Review Article

AN EXTENSIVE REVIEW: INDUSTRIALLY IMPORTANT ENZYMES, THEIR CLASSIFICATION AND DEPICTING THE CONSORTIUM OF INDUSTRIAL APPLICATIONS

ABSTRACT

Microorganism has been used since the start of human society. Enzyme processing processes are quickly gaining the attention because of their short time of processing, cost effective, non-toxic, low energy input and environment friendly characters as well. Moreover, through protein engineering and recombinant DNA technology, a microorganism can be easily manipulated and cultured in large scale to meet increased demand in different sectors. Therapeutic enzymes have a huge variety of selective uses such as anticoagulants or thrombolytics, oncolytics and also as replacement for various metabolic deficiencies. Proteolytic enzymes are excellent anti-inflammatory agents. There are various factors that decrease the potential of microbial source enzymes, once we enter to medical sciences due to high molecular size of catalyst which stop their distribution within somatic cells. In industrial processes the quantity of enzymes should be high, while in therapeutic case the purity and specificity should be excellent, if the quantity level is less no matter. The kinetics of such enzymes is high and low so that it is maximally efficient even at low concentration of substrate and enzymes. The source of such enzymes should be designated with high care to minimize or prevent the chances of undesirable growth by mismatched material and also to enable ready purification.

INTRODUCTION

Microorganism has been used since the start of human society. The first reported microorganism is the uses of yeast to make alcoholic products from barley by the Sumerians and Babylonians as early as 6000 BC.Microbes enzymes catch their attention globally for their great application in distinguish sectors of industries, such as in chemical, energy, food, medicine and agriculture.

Enzyme processing processes are quickly gaining the attention because of their short time of processing, cost effective, non-toxic, low energy input and environment friendly characters as well. Moreover through protein engineering and recombinant DNA technology a microorganism can be easily manipulated and cultured in large scale to meet increased demand in different sectors. Related driving factors that enhance the use of microbes enzyme products in industrial uses are increasing demand of user, natural resources reduction, necessity of cost reduction and eco-friendly. World market for industrial microbial enzymes was projected nearly about $4.2-4.5 billion in 2014 and predictable to make at a compound AGR of nearly 8% over the time from 2016 to 2020 to influence $6.4 billion (Kour et al., 2019). Microbial enzymes are biological compounds, protein in nature except catalytic ribonucleic acid molecules and act as a catalyst to speed up the chemical reaction and to support almost all of the chemical reaction needed for the life (Gurung, Ray, Bose, & Rai, 2013). Enzymes are highly specific in nature; speed up the chemical reaction by decreasing the activation energy of the chemical reaction with any permanent change in the reaction, and therefore major molecules to support life (Anastas, Bartlett, Kirchhoff, & Williamson, 2000). For enzyme activity they need optimal temperature and pressure for catalyzing reaction, and are uses in substitute toxic chemical pollutant owing to their non toxic and biodegradable nature (Anastas et al., 2000).

In addition to advantages of enzymes over conventional methods, there are some drawbacks of using enzymes in healthcare and other industries. For many mammalian enzymes, 37 °C and 7.4 are the optimal temperature and pH, respectively, and their activity is highly sensitive to any change in these parameters. Higher temperature (>40 °C), and a large deviation from the physiological pH (7.4) lead to their denaturation, which limits the use of these macromolecules in non-physiological conditions. Additionally, they are susceptible to substrate or product inhibition and their products may cause allergic reactions. The high cost of isolation and purification of enzymes and their difficult recovery for subsequent reuse may discourage their use (Singh, Kumar, Mittal, & Mehta, 2016).

ENZYMES

Enzyme are large molecules consist of AA attached through amide NH2 bonds, range between kilo-Dalton to mega-Dalton in molecular mass. In biochemistry area a great advancement achieved after 1940, a huge number of enzymes were isolated and identified, for this purpose it was mandatory to legalize enzyme nomenclature. IUBMB in discussion with International Union of Pure and Applied Chemistry develop an enzyme Commission to be in charge of managing systemic classification and naming to enzymes (Almeida et al., 2010). Enzyme was classified into six different groups on the basis of the reaction they catalyzed. For industrial enzymes, microbes are the best choice because of their easily availability, and fast growth rate of microbes. Through recombinant DNA technology genetic changes can easily be done in microbes for the production of enzymes and scientific development (Liu, 2015). Microbial enzymes production is mandatory event in industrial zone, due to the loftier and great performance of enzymes from various microorganisms, because they performed well in wide range of chemical and physical condition (Anbu et al., 2015). Microbial enzymes help in the cure of many disorder linked with the absence of human enzymes caused due to genetic changes. Some enzymes are greater than the substrate they act on, and only a very small part of enzymes is directly involved in catalysis (Almeida et al., 2010). Enzymes also have a site that binds to the cofactors, which are required for catalysis of the reaction (Bartlett et *al.*, 2002). Like protein enzymes have linear chain of AA, long that fold to make a 3D products. Each AA sequence makes a selective structure, which has unique properties. Most enzymes are denatured that is outspread and deactivated by chemical or heating, which disrupt 3D structure of the protein. Denaturation may be irreversible or reversible dependent on the enzymes (Kotera et al., 2004).

Amylase enzyme

Amylase is an enzyme that break starch molecule into sugars. Amylase enzyme is commonly found in saliva that breakdown the food and help in digestion. Some foods contain high amount of starch and contain slight sugar, such as potato and rice, taste somewhat as they are masticated because amylase cracks a few of their starch into sugar in the human mouth. Pancreas also produces amylase which hydrolyze dietary starch into disaccharide carbohydrates and trisaccharides which are rehabilitated by other enzymes. Bacteria and some plants also produce amylase. In 1833 amylase was the first enzyme isolated and identified by Anselme Payen. Entirely amylases act on α-1, 4-glycosidic and are glycoside hydrolases. Amylases have nearly 25 % enzyme market and are widely used in industry (Edner et al., 2007). In the present area chemical hydrolysis of starch in starch processing industry is almost replaced by amylases. Amylases isolated from microbes are more stable than plants and animals and for that reason they have a broad spectrum of industrial uses. Microbes are easy to change their nature and desired enzymes, so the major benefit of microbes for the production of enzymes is the mass production and economical. In biotechnology sector starch hydrolyzing amylolytic enzymes are of high advantages ranging from paper, textile, fermentation and food industries. (Souza, 2010).

Types

Amylase has three sub-classes —*α*- *β*- *γ*-amylase.

***α*-Amylase**

Alpha amylase enzymes help in the hydrolysis or breakdown of the internal *α*-1,4-glycosidic linkages in low molecular weight compounds such as maltotriose, maltose and glucose (Sundarram & Murthy, 2014). *α*-Amylase are important form of amylase found in human, cow, sheep, and in other mammals (Butterworth et al., -2011). It is also present in those seeds of plants that comprise of starch as food reserve and is released by numerous fungi. Amylase is also present in high amount in saliva and pancreatic juice, each of which has itself is form of human amylase.

Uses of *α*-Amylase

α-Amylase is used in production of ethyl alcohol by break down the starch in grains into sugars. The intital phase in the making of high fructose corn syrup is the treatment of cornstarch with *α*-amylase, making a smaller chain of sugars known as oligosaccharides. Some microbial source alpha amylases help in removing starch detergent Alpha amylases from microbial sources are being used for the removal of starch detergent (Butterworth et al., 2011).

***β*-Amylase**

β-amylase is another form of amylases synthesize by plants, fungi and bacteria. The alternative name for β-amylase is 1, 4-*α*-D-glucan maltohydrolase; saccharogen amylase; glycogenase). B-amylase, working from a non-reducing end, catalyzes the breakdown of the 2nd *α*-1, 4 glycosidic bond, breaking two glucose units at the same time. *β*-amylase breaks the starch into maltose during the ripening of fruits, resulting in the sweet flavor of ripe fruits. α-amylase and β-amylase are found in seeds, β-amylase are inactive before the germination while proteases and α-amylase seen once germination has started. For the production of malt cereal grain amylase is the key. Different microorganisms also produce amylases that help in the degradation of extracellular starches. Animal cells and tissues do not have β-amylase, although microorganisms present in the gut of animal produce β-amylase.

Use of β-amylase

Both alpha and beta amylases are significant in making liquor and beer prepared from sugars consequent from starch. In microbial fermentation process, mushrooms ingest sugars and produce ethyl alcohol. In liquors and beer, sugars that exist at the start of fermentation have been formed by “squashing" grains or other starch cradles.

γ-Amylase

Gamma-amylase is an amylase which break α(1-4) glycosidic and α(1-6) glycosidic linkages at the non-reducing end of amylopectin and amylose, resulting in glucose. The γ-amylase has high acidic pH because they are highly active at pH 3. The alternative name for γ-amylase is 1, 4-α-glycosidase.

Uses γ-Amylase

γ-Amylase are used in chemical, pharmacological, drugs distribution and food industry, as well as environmental and agriculture engineering. Hydroxypropyl beta cyclodextrin is the main compound present in Gamble’s and Procter Freshening products.

Bacterial spp amylase

Amylase can be extracted from various species of microbes, but for commercial use, α-amylase derived from *Bacillus amyloliquefaciens*, *Bacillus licheniformis* and *Bacillus stearothermophilus*, has wide range of application in various industries such as in paper, textile, fermentation and food industries (Souza, 2010). Industrial enzymes has major and desirable characters known as thermostabality. Temperature stable enzymes have found huge number of commercial significance due to their stability in high temperature. The production of valuable industrial products such as glucose, dextrose syrup, maltose, crystalline dextrose and maltodextrins are improving by using thermo stable amylolytic enzymes (Guzmán- Maldonado et al., 1995).*Bacillus amyloliquefaciens*, *Bacillus subtilis*,*Bacillus licheniformis* and *Bacillus stearothermophilus* are widely used for industrial level production of enzymes for huge application. In present temperature resistance enzymes of *Bacillus licheniformis* or *Bacillus stearothermophilus* are used in starch degrading/-processing industry (Prakash & Jaiswal, 2010). In some industries high levels of harsh processes occur and contain huge amounts of salt solution which stop/ inhibit the many enzymatic reactions. For this purpose halophilic microbes have excellent activity at high salt concentration and enzymes produced could be used in such processes(Ventosa & Nieto, 1995). Besides such a good character, halobacterial enzymes are temperature stable and can survive for longer period of time at high temperature. Halophilic amylases have been resulting from bacteria such as and *Bacillus dipsosauri*, *Chromohalobacter* sp., *Haloarcula hispanica* (Caton et al., 2004) and *Halomonas meridian*(Edbei, 2016).

Amylase

Many researchers work on fungi to produce amylases and choose the best strains to produce amylase commercially. Previously, mesophilic fungi were reported that produce alpha-amylase.

Terrestrial isolates of fungi such as *Penicillium* and Aspergillus produce various kinds of enzymes (Kango et al., 2019). *Aspergillus* spp mainly make different varieties of extracellular enzymes, and amylases are the ones with most important industrial value. *Aspergillus niger*, and *Aspergillus oryzae* are filamentous fungi that produce huge amount of enzymes that can be greatly use in industry. *A. oryzae* is well-thought-out to be the satisfactory host for the manufacture of heterologous protein as it has capability to conceal a huge amount of high valuable proteins and industrial products (Machida et al., 2005). *A. oryzae* has been greatly used in commercial enzymes containing α-amylase and food such as soy sauce and organic acid such as acetic and citric acid (Hajar-Azhari et al.,2018). *A. niger* has a significant hydrolytic capacities in the α-amylase production, because of low pH/ acid tolerant, and they also avoid bacterial growth/contamination (Djekrif-Dakhmouche et al., 2006). Fungal enzymes have generally safe status and that's why they are preferred over other microbial enzymes. The high temperature resistance fungus *Thermomyces lanuginosus* is a great producer of amylase (Tiwari et al., 2015).

LIPASE

Lipases are enzymes that breakdown the fats. Lipases are the sub-class of the esterase's (Arreaza et al., 1997). These enzymes play a significant role in processing of dietary lipids, digestion and transport. Certain viruses also contain genes that encode lipases. Especially in the small intestine, most lipases enzymes act in a precise position on the glycerol backbone of lipid substrate (Rahman & Basri, 2006). Several other kinds of lipase activities present in nature, such as sphingomyelinases and phospholipase, however, these are frequently treated distinctly from orthodox lipases. Some pathogenic microbes also produce lipases during infection, especially *Candida albicans which contain a huge* number of lipases. In the biotechnology sector lipases play a significant role and are valuable enzyme in these sectors (Saini et al., 2017). Microbial enzymes have a wide range of applications at industrial level, because of their differentiated enzymatic properties and substrate specificity (Goodman, 2010). Bacterial lipases enzymes are more stable than plant and animal lipases (Burhan et al., 2003).

Types

There is no specific kinds of lipases, but mainly they are classified according to their use, namely; hepatic lipase, human digestive lipase and pancreatic lipase.

Uses of Lipase

Lipases carry various biological processes ranging from the daily metabolism process of dietary triglycerides to inflammation and cell signaling (Pascoal et al., 2018). Some lipases have specific selective function, while others play a significant role in extracellular compartments. In lysosome lipase enzyme is present in lysosomes, while other lipases such as pancreatic lipases, they veiled into extracellular compartments where they do the breakdown of dietary lipids into simpler forms that can be easily transported throughout the body and absorbed. Bacteria and fungus may also conceal lipases to ease nutrient preoccupation from external medium. Certain bees and wasps contain phospholipases that increase biological processes of inflammation and wound delivered by sting. Dandruff in humans is caused by a fungus known as *Malassezia globosa*, this fungus usually uses lipase to do the breakdown of sebum into oleic acid and enhance skin cell production causing dandruff. Lipases play a significant role in cheese and yogurt fermentation. In modern area lipases are used as a versatile and cheap source to degrade lipid. In biotechnology sector, recombinant lipases are used in laundry, baking and even as catalysts, also used as alternative approaches to change vegetable oil into fuel. Lipases are safer and environmentally friendly and also replace traditional catalysts in biodiesel processing (Gurung et al., 2013).

Bacterial Lipase

The lipase producing bacterial genera comprises of; *Burkholderia*, *Pseudomonas and Bacillus*. The bulk production of bacterial lipase are easy and they are commercially very significant because of extracellular production (Gupta et al., 2004). Only a few bacterial species are used commercially for the production of lipases. The bacterial genera used commercially include, *Pseudomonas*, *Achromobacter, Corynebacterium, Alcaligenes, Enterococcus, Arthrobacter, Chromobacterium, Burkholderia* and *Bacillus,* and in various biotechnology applications lipases from *Pseudomonas* are greatly used. Different products launched in the market are bacterial lipases based in a few years, such as Lipomax and lumafast extracted from *Pseudomonas,* and their major application in detergent (Verma, 2019).

Fungal Lipase

Fungi that produce lipases are present in different habitats, such as dairy byproducts, soil contaminated with waste of vegetables, deteriorated food and seeds (Mehta et al., 2017). One of the important fungus known as *Candida rugosa lipases* have been greatly use in biotechnology sector, (Akoh, Lee, & Shaw, 2004). It is previously reported that, *Thermomyces lanuginosus* lipases has wide range of applications in the field biotechnology and detergents. Other major lipase making fungi includes, *Rhizopus,Mucor,Humicola, Candida,Geotrichum, Aspergillus, Penicillium,Rhizomucor* and *Rhizopus* (Gurung et al., 2013).The temperature stable lipase is produced by thermophilic *Aspergillus terreu*s, *Mucor pusillus* and *Rhizopus homothallic.* A few studies reported about molds with thermostable and alkaliphilic lipase (Mehta et al., 2017).

Applications of other different enzymes

Therapeutic enzymes have a huge variety of selective uses such as anticoagulants or thrombolytics, oncolytics and also as replacement for various metabolic deficiencies. Proteolytic enzymes are excellent anti-inflammatory agents. There are various factors that decrease the potential of microbial source enzymes, once we enter the medical sciences due to the high molecular size of catalysts which stop their distribution within somatic cells. In industrial processes the quantity of enzymes should be high, while in therapeutic case the purity and specificity should be excellent, if the quantity level is less no matter. The kinetics of such enzymes is high and low so that it is maximally efficient even at low concentration of substrate and enzymes. The source of such enzymes should be designated with high care to minimize or prevent the chances of undesirable growth by mismatched material and also to enable ready purification. In market therapeutic enzymes are usually in lyophilized form with biocompatible mannitol diluent and buffering salts. Cost of such enzymes is usually high in comparison to treatment or therapeutic agents. Urokinase is an enzyme that is extracted from urine and used for blood clots as an example of such enzymes. In the current research area therapeutic enzymes are used in cancer treatment such as acute lymphocytic leukemia, example asparaginase enzyme has proved an excellent activity. The activity of such enzymes depend upon the fact that tumour cells lack aspartate-ammonia ligase activity, which inhibit/stop the synthesis of non-essential amino acids. The asparaginase does not have an impact on normal cells which are capable of manufacturing sufficient for their own necessities, but they reduce the free exogenous attentiveness, so in tumor cells they cause a state of fatal starvation. The enzymes can be directed intravenously and are only operational in decreasing asparagine levels inside the bloodstream, showing a half-life of about a day. This half-life can be enhanced by 20-fold with use of polyethylene glycol-modified asparaginase (Vellard, 2003).

Conclusion

Enzymes are being known to mankind since the ancient human civilization. The use of enzymes had been done intensively in different fields especially, in ancient brewing and other uses. But since the 18th century it has been technically known to us as enzymes. Many scientists had tried to study the use of enzymes, and from their pioneer work, we have come to know about its power and utility in our daily life. Today different types of enzymes are being manufactured by many big companies and being sold for their important role in different industries like food, dairy, detergent, and chemical as well as for their important lifesaving therapeutically application. Due to advancement of modern biotechnology and protein engineering a new area of enzyme engineering, has evolved which mainly deals with the purification and stability of these important enzymes. Different microbes as well as other model systems are extensively used for the production of these important biomolecules. Enzymes industry is one among the major industries of the world, and there exists a great market for further improvement in this field. Amylase and lipase are few of these mentionable enzymes that have a wide spectrum role in this sector. Its use is almost done in every industry whether it may be detergent, dairy, food, or medicine. This review especially emphasizes the important wide spectrum role of amylase and lipase in various sectors of industries and also discussed the role of other enzymes in therapeutic field. There is an indeed need of future research in these biomolecules which will later be beneficial for the mankind in their relevance.

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