

## **Fermentation , fermentation products and methods of extraction of Fermentation products .**

Fermentation is derived from the Latin verb *fervere* , (to boil), thus describing the action of yeasts on extracts of fruits or malted grain. The boiling appearance is due to the production of carbon dioxide bubbles caused by the anaerobic catabolism of the sugar present in the extract.

However fermentation has come to have different meanings to biochemists and industrial microbiologists .

Its biochemical meaning relates to the generation of energy by the catabolism of organic compounds, whereas its meaning in industrial microbiology to be much boarder.

### **Definitions of fermentation**

#### **From biochemistry view**

Is the energy production as a result of catabolic metabolism of organic compounds.

#### **From Industrial microbiology view**

Is a processes in which microorganisms is used in order to produce a beneficial economical product.

#### **From Biotechnology view**

Is the metabolic activity of living cells or a part of living cells or enzymes which is extracted from.

### **Fermentation products**

Fermentation products are many , most of them produced as a result of carbohydrate metabolism.

The production of a specific product depends on the presence or absence of certain enzymes in the microorganisms.

## 1-Microbial biomass

Microbial biomass is produced commercially as single cell protein (SCP) using such unicellular algae as species of *Chlorella* or *Spirulina* for human or animal consumption, or viable yeast cells needed for the baking industry, which was also used as human feed at one time.

Bacterial biomass is used as animal feed.

The biomass of *Fusarium graminearum* is also produced, for a similar use.

## 2. Microbial metabolites.

### i) Primary metabolites:

During the log or exponential phase organisms produce a variety of substances that are essential for their growth, such as nucleotides, nucleic acids, amino acids, proteins, carbohydrates, lipids, etc., or by-products of energy yielding metabolism such as ethanol, acetone, butanol, etc.

This phase is described as the trophophase, and the products are usually called primary metabolites. Commercial examples of such products are given in Table below.

### Examples of commercially produced primary metabolites

<u>Primary Metabolite</u>	<u>organism</u>	<u>Significance</u>
Ethanol	<i>Saccharomyces cerevisiae</i> <i>Kluyveromyces fragilis</i>	alcoholic beverages
Citric acid	<i>Aspergillus niger</i>	food industry
Acetone and butanol	<i>Clostridium acetobutyricum</i>	solvents
Lysine	<i>Corynebacterium</i>	nutritional additive
Glutamic acid	<i>glutamacium</i>	flavour enhancer
Riboflavin	<i>Ashbya gossipii</i> <i>Eremothecium ashbyi</i>	nutritional

Vitamin B12	Pseudomonas denitrificans	nutritional
	Propionibacterium shermanii	
Dextran	Leuconostoc mesenteroides	industrial
Xanthan gum	Xanthomonas campestris	industrial

## ii) Secondary metabolites:

Organisms produce a number of products, other than the primary metabolites. The phase, during which products that have no obvious role in metabolism of the culture organisms are produced, is called the idiophase, and the products are called secondary metabolites.

In reality, the distinction between the primary and secondary metabolites is not a straight jacket situation.

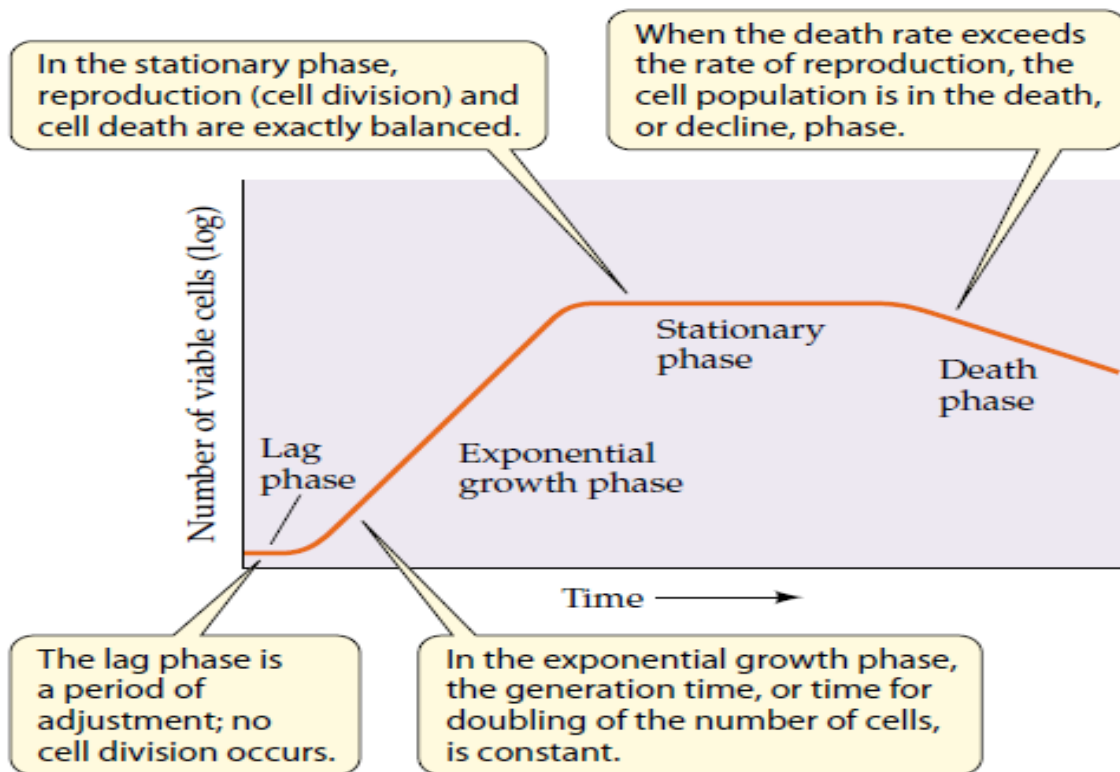
Many secondary metabolites are produced from intermediates and end products of secondary metabolism.

Some like those of the Enterobacteriaceae do not undergo secondary metabolism. Examples of secondary metabolites are given in Table below.

Secondary metabolism may be repressed in certain cases. Glucose represses the production of actinomycin, penicillin, neomycin and streptomycin; phosphate represses streptomycin and tetracycline production. Hence, the culture medium for secondary metabolite production should be carefully chosen.

## Examples of commercially produced secondary metabolites

Metabolite	Species	Significance
Penicillin	<i>Penicillium chrysogenum</i>	antibiotic
Erythromycin	<i>Streptomyces erythreus</i>	antibiotic
Streptomycin	<i>Streptomyces griseus</i>	antibiotic
Cephalosporin	<i>Cephalosporium acrimonium</i>	antibiotic
Griseofulvin	<i>Penicillium griseofulvin</i>	antifungal antibiotic
Cyclosporin A	<i>Tolypocladium inflatum</i>	immunosuppressant
Gibberellin	<i>Gibberella fujikuroi</i>	plant growth regulator



**Figure 6.2 Growth curve**

Growth curve of a typical bacterial population growing in batch culture, illustrating the four growth phases.

## PRIMARY & SECONDARY METABOLITES

Primary metabolites are produced during active growth  
e.g. alcohol, lactic acid



Secondary metabolites are produced after the growth phase nears completion  
e.g. penicillin, streptomycin



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Basis for comparison	Primary metabolite	Secondary metabolite
Meaning	The metabolism products that are produced during the growth phase of an organisms in order to perform the physiological functions and supports in overall development of the cell are called primary metabolites.	The end products of primary metabolism that are synthesized after the growth phase has been completed and are important in ecological and other activities of the cell are known as secondary metabolites
Also known as	Trophophase.	Idiophase.
It occurs at the	Growth phase.	Stationary phase.
Production	These are produced in large quantities, and their extraction is easy.	These are produced in small quantities, and their extraction is difficult.
Occurrence	Same in every species, which means they produce the same products.	Varies in different species.
Importance	1. These products are used in industries for various purpose. 2. Primary products play the significant role in the cell growth, reproduction and development.	1. Secondary metabolites such as antibiotics, gibberellins are also important. 2. They also indirectly support the cell, in sustaining their life for long duration.
Examples	Vitamins, carbohydrates, proteins and lipids are some of the examples.	Phenolics, steroids, essential oils, alkaloids, steroids are few examples.

### 3- Microbial enzymes

Industrial production of enzymes is needed for the commercial production of food and beverages.

Enzymes are also used in clinical or industrial analysis and now they are even added to washing powders (cellulase, protease, lipase).

Enzymes may be produced by microbial, plant or animal cultures. Even plant and animal enzymes can be produced by microbial fermentation.

While most enzymes are produced in the trophophase, some like the amylases (by *Bacillus stearothermophilus*) are produced in the idiophase, and hence are secondary metabolites.

Examples of enzymes produced through fermentation processes are given in Table below.

<u>Organism</u>	<u>Enzyme</u>
<i>Aspergillus oryzae</i>	Amylases
<i>Aspergillus niger</i>	Cellulase
<i>Saccharomyces cerevisiae</i>	Invertase
<i>Kluyveromyces fragilis</i>	Lactase
<i>Saccharomycopsis lipolytica</i>	Lipase
<i>Aspergillus</i> species	Pectinases and proteases
<i>Bacillus</i> species	Proteases
<i>Mucor pusillus</i>	Microbial rennet
<i>Mucor meihei</i>	Microbial rennet

### 4-Microbial bioconversion.

Production of a structurally similar compound from a particular one, during the fermentation process is transformation, or biotransformation, or bioconversion. The oldest instance of this process is the production of acetic acid from ethanol.

## **5-Microbial recombinant products.**

Recombinant DNA technology has made it possible to introduce genes from any organism into micro-organisms and vice versa, resulting in transgenic organisms and the latter are made to produce the gene product.

Genetically manipulated *Escherichia coli*, *Saccharomyces cerevisiae*, other yeasts and even filamentous fungi are now being used to produce interferon, insulin, human serum albumin, and several other products.

### **1. Food industry products**

A very wide range of innumerable products of the food industry, such as sour cream, yoghurt, cheeses, fermented meats, bread and other bakery products, alcoholic beverages, vinegar, fermented vegetables and pickles, etc., are produced through microbial fermentation processes.

The efficiency of the strains of the organisms used, and the processes are being continuously improved to market quality products at more reasonable costs.

### **Methods of extraction of fermentation products**

Method of extraction depend on the type of product and available instruments.

Extraction of non-soluble products

Extraction of soluble products.

Extraction of intracellular products

## **Removal of non-soluble substances**

Non-soluble substances includes mainly the microorganisms cells which represent 10% or more of culture media.

- 1-Centrifuge.
- 2- flocculation
- 3- Filtration
- 4- Flootation.
- 5- Adsorption

## **Extraction of soluble products**

This is in the case the byproduct is present as soluble in liquid phase of the culture media and it can be obtained after removal of non-soluble substances by the followings methods:-

- 1- solvent extraction.
- 2- Adsorption and elution.
- 3- Precipitation .
- 4- liquid ion exchange.
- 5- Gel filtration.
- 6- Membrane methods

## **Extraction of Intracellular product**

The product may occur inside the cell wholly or partially .

There are several methods for extraction of the intracellular product:-

- 1- Modification of PH
- 2- Solvent
- 3- Enzymes.
- 4- Heat shock.
- 5- Osmotic shock.
- 6- Freezing and thawing .
- 7- Crushing the freezed cells at 30 oC.
- 8- Grinding with glass balls.

Then other methods of extraction are used after removal of cell wall or damaging the cell.