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Time: 2 Hours
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Final Exam of Biotransformation and Fermentation.
For Diploma of Biocontrol Treatment

Q1) write in details on the following:

1. Differences between primary and secondary metabolites
2. Properties of microorganism that used industrially.
3. Upstream and downstream processing in fermentation.
4. Batch, Fed-batch and continuous fermentation.
5. Biotransformation using whole cells and isolated enzymes.
6. Biotransformation using growing and resting cells.

With My Best Wishes

Model Answer

Q1) write in details on the following:

1. **Differences between primary and secondary metabolites**

The main difference **between primary metabolites and secondary metabolites is that** primary metabolites are directly involved in primary growth development and reproduction **whereas** secondary metabolites are indirectly involved in metabolisms while playing important ecological functions in the body.

2. **Properties of microorganism that used industrially.**

The success of an industrial fermentation process chiefly depends on the microorganism strain used. An ideal producer or economically important strain should have the following characteristics.

1. It should be pure, and free from phage.
2. It should be genetically stable, but amenable to genetic modification.
3. It should produce both vegetative cells and spores; species producing only mycelium are rarely used.
4. It should grow vigorously after inoculation in seed stage vessels.
5. Should produce a single valuable product, and no toxic by-products.
6. Product should be produced in a short time, e.g., 3 days.
7. It should be amenable to long term conservation.
8. The risk of contamination should be minimal under the optimum performance condition

3. **Upstream and downstream processing in fermentation.**

The upstream process is defined as the entire process from early cell isolation and cultivation, to cell banking and culture expansion of the cells until final harvest (termination of the culture and collection of the live cell batch). The upstream part of a bioprocess refers to the first step in which microbes/cells are grown, e.g. bacterial or mammalian cell lines, in bioreactors. Upstream processing involves all the steps related to inoculum development, media

development, improvement of inoculum by genetic engineering process, optimization of growth kinetics so that product development can improve tremendously.

The downstream part of a bioprocess refers to the part where the cell mass from the upstream are processed to meet purity and quality requirements. Downstream processing is usually divided into three main sections: cell disruption, a purification section and a polishing section. The steps of downstream processing are:

I. Separation of biomass: separating the biomass (microbial cells) generally carried out by centrifugation or ultra-centrifugation. If the product is biomass, then it is recovered for processing and spent medium is discarded. If the product is extra cellular the biomass will be discarded. Ultra-filtration is an alternative to the centrifugation.
II. Cell disruption: If the desired product is intra cellular the cell biomass can be disrupted so that the product should be released. The solid-liquid is separated by centrifugation or filtration and cell debris is discarded.
III. Concentration of broth: The spent medium is concentrated if the product is extracellular.
IV. Initial purification of metabolites: According to the physico-chemical nature of the product molecule several methods for recovery of product from the clarified fermented broth were used (precipitation, etc.)
V. De-watering: If low amount of product is found in very large volume of spent medium, the volume is reduced by removing water to concentrate the product. It is done by vacuum drying or reverse osmosis.
VI. Polishing of metabolites: this is the final step of making the product 98 to 100% pure. The purified product is mixed with several inert ingredients called excipients. The formulated product is packed and sent to the market for the consumers.

4. **Batch, Fed-batch and continuous fermentation**.

Characteristics	Batch culture	Fed-batch culture	Continuous culture
Cultivation system	Closed type	Semi-closed type	Open type
Addition of fresh nutrition	No	Yes	Yes
Volume of culture	Constant	Increases	Constant
Removal of wastes	No	No	Yes
Chance of contamination	minimum	Intermediate	Maximum
Growth phase	Lag, log, stationary and decline phase	Lag, log , stationary and decline phase	Lag and log phase
Log phase	Shorter	longer	Longest and Continuous
Density of bacteria	Change with time	Change with time	Remain same
Product yield	Low	Medium	High