until the solidified solid (gel)

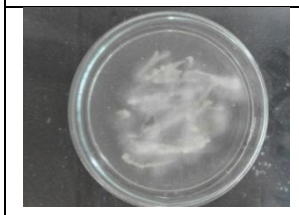
We put yogurt on the gel obtained and put it in the incubator for 48 hours, we read.

1 tube de tryptone		
0.5g Nacl		
10 ml eau distillee		

0.5gNaCl water10ml Becher containing water to warm the tube of tryptone 	
	

spread of yoghurt

spreading penicillin



After incubation from 26.4.2018 until 28.4.2018

25.2 Experiment 2: Preparation of penicillium colony

weigh glucose

sterilization of glassware

heat the tube tryptone agar yeast extract

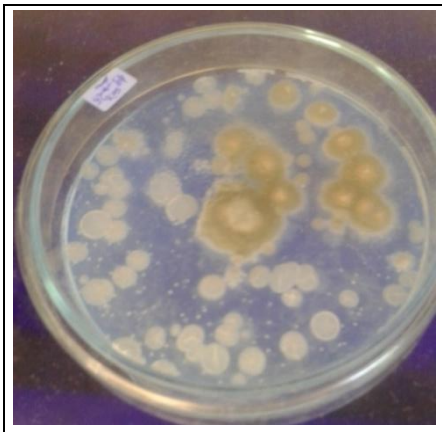
prepare the petri dish 19-04-2018

25-4-2018

25.3 Experiment 3: Preparation of penicillin cristal by amino acids

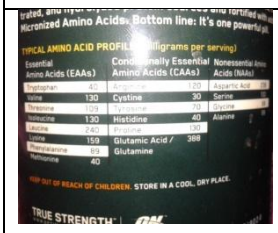
Uv machine 

Materials used for the manufacture of liquid medium:

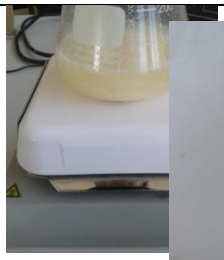


UNDER UV observation blue toxin secreted by penicillium

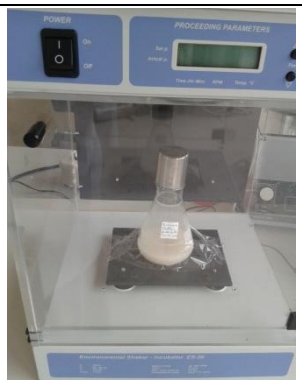
2g glucose + 2g lactose (milk) + 1g Amino
0.1g MgCl2 + 0.1g kcl + 0.5g KH2PO4 +
100ml distilled water



Amino acids



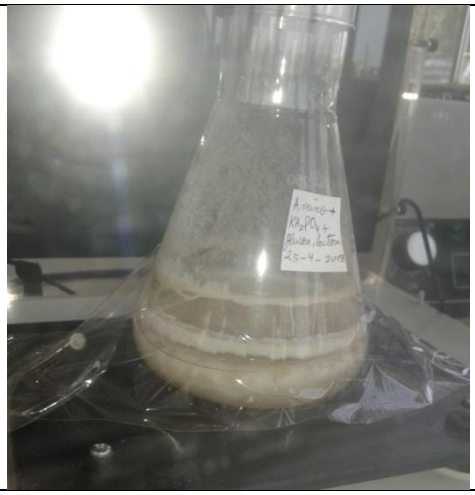
25-04-2018

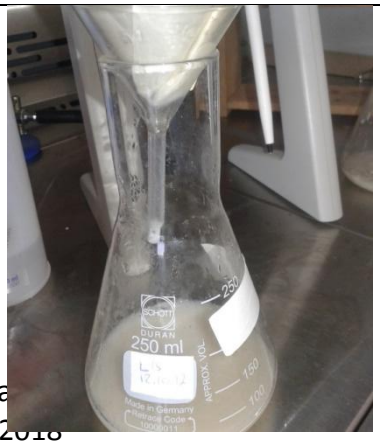


incubation

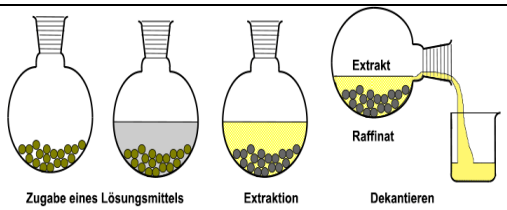
0.43 g charcoal treatment+ 0.5g KH2PO4

acid such as phosphoric acid are introduced as pH will be as high as 8.5. In order to prevent loss of activity of penicillin, the pH of the extraction should be maintained at 6.0-6.5.



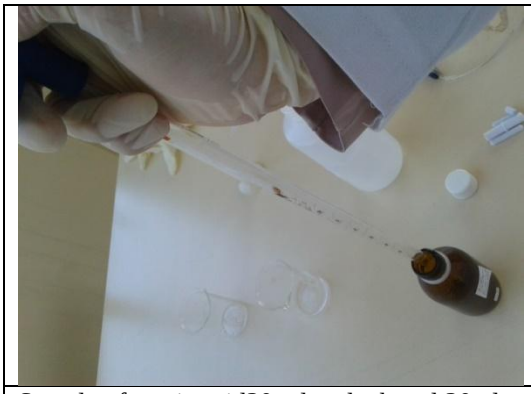


Filtra
4-5-2018

After 10 days from development of *penicillium*	
	 Charcoal treatment incubation +KH2PO4 to regulate the pH
Inc  acétate (vinaigre +éthanol not pure) incubation in refrigerator	 26-5-2018 Following the protocol we put 5g of sodium bicarbonate we note an effervescence So the ethyl acetate that we prepare contains More vinegar which allows this result
Saturday, June 30, 2018 1:28 PM  long time incubation in the refrigerant after filtration obtaining penicillin crystals	

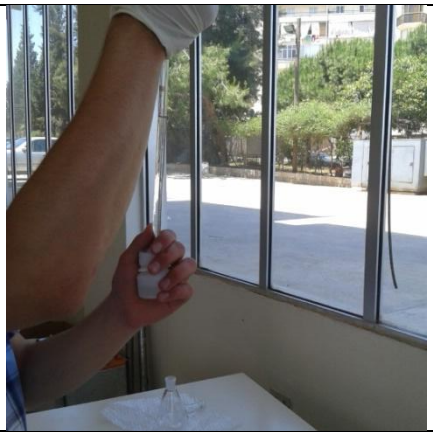
25.4 Experiment 4: Preparation of ethyl acetate

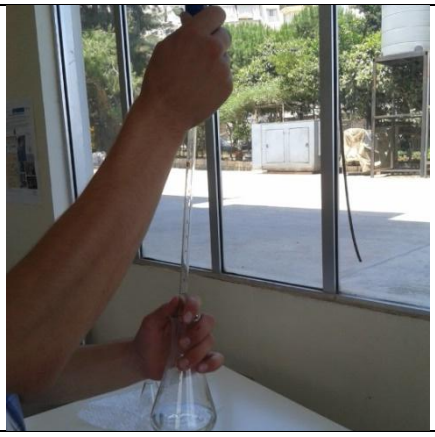
2-6-2018



Sample of acetic acid30 ml and ethanol 30ml

acetic acid prepared by heating the vinegar from 250ml to 15ml





Sample of sulfuric acid (37% acid +water) 17 ml

for the reaction to take place, the contents are heated with condensation

mount

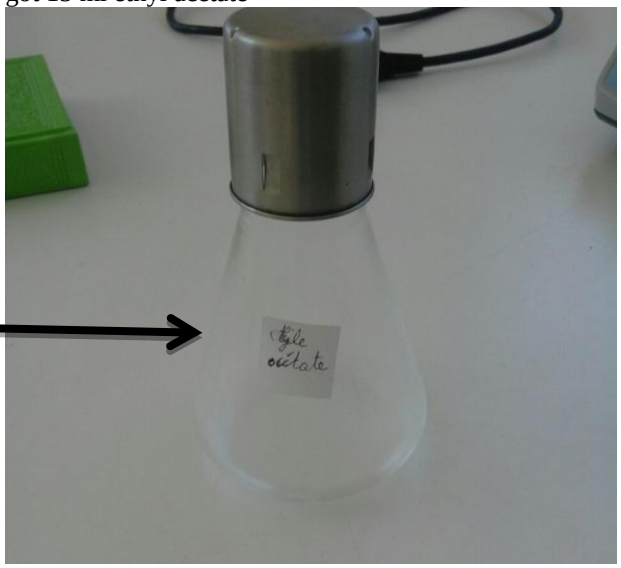
Heated to 88 °C

the change of smell shows that ethyl acetate is formed

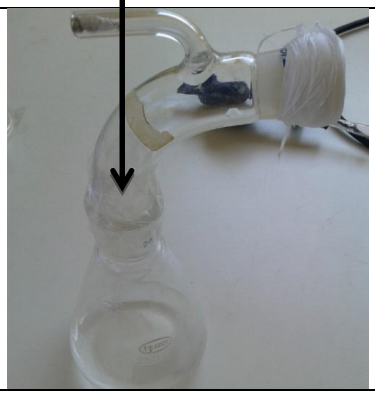
9-6-2018

Of 30ml acetic acid +30ml ethanol+17ml sulfuric acid we got 15 ml ethyl acetate

condensation of ethyl acetate 88° C



We tested that by sodium carbonate



	effervescence[9] This means that we have acetic acid (Because there was a reaction with pure sodium bicarbonate)	no effervescence This means that we **do not have** an acetic acid. There is pure ethyl acetate. (Because there was no reaction with pure sodium bicarbonate)

25.5 Experiment 5:Preparation of ethyl acetate with the spirit of vinegar

100ml sprint vinegar,100ml ethanol

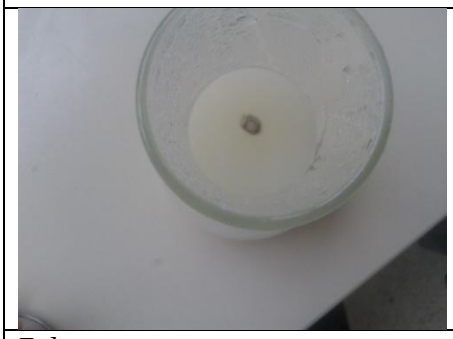
higher yield of ethyl acetate

25.6 Experiment 6: Preparation of liquid medium with peptone

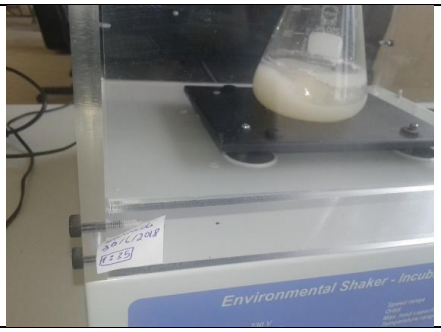
Le 30-6-2018	

[9] effervescence: escape of gas from an aqueous solution

1g peptone,2g glucose ,2g lactose 0.1gKCl.,0.1gMgCl2,0.5g KH2PO4 ,100ml distilled water



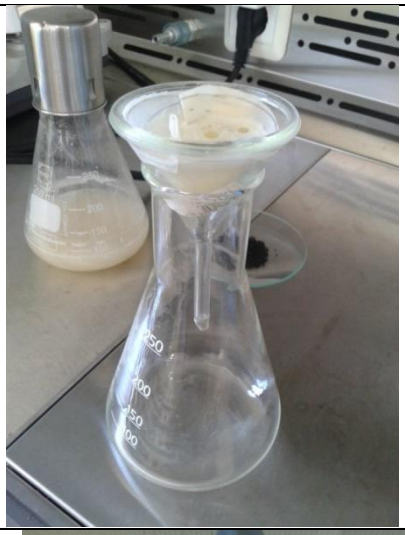
7 days



incubation

After 7 days Purification of penicillin

0.5 g



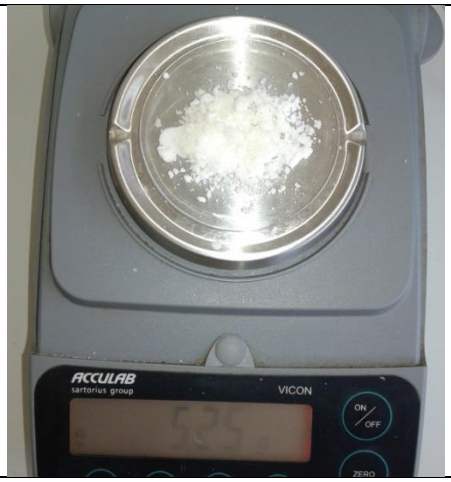
filtration

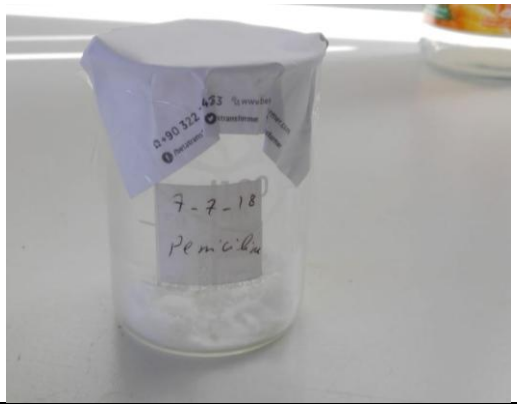
addition of pure ethyl acetat

remove the content from the tubes

centrefigation

From 100 ml of medium we obtained 5.25 g of penicillin





25.7 Some Experiments done once again[10]

25.7.1 Preparation of Agarose Gel

Materials:

-Tube of tryptone

-Beaker

-Erlenmeyer

-Distilled water

-Glucose

-Ethanol

-Petri dish

-Gloves

[10] Samar Youssef, Report 10.12.2019

-Spatula

-Lighter

-Heater

-Digital balance

-Graduated cylinder

Procedure:

- First step: we put an orange in a fermentation conditions until we become able to see a fermented region.

- Step 2: preparation of agarose gel:

1-we put the tryptone tube into a 250 ml beaker full of water

2- we heat the beaker using a lab heater until the gel melt

3-we measure 10 ml of water using a graduated cylinder

4- we weight 0.5 g of glucose powder using a digital balance.

5-we mix the Tryptone Gel, the Glucose and the Water in the Erlenmeyer.

We keep heating until we get a homogeneous mixture.

Then we fill the mixture in the petri dish.

And we wait around 30 mins until the gel become totally solidified.

Remark:

A plate which has been streaked showing the colonies thinning as the streaking moves clockwise.

	In microbiology, streaking is a technique used to isolate a pure strain from a single species of microorganism, often bacteria. Samples can then be taken from the resulting colonies and a microbiological culture can be grown on a new plate so that the organism can be identified, studied, or tested.

25.7.2 Preparation of liquid medium

Materials:

-Beaker

-Spatula

-Glucose

-Lactose

-Peptone

-$MgCl_2$

-KCl

-KH_2PO_4

-Distilled water

-Erlenmeyer

-Metallic paper

-Ethanol or Ethyl alcohol

-Graduated cylinder

-Digital balance

-Magnetic hot plate stirrers

-Shaker.

Procedure:

-First step: sterilization.

We put 2 ml of distilled water in the Erlenmeyer we close it with metallic paper then we heat until the solution start boiling (so now T~100˚C).

-Step 2: preparation of liquid medium.

we weight: -2 g of Glucose powder.

-2 g of Lactose

-1 g of Peptone

-0.1 g of $MgCl_2$

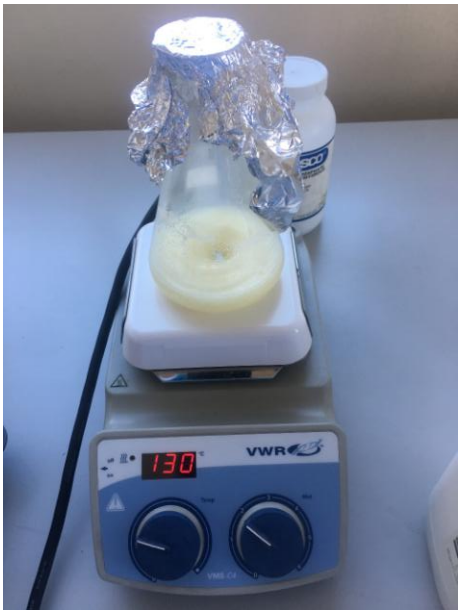
-0.1 g of KCl

-0.5 g of KH_2PO_4

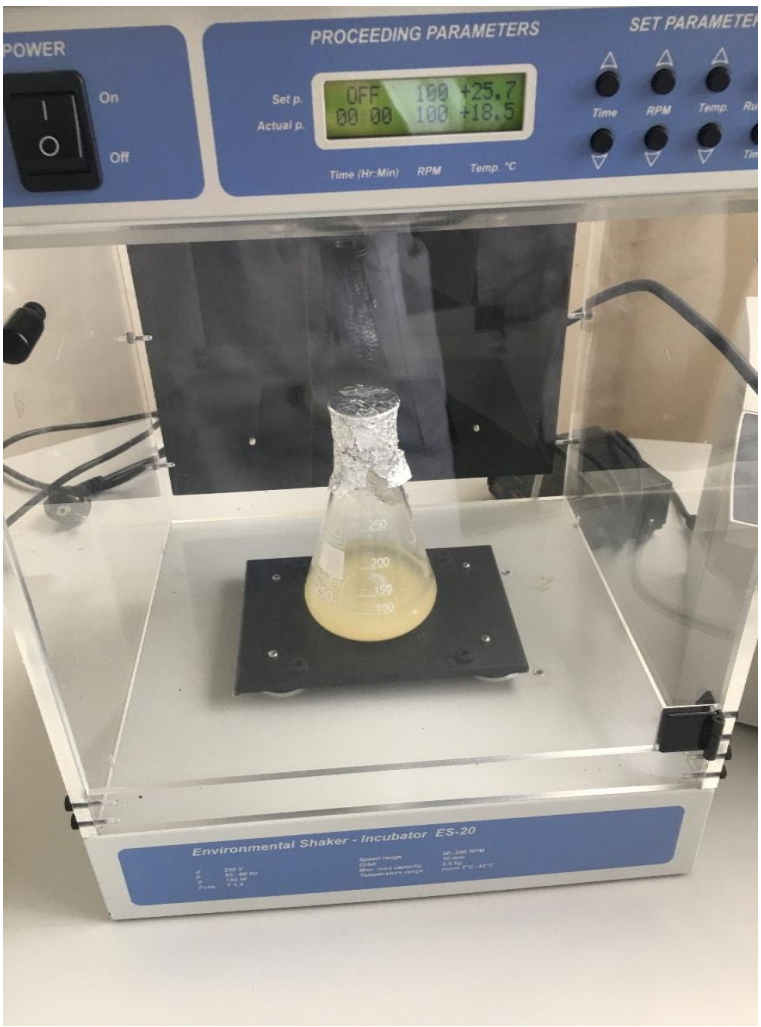
using a digital balance.

We fill the previous measurements in the Erlenmeyer, then we add 100 ml of distilled water.

Then we heat and mix at the same time using a magnetic hot plate stirrer for 15 mins (to obtain perfect mixing during the reaction which will increase our reaction rate).



Further, we will need to wait for 30 mins in order to cool down the mixture.

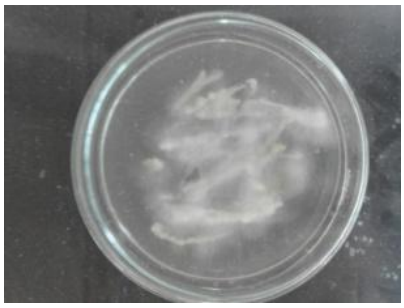


After cooling, we add a portion of the colony. Then we put the mixture on shaker for 7 days.

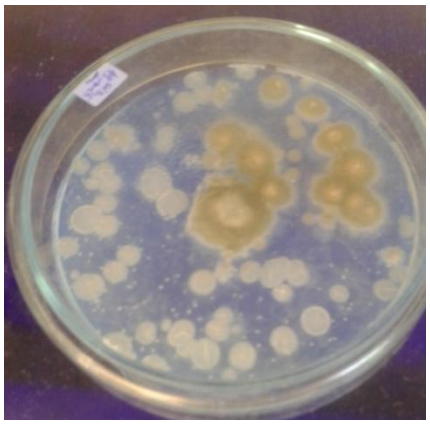
26 Results of experimental lab scale production of penicillin

26.1 From experiment 1

The bacteria of yogurt is living. This means that penicillin preparation is incorrect or incomplete



26.2 From experiment 2: preparation of penicillium colony



26.3 From experiment 3: Preparation of penicillin crystal by amino :

We get after the incubation in the fridge a few weeks of penicillin crystals

Saturday, June 30, 2018 1:28 PM long time incubation in the refrigerant: After filtration obtaining penicillin crystals 	

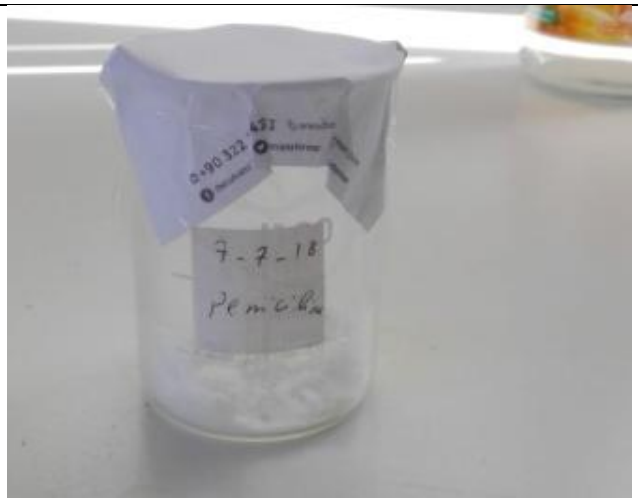
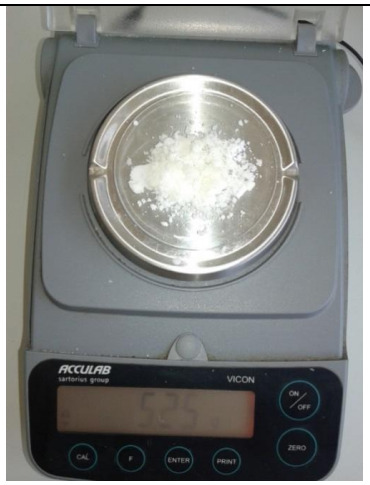
26.4 Experiment 4 preparation of ethyl acetate

	we have a small percentage of acetic acid

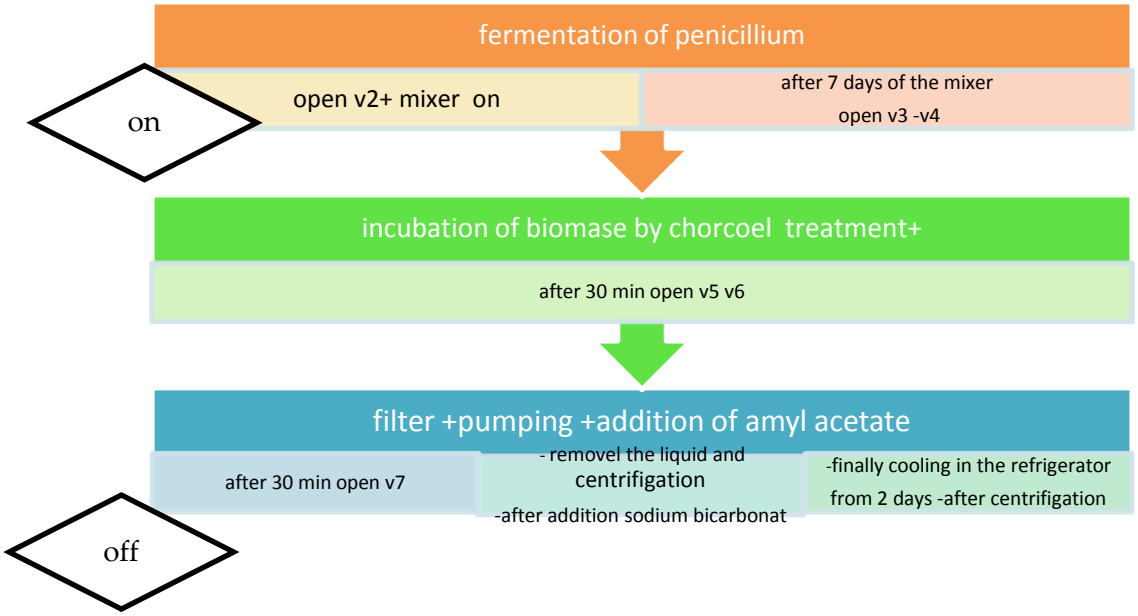
26.5 Experiment 5: Preparation of ethyl acetate with the spirit of vinegar

	We have a high percentage of acetic acid

26.6 Experiment 6: preparation of liquid medium with peptone

	
We obtain the 5.25 g of penicillin powder	Filtration. Freeze-dry the contents

27 Program (Flow Diagram) for Automatic Synthesis of penicillin in machine



time h	open valves	closed valves	mixer on/of	pump
0:00	2		on	
0:45		2		
168	3		of	
168	4			
168:30:00	5	3		
168:30:00	6	4		
169	7	5		on
169		6		

28 Pilot scale plant for penicillin production

1 incubation 7 days

Inoculum Penicillium + medium (peptone lactose glucose eau distillee KH2PO4 MgCl)

Materials used for the manufacture of liquid medium:

2g glucose + 2g lactose (milk) + 1g Amino

0.1g MgCl2 + 0.1g kCl + 0.5g KH2PO4 + 100ml distilled water (small scale)

4

filtration

5

Incubation with éthyle acétate (vinaigre +éthanol)

Dumping the content

incubation in refrigerator some days

Following the protocol we put (5gsmall skill) of sodium bicarbonate

2

Charcoal treatment

Incubation 1 hour

0.43 g charcoal treatment+ 0.5g KH2PO4 acid such as phosphoric acid are introduced as pH will be as high as 8.5. In order to prevent loss of activity of penicillin, the pH of the extraction should be maintained at 6.0-6.5.

3

pumping

1 incubation 7 days

Inoculum Penicillium + medium (peptone lactose glucose eau distillee KH2PO4 MgCl)

Materials used for the manufacture of liquid medium:

2g glucose + 2g lactose (milk) + 1g Amino

0.1g MgCl2 + 0.1g kCl + 0.5g KH2PO4 + 100ml distilled water (small scale)

4

filtration

5

Incubation with éthyle acétate (vinaigre +éthanol)

Dumping the content

incubation in refrigerator some days

Following the protocol we put (5gsmall skill) of sodium bicarbonate

2

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Incubation 1 hour

0.43 g charcoal treatment+ 0.5g KH2PO4 acid such as phosphoric acid are introduced as pH will be as high as 8.5. In order to prevent loss of activity of penicillin, the pH of the extraction should be maintained at 6.0-6.5.

3

pumping

29 Operating the Control System, Version 2020

29.1 HMI Program

29.1.1 Auto mode

Press “Start”

- Start Timer 1 of tank 1, Mixer ON
- Delay 168 hours (7 days)
- If Timer 1= 168 hours, Open Valve 1
- Start Timer 2 of tank 2
- If Timer 2 = 1 hour, Open Valve 2
- Pump 2 ON for 5 min after Valve 2 is open

29.1.2 Manuel mode (interactive)

Press “Start”

Fermentation pen cilium :

c) Mixer :
- Press “Manual”
- for OFF Press “Manual OFF”
- for ON press “Manal ON”

d) Valve :
- Press “Manual”
- for Open Press “Manual Open”
- for Close press “Manal Close ”

Charcoal treatment :

c) Valve :
- Press “Manual”
- for Open Press “Manual Open”
- for Close press “Manal Close ”

d) Pump : (if valve 2 Close, Pump not working)
- Press “Manual”
- for OFF Press “Manual OFF”
- for ON press “Manal ON”

Autoclave system:

c) Heater :

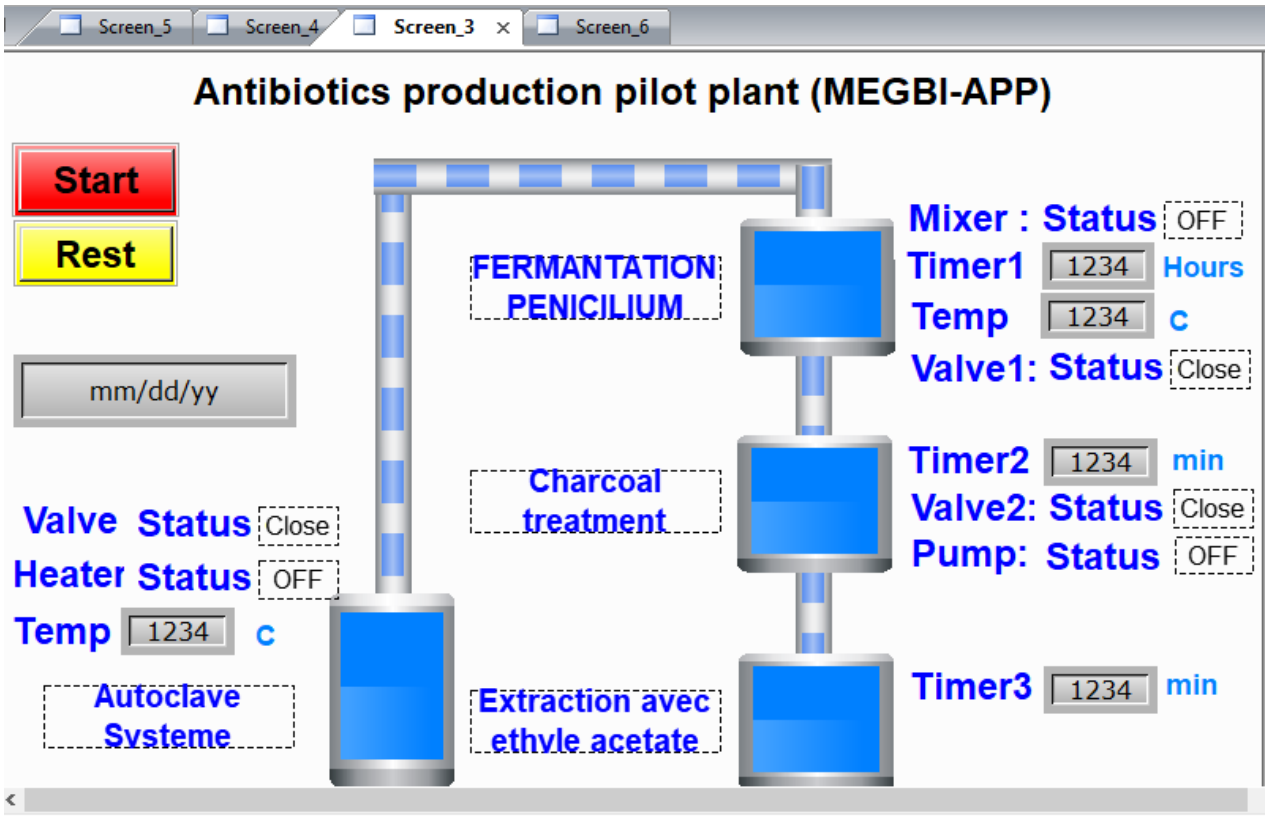
- Press “Manual”
- for OFF Press “Manual OFF”
- for ON press “Manal ON” (if Temperature > 122^0 C Heater OFF)

d) Solenoid valve

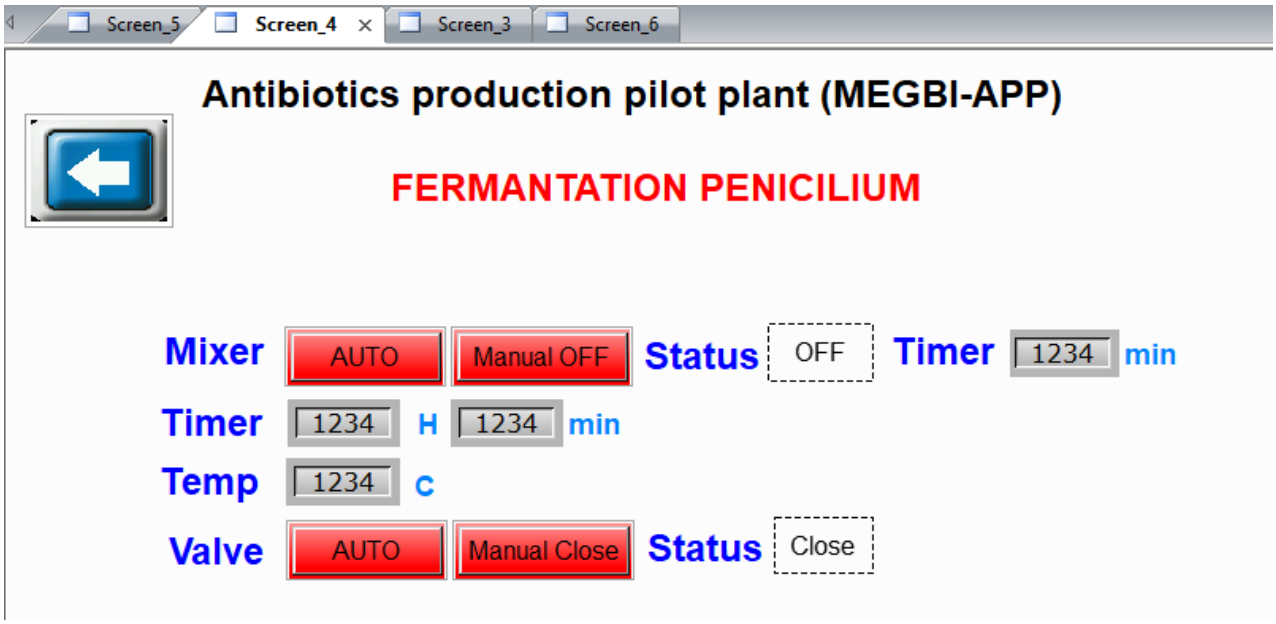
- Press “Manual”
- for Open Press “Manual Open”
- for Close press “Manual Close”

29.1.3 HMI pages

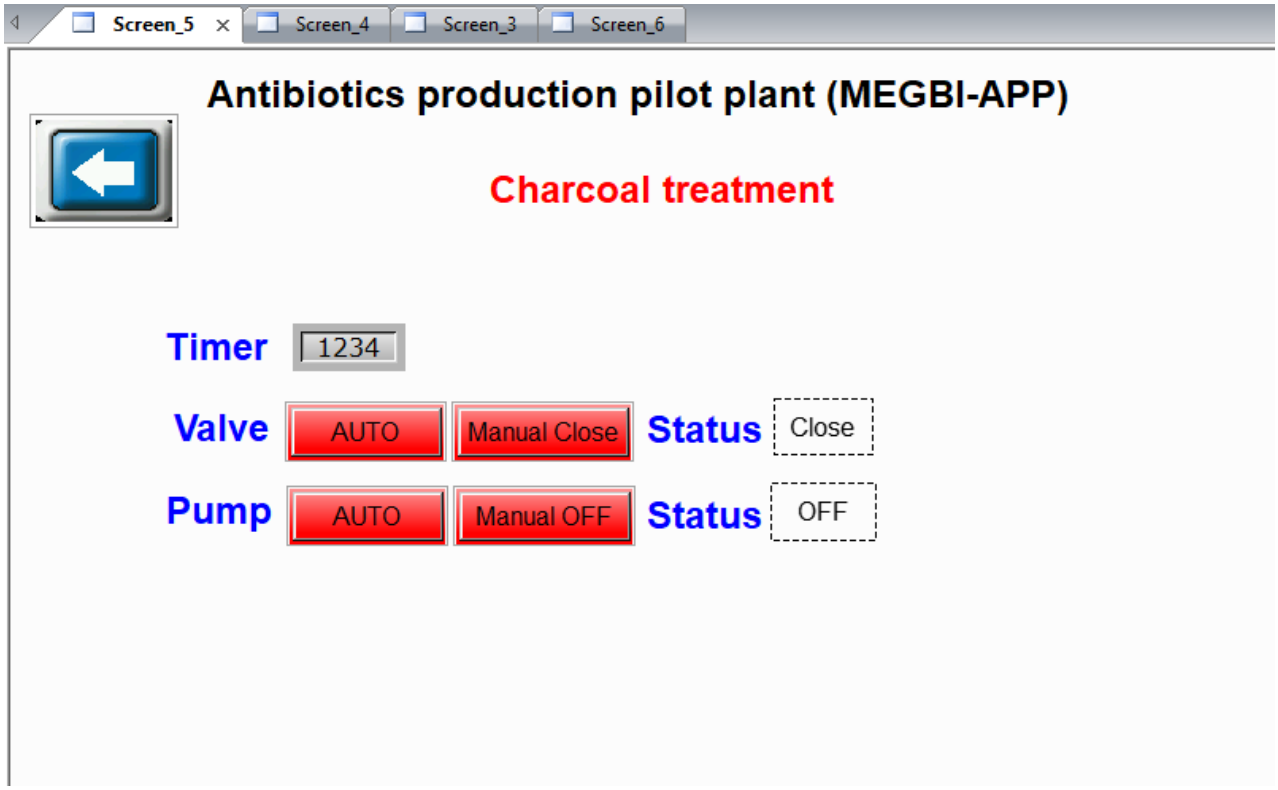
29.1.3.1 Main page



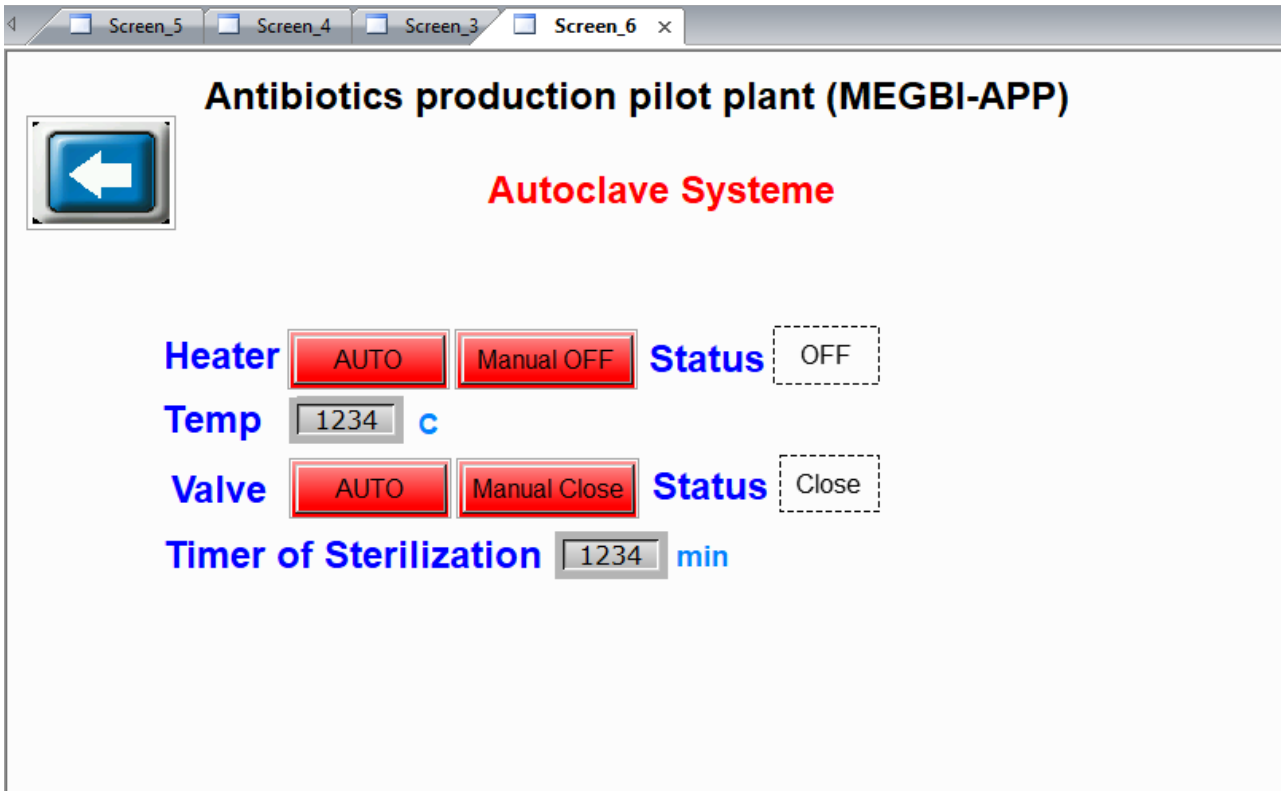
29.1.3.2 Fermentation pencilium page



29.1.3.3 Charcoal treatment page



29.1.3.4 Autoclave system page



30 Suppliers

30.1 Chemicals, Devices, Molecular Biology

30.1.1 Burhan Kabbara, Tripoli, Tel. 03/339523

30.1.2 Jaudat al-Khatib, Tel. 70916173

RC.TRADING
Tel :961 3 888 809 Fax:00961 7 739 333
Email:jawdathkatib80@gmail.com

Quality Assurance: Determination of penicillin (quantitive diagnostic)[11]

[11] from [MEGBI-APP 2019]

31 Determination of sensibility of penicillin production

Based on practical work of Maryam Khodor (originally planned as master thesis)

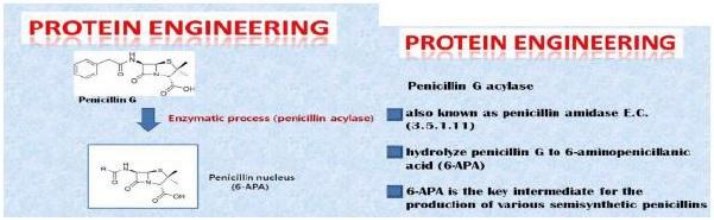
31.1 Master Thesis Task

بسم الله الرحمن الرحيم

Ras Nhache/Batroun - Tripoli, 5th April 2016

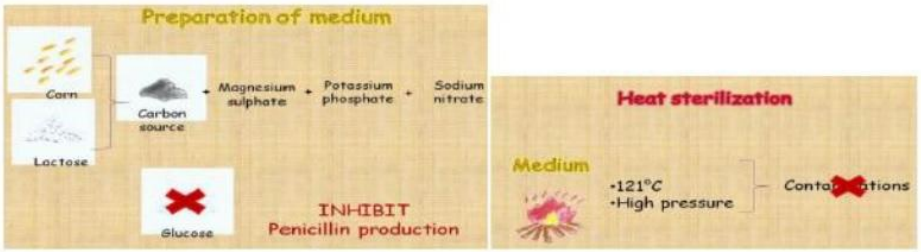
MEGBI Antibiotics Pilot Plant Process:

2.4.1.2 Production of semisynthtic penicillins



Master Thesis: Sensitivity Determination of Process Intermediate Products of the MEGBI Antibiotics Pilot Plant

- Preparation of Medium for Seed Culture



- Seed Culture of Penicillium chrysogenum
- Penicillin Sensitivitiy Test
- Process Culture in Bioreactor
- Penicillin Sensitivitiy Test
- Documentation (3 weeks)

Keywords: Antibiotics, Penicillin, Fungus, Biotechnology

31.2 List of materials:

- Glucose
- Lactose
- Peptone
- NaNo3
- K2HPO4
- KCl
- MgSO47H2O
- FeSO47H2O
- Sucrose
- ZnSO47H2O
- CuSO45H2O
- Corn steep liquor
- Beef extract
- (NH4)2SO4
- Parafilm
- Amyl acetate
- Phosphate buffer
- Chloroform
- Lacto phenol cotton blue stain
- Butyl acetate

Reference	1	2	3	4	5	6
Souche+ origine	5031,5037	Wild Fruits+vegetables	W49-133 Spore from dry sterile soil	DS17690 DSM, The Netherlands	Q176 (Carnegie institution)	W50-935/W50-1583 W51-20 /W51-616 W50-20F3/W51-20F3-64
Medium	PDB:200g potatoes 1L H2O 20g dextrose 20g agar powder	Sabouraud's glucose agar: glucose 40.0g, peptone 10.0g, agar 15.0g dissolved in 1000ml H2O	Standard spore plate medium inoculum: 3% corn steep liquor- 5% dextrin medium with 5 ml spore	YGG: KCl, 10.0; glucose, 20.0; yeast nitrogen base (YNB), 6.66; citric acid, 1.5;K2HPO4, 6.0; and yeast	Standard fermentation media :lactose, 30 (in control only); glucose, 10; ammonium acetate, 3.5;ammonium lactate, 6.0;	Media I-III

				extract, 2.0.	KH2PO4, 6.0; MgSO4 7H20, 0.25; ZnSO4c7H20, 0.02; FeSO4, 0.02; MnSO4, 0.02; and Na2SO4, 0.5.	
Medium 2	3g yeast extraction 21g sucrose 1L H2O	CYA:NaNO3, 3.0; K2HPO4, 1.0; KCl, 0.5; MgSO4.7H2O, 0.5; FeSO4.7H2O, 0.01; yeast extract 5.0; sucrose, 30.0; agar, 15.0 and trace metal solution, 1.0ml. Trace element solution : ZnSO4.7H2O, 1.0g and CuSO4.5H2O, 0.5g in 100ml H2O	Fermentation media : corn steep liquor, dry basis (CSL), 1.5% lactose, 2.5%; CaCO3,0.2%; Na2SO4,0.05%.	Penicillin production medium glucose, 5.0; lactose, 75; urea, 4.0; Na2SO4, 4.0; CH3COONH4, 5.0; K2HPO4, 2.12; KH2PO4, 5.1; and phenoxyacetic acid, 2.5.:		6% dextrin 2%corn steep solids
PH	2	5.4	5.8-6.0		6.5	5.2-5.6
Temperature	Room temperature	25	25-30	25	25	24-25
Extraction	Chloroform + butyl acetate	Amylacetate Phosphate buffer Chloroform H2O			Sugar solution	ammoniu m acetate
Precurseur			Potassium phenylacetate at PH =6.8-7		Sodium phenylacetate 0.05%	Phenylacet ic acid 0.05%`
		Shake flask cultivations : glucose, 20.0; yeast extract, 10.0; Corn Steep Liquor		Primers gene: penDE, phl		Lard oil 3% octadecano l : antifoam agent

		(CSL), 5.0; beef extract, 0.075; peptone, 0.125; (NH4)2SO4, 4.0; KH2PO4, 3.0; ZnSO4.7H2O, 0.01; MgSO4.7H2O, 2.3.				
				Promoter : pCBC Selrction marker : acetamidase		

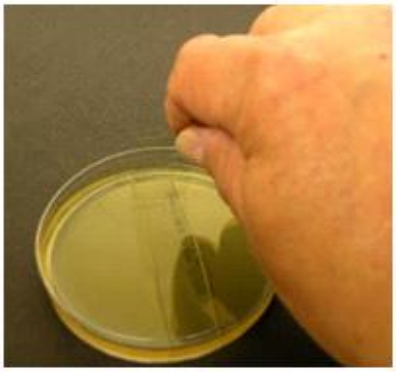
31.3 Methods

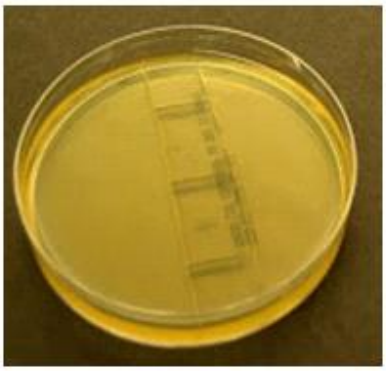
31.3.1 Slide culture method

- It used in the study and identification of an unknown fungal isolate .
- ***Steps:***

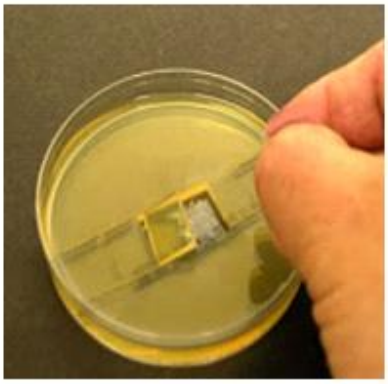
- getting a plate of fungal media (Sabouraud's agar)
- cutting the agar with a sterile scalpel .
- plunge or drag the edge of a cover slip into the agar surface .
- cutting out small blocks of agar (1/2 to 3/4 of an inch square .

Using a glass cover slip as a knife , sliced the agar into squares

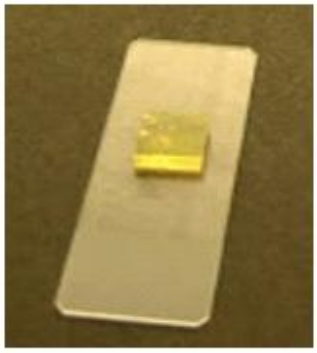




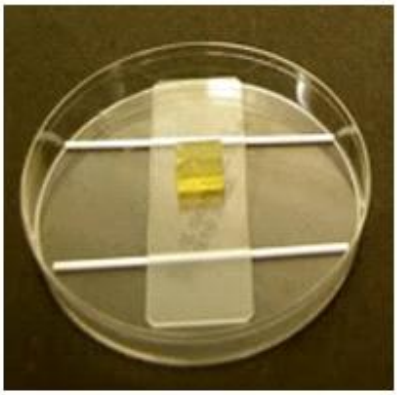
Remove an agar into the plate using the same cutting tool (scalpel, cover slip)



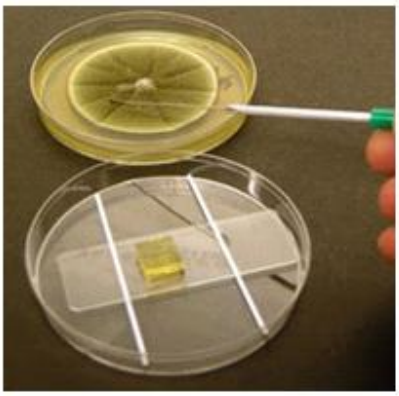
Place the agar block onto a clean glass microscope slide



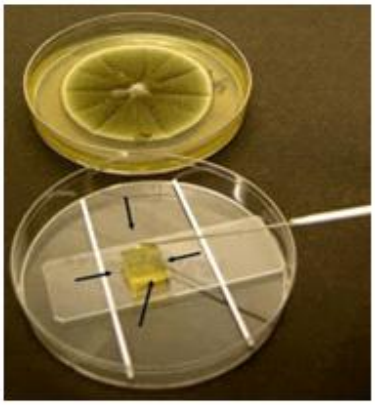
The slide can then be placed in a clean petrie dish which will prevent contamination and preserve moisture during incubation



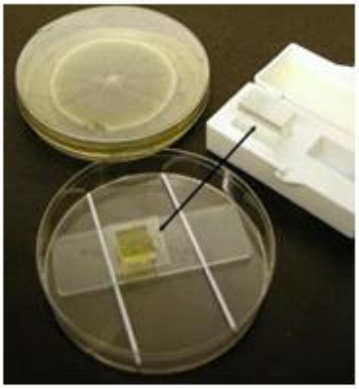
Using a sterile instrument (loop, needle) transfer some of the fungus from the specimen being cultured to each of the four sides of the agar block



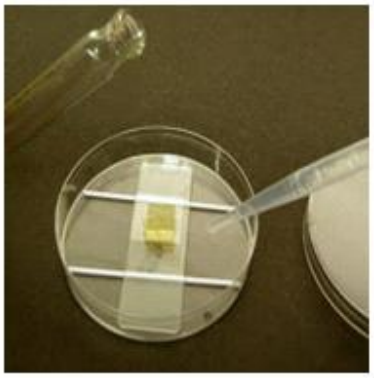
Transfer the fungus to the agar block's sides.



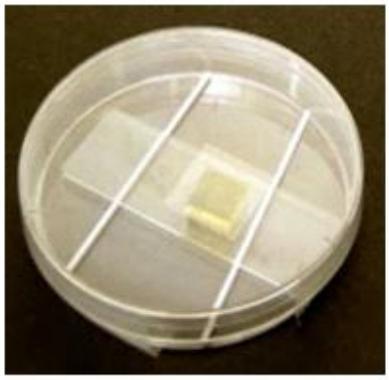
After inoculation place a clean cover slip on the surface of the agar block



-A few drop of a sterile water can be added to the petrie dish as an additional source of moisture
- which may be beneficial to slow growing fungi which may dry out with prolonged incubation



-The plate is partially sealed with parafilm or a bit of cellulose tape
-If fully sealed the plate may fog up and moisture condense on specimen



- Incubate the slide at room temperature to 30°c for most fungi and for an appropriate length of time
- Fast growing fungi can overgrow the agar block very quickly
- To examine the slide culture remove the slide from the petrie dish
- Then remove the cover slip from the agar block using plastic forceps or gloved finger .
- Place a drop of lacto phenol cotton blue stain onto a clean microscope slide and then place the cover slip from the slide cultured onto the LPCB.
- The slide is ready for examination under the light microscope .

31.4 Time Plan

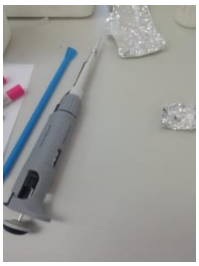
Name	Period	Begning date	End date
Culture and incubation	7 days	26 April	3 may
Identification / diagnosis	3 days	3 may	5 may
Purification of seed culture	7 days	6 may	13 may
Re identification	3 days	13 may	15 may
Production of penicillin	13 days (300h)	16 may	29 may
Extraction			
Sensitivity			

31.5 Preparation of Media

31.6 Aimed Results

In this study, we aim to produce natural penicillin from bread, fruits and vegetables, and determine its sensitivity to prevent the growth of bacteria.

32 Devices for diagnoses of penicillin[12]




HCl ,iodine
silica gel paper
KI , ethylacetate ,ethanol
Penicilline stander
Micropipet,

[12] from [MEGBI-APP 2019]

33 Working methods in diagnoses to penicillin

33.1 Thin-layer chromatography to reveal presence of penicillin[13]

Purpose: The paper describes some thin layer chromatographic procedures that allow simple and rapid separation and identification of penicillins and cephalosporins from complex mixtures. ***Methods:*** Using silicagel GF254 as stationary phase and selecting different mobile phases we succeeded in the separation of the studied beta-lactamins. Our aim was not only to develop a simple, rapid and efficient method for their separation but also the optimization of the analytical conditions. ***Results:*** No system will separate all the beta-lactams, but they could be identified when supplementary information is used from color reactions and/or by using additional chromatographic systems. ***Conclusion:*** The right combination of solvent system and detection method allows the identification of the studied penicillins and cephalosporins and can be successfully used in the preliminary analysis beta-lactam antibiotics.

Materials and Methods

Instrumentation

The TLC system consisted of a Camag Nanomat III automatic sampler, a Camag Linomat IV semiautomatic sampler (Camag, Switzerland), a 2-ml Hamilton microsyringe (Hamilton, USA), a Camag Normal Development Chamber and a Camag fluorescence inspection lamp (Camag, Switzerland). As stationary phase we used 10x20 and 20x20 cm pre-coated silicagel GF254 HPTLC glass plates (Merck, Germany).

Reagents

Penicillins: amoxicillin trihydrate, ampicillin trihydrate, benzylpenicillin sodium, oxacillin sodium (Antibiotice Iaşi, Romania). Cephalosporins: cefalexin monohydrate, cefadroxil monohydrate, cefaclor monohydrate (Sandoz, Romania), cefuroxim sodium (Medochemie, Cyprus), ceftazidim pentahydrate, ceftriaxon sodium (Antibiotice Iaşi, Romania). All the studied beta-lactams were of pharmaceutical grade.

Reagents: acetone, acetic acid, benzene, butanol, ethanol, ethyl acetate, formaldehyde, methanol, sulphuric acid (Reactivul Bucureşti, Romania). All reagents were of analytical grade.

Samples

PEN and OXA, were used as sodium salts, consequently samples were prepared in water at a concentration of 0.2%. AMP and AMO, used as trihydrates, exhibit poor solubility in water; consequently samples of 0.2% were prepared in a 2% sodium bicarbonate solution. Cephalosporin

[13] Source ?

samples were prepared by dissolving the substances in methanol and then diluting with water (1:1). Amounts of 0.5 ml were applied on the chromatoplates using a Hamilton syringe.

Method

The chromatographic chambers were saturated with the mobile phase for 30 minutes. The plates were developed over a distance of 15 cm in filter-paper-lined chromatographic chambers, dried in a stream of hot air, and examined under UV radiation at wavelengths of 254 and 366 nm. The spots were then visualized by placing the plates in a chromatographic chamber saturated with iodine vapors. Some specific in situ color reactions were used in order to increase specificity of the method. All experiments were carried out at room temperature. Photographs of the chromatoplates were taken with a Nikon D-3100 camera, equipped with a UV filter.

Chromatographic detection procedure

Three detection procedures were used; first with iodine vapors and then using in situ plate color reactions with iodine and ninhydrine, after an alkaline hydrolysis.

A few iodine crystals were placed on the base of tightly sealed chromatographic chamber, stored in a fume cupboard. After a few hours during which violet iodine vaporizes and distributes itself homogenously throughout the interior of the chamber, the chromatographic plates were introduced in the chamber. After 30 minutes the plates were sprayed with a 1% starch solution.

Chromatograms were first sprayed with a 1N sodium hydroxide solution, in order to hydrolyze the beta-lactam ring, and after 15 minutes with a solution containing 0.2 g potassium iodine, 0.4 g iodine dissolved in 20 ml ethanol and 5 ml 10% hydrochloric acid.

Chromatoplates were first sprayed with a 1N sodium hydroxide solution, in order to hydrolyze the beta-lactam ring, and after 15 minutes with a 0.1% ninhydrine solution in ethanol, and heated in an oven at 120 °Cfor 10 minutes.

Results and Discussion

The purpose of the method (simultaneous separation of a multicomponent mixture), and the information about the samples (structure, polarity, solubility, stability) were important as initial hints for the choice of the chromatographic system, using the rule of the Stahl's triangle.[2,10,11]

The most widely used stationary phase for the analysis of beta-lactam is silicagel, but if we consult the literature reversed-phase or cellulose plates have also been used. Silicagel surface bears Si-OH groups capable of hydrogen bonding with polar substances. Mobile phases for the separation of both penicillins and cephalosporins are polar, usually containing variable quantities of water.[5-7,12]

An acid (acetic acid) was added to the mobile phase in order to avoid decomposition of the beta-lactam ring on silicagel.

Around twenty solvents were tested and six mobile phases were selected (Table 1)

Table 1

The selected mobile phases

NoMobile phases (V/V)

I butanol – water – ethanol – acetic acid 50:20:15:15

II butanol – water –acetic acid 60:20:20

III ethyl acetate – water – acetic acid 60:20:20

IV ethyl acetate – methanol – acetic acid 45:50:5

V acetone – acetic acid 95:5

VI acetone – benzene – water – acetic acid 65:14:14:7

All beta-lactams can be detected in UV light at 254 nm (green fluorescence) and 366 nm (blue fluorescence). Applying reagents such as ninhydrin or exposing the chromatoplate to iodine vapor can diminish the detection limit.

(Hancu et al., 2013)

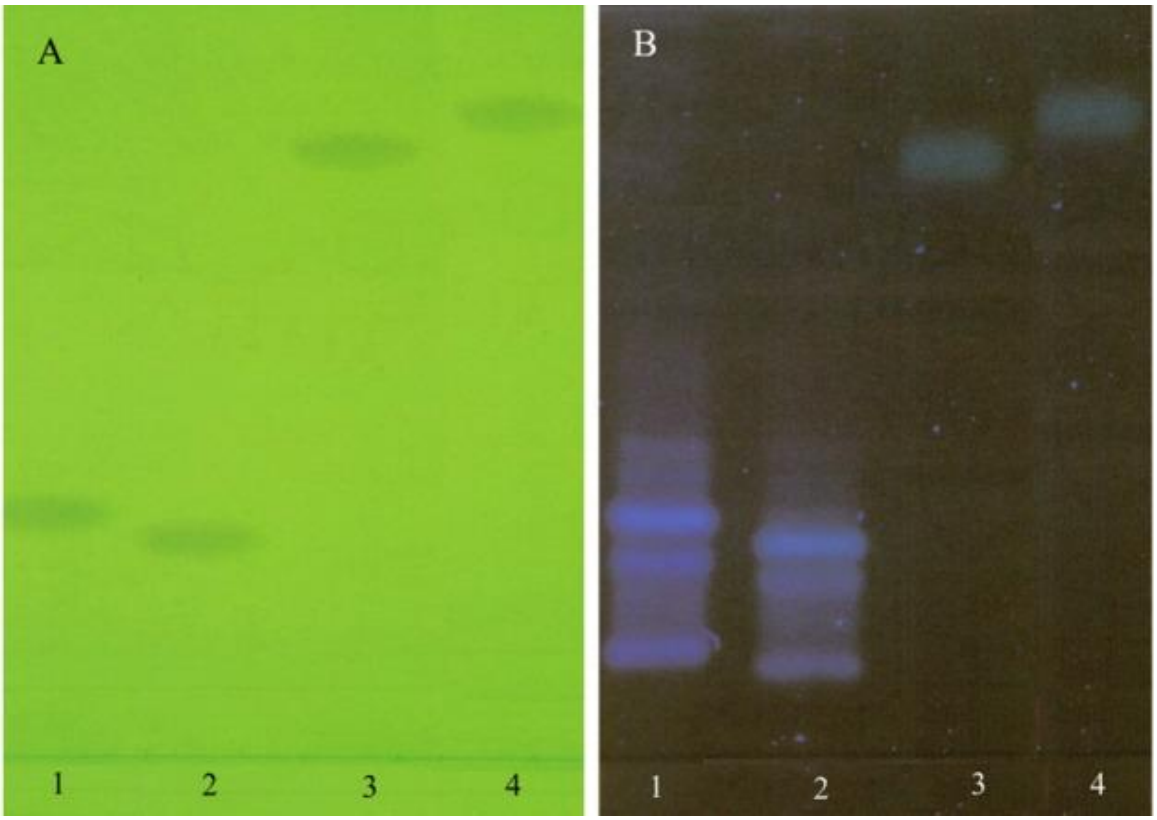


Fig 1 Chromatogram obtained at the separation of penicillins using mobile phase III (ethyl acetate – water – acetic acid 60:20:20), detection in UV light at 254 nm(A) and 366 nm(B) (1 - AMP, 2 - AMO, 3 – PEN, 4 - OXA)

(Hancu et al., 2013)

33.2 PREPARATION OF THE STOCK TEMPLATE SOLUTION

For the preparation of the stock control solution, benzyl penicillin potassium is required as a pure powder commercially available, a finished product or suitable raw material of good quality (> 85%) for reference purposes. . Place an aluminum foil on the measuring plate of the supplied electronic pocket scale, set the zero and measure approx. 0.3 g appropriately. Of benzyl penicillin potassium using a spatula. Carefully empty the aluminum foil over a 10 ml laboratory glass vial and rinse all the resulting powder with 5.7 ml of water using a graduated pipette. Record each time the exact weight obtained and adjusts the amount of water suitable for dissolution using, for example, 5.5 ml of water for 0.29 g or 6.1 ml of water per 0.32 g of water. Control substance collected from the main container respectively. Close the laboratory bottle and shake until

Dissolution the solids. The final solution obtained should contain 50 mg of benzyl penicillin sodium equivalents per ml and be labeled as a Penicillin G Stock Control Solution. Prepare this solution only just before each test. Important Note: The scales supplied cannot weigh exactly less than 0.25 g. The relative standard deviation of +/- 2% is considered too high. For the measurement of higher quantities, the difference is only about +/- 1%. The scale will not record changes of a few milligrams added or subtracted approaching the target weight of 0.3 g. Then remove the aluminum foil or lightly pat the scale pan with a pencil or spatula whenever a few milligrams have been added or subtracted to compensate for dynamic inertia and ensure correct readings.

(Jähnke, s. d.)

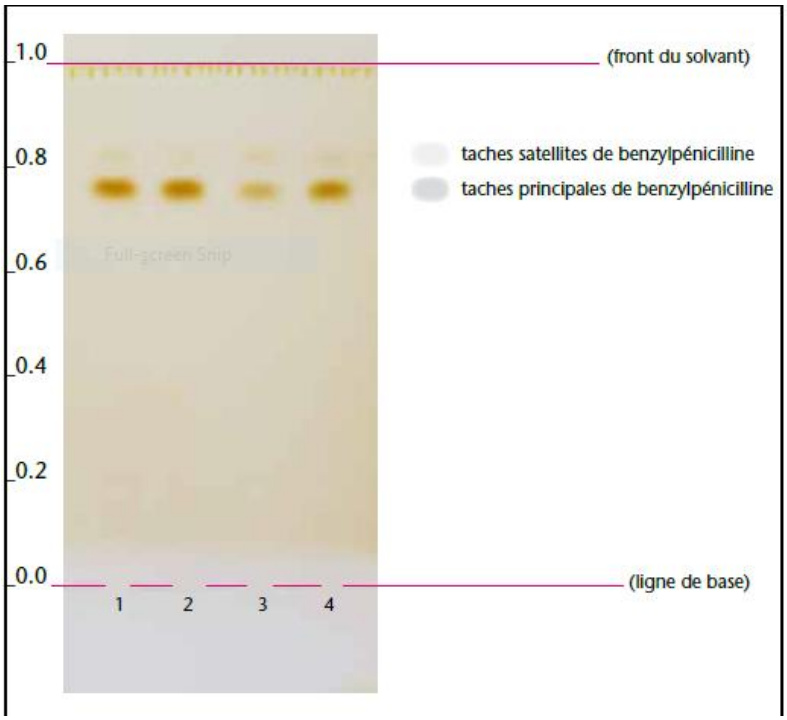


Fig3 : chromatoplaque observed in the light of the day after coloring in iode (Jähnke, s. d.)

33.3 Thin layer chromatography: the Rf or retention factor

In given conditions

nature (and composition) of the solvent
nature of the adsorbent
thickness of the absorbent layer
amount of sample deposited

the Rf of a substance is a characteristic constant as well as a melting temperature for example. His determination can therefore be valuable for identification.
Rf is determined by the ratio Rf in which

$$Rf = \frac{d}{d_s}$$

d represents the distance covered by the substance
and ds the distance traveled by the solvent.

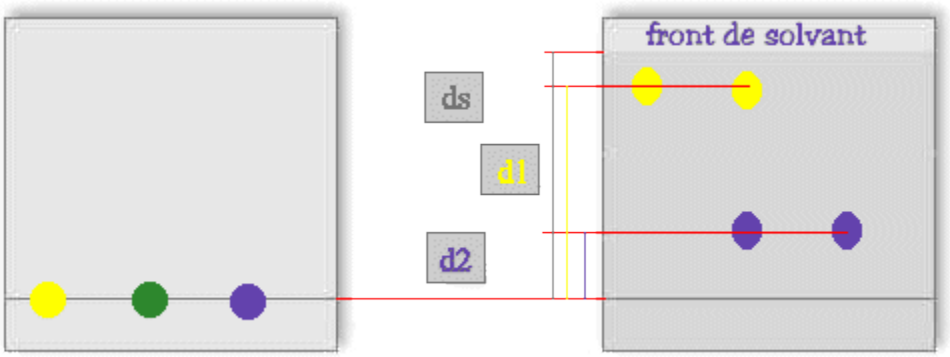
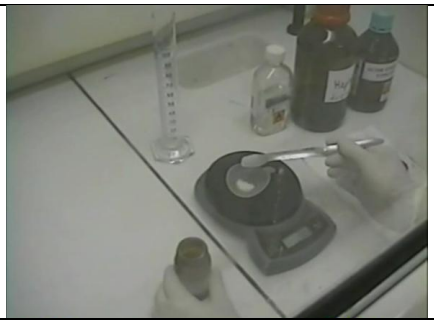
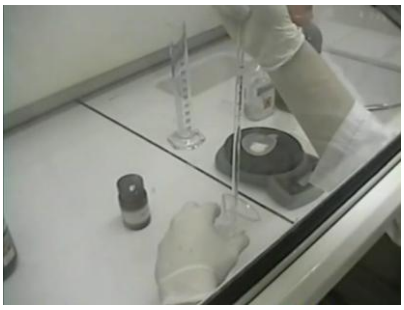
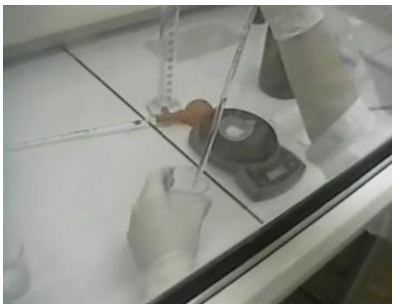
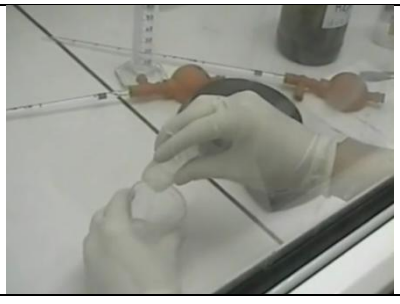


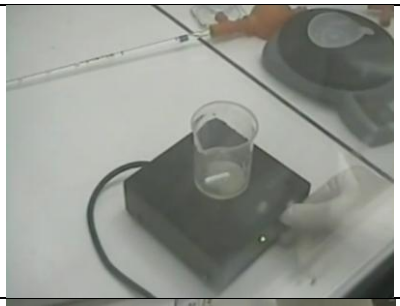
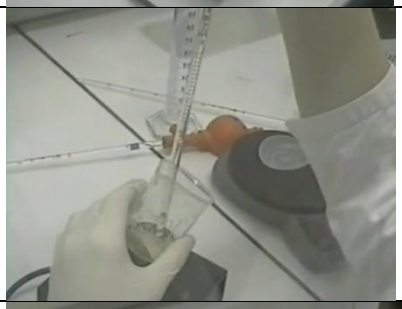
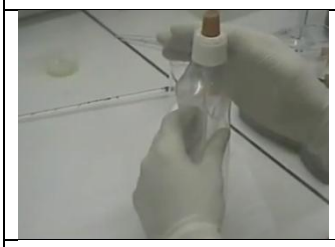
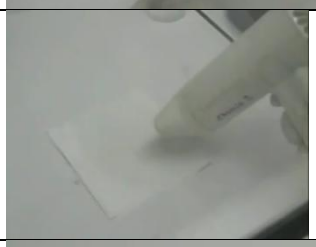
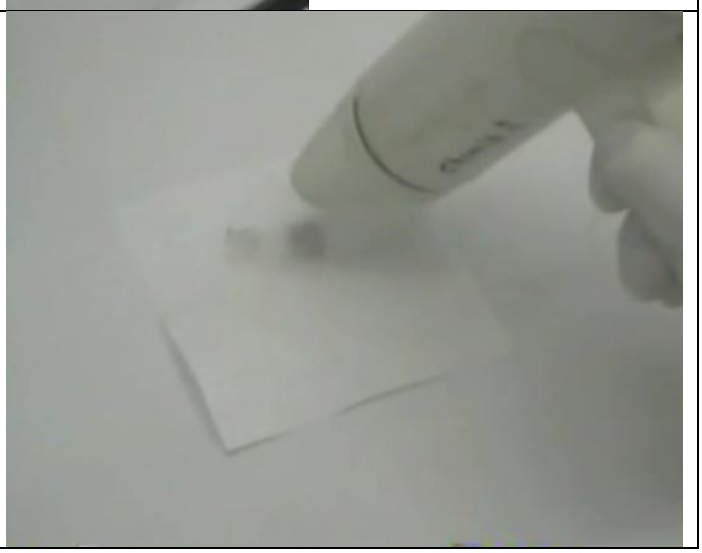
Fig 2: This technique has limitations related to the difficulty of obtaining reproducible conditions

(« CCM : Calcul du facteur de retention Rf », s. d.)

33.4 Preparation de colorant ninhydrine

On commence par peser 1g de ninhydrine	
Puis on prélève 2ml d'acide acétique cristallisant	
Ensuite on prélève 4ml d'éthanol	
On l'ajoute à l'acide acétique	

On ajoute la ninhydrine à la solution de travail	

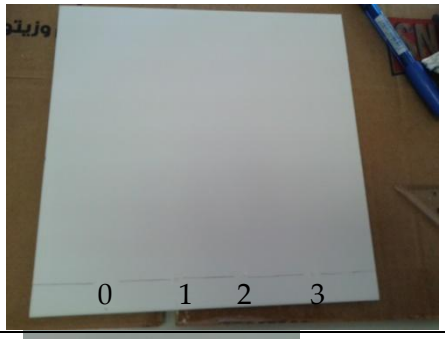
On homogénéise la solution avec un agitateur magnétique	
On prélève 2ml de la solution de travail	
On ajoute 3,33ml d'éthanol à la solution de travail	
	
Enfin on obtient des empreintes colorées au pourpre de Ruhemann	

https://youtu.be/Gz6Vlu4h72M

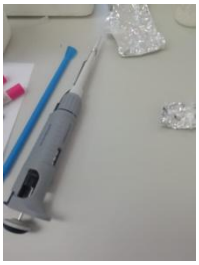
34 Results: Diagnostic

Done in the laboratory of chemistry in Lebanese university (LU)

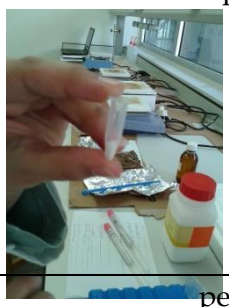
34.1 Experiment 1: Preparation of TLC silica gel



echantion of penicilline 0 1 2 et 3



micropipette



penicilline solution 0 stander

penicilline solution 1

penicilline solution 2

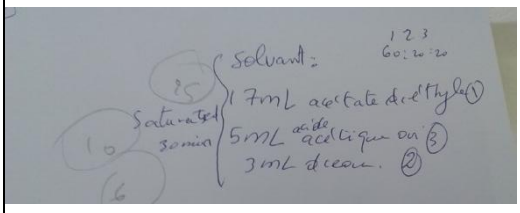
penicilline solution 3

prelevation of penicilline solution

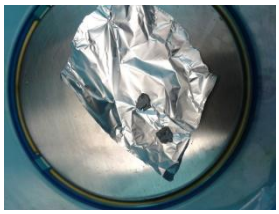
depot of penicilline

0 1 2 3

34.2 Experiment 2: preparation of solvant



34.3 Experiment 3: Preparation of color revelution



containing 0.2 g potassium iodine, 0.4 g iodine dissolved in 20 ml ethanol and 5 ml 10% hydrochloric acid.

hydrochloric acid

ethanol

35 Discussion

- Experiment n 1

We left the silicagel paper two days and this led to the rise of the solvent in a zigzag line also due to the lack of equivalent a quantity of solvent with the size of the silicagel paper

تركنا ورقة السيليس يومين وهذا ادى الى صعود المذيب بخط متعرج السبب يعود ايضا الى عدم تكافئ كمية المذيب مع حجم ورقة السيليس

This penicillin that we used as a reference wasn't powder, but it was a small, thin disc that was used for microbiology (antibiograme)

هذا والبنسلين الذي استعملناه ك مرجع لم يكن بودرة ولكن كان عبارة عن ديسكات صغيرة ورفيعة ك التي تستعمل للميكروبيولوجي

- Experiment n 2

The lack of equivalent a quantity of solvent with the size of the silica gel paper

- Experiment n 3

The quantity of HCl used was too much.
I didn't spray the paper with it
Because the acid damages the silicagel

We can use uv rays instead of color. Examined under UV radiation at wavelengths of 254 and 366 nm.

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[Braun 2001] Fluoreszenz-optimierte Detektion heterologer Protein-Protein Interaktionen in *Saccharomyces cerevisiae* als Alternative zur Yeast Two Hybrid Methode DISSERTATION der Fakultät für Chemie und Pharmazie der Eberhard-Karls-Universität Tübingen zur Erlangung des Grades eines Doktors der Naturwissenschaften 2001, vorgelegt von Manuela Braun, Datum der mündlichen Prüfung 14. Mai 2001, Dekan Prof. Dr. H. Probst, 1. Berichterstatter Prof. Dr. J. P. Ruppersberg, 2. Berichterstatter Prof. Dr. D. Mecke

[AKTA Process Technical Manual]

[1]http://www.aecenar.com/publications

[2] http://www.aecenar.com/downloads/cat_view/7-megbi-institute

[3] http://www.aecenar.com/downloads/cat_view/3-meae-institute?start=10

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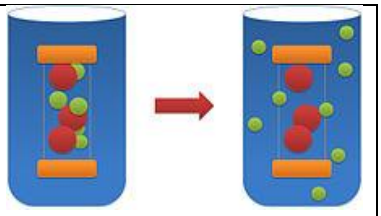
dialysis	in biochemistry, **dialysis** is the process of separating molecules in solution by the difference in their rates of diffusionthrough a semipermeable membrane, such as dialysis tubing.	
cellular		خلوية
detergents	Reinigungsmittel	المنظّفات
dialysis		
protein expression		تعبير بروتيني
heterologous protein		بروتين لجين خارجي
thioredoxin		ذيوريدوكسين
plasmid		بلازميد
fusion protein		البروتين الانصهاري
Medium (Media)/ Culture Medium	Medium	مستنبت
purification		تنقية
incubation		احتضان
hydrophobic		هيدروفوبية
stimulated		محفز
Ammonium sulfate precipitation	**Ammonium sulfate precipitation** is a method used to purify proteins by altering their solubility. It is a specific case of a more general technique known as salting out.	ترقيد كبريتات الأمونيوم
recombinant		مؤتلف
glycoproteins		البروتينات السكّرية
Mannose		مانُّوز

immunogenic		
initialization		الاستهلال
sensors	Sensoren	اجهزة الاحساس

to research : Ti 15 rotor from Beckman