AMYLOLYTIC-EXTREMOENZYMES: SAVIOUR OF ENVIRONMENTS.

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ABSTRACT

There is no doubt Industries run a nation and developing countries like India are facing a challenge to make a choice, either to make compromise with the environments or with development pace. Many chemical transformation processes used in various industries have inherent drawbacks from a commercial and environmental point of view. In particular, with greater awareness of conservation issues has forced industries to consider alternative, cleaner methods. As an efficient alternative the use of enzymes as industrial catalyst is becoming the best option, and enzymes are gradually replacing chemical catalysts in many areas of industry. Enzymes are highly specific and efficient in catalysis; they are biodegradable and carryout catalytic reactions under mild conditions to save energy and resources. Not just this, amylase produced by these high salinity tolerant microbes can be used for treatment of effluents with high salinity containing starch or cellulosic residues in pollution control mechanism. Our non-renewable resources are limited and scientists have estimated with the current rate of their consumption, these fuels won’t last this century end. Use of bio-fuels which are produced from maize or any other plant based product is another alternate but in many countries it has led to scarcity of food for common man and global food prices are touching the sky. Dead plants or trees near high salinity area can be used to produce bio-fuels in the presence of these salinity tolerant alkalophilic bacteria.
1. INTRODUCTION
Extremoenzymes of amylase category include amylases which can withstand extreme salinity; pH, temperature and presence of detergents. Amylases are the enzymes which have capabilities to hydrolyze starch to give diverse products like dextrins and progressively smaller polymers made up of glucose units (Reddy et al, 2003). Starch is the most abundant form of storage polysaccharide in plants and is an inexpensive source for production of various syrups containing glucose, fructose, maltose etc. and is widely used in food industries (Roy and Gupta, 2004). Not only this sugars produced from starch on fermentation can produce bio-ethanol, amino acids, organic acids and others (Polakovic and Bryjak, 2004). Conventionally, conversion of starch to glucose requires primarily two steps one is liquefaction and second saccharification. These processes are quite energy consuming and so increases the cost of starch based products so to reduce the energy consumption lots of research is going on amylase enzymes (Haiyan et al, 2009). Amylases have several interesting potential applications in the food, detergent, pharmaceutical, leather, textile, cosmetic, paper industries and in bio-fuels production. Microbial enzymes are becoming increasingly important for their technical and economical advantages (Cherry et al., 2004).

With annual growth rate of about 3.3 %, the global market for enzymes reached about $2 billion in 2004 (Sivaramakrishnan et al., 2006). Amylases constitute a class of industrial enzymes having nearly 25% of the enzyme market (Sindhu et al., 1997; Rao et al., 1998). Amylases can be obtained from microbes which can with stand extreme conditions and with the availability of thermostable enzymes, a no. of possible industrial uses has also emerged (Haki and Rakshit, 2003) and the most widely used thermo stable enzymes are the amylases used in the starch industries (Poonam and Dalel, 1995; Crab and Mitchinson, 1997; Sarikya et al., 2000).

2. Types of amylolytic enzymes: On the basis of their mode of actions enzymes which hydrolyze starch can be divided in to endoamylases, exoamylases and debranching amylases (Gomes et al., 2005).

2.1 Endoamylases: Enzymes which randomly cleave alpha -1,4 glycosidic bonds in amylose, amyllopectin and related polysaccharides and produce oligosaccharides of different chain lengths with alpha-configuration on the C1 of the reducing glucose unit produced.
(Guzman and Paredes 1995; Van der Maarel et al, 2002; Reddy et al 2003). Alpha -amyloses (EC 3.2.1.1) are the well-known endoamylases.

2.2 Exoamylases: The exoamylases act on alpha -1, 4 linkages from the non-reducing end successively, result in low molecular weight products. Microbial exoamylases are of various types with respect to bond and substrate preference as well as products formed. These enzymes either exclusively cleave alpha -1,4 glycosidic bonds as beta -amylase (EC 3.2.1.2) or cleave both alpha- 1,4 and alpha-1,6 glycosidic bonds like glucoamylase (EC 3.2.1.3) and alpha -glucosidase (EC3.2.1.20) (Sivaramakrishnan et al, 2006).

2.3 Debranching Enzymes: Debranching amylases include isoamylase (EC3.2.1.68) pullulanase (EC 3.2.1.41). Pullulanase cleaves alpha-1, 6 linkages in pullulan, starch, amylopectin and related oligosaccharides, whereas isoamylase (EC 3.2.1.68) hydrolyses alpha-1, 6 linkages in amylopectin. Debranching enzymes result in long linear polysaccharides (Israilides et al., 1999; Sivaramakrishnan et al 2006).

3. Sources: Alpha amylases are universally distributed throughout the microbial, plants and animal kingdoms. But enzymes from fungal and bacterial sources dominate in their applications in industrial sectors (Reddy et al., 2003). A search for highly active amylolytic enzymes with novel properties is necessary to improve biotechnological processes. Extracellular amylases stable at high salt concentration is one of these new frontiers. It was believed that archaea, bacteria and other eukaryotic species Dunaliella salina are good sources for the high salinity tolerant amylases. Microbial diversity in hyper saline regions and environments have found the presence of melanised fungi, which forms a new group-“Eukaryotic Halophiles” (Butinar et al., 2005). Several fungi including Pencillium spp. are also good sources of halotolerant spp.( Marbaniang et al.,2007;Zalari et al., 2005). Extracelluar amylase obtained from B. dipsoosauri strain, purified enzyme has extraordinary salt tolerance (Deutch, 2002). Halotolerant amylase was also obtained from a novel bacterial strain Rheinheimera aquimaris (Ghasemi et al., 2010). Chromohalobacter sp. TVSP 101 is producer of two alkaliplhilic, thermophilic halotolerant amylases (Prakasham et al., 2007). Thermostable amylases from hyperthermophilic microbes are mostly used in industries AS they are active at the high temperatures of gelatinization (100-110°C) and liquefaction (80-90°C) to economize processes, therefore there has been a need for more thermophilic and thermostable α-amylases (Sindhu et al., 1997). Extra cellular amylases are also obtained from
hyperthermophillic Archaeon *Thermococcus profundus* DT5432 by Chung *et al.*, 1995, anaerobic thermophilic bacteria (klingeberg *et al.*, 1990), from *Bacillus licheniformis* strain (Tsurikova *et al.*, 2002), *Bacillus amyloliquifaciens* (Underkofler, 1976), bacillus lentus (Elaasser *et al.*, 1992), *Myceliophthora thermophila* (Ramakrishna *et al.*, 1993). In various industrial processes enzymes are required to work at extreme pH either alkaline or alkali. Acid stable alpha amylase was produced by submerged culture of *Aspergillus kawachii* NBRC4308 (Hiroshi *et al.*, 2007), acidic amylase from *Aspergillus awamori* was also produced (Prakasham *et al.*, 2007), highly acidic and thermo stable amylase was isolated from thermophilic *Bascillus* strain HUTBS62 under different environmental conditions. Alkali tolerant, halophilic, surfactant and detergent stable alpha amylase isolated from halophilic Bacillus sp. Strain TSCVKK (kiran *et al.*, 2007).

4. **Applications:** Applications of amylases dates back to thousands of years, indirectly amyalase was used as various microbes use them to carry out biochemical reactions to produce fermented products like breads, wines, beer and also to carry out solid state fermentations. In 1894 it was Dr. Jhokichi Takamine who prepared digestive enzymes from koji fermentations of wheat bran by *Aspergillus oryzae* (Prasanna, 2005).

Alpha amylase has 30% global enzyme share (Maarel *et al.*, 2002) and annual sale of about $11 million (Kilara and Desai 2002). Amylases are capable of degrading starch related polymers.

4.1 **Detergents additives:** Detergents are used for laundry purposes, dishwashing and cleaning purposes. Both basic and acidic alpha amylases are used in detergents. Alkali amylases are obtained from alkaliphilic Bacillus sp. (Horishiki, 1996). Detergents which contain alkaline alpha amylase also contain chelating agents and usually calcium is used (Nonaka *et al.*, 2003). With the use of enzymes in the detergents biological detergents are been made which are more environment friendly.

4.2 **Textiles desizing:** Fabrics are made from yarns and wrap yarns are coated with a removable sizing agent which lubricates and protects yarn from abrasions during weaving. And starch has been the first choice for this purpose from centuries because of its efficient film forming capacity. Applied sizing agent has to be removed so that fabric can be dyed and for that chemicals were used. Chemical treatment does not remove complete starch and also
harm fibres and environment. With the use of amylase enzyme to remove starch from the yarn appropriate desizing is achieved. (Kumar et al., 1995; Tsurikova et al 2005).

4.3 Paper desizing: Like textiles sizing of paper is done to protect paper from damage during processing. So to desize starch from coated paper alpha amylase find application in pulp and paper industries.

4.4 Beverage ethanol: Alcholos are consumed by humans since ages. In beer, wine and other alcohol industries. In case of beer industries microbial amylases are used to enhance the production of fermented sugar. Microbial amylases are added in aid to cereal amylases. These enzymes are also used for the direct fermentation of the starch to ethanol (Aiyer et al., 2005). Moulds amylases are used in beer, alcohol production and brewing industries. The advantages of such system are their uniform enzyme action in mashes and increase in their rate of saccharification and alcohol yields (Van lenin and Smith, 1968).

4.5 Bio fuel production: Fossil fuels cause a lot or pollution they do more harm than the benfit. Over the last few decades due the environmental concern and high prices of the fuels, bio fuels have generated so much interest. Bio fuels mainly include ethanol fuel. Ethanol can be derived from renewable resources such as waste generated from the agriculture crops and by products. Enzymes such as alpha amylase and others like glucoamylase and cellulose are important to produce fermentable sugars to produce ethanol (Kirk et al., 2002).

4.6 Treatment of starch processing waste water: Waste from the food industries have very high biological demand and chemical oxygen demand. They have high quantity of carbon and nitrogen. Nutrients such as phosphorous and nitrogen are required by the algae and other plants. Aquatic life depends on these photo synthesizers and these are generally present on the surface at low level. Excessive availability of the nutrients can create an overstimulating environment and cause overgrowth of the aquatic plants and algae. Bacterial respiration and algal growth consume up the available oxygen and create oxygen deprived environment for the aquatic animals and can lead to their death (Brown and Caldwall). Biotechnological treatment of these waste products can produce proteins and also purifies the effluents (Aiyer., 2005).

4.7 Baking: Cereals have natural amylase enzymes. But food processes rely on both endogenous as well as on microbial amylases for the complete degradation of starch and like
polymers. Alpha amylases are used to supplement the natural enzymes of cereals and also improve the shelf life of the fermented and baked products (Sahlstrom and Brathen, 1997).

4.8) Production of branched dextrins: Hydrolysis of corn starch with alpha amylases produces branched dextrins of high molecular weight. Extent to which starch degradation occur also depends upon the type and the physical properties desired. Branched dextrins can be converted into powder form by chromatography followed by the spray drying. High molecular weight branched dextrins are used as an extender, glozing agent in the powdery foods (Aiyer, 2005).

4.9 Liquefaction: It’s a process of dispersion of insoluble starch granules present in the aqueous solution by doing thermo stable enzymatic partial hydrolysis. Enzymatic hydrolysis has several advantages as it’s more specific and few by products are produced. Also as enzymes need milder condition so there is less energy consumptions and also its environmental friendly (Aiyer, 2005).

4.10 Production of the Malto-oligomer mixtures: It is obtained by the digestion of the corn starch with alpha amylase, beta amylase and pullanase (Aiyer, 2005). Malto oligomer mix is considered as the new product for commercial purpose and it has composition of the glucose (2.2%), maltose (37.5%), maltotriose (46.4%) and maltotetrose and malto oligosaccharides (14%).

4.11 Starch processing: Sugar syrups are important and major part of the starch processing industries and include glucose, maltose, maltotriosctose, dextrins sugars, fructose syrups etc. the hydrolysis of the starch may be carried out by the use of either chemical method by use of acid or by the use of biocatalysts like amylase enzymes. Chemical conversion based on acid has numbers of disadvantages like cost and harm to the nature but on the other hand enzymes based bioconversion is environment friendly (Crabb and Shetty, 1999).

4.12 Other fields: Amylase applications has touched the fields of clinical, medica and analytical chemistries (Pandey et al., 2000; Cherry et al., 2004). They are also used as digestive aids in animal feed (Kumar et al., 1995).

5. CONCLUSIONS

Earth is our home and uncontrolled irregular human activities, industrial revolutions, increased consumption of fossil fuels and increase in population has posed a threat to our
environment. To save our earth, our without wasting any second we should find alternate new green technologies like use of bioconversion methods instead of chemical methods. We can use biocatalysts. Use of enzymes is one important step toward achieving that goal. Sustainable development for present and future generation is the need of the hour. And amylase enzyme are playing there important role in fulfilling this dream.

REFERENCES