Fermentation: When the microbes are the main Laborers in the industry

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Introduction: Use of fermentation has been dated back to the prehistoric times. Fermented beverages were in use as early as 5000 BC in Babylon. The term 'fermentation' stems from the Latin word 'fervere' which means boiling- an observation from action of yeast on fruit extracts due to carbon di oxide effervescence. Although traditionally fermentation used to mean conversion of the substrate to a different compound by action of microbes like yeast under anaerobic conditions, with fermentation being highly used industrially from last century, the definition has been extended to the use of mass culture of microorganisms under either anaerobic or aerobic conditions for production of a plethora of chemicals . Although use of fermentation dates back to thousands of years, the current chemical understanding of the fermentation process has been from the works of the eminent French chemist Louis Pasteur in 1857 where he experimentally demonstrated the conversion of glucose to ethanol by the action of the microbe, yeast.

Fig 1. Louis Pasteur in his laboratory

Types of fermentation products: Apart from food, beverages and chemicals, fermentation is now used industrially to produce important microbial metabolites which includes vitamins, amino acids and antibiotics. Different microbial enzymes like proteases and restriction endonucleases are produced in biotech industries. The process that produces the microbial cells or biomass itself constitutes an important part of industrial fermentation.

Fermentation produces useful chemicals: Chemicals like Citric acid, ethanol is mainly produced in industry by fermentation. Acetone, acetic acid and butanol were also produced mainly by fermentation before being substituted by low-cost chemical processes with hydrocarbons and petrochemicals as starting materials.

Ethanol has been one of the foremost products produced by fermentation on an industrial scale. The process involves the action of microorganism *Saccharomyces cerevisiae* (yeast) on glucose.

$$C_6H_{12}O_6 \xrightarrow{\text{yeast}} 2C_2H_5OH + 2CO_2$$

(glucose) (ethanol)

For production of ethanol fuel, the feedstock depends on the availability in that region, for e.g., in USA it is corn whereas in Brazil, sugarcane. These are first converted to glucose by enzymes such as amylase before yeast acts on it.

Citric acid is industrially produced by action of *Aspergillus niger* on substrates like beet molasses. American food chemist James Currie discovered this strain of Aspergillus for efficient citric acid production. The fermentation process is followed by filtering out the microbes and consequent purification of the fermented product.

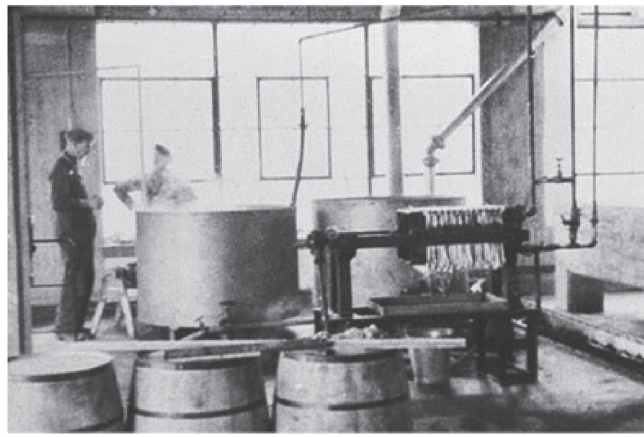

Fig. 2 Citric acid fermentation at Pfizer's Brooklyn facility during 1920s

Production of antibiotics:

"An antibiotic is a chemical substance, produced by micro-organisms, which has the capacity to inhibit the growth of and even to destroy bacteria and other micro-organisms." - Waksman

The first antibiotic was discovered in 1928 by Alexander Fleming when he discovered that contamination with mold has led to inhibition of bacterial growth around the mold (Fig 3). He found that the mold is from *Penicillium notatum* and he named the antibiotic released as Penicillin.

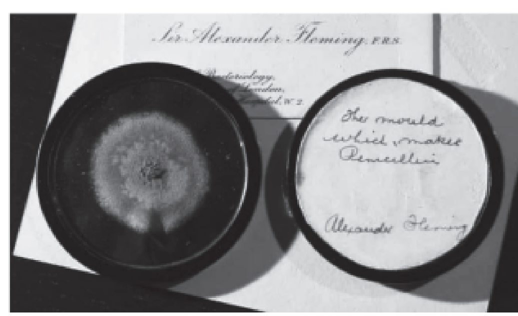

Fig 3. The famous mould and a note from Alexander Fleming

Antibiotics are commonly classified based on chemical structure (Fig.4) or based on spectrum of activity. Broad spectrum antibiotics inhibit or act on a wide range of more than one species of microorganisms e.g., Cephalosporin, Chloromycetin, Tetracycline while narrow spectrum antibiotics are effective against mainly one species of microorganism e.g. bacitracin.

The general scheme for commercial production of antibiotics include :i) preparation of a pure culture of the required microorganism for inoculation (ii) fermentation process producing the antibiotic (iii) isolation of antibiotic from the culture medium (iv) assays for potency (v) formulation into acceptable doses.

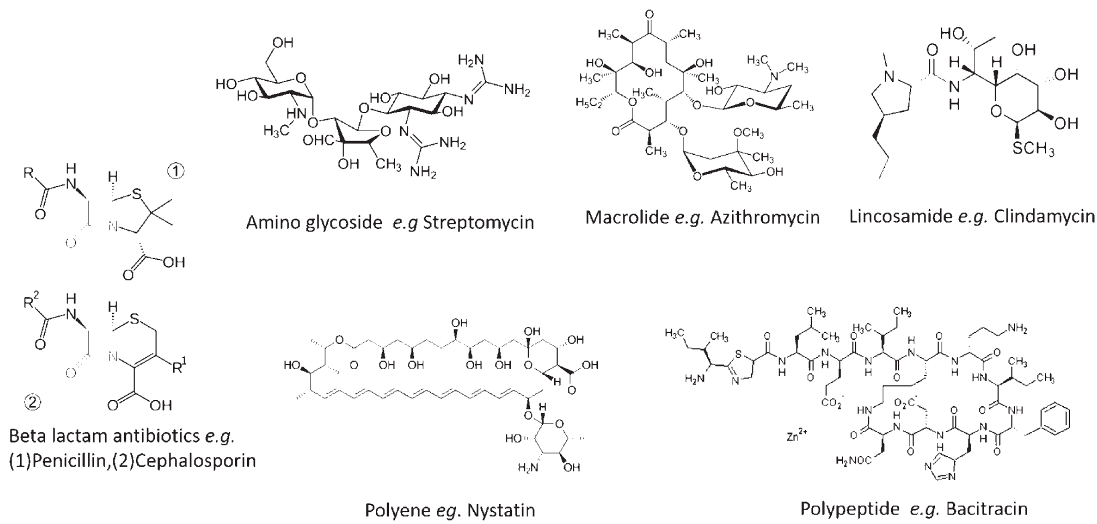

Fig 4. Various classes of antibiotics based on their chemical structures.

Although first naturally occurring penicillin was isolated from *Penicillium notatum*, it is currently isolated from high yielding strain *Penicillium chrysogenum*. Bacterial infections that may be treated with penicillin include pneumonia, sore throat, meningitis, syphilis and gonorrhea.

In 1942, another important antibiotic streptomycin was isolated by Waksman's group that can be used for treatment of pulmonary tuberculosis, 60 years after Robert Koch found in 1882 that tuberculosis is caused by bacteria *Mycobacterium tuberculosis*. The microorganism used for streptomycin production is *Streptomyces griseus*.

Production of amino acid by fermentation: Amino acids play central role as building blocks in protein synthesis. For more than 50% of the amino acids, fermentation is the favored process for industrial production (Table 1). Amino acids are produced as a primary metabolite by the microorganisms.

90% of world's demand of glutamic acid is met by microbial fermentation. The bacterial strain used is *Cornybacterium glutamicum*. Biosynthetic pathway for lysine production, on the other hand, is more complex and certain improvements have been made in strains of *C.glutamicum* and *B. flavum* for industrial production of lysine.

Table 1. Estimated global production of amino acids (1996) *

Amino acid	Amount (ton/y)	Process	Uses
L-glutamate	1,000,000	Ferm	Flavor enhancer
D, L Methionine	350,000	Chemical	Food, Feed Pharm
L-Lysine HCL	250,000	Ferm	Feed Supplement
Glycine	22,000	Chemical	Pharm, soy sauce
L-Phenylalanine	8,000	Ferm, Synthesis	Aspartame
L-Aspartic acid	7,000	Enzymatic	Aspartame, Pharm.
L-Threonine	4,000	Ferm	Feed supplement
L-Cysteine	1,500	Extraction, Enzyma.	Pharm
D, L –Alanine	1,500	Chemical	Flavor, sweetener
L- Glutamine	1,300	Ferm.	Pharmaceuticals
L-Arginine	1,200	Ferm.	Flavor, pharm.
L- Tryptophan	500	Ferm., Enzymatic	Feed suppl., Pham
L – Valine	500	Ferm.	Pharmaceuticals
L –Leucine	500	Ferm, Extraction	Pharmaceuticals
L –Alanine	500	Enzymatic	Pharm.
L –Isoleucine	400	Ferm	Pharmaceuticals
L – Histidine	400	Ferm.	Pharmaceuticals
L – Proline	350	Ferm	Pharmaceuticals
L – Serine	200	Ferm	Pharmaceuticals
L - Tyrosine	120	Extraction	Pharmaceuticals

*Adapted from Ikeda M. 2003 Adv. Biochem. Eng. Biotech 79:1-35

Production of vitamins: Vitamins are the essential micronutrients required by organisms in very small quantities for proper functioning of its metabolism. Some notable vitamins produced industrially by fermentation are Vitamin B2, B12 and Vitamin C.

For Vit B2 production by fermentation, three microorganisms are currently used: filamentous fungus *Ashbya gossypii*, yeast *Candida famata* and genetically engineered strain of *Bacillus subtilis.*

Pseudomonas dentrificans is the most productive microbe for Vit B12 production.

For industrial scale production of vitamin C, Reichstein–Grüssner process was first used where D-sorbitol is converted to L-ascorbic acid using a fermentation step (from D-sorbitol to L-sorbose by *Gluconobacteroxydans*) and several chemical steps (from L-sorbose to L-ascorbic acid). Currently, a two-step fermentation process is widely used where a mixed fermentation consisting *Ketogulonicigenium vulgare* and *Bacillus spp.* is conducted to convert L-sorbose to the intermediate 2-keto-L-gulonic acid (2-KLG) in the two-step fermentation process. (Fig 5).

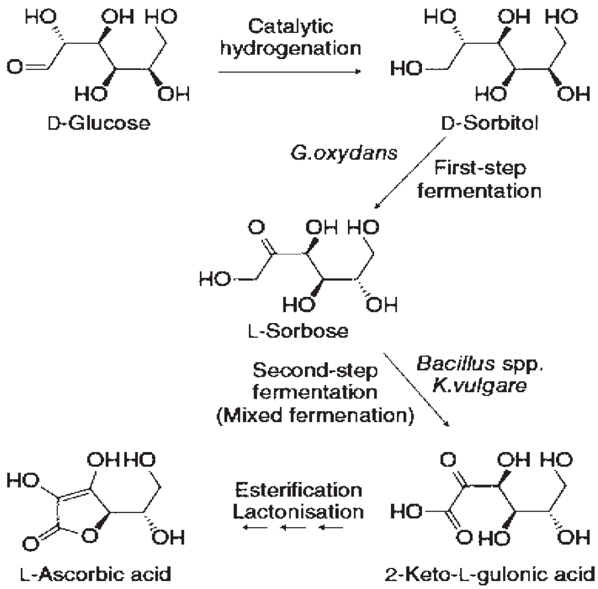

Fig. 5 Current method for industrial production of Vitamin C (fig adopted from Ref. 5)

Process of fermentation has been in use since prehistory and continues to be a very important process for industrial production of a range of products from food, beverages, chemicals to vitamins, amino acids and antibiotics. Starting from the works of Louis Pasteur till date, scientists have got better and better understanding of the molecular mechanisms behind the fermentation process and have been able to engineer the process more effectively.

References and further reading

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