Module-2: The Range of fermentation processes

Fermentation Processes can be classify into five different categories

1. Processes Producing Microbial Enzymes
2. Processes Producing Microbial Metabolites
3. Processes Producing Microbial Cells (Biomass) as the Products
4. Processes Producing Recombinant Products
5. Processes modifying Substrates (Transformation Process)

1. Processes that produce microbial enzymes

- Microbes, plants and animal are the major source of enzymes
- Commercial production of many enzymes exploiting these sources have been achieved
- As being produced in large quantities by the fermentation processes, microbial enzymes have the enormous economic potential
- Microbes are more prone to change in its genetics to enhance its productivity compared to plant or animal system
- It is possible to produce enzymes of eukaryotes into the prokaryote systems with the help of recombinant DNA technology
- It is possible to control and improve microbial enzyme production by introducing inducers and activators in the production medium
- It is also possible to increase the copy number of gene coding for the a specific enzyme using principles of recombinant DNA technology
<table>
<thead>
<tr>
<th>Industry</th>
<th>Applications</th>
<th>Enzymes</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baking &amp; Milling</td>
<td>• Reduction of bulk viscosity</td>
<td>Amylase</td>
<td>Fungal</td>
</tr>
<tr>
<td></td>
<td>• Acceleration of fermentation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Increase in loaf (unoccupied) volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Improvement of crumb (fragments) softness</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Maintenance of freshness</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Improvement of dough texture, reduction of mixing time, increase in loaf volume</td>
<td>Protease</td>
<td>Fungal/Bacterial</td>
</tr>
<tr>
<td>Brewing</td>
<td>Mashing (Crushing, Squashing, Smashing)</td>
<td>Amylase</td>
<td>Fungal/Bacterial</td>
</tr>
<tr>
<td></td>
<td>Chill proofing</td>
<td>Protease</td>
<td>Fungal/Bacterial</td>
</tr>
<tr>
<td></td>
<td>Improvement of fine filtration</td>
<td>β-glucanases</td>
<td>Fungal/Bacterial</td>
</tr>
<tr>
<td>Cereals</td>
<td>Precooked baby foods, breakfast foods</td>
<td>Amylase</td>
<td>Fungal</td>
</tr>
<tr>
<td>Chocolate &amp; Coca</td>
<td>Manufacture of syrup</td>
<td>Amylase</td>
<td>Fungal/Bacterial</td>
</tr>
<tr>
<td>Coffee</td>
<td>Coffee bean fermentation</td>
<td>Pectinase</td>
<td>Fungal</td>
</tr>
<tr>
<td></td>
<td>Preparation of coffee concentrates</td>
<td>Pectinase</td>
<td>Fungal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hemicellulase</td>
<td></td>
</tr>
<tr>
<td>Confectionery</td>
<td>Manufacture of soft Centre candies</td>
<td>Invertase</td>
<td>Fungal/Bacterial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pectinase</td>
<td></td>
</tr>
<tr>
<td>Corn syrup</td>
<td>• Manufacture of high maltose syrups</td>
<td>Amylase</td>
<td>Fungal</td>
</tr>
<tr>
<td></td>
<td>• Production of low D.E. syrup</td>
<td>Amylase</td>
<td>Bacterial</td>
</tr>
<tr>
<td></td>
<td>• Prod. of low glucose from corn syrup</td>
<td>Amyloglucosidase</td>
<td>Fungal</td>
</tr>
<tr>
<td></td>
<td>• Manufacture of fructose syrup</td>
<td>Glucose isomerase</td>
<td>Bacterial</td>
</tr>
<tr>
<td>Category</td>
<td>Process</td>
<td>Enzymes</td>
<td>Type</td>
</tr>
<tr>
<td>---------------</td>
<td>-------------------------------------------------------------------------</td>
<td>------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Dairy</td>
<td>Manufacture of protein hydrolysates</td>
<td>Protease</td>
<td>Fungal/Bacterial</td>
</tr>
<tr>
<td></td>
<td>Stabilization of evaporated milk</td>
<td>Protease</td>
<td>Fungal</td>
</tr>
<tr>
<td></td>
<td>Production of whole milk concentrates, ice-cream &amp; frozen desserts</td>
<td>Protease</td>
<td>Yeast</td>
</tr>
<tr>
<td></td>
<td>Curdling milk</td>
<td>Protease</td>
<td>Fungal/Bacterial</td>
</tr>
<tr>
<td>Eggs, dried</td>
<td>Glucose removal</td>
<td>Glucose oxidase</td>
<td>Fungal</td>
</tr>
<tr>
<td>Fruit juices</td>
<td>Clarification</td>
<td>Pectinases</td>
<td>Fungal</td>
</tr>
<tr>
<td></td>
<td>Oxygen removal</td>
<td>Glucose oxidase</td>
<td>Fungal</td>
</tr>
<tr>
<td>Laundry</td>
<td>Detergents</td>
<td>Protease, Lipase</td>
<td>Bacterial</td>
</tr>
<tr>
<td>Leather</td>
<td>Dehairing, Baiting (attract, temp)</td>
<td>Protease</td>
<td>Fungal</td>
</tr>
<tr>
<td>Meat</td>
<td>Tenderization (Smoothining)</td>
<td>Protease</td>
<td>Fungal</td>
</tr>
<tr>
<td>Pharmaceutical</td>
<td>Digestive aids</td>
<td>Amylase, Protease</td>
<td>Bacterial</td>
</tr>
<tr>
<td></td>
<td>Anti-blood clotting</td>
<td>Streptokinase</td>
<td>Bacterial</td>
</tr>
<tr>
<td></td>
<td>Various clinical tests</td>
<td>Numerous</td>
<td>Fungal/Bacterial</td>
</tr>
<tr>
<td>Photography</td>
<td>Recovery of silver from spent film</td>
<td>Protease</td>
<td>Bacterial</td>
</tr>
<tr>
<td>Protein</td>
<td>Manufacture</td>
<td>Protease</td>
<td>Fungal/Bacterial</td>
</tr>
<tr>
<td>hydrolysates</td>
<td>Soft drinks</td>
<td>Glucose oxidase,</td>
<td>Fungal</td>
</tr>
<tr>
<td></td>
<td>Stabilization</td>
<td>Catalase</td>
<td></td>
</tr>
<tr>
<td>Textiles</td>
<td>Desizing (Reducing volume, Demassing ) of fabrics</td>
<td>Amylase</td>
<td>Bacterial</td>
</tr>
<tr>
<td>Vegetables</td>
<td>Preparation of purees and soups</td>
<td>Pectinase, Amylase, Cellulase</td>
<td>Fungal</td>
</tr>
</tbody>
</table>
2. Processes that produce microbial metabolites

The growth of a microbial culture can be divided into major four phases. These are.

1. Lag phase
2. Log phase
3. Stationary phase
4. Death phase

1. Lag phase

- Once the inoculation of the cells into fresh medium is done, the bacterial population remains temporarily unchanged
- There is no cell division during this phase
- The cells grow in volume and mass by synthesizing the population remains temporarily unchanged etc.
- Metabolic activity is at high rate
- This period is known as the period of adaptation
- There are various factors that affects the this phase are size of inoculum, time required to recover shock in the transfer, time required for synthesizing essential coenzymes and other factors
- Time required for synthesis of necessary new enzymes to metabolize the substrates present in the medium

2. Phase of exponential growth

- This period is also known as the phase of exponential growth
- During this period, the growth rate of the cells gradually increases
- The cells grow at a constant, maximum rate
- Cells are growing in geometric progression dividing by binary fission
- The incubation conditions and composition of the growth medium control the rate of cell division

3. Stationary phase

- During this phase growth cease
- In a batch culture (in test tube or EM flask), exponential growth cannot be continued forever
- Various factors like exhaustion of available nutrients, accumulation of inhibitory, metabolites or end products and lack of biological space limit the growth during this phase
- During this phase the number of dividing cells equals the number of dyeing cells
- This is not a quiescence period like lag
• This is the phase during which bacteria produce secondary metabolites, such as antibiotics

4. Death phase

• This phase is the reverse of the log phase
• The viable cell population declines exponentially during this phase

Based on the various products produced, the phases of bacterial growth can be categorized into two phases. These are

(i) The Trophophase

(ii) The Idiophase

1. Trophophase

• Metabolites which are essential to the growth of the cells like amino acids, nucleotides, proteins, nucleic acids, lipids, carbohydrates are produced during the log phase of the growth
• The products (metabolites) produced during this phase (log phase) are known as primary metabolites and the phase in which they are produced (equivalent to the log, or exponential phase) is referred to as the trophophase (Bu'Lock et al., 1965)
• The primary metabolites are also known as central metabolites
• Several primary metabolites are of economic importance and can be produced in large quantity by fermentation process
• The synthesis of primary metabolites by wild-type micro-organisms aims to meet the requirements of the organism
• The industrial production these metabolites can be achieve by providing appropriate cultural conditions to the wild-type organism to increase and improve the productivity of these compounds
• Productivity can also be improve by modifying interested genes by the help of recombinant DNA technology
• Following are few economically important primary metabolites which can be produced at large scale
<table>
<thead>
<tr>
<th>Primary metabolite</th>
<th>Commercial importance</th>
</tr>
</thead>
</table>
| Ethanol            | 'Active ingredient' in alcoholic beverages  
                     | Used as a motor-car fuel when blended with petroleum                                    |
| Citric acid        | Various uses in the food industry                                                     |
| Glutamic acid      | Flavour enhancer                                                                       |
| Lysine             | Feed supplement                                                                        |
| Nucleotides        | Flavour enhancers                                                                      |
| Phenylalanine      | Precursor of aspartame, sweetener                                                     |
| Polysaccharides    | Applications in the food industry, Enhanced oil recovery                                |
| Vitamins           | Feed supplements                                                                       |

2. Idiophase

- During the stationary phases several microbial cultures produce certain compounds (these compounds are not produced during the “trophophase” and which do not appear to have any obvious function in cell metabolism). These compounds are called the secondary compounds of metabolism. The phase during which these compounds are produced (equivalent to the stationary phase) as the “idiophase” (Bu'Lock et al., 1965)
- The secondary metabolism is also known as “special metabolism”
- The products of secondary metabolism are not absolutely required for the survival of the organisms
- All microorganisms do not undergo secondary metabolism. It is common amongst the filamentous bacteria and fungi and the spore forming bacteria
- The taxonomic distribution of secondary metabolism is different from that of primary metabolism
- The physiological role of secondary metabolism and hence secondary metabolites in the producer cells has been the subject of considerable debate
- The large scale production of secondary metabolites focus on the importance of these metabolites on organisms other than those that produce them
- Secondary metabolites play an important physiological role several ways. Many secondary metabolites possess antimicrobial activity, some acts as specific enzyme inhibitors and growth promoters and many have pharmacological properties
- Thus, due to a huge economic potential, the industrial production of these metabolites have formed the basis of a number of fermentation processes
• As the wild-type microorganisms produce very low concentrations of secondary metabolites, the large scale synthesis can be controlled by induction, catabolite repression and feed-back systems
• Following is the outline of inter-relationships between primary and secondary metabolism and their respective products

3. Processes that produce microbial cells (or biomass) as the product

The commercial microbial biomass production can be divided into two major processes:

1. The production of yeast to be used in the baking industry and
2. The production of microbial cells which can be used as human and/or animal food (single-cell protein)

• Bakers' yeast has been produced on a large scale since the early 1900s and yeast was produced as human food in Germany during the First World War
• However, it was not until the 1960s that the production of microbial biomass as a source of food protein was explored to any great depth
• A few large-scale continuous processes for animal feed production were established in the 1970s. These processes were based on hydrocarbon feedstocks which could not compete against other high protein animal feeds, resulting in their closure in the late 1980s

4. Recombinant products

• Recombinant DNA molecules are also known as chimeric DNA, as they consist genes (DNA) of two different species
• The nucleotide sequences used in the construction of recombinant DNA (rDNA) molecules can be from any species. For instance, plant or human DNA may be combined with bacterial DNA, or human DNA may be joined with fungal DNA
• Genes from higher organisms can be inserted into microbial cells in such a way that the recipients are capable of synthesizing foreign proteins
• The advancement in the application of rDNA technology has made possible to produce a range of recombinant products by the fermentation process
• A wide range of microbial cells have been used as hosts for such systems including Escherichia coli, Saccharomyces cerevisiae and filamentous fungi
• Recombinant DNA is widely used in research, agriculture, medicine and biotechnology
• Several products that result from the use of rDNA technology are found in almost every pharmacy, medical testing laboratory, doctor’s as well as and veterinarian’s office, and biological research laboratory
• Following are the recombinant products that produced by genetically engineered organisms organisms
✓ Human Growth Hormone (rHGH)
✓ Biosynthetic Human Insulin (BHI)
✓ Envelope protein of the Hepatitis B virus
✓ Follicle Stimulating Hormone (FSH)
✓ Blood clotting Factor III
✓ Erythropoietin (EPO)
✓ Granulocyte Colony-Stimulating Factor (G-CSF)
✓ Alpha-galactosidase
✓ Alpha-L-iduronidase
✓ N-acetylgalactosamine-4-sulfatase
✓ Dornasealfa
✓ Tissue Plasminogen Activator (TPA)
✓ Glucocerebrosidase
✓ Interferon (IF)
✓ Insulin-like growth factor 1 (IGF-1)
✓ Bovine somatotropin (bST)
✓ Porcine somatotropin
✓ Bovine chymosin

5. Processes modifying substrates (Transformation Process)

- Many microbial cells may be exploited to convert a compound into a structurally related, financially more valuable compounds
- As microbes can behave as catalysts with high positional specificity and stereo-specificity, microbial processes are more specific than purely chemical ones
- These microbial processes enable the removal, addition and/or modification of various functional groups at predefined specific sites on a complex molecule without the use of chemical protection
- The reactions which may be catalyzed include Dehydrogenation, Oxidation, Hydroxylation, Dehydration and Condensation, Decarboxylation, Amination, Deamination and Isomerization
- As microbial processes can be operated at a relatively low temperatures and pressures have the additional advantage over chemical processes which require high temperatures, more pressures and presence of heavy-metal catalysts-a potential environmental pollutant
- Production of vinegar is the most well-established microbial transformation process (conversion of ethanol to acetic acid)
- Many transformation processes have been rationalized by immobilizing either the whole cells, or the isolated enzymes on an inert support which catalyze the reactions
- The immobilized cells or enzymes may be reused many times
References

- **Industrial Microbiology**: (By Casida L. E.New Age international (P) ltd publications)
- **A Text Book of Industrial Microbiology**: (2nd edition By Wulf Crueger & Anneliese Crueger)
- **Biotechnology**: Food Fermentation Microbiology, Biochemistry & Technology Vol. 1 & 2:(By V.K. Joshi & Ashok Pandey)
- **Industrial Microbiology-An introduction**: By Michael J. Waites, Neil L. Morgan, John S. Rockey and Gary Highton)
- **Comprehensive Biotechnology-The Principles, Applications and Regulations of Biotechnology in Industry, Agriculture and Medicine**: (By Murray Moo Young)
- **Fermentation Technology**: Up Stream Fermentation Technology- Vol-I: (By H. A. Modi-Pointer Publications)
- **Fermentation Technology**: Down Stream Fermentation Technology- Vol-II: (By H. A. Modi-Pointer Publications)
- **Industrial Microbiology by Prescott and Dunn's**: (4th edition, edited by Gerald Reed, CBR publications)
- **Fermentation Technology**: (By M.L. Srivastava, NAROSA publications)
- **Industrial Microbiology**: (By A.H. Patel)
- Bacteriological Techniques: (By F.J. Baker)
- Introduction to Microbial Techniques: (By Gunasekaran)

Web references

- [http://www.homebrew.net/ferment/](http://www.homebrew.net/ferment/)
- [http://scialert.net/fulltext/?doi=jm.2007.201.208](http://scialert.net/fulltext/?doi=jm.2007.201.208)
- [http://aem.asm.org/content/7/1/57.full.pdf](http://aem.asm.org/content/7/1/57.full.pdf)
- [http://www.slideshare.net/yongkangbirdnest/lecture-4-sterilization](http://www.slideshare.net/yongkangbirdnest/lecture-4-sterilization)
- [http://www.wiley-vch.de/books/sample/3527318194_e01.pdf](http://www.wiley-vch.de/books/sample/3527318194_e01.pdf)
- [www.vobrew.co.uk/fermentation.php](http://www.vobrew.co.uk/fermentation.php)
- [http://download.bioon.com.cn/upload/month_0902/20090223_b809d1c59ba2a6e2abfdJtWJOgFدم02.attach.pdf](http://download.bioon.com.cn/upload/month_0902/20090223_b809d1c59ba2a6e2abfdJtWJOgFدم02.attach.pdf)
- [http://www.rsc.org/ebooks/archive/free/BK9780854046065/BK9780854046065-00001.pdf](http://www.rsc.org/ebooks/archive/free/BK9780854046065/BK9780854046065-00001.pdf)
- [http://www.biotchreresources.com/services-strain.shtml](http://www.biotchreresources.com/services-strain.shtml)
- [http://www.idosi.org/wjc/4%281%2909/14.pdf](http://www.idosi.org/wjc/4%281%2909/14.pdf)
- [http://cheserver.ent.ohiou.edu/Paper-gu/DualFeed.pdf](http://cheserver.ent.ohiou.edu/Paper-gu/DualFeed.pdf)