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Amylases- Bioprocess and Potential Applications: A Review

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ABSTRACT

Amylases are enzymes which hydrolyze starch molecules to give diverse products including dextrin and progressively smaller polymers composed of glucose units. These enzymes are of great significance in present day industries ranging from food, fermentation, textile, paper to pharmaceutical industries. Based on growing concern and aware of environmental issues, industries find enzymes as a good alternative over other chemical catalysts. Enzymes from fungal and bacterial sources have dominated applications in industrial sectors. The ease of production and broad spectrum of applications make α-Amylase an industrially valuable biocatalyst. This review focuses on the production of bacterial and fungal α-amylases, their distribution, structural-functional aspects and their use in industrial applications.

Keywords: Amylase, Enzymes, Starch, microbial sources, applications

Enzymes are chemical substances produced by living cells that are capable of initiating a chemical reaction without being utilized them in that reaction. They enhance the rate of a chemical reaction (Oyeleke *et al*., 2009). Enzymes are produced by plants, animals but microbial enzyme production is of great importance as these are more economical to produce, calculable, tractable and stable (Burhan *et al*., 2003). Enzymes are the most important substances used to day in so many areas either in research, medicine or commonly in industries. Amylases are one of the most important industrial enzymes, which hydrolyze starch molecules to fine products such as dextrin, maltose etc. In recent years, interest in the microbial production of enzyme has increased dramatically due to its wide spread use in baking, food, textile, detergent and pharmaceutical industry. With the advent of new frontiers in biotechnology, the spectrum of amylase application has widened in many other fields. Currently, three-dimensional (3D) structures of a few members of the alpha-amylase family have been determined using protein crystallization and X-ray crystallography. These data in combination with site-directed mutagenesis studies have helped to better understand the interactions between the substrate or product molecule and the different amino acids found in and around the active site.

There are two major classes of amylases, mostly identified among microorganisms are α-amylase and gluco-amylase. α-Amylases (endo-1, 4-a-D-glucan glucohydrolase) are extracellular enzymes that randomly cleave the 1,4-α-D-glucosidic linkages between adjacent glucose units in the linear amylase chain. Glucoamylase (exo-1,4-α-D-glucan glucanohydrolase) hydrolyzes single glucose units from the nonreducing ends of amylose and amylopectin in a stepwise manner. Amylases are widely distributed in microbial, plant and animal kingdoms. They degrade starch and related polymers to yield products characteristic of individual amylolytic enzymes. Initially the term amylase was used originally to designate enzymes capable of hydrolysing α-1, 4-glycosidic bonds of amylose, amylopectin, glycogen and their degradation products

(Damien *et al.,* 2010). They act by hydrolysing bonds between adjacent glucose units, yielding products characteristic of the particular enzyme involved (Dhanya *et al.,* 2009). In recent years a number of new enzymes associated with degradation of starch and related polysaccharides structures have been detected and studied. The hydrolysis of starch can be carried out by using either acid or enzyme as catalyst. Enzyme hydrolysis has several advantages; it is more specific, therefore fewer by products are formed, and hence yields are higher. The enzymatic hydrolysis of starch has been practiced on an industrial scale for many years and is gradually replacing the traditional acid hydrolysis process (Kazunari and Imanaka, 2011).

Starch is a major reserve of carbohydrate of all higher plants (Encarnacion *et al.,* 2011). In some cases it accounts for as high as 70% of the undried plant material. It occurs in the form of water insoluble granules. The size and shape of the granules is often characteristic of the plant species from which they are extracted (Guadalupe *et al*., 2011). Starch is produced commercially from the seeds of plants, such as corn, wheat, sorghum or rice, from the tubers and roots of the plants such as cassava, potato, arrowroot and the pith of sago palm (Cynthia *et al.,* 2011).The major commercial source of starch is corn from which it is extracted by a wet milling process (Dhanya *et al.,* 2009).

Starch, the storage form of energy in plants, consists of two polymers composed of glucose units: amylose (linear) and amylopectin (branched). Amylose is composed of linear chains of α-1,4 linked D-glucose residues (Chi-Wen Lin *et al.,* 2011). Hence it is extensively degraded by α-amylase. Amylose has a degree of polymerization of several thousands of glucose units. Amylopectin may account for 75 to 80% of most starches. It has molecular weight in excess and has a branched structure composed of chains about 20-25 α-1,4 linked D-glucose residues. Amylopectin is branched by α-1,6 D-glucosidic bonds. In aqueous solutions, amylopectins are relatively stable due to branched molecules and are not able to form compact aggregates. Starch is a major component of waste produced from food processing plants. Biotechnological treatment of food processing waste water can produce valuable products such as microbial biomass, proteins and also can purify the effluent.

Microbial Amylases

Microorganisms are good source of industrially important enzymes. Microbial amylase has almost surpassed the synthetic sources in different industries (Pandey *et al*., 2000). The major advantage of using microorganisms for the amylase production is economical bulk production capacity and microbes can be easily manipulated to obtain enzymes of desired characteristics (Ramesh and Lonsane, 1990). Amylolytic enzymes are widely distributed in bacteria and fungi. They are categorized in to exo-acting, endo-acting and debranching enzymes. Selection of the right organism plays a key role in high yield of desirable enzymes. For production of enzymes for industrial use, isolation and characterization of new promising strains using cheap carbon and nitrogen source is a continuous process. Starch degrading bacteria are mostly important in food, textile, fermentation and paper industries. The isolation and manipulation of pure culture of starch degrading microorganisms from soil have a great importance on biotechnology field. Thus isolating and manipulating pure culture from various waste materials has manifold importance for various biotechnology industries (Rwarinda *et al.,* 2013).

Among bacteria *Bacillus* species is widely reported for the production of amylases. Species like *B.subtilis, B.stearothermophilus, B.licheniformis,* and *B.amyloliquefaciens* are known to be good producers of alpha amylase. Other species which have been explored for production of the enzyme include *B.cereus* and *B. subtilis* to name a few. Amylases produced from *Bacillus licheniformis*, *Bacillus stearothermophilus*, and *Bacillus amyloliquefaciens* show promising potential in a number of industrial applications in processes such as food, fermentation, textiles and paper industries. *Bacillus subtilis, Bacillus stearothermophilus*, *Bacillus licheniformis* and *Bacillus*

amyloliquefaciens are known to be good producers of thermo stable α-Amylase (Harshemi *et al.,* 2011).

Filamentous fungi have been widely used for the production of amylases. Being prolific producers of extracellular proteins, they are widely exploited for the production of different enzymes including alpha amylases (Kazunari *et al.,* 2011). Fungi belonging to the genus Aspergillus have been most commonly employed for the production of alpha amylase. Production of enzymes by solid state fermentation using these moulds turned a cost effective production technique (Parveen *et al.,* 2011). Fungal amylases are widely used in preparation of oriental foods (Popovic *et al.,* 2009). Fungal and bacterial amylases are mainly used for industrial applications due to their cost effectiveness, consistency, less time and space requirement for production and ease of process optimization and modification (Ellaiah *et al.,* 2002).

Fungal sources are mostly terrestrial isolates such as Aspergillus species. Generally fungi secrete alpha amylase (dextrinizing enzymes) although a few fungi have been known to secrete alpha amylase and beta amylase (saccharifying enzymes). *A. oryzae* EI 212 secrete alpha and beta amylase or both depending upon the composition of media and fermentation conditions. The nature and amount of extracellular amylase produced by *Aspergillus* species determine the efficiency of conversion of starch to oligosaccharides. Filamentous fungi are suitable microorganisms for solidstate fermentation (SSF), especially because their morphology allows them to colonize and penetrate the solid substrate (Rahardjo *et al*., 2005). The fungal α-amylases are preferred over other microbial sources due to their GRAS (Generally Recognized as Safe) status (Gupta *et al*., 2003).

Production and Purification of Amylase

Alpha Amylase (α-1,4 glucan-glucanohydrolase EC 3.2.1.1) is an enzyme that is used in various industries to rapidly degrade complex polysaccharides (e.g. starches) into smaller oligosaccharides. This extracellular enzyme hydrolyses α-1,4 glucosidic linkages randomly throughout the starch molecule in an endo-fashion producing oligosaccharides and monosaccharides. α- Amylase can be produced by plant or microbial sources. Due to the advantages that microbial production offers, α-Amylase from microorganisms has been focused upon and preferred to other sources for production. The ubiquitous nature, ease of production and broad spectrum of applications make α-Amylase an industrially important enzyme (Leveque *et al*., 2000). Currently, these enzymes comprise about 30 % of the world's enzyme production. Industrial production processes are designed to meet the demand of industries and economic aspects. Generally amylases are produced by submerged cultures because of easy handling and greater control of environmental factors such as temperature and pH (Xusheng *et al.,* 2011). Mostly synthetic media have been used for the production of bacterial amylase through submerged fermentation. The contents of synthetic media such as nutrient broth, soluble starch, as well as other components are very expensive and these could be replaced with cheaper agricultural by products for the reduction of the cost and the medium (Solange *et al.,* 2010). Gebreselema (2015) observed maximum amylase activity in submerged state fermentation under optimum conditions and then declined. The optimum temperature reported for maximum amylase activity of Bacillus was 40°C and Streptomyces at 37°C. The highest amylase activity was observed at neutral pH and 4% of starch concentration.

Esfahanibol and balaie *et al*. (2008) reported the effect of many chemical and physical factors on alpha amylase production by *A. oryzae* in shake flasks fermentation via an Adlof-Kuhner orbital shaker. The impact of varying pH of medium ranging from 4-7 was studied. The maximum alpha amylase production was obtained at pH 6.2. Carbon and nitrogen source has discernible effect on the enzyme production. The corn starch at level of 15 g/l proved to be best carbon source for alpha amylase synthesis while glucose represses the alpha amylase production. The medium consist of corn starch, sodium nitrate as inorganic nitrogen resulted in significant enzyme production. Among the organic nitrogen sources yeast extract at the level of 2.5g /l was excellent nitrogen source.

The maximum activity was obtained at 35°C and 180 rpm. Planchot and Colonna (1995) purified *A. fumigatus (Aspergillus sp.* K-27) extracellular alpha amylase to homogeneity by using anion-exchange DEAE-cellulose and affinity α-cyclodextrin-Sepharose chromatography. The purified enzyme, a glycoprotein with 15% carbohydrate content, showed an isoelectric point of 3.7, a molecular weight of 65,000 (as estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis), and an amino acid composition with a high number of neutral hydrophobic residues. Alpha-amylase activity on in solution was optimal at pH 5.5, and the enzyme was stable at 40°C. It hydrolyzed amylose and amylopectin, with respective Km of 0.42 and 7.7 mg mL−1 and kcat/Km of 3.4 and 2.5 mL mg−1 min−1. The major end-products of maltohexaose, degradation were glucose and maltose.

Singh *et al*., (2009) investigated the effect of various agricultural by products as a substrate such as wheat bran, wheat straw, rye, straw on the alpha amylase production by *Humicola lanuginose* in solid state fermentation. It was noted the optimum condition for the alpha amylase production by *Humicola lanuginose* in SSF was incubation period 144 h, initial moisture content 90%, initial pH of medium 6, incubation temperature 50°C, size of inoculum 20 % and soluble starch as best carbon source. Rasooli *et al*. (2008) reports that among defined carbohydrates, tested starch and maltose supported good growth and amylase production, with the highest productivity recorded in the presence of starch. The productivity remained constant up to 8% starch level after which it gradually declined. Tryptophan was found to enhance the enzyme productivity to 202% as compared to the basal medium whereas peptone and lysine at 0.5% level showed a strong repression.

Optimization of the various parameters and manipulations of media are the techniques, which can be done for the over expression of amylase. Various physical and chemical factors have been known to effect the production of alpha amylase such as temperature, pH, Incubation period, carbon, nitrogen sources, surfactants, phosphate, different metal ions, moisture and agitation (Ellaiah and Rakshit, 2002). The influence of temperature on amylase production depends on whether the culture is mesophilic or thermophilic. Among the fungi most amylase production studies have been done with mesophilic fungi within the temperature range of 25-37°C. A raw starch degrading amylase was produced by *Aspergillus ficum* at 30°C by Hayashida *et al* in 1986. Yeast such as *Saccharomyces kluyveri* and *S.cerevisiae* was reported to produce alpha amylase at 30°C (Moller *et al*., 2004). Ahmad *et al*., (2010) reported optimum level of amylase production between 50-55°C for the thermophilic fungal cultures such as *Talaromyces emersonni*, *Thermomonospora fusca* etc.

The growth of microorganisms and production of extracellular enzymes are greatly affected by the chemical and physical nature of their surroundings. An understanding of these influences aids in the control and optimization of microbial growth. These factors include pH, temperature, nutritional supply, presence of metal ions and physical factors such as humidity. It refers to the acidity or alkalinity of a solution. It is a measure of the hydrogen ion activity of a solution and is defined as the negative logarithm of the hydrogen ion concentration. pH is one of the important factors that determines the growth and morphology of microorganisms as they are sensitive to the concentration of hydrogen ions present in the medium. Earlier studies have revealed that fungi required slightly acidic pH and bacteria required neutral pH for optimum growth. pH affects the synthesis, secretion and stability of α-amylase. Bacterial cultures such as *B. amyloliquefaciens, B. licheniformis* and *B.subtilis* required an initial pH of 7 (Syu and Chen, 1997; Tanyildizi *et al*., 2005; Haq *et al*., 2005). Studies have revealed that fungi required slightly acidic pH and bacteria required neutral pH for optimum growth. pH is known to effect the synthesis and secretion of alpha amylase and its stability. Fungi of *Aspergillus* sp. such as *A.oryzae, A.ficuum* and *A.niger* were found to give significant yields of alpha amylase at pH equal to 5.0 to 6.0 in SmF (Djekrif-Dakhmouche 2005). Alpha amylase producing yeast strains such as *S.cerevisiae* and *S.kluyveri* exhibited maximum enzyme production at pH 5.0 (Samrat *et al*., 2011).

Nature of carbon and nitrogen sources is found to influence the bacterial biomass and enzyme production. Presence of glycerol in starch medium reported to increase enzyme production in *B.subtilis* (Maryam *et al.,* 2010). Soluble starch has been found as the best substrate for the production of alpha amylase by *B.stearothermophilus*. Bacillus sp. was noted to give a maximum raw starch digesting amylase in a medium containing lactose (1%) and yeast extract (15%). Galactose, glycogen and Inulin were noted as the suitable substrates for the production of amylases by *B.licheniformis* and *Bacillus.sp.* (Xusheng *et al.,* 2011). The cost of fermentation media can be reduced by using agricultural wastes such as orange waste, peer millet starch, potato, corn, tapioca, wheat and rice as flours for both liquid and solid fermentation. This will provide both carbon and nitrogen sources necessary for the growth and metabolism of organism. Arpana *et al.,* (2011) reports soybean meal was found as the best nitrogen source for alpha amylase by Bacillus sp. Strains of *Bacillus stearothermophilus* and *B.amylolyticus* secreted maximum alpha amylase in a medium supplemented with 1% peptone, 0.5% yeast extract and 0.5% maltose under vigorous shaking conditions. Elif *et al.,* (2005), compared the influence of organic and inorganic nitrogen sources and reported peptone to be a better nitrogen source for enzyme production by *B. licheniformis* than ammonium phosphate, the common inorganic nitrogen source.

Supplementation of salts of certain metal ions provided good growth of microorganisms and thereby better enzyme production as most alpha amylases are known to be metalloenzymes (Zoe *et al.,* 2006). Most of the amylases are known to be metal ion dependent enzymes, namely divalent ions like Ca^{2+} , Mg^{2+} , Mn^{2+} , Zn^{2+} , Fe^{2+} etc. (Pandey *et al*., 2000). Presence of Ca^{2+} was reported to increase amylase activity of an alkaliphilic *Bacillus* sp. (Burhan *et al*., 2003). Ca^{+2} are reported to be present in majority of these enzymes. Arthur *et al*., (1996) observed that addition of calcium chloride to the fermentation media increased the enzyme production. $LiSO_4$ (25 mM) and $MgSO_4$ (1mM) increased alpha amylase production by *Bacillus* sp., while $FeCl_3$ and $MgSO_4$ exhibited negative influence on alpha amylase production. The metal ions may change the enzyme activity via change in electrostatic bonding which could change the tertiary structure of enzymes (Reeta *et al.,* 2009).

Environmental parameters such as temperature and moisture are also found to have influence on enzyme production. Amylases from *B. subtilis* AX 20 (Najafi *et al*., 2005), *Geobacillus thermodenitrificans* HRO 10 (Ezeji and Bahl, 2006), *Thermomyces lanuginosus* (Kunamneni *et al*., 2005) and yeast *Cryptococcus flavus* (Wanderley *et al*., 2004), have temperature optimum in the range of 50-55°C. Higher temperature optimum of 75-85°C was reported by Mamo and Gessesse (1999) for Bacillus sp.WN11. Bernhardsdotter *et al*. (2005) reported 40°C as optimum temperature for amylase from *Bacillus* sp. There has been a need and continual search for more thermophilic and thermostable amylases since thermostability is considered as an important and useful criterion for industrial application of α-amylase.

Low and high moisture levels of the substrate effect the growth of the microorganisms resulting in lower enzyme production in SSF (Ellaiah *et al.,* 2002). High moisture content leads to reduction in substrate porosity, changes in the structure of substrate particles and reduction of gas volume. Bacteria are generally known to require initial moisture of 70-80%. Alpha amylase production by *Bacillus licheniformis* M27 was highest with 65% initial moisture content in an SSF system (Namita *et al.,* 2007). Significant decrease in enzyme production was observed with high increase in moisture content which was due to the decrease in the rate of oxygen transfer. Studies indicated that enzymes titres could be increased significantly by agitation of the medium with high moisture content (Lonsane *et al.,* 1990). Surfactants in the fermentation medium are also found to increase the secretion of proteins by increasing cell membrane permeability. Addition of tween 80 (1.3%) to the fermentation medium increased alpha amylase production by 2 fold in *Thermomyces lanuginosus* (Sivaramakrishnan *et al.,* 2006).

Purification process in downstream processing after fermentation strongly depend on the market, processing cost, final quality and available technology. Most enzymes are purified by Chromatographic techniques after crude isolation by precipitation and membrane separations (Prakash *et al.,* 2009). The need for large scale cost effective purification of proteins has resulted in evolution of techniques that provide fast, efficient and economical protocols in fewer processing steps (San-Lang *et al.,* 2011). Different strategies for purification of enzymes have been investigated, exploiting specific characteristics of the target biomolecule. Laboratory scale purification for α-amylase includes various combinations of ion exchange, gel filtration, hydrophobicity interactions and reverse phase chromatography. Alternatively, α-amylase extraction protocols using organic solvents such as ethanol, acetone and ammonium sulfate precipitation (Khoo *et al*., 1994; Hamilton *et al*., 1999) and ultrafiltration have been proposed (Moraes *et al*., 1999). These conventional multi-step methods requires expensive equipments at each step, making them laborious, time consuming, barely reproducible and may result in increasing loss of the desired product. Liquid-liquid extractions are widely employed in the chemical industry due to its simplicity, low costs, and ease of scale up. Liquid-liquid extraction is the transfer of certain components from one phase to another when immiscible or partially soluble liquid phases are brought into contact with each other (Paula and Pérola, 2010).

Industrial applications of amylases

A large-scale starch processing industry has emerged in the last century. In the past decades, we have seen a shift from the acid hydrolysis of starch to the use of starch-converting enzymes in the production of maltodextrin, modified starches, or glucose and fructose syrups. Among amylases α-amylase is in maximum demand due to its wide range of applications in the industrial front. With consumers growing increasingly aware of environmental issues, industries find enzymes as a good alternative over other chemical catalysts. Amylases have a quarter of the world enzyme market and thermostable α-amylases possess extensive commercial applications. Thermostable α-amylases have had extensive commercial applications in starch processing, brewing and sugar production (Leaveque *et al.,* 2000). Thermophilic processes appear more stable, rapid and less expensive and facilitate reactant activity and product recovery. The introduction of thermostable α-amylases has resulted in milder processing conditions, reduced the formation of byproducts and lowered the refining and recovery costs.

Food industry

Amylases play important role in bakery products. For decades, enzymes such as malt and fungal alpha-amylases have been used in bread-making. These enzymes can be employed to replace chemical additive such as potassium bromate, which has been prohibited in several countries. The quantities, taste, aroma and porosity of the bread are improved by using the enzyme in the flour. Amylases can degrade starch in the wheat flour and produce small dextrin, which allows yeast to work continuously during dough fermentation, proofing and the early stage of baking. This results in improved bread volume and crumb texture. In addition, the small oligosaccharides and sugars such as glucose and maltose produced by these enzymes enhance the Maillard reactions responsible for the browning of the crust and the development of an attractive baked flavour (Lundkvist *et al*., 2007).

Chocolate syrup can be produced by the treatment of cocoa slurries with amylases, in which chocolate starch will dextrinize and thus syrup does not become thick. Cocoa flavored syrups having a high cocoa content and excellent stability and flow properties at room temperature can be produced by using amylolytic enzymes. The stabilized cocoa flavored syrups may be added at room temperature to conventional non-acid confection mixes for use in the production of quiescently frozen chocolate flavored confectioneries (Ismail *et al.,* 1992). Currently, a thermostable maltogenic amylase of *Bacillus stearothermophilus* is

used commercially in the bakery industry. Amylases are also used for the clarification of beer or fruit juices, or for the pretreatment of animal feed to improve the digestibility of fiber (Gavrilescu and Chisti, 2005; Ghorai *et al*., 2009).

Glucose and fermentation industries

The presence of starch and other polysaccharides in sugar cane creates problem throughout the sugar manufacturing which is minimized or eliminated by the action of alpha amylase. Many industries use alpha amylases for the production of glucose. Enzyme hydrolyzes the starch and converts it into glucose. They hydrolyze α-1,4 glucosidic linkage in the starch polymer in a random manner to yield glucose and maltose. Therefore, alpha amylase is extensively used in many industries for the production of glucose and water-soluble dextrin (Akiba *et al*., 1998, Shetty and Crab, 1990).

Alpha amylases are also used for the production of biofuels such as ethanol. Alpha amylases can convert starches present in grains and potatoes into fermentable sugars there by ethyl alcohol. The bioconversion of starch into ethanol involves liquefaction and saccharification, where starch is converted into sugar using an amylolytic microorganism or enzymes such as α-amylase, followed by fermentation, where sugar is converted into ethanol using an ethanol fermenting microorganism such as yeast *Saccharomyces cerevisiae.* The use of bacterial enzyme partly replaces malt in brewing industry, thus making the process more economically significant. Moulds amylases are used in alcohol production and brewing industries. The advantages of such systems are uniform enzyme action in mashes, increase rate of saccharification, alcohol yield and yeast growth (Santamaria *et al*., 1999).

Paper industry

The starch coating treatment of paper serves to make the surface of paper sufficiently smooth and strong, to improve the writing quality of the paper. In this application, the viscosity of the natural starch is too high for paper sizing and this can be altered by partially degrading the polymer with α-amylases in a batch or continuous processes. Starch is a good sizing agent for the finishing of paper, improving the quality and erasebility, besides being a good coating for the paper. The size enhances the stiffness and strength in paper (Bruinenberg *et al*., 1996). Starch paste when used as a mounting adhesive modified with additives such as protein glue or alum, frequently, causes damage to paper as a result of its embrittlement. Starch digesting enzymes, e.g. alpha amylase, in immersion or as a gel poultice are applied to facilitate its removal. Alpha amylase will hydrolyze the raw starch that is used for sizing and coating the paper instead of expensive chemically modified starches (Okolo *et al*., 1996).

Textile and detergent industries

Textile industries are extensively using alpha amylases to hydrolyze and solubilize the starch, which then wash out of the cloth for increasing the stiffness of the finished products. Fabrics are sized with starch. Alpha amylase is used as desizing agent for removing starch from the grey cloth before its further processing in bleaching and dyeing. Sizing agents like starch are applied to yarn before fabric production to ensure a fast and secure weaving process. Desizing involves the removal of starch from the fabric which serves as the strengthening agent to prevent breaking of the warp thread during the weaving process. The α-amylases remove selectively the size and do not attack the fibres (Iqbal *et al.,* 1997).

In detergent industries, the enzyme alpha amylase plays a vital role. The use of enzymes in detergents formulations enhances the detergents ability to remove tough stains and making the detergent environmentally safe. Amylases are the second type of enzymes used in the formulation of enzymatic detergent. Amylases derived from *Bacillus* and *Aspergillus* are in general use. Removal of starch from surfaces is also important in providing a whiteness benefit, since starch can be an attractant for many types of particulate soils (Mitidieri *et al*., 2006). It is widely used for improvement of detergency of laundry bleach composition and bleaching without

color darkening (Borchet *et al*., 1995; Atsushi and Eiichi, 1998). The addition of enzyme stabilizes the bleach agent and preserves effectiveness of the bleach in laundry detergent bar composition (Onzales, 1997). The enzymes from the *Bacillus* species are of special interest for large-scale biotechnological processes due to their remarkable thermostability and because efficient expression systems are available for these enzymes (Prakash and Jaiswal, 2009).

CONCLUSION

In recent years, the potential of microbes as biotechnological source industrially relevant enzymes has stimulated the interest in the exploration of extracellular enzymatic activity in several microorganisms. The use of α-amylase in starch based industries has been prevalent for many years and a number of microbial sources have been utilized for the efficient production of this enzyme. In the past decades, we have seen a shift from the acid hydrolysis of starch to the use of starch-converting enzymes in the production of maltodextrin, modified starches, or glucose and fructose syrups. Exploration of new microbial sources, application of biophysical techniques in characterization of these enzymes and use of genetic engineering tools will help to improve its performance and applicability. Demand for microbial a-amylases has increased due to their specificity of reaction, mild conditions required for reaction and less energy consumption than the conventional chemical methods. The search for new microorganisms that can be used for amylase production is a continuous process. Screening of microorganisms with higher amylase activities could therefore facilitate the discovery of novel amylases suitable to new industrial applications.

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