Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(1): 198-205

Received: 23-11-2018
Accepted: 27-12-2018

**Nisha Kumari**
Department of Biochemistry, CCS Haryana Agricultural University, Hisar, Haryana, India

**Sushil**
Department of Chemistry, CCS Haryana Agricultural University, Hisar, Haryana, India

**Babita Rani**
Department of Biochemistry, CCS Haryana Agricultural University, Hisar, Haryana, India

**Kamla Malik**
Department of Microbiology, CCS Haryana Agricultural University, Hisar, Haryana, India

**Ram Avtar**
Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar, Haryana, India

**Correspondence**
**Nisha Kumari**
Department of Biochemistry, CCS Haryana Agricultural University, Hisar, Haryana, India

# Microbial amylases: An overview on recent advancement

**Nisha Kumari, Sushil, Babita Rani, Kamla Malik and Ram Avtar**

**Abstract**
Amylase is an extracellular enzyme, which is involved in the starch processing industries where it breaks starch into simple sugar constituents. Two major classes of amylases namely α-amylase and glucoamylase have been identified in microorganisms. In addition, β-amylase which is of plant origin has also been reported from few microbial strains. These amylases are usually extracellular and are widely distributed in bacteria, actinomycetes and fungi. Amylases are of ubiquitous occurrence and holding maximum market share of enzyme sales. Compared to plant and animal origins, microbial α-amylase is the most popular source of industrial α-amylase. Microbial sources of α-amylases are cost effective and appropriate for industrial demands. These microbial amylases are now available commercially and they have almost completely replaced acid hydrolysis of starch in starch processing industry because of number of advantages such as specificity of the reaction, stability of the generated products, lower energy requirements and elimination of neutralization steps. Amylase has wide application in starch processing, baking, brewing, sugar production, textile industries and in detergent manufacturing processes. Interestingly, the first enzyme produced industrially was an amylase. This review focuses on the production, purification, characterization and immobilization of microbial amylase.

**Keywords:** Amylase, baking, glucoamylase, microbial, starch

## 1. Introduction
Enzymes, the natural catalysts which boost the rate of chemical reaction several folds have become an important and indispensable tool in many industries all over the world. Industrialization of biotechnological processes has led to the rapid use of enzymes in staggering quantities in food processing, animal and poultry feed, detergents, leather, tanning, textile and pharmaceutical industries etc. [1], making enzyme production an emerging field of biotechnology. On an industrial scale, amylases are produced using both solid state (SSF) and submerged fermentation (SmF) systems. Solid state system is more effective for amylase production [2]. However, on laboratory scale, submerged fermentation is more suitable due to its ease of operation and regulation [3].

Amylases are enzymes, which hydrolyze starch molecules to give diverse products including dextrins and progressively smaller polymer composed of glucose units. They can be categorized by their mode of action into four groups: a) α-amylase (1, 4- α -D-glucan glucanohydrolase), an endoenzyme randomly cleaves the α, 1-4 glucosidic linkage, b) β-amylase (1, 4- α -D-glucan maltohydrolase), an exoenzyme hydrolyzes alternate α 1-4 glucosidic linkage from the non-reducing ends to form maltose but can't bypass the α 1- 6 linkage, c) glucoamylase (GA) (exo- α -1, 4-D-glucan glucanohydrolase), also called γ-amylase, cleaves α,1- 4 as well as α 1-6 linkage releasing single glucose units from the non-reducing and d) debranching enzymes, hydrolyzes the α 1-6 glucosidic linkage of starch [4]. Amylases can be derived from several sources such as plants, animals and microorganisms. However, microbial sources are the most preferred one for large scale production meeting industrial demands [5]. The major advantage of using microorganisms for the enzyme production is that the process is economically viable and microbes are easy to manipulate to obtain enzymes of desired characteristics [6]. Two major classes of amylases namely α-amylase and glucoamylase have been identified in microorganisms. In addition, β-amylase which is of plant origin has also been reported from few microbial strains. These amylases are usually extracellular and are widely distributed in bacteria, actinomycetes and fungi [7]. Amylases were produced by number of microorganisms such as *Bacillus* [8], *Penicillium rugulosum* [9], and *Trichoderma pseudokoningii* [10] and *Streptococyces aurofasciculans* [11]. These microbial

amylases are now available commercially and they have almost completely replaced acid hydrolysis of starch in starch processing industry [12, 13] because of number of advantages such as specificity of the reaction, stability of the generated products, lower energy requirements and elimination of neutralization steps [14].

First enzyme produced industrially was an amylase from a fungal source, which was used as a pharmaceutical aid for the treatments of digestive disorders [15]. Amylases are useful in a broad range of industrial applications ranging from baking, brewing, fermentation, textile, paper and detergents industries [16]. Amylases that are active at acidic pH are generally used in the glucose syrup industry, whereas those active at basic pH are explored in detergents. Due to the industrial importance of amylases, there is an ongoing interest in the isolation of new bacterial strains producing enzymes suitable for industrial applications such as alkaline amylases for the decrement industry and starch saccharifications [17]. A search for highly active amylolytic enzyme with novel properties is necessary to improve biotechnological processes. Properties such as thermostability [18], capacity to hydrolyze native starch [19], stability to high salt concentration [20] and activity over a wide range of pH attract particular attention. Although several microorganisms have been reported to produce amylases, increased use of microbial amylases has created the need to isolate strains producing amylases with characteristics that are more amenable to industrial and other useful applications [21].

## 2. Microorganisms producing amylases

Amylolytic enzymes from numerous sources degrade starch, the primary polysaccharide in plants. Amylases can be derived from plants, animals and microbes, but its production from microbial sources is cost effective and fulfills the industrial demands [5]. Amylase producing microorganisms mainly bacterial [22-26], fungi [11, 16] and yeast [27] has been isolated and identified. Among the bacteria, *Bacillus* species produce a large variety of extracellular enzymes such as amylases. Bacterial amylases are known to possess more thermostability than fungal amylases [28] therefore bacterial α-amylases are preferred over fungal amylases. The α-amylases have generally been obtained from plant sources. Filamentous fungi apparently constitute the major source of glucoamylase among all microbes.

## 3. Fermentation conditions

There are two types of fermentation processes used for production of amylases i.e. Solid state fermentation (SSF) and submerged fermentation (SmF). Submerged fermentation technique is traditionally used because of ease of handling and great control of environmental factors such as temperature and pH [29]. The use of the submerged culture is advantageous because of the ease of sterilization and process control is easier to engineer in these systems [3].The SmF involves the growth of microorganisms as a suspension in a liquid medium whereas in SSF, microorganisms grows on the solidified medium [30]. Though amylase production has been carried out mainly under SmF [31, 32], SSF has been found effective for amylase production by various microorganisms *viz. Aspergillus oryzae* [2] and *Humicola lanuginosa* [16]. Optimization of various growth conditions *viz.*, pH, size of inoculum, temperature and media like carbon and nitrogen sources are one of the most important techniques, used for the overproduction of enzymes in large quantities to meet industrial demands [33].

### 3.1 Inoculum size

The inoculum size is an important factor for the production of microbial amylase. Higher inoculum concentration significantly increases the moisture content and leads to decrease in growth and enzyme production [34]. Lower inoculum size results in a lower number of cells in the production medium and requires a longer time to grow to an optimum number to utilize the substrate and to form the desired product [35]. It has been reported that 2% (*v/v*) inoculum size was optimum for production of amylase from *Bacillus thermooleovorans* NP54 [36]. Maximum amylase production was obtained from *Aspergillus oryzae* using 2% inoculum size having concentration of 6 x $10^7$spore/ml [2]. Use of 3% inoculum size having $10^5$ CFU/ml was optimum for amylase production [37]. The 10% inoculum size was proved to be best for amylase production from *Bacillus* sp. I-3 [38].

### 3.2 Incubation period

The incubation period for maximum amylase production varies with the type of the microorganisms used and it varies from 12h as reported for *Bacillus thermooleovorans* NP54 [36] to 6 days as reported for *Humicola lanuginosa* [16] for maximum enzyme production. Highest amylase production (3700 U) was obtained after 36h of incubation of *Streptomyces erumpens* [39]. Amylase production by *Bacillus subtilis* increased upto 72h of incubation period; thereafter the activity started declining [32]. However, in *Bacillus* sp.SMIA-2, the enzyme production began in the exponential growth phase reaching a maximum at 32h, remained more or less the same up to 36h and then dropped to 30 U/ml at 48h [40]. Maximum amylase production from *Bacillus* sp. AS-1 was obtained at 24h of incubation. After 24h cell mass was increased but enzyme production declined and after 72h no activity was observed [41]. The productivity decreased significantly at the 8$^{th}$ day (1800 U). Similarly it was found that 19h incubation of *Bacillus subtilis* [34] and 72h incubation of *Nocardiopsis* sp. [42] were optimum for amylase production.

### 3.3 pH of the medium

The pH is one of the important factors that determine the growth and morphology of microorganisms as they are sensitive to the concentration of hydrogen ions present in the medium [43]. The pH change observed during the growth of microbes also affects product stability in the medium [12]. Each microorganism has a pH range for its growth and activity with an optimum pH range. Amylase synthesis from *Bacillus* sp. I-3 occurred at a wide pH range from 4.0 to 8.0 with an optimum of 7.0 [38]. The amylase from *Lactobacillus manihotivorans* LMG18010$^T$ showed higher activity from pH 4 to 6 with a maximum observed at 5.5 [44]. However, *Bacillus thermooleovorans* NP54 grown at varied pH levels (4 to 8) showed maximum enzyme production at pH 7.0 [36]. Similarly, neutral pH (7.0) was optimum for high amylase production by *Bacillus* sp. AS-1 [41], *Bacillus subtilis* [45] and *Penicillium rugulosum* [9].The influence of pH of medium on amylase production by different species of *Bacillus* showed maximum activity by *Bacillus* SMIA-2 at pH 8.5 [40], by *Bacillus subtilis* at pH 7.5 [46]. The cultivation of microorganism at an unfavorable pH value may limit the growth rate and amylase production.

### 3.4 Incubation temperature

The influence of temperature on amylase production is related to the growth of the organism. Hence, the optimum

temperature depends on whether the culture is mesophillic or thermophilic [43]. The maximum release of amylase occurred at 50 ºC (266 U/g) when *Humicola lanuginosa* was subjected to varied temperature (30-80 ºC) [16]. It has been showed that the production of amylase by *Bacillus* sp. I-3 on varying the fermentation temperature from 30-60 ºC, with an optimum of 37 ºC (900 U/ml) [38]. The optimum temperature for amylase production by *Streptomyces erumpens* was 50 ºC with amylase activity of 3480 units. Beyond 50 ºC, there was sudden a decrease in enzyme production [39].

### 3.5 Carbon source

Amylase is an inducible enzyme and is generally induced in the presence of carbon sources such as starch, its hydrolytic product, or maltose [47]. However, constitutive production of amylase has also been reported [36]. Glucose, xylose, galactose, sucrose, mannitol, fructose and lactose were found to be good sources for production of amylase [46]. Starch was found to support amylase synthesis in *Bacillus* sp. K-12 [48], *Bacillus subtilis* [46] and *Streptomyces erumpens* MTCC 7317 [39]. Avdiiuk and Varbanets [49] reported that 0.1% insoluble starch was best to enhance the production of amylase from *Bacillus subtilis* US147. Galactose at the concentration of 1.0% proved to be the best inducer for amylase production (324 U/ml) by *Bacillus subtilis* sp. I-3 [38], *Penicillium rugulosum* [9]. Agricultural wastes are used for both liquid and solid fermentation to reduce cost of fermentation media. These wastes consist of carbon sources necessary for the growth and metabolism of organisms. These sources include orange waste, pearl millet, potato, corn, tapioca, wheat and rice as flours [50]. Wheat bran has been used for the economic production of α -amylase by SSF [6].

### 3.6 Nitrogen source

It has been reported that more amylase was produced when organic nitrogen compounds were used [51]. Aiyer [52] compared the influence of organic and inorganic nitrogen sources and reported peptone to be a better nitrogen source for enzyme production by *B. licheniformis* SPT 278 than ammonium hydrogen phosphate. Maximum production of amylase was achieved when beef extract (1%) was used as nitrogen source in production medium [39]. Malhotra *et al*. [36] observed that tryptone is best nitrogen source for maximum amylase production by *B. thermooleovorans* NP54. Supplementation of casein hydrolysate to the medium resulted in 143% higher yield α-amylase productivity by *Aspergillus oryzae* A-1560 as compared to ammonia. Yeast extract along with ammonium sulphate also gave significant enzyme productivity (110%) by *A. oryzae* [53]. Apart from this, various inorganic salts such as ammonium sulphate and ammonium nitrate have been reported to support better α-amylase production in various species of Aspergillus, *viz. A. nidulans* [47] and *A. oryzae* [54].

## 4. Purification of amylase

The enzyme application in pharmaceutical and clinical sectors requires high purity amylases. The enzyme in purified form is also a pre-requisite in studies of structure function relationships and biochemical properties [12]. As purification of extracellular protein is easier than the intracellular proteins, extracellular amylases have been purified to homogeneity by several researchers [5, 39, 44, 55]. Traditionally the purification of amylases from fermentation media has been done in several steps, which include centrifugation of the culture, selective concentration of the supernatant usually by ultra filtration and selective precipitation of the enzyme by ammonium sulphate. Then the enzyme is subjected to chromatographic techniques such as ion exchange and gel filtration chromatography [5]. 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.

Using this purification strategy, amylase has been purified 5.4 fold from *B. subtilis* [56], 4.3 fold from *B. acidocaldarius* [57], 5 fold from *B. cohnii* US 147 [58] and 203.29 fold from *B. licheniformis* [21]. Thermostable amylase from *streptomyces* sp. has been purified 2130 fold with specific activity 11.7 Units $mg^{-1}$ protein and 60% yield [59]. Amylase enzymes was purified 25.14 fold by acetone prepitation, column chromatography on sephadex G-100 and DEAE-cellulose from *Penicillium olsonii* with specific activity 23.45 U $mg^{-1}$ protein and 36.5% recovery [60]. Kaur and Satyanarayana [61] reported, 2.5 fold purification of amylase from thermophilic mould, *Thermomucor indicae-seudaticae*. Purification fold of 2.7 was obtained in the successive chromatographics from the *Bacillus* strain GM 8901 [62]. Yoshigi *et al*. [63] found 1101 fold purification of amylase from *B. cereus* NY-14 with specific activity 315 $Umg^{-1}$ proteins and 30% yield. Alpha-amylase was partially purified 2.6 fold from *Streptomyces erumpens* MTCC-7317 with specific activity on 3912.92 $Umg^{-1}$ proteins with a yield of 20.70% [39]. The α-amylase secreted by *Geobacillus thermodenitrificans* HR010 was purified to homogeneity (13.6 fold purification with 11.5% yield) by using ammonium sulfate precipitation, ion-exchange chromatography and gel-permeation chromatography [64].

## 5. Characterization of amylase

### 5.1 Molecular weight

Microbial amylases have been extensively characterized for their physico-chemical properties. Krishnan and Chandra [65] noted that many amylases fall into a pattern of low molecular mass and high molecular mass value. Amylase of low molecular weight of 10 kDa [66] and high molecular weight of 100 kDa [21] and 135 kDa [44] have also been reported. Endoamylases are almost exclusively single subunit proteins; however, some amylases especially those having large molecular weight are found to possess more than one subunit as reported in *Aureobasidium pullulans* N13d [27] and *Pyrococcus furiosus* [67].

### 5.2 Temperature optima and thermostability

Thermostable α-amylases have many commercial applications. These enzymes are used in paper and textiles industries, starch liquefaction, food adhesive and sugar production [5]. The optimum temperature of majority of amylases lies in the range of 30 ºC [68] to 100 ºC [36]. However, thermostable amyalses have been isolated from a number of thermophilic organisms. Thermostable amylases having temperature optima of 90 ºC have been isolated from a number of sources including *Bacills licheniformis* NCIB 6346 [69] and *Bacillus licheniformis* [21]. The enzyme from *Thermus* sp. [70], *Nocardiopsis* sp. [42], *Bacillus* sp. A3-15 [71], *B. cohnii* US147 [58] exhibited maximum activity at 70 ºC.

### 5.3 pH optima and stability

For the application of amylase in industries, screening criteria for amylases from different microbial strains involves good thermostability and broad pH range. Amylases that are active at acidic pH are generally used in the glucose syrup industry,

whereas those active at basic pH are explored in detergent industry [17]. An extracellular amylase purified from *Bacillus cereus* NY-14 exhibited optimal pH of 6.0 [63] while that from *Bacillus licheniformis* NCIB 6346 exhibited pH optima of 7.0 [69]. The pH optima of amylases vary from 2 to 12 [72]. Amylases from most bacteria and fungi have pH optima in the acidic to neutral range. Amylase from *Alicyclobacillus acidocaldarius* showed an acidic pH optima of 3 [73] in contrast to the alkaline amylase with optima of pH 9-10.5 reported from an alkalophilic *Bacillus* sp. [74]. Amylase had an optimal pH of 7.5 from *B. licheniformis* [21] and *B. subtilis* [46].

**5.4 Substrate specificity and kinetic parameters**

The amylases differ in their specificity towards substrate. It varies from microorganism to microorganism [12]. The substrate specificity of the amylase was evaluated using soluble starch, amylase, amylopectin, glycogen, maltodextrins and α-and β-cyclodextrins. Amylose was found to be the best substrate for α-amylase from *Lactobacillus manihotivorans* LMG 18010$^T$ [44], *Bacillus licheniformis* CUMC 305 [65]. Uyar *et al* [75] reported that starch was best substrate for α -amylase from *B. subtilis*. Raw potato starch was best substrate for extracellular amylase from *Aureobasidium pullulans* N13d [27]. Amylases differ in their kinetic properties depending on the source of their production and the substrate used for the reaction. The enzyme showed Km values in the range of 0.19 mg ml$^{-1}$ [76] to 5.75 mg ml$^{-1}$ [27] and $V_{max}$ in the range of 0.016 U/ml [75] to 7.35 U ml$^{-1}$ [64]. The amylase of *B. cohnii* US 147 had Km and Vmax values of 0.7 mg/ml and 2.2 U/ml respectively [58].

**5.5 Effects of additives**

The effect of metal ions, chemical modifiers, detergents and metal chelators on the stability and activity of purified amylases have been reported by many workers. Most of amylases are known to be dependent on metal ions namely, divalent ions like $Ca^{2+}$, $Mg^{2+}$, $Mn^{2+}$, $Fe^{2+}$ for their activities. $Ca^{2+}$ has been reported to increase α-amylase activity of an alikaliphilic *Bacillus* sp. ANT-6 [77]. The stabilizing effect of $Ca^{2+}$ on thermostability of the enzyme could be due to the salting out of hydrophobic residues by $Ca^{2+}$ in the protein [38]. Contrarily Malhotra *et al*. [36] reported that α-amylase from *B. thermoolevorans* NP54 and *B. cereus* NY-14 [63] didn't require calcium ions for their activity or production. The inhibition with $Zn^{2+}$ was found to be an important parameter determining the thermostability of the amylase. Infact, it had a potent inhibitory effect on the amylases from *B. cereus* NY-14 [63], *Bacillus* sp. ANT-6 [77], whereas it had no effect at all on the amylase of *Aspergillus kowachii* [78]. Mahmoud *et al* [79]explained metal ions might have stimulated the enzyme activity by acting as a binding link between enzyme and substrate, combining with both and so holding the substrate and the active site of the enzyme.

**6. Immobilization of amylase**

The catalytic activity of an enzyme depends on its three dimensional structure and conformation. Any permanent or temporary change in its natural conformation caused by any physical or chemical agent affects its catalytic function. Thus, it becomes necessary to protect enzymes to sustain its catalytic activity. Inspite of their excellent catalytic properties, enzymes properties have to be usually improved before their implementation at industrial scale. Soluble enzymes have to be immobilized to be reused for a long time in industrial reactors. Immobilization of enzyme is one of the methods for protecting and stabilizing the enzymes, thereby enhancing their properties and operational stability. Immobilized enzymes are used in food technology, biotechnology, biomedicine and analytical chemistry. They have various advantages over free enzymes including easy separation of the reactants, products and reaction media, easy recovery of the enzyme, and repeated or continuous reuse [80]. In addition, the reaction product is not contaminated with the enzyme (especially useful in the food and pharmaceutical industries). Furthermore, the immobilized enzyme has a longer half-life and predictable decay rate [81]. Effective enzyme immobilization can be achieved using several techniques including adsorption to insoluble materials, entrapment in polymeric matrix encapsulation, cross linking with a bifunctional reagent, or covalent linking to an insoluble carrier [82]. The nature of the solid support or matrix plays an important role in retaining the actual conformation and activity of enzyme in the processes that utilized immobilized biocatalysts. Therefore, amongst the various methods used, entrapment is taken as the most preferable method because it prevents excessive loss of enzyme activity after immobilization, increase enzyme stability in microenvironment of matrix, protects enzyme from microbial contamination. Milosavic *et al*. [83] immobilized the glucoamylase via its carbohydrate moiety on macroporous poly (GMA-CO-EGDMA).

Alpha-amylase from *Bacillus cereus* MTCC 10205 was immobilized by entrapment in alginate beads and carrageenan as well as via adsorption on charcoal. The immobilized α-amylase enzyme displayed higher pH (6.0) and temperature optima (60ºC) as compared to free enzyme. Thermostability and storage ability of enzyme was also improved after immobilization [84]. Amylase from *B. acidocaldarius* was immobilized by four method *viz.* covalent binding, ionic binding, entrapment and adsorption with immobilization yield of 86.4%, 85.3%, 60.3% and 53.3% respectively [57]. Immobilization yield of alignate entrapped amylase from *B. circulans* [85]. Immobilized enzyme showed enhances resistance to thermal and chemical denaturation, increased temperature optima and rapid rate of substrate saturation. The specific activity of α-amylase on the zirconium dynamic membrane was 21.85 U/mg protein at its optimum pH 5.5 and temperature 41 ºC, compared with the highest specific activity of 1.91 U/mg protein of the free enzyme at an optimum pH 6.6 and 63 ºC temperature [86].

The kinetic properties of the enzyme were found altered upon immobilization of enzyme. Immobilized enzymes generally show increase in Km values and decreased $V_{max}$ values. The Km value for immobilized amylase on alumina was 4.67 U/ml, which was 1.8 fold higher than that of free enzyme. The $V_{max}$ was 0.99 U/min while that of free enzyme is 1.02 x $10^{-4}$ U/min [81]. Contrarily, Both Km and Vmax of immobilized amylase were found to be higher than of free one [46]. This increase is a consequence of either structural change in the enzyme introduced by the applied immobilization procedure and/or lower accessibility of the substrate to the active site of the immobilized enzyme.

The operational stability of enzymes has been reported to increase upon immobilization and suggested to be exploited on industrial scale. The alginate entrapped amylase was active upto 7 cycles containing 85% activity [85]. Gangadharan *et al*. [87] reported that aliginate entrapped amylase retained 40% of efficiency in 6$^{th}$ and 7$^{th}$ batch run of 6h duration and greater

than 60% of their initial efficiency after 5 batches of successive use. Kahraman *et al*. [88] found that covalently bound enzyme demonstrated greater than 98% activity after 6 runs and 81.4% activity after 25 runs. It was found that immobilized barley β-amylase stored at 4 ºC retained approximately 90% of its original activity after 30 days, whereas free β-amylase retained only 47% of its activity [89].

## 7. Commercial application of amylases

Amylases are among the most important hydrolytic enzymes for all starch based industries and were first commercialized in 1984 as a pharmaceutical aid for the treatment of digestive disorders [15]. Conversion of starch into sugar, syrups and dextrins forms the major part of the starch processing industry [53]. Various applications of amylases are dealt here in brief.

### 7.1 Bread and baking industry and as an antistaling agent

These enzymes were used in bread and rolls to give these products a higher volume, better colour, and a softer crumb [90]. Alpha-Amylase supplementation in flour not only enhances the rate of fermentation and reduces the viscosity of dough but also generates additional sugar in the dough, which improves the taste, crust colour and toasting qualities of the bread [91]. One of the new applications of α-amylase in the industry has been in retarding the staling of baked products, which reduces the shelf life of these products.

Recently emphasis has been given to the use of enzymes in dough improvement/as anti-staling agents, *e.g*. α-amylase, branching enzyme, debranching enzymes and maltogenic amylases. Pullulanses and α-amylase combination are used for efficient antistaling property.

### 7.2 Starch liquefaction and saccharification

Liquefaction is a process of dispersion of insoluble starch granules in aqueous solution followed by partial hydrolysis using thermos table amylases [52]. It is the first and most important step in starch processing. The purpose is to provide hydrolyzed starch suspension of low viscosity, stable to retrogradation and suitable for further processing, *i.e*. saccharification. Hattori [92] found that the most important factor for ideal liquefaction of starch is that the starch slurry which contains suitable amount of α-amylase is treated at 105 to 107ºC as quickly and uniformly as possible.

### 7.3 Paper industry

The use of α-amylase for the production of low viscosity, high molecular weight starch for coating of paper has been reported. The viscosity of the natural starch being too high for paper sizing, could be adjusted by partially degrading the polymer with α-amylases in a batch or continuous processes. The conditions depend upon the source of starch and the α-amylase used [93].

### 7.4 Detergent applicants

Amylases have been used in powder laundry detergents since 1975. Now days, 90% of all liquid detergents contain α-amylase and the demand for α-amylase for automatic dishwashing detergents is growing. Most wild type α-amylases were sensitive to oxidants which are generally a component of detergent formulations. Stability against oxidants in household detergents was achieved by utilizing successful strategies followed with other enzymes such as protease. Amylases from *Bacillus* sp. A3-15 [71], *B. lichonformis* NH1 [94] are used in detergent industry.

### 7.5 Analysis in medicinal and clinical chemistry

There are several processes in the medicinal and clinical areas that involve the application of amylases. A process for the detection of higher oligosaccharides, which involved the application of amylase was also developed [95].This method was claimed to be more efficient than the silver nitrate test.

## 8. Future perspectives and conclusion

Microbial α-amylase is one of the most important industrial amylases, occupying approximately 30 % of the global enzyme market [25]. As the above review shows, amylases are one of the most important enzymes in industrial processes. Although the use of amylases, especially α-amylases, has been prevalent for many decades in starch liquefaction and other starch based industries and for the efficient production of this enzyme a number of microbial sources exist. In addition, the demand for these enzymes is also limited to specific applications, such as in the food industry, where fungal α -amylases are preferred over other microbial sources because of their more accepted generally recognized as safe. In addition, more efficient α -amylases are needed in different sectors, which can be achieved either by chemical modification of existing enzymes or by means of protein engineering. Alpha-amylases are now becoming more important in biopharmaceutical applications. However, their application in the food and starch industries is the main market and therefore the demand for amylases in these sectors would always be high. It is reasonable to believe that microbial amylase plays a more important and diverse role in our daily lives by transforming academic achievements in biomolecular research into commercialized products.

## 9. References


1. Uddin SN, Hasan AM, Anower MR, Salam MA, Alam MJ, Islam S. Commercial enzymes production by Recombinant DNA Technology: A conceptual works. Pakistan Journal of Biological Sciences. 2005; 8:345-355.
2. Ramachandran S, Patel AK, Nampoothiri KM, Chandran S, Szakacs G, Soccol CR *et al*. α-amylase from a fungal culture grown on oil cakes and its properties. Brazilian Archives of Biology and Technology. 2004; 47: 309-317.
3. Vidyalakshmi R, Paranthaman R, Indhumathi J. Amylase production on submerged fermentation by *Bacillus* sp. World Journal of Chemistry. 2009; 4:89-91.
4. Reddy LVA, Reddy OVS, Basappa SC. Potentiality of amylolytic yeast for direct fermentation of starchy substrates to ethanol. Indian Journal of Microbiology. 2005; 5:1-15.
5. Rao DM, Swamy AVN, Siva Ramakrishan G. Bioprocess technology strategies, production and purification of amylases: An overview. The Internet Journal of Genomics and Proteomics. 2007; 2:342-351.
6. Lonsane BK, Ramesh RV. Production of an extracellular thermostable α-amylase by solid state fermentation; a potetnail tool for achieving economy in enzyme production and starch hydrolysis. Advance in Applied Microbiology. 1990; 32:31-35.
7. Mishra BK, Arora A, Lata. Use of amylolytic cultures of *Aspergillus* for treatment of waste water from potato processing. Indian Journal of Microbiology. 2003; 43:57-59.
8. Singh V, Sharma R, Sharma P. Isolation, screening and optimization of amylase producing Bacillus sp. from soil.

Asian Pacific Journal of Health Science. 2015; 2:86-93
9. Tiwari KL, Jadhav SK, Fatima. Culture condition for the production of thermostable Amylase by *Penicillium rugulosum*. Global Journal of Biotechnology and Biochemistry. 2007; 2:21-24.
10. Abdulaal WH. Purification and characterization of α-amylase from *Trichoderma pseudokoningii*. BMC Biochemistry. 2018; 19:4-6.
11. Poornima R, Sahu MK, Sivakumar K, Pushpavalli V. Optimization of α-amylase production by Actinomycete strain AE-19 isolated from shrimp pand. Trends in Applied Sciences Research. 2008; 3:45-52.
12. Gupta R, Gigars P, Mohapatra H, Goswami VK, Chauhan B. Microbiol. A-amylase: a biotechnological perspective. Process Biochemistry. 2003; 38:1599-1616.
13. Gopinath SCB, Anbu P, Arshad MKM, Lakshmipriya T, Chun Hong Voon CH, Uda Hashim U *et al*. Biotechnological Processes in Microbial Amylase Production. BioMed Research International. 2017; Article ID 1272193: 1-9.
14. Satyanarayana T, Rao JLUM, Ezhilvannan M. α-amylases. In: *Enzyme Microbiol Technol*. A. Pandey, (Webb, C.R. Soccol, C. Larroche (eds.), Asiatech Publishers Inc., New Delhi, India, 2005, 89-220.
15. Cruger W, Cruger A. Industrial Microbiol. Sinaver Association, Sundreland, M.A. 1989, 189-218.
16. Singh RK, Kumar S, Kumar S. Production of α-amylase from agricultural byproducts by *Humicola lanuginosa* in solid state fermentation. Current Trends in Biotechnology and Pharmacy. 2009; 3:184-197.
17. Ben-Ali M, Mezghani M, Bejar S. A thermostable α-amylase producing maltohexose from a new isolated *Bacillus* sp. US 100: study of activity and molecular cloning of the corresponding agent. Enzyme and Microbial Technology. 1999; 24:548-556.
18. Kunamneni A, Kuttanpillai SK, Singh S. Response surface methodological approach to optimize the nutritional parameters for enhanced production of α-amylase in solid-state fermentation by *Thermomyces lanuginosus*. African Journal of Biotechnology. 2005; 4:708-716.
19. Wijbenga DJ, Beldman G, Veen A, Binnema DJ. Production of native starch degrading enzymes by a *Bacillus firmusllentus* strain. Applied Microbiology and Biotechnology. 1999; 35:180-184.
20. Chessa JP, Feller G, Gerday C. Purification and characterization of the heat-labile α-amylase secreted by the *psychrophilic* bacterium TAC 240 B. Canadian Journal of Microbiology. 1999; 45:452-457.
21. Adeyanju MM, Agboola FK, Omfuvbe BO, Oyefuga OH, Adebawo OO. A themostable extracellular α-amylase from *B. licheniformis* isolated from cassava steep water. Biotechnology. 2007; 6:473-480.
22. Karnwal A, Nigam V. Production of amylase enzyme by isolated microorganisms and its application. International Journal of Pharmacy and Biological Sciences. 2013; 3:354-360.
23. Kumari N, Jain V, Malik M, Sushil. Production and Optimization of Amylase from *Bacillus cereus* Using Submerged Fermentation. International Journal of Current Microbiology and Applied Science. 2017; 6:263-271.
24. Murali Krishnan S, Arun Nagendran N, Pandiaraja D, Vinayaga Moorthi P. Isolation and characterization of amylase producers and optimization of enzyme production. International Journal of Development Research. 2017; 7:18128-18134.
25. Ekka A, Namdeo N. Screening, Isolation and Characterization of Amylase Producing Bacteria and optimization for Production of Amylase. IOSR Journal of Biotechnology and Biochemistry. 2018; 4:50-56.
26. Indriati G, Megahati RRP. Isolation of Thermophilic Bacteria and Optimizing the Medium Growth Conditions. International Journal of Current Microbiology and Applied Sciences. 2018; 7:1457-64.
27. Li H, Chi Z, Wang X, Duan X, Ma L, Gao L. Purification and characterization of extracellular amylase from the marine yeast *Aureobasidium pullulans* N13d and its raw potato starch digestion. Applied and Environmental Microbiology. 2007; 40:1006-1012.
28. Eke OS, Oguntimehin GB. Partial purification and characterization of α-amylase from *Bacillus cereus* B.C. Journal of Agricultural Science and Technology. 1992; 2:152-157.
29. Singh R, Sharma DC, Gupta MK. Optimization of critical process parameters for amylase production by Bacillus sp. using statistical approach (RSM). Journal of Microbiology and Biotechnology Research. 2017; 7:7-15.
30. Joshi VK, Parmar M, Rana NS. Pectin-esterase production from apple pomace in solid state and submerged fermentation. Food Technology and Biotechnology. 2006; 4:253-256.
31. Aygan A, Arikan B, Korkmaz H, Dincer S, Colak O. Highly thermostable and alkaline α-amylase from a halotolernat alkaliphillic *Bacillus* sp. AB-68. Brazilian Journal of Microbiology. 2008; 39:1467-1470.
32. Oyeleke SB, Oduwole AA. Production of amylase by bacteria isolated from a cassva waste dump site in Minna, Niger state, Nigeria. African Journal of Microbiology. 2009; 3:143-146.
33. Tanyildizi MS, Ozer D, Elibol M. Optimization of α-amylase production of *Bacillus* sp. using Response Surface Methodology. Process Biochemistry. 2005; 40:2291-2296.
34. Baysal Z, Uyar F, Ayteki C. Production of α-amylase by thermotolerant *Bacillus subtilis* in the presence of some carbon, nitrogen containing compounds and surfactants. Annals of Microbiology. 2003; 53:323-328.
35. Kashyap P, Sabu A, Pandey A, Szakas G, Soccol CR. Extracellular L-glutaminase production by *Zygosaccharomyce rouxii* under solid state fermentation. Process Biochemistry. 2002; 38:307-312.
36. Malhotra R, Noorvez SM, Satyanarayana T. Production and partial characterization of thermostable and calcium independent α-amylase of an extreme thermophile *B. thermooleovorans* NP54. Letters of Applied Microbiology. 2000; 31:378-384.
37. Abou-Elela GM, El-Sersy NA, Wefky SH. Statistical optimization of cold adapted α-amylase production by free and immobilized cells of *Nocardiopsis aegyptia*. Journal of Applied Sciences Research. 2009; 5(3):286-292.
38. Goyal N, Gupta JK, Soni SK. A novel raw starch digesting thermostable α-amylase from *Bacillus* sp. I-3 and its use in the direct hydrolysis of raw potato starch. Enzyme and Microbial Technology. 2005; 37:723-734.
39. Kar S, Ray R. Statistical optimization of α-amylase production by *Streptomyces erumpens* MTCC 7317 cells

in calcium alginate beads using Response Surface Methodology. Polish Journal of Microbiology. 2008; 57:49-57.
40. de-Carvalho RV, Correa TLR, Matos da Silva JC, Mansur LRC, Martins MLL. Properties of an amylase from thermophillic *Bacillus* sp. *Brazilian* Journal of Microbiology. 2008; 39:1517-1525.
41. Qader SA, Bano S, Aman A, Syed N, Azhar A. Enhanced production and extracellular activity of commercially important amylolytic enzyme by a newly isolated strain of *Bacillus* sp. AS-1. Turkish Journal of Biochemistry. 2006; 31:135-140.
42. Stamford TLM, Stamford NP, Coelho LCBB, Araujo JM. Production and characterization of a thermostable α-amylase from *Nocardiopsis* sp. endophyte of Yam bean. Bioresource Technology. 2001; 76:137-141.
43. Sivaramakrishanan S, Gangadharan D, Nampoothrii MK, Soccol CR, Pandey A. α-amylase from microbial soruce - An overview on recent developments. Food Technology and Biotechnology. 2006; 44:173-184.
44. Aguilar G, Marlon-Guyot J, Trejo Aguilar B, Guyot JP. Purification and characterization of an extracellular α-amylase produced by *Lactobacillus manihotivorans* LMG 18010$^{T}$, an amylolytic lactic acid bacterium. Enzyme and Microbial Technology. 2000; 27:406-413.
45. Ashger MM, Javaid Asad SU, Rahman RL, Legge. A thermostable α-amylase from a moderately thermophilic *B. subtilis* strain for starch processing. Journal of Food Engineering. 2007; 79:950-55.
46. El-Banna TE, Ahmed A, Abd-Aziz AA, Abou-Dobara MI, Ibrahim RI. Production and immobilization of α-amylase from *Bacillus subtilis*. Pakistan Journal of Biological Science. 2007; 10:2039-47.
47. Lachmund AK, Das S, Gupta TK. Regulation of α-amylase formation in *Aspergillus oryzae* and *A. nidulans* transformants. Current Microbiology. 1993; 26:47-51.
48. Kiran O, Comlekciglu U, Arikan B. Effects of carbon sources and various chemicals on the production of a novel-amylase from a thermophillic *Bacillus* sp. K-12. Turkish Journal of Biology. 2005; 29:99-103.
49. Avdiiuk KV, Varbanets LD. Optimization of cultivation conditions of the alpha-amylase producer *Bacillus subtilis*, 147. Journal of Microbiology. 2008; 70:10-16.
50. Haq I, Ashraf H, Qadeer MA, Iqbal J. Pearl millet, a source of α-amylase production by *B. licheniformis*. Bioresource Technology. 2005; 96:1201-1204.
51. Lin LL, Chyau CC, Hsu WH. Production and properties of a raw-starch degrading amylase from thermophilic and alkaliphilic *Bacillus* sp. TS-23. Biotechnology and Applied Biochemistry. 1998; 28:61-68.
52. Aiyer PVD. Effect of C. N ratio of α-amylase production by *B. licheniformis* SPT27. African Journal of Biotechnology. 2004; 3:519-522.
53. Pederson H, Nielson J. The influence of nitrogen sources on the α-amylase productivity of *A. oryzae* in continuous cultures. Applied Microbiology and Biotechnology. 2000; 53:278-281.
54. Morkeberg R, Carlsen M, Neilsen J. Induction and repression of α-amylase production in batch and continuous clusters. *A. oryzae.* Microbiology. 1995; 14:2449-2454.
55. Kumari N, Jain V, Malhotra S. Purification and characterization of extracellular acidophilic α-amylase from *Bacillus cereus* MTCC 10205 isolated from soil. African Journal of Microbiology Research. 2013; 7:5440-448.
56. Bezerra RP, Borba FKSL, Moreira KA, Lima Filho JL, Porto ALF, Chaves AC. Extraction of amylase from fermentation broth in poly (*Ethylene glycol*) salt aqueous two-phase system. Brazilian Archives of Biology and Technology. 2006; 49:569-574.
57. Ahmed SA, Mahdy EME, Hassan O, Saleh NA, Goda HA. Studies on the activity and stability of immobilized *B. acidocaldarius* α-amylase. Australian Journal of Basic and Applied Sciences. 2008; 2:466-474.
58. Ghorbel RE, Maktouf S, Massoud EB, Bejar S, Chaabouni SE. New thermostable amylase from *B. cohnii* US 147 with broad pH applicability. Applied Biochemistry and Biotechnology. 2008; 35:649-654.
59. Kaneko T, Ohno T, Ohisa N. Purification and characterization of a thermostable raw starch digesting amylase from a *Streptomyces* sp. isolated in a milling factory. Bioscience, Biotechnology, and Biochemistry. 2005; 69:1073-1081.
60. Afifi AF, Kamel EM, Khalil AA, Foaad MA, Fawzi EM, Houseny MM. Purification and characterization of α-amylase from *Penicillium olsonii* under the effect of some antioxidant vitamins. Global Journal of Biotechnology and Biochemistry. 2008; 3:14-21.
61. Kaur P, Satyanarayana T. Partial purification and characterization of GA of thermophillic mould. Indian Journal of Microbiology. 2001; 41:195-199.
62. Kim TU, Gu BG, Jeong JY, Byun SM, Shine YC. Purification and characterization of a maltotetraose forming alkaline α-amylase from an alkalophilic *Bacillus* sp. GM8901. Applied and Environmental Microbiology. 1995; 61:3105-12.
63. Yoshigi N, Chikano T, Kamimura M. Purification and properties of an amylase from *B. cereus* NY-14. Agricultural and Biological Chemistry. 1985; 49:3369-3376.
64. Ezeji TC, Bahl H. Purification, characterization and synergistic action of phytate-resistant α-amylase and α-glucosidase from *Geobacillus thermodenitrificans* HR010. Journal of Biotechnology. 2006; 125:27-38.
65. Krishnan T, Chandra AK. Purification and characterization of α-amylase from *Bacillus licheniformis* CUMC-305. Applied and Environmental Microbiology. 1983; 46:430-437.
66. Grootegoed JA, Lauwers AM, Heinen W. Separation and partial purification of extracellular amylase and protease from *B. caldolyticus*. Archives of Microbiology. 1973;90: 223.
67. Laderman KA, Davis BR, Krutzsch HC, Lewis MS, Griko YV, Privalou PL *et al*. The purification and characterization of an extremely thermostable α-amylase from the hyperthermophilic archaebacterium *Pyrococcus furiosus*. Journal of Biological Chemistry. 1993; 268:24394-24401.
68. Illori MO, Amund OO, Imidiji O. Purification and properties of an α-amylase produced by a cassava fermenting strain of *Micrococcus luteus*. Folio Microbiologica. 1997; 42:445-446.
69. Morgan FJ, Priest FG. Characterization of a themostable α-amylase from *B. licheniformis* NCIB 6346. Journal of Applied Bacteriology. 1981; 50:107-114.
70. Shaw JF, Lin FP, Chen SC, Chen HC. Purification and properties of an extracellular α-amylase from *Thermus*

sp. *Botanical Bulletin-Academia Sinica*. 1995; 36:195-200.
71. Arikan B. Highly thermostable, thermophilic, alkaline, SDS and chelator resistant amylase from a thermophilic *Bacillus* sp. isolates A3-15. Bioresource Technology. 2008; 99:3071-3076.
72. Vihinen M, Mantsala P. Microbial amylolytic enzymes. Critical Reviews in Biochemistry and Molecular Biology. 1989; 24:329-418.
73. Schwermann B, Pfau K, Liliensick B, Schleyer M, Fischer T, Bakker EP. Purification, properties and structural aspects of a thermo acidophilic α-amylase from *Alicylobacillus acidocaldarius* ATCC27009. Insight into acidostability of proteins. European Journal of Biochemistry. 1994; 226:981-991.
74. Shinke R, Aoki K, Murokam S, Inoue T, Babat T. Alakline and thermophilic amylases of industrial use. In: Dordick J.S. Russell, A.J. editors. *Enzyme engineering* XIII, New York: Annals of the New York Academy of Sciences. 1996; 799:332-40.
75. Uyar F, Baysal Z, Dogru M. Purification and some characteristics of an extracellular α-amylase from a thermo tolerant *B. subtilis*. Annals of Microbiology. 2003; 53: 315-322.
76. Olutiola PO. A-amylolytic activity of *A. chevalieri* from mouldy maize seeds. Indian Phytopathology. 1982; 35:428.
77. Burhan A, Nisa U, Gokhan C, Omer C, Ashabil A, Osman G. Enzymatic properties of a novel themrostable, thermophilic, alkaline and chelator resistant amylase from an alkaliphilic *Bacillus* sp. isolate ANT-6. Process Biochemistry. 2003; 38:1397-403.
78. Saxena RK, Dutt K, Agarwal L, Nayyar P. A highly thermostable and alkaline amylase from a *Bacillus* sp. PN-5. Bioresource Technology. 2007; 98:260-265.
79. Mahmoud SAZ, Taha SM, Attia RM. Effect of metal ion activators on the reaction velocity of bacterial α-amylase. Journal of Botany UAR. 1968; 11:41-48.
80. Varavinit S, Chaokasem N, Shobsngob S. Immobilziaton of a thermostable α-amylase. Science Asia. 2002; 28:247-251.
81. Reshmi R, Sanjay G, Sugunan S. Enhanced activity and stability of α-amylase immobilized on alumina. Catalysis Communication. 2006; 7:460-465.
82. Kara K, Osman B, Yavuz H, Besirilana N, Denizli A. Immobilization of α-amylase on $Cu^{2+}$ chelated poly (ethylene glycol dimethacrylate-n-Vinylimidazole) matrix via adsorption. Reactive and Functional Polymers. 2005; 62:61-68.
83. Milosavic N, Prodanovic R, Jovanovic S, Vujic Z. Immobilization of glucoamylase via its carbohydrate moiety on macroporous poly (GMA-CO-EGDMA). Enzyme and Microbial Technology. 2007; 40:1422-1426.
84. Kumari N, Pal A, Jain V. Immobilization of α-amylase purified from bacillus cereus MTCC 10205 by entrapment and adsorption on various support systems. Applied Biological Research. 2014; 16:21-30.
85. Dey G, Singh B, Banerjee R. Immobilization of α-amylase produced by *B. circulans* GRS 313. Brazilan Archives of Biotechnology. 2003; 46:167-176.
86. Tien CJ, Chiang BH. Immobilization of α-amylase on a Zirconium dynamic membrane. Process Biochemistry. 1999; 35:377-383.
87. Gangadharan D, Namporthiri KM, Sivaramakrishan S, Pandey A. Immobilized bacterial α-amylase for effective hydrolysis of raw and soluble starch. Journal of Food Research. 2008; 158:653-662.
88. Kahraman MV, Bayramoglu G, Kayaman-Apohan N, Gungar A. α-amylase immobilization on functionalized glass beads by covalent attachment. Food Chemistry. 2007; 104:1327-1364.
89. Atia KS, Ismail SA, Dessouki AM. Immobilization of α-amylase using polyacrylamide polymer derivatives. Journal of Chemical Technology & Biotechnology. 2003; 78:891-898.
90. Pintauro ND. Bread and baked goods. In: Pintauro N.D., Editor. Food processing enzymes recent developments. *Food Technology Review* No. 52. Park Ridge, N.J., USA: Noyes Data Corporation, 1979.
91. Van Dam HW, Hill JDR. Yeast and enzymes in bread making cereals. Foods World. 1992; 37:245-252.
92. Hattori F. Japan Patent No. 1203977, Japan Kokai Tokyo, Koho, 1984, 51-44652.
93. Tolan JS. Pulp and Paper. In: Godfrey, T West, S editors. Industrial Enzymology, 2nd ed. New York: Stockton Press, 1996, 327-38.
94. Hmidet N, Ali NE, Haddan A, Kanoun S, Alya SK, Nasri M. Alkaline proteasees and thermostable α-amylase co-produced by *B. licheniformis* NH1: Characterization and potential application as detergent additive. Journal of Biochemical Engineering. 2009; 47:71-79.
95. Giri NY, Mohan AR, Rao LV, Rao CP. Immobilization of α-amylase complex in detection of higher oligosaccharides on paper. Current Science. 1990; 59:1339-40.