

**SEMINAR
ON
UPSTREAM & DOWNSTREAM PROCESSING OF
ANTIBIOTICS, HORMONES, VACCINES.**

**SUBMITTED
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TOPIC–UPSTREAM & DOWNSTREAM PROCESSING OF ANTIBIOTICS,HORMONES,VACCINES

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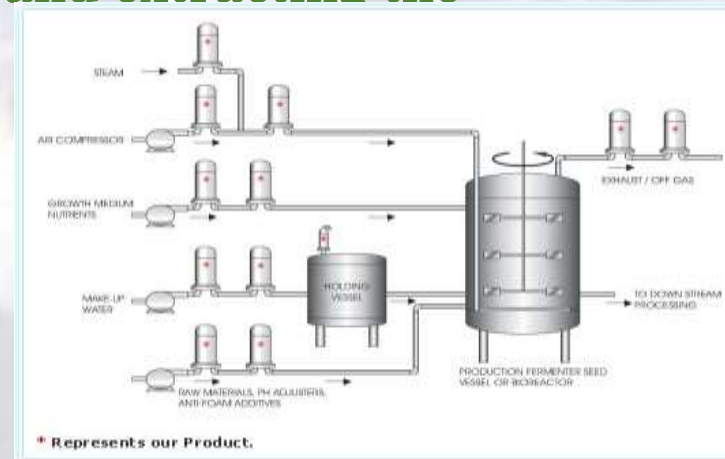
- **Definitions**
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- **Up stream process**
- **Fermentation**
- **Down stream process**
- **Penicillin**
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What do you mean by upstream & downstream process?

❖ **The upstream processing in biotechnology involves identifying and extracting the raw materials. This forms the initial process of fermentation.**

➤ **Upstream process**-it deals with the:

- Inoculum preparation which includes screening or microorganisms and selection of suitable strain and genetic modification of the organism if needed.
- Preparation of culture media having suitable growth parameters at laboratory scale
- Scale up of the entire process.
- Inoculation.



Downstream process-

- ❖ When the products are subjected to a series of processes including separation and purification which are collectively known as **Downstream processing**.
- ❖ It is also known as **product recovery**.
- ❖ **Materials –upstream-finished products**

The downstream processing deals with:

- Solid-liquid separation
- Release of intracellular products
- Concentration
- Purification
- Formulation

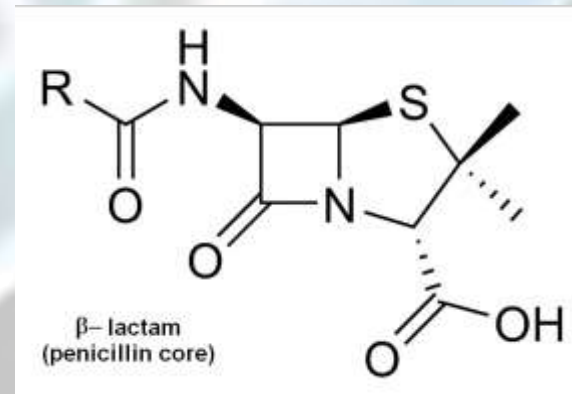
Upstream & downstream process of

- ❖ Antibiotics
- ❖ Hormones
- ❖ Vaccines

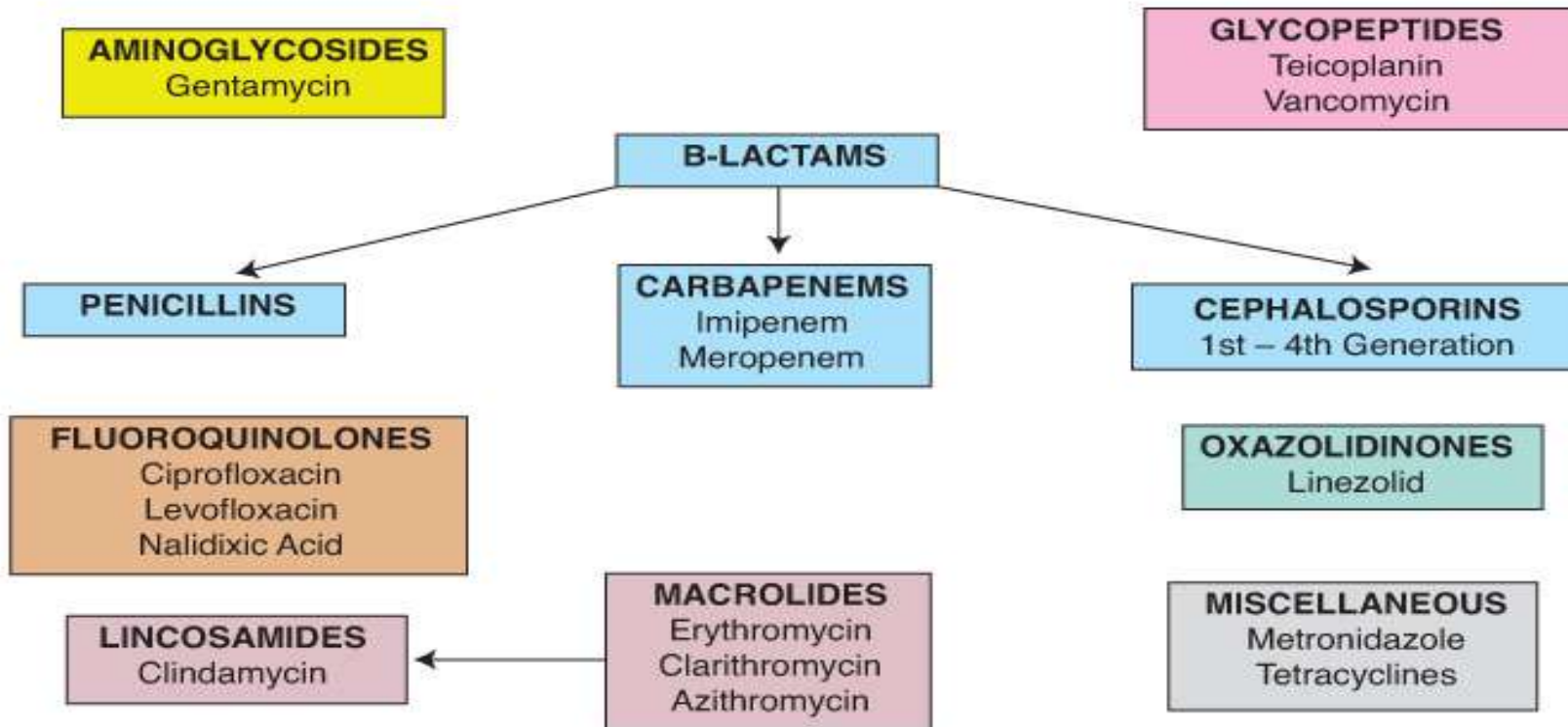


Antibiotics:-it is a chemical drug used to treat bacterial infections.

- **A medicine (such as penicillin or its derivatives) that inhibits the growth of or destroys microorganisms.**
- **Originally, an antibiotic was a substance produced by one **microorganism that selectively inhibits the growth of another.****
- **Eg: penicillin, streptomycin, etc.**



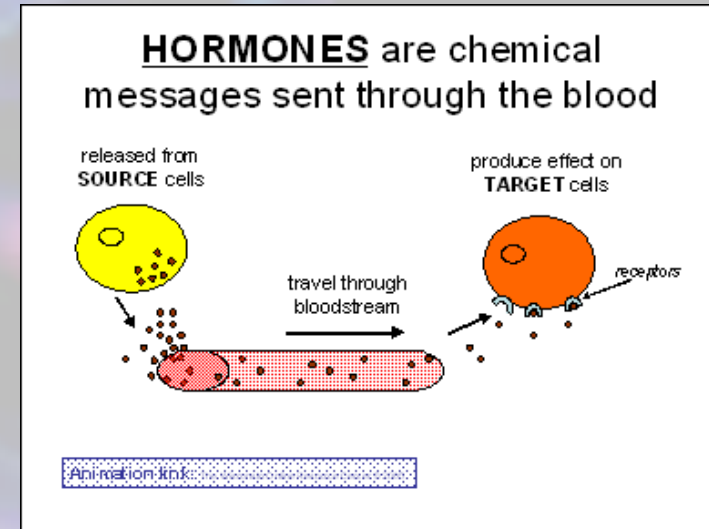
CLASSIFICATION OF ANTIBIOTICS



Hormones-A chemical substance produced in the body that controls and regulates the activity of certain cells or organs.

- ❖ it act like **messenger molecules** in the body.
- ❖ It is carried out by blood.

- Hormones are essential for every activity of life, including the processes of digestion, metabolism, growth, reproduction, and mood control.
- Eg: thyroid hormone, insulin, GSH.



Vaccines- A vaccine is a biological preparation that improves **immunity to a particular disease.**

- ❖ a preparation of killed microorganisms, **living attenuated organisms**, or living fully virulent organisms that is administered to produce or artificially increase immunity to a particular diseases.
- ❖ Eg: Diphtheria vaccine, Polio vaccine, etc.



Stages & steps:

☐ Upstream process.

**INNOCULUM
DEVELOPM
ENT**

**PREPARATI
ON OF
CULTURE
MEDIA**

STERILISATION

☐ Fermentation.

☐ Downstream process

**PRODUCT
RECIVERY**

FORMULATION

**FORMULATION
& EFFLUENT
TREATMENT**

Upstream process:-inoculum development

A. Characteristic's:

The microorganisms must fulfill the industrial useful characteristics like:

- i. Carry out fermentation using cheaper medium**
- ii. Genetic stability**
- iii. High yield**
- iv. Easy recovery of formed product.**

B. Source

- i. **Culture collection:** from the process of microbial technology
- ii. **Isolation:** By method of screening that is based on use of ELISA, molecular probes, inhibition of enzymes
- iii. **Improvement:** Done by genetic machinery of microorganisms.

By following methods

- ❖ Selection of natural variant
- ❖ Mutation -alkylating agents, exciting radiation, ionizing radiation
- ❖ Genetic recombination-r DNA technology, protoplast fusion

C. Preservation: By lyophilization, cryopreservation, agar slope.

D. Development of inoculum:

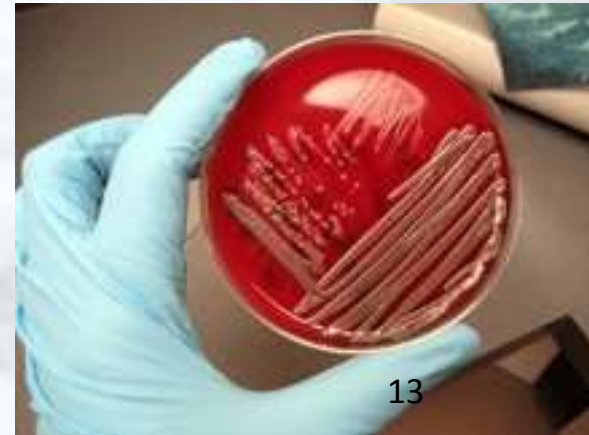
It is to produce **active biomass of cell**

- The size of inoculum is generally 1-10% of production media
Should be morphologically suitable, pure culture.

Preparation of culture medium

Culture medium may be simple or complex:

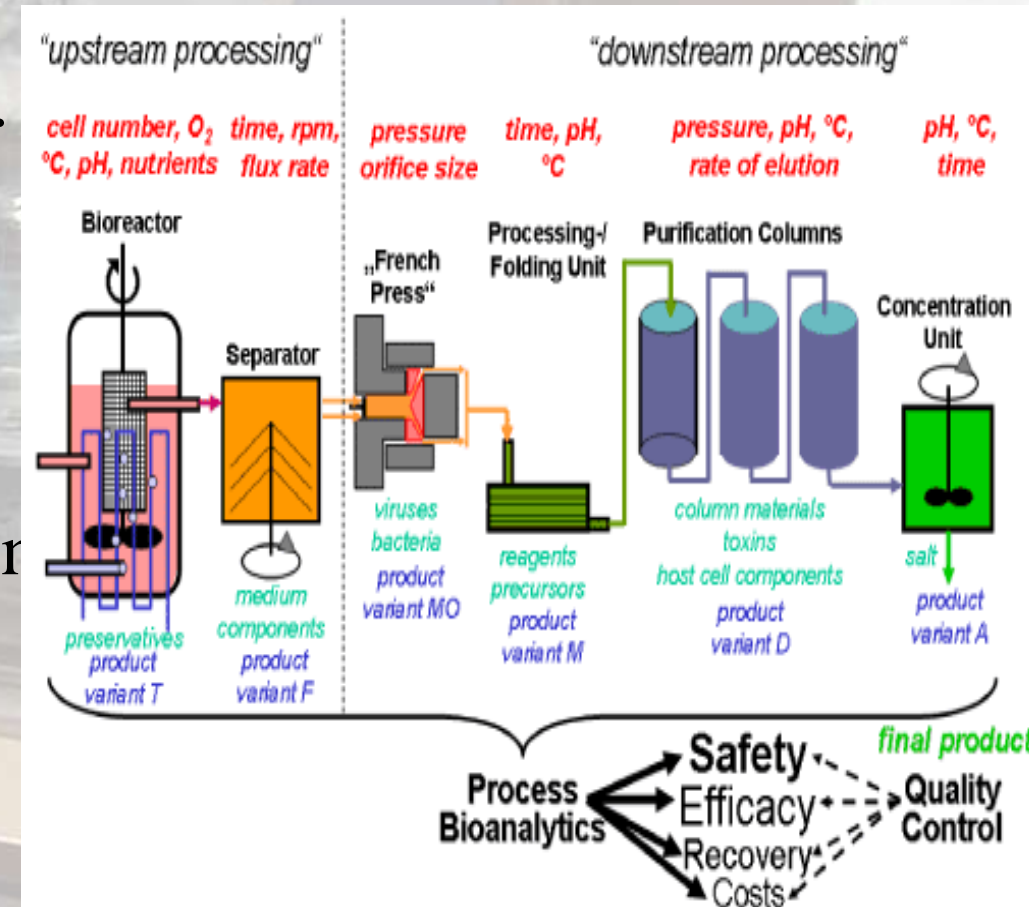
- Carbon source- starch, lactose, sulfite waste liquor, molasses.
- Nitrogen source-corn steep liquor, ammonia, fish meal, soya bean.
- Minerals- macro-Mg, K, Ca, Cl
micro- MN, Cu, Zn, MO
- Anti foaming agents-silicon oil, fatty acids, alcohol.



- Metabolic regulators-
 - Precursors: inorganic cobalt, PAA, Cl
 - Inducers: starch, pectin
 - Inhibitors: sodium bisulfite, alkali metals.

Sterilization

- culture medium – steam
- By batch process or continuous process
- fermenter vessel-steam at 15psi for 20 min followed by sterile air at positive pressure.



FERMENTATION:

- It's a process of growing culture of microbes in nutrient medium at maintained physicochemical conditions and thereby converting feed into desired end product.

Controlling parameters	Device /sensor
Temperature	Electrical resistance thermometer Hg in glass thermometer
Pressure	Pressure gauge
pH	PH meter
Agitator speed control	Tachometer
Air flow	Flow meter

DOWNSTREAM PROCESS:

Recovery

A. Separation: Done by centrifugation, filtration



Laboratory

Small tube

High speed chilled

Ultra centrifuge

Industrial

Tubular

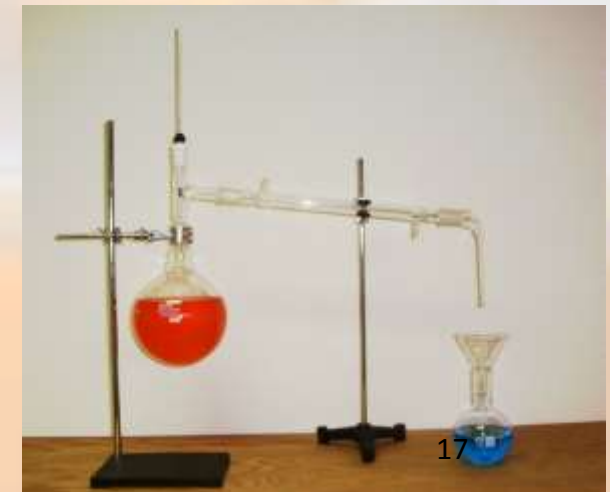
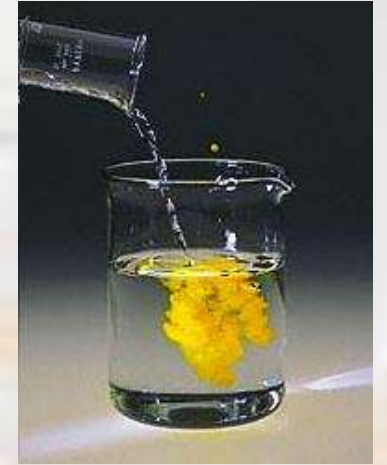
Disk track

Decanter

B. Concentration: Removal of broth water so, also called Dewatering
To reduce handling volume to next step

Methods-

- Precipitation: acids, bases, organic solvents.
- extraction,
- flocculation,
- sedimentation,
- fractionation/distillation



C. CELL DISRUPTION

MECHANICAL TYPE

- SOLID SHEAR**
- LIQUID SHEAR**
- FREEZE THAW**
- ULTRASONICATION**
- AGITATION WITH ABRASIVES**

CHEMICAL TYPE

- DETERGENTS**
- OSMOTIC SHOCKS**
- ALKALI TREATMENT**

ENZYMATIC

- LYSOZYME**
- GLUCONASE**

PURIFICATION:

Purification-

To obtain product in purest form

- i. Gel permeation: separation is on basis of size gels used are cross linked dextran (sephadex and sephacryl) cross linked agarose(sepharose).
- i. Adsorption chromatography: In this the stationary phases or column is packed with the mixture and followed by mobile phase.



FORMULATION AND EFFLUENT TREATMENT

As solution, suspension, dry powders stabilizers are added.

Eg-ammonium sulphate, sorbitol, glycerol, PEG.

Methods for effluent treatment-

- a) Physical:- sedimentation, incineration of solid waste
- b) Chemical:- coagulation
- c) Microbiological:- bio filtration, oxidation by microbes.

PENICILLIN:

1. Up stream process

❖ Inoculum development: micro organism-**P.chrysogenum**

Strain development: **P.chrysogenum** NRRL 1951

planting & selection

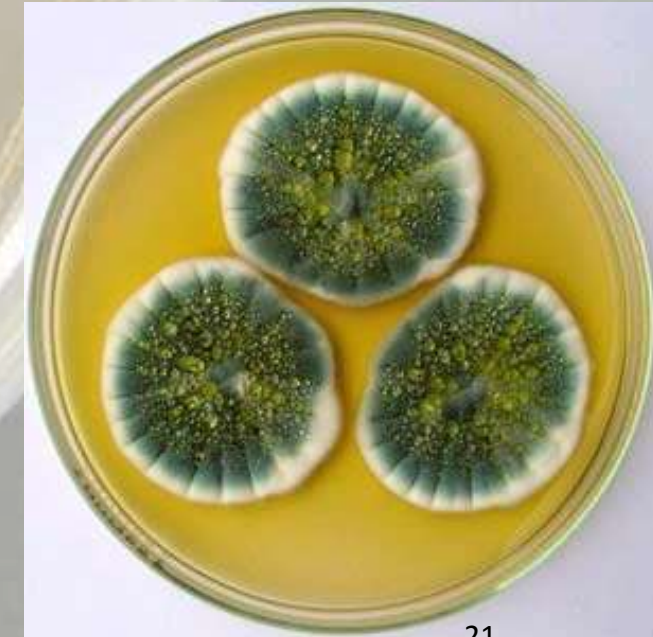


NRRL 1951 B.25

x-ray, uv, mutagen



commercial strain



❖ **Preparation of culture medium:**

- **Carbon source-**lactose, glucose, sucrose
- **Nitrogen source-**corn steep liquor, ammonium sulphate
- **Precursor-** phenyl acetic acid, phenoxy acetic acid
- **Buffer-** calcium carbonate



Ingredient	Conc.
Corn steep liquor	3.5%
Sucrose / glucose	2%
Calcium carbonate	0.5-1%
Inorganic salts	-

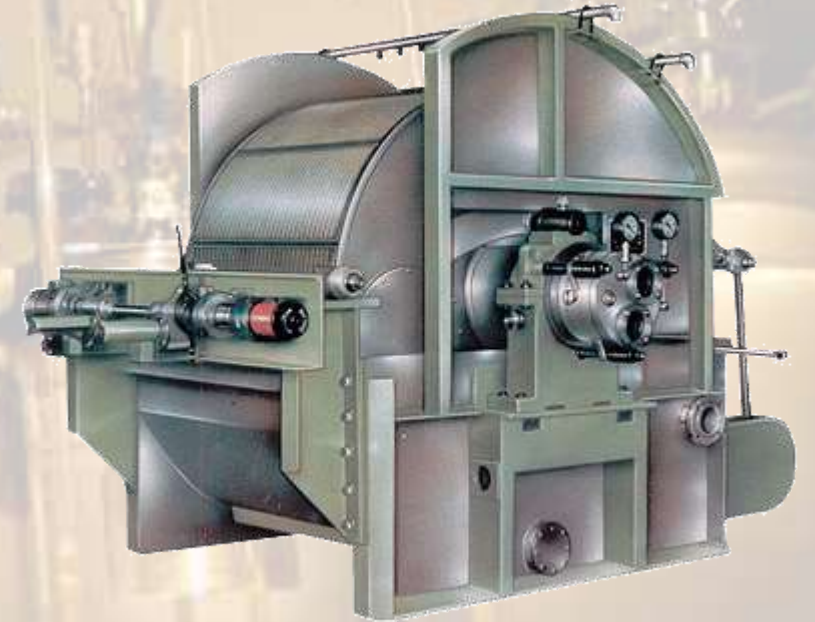
❖ **Sterilization-** steam under pressure

2. FERMENTATION- suspended growth method pH -6.2 to 6.8 .

- Aeration rate- 0.5- 1 volumes of air/ min
- Temp-23to25°C
- Time- 5 to 6 days

3. Down stream-

- Separation- filtration(rotary vacuum)
- concentration-extraction method
- Purification – crystallization
- Drying- on horizontal belts



CONCLUSION

- **This is a type of sequence process, that are involved in extraction, separation & purification to get a desired product.**
- **Upstream & downstream process ensures the efficiency of finished product.**
- **By upstream & downstream process a fine biopharmaceutical products are obtained by using of some microbial organisms through different steps involved in it.**

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A close-up photograph of a hand holding a dark green ballpoint pen, writing the words "Thank you!" in a cursive script on a white surface. The pen is positioned at the end of the word "you!".

Thank you!