Al-Farabi Kazakh National University

Culture Preservation and Inoculum Development

Lecture 6

Meiramkul Narmuratova

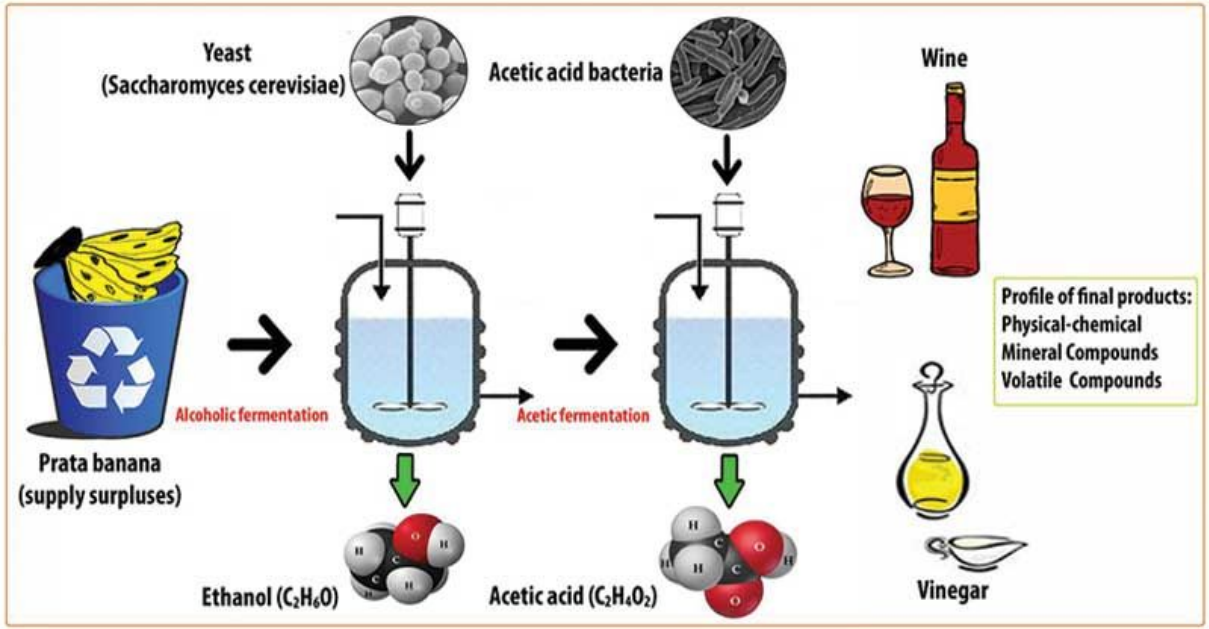
Importance of Fermentation

- Production and yield of fermentation product depends upon number of factors that control fermentation process directly or indirectly
- It is science as well as art to extract maximum productivity and yield from a given fermentation process
- A good fermentation always start with a good fermenting strain and culture of such strain in appropriate density and healthy state
- Therefore successful fermentation process start with a excellent inoculum development to obtain maximum possible for productivity and yield

Types of Fermentation

A classification, based on the product formation in relation to energy metabolism.

1. Type I fermentation
2. Type II fermentation
3. Type III fermentation



The Biology notes

Type I Fermentation:

When the product is formed directly from the primary metabolism used for energy production, it is referred to as type I and may be represented as.

Substrate A → Product

Substrate A → B → C → D → Product

Growth, energy metabolism and product formation almost run in a parallel manner

Type II Fermentation

The product is also formed from the substrate used for primary energy metabolism. However, the product is produced in the secondary pathway

Substrate A → B → C → D ….Primary metabolism → E → F G → Product

At the beginning, the growth of the microorganisms is accompanied by high substrate utilization with little or no product formation. Now the growth is slowed down but the substrate consumption is high, and this is coupled with product formation.

Type III Fermentation

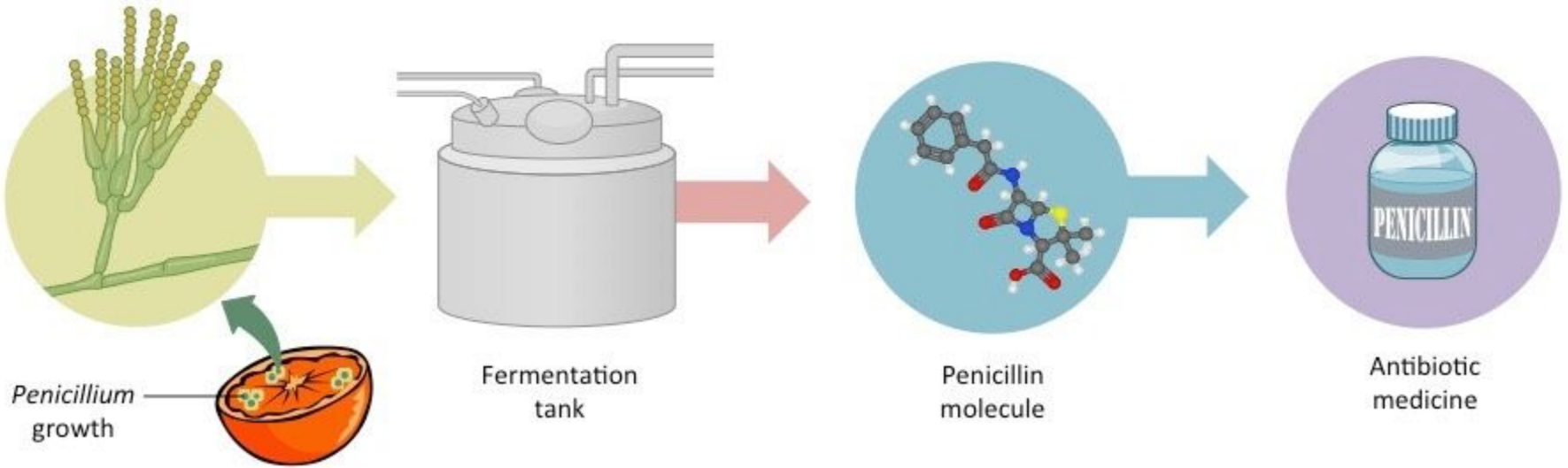
There is a clear distinction between the primary metabolism and product formation in type III fermentation as they occur at separate times.

Substrate consumption and rapid growth occur in the first phase and the product formation occurs in the second phase.

The product is formed from amphibolic metabolic pathways and not from primary metabolism e.g. production of vitamins and antibiotics.

Production of Penicillin via Batch Fermentation

1. *Penicillium* mold produces the antibiotic penicillin
2. Scientists grow mold in deep batch fermenters by adding sugar and other key ingredients
3. Scientists separate the penicillin from the mold
4. Penicillin is purified for use as an antibiotic medicine



https://ib.bioninja.com.au/options/untitled/b1-microbiology-organisms/batch-versus-continuous.html

Four stages of fermentation

(1) Inoculum Preservation

(2) Inoculum Build-up

(3) Pre-Fermenter Culture

(4) Production Fermentation

https://www.alfa.com/en/bacterial_culture_media/

What is Inoculum?

- Inoculum we use for industrial fermentations
- Inoculum is the mixture of cultured microbes along with in which it is growing
- Transfer about 0.5-5% inoculum.
- In its active, healthy, and exponential growth phase.
- Available free of contamination required large volumes.
- Retain its capability of the formation of desired product formation

What is Inoculum Preservation?

- Industrial important microorganism have the capability to produce high yields of desirable metabolites in large scale-up production fermentation

- Highly productive mutant strains are preserved for long periods free from phenotypic change

- With particular respect to the capability of high production of a primary or secondary metabolic product

Methods of Cell Culture Preservation

1. Storage at low temperature

- **In the freezers**, the preservation can be done at -18°C or, at -80°C.
- **Cryopreservation.** For preservation at -196°C, liquid nitrogen must be used.

2. Storage in a dehydrated form:

- **Dried cultures.** The technique has been used extensively for the storage of fungi and actinomycetes
- **Lyophylization** or freeze-drying, is suitable for the preservation of microorganisms but not for animal cell cultures. It involves the freezing of a culture followed by its drying under vacuum, which results in the sublimation of the cell water.

Inoculum Build Up

The preserved cultures have to be revived for their industrial use. This can be done by growing the cultures in liquid or on solid media.

The actual process and the conditions used for inoculum build-up largely depend on the preservation technique used.

Refrigerated cultures (2-6° C)	**Frozen cultures (-18°C, -80° C, -196°C):**	**Lyophilized cultures:**
Bacteria 6-24 hours Actinomycetes 1-3 days Fungi 1-5 days	Bacteria 6-48 hours Actinomycetes 1-5 days Fungi 1-7 days	For all organisms 4-10 days

Pre-fermenter Culture

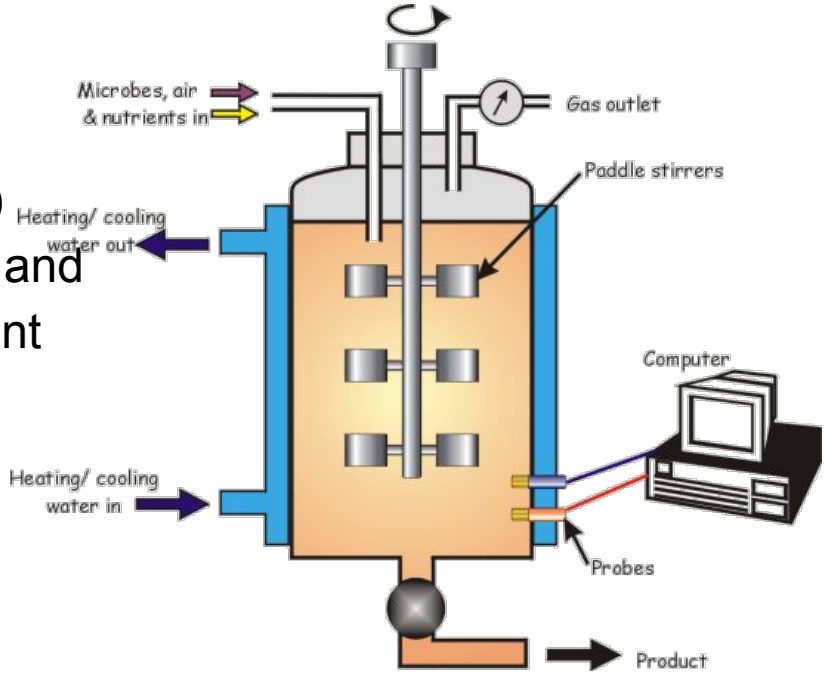
- Fermenter pre-culture or pre-fermenter culture is often required for inoculating large sized bioreactors.
- Inadequate quantity of inoculum will not only delay the product formation, but also reduce the yield drastically.
- By culturing the microorganisms (the inoculum build-up) in small fermenters, the size of the inoculum can be increased for large-scale industrial use.

***For example*,** for bacterial fermentation, the inoculum concentration should be between 0.2 to 3.0%; for fungal fermentation, it is in the range of 5-10%.

Production Fermentation

The size of the fermenter used mainly depends on the product.

For example, a small bioreactor (1-20 litre size) can be used for producing diagnostic enzymes and substances for molecular biology by recombinant microorganisms, while large bioreactors (≥450 litres) are employed for producing single-cell protein and amino acids.



https://moodle.beverleyhigh.net/mod/resource/view.php?id=6169