بسم الله الرحمن الرحيم

MEGBI Antibiotics Pilot Plant (MEGBI-APP) - 4th Project Report (2016)

- Producing Penicillin
- Simplified chemical engineering process implementation for producing amoxillin from penicillin
- ~~Diagnostic Station: Penicillin/Amoxillin Concentration~~

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مركـز أبحـاث الشـرق الأوسـط للجينـات والتقنيـة البيولوجية

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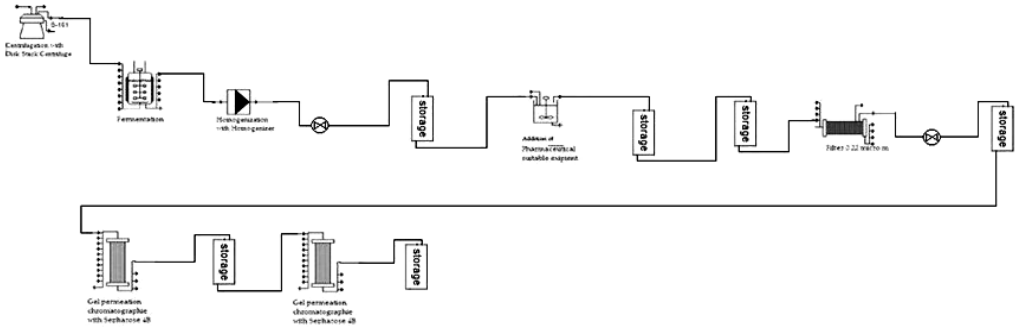
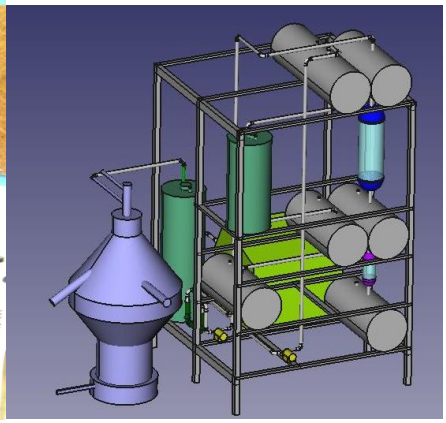
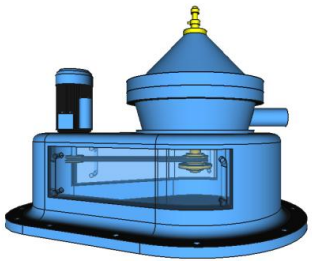
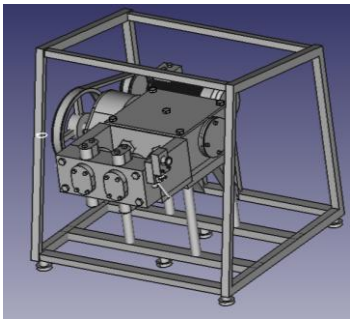
المضمون

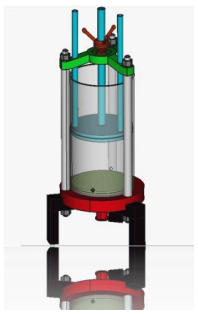
Project Status at beginning of this project phase











- Automation system

- Mechanical parts of minimal USP-DSP manufactored

1 إدارة المشروع (Project Management)

1.1 هدف /العمل (Project phase goal)

~~The goal is to install a minimal biotechnological USP – DSP production plant for monoclonal antibody (MAB) production in E.coli.~~

The goal is to install a pilot plant for producing semi-synthetic penicillin.

1.2 Budget Planning

From 3rd project report, Ch. 1.4: Azm Association (Faisal Maulawi, Dr Dani Saaduddin, Dr Kifah Tout) visited AECENAR Center at Ras Nhache on 6th March 2015 and Business Plan 2 was discussed. Result (Status 17th March 2015): Azm wants a more **detailed business plan with detailed market strategy.**

This is to be done in 2017.

1.3 Time Schedule / الجدول الزمني/

Originally planned:

Nov/Dec 14: Financement and Concept Phase

Jan – June 15: Finishing of Development of MEGBI Vaccine Production Pilot Plant (MEGBI-VPP)

Actually:

March-May 2016: Migration of specification to semi-synthetic penicillin plant

2 Basics

2.1 Chemical Engineering Basics[1]

To master chemical process technology five crucial steps are involved namely:

a) Raw-Materials and reactions: A chosen process route to manufacture desired chemicals with appropriate purities will eventually lead to preparing a list of raw-materials and utilities. Thereby, prominent reactions can be also known.

b) Conceptual process flow-sheet: A conceptual process flow-sheet where a chemical engineer has an abstract representation of the actual process flow-sheet will enable quicker learning. A conceptual process flow-sheet typically constitute the following attributes:

- Raw-material purification (Solid-fluid operations such as cyclone separators, bag filters etc.)
- Raw-material processing (Heat exchange operations such as furnace heating, cooling etc.)
- Raw-material to product transformation (Reaction operations using CSTR, PFR, PBR and Batch reactors)
- Product purification (In separation processes such as flash, distillation, absorption and extraction)
- Product processing (heat exchangers, phase change units)
- Recycle of un-reacted raw-materials as recycle streams to the reaction operations.

c) Process intensification in the form of heat-integration, stream utilization and waste reduction and multiple recycle streams: These options are in fact optional and they enrich the energy enhancement and waste reduction efficiency of a process plant. Originally, chemical plants developed without such process intensification policies have been subjected to rigorous research and case study investigations to identify opportunities for cost reduction and better energy/waste management.

d) Additional critical issues related to various unit operations/processes

- Safety issues: What safety issues are most relevant and need frequent monitoring

e) Alternate technologies: For a desired function of a process unit, can thereby alternate technologies that could reduce the cost and even then provide the same functional role and desired flow rates and compositions of the emanating streams.

2.1.1 Prominent unit-operations and unit-processes in chemical industry

A detailed summary of various prominent unit operations/processes and their functional role in the chemical plant are summarized in Table **0.1** along with suitable figures.

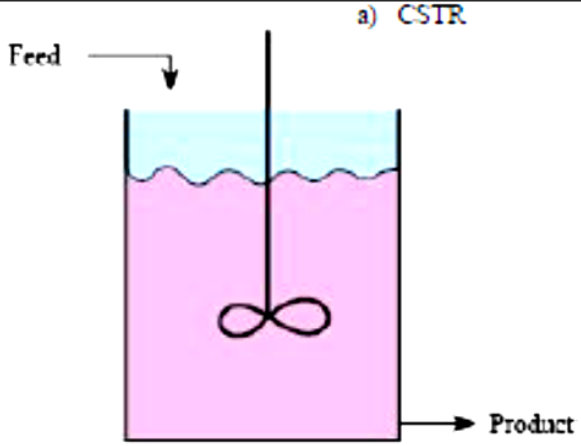
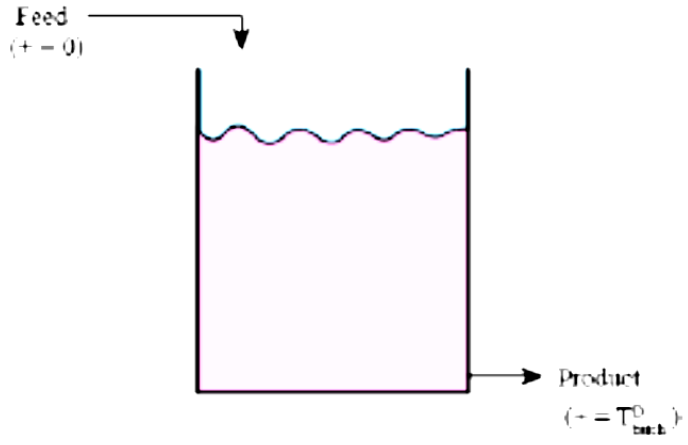
[1] from [5]

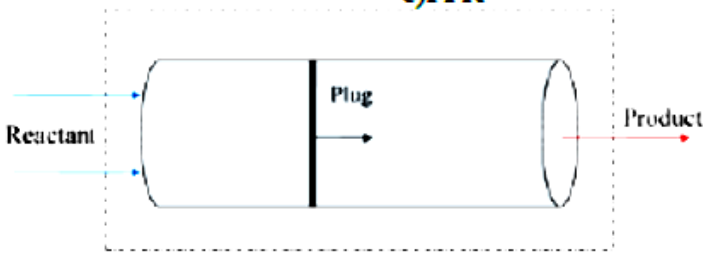
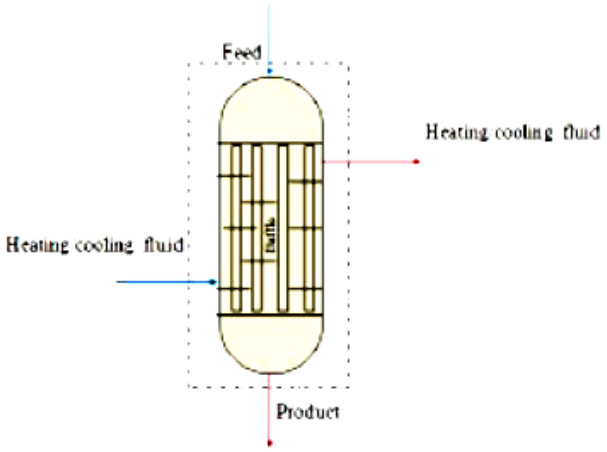
Category	Unit operations/processes	Functional role
fluid operations	a) Centrifugal pump b) Reciprocating pump c) Compressor d) Expander	a) To pressurize liquids and gases. b) To depressurize gases
solid operations	a) Crusher b) Grinder	a) To reduce the size of solids
Solid-fluid separators	a) Cyclone separator b) Centrifuge c) Electrostatic precipitator d) Classifier & Thickener e) Liquid-liquid separator	a) To separate solid particles from solid-liquid/gas mixtures
Heat exchangers	a) Shell & Tube heat exchangers b) Fired heaters and furnaces c) Coolers	a) To either remove or add heat to process streams so as to meet desired conditions in other units. b) Either utilities or other process streams are used to carry out heating/cooling requirements.
Mass transfer units	a) Phase separation b) Distillation c) Absorption d) Stripping e) Adsorption f) Extraction g) Leaching h) Crystallization i) Membrane	a) To separate a feed into products with different compositions. b) A third agent (heat or compound) is usually used to carry out separation.
Reactor units	a) Completely stirred tank reactor (CSTR) b) Plug flow reactor (PFR) c) Packed bed reactors (PBR) d) Slurry & Trickle bed reactors	a) To carry out reactions in homogenous fluids (gases/liquids). b) To carry out catalytic and multi-phase reactions.

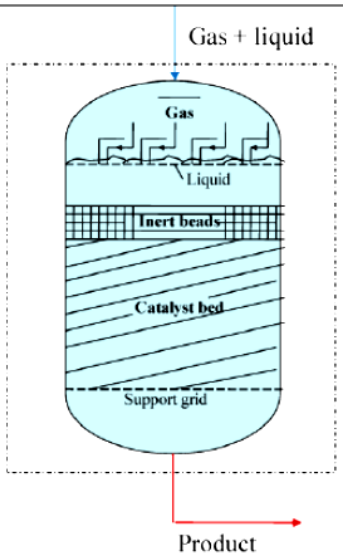
Table 0.1: Important unit operations/unit processes and their functional role in chemical process technology.

References:

Dryden C. E., Outlines of Chemical Technology, East-West Press, 2008

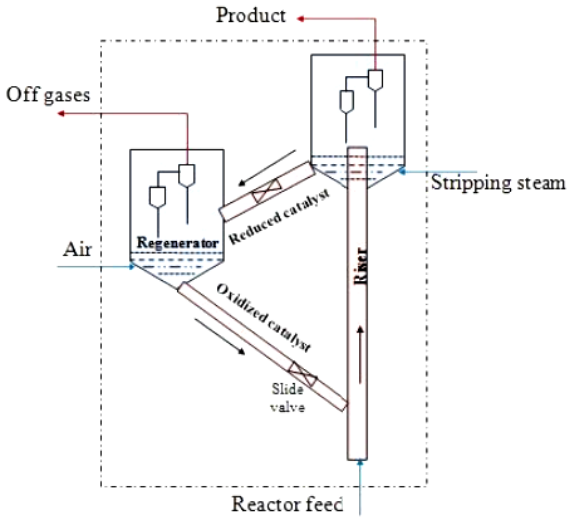
Process Technology	Functional Role
Reactors a) CSTR b) Batch Reactor c) PFR d) Packed bed reactor e) Trickle bed reactor f) Fluidized bed reactor	- Central and most important process technology in process flow sheets - Carry out desired reactive transformations
a) CSTR  CSTR	- Well mixed reaction system **set alignment** - Homogenous liquid/gas phase reaction - Most easy configuration - Temperature control through Jacket - Reactant instantaneously reaches lowest concentration - Most inexpensive to design and operate
b) Batch Reactor  	- Has a simple design, with the requirement of very little supporting equipments - Ideal for small scale experimental studies on reactor kinetics - Can be used industrially for treatment of very small quantities of materials.

c)PFR  	- Homogenous liquid/gas phase reaction - Reactant gradually reaches low concentrations - Good control over temperature - Temperature control through jacket (not shown)
d)Packed Bed Reactor (PBR)  	- Heterogeneous reaction - Packing to act as catalyst - Packing packed in tubes - Shell fed with cooling/heating fluid (optional) set alignment - continuous sentence
e)Trickle Bed Reactor	- Multi-phase reaction - If the reaction is not catalytic, packing serves to enhance interfacial area

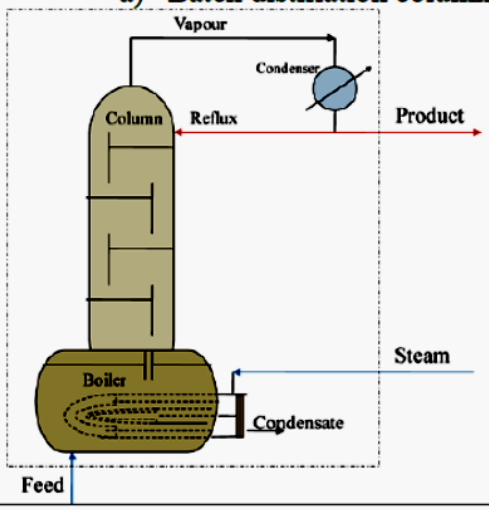


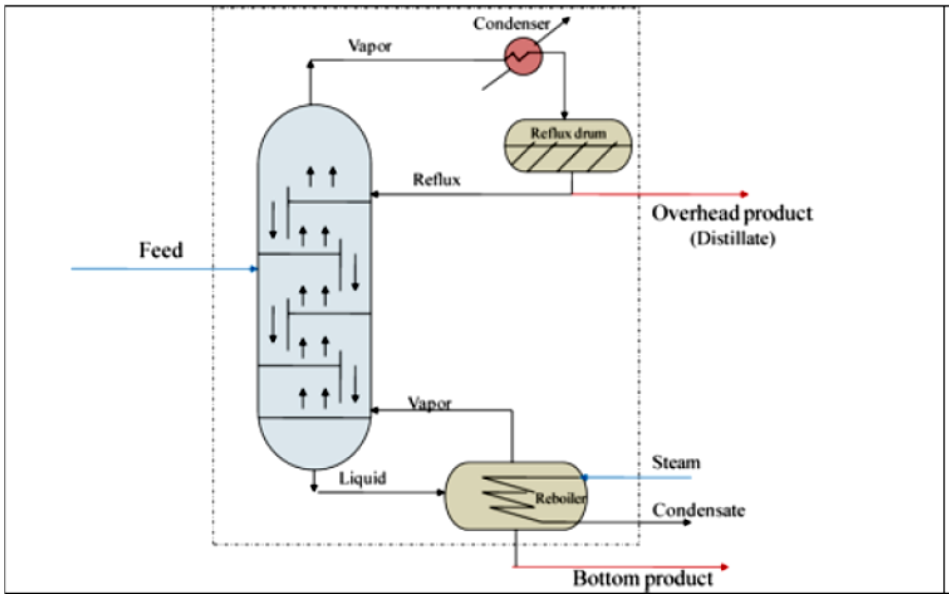
- If the reaction is catalytic, packing acts as a catalyst as well
- Complicated design

f) Fluidized bed reactor



- Provides highest mass, heat and hence reaction rates for solid-fluid reactions
- Very commonly deployed in petroleum refineries (catalytic cracking)
- Complicated accessories (shown) and control system required
- The accessories are for catalyst re-generation and transport.

Separators: a) Batch distillation b) Continuous distillation c) Absorption d) Stripping e) Liquid-liquid extraction f) Leaching g) Crystallization h) Drying i) Flash separator j) Membrane separator k) Packed bed contactor	- Most important process technology - Provides desired separation between phases and streams - Located next to the reactor as 100 % conversions are very rare in industrial practice
a) Batch distillation column  	**- Used to separate a liquid mixture based on relative volatility (differences in boiling points)** **- Operated in batch mode**
b) Continuous distillation (Fractionator) column missed	**- The most important separation technology in process flow sheets** **- Provides very pure products** **- Differences in boiling points is the working principle**



- Energy intensive operation
-

2.2 Nitrogene basic products

2.2.1 liquid nitrogene (N2)

Market

medical/laboratories

argriculture

automobile

Producer in Lebanon

http://lb.kompass.com/c/chehab-industrial-medical-gases-sal/lb001453/

2.2.2 Ammoniumnitrat(NH_4NO_3)

Market

medical

Producer in Lebanon

no known

Manufaturing

2.2.3 N2O (nitrous oxide)

Market

medical

Producer in Lebanon

no known

Manufaturing

Lachgas (Distickstoffmonoxid; N_2O) wird industriell aus Ammoniumnitrat(NH_4NO_3) hergestellt. Dabei entsteht in einem Zwischenschritt Salpetersäure(H_2NO_3) und Ammoniak (NH_4) nach folgender chemischer Formel:

$NH_4NO_3 \leftrightarrow H_2NO_3 + NH_4 \leftrightarrow N_2O + 2\ H_2O$

Ammoniumnitrat ist unter Hitzeeinwirkung hochexplosiv. Mehrere großeExplosionsunfälle mit mehreren hundert Todesopfern sind aus der Geschichte bekannt (z.B. Oppau 1921, Toulouse 2001 und zuletzt am 17.04.2013 in West, USA).

2.3 Penicillin

2.3.1 Definition

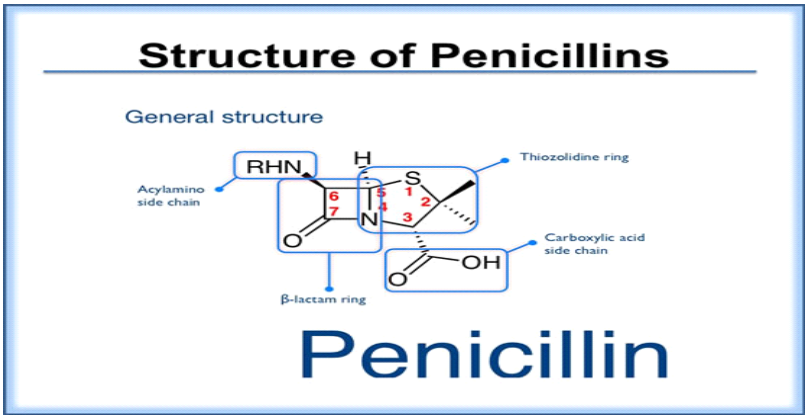
Antibiotics are a type of antimicrobial drug. They are one of the secondary metabolites produced by some fungi and bacteria.

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.

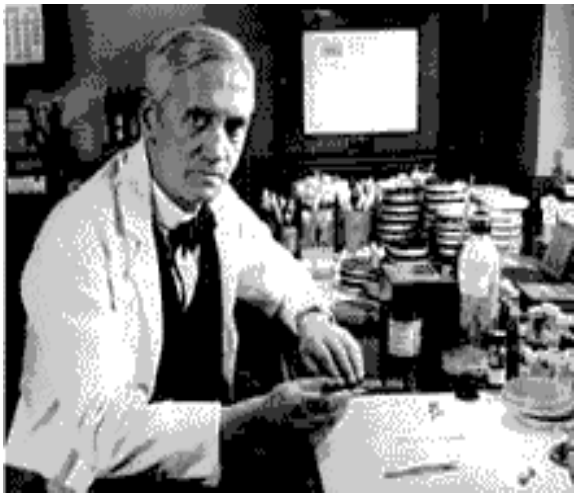
2.3.2 The structure of the penicillins:

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.



2.3.3 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



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.*

2.3.4 Strains of penicillium:

In the early days of penicillin production (1928)

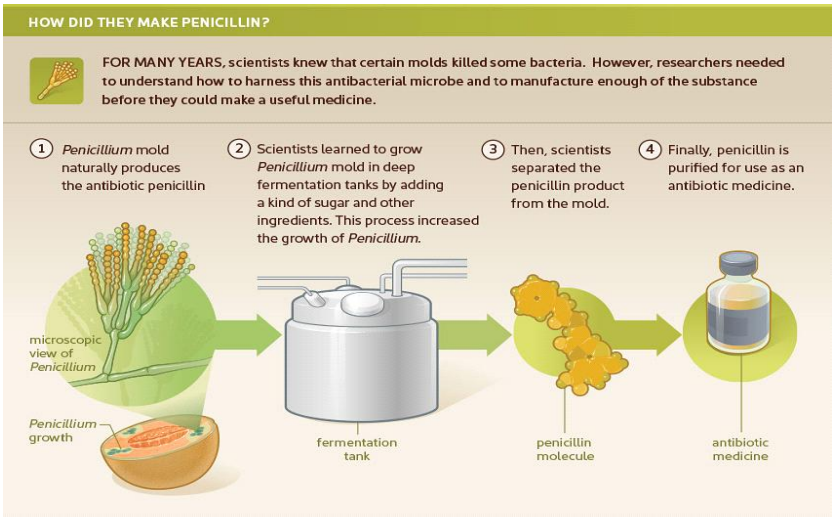
Penicillium Notatum strain was employed. After a few years, a new strain of Penicillium Chrysogenum discovered in 1943 was employed for penicillin production.

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*.

2.3.5 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 H_2SO_4 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.

2.3.6 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.

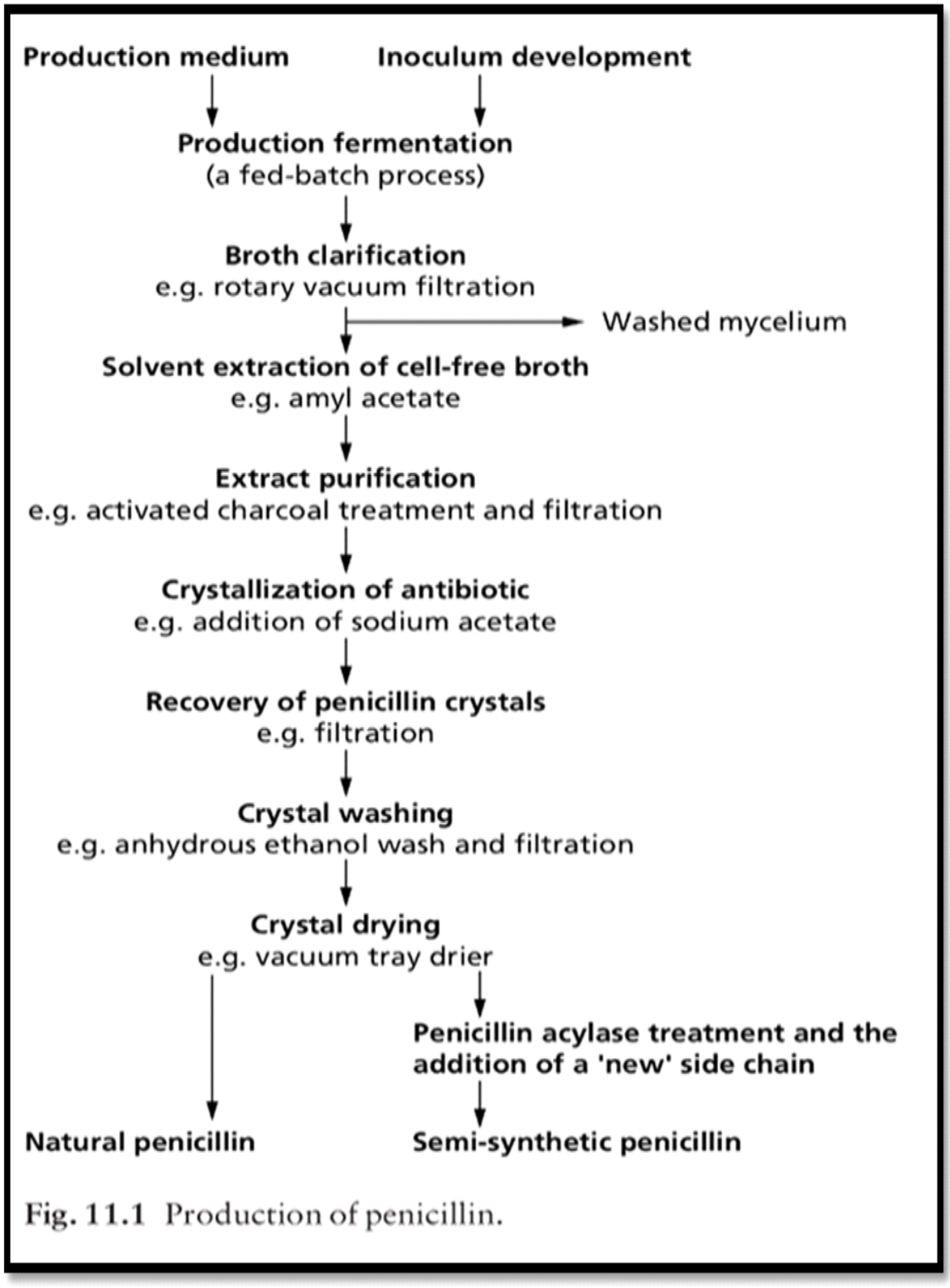


Fig. 11.1 Production of penicillin.

2.3.7 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.

2.3.8 Mechanism of action:

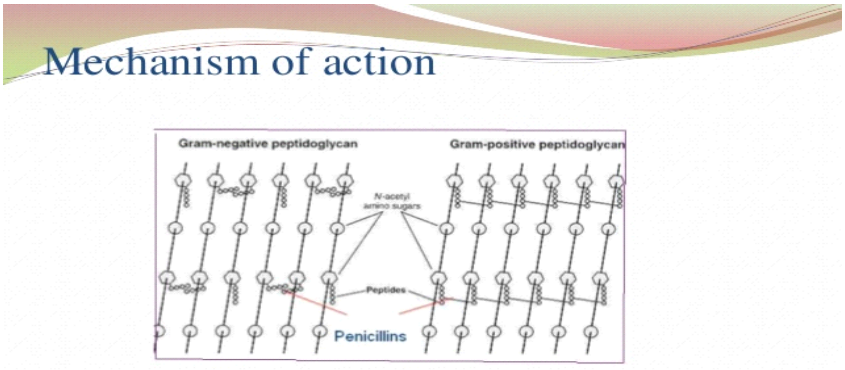
Beta- lactam antibiotics inhibit the formation of peptidoglycan an essential part of the cell wall.

All penicillins work in the same way:

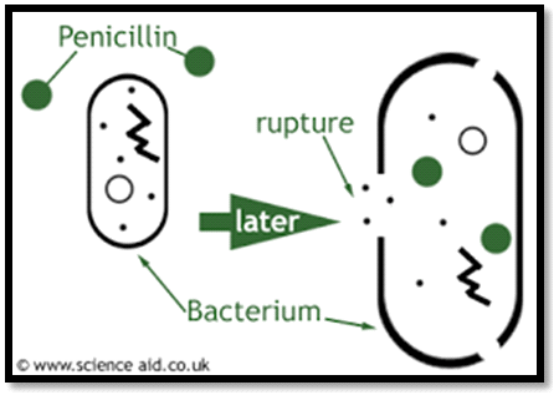
They interfere with cell wall synthesis by binding to penicillin-binding proteins (PBPs) which are located in bacterial cell walls, and by activating other enzymes to break down the protective wall of the microorganism. Then, inhibition of PBPs leads to inhibition of peptidoglycan synthesis then, inhibition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

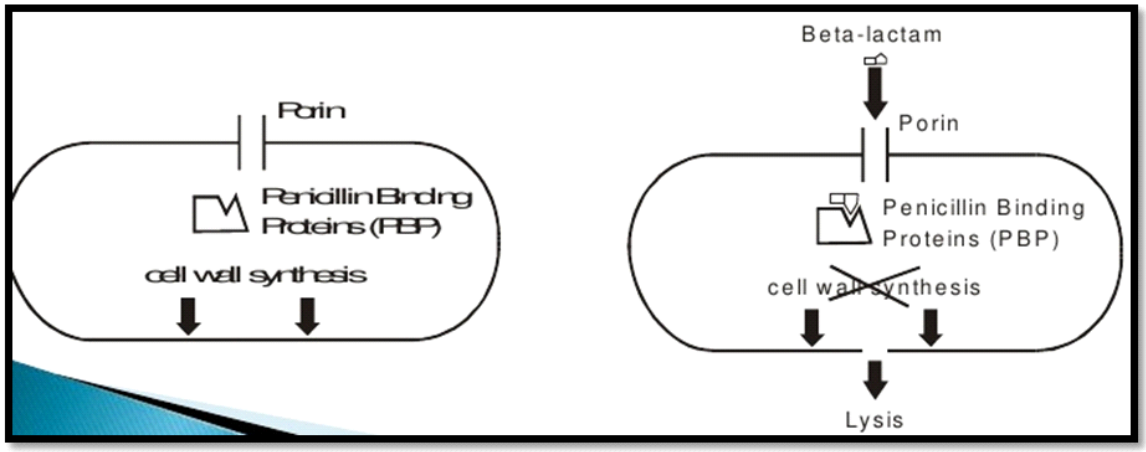
Since human cells do not contain a cell wall, penicillin treatment results in bacterial cell death without affecting human cells.

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.



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.





2.3.9 Resistance to beta lactams :

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

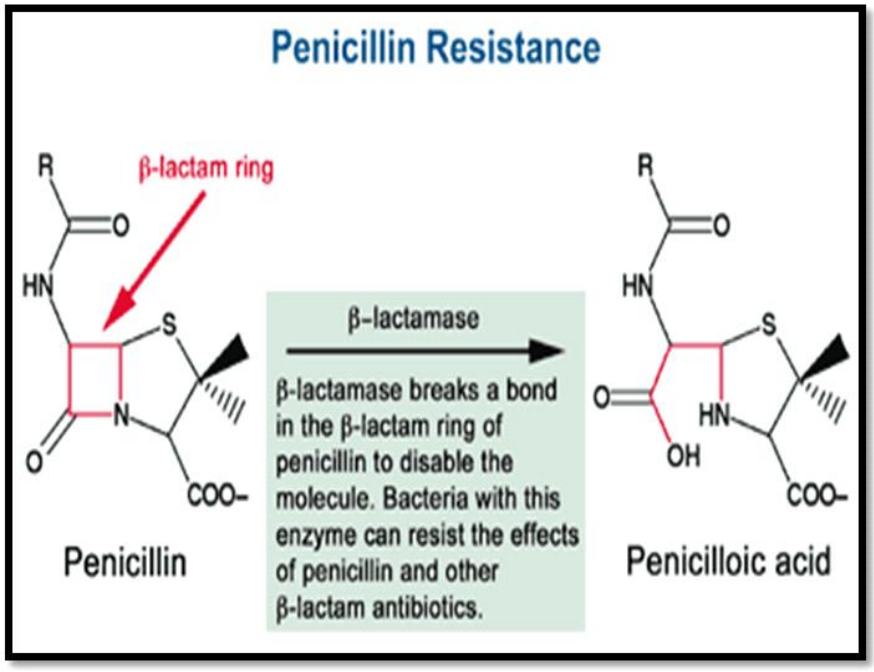
Bacteria are constantly finding ways to counteract antibiotics, one of the most important bacterial defense mechanisms is the production of enzymes B lactamase.

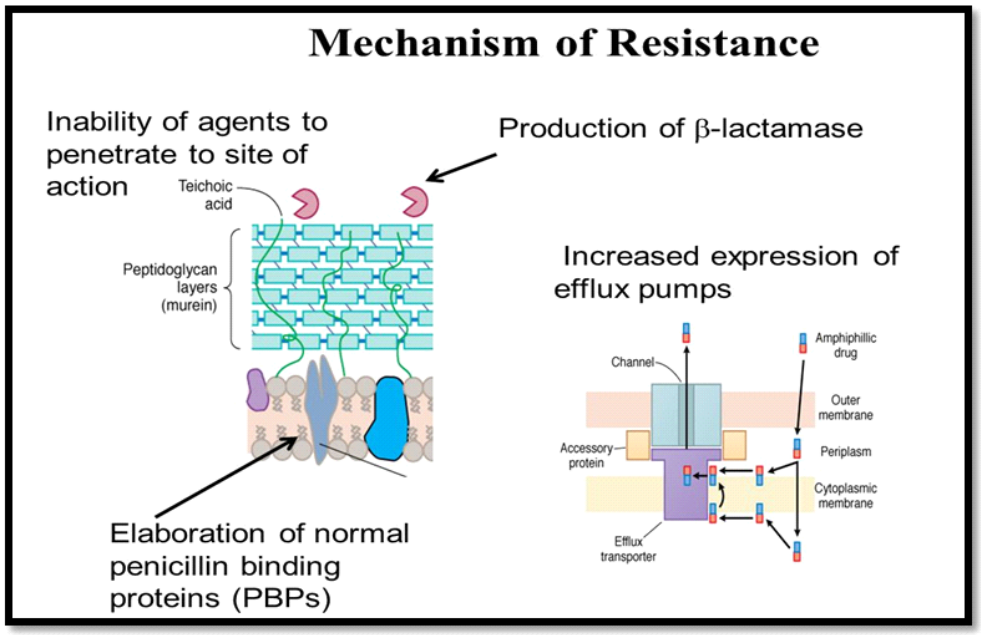
Organisms that produce B lactamase are resistant to penicillin by hydrolyses beta-lactam ring.

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.





2.3.10 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

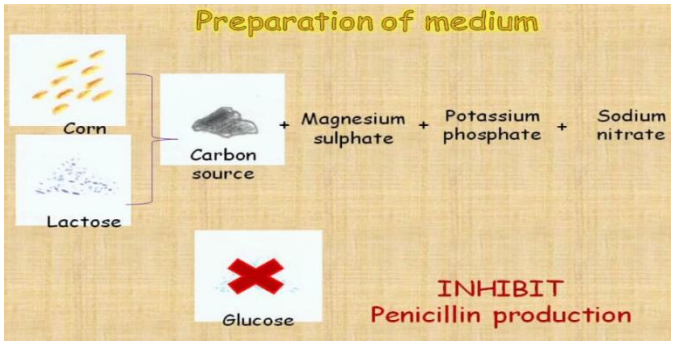
2.4 Production of Semisynthetic Penicillins

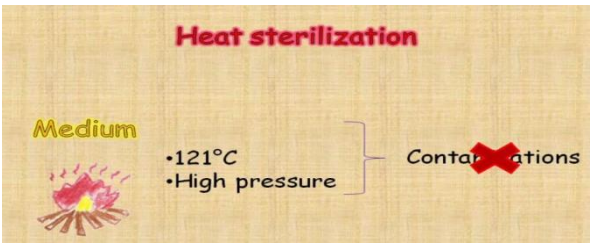
Semisynthetic penicillins:

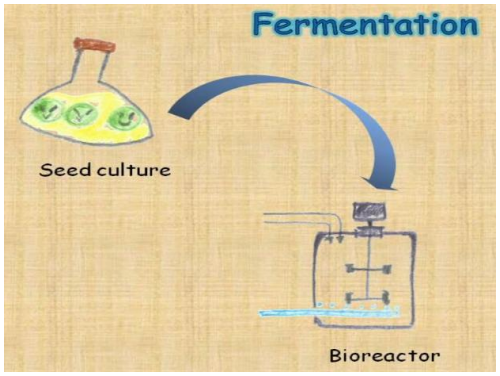
Ampicillin

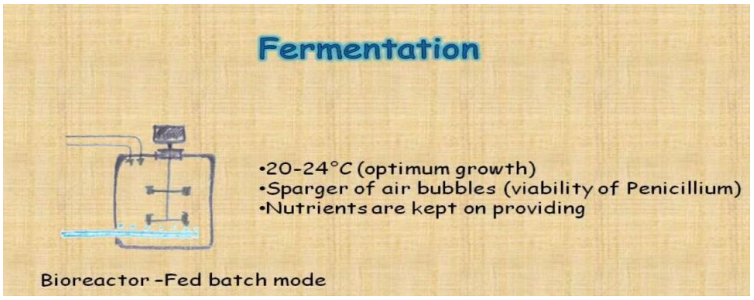
Amoxillin

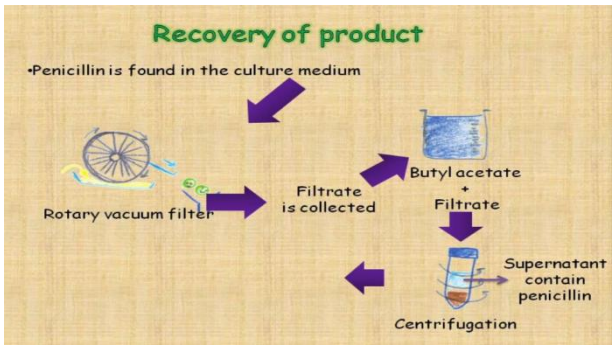
2.4.1 Penicillin in culture

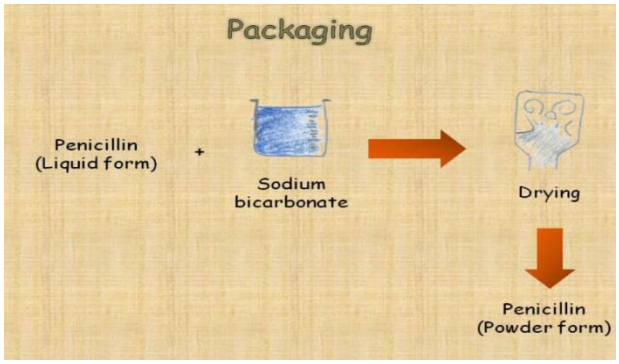


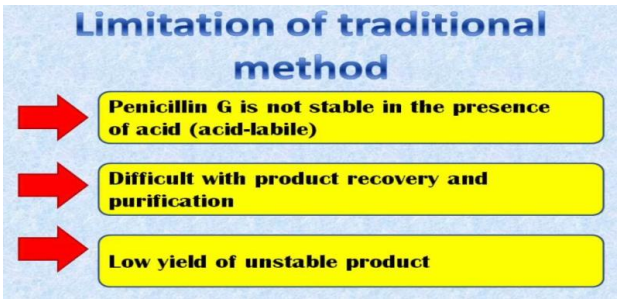




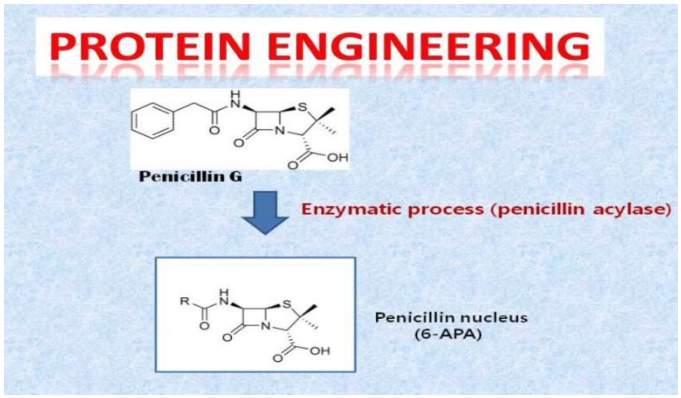


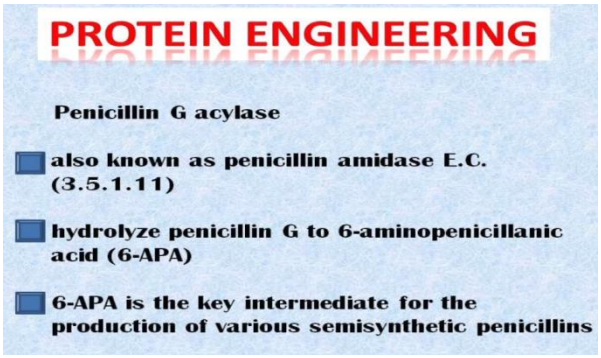


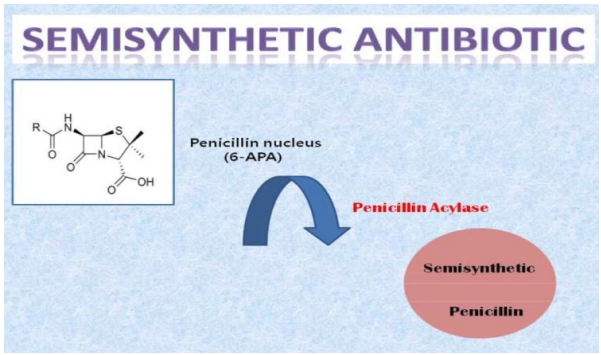


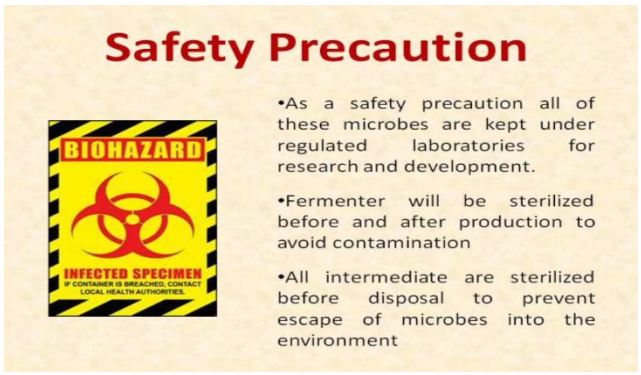


2.4.2 Production of semisynthetic penicillins

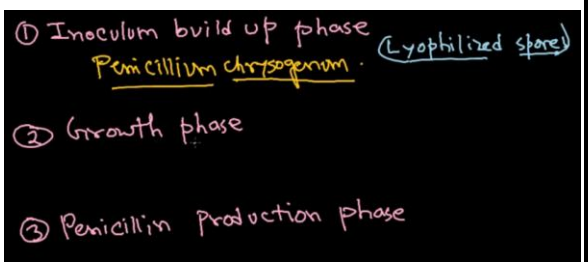


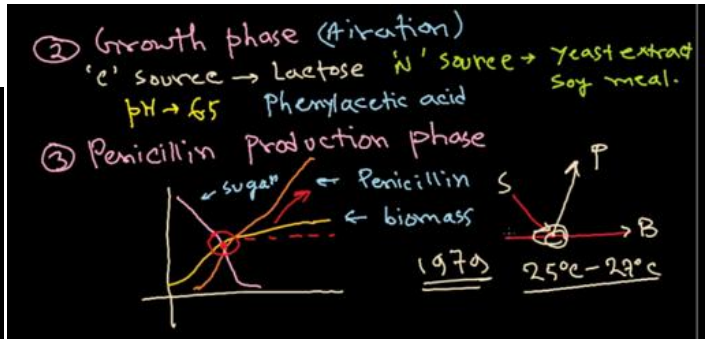


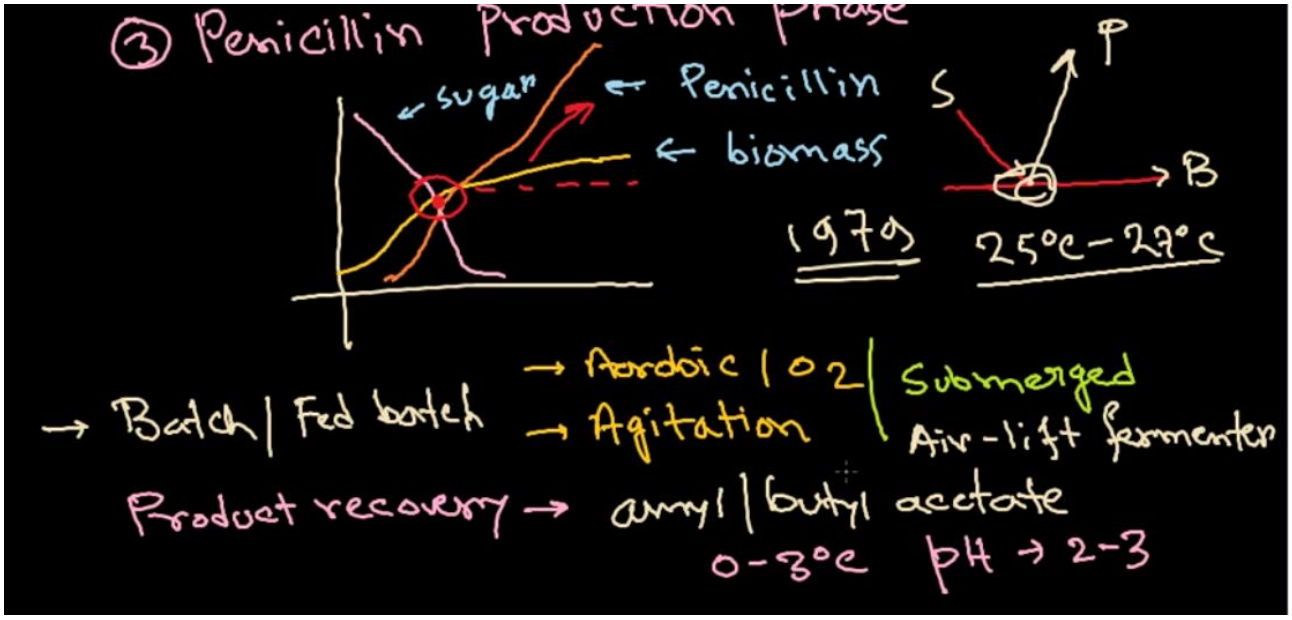




2.4.3 Industrial Prodution of penicillin







2.4.4 Devices

2.4.4.1 Rotary vacuum drum

2.4.5 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 MlBK-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 (MlLA) 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.

2.5 قائمة منظمة الصحة العالمية للأدوية الأساسية (WHO Model List of Essential Medicines)

WHO Model List of Essential Medicines is published by the World Health Organization (WHO). The first list, published in 1977, included 204 pharmaceutical drugs.[1] The WHO updates the list every two years and it currently has about 400 medicines. The WHO later added a separate **WHO Model List of Essential Medicines for Children** up to 12 years of age.

As of 2016, at least 156 countries have created national lists of essential medicines based on the WHO's model list.[2] The national lists contain between 334 and 580 medications.[3]

In April 2015, the WHO published the 19th edition of the adult list and 5th edition of the list for children.[4][5]

2.5.1 مجالات الطب والادوية (Areas of medicine)[2]

1Anaesthetics

- 1.1General anaesthetics and oxygen
- 1.2Local anaesthetics
- 1.3Preoperative medication and sedation for short-term procedures

2Medicines for pain and palliative care

- 2.1Nonopioids and nonsteroidal anti-inflammatory drugs (NSAIDs)
- 2.2Opioid analgesics
- 2.3Medicines for other common symptoms in palliative care

3Antiallergics and medicines used in anaphylaxis

4Antidotes and other substances used in poisonings

- 4.1Nonspecific
- 4.2Specific

5Anticonvulsive medication

6Anti-infective medicines

- 6.1Antihelminthics
- 6.2Antibiotics
- 6.3Antifungal medicines
- 6.4Antiviral medicines
- 6.5Antiprotozoal medicines

7Antimigraine medicines

- 7.1Acute attack
- 7.2Prevention

8Antineoplastic and immunosuppressives

- 8.1Immunosuppressive medicines
- 8.2Cytotoxic and adjuvant medicines
- 8.3Hormones and antihormones

9Antiparkinsonism medicines

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[2] https://en.wikipedia.org/wiki/WHO_Model_List_of_Essential_Medicines#Anti-infective_medicines

2.6 WHO List of Anti-Infective medicines[3]

2.6.1 Antihelminthics

2.6.1.1 Intestinal antihelminthics

- Albendazole
- Levamisole
- Mebendazole
- Niclosamide
- Praziquantel

[3] https://en.wikipedia.org/wiki/WHO_Model_List_of_Essential_Medicines#Anti-infective_medicines

- Pyrantel

2.6.1.2 Antifilarials

- Albendazole
- Diethylcarbamazine
- Ivermectin

2.6.1.3 Antischistosomals and other antinematode medicines

- Praziquantel
- Triclabendazole
- Oxamniquine†

2.6.2 Antibiotics

2.6.2.1 Beta Lactam medicines

- Amoxicillin
- Amoxicillin/clavulanic acid (amoxicillin + clavulanic acid)
- Ampicillin
- Benzathine benzylpenicillin
- Benzylpenicillin
- Cefalexin
- Cefazolin[note 6]
- Cefixime[note 7]
- Ceftriaxone[note 8]
- Cloxacillin
- Phenoxymethylpenicillin (penicillin V)
- Procaine benzylpenicillin[note 9]
- Cefotaxime†[note 10]
- Ceftazidime†
- Imipenem/cilastatin†[note 11]

2.6.2.2 Other antibacterials

- Azithromycin[note 12]
- Chloramphenicol
- Ciprofloxacin
- Clarithromycin[note 13]
- Doxycycline
- Erythromycin
- Gentamicin
- Metronidazole
- Nitrofurantoin
- Spectinomycin
- Trimethoprim/sulfamethoxazole
- Trimethoprim
- Clindamycin†
- Vancomycin†

2.6.2.3 Antileprosy medicines

- Clofazimine
- Dapsone
- Rifampicin

2.6.2.4 Antituberculosis medicines

- Ethambutol
- Ethambutol/isoniazid (ethambutol + isoniazid)
- Ethambutol/isoniazid/pyrazinamide/rifampicin (ethambutol + isoniazid + pyrazinamide + rifampicin)
- Ethambutol/isoniazid/rifampicin (ethambutol + isoniazid + rifampicin)
- Isoniazid
- Isoniazid/pyrazinamide/rifampicin (isoniazid + pyrazinamide + rifampicin)
- Isoniazid/rifampicin (isoniazid + rifampicin)
- Pyrazinamide
- Rifabutin[note 14]
- Rifampicin
- Rifapentine[note 15]
- Amikacin†
- Bedaquiline†
- Capreomycin†
- Cycloserine†[note 16]
- Delamanid†
- Ethionamide†[note 17]
- Kanamycin†
- Levofloxacin†[note 18]
- Linezolid†
- p-aminosalicylic acid†
- Streptomycin†

2.6.3 Antifungal medicines

- Amphotericin B
- Clotrimazole
- Fluconazole
- Flucytosine
- Griseofulvin
- Nystatin
- Potassium iodide†

2.6.4 Antiviral medicines

2.6.4.1 Antiherpes medicines

- Aciclovir

2.6.4.2 Antiretrovirals

Nucleoside/nucleotide reverse transcriptase inhibitors

- Abacavir (ABC)
- Lamivudine (3TC)
- Stavudine (d4T)
- Tenofovir disoproxil fumarate (TDF)
- Zidovudine (ZDV or AZT)

Non-nucleoside reverse transcriptase inhibitors

- Efavirenz (EGV or EFZ)
- Nevirapine (NVP)

Protease inhibitors

- Atazanavir
- Darunavir
- Lopinavir/ritonavir (LPV/r)
- Ritonavir
- Saquinavir (SQV)

Fixed-dose combinations

- Abacavir/lamivudine (abacavir + lamivudine)
- Efavirenz/emtricitabine/tenofovir[note 19]
- Emtricitabine/tenofovir[note 19]
- Lamivudine/nevirapine/stavudine
- Lamivudine/nevirapine/zidovudine
- Lamivudine/zidovudine

Other antivirals

- Oseltamivir[note 20]
- Ribavirin[note 21]
- Valganciclovir

Antihepatitis medicines

Medicines for hepatitis B

—Nucleoside/Nucleotide reverse transcriptase inhibitors

- Entecavir
- Tenofovir disoproxil fumarate (TDF)

Medicines for hepatitis C

—Nucleotide polymerase inhibitors

- Sofosbuvir

—Protease inhibitors

- Simeprevir

—NS5A inhibitors

- Daclatasvir

—Non-nucleoside polymerase inhibitors

- Dasabuvir

—Other antivirals

- Ribavirin[note 22]
- Pegylated interferon alpha (2a or 2b)‡[note 23]

—Fixed-dose combinations

- Ledipasvir/sofosbuvir
- Ombitasvir/paritaprevir/ritonavir

2.6.5 Antiprotozoal medicines

2.6.5.1 Antiamoebic and antigiardiasis medicines

- Diloxanide
- Metronidazole

2.6.5.2 Antileishmaniasis medicines

- Amphotericin B
- Miltefosine
- Paromomycin
- Sodium stibogluconate or meglumine antimoniate

2.6.5.3 Antimalarial medicines

For curative treatment[edit]

- Amodiaquine[note 24]
- Artemether[note 25]
- Artemether/lumefantrine[note 26]
- Artesunate[note 27]
- Artesunate/amodiaquine[note 28]
- Artesunate/mefloquine
- Chloroquine[note 29]
- Doxycycline[note 30]
- Mefloquine[note 31]
- Primaquine[note 32]
- Quinine[note 33]
- Sulfadoxine/pyrimethamine[note 34]

For prevention

- Chloroquine[note 35]
- Doxycycline
- Mefloquine
- Proguanil[note 36]

2.6.5.4 Antipneumocystosis and antitoxoplasmosis medicines

- Pyrimethamine
- Sulfadiazine
- Sulfamethoxazole/trimethoprim
- Pentamidine†

2.6.5.5 Antitrypanosomal medicines

African trypanosomiasis

Medicines for the treatment of 1st stage African trypanosomiasis

- Pentamidine[note 37]
- Suramin sodium[note 38]

Medicines for the treatment of second stage African trypanosomiasis

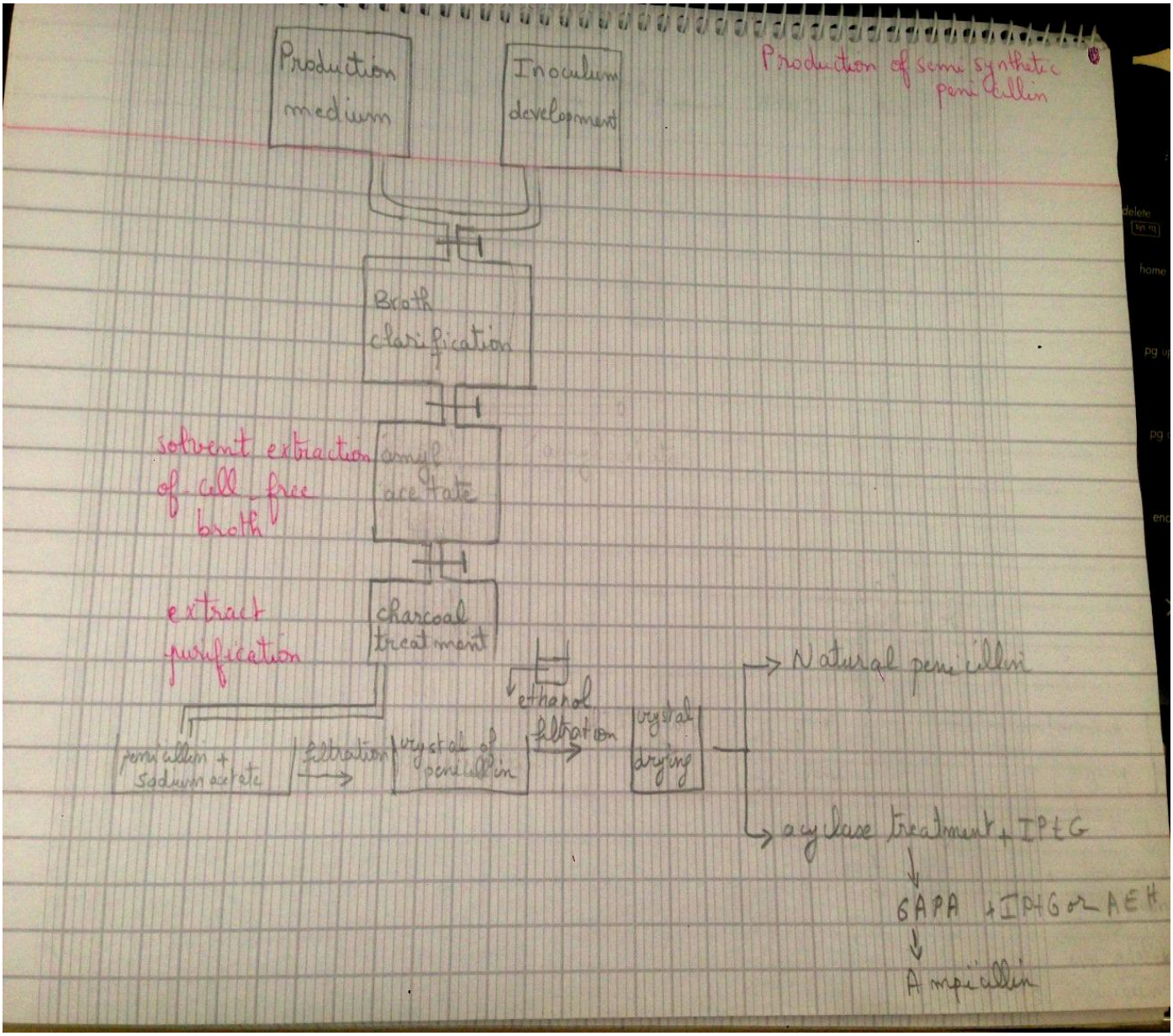
- Eflornithine[note 39]
- Melarsoprol
- Nifurtimox[note 40]

2.6.6 American trypanosomiasis

- Benznidazole
- Nifurtimox

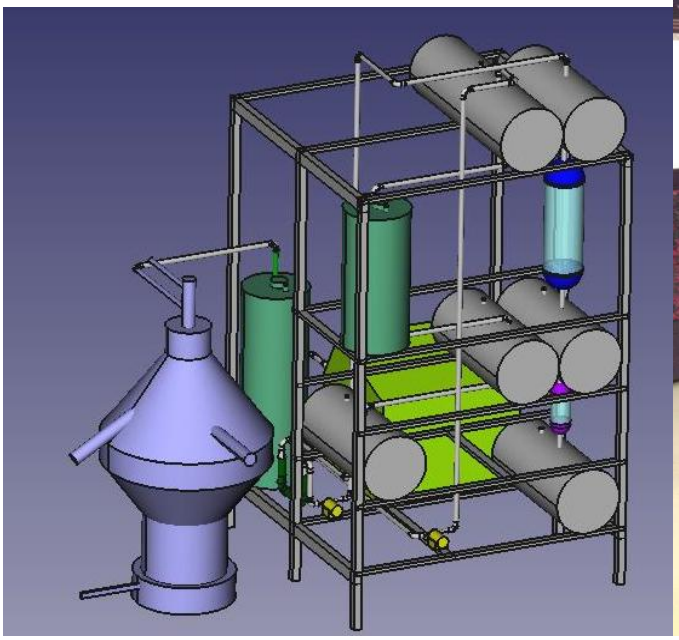
3 Concept

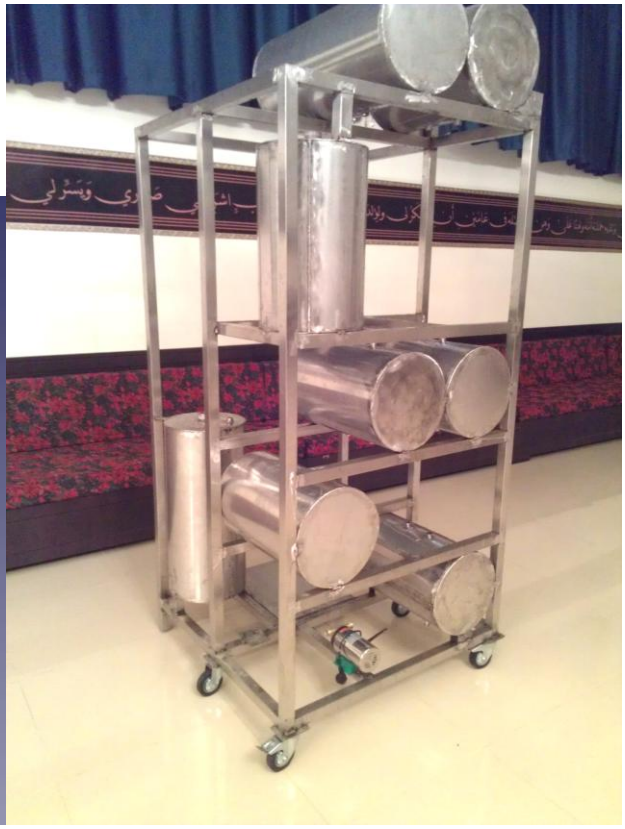
3.1 Flow diagram



3.2 Mechanical structure

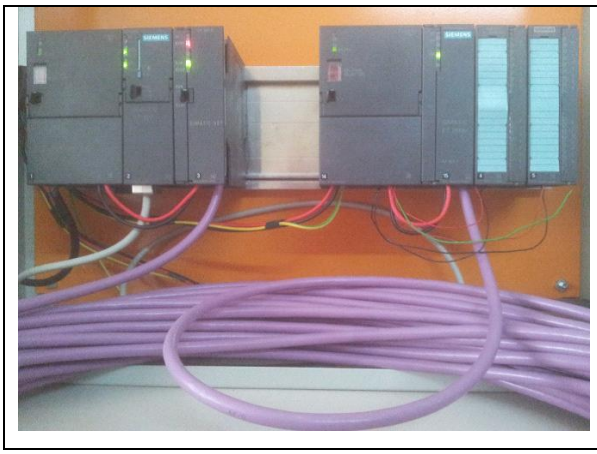
The concept is to install a simplified semi-synthetic penicillin production line based on the already existing mechanical structure (see picture below on the right).

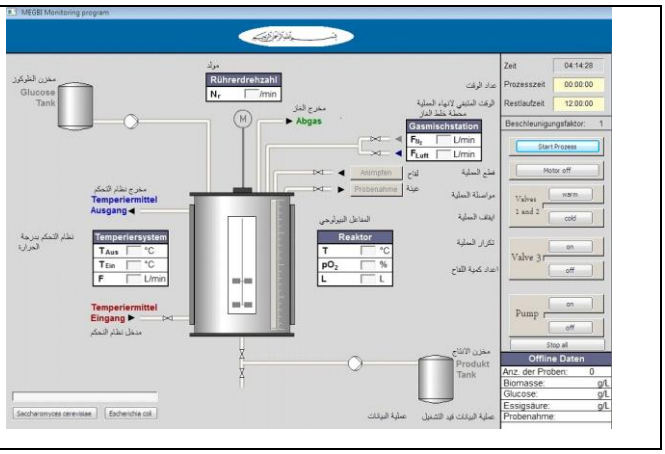


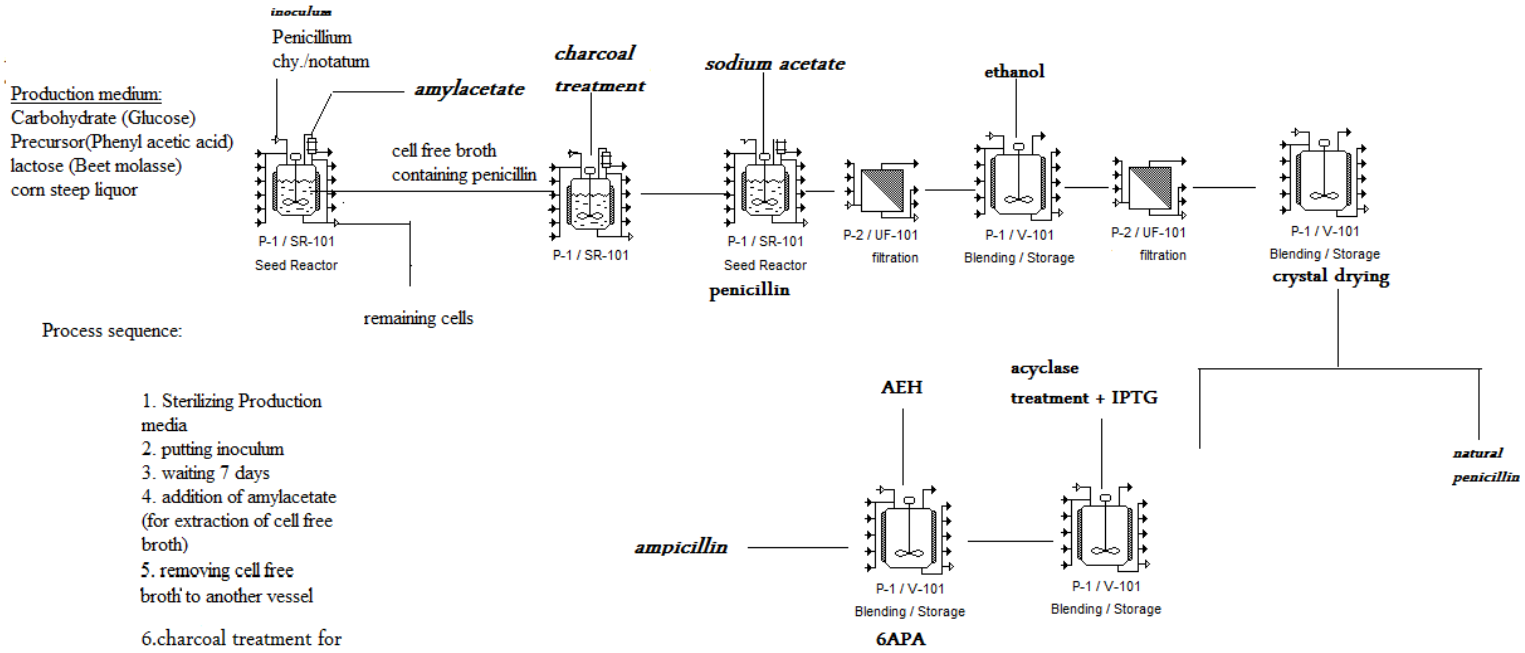


3.3 Automation System

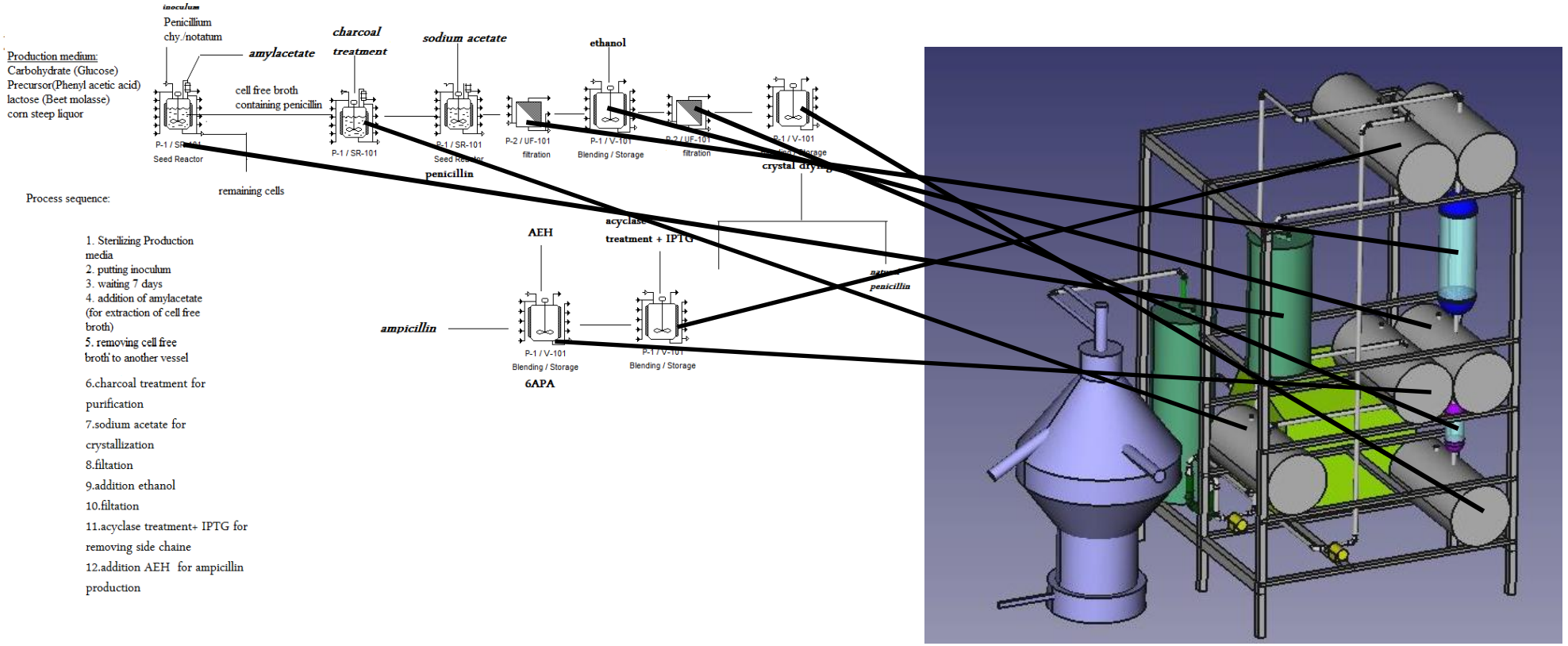
The automation system shall have a C++/phython user interface and a Simatic S7 interface to the sensors/actuators.







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



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
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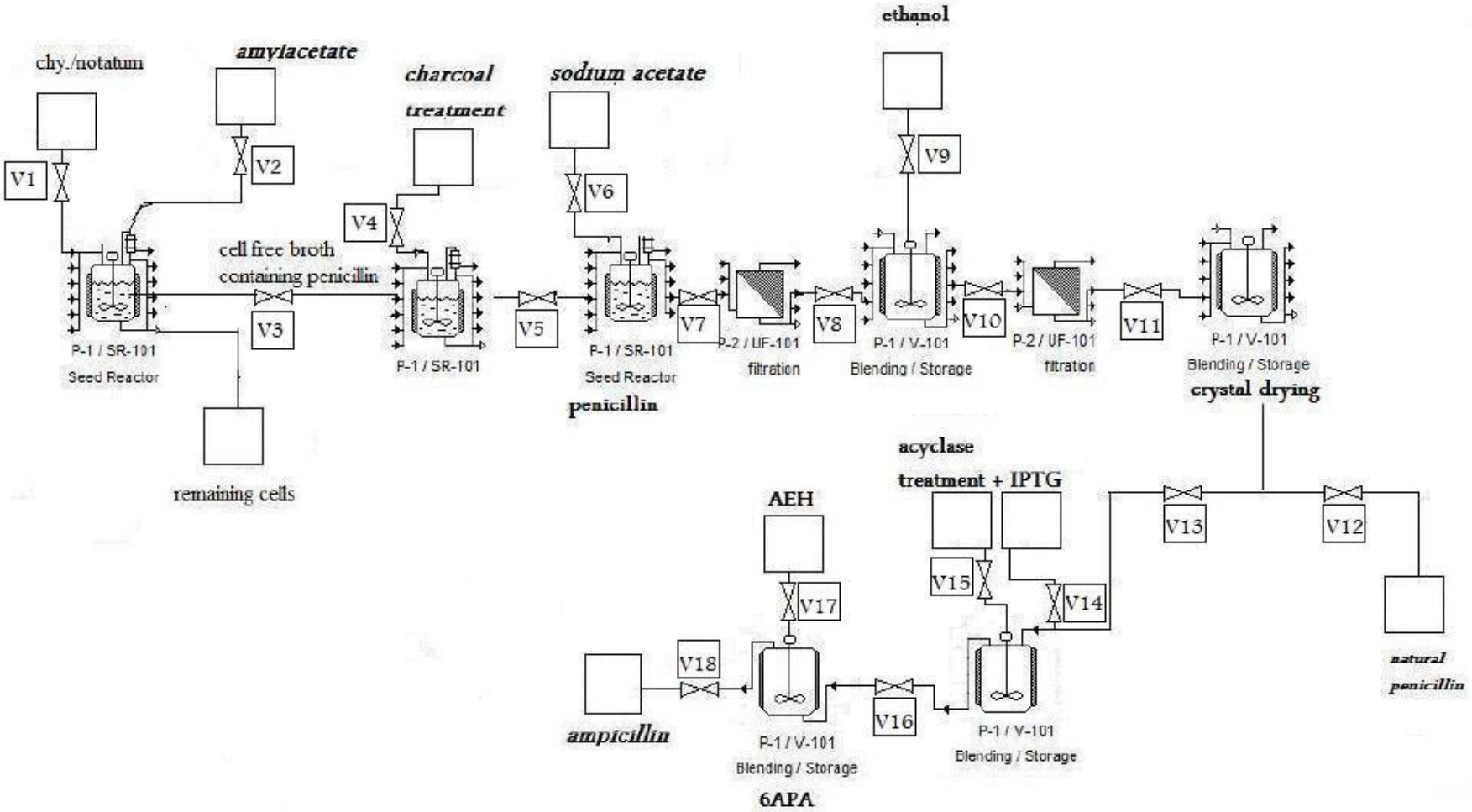

chy./notatum
amylacetate
charcoal
treatment
sodium acetate
ethanol
V1
V2
V4
V6
V9
cell free broth
containing penicillin
V3
V5
V7
V8
V10
V11
P-1 / SR-101
Seed Reactor
P-1 / SR-101
P-1 / SR-101
Seed Reactor
P-2 / UF-101
filtration
P-1 / V-101
Blending / Storage
P-2 / UF-101
filtration
P-1 / V-101
Blending / Storage
penicillin
crystal drying
remaining cells
acyclase
treatment + IPTG
AEH
V13
V12
V15
V14
V17
V18
V16
ampicillin
P-1 / V-101
Blending / Storage
P-1 / V-101
Blending / Storage
6APA
natural
penicillin

4.1.1 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.

4.1.2 Filtration of sodium acetate and after adding of ethanol

...

4.1.3 Package 1: vessels for storing and mixing

Placing of storages based on flowdiagram

Costs: 1500$

4.1.4 Package 2: Chromatographic Columns

Costs: 1400$

4.1.5 Package 3: Pumps & Valves

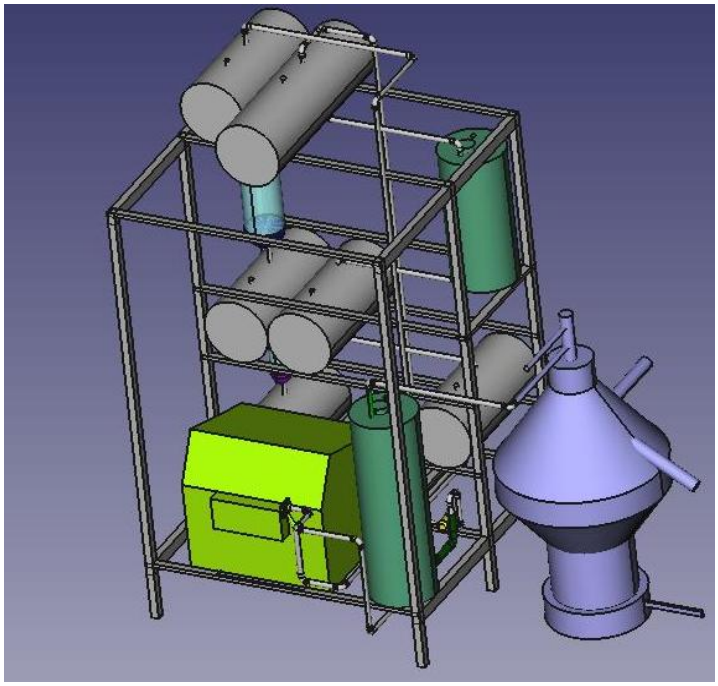
Costs: 3500$

4.1.6 Package 4: Piping

Costs: 1500$

4.2 Manufactored 24.12.-30.12.2015 (based on minimal system)

4.2.1 Design



4.3 Simpliyfied System

4.4 Still missing (to be developed/manufactored/buyed in 2017 insha Allah)

- automatic control valves
- piping
- ~~disc stack cetrifuge, homoginizer (for manufactoring disc stack centrifuge and homoginizer a CNC machine is needed)~~
- connecting to automation system

5 Determination of sensibility of penicillin production

Based on practical work of Maryam Khodor (originally planned as master thesis)

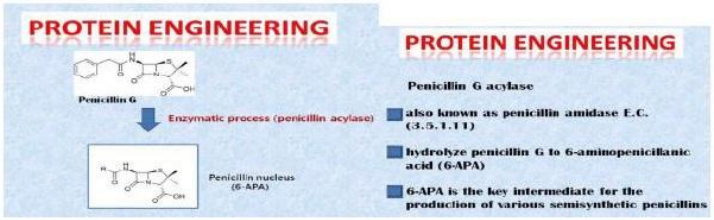
5.1 Master Thesis Task

بسم الله الرحمن الرحيم

Ras Nhache/Batroun - Tripoli, 5th April 2016

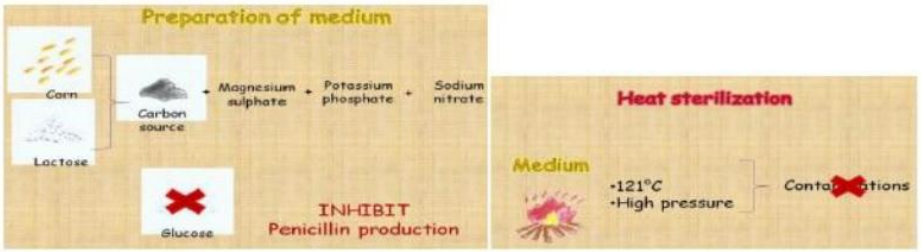
MEGBI Antibiotics Pilot Plant Process:

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

5.2 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 extract, 2.0.	Standard fermentation media :lactose, 30 (in control only); glucose, 10; ammonium acetate, 3.5;ammonium lactate, 6.0; KH2PO4, 6.0; MgSO4	Media I-III

					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 (CSL), 5.0; beef extract, 0.075; peptone, 0.125;		Primers gene: penDE, phl		Lard oil 3% octadecano l : antifoam agent

		(NH4)2SO4, 4.0; KH2PO4, 3.0; ZnSO4.7H2O, 0.01; MgSO4.7H2O, 2.3.				
				Promoter : pCBC Selrction marker : acetamidase		

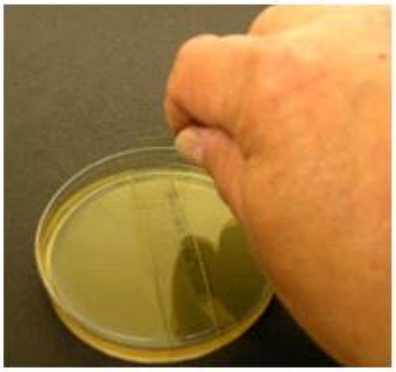
5.3 Methods

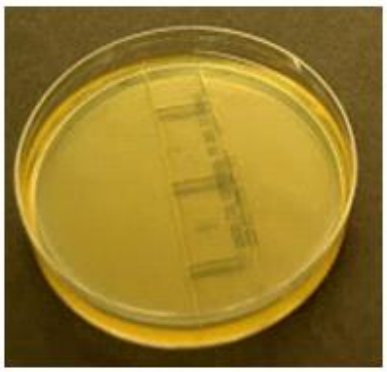
5.3.1 Slide culture method

- It used in the study and identification of an unknown fungal isolate .
- ***Steps:***

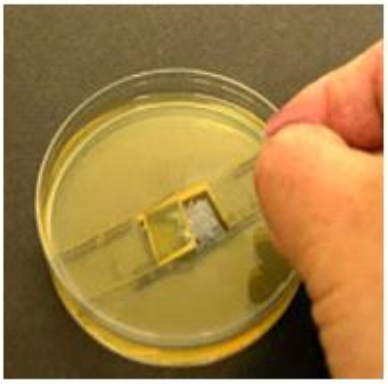
- getting a plate of fungal media (Sabouraud's agar)
- cutting the agar with a sterile scalpel .
- plunge or drag the edge of a cover slip into the agar surface .
- cutting out small blocks of agar (1/2 to 3/4 of an inch square .

Using a glass cover slip as a knife , sliced the agar into squares

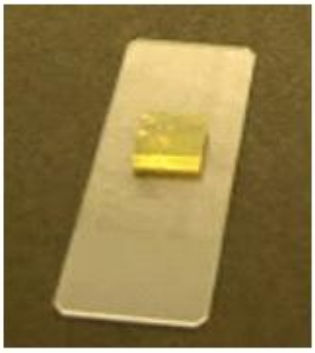




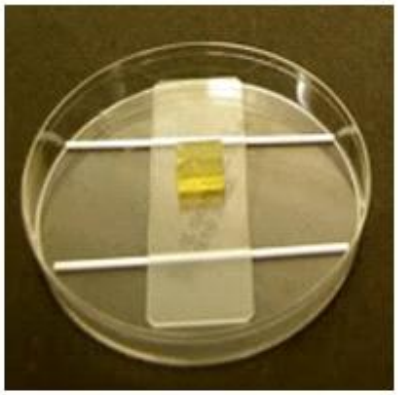
Remove an agar into the plate using the same cutting tool (scalpel, cover slip)



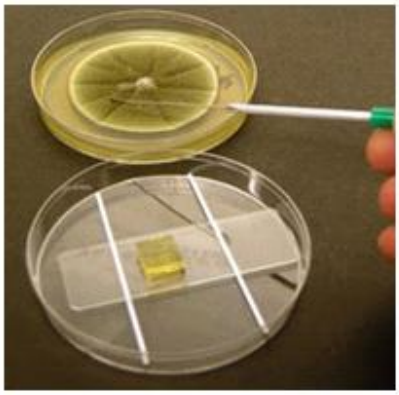
Place the agar block onto a clean glass microscope slide



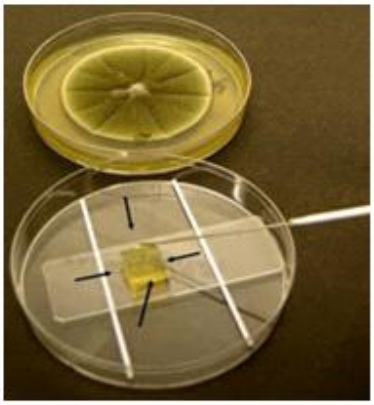
The slide can then be placed in a clean petrie dish which will prevent contamination and preserve moisture during incubation



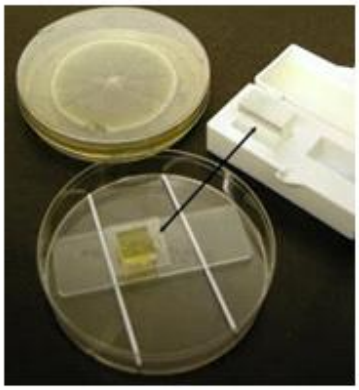
Using a sterile instrument (loop, needle) transfer some of the fungus from the specimen being cultured to each of the four sides of the agar block



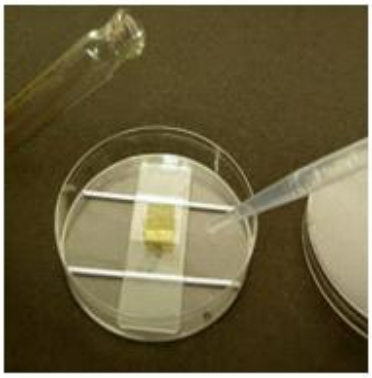
Transfer the fungus to the agar block's sides.



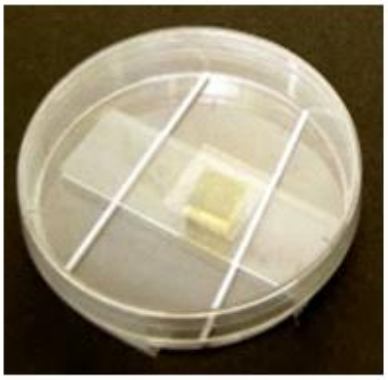
After inoculation place a clean cover slip on the surface of the agar block



-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 .

5.4 Time Plan

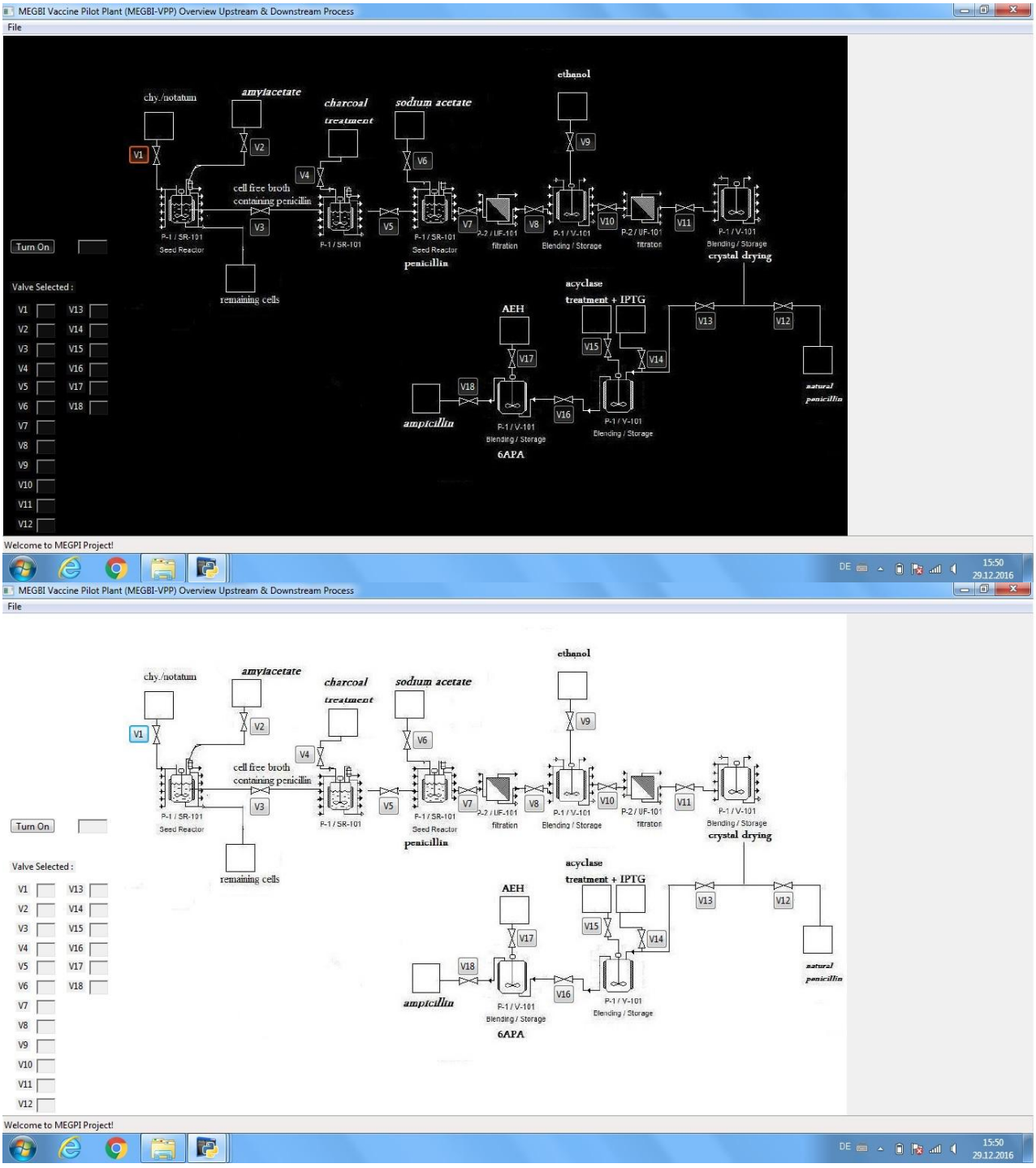
Name	Period	Begning date	End date
Culture and incubation	7 days	26 April	3 may
Identification / diagnosis	3 days	3 may	5 may
Purification of seed culture	7 days	6 may	13 may
Re identification	3 days	13 may	15 may
Production of penicillin	13 days (300h)	16 may	29 may
Extraction			
Sensitivity			

5.5 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.

6 Process Control System

6.1 Graphical User Interface



05121621uhr_MEGBI
-APP_automation.py

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