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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مركز أبحاث الشرق الأوسط للجينات والتقنية البيولوجية

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المضمون

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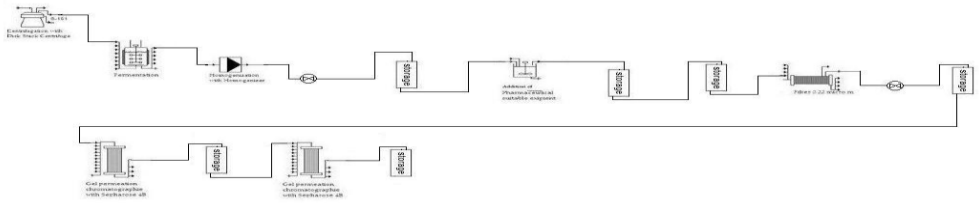
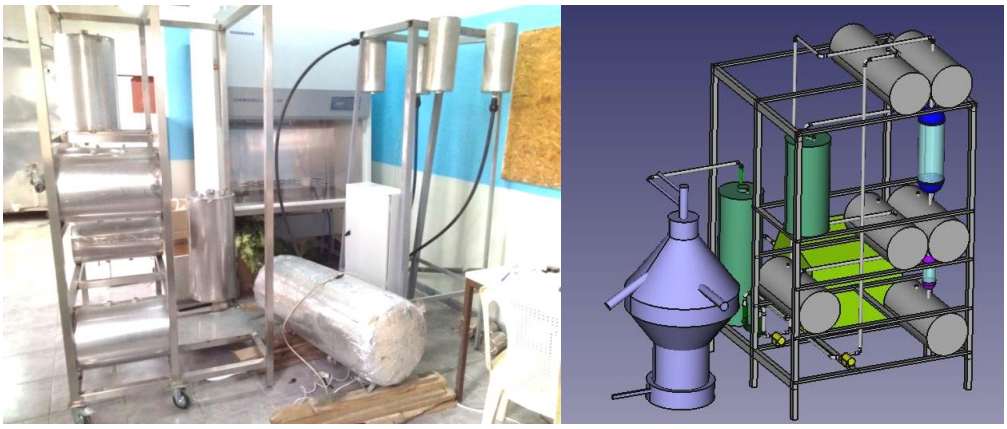
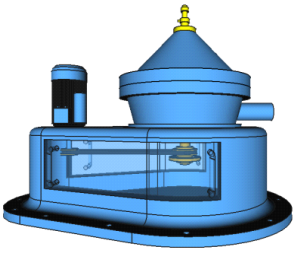
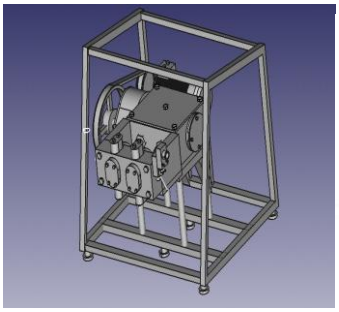
Project Status at beginning of this project phase









- Automation system, - Mechanical parts of minimal USP-DSP manufactored

1 Project Management / إدارة المشروع

1.1 Project phase goal / هدف العمل/

~~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.**

1.3 Time Schedule / الزمني/الجدول

Nov/Dec 14: Financement and Concept Phase

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

	Planned	Staff
Manufactoring a low cost version of MEGBI-VPP (	16.3.-30.7.15	

2 Basics

2.1 Chemical Engineering Basics

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

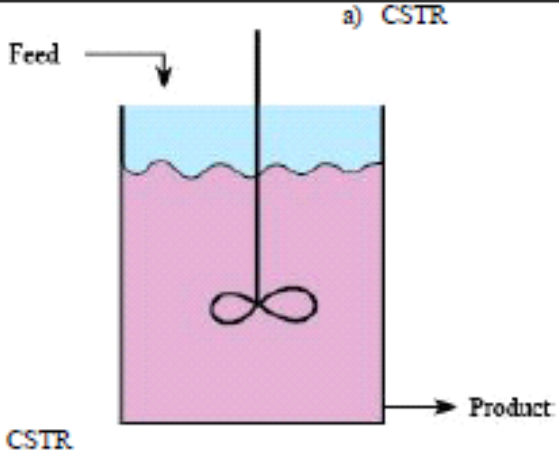
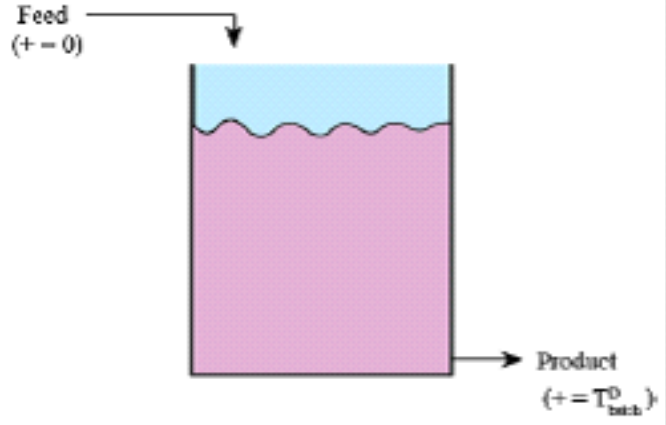
A detailed summary of various prominent unit operations/processes and their functional role in the chemical plant are summarized in Table **0.1** along with suitable figures.

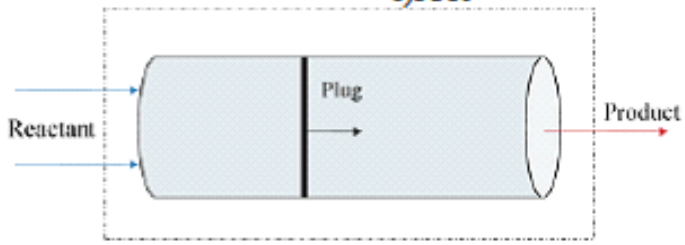
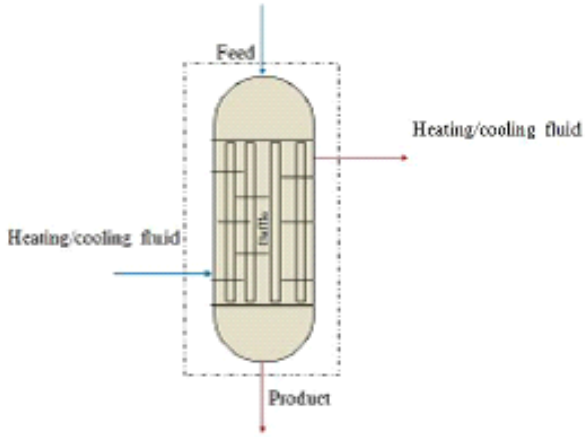
Category	Unit operations/processes	Functional role
fluid operations	a) Centrifugal pump b) Reciprocating pump c) Compressor d) Expander	a) To pressurize liquids and gases. b) To depressurize gases
solid operations	a) Crusher b) Grinder	a) To reduce the size of solids
Solid-fluid separators	a) Cyclone 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.

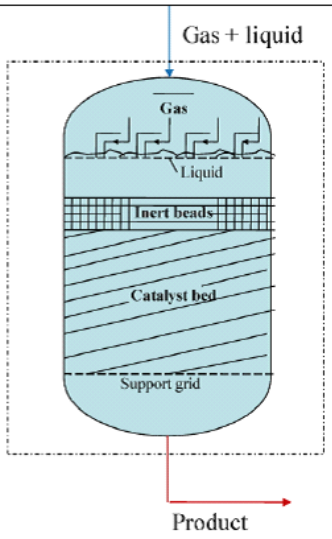
Table 0.1: Important unit operations/unit processes and their functional role in chemical process technology.

References:

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

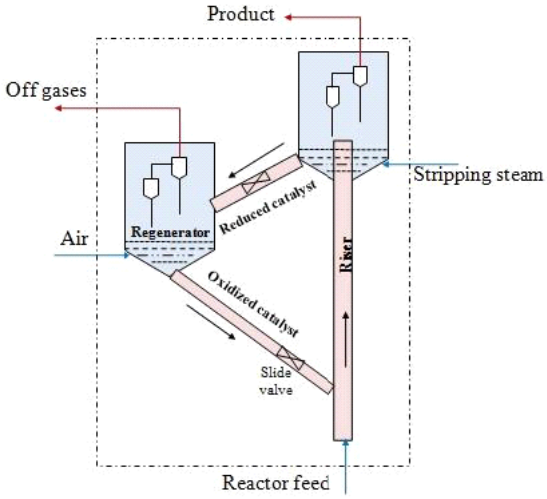
Process Technology	Functional Role
Reactors a) CSTR b) Batch Reactor c) PFR d) Packed bed reactor e) Trickle bed reactor f) Fluidized bed reactor	- Central and most important process technology in process flow sheets - Carry out desired reactive transformations
a) CSTR  CSTR	- Well mixed reaction system 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

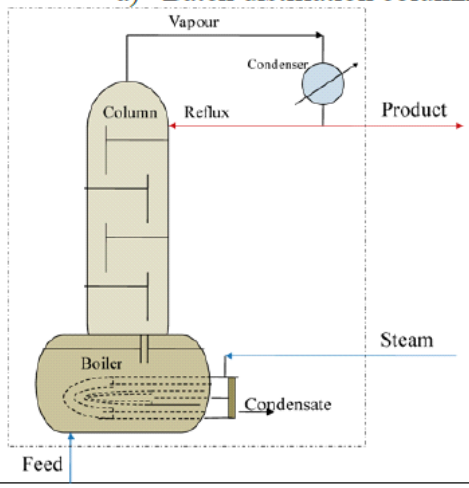


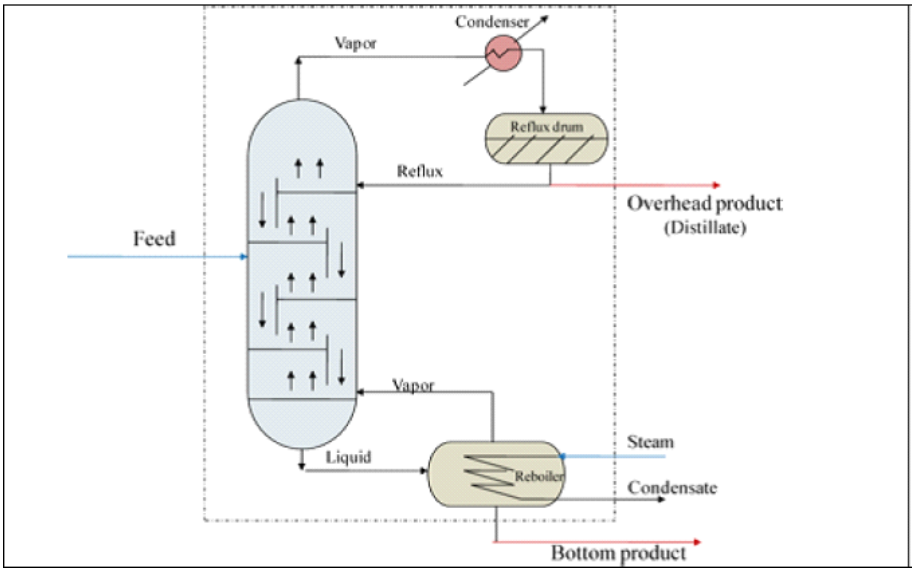
- If the reaction is catalytic, packing acts as a catalyst as well
- Complicated design

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 kwown

Manufaturing

- N2O (nitrous oxide)

Market

medical

Producer in Lebanon

no kwown

Manufaturing

Lachgas (Distickstoffmonoxid; N_2O) wird industriell aus Ammoniumnitrat(NH_4NO_3) hergestellt. Dabei entsteht in einem Zwischenschritt 34Salpetersäure(H_2NO_3) und Ammoniak (NH_4) nach folgender chemischer Formel:

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

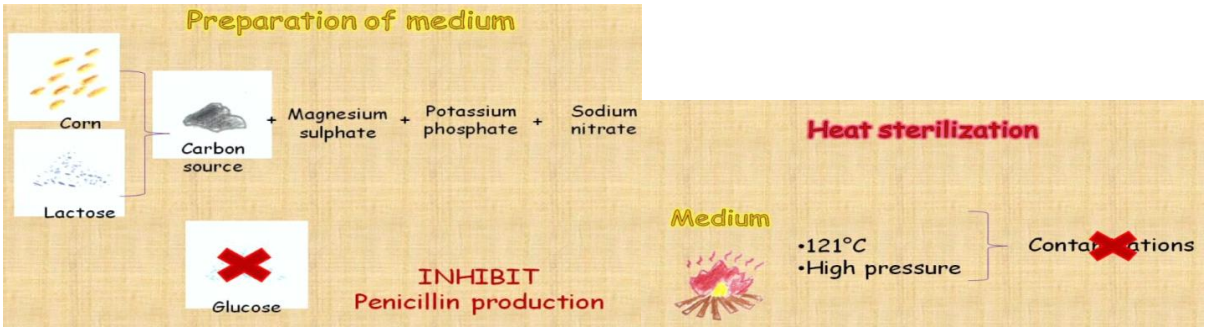
Ammoniumnitrat ist unter Hitzeeinwirkung hochexplosiv. Mehrere großeExplosionsunfälle mit mehreren hundert Todesopfern sind aus der Geschichte bekannt (z.B. Oppau 1921, Toulouse 2001 und zuletzt am 17.04.2013 in West, USA).

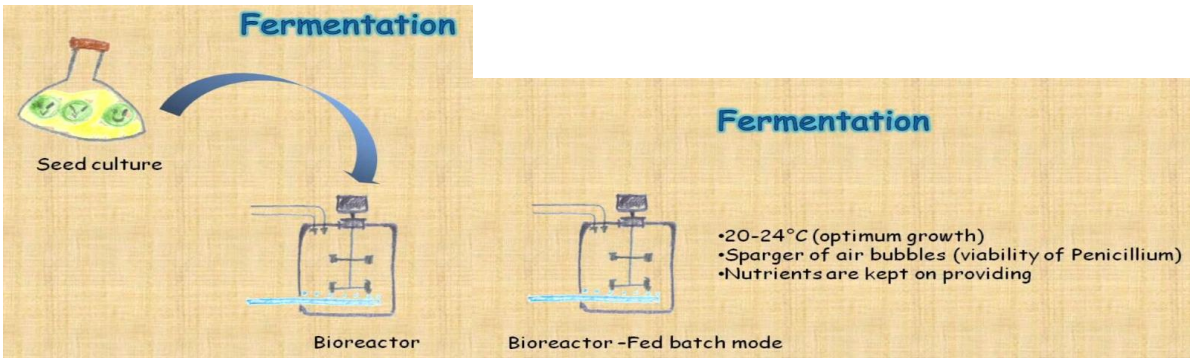
2.3 Antibiotics

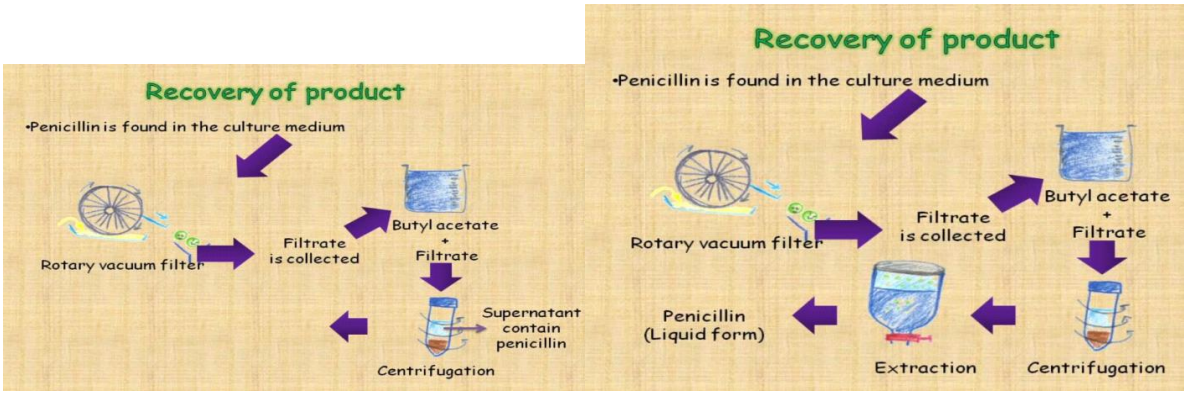
Ampicillin

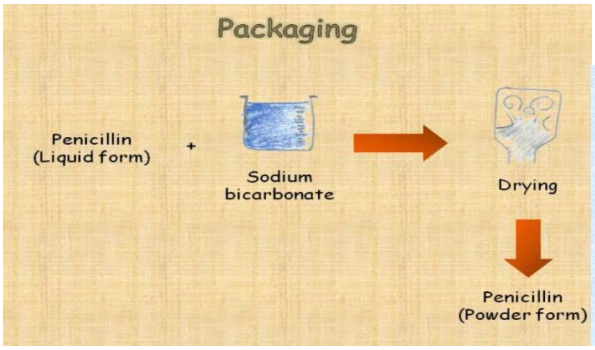
Amoxillin

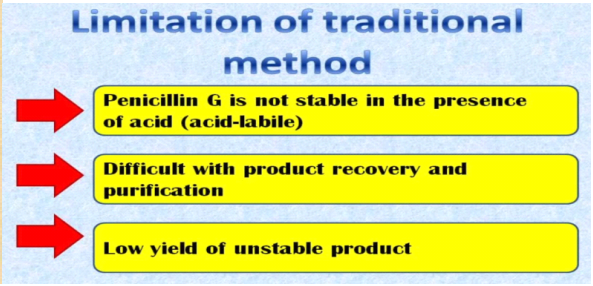
2.3.1 Penicillin in culture



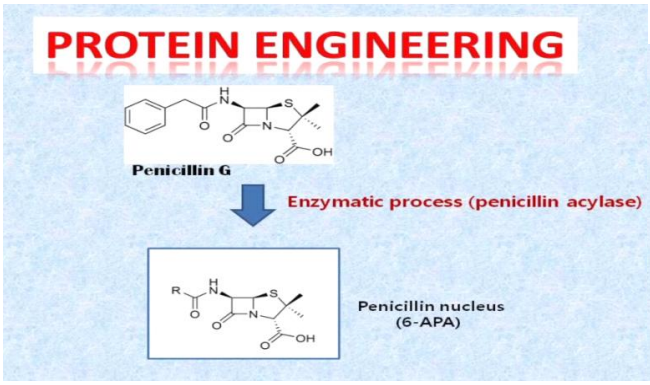


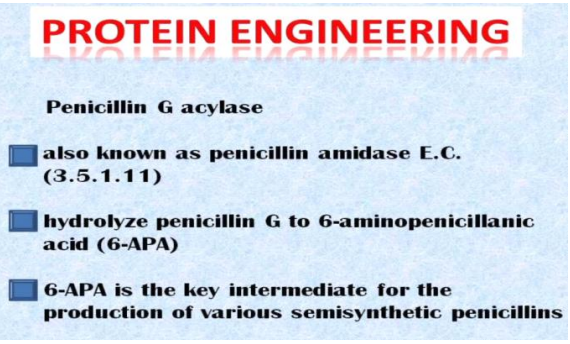


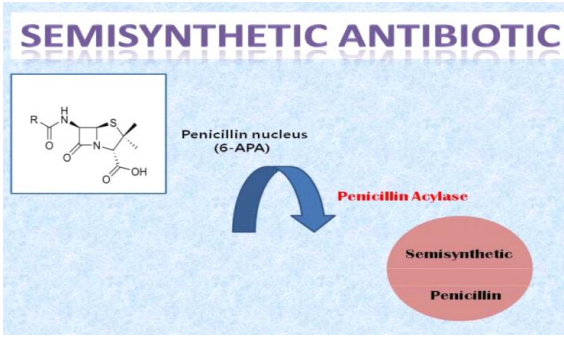


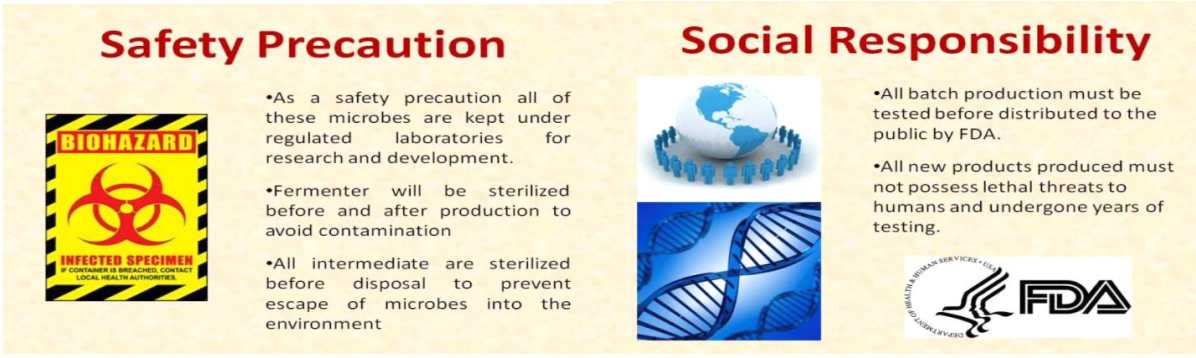


2.3.2 Production of semisynthtic penicillins

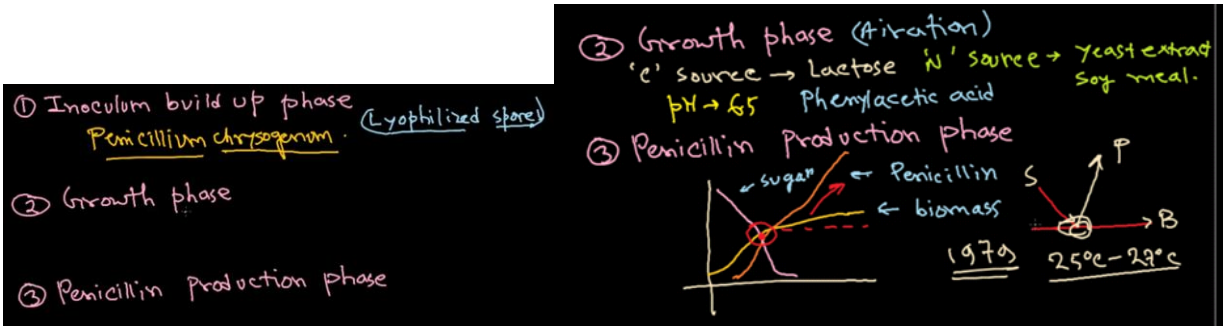


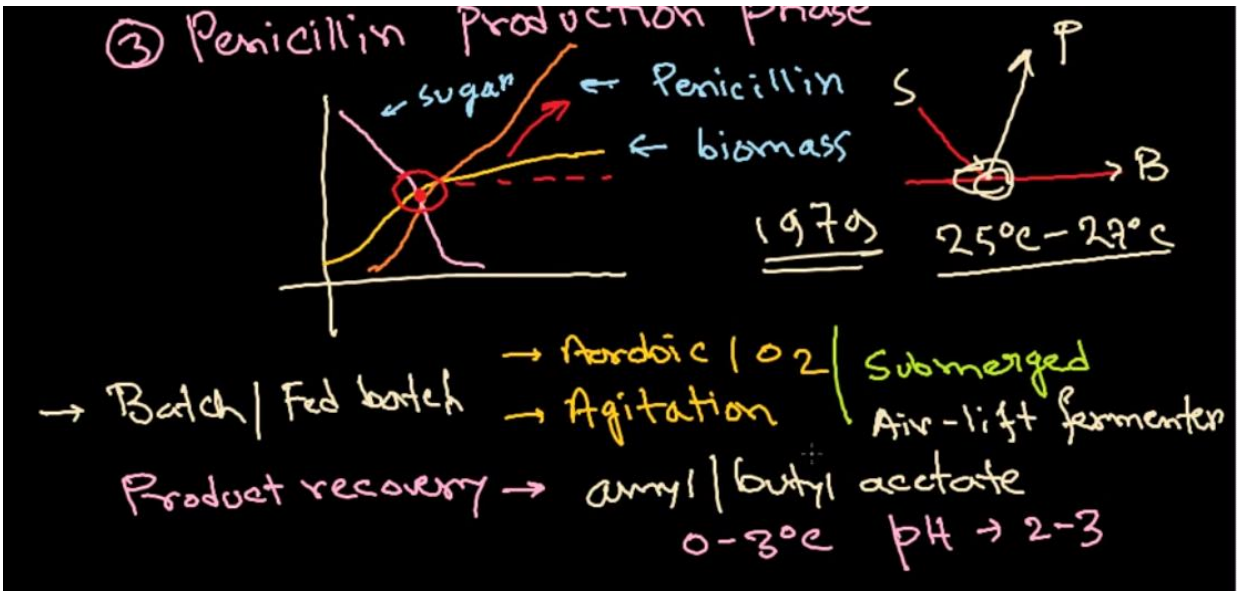






- Industrial Prodution of penicillin





2.3.3 Amoxillin

is a semisynthetic penicillin

2.4 Devices

2.4.1 Rotary vacuum drum

3 Concept

3.1 Mechanical structure

The concept is to install a simplified semi-synthetic penicillin production line.

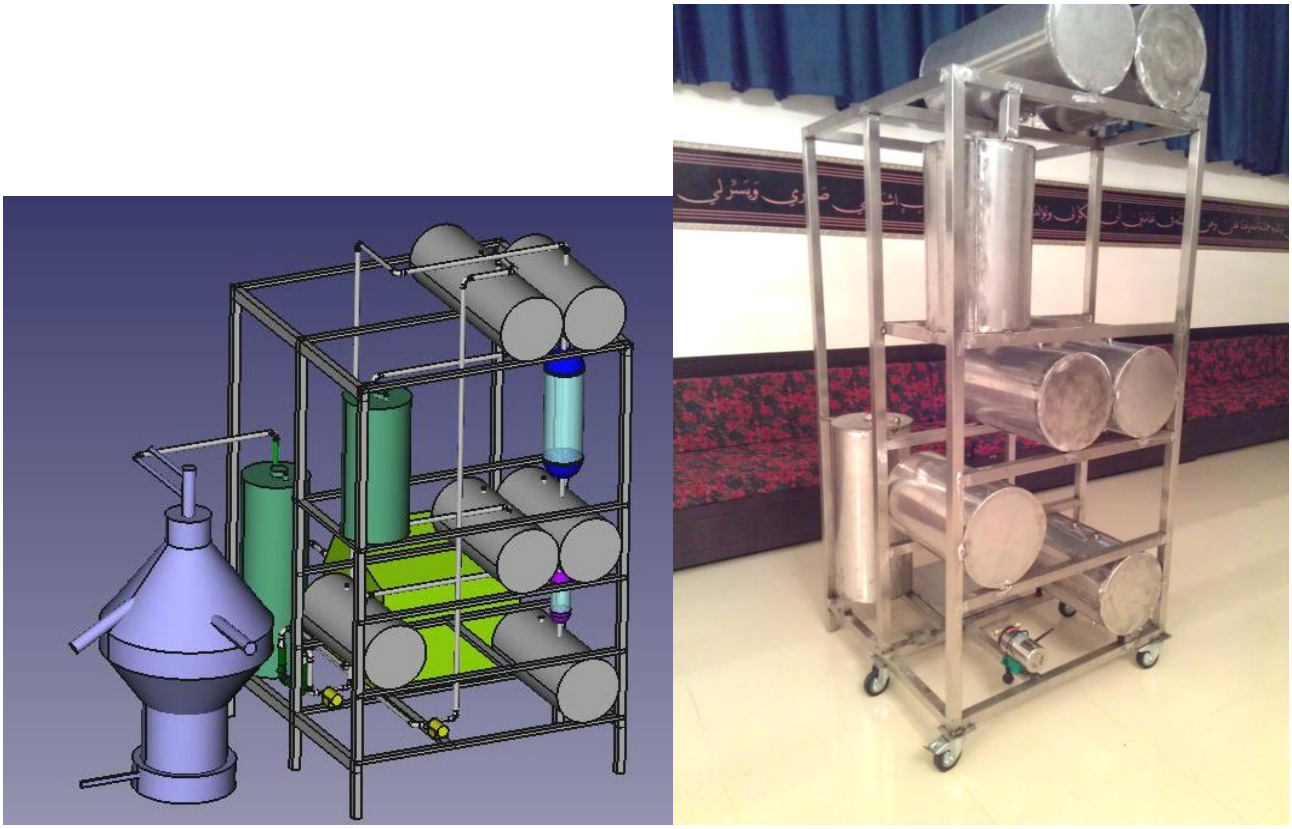
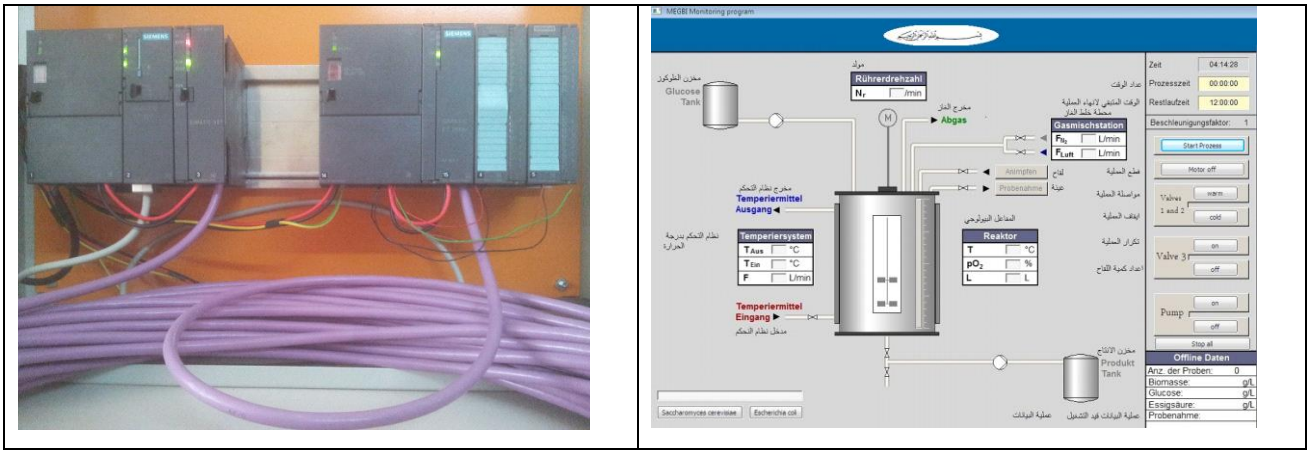


Fig.: Solaris downstream line. The MEGBI-VPP downstream line shall be similar to this.

3.2 Automation System

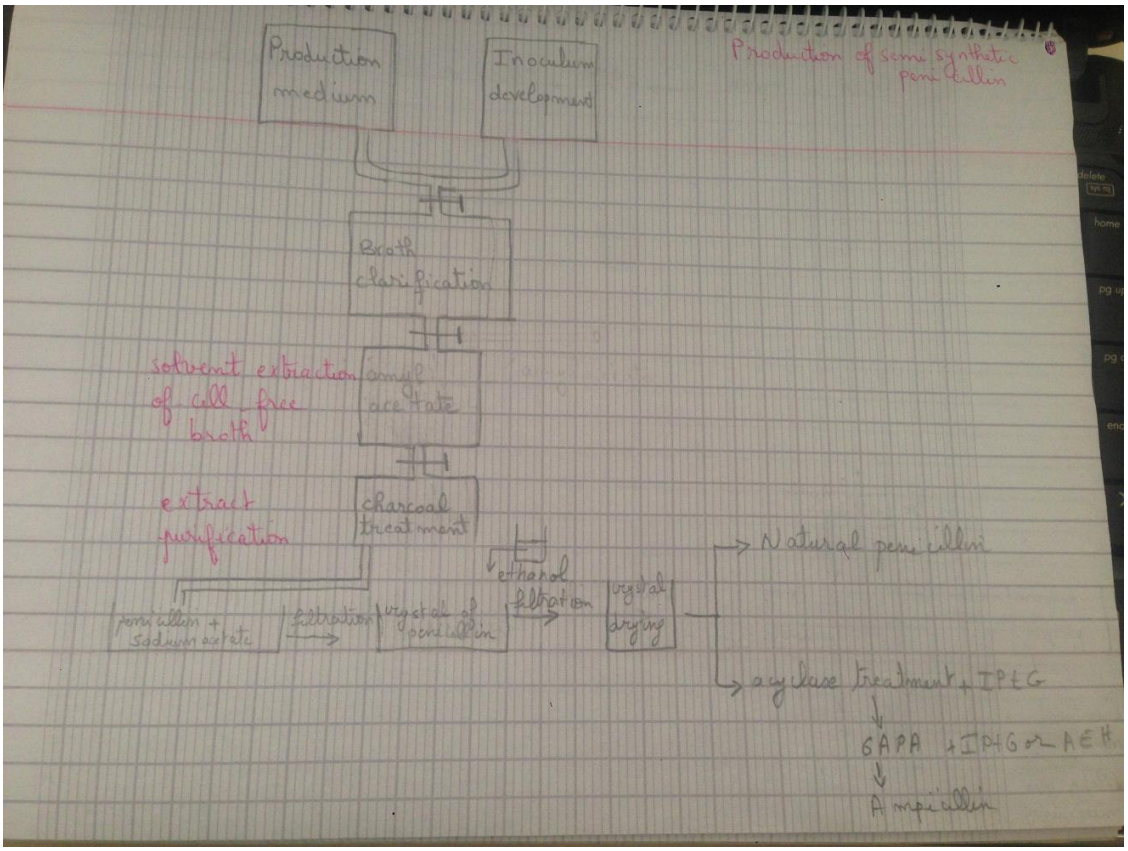
The automation system shall have a C++/phython user interface and a Simatic S7 interface to the sensors/actuators.

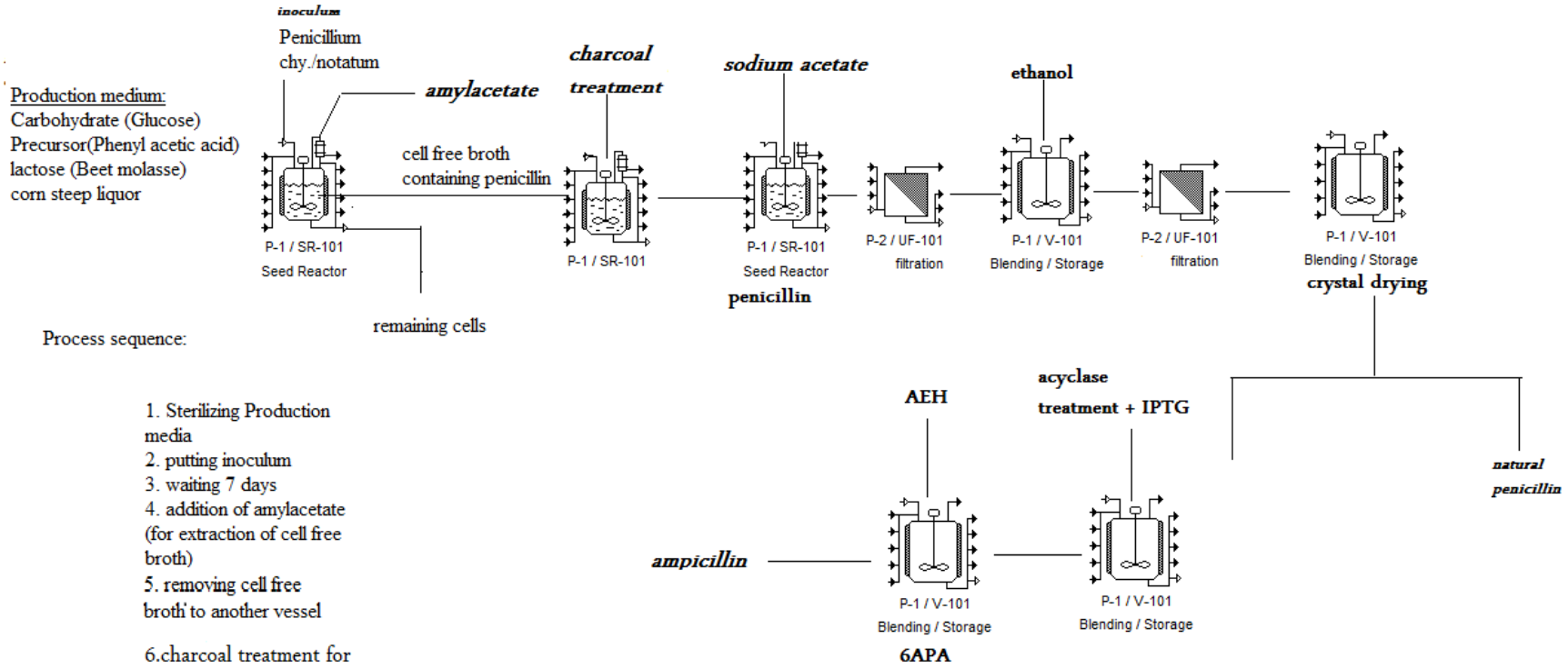


4 System Design

4.1 Overview of MEGBI-APP

4.1.1 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

4.1.2 Package 1: vessels for storing and mixing

Placing of storages based on flowdiagram

Costs: 1500$

4.1.3 Package 2: Chromatographic Columns, Disc Stack Centrifuge, Homogenizer

Costs: 1400$

4.1.4 Package 3: Pumps & Valves

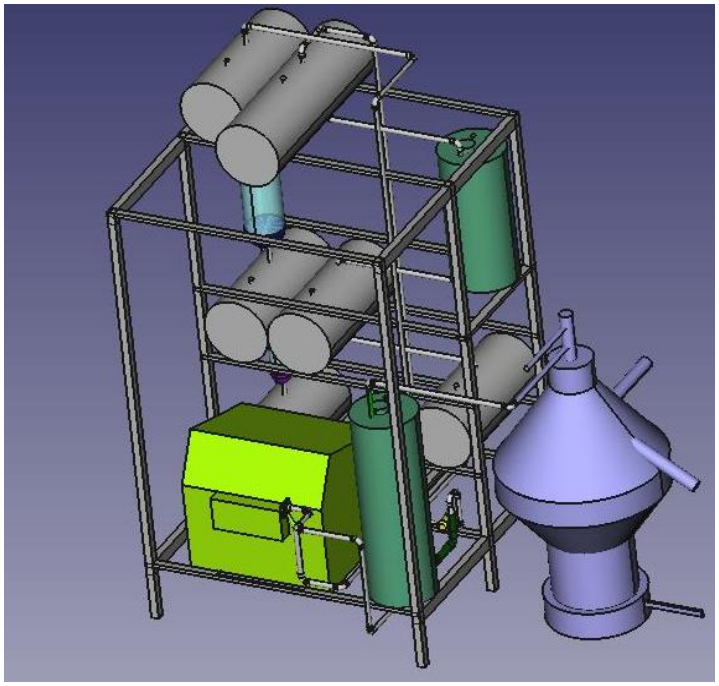
Costs: 3500$

4.1.5 Package 4: Piping

Costs: 1500$

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

4.2.1 Design



4.3 Still missing (to be manufactored/buyed in 2016 insha Allah)

piping

For manufactoring disc stack centrifuge and homoginizer a CNC machine is needed.

4.3.1 References / مراجع

[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

[4] Akademie für Lehrlingsausbildung, Projektarbeit für den Weiterbildungspass, Stufe 2, CNC-Maschinen Grundlagen, VEM-Ausbildungsbetrieb Doppelmayr

[5] NPTEL – Chemical – Chemical Technology II, Joint initiative of IITs and IISc,

5 Determination of sensibility of penicillin

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

5.1 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	YGG: KCl, 10.0; glucose, 20.0; yeast nitrogen base (YNB), 6.66; citric acid,	Standard fermentation media :lactose, 30 (in control only); glucose, 10; ammonium acetate,	Media I-III

			ml spore	1.5;K2HPO4, 6.0; and yeast extract, 2.0.	3.5;ammonium lactate, 6.0; 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		

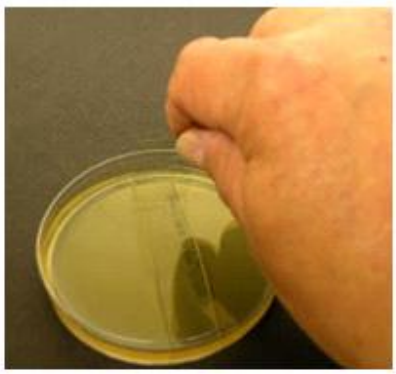
5.2 Methods

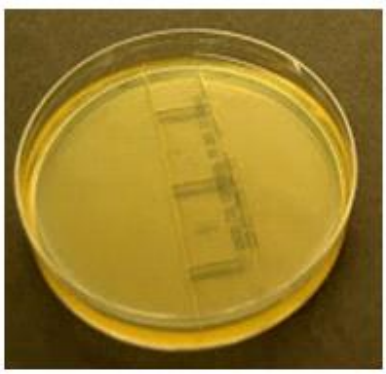
5.2.1 Slide culture method

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

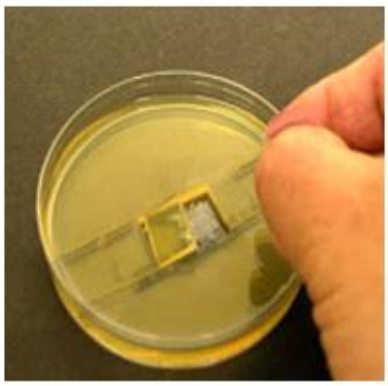
- getting a plate of fungal media (Sabouraud's agar)
- cutting the agar with a sterile scalpel .
- plunge or drag the edge of a cover slip into the agar surface .
- cutting out small blocks of agar (1/2 to 3/4 of an inch square .

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

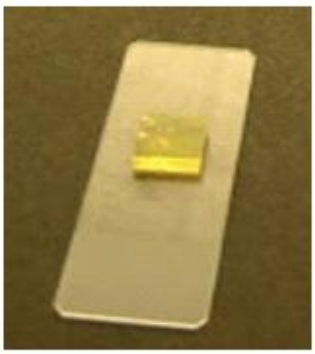




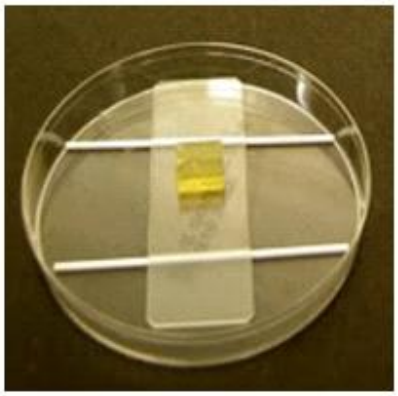
Remove an agar into the plate using the same cutting tool (scalpel, cover slip)



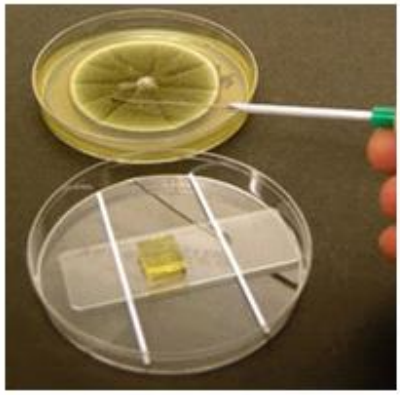
Place the agar block onto a clean glass microscope slide



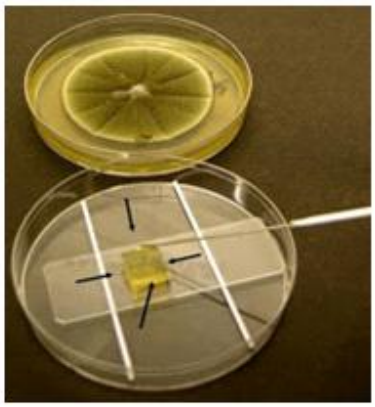
The slide can then be placed in a clean petrie dish which will prevent contamination and preserve moisture during incubation



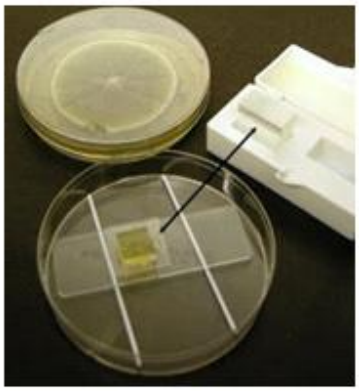
Using a sterile instrument (loop, needle) transfer some of the fungus from the specimen being cultured to each of the four sides of the agar block



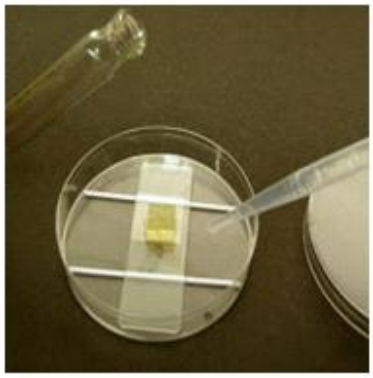
Transfer the fungus to the agar block's sides .



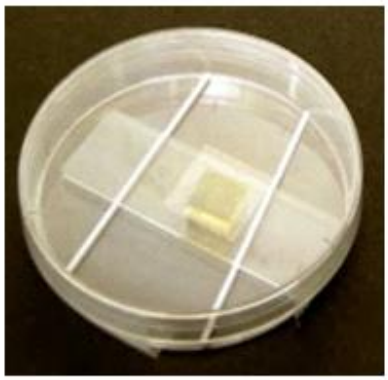
After inoculation place a clean cover slip on the surface of the agar block



-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 .

5.3 Basics

5.3.1 Definition:

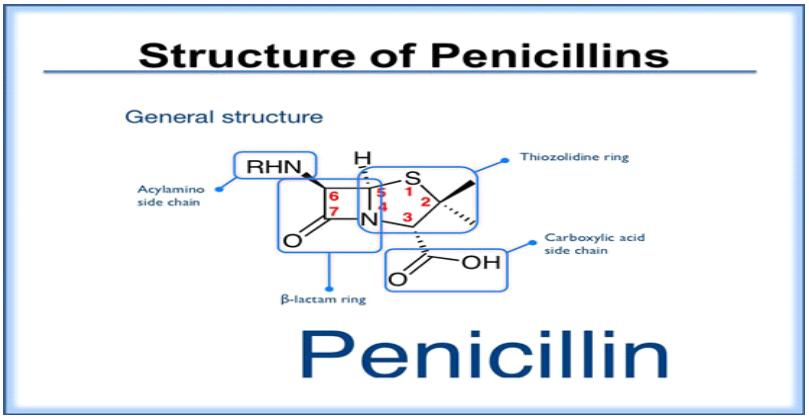
Antibiotics are a type of antimicrobial drug. They are one of the secondary metabolites produced by some fungi and bacteria.

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.

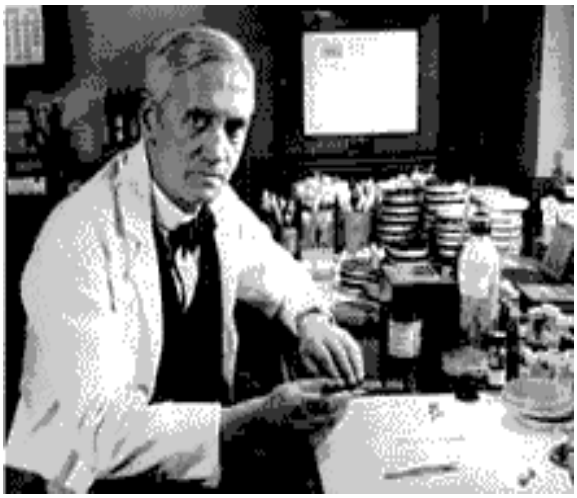
5.3.2 The structure of the penicillins:

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.



5.3.3 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.*

5.3.4 Strains of penicillium:

In the early days of penicillin production (1928)

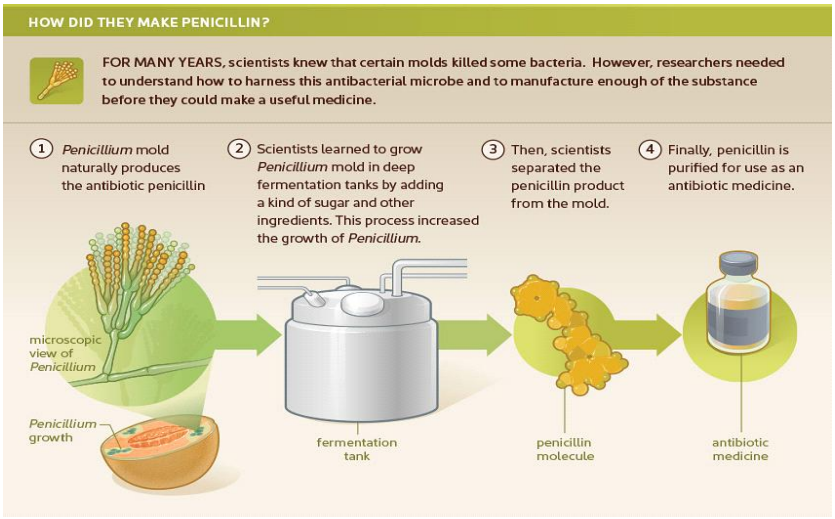
Penicillium Notatum strain was employed. After a few years, a new strain of Penicillium Chrysogenum discovered in 1943 was employed for penicillin production.

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*.

5.3.5 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 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.

5.3.6 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.

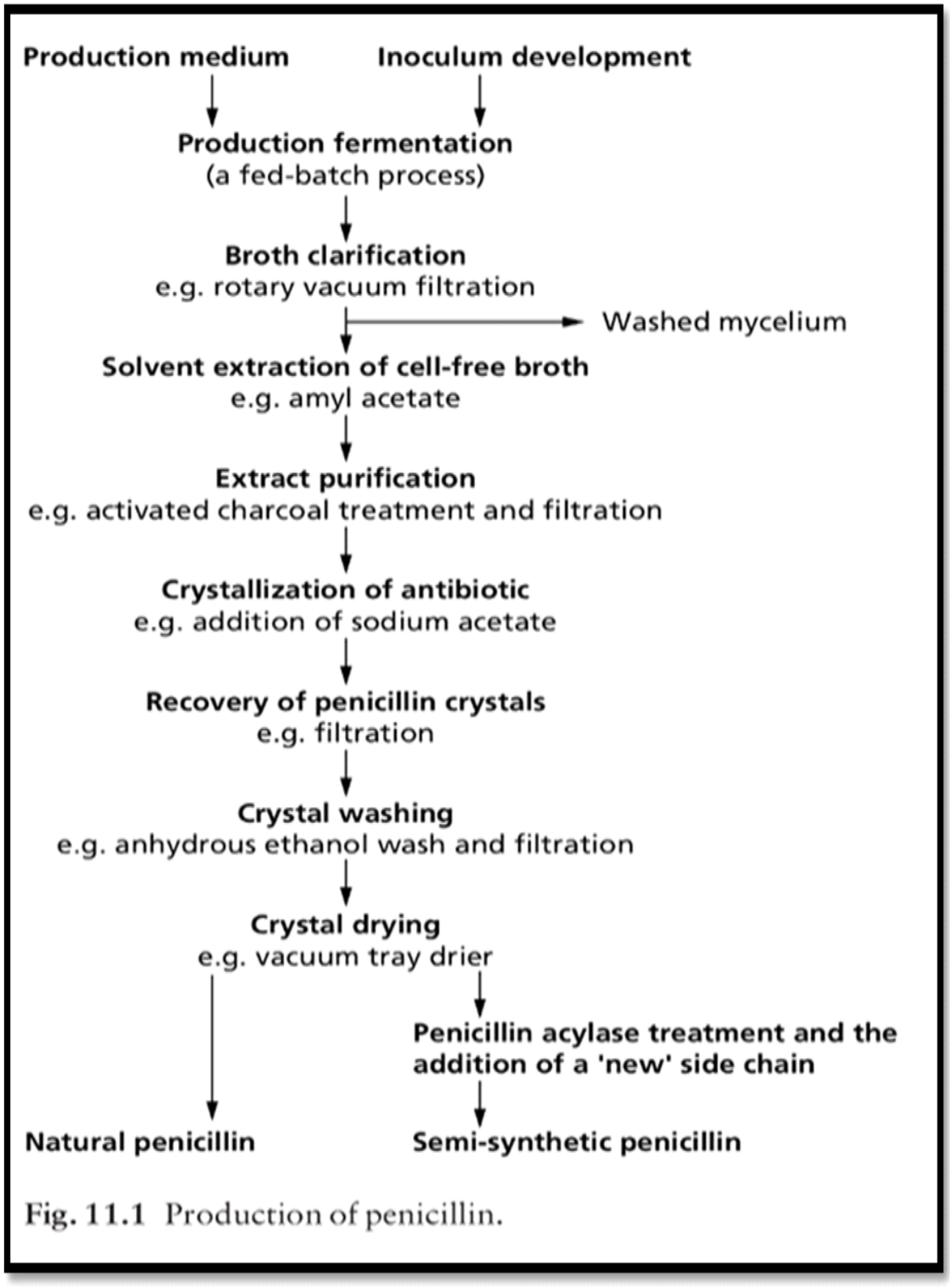


Fig. 11.1 Production of penicillin.

5.3.7 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.

5.3.8 Mechanism of action:

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

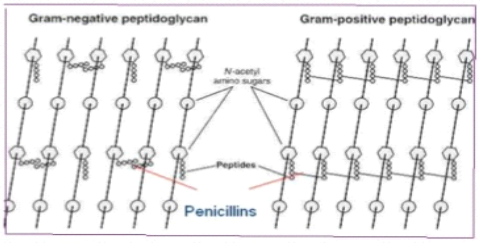
All penicillins work in the same way:

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.

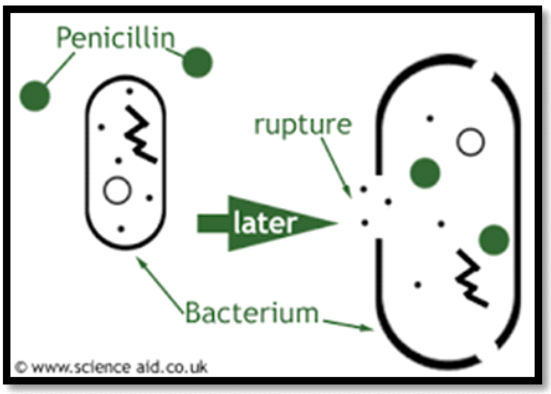
Since human cells do not contain a cell wall, penicillin treatment results in bacterial cell death without affecting human cells.

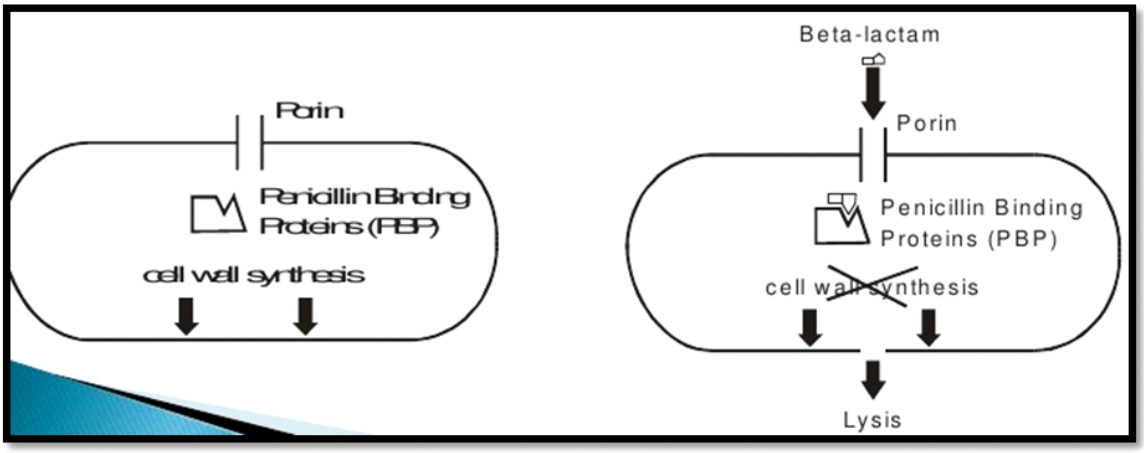
Gram positive bacteria have thick cell walls containing high levels of peptidoglycan, while gram negative bacteria are characterized by thinner cell walls with low levels of peptidoglycan. The cell wall of gram negative bacteria is surrounded by a lipopolysaccharide (LPS) layer than prevents antibiotic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

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.





5.3.9 Resistance to beta lactams :

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

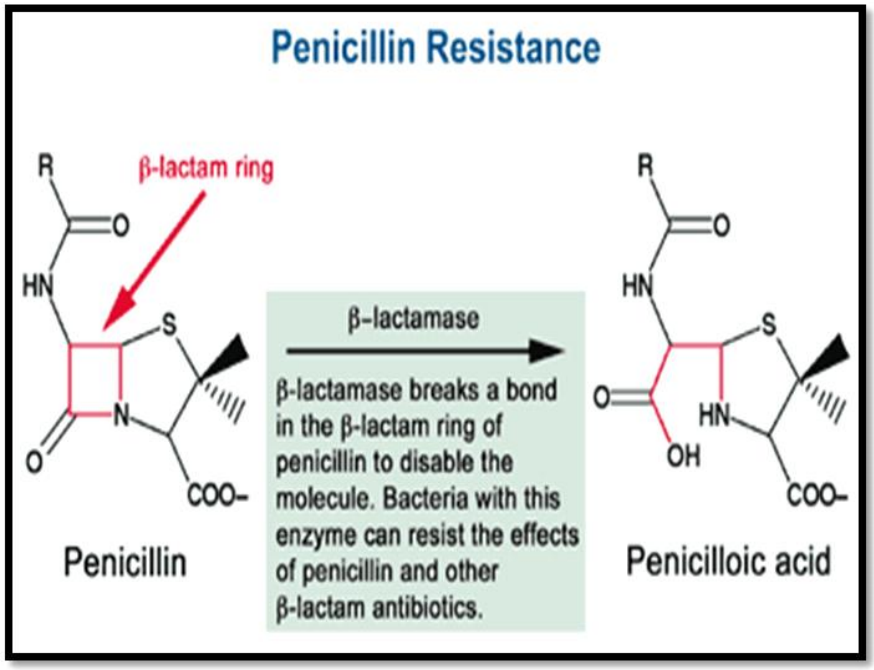
Bacteria are constantly finding ways to counteract antibiotics, one of the most important bacterial defense mechanisms is the production of enzymes B lactamase.

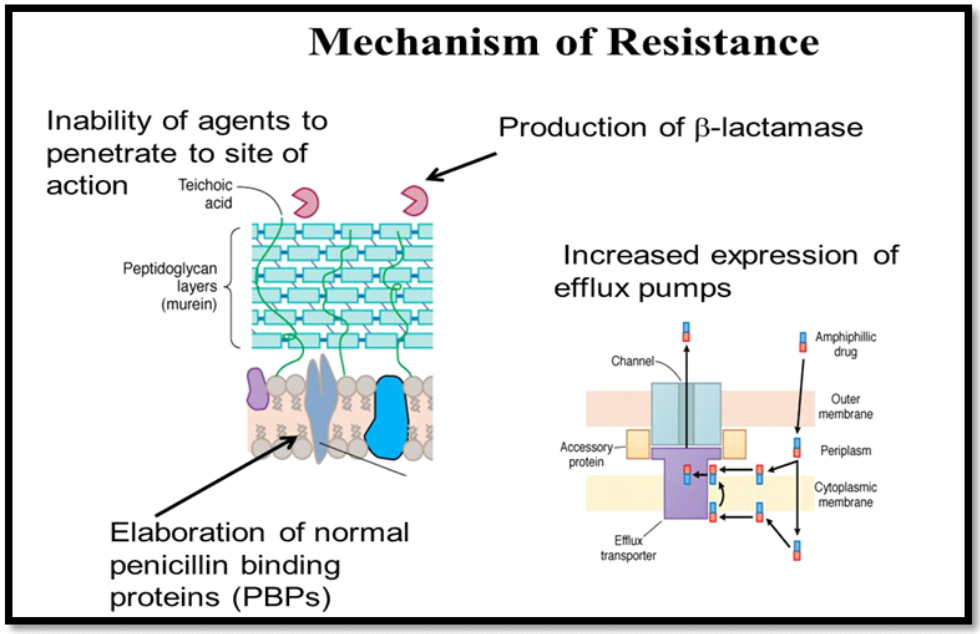
Organisms that produce B lactamase are resistant to penicillin by hydrolyses beta-lactam ring.

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.





5.3.10 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

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 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.