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Enzyme Engineering

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Bioprocess Technology Governs Enzyme Use and Production in Industrial Biotechnology: An Overview

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Abstract

The present paper describes the bioprocess strategies involved in the application and production of enzymes from industrial biotechnology perspectives. Several enzymes are produced from different microbial sources and there are several chemical reactions occur with the help of enzymes within very short time. Bioprocess technology is the key factor to use enzymes in different purposes and also produce under different circumstances. Therefore, it is very important to discuss the synopsis of these issues on a same platform for future references.

Keywords: Bioprocess; Enzyme; Marine; Bioreactor; k_La; Biochemical; Kinetics

Introduction

Catalyst in a chemical reaction used to fast the process. Enzymes are using as biocatalyst. Enzymes are proteins that used to fast the reaction rate to form product. Enzymes are available from different sources such as from extreme environment or from normal microorganism and also from different species. Therefore, enzymes are the key factor to build and maintain the cells such as bacteria or fungi and also all living organisms including human beings. Enzymes are so called very small machines evolved millions of years to perform very specific biochemical tasks. Some enzymes have been designed by nature to build chemical compounds and others are either breaking them or modifying them over time. These breaking or modifying bond between molecules called reactions. Enzymes are used as catalyst for the reaction so that the reaction is very fast (million times faster than normal reactions) and effective.

Enzymes are very specific with the substrate and once if enzymes have the right substrate to act then the process becomes very faster. This system has been described by lock (enzyme) and key (substrate) model. In the old time, people use natural process by using hand to make yoghurt without knowing the enzyme involved in the process. In this line, enzyme engineering was developed to use enzymes in industrial processes and also production of enzymes. Not surprisingly, industries were among the first to recognize and use this most potential of enzymes in different fields. The first field was food industry where enzymes have a big impact such as beer, milk, cheese, bread, juice industry and later the process have been developed to other sectors such as detergents, pharmaceutical industry.

Today the global market for industrial enzymes is growing rapidly and is currently worth more than two billion euro per annum where the most potential sectors are detergent, starch processing, food and feed industries and the recovery of oil and gas. Finally, enzyme using is very efficient, most cost effective, less time consuming or the fastest process in the industry, and moreover, it is regarded as green or environmental friendly technology ever.

Bioprocessing for enzyme engineering

Bioprocessing for enzyme engineering is a tool that provided the knowledge base for fundamental development of innovative approaches or methods from research area. Several processes used for food or feed beverages, biofuels company, pharmaceutical companies will also provide the technological solutions for upgrading processes and also look for value added compounds. These also provide the knowledge of kinetics reaction/s, enzyme robustness in several industrial actions and on complex substrates. Finally, several new enzymes can also be developed for different process in food, biorefinery, agricultural and pharmaceutical applications.

Enzyme application

With the advent of new frontiers in biotechnology and bioprocess engineering, the spectrum of enzymes application has widened in many different fields, such as medicinal, food and additives, brewing, distilling, clinical and textiles industries. Enzymes in the bioprocessing are fast and effective technology to get quick and efficient product. There are several industrial processes where enzymes use in commercial purposes. Proteases in face cream for hair removing, amylases in brewing to germinate barley, rennin in cheese production for coagulation are some specific example of enzyme used in commercial purposes. Enzymes are most preferable in industries for various advantages. Enzymes always use as a catalyst in many industries in a small quantity in a reaction and make a pure product. Therefore, there is no requirement for extraction of by-product from the desired pure product. All the reactions with enzymes in general work in normal condition under low temperature, neutral pH and normal atmospheric pressure which saves time, cost and therefore, energy.

Biochemical reaction engineering principles are the main platform for using enzymes. Bioprocesses especially fermenters are the best possible process to use or produce enzymes. Enzymes can be used to get volumetric mass transfer coefficient (k_La) experimentally in any volume of the reactor to check the performance end efficiency of the process. Enzyme-catalyzed reaction is also related to the basic concept of transport phenomena. Enzyme kinetics is the example for the reaction of substrate and enzyme to form product under different condition [1]. Enzymes are working in the cytoplasm under a stable environment of the cell called intracellular and those secreted from

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the cell and work in the surrounding environment called extracellular. Extracellular enzymes are most robust because they have wide range of pH and temperature and therefore much cheaper and therefore, long shelf life for commercial use. All the enzymes used in different sector are summarized in (Table 1).

As discussed before, the industrial enzymes are used mostly in food industry. First time glucose is produced from starch in 1960s with the use of glucoamylase with a greater yield and higher purity with easier crystallization technology. This application replaced traditional acid hydrolysis method and the steam cost reduced by 30%, ash production by 50% and by-product by more than 90%. In 1973 immobilized glucose isomerase was invented (high fructose corn syrups from starch). Furthermore, amylase was also used to increase the bread, cake shelf life and beta-galactosidase in the production of cheese and other dairy products. Industrial enzymes have also a big impact on beverage industry. In 1930 RohM & Haas launched the pectinase (Pectinol K) in the market for higher yield of juice products (apple juice). Now-a-days detergent industry is the largest user of industrial enzymes (proteases, lipases, amylases and cellulases). These enzymes are using based on their efficiency to remove dirt from the cloths quickly under lower temperature which save energy and prevent wastage of lot of chemical detergents over a longer time period under higher temperature. In 1960s research scientists found the great potential of using enzymes in the field of medical diagnostics, food and beverage analysis. Several test kits and reagents are produced from different industry for different analysis. Reagents for medical diagnostics have been developed and manufactured in different industry (Roche, Trinity Biotech and Merck). Dairy, food and feed beverage analysis test kits and reagents are also developed and manufactured from different industry (Megazyme, Boehringer, and Merck). Several microorganisms have been selected to get different flavor compounds by enzymatic methods [2]. New advanced literatures or studies have shown the current progress in the application of the enzyme engineering (Table 2) [3].

Enzyme production

In the other hand, enzymes are produced from the microbes in fermenter and the major producer of industrial enzymes is Novozymes [1]. One of the examples is that, on-site enzyme productions in biorefineries have always some optimal strategy for lowering the enzyme conversion cost. There are known industrial enzyme producers such as *Aspergillus niger*, *Aspergillus oryzae*, *Tricoderma reesei* [4-6]. The first enzyme produced industrially were an amylase in 1894 from fungal microorganism which was used as a pharmaceutical product for the digestive disorder treatment [7]. Bacillus species are considered to be the most important sources of amylase and have been used for enzyme production using solid state fermentation (SSF). SSF is used to produce glucoamylase using *A. niger* [8,9]. Producing enzymes in bioprocess technology has been performed by SSF and liquid fermentation. But in both conditions, a strict rule is followed known as fermentation process such as sterile bioreactor to avoid contamination, sterile air to aerate, with a constant stirring speed for bacterial or fungi growth or maximize yield, maintain a constant pH and constant temperature for cooling effect to enzyme denaturing effect and finally nutrient requirements for energy and protein required for cell growth and maximize yield.

Therefore, enzymes that stable and work quickly are preferred most. Hot springs bacteria are most preferred due to this reason. The enzymes from hot springs are thermostable and can tolerate a wide range of pH variation and therefore use efficiently. Extracellular industrial enzymes are extracted from the microbes by centrifuging or filtering and then evaporate the water or using ultra filtration method and finally dried for the customer. On the other hand, intracellular enzymes are much more expensive and very difficult to obtain.

Most enzymes are produced by submerges cultivation in fermentor/s. Industrial enzymes production process is commonly

Enzyme/s	Sector/Industry	Potential market value
Lipase, Protease, Cellulase, Amylase	Detergent (Novozymes)	$2.767 M by 2019 (http://www.marketsandmarkets.com/PressReleases/protein-hydrolysis-enzymes.asp)
Amylase, Glucoamylase, Glucose Isomerase	Processing Starch (Novozymes in Denmark, DuPont in U.S., Rouquette Freres in France etc.)	$2.238 M by 2018 (http://www.prnewswire.com/news-releases/alcohol-and-starchsugar-enzyme-market-by-type-carbohydrase-protease-and-lipase-by-application-industrial-specialty--geography---global-trends--forecasts-to-2018-256229371.htmlV)
Protease, Phytase, Xylanase	For animal feed (Royal DSM NV)	$1.371 M by 2020 (http://www.marketsandmarkets.com/PressReleases/feed-enzyme.asp)
Xylanase	Pulp and Paper (Novozymes)	200 M (www.novozymes.com)
Arbinanase, Amylase, Polygalacturonanase	Processing of fruits or vegetables (Novozymes, DSM)	Food enzymes: $ 2.3 B by 2018 (http://www.iisc.ernet.in/currsci/jul10/articles22.htm)
Hydrolase	Oil and Gas (Novozymes)	$330 B by 2015 (Petrochina, China)
Chymosin	Dairy (Pfizer, Chr. Hansen)	Food enzymes: $ 2.3 B by 2018 (http://www.iisc.ernet.in/currsci/jul10/articles22.htm)
Urease, Pectinase	Wine (Carlsberg)	Feed enzymes: $ 1.37 M by 2020 (http://www.marketsandmarkets.com/PressReleases/feed-enzyme.asp)
Protease	Meat (Novozymes)	Feed enzymes: $ 1.37 M by 2020 (http://www.marketsandmarkets.com/PressReleases/feed-enzyme.asp)
Amylase	Textiles (Novozymes)	$2.7 M by 2019 (www.marketsandmarkets.com)
Amylase	Baking (Novozymes)	$695.1 M by 2019 (www.marketsandmarkets.com)
Beta-glucanase	Brewing (Carlsberg)	Feed enzymes: $ 1.37 M by 2020 (http://www.marketsandmarkets.com/PressReleases/feed-enzyme.asp)
Protease	Tanning (Novozymes)	Feed enzymes: $ 1.37 M by 2020 (http://www.marketsandmarkets.com/PressReleases/feed-enzyme.asp)

Table 1: Enzymes are using in different commercial applications.

Industry product/s	Enzyme	Known Company	Application/Benefits
Detergents	Proteinases	Novozymes, Genencor	Remove protein based stains in fabrics into soluble amino acids.
	Lipases		Digest oils and fats like butter, sauces etc.
	Amylases		Remove resistant starch residues.
	Cellulases		Remove the fuzz and microfibers that gives fabric a glossier appearance and soften the cotton.
Starch	Amylases, amyloglucosidases and glucoamylases	Novozymes	Help to convert starch to glucose and other sugar syrups
	α-amylases		Increasing maltose and glucose content.
	β-amylases		Producing low molecular weight carbohydrates for example, maltose.
	β-glucanases		Improving wort seperation.
	Pillulanases		This secure maximum fermentability of the wort.
	Glucose isomerases		Help to convert glucose to fructose
Dairy	Lipases, chymosin,lysozymes, Rennin	Chr. Hansen	Cheese manufacturing
	Lactases, β-galactosidases		Used for breaking lactose to glucose and galactose in milk processing to avoid lactose intolerance.
Juice	Amylases, glucoamylases	AB Enzymes	Help to break starch into glucose.
	Laccase		Susceptibility of browning during storage increased.
	Naringinase and limoninases		Cause bitterness in citrus juices.
	Cellulases, hemicellulases		Maintain the texture and lower the viscosity.
	Pectinases		Increasing the juice production.
Textiles	Amylases	Genencor	Used to remove starch from woven fabrics
	Cellulases		Fuzz and microfibers remove from fabric to give glossier and smoother appearance.
	Laccases, glucoseoxidases		Creating bleaching agent in whiteness.
	Pectinases		Reduce the outer cell layer to improve fiber extraction.
Cosmetic	Oxidases, peroxidases, polyphenol oxidases	DSM	Hair dyeing.
	Papain, bromelain, subtilisin		Peeling effects in skin care.
	Amyloglucosidase, glucose oxidases		Toothpaste and mouthwash.
	Transglutaminases		Hair waving.
Brewing	α-acetolactate-decarboxylases (ALDC)	Carlsberg	Fermentation time reduced where prevent of diacetyl formation and make the beer test correct.
	Amylases, glucanases, proteinases		Make polysaccharides and proteins in malt.
	Pentosanases, xylanases		Improving extraction and beer filtration process, hydrolyze pentosans of malt, barley and wheat.
	Proteinases		Improve the yeast growth and reducing the clouding of beers.
	Amyloglucosidases		Help for low calorie beer production ("light beer") and increasing the glucose content.
	β-glucanases		Used for improvement of filtration
Baking	Lipases	Novozymes	Gas cells stability improved in dough.
	Oxidoreductases		Gluten strength is improved.
	α-amylases		Degrading starch (catalyses) in flours and bread structure is controlled.
	β-xylanases		Using for control the handling and stability issues of dough and rising bread.
	Proteinases (Biscuit)		Reduces the protein in flour
Leather	Proteinases	Novozymes	Trypsin is used here to treat the leather with proteinase to make it more pliable. (process is known as bating)
Pulp and Paper	Mannanases	AB Enzymes	Increasing brightness by degrade residual glucomannan.
	Amylases		Cleaving the starch molecules which reduce viscosity for surface sizing in coatings.
	β-xylanases		Enhance the pulp-bleaching efficiency.
	Cellulases		Make flexible fibers and softness improved by cellulose hydrolyzing.
	Laccases		Improving brightness by bleaching.
	Lipases		Reduces or control pitch for stickiness of paper in pulping processes.

Table 2: Current progress in the application of enzyme engineering [3] is divided in maincategories and corresponding one known company is assigned with their applications.

follow the basic approach. The approach is described in short and summarized as follows:

1. Enzyme selection,
2. Production strain selection,
3. Using genetic engineering, overproducing strain can be constructed,
4. Medium optimization and process parameters conditions,
5. Downstream process (recovery) optimization, and

6. Production of stable and efficient (long shelf life) enzyme (Formulation).

In addition, after fermentation the broth is stored in a tank and micro filtrate the broth. After this stage, the broth is send to homogenizer and then to centrifugation. It is also known that ultra filtration can be used here. After the ultra filtration, freeze drying is the important last step is used commonly. There are several other unit operations and processes in the formulation and the process stages can be changed according to the need (Figure 1).

Batch, Fed-batch and continuous cultivations are three major mode of operation for production of enzymes mainly from marine bacteria and fungi in the laboratory and pilot plant scales. Marine bacteria are used in batch mode under different processes such as submerged, immobilized and solid-state. But continuous process is used only with submerged or with immobilized cells. Marine enzymes are unique in terms of stability and having novel properties because they found from extreme environment. Apart from microorganisms such as bacteria and fungi, marine organisms like fishes, prawns, snakes, algae and other species were also studied to get the enzymes from different environment like salt tolerance or cold adaptivity and other extreme conditions [10].

The industrial enzymes are produced from different organisms and their production processes are summarized in the following (Table 3).

Scale-up and optimization of enzyme production processes at the pilot scales was also discussed based on 600-L fermenter where the economical process for enzyme production has been achieved [11,12]. Scale-up issues is very important in commercial purpose from laboratory scale fermenters. Process optimization for the scale-up required the medium optimization to determine the effect of various defined ingredients as well as the complex nitrogen sources on enzyme production. Other fermentation conditions such as inoculum transfer, agitation, temperature for cultivation have also effect on optimized enzyme production for synthetic use.

Quality Control (QC) of Enzymes

Performance of enzymes can be tested in lab scale based on the efficiency, stability and robustness. Industrially, microorganisms are sorted based on high throughput screening (HTS) producing specific enzymes. HTS plays a crucial role in this phase where large numbers of enzymes are exposed to the particular target like as detergent. Few enzymes showing in terms of degree of confidence are processed further as lead enzymes for laboratory scale and finally one or two enzyme process to the industrial scale. The experiments are performed in the laboratories and the positive results are then optimized in terms of potency and selectivity. Physiology of the bacteria and the industrial enzyme produced from it should be very robust and stable for long shelf life for future use for the customers.

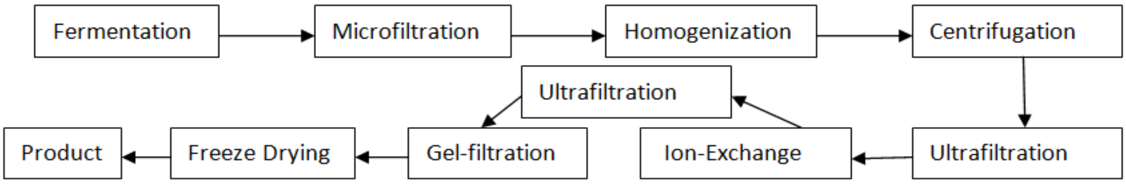

Figure 1: Block diagram to illustrate the enzyme production process.

Source	Enzymes	Microorganisms	Method/s
Fungal	Amylase	*Aspergillus oryzae, Trichoderma longibrachiatum*	Submerged cultivation (Batch, Fed-batch and Continuous)
	Glucosidases	*Aspergillus flavus*	
	Proteases	*Aspergillus niger*	
	Pectinases	*Aspergillus niger*	
	Glucose oxidase	*Penicillium notatum*	
	Catalase	*Aspergillus niger*	
	α- Galactosidase	*Aspergillus niger*	
	Cellulase	*Trichoderma longibrachiatum*	
	β-Glucanase	*Aspergillus niger, Trichoderma longibrachiatum*	
	Lipase	*Aspergillus niger*	
	Phytase	*Aspergillus niger*	
	Xylanase	*Aspergillus niger, Trichoderma longibrachiatum*	
Bacterial	Amylases	*Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus licheniformis*	
	β- Glucanase	*Bacillus subtilis*	
	Proteases	*Bacillus subtilis*	
	Penicillinase	*Bacillus subtilis*	
	Asperginase	*Escherichia coli*	
	Pullulanase	*Bacillus acidopullulyticus*	
	Maltase	*Bacillus subtilis*	
	Mannanase	*Bacillus leutus*	
	Xylanase	*Bacillus subtilis*	
Yeast	Invertase	*Saccharomyces cerevisiae*	
	Lactase	*Saccharomyces fragilis*	

Table 3: The industrial enzymes production from different sources [11].

Life cycle assessment (LCA) is an analytical tool developed to facilitate the measurement of the relative environmental impacts by comparing two or more processes. LCA has been applied different processes in oil and fats industry under different enzymatic bioprocesses to compare the environmental impact. Mainly LCA is using to compare different CO2 emission savings from different processes and very efficient tool to describe [13].

Conclusions

Enzymes are catalyzed all kind of chemical reactions. Industrial and household catalysis becomes more and more dependent on enzymes. Industrial enzymes with the desired activity can be obtained by optimizing bioprocess technology conditions and also by protein engineering. Enzyme engineering provides higher product quality, low manufacturing cost, low energy consumption and save time. This drives the market growth to enhance cost efficiencies and productivity which grow the interest among consumers or customers. In this paper several industrial and laboratory approaches have been summarized in terms of enzyme production, application and uses. These approaches will help us in future to get an overall idea about enzymes and can be useful for further research and development.

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