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Dr. Chinky Goyal

Assistant Professor, Department of Rasa Shastra & Bhaishjya Kalpana, Shri Dhanwantry Ayurvedic College and Hospital, Chandigarh, India

Dr. Amrit Malik

Associate Professor, Dept. of Agad Tantra, Shri Dhanwantry Ayurvedic College, Chandigarh, India

Dr. Sudhir Malik

Assistant Professor, Dept. of Kaumarbhritya, Shri Krishna Ayush Govt. College, Kurukshetra, Haryana, India

Dr. Arun Kumar Gupta

Assistant Professor, Dept. of Panchkarma, LN Ayurveda College Bhopal, Madhya Pradesh, India

Dr. Rimpaljeet Kaur

Assistant Professor, Dept. of Roga Nidan, Dayanand Ayurvedic College and Hospital, Punjab, India

Corresponding Author:

Dr. Chinky Goyal

Assistant Professor, Department of Rasa Shastra & Bhaishjya Kalpana, Shri Dhanwantry Ayurvedic College and Hospital, Chandigarh, India

A Bird's eye view on fermentation

Dr. Chinky Goyal, Dr. Amrit Malik, Dr. Sudhir Malik, Dr. Arun Kumar Gupta and Dr. Rimpaljeet Kaur

Abstract

Brewery industry is one among huge, enormously profitable agricultural industries which developed from ancient knowledge from many different cultures around the world. Fermentation is a chemical process by which molecules such as glucose are broken down anaerobically. Several types of microorganisms (including yeast and some bacteria) have been known to be involved in the process of fermentation. Present review of Fermentation include the etymology, definition, various theories propounded for fermentation, detailed classification, the science of fermentation engineering is detailed in terms of range of fermentation process, component parts of fermentation process with a schematic diagram and steps of fermentation in both alcoholic and acidic fermentation.

Keywords: Fermentation, germ theory, aerobic fermentation, anaerobic fermentation

Introduction

The term Fermentation is derived from the Latin word 'Fervere' which means to boil, the appearance that is due to the liberation of CO₂ in the form of bubbles which at the height of reaction may cause a marked agitation or movement of the liquid medium, sufficient to give it an appearance of boiling liquid. Fermentation is a metabolic process by which chemical changes are induced in a complex organic compound by the action of enzymes there by splitting it into more simple compounds. Such a chemical change depends upon the type of organism involved, the kind of substrate and other factors such as pH and Oxygen supply. In strict biochemical sense, the term fermentation applies only to reduction of organic substrates performed under anaerobic conditions for the generation of energy. In industrial microbiology, it refers to any large scale process regardless of whether it is aerobic or anaerobic ^[1].

Theories of fermentation-² Regarding fermentation, either Alcoholic or Acidic, several theories have been postulated by various scientists. Important ones are-

1. Germ Theory

It considers fermentation to be a natural process in which alcohol and organic acids are formed from dissolved sugar in the presence of microbes and in the absence of air. Yet, despite the recorded descriptions of micro organisms by Leeuwenhoek, the biological basis of fermentation was not formulated until well into the nineteenth century. Basically, two viewpoints evolved to explain these processes viz. Non-vital (non-biological) and the vital (biological) theory.

According to non – vital view, some unstable chemical entities called as ferments, produces the fermentation reactions by acting as catalysts or enzymes, and not the yeasts are held responsible for the same. These unstable ferments were formed by the action of air on sugar containing fluids that passed their instability to sugar molecules, which in turn get decomposed to form the products of fermentation. Liebig used as support for the non-vital theory the absence of any yeasts in acetic acid and lactic acid fermentations. Non-vitalists neglected to consider the possibility that other micro-organisms could produce these 'essential ferments', several years later these reactions were known to be caused by bacteria.

2. Biological theory

It was strengthened by the work of French and German scientists in the 1830's who clearly demonstrated the role of yeast in alcoholic fermentation. He also described budding of yeasts and showed that this 'sugar fungus' *Saccharomyces cerevisiae* was needed in large numbers for fermentation to proceed. These observations were not readily accepted by the non vitalists

and the stage was set for a bitter controversy that was not settled until Louis Pasteur provided experimental proof for the biological theory in 1857.

Classification of fermentation

1. Depending on the availability and non – availability of oxygen it is classified as

- a) **Anaerobic:** Oxygen does not take part in the fermentation but other entities such as aldehyde, pyruvic acid serves as hydrogen acceptor. E.g. Alcoholic fermentation
- b) **Aerobic:** Dissimilation of the substrate is accomplished by absorption of Oxygen, which acts as hydrogen acceptor. E.g Acetic acid and Lactic acid fermentation

2. Based on the addition of nutrition, it is divided as

- a) **Batch:** Microbial inoculum is introduced into the fermentation medium and is left undisturbed until completed. The course of which is similar to that of microbial growth cycle. Cell numbers increase rapidly during exponential growth and reach stationary levels when the nutrients in the growth medium are exhausted. Desired fermentation products generally appear only after the stationary phase begins.
- b) **Fed batch:** Nutrient is fed incrementally or batch-wise to a growing yeast culture without removal of culture fluid.
- c) **Continuous:** Fresh growth medium is continuously pumped into the medium causing a steady overflow of fluid containing the desired fermentation product. The process may be carried out for several days or weeks and left at the maximum stage for long periods of time, thus making continuous collection of the product is possible.

3. Depending on the number of organisms involved, it is classified as

- a) **Dual/multiple or indirect fermentation:** More than one micro-organism is employed that may be grown first in the medium followed by inoculation and growth of a second organism. The basic concept is that two or more organism accomplishes something that neither a single organism can do alone. E.g. Acetic acid where initially yeast is inoculated for production of alcohol and later Acetobacter is used to convert ethanol to acetic acid.
- b) **Single/Direct fermentation:** This is carried out by a single species of micro organism e.g yeasts in alcoholic fermentation. It is also called as direct fermentation.

4. Depending upon the state of fermentation

- a) **Solid state fermentation:** It is defined as the growth of the micro organism on (moist) solid substrates in the

absence or near absence of free water.

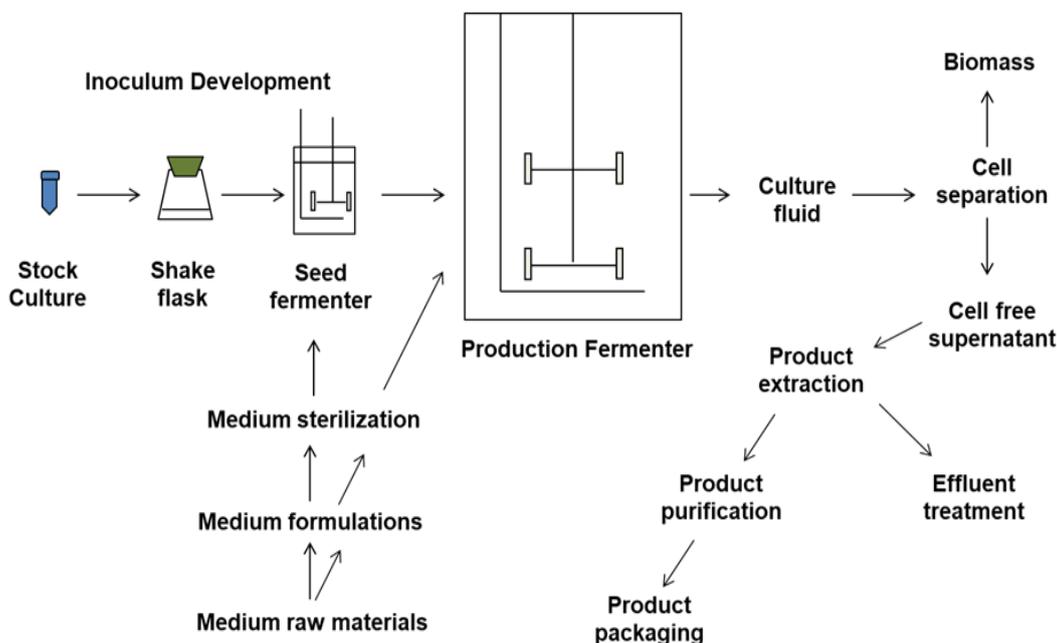
- b) **Submerged fermentation:** In submerged type of fermentation the fermenting substance is in the liquid form. All types of fermentation except solid state fermentation come under this type.

5. Depending upon the types of product obtained: It can of several types viz. ethanol, acetic acid, lactic acid, gluconic acid, citric acid, glycol fermentation etc.

Range of Fermentation process:³ There are five major groups of commercially important fermentations as given below-

1. **Those that produce microbial cells (or biomass):** Production of yeast as well as microbial cells are the two major examples of commercial production of microbial biomass.
2. **Those that produce microbial enzymes:** Various enzymes viz. amylase, protease, lactase, lipase etc have been produced from plants. Furthermore, the arrival of recombinant DNA technology has empowered animal origin enzymes to be harmonized by microorganisms.
3. **Those that produce microbial metabolites:** The specific compounds extracted as intermediates or as final outcome of the fermentation form this group. The primary and the secondary metabolites form the two broad divisions of this group. The primary biosynthetic products are ethanol, citric acid, glutamic acid, vitamins etc. The secondary metabolites tend to have antimicrobial activity, others are specific enzyme inhibitors, growth promoters and many have certain pharmacological properties. Therefore, the products of subsidiary metabolism have formed the bedrock of a number of fermentation processes.
4. **Those that produce recombinant products:** Genes from higher organisms may be introduced into microbial cells for synthesizing 'foreign' (heterologous) proteins. E.g interferon, insulin, serum albumin, factors VIII and IX, epidermal growth factor etc.
5. **Those that to alter a compound appended to the fermentation transformation process-** Microbial cells may be used to convert a compound into a structurally related, financially more valuable, compound due to their high positional specificity and stereo specificity. E.g. production of vinegar, steroids, antibiotics, prostaglandins etc.

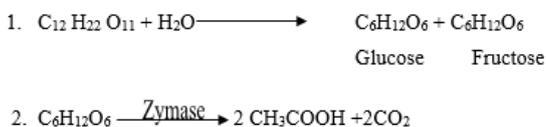
Component parts of a typical fermentation process



Steps of fermentation-⁴ The process of fermentation takes place in two steps.

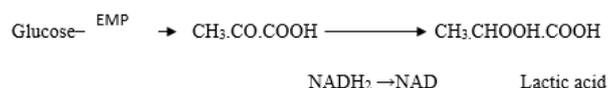
1. Glucose is broken down to pyruvate with the release of two pairs of hydrogen atom.
2. Pyruvate or compounds derived from it are reduced by hydrogen, released in first stage.

Alcoholic fermentation: Pyruvate is converted into ethanol and CO₂ which is characteristic of yeasts, particularly *Saccharomyces cerevisiae*. Two steps are as given below-
 1-Pyruvate is first decarboxylated yielding acetic acid and CO₂ catalyzed by the enzyme, pyruvate decarboxylase with thiamine pyrophosphate (TPP) as the co-enzyme.
 2-Acetaldehyde is then reduced to ethanol by NADH + H⁺ (NADH₂) and NAD⁺ is regenerated catalyzed by alcohol dehydrogenase. Regeneration of NAD takes place when acetaldehyde is reduced to ethanol. The chemical reactions can be summarized as follows-

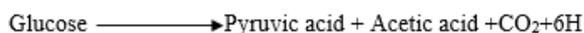


Lactic acid fermentation: It is one step reaction similar to glycolysis of mammalian cells. Pyruvic acid is reduced to lactic acid catalyzed by pyruvate reductase which is characteristic of the lactic acid bacteria which causes spoilage of the food. Lactobacilli are divided into 2 groups, Homofermentative and Heterofermentative. In homolactic fermentation, only lactic acid is produced. In heterolactic fermentation products like Ethanol, Glycerol, Acetate, Propionic acid and CO₂ and Butyric acid are formed in addition to Lactic acid.

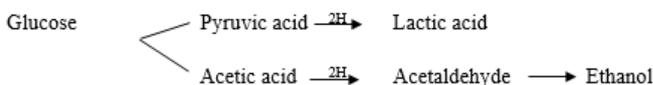
Homolactic fermentation



Heterolactic fermentation- Glucose is first metabolized to Pyruvic acid, Acetic acid and CO₂



Pyruvic acid is then reduced to lactic acid and to ethanol through Acetaldehyde.



Heterolactic one produces only half the energy as produced by homolactic fermentation.

Discussion: During the operation of fermentation biotic matter is degraded in the absence of oxygen, hence, there is always an aggregation of reduction products, or partial oxidation products (for example, alcohol and lactic acid) which are of relevance to humans, and fermentation has therefore been used for their manufacture on an industrial scale. The yeast-oriented fermentations are dealt under the branch of Zymology which is most frequently isolated from sugar-rich samples with a favourable pH of ≤ 5.5. As a part of their enzymatic activity yeast metabolize their substrates which are responsible for the physical, chemical, and sensory properties. Fermentation in a broader way is the foaming which occurs during the production in brewery industry. The frothing is a consequence from the development of carbon dioxide gas, though this was not recognized until the 17th century. French chemist and microbiologist Louis Pasteur in the 19th century used the term *fermentation* in a narrow sense the changes brought about by yeasts and other microorganisms evolving in the trauncy of air (anaerobically); he also recognized that ethyl alcohol and carbon dioxide are not the only products of fermentation. The term *fermentation* now denotes the enzyme-catalyzed, energy-yielding pathway in cells involving the anaerobic breakdown of molecules such as glucose. In most cells the enzymes occur in the soluble portion of the cytoplasm. The reactions leading to the

generation of ATP and pyruvate are thus similar to sugar transformation in muscle, yeasts, some bacteria, and plants.

Conclusion: Fermentation is one of the oldest human technologies using either plant carbohydrates or yeasts as a source of fermentation. *It* is a metabolic process which generates chemical changes in biotic substrates through the accomplishment of enzymes. As per biochemistry, it can be precisely defined as the unsheathing of energy from carbohydrates in the trauncy of oxygen.

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