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AN OVERVIEW OF THE ENZYME: AMYLASE AND ITS INDUSTRIAL POTENTIALS

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ABSTRACT
Amylases are group of enzymes produced by plants, animals and microorganisms, the enzyme has the ability to hydrolyze or degrade starch molecules into polymers containing units of glucose, thus, it is one of the most useful enzymes used by industries dependent on starch in their production processes. The enzyme has varying applications in food, fermentation, textile, pharmaceutical industries among others. Generally, amylase from microbial sources (i.e. fungal and bacterial origin) has over shadowed others in industrial usage. As such, this Paper aimed at reviewing amylase enzyme as a whole and some of its common industrial applications. The review visited the types of amylase based on hydrolases classification, its sources with emphasis to microorganisms, methods of production as well as effects of some chemical and physical parameters. The review also discusses some of the most common industrial application or uses of amylase enzyme in food, brewing, chemicals, paper, pharmaceutical, textile industries to mention but few. In conclusion, the reviewers suggest the use of microbial amylase due to it easy and simple technique in production, lower capital investment, lower energy requirement and high yield during production, exploration of more microbes with enzyme production potentials as well as improved industrial Scale production of the amylase for the betterment of the economy and improved industrial production of products.
Key: Amylase, Application, Enzyme, Industry, Microbes and Starch.

INTRODUCTION

Industries use Amylase as one of their main and most valuable enzyme. This enzyme has the ability to hydrolyze starch molecules into polymers containing units of glucose. Several industries such as food, fermentation and pharmaceutical industries utilize amylase for its potential application in their several production processes. Amylases can be obtain from living organisms (i.e. plants, animals and microorganisms) (Souza, 2010).

Generally, enzymes from microorganisms (fungal and bacterial origin) are more useful and have over shadowed others in industrial applications (Saini *et al.,* 2017). More so, enzymes from microorganisms have a broad spectrum of industrial uses than those from plant and animal due to their stability (Tanyildizi, 2007). Bulk production capacity and ease of manipulation to obtain enzymes of desired character are considered major advantage to others(Souza and Magalhães, 2010).

According to Noomen *et al*., (2009), Amylases are among the most studied enzymes. In contrast to specificity of their action, their diversity attracted worldwide attention in attempts to exploit their potential applications in biotechnological and other industries (San-Lang *et al*., 2011).

Sources of Amylase Enzyme

Enzymes can be produced by plants, animals and microorganisms, those of Microbial source have been reported by Scientists to meet industrial demand (Pandey *et al*., 2000). Amylases of bacteria, fungi and viruses are area of recent researches and are studied more often than others, this is attributed to their relative ease of large-scale production as well as low downstream cost as they are extracellular in nature (Ashis *et al*., 2009). Amylase (e.g. alpha amylase) have been isolated from fungi, yeasts, bacteria and actinomycetes, however, enzymes of fungal and bacterial origin has more industrial applications and dominated industries than others, *Aspergillus, Rhizopus* as well as *bacillus* species are among the high producers of the enzyme amylase (Pandey *et al.,* 2000; McVey,2002). Consistency, cost effectiveness, less time and space requirement for production and ease of process optimization and modification are among the reasons why enzymes of bacterial and fungal sources are used in industries (Ellaiah *et al*., 2002).

Types of Amylase and their major functions

Amylases can be broadly classified based on either class of hydrolases (Enzyme commission, EC 3) or transferases (Enzyme Commission, EC 2)

Based on their hydrolases class, they are divided into three (3) main groups; group one, two and three which comprises of the Endoamylases, Exoamylases and debranching enzymes respectively. This classification is based on their specificity for bond cleavage of polysaccharides (Castro *et al*., 2018).

The first group of amylases known as the Endoamylases is made up of the α-amylases enzyme with EC 3.2.1.1. This enzyme has the ability to cleave or attack α-1, 4 bonds in internal regions of long-chain substrates, leading to the release of oligodextrins. The second group of amylases known as Exoamylases comprises of the glucoamylase with EC 3.2.1.3., α-glucosidase with EC 3.2.1.20, and b-amylase with EC 3.2.1.2). Enzymes in this group represent enzymes responsible for the breakage of either long-chain or short-chain substrates to release the low molecular weight products (e.g., glucose, maltose). Lastly, debranching amylases which includes enzymes like isoamylases (EC 3.2.1.68) and pullulanase (EC 3.2.1.41). These third group of Amylases enzyme act only on α-1,6 linkages in the structure of amylopectin and other related small substrates (e.g., isomaltose) Pullulanase has been reported to catalyze the breakdown of α-1,6 linkages in pullulan and glycogen (Castro *et al*., 2018; Cinelli *et al*., 2015).

So also, Glucoamylase and α-glucosidase (both exoamylase) has been proven to catalyze the hydrolysis of α-1,6 linkages in isomaltooligosaccharides, in addition to α-1,4 bonds **(**van der Maarel *et al*., 2002).

In industries, the main enzymes used are the, carbohydrases, proteases, and lipases groups. Amylase belongs to the carbohydrase group **(**Castro *et al*., 2018). α-amylase and glucoamylase represent the most marketable form of amylolytic enzymes, microorganisms belonging to bacteria (*Bacillus licheniforms)* and fungal (*Aspergillus oryzae* and *Aspergillus niger*) origin has been reported by McVey (2002) as the main sources of α-amylase and glucoamylase use in various industrial processes.

Amylases have been effectively used in fuel, beverage (beer and distilled spirits), and industrial ethanol production; cleaning products; bakery products; textile processing; pulp and paper processing; animal feed; and digestive pharmaceuticals. Glucoamylase are mainly used industrially for starch-based ethanol. In 2014, α-amylase and glucoamylase account for about 60.7% of the total carbohydrase market, additionally, α-amylases a class of Amylase have been proven useful in production of cleaning products, bakery products, textile processing, paper processing ,animal feeds, pharmaceuticals, ethanol profuction while glucoamylase is used in sweetener production, starch-based ethanol production among others (Freedonia, 2010).

Although α-amylase can be produced from plant and fungi, however bacteria account for 83% of total α-amylase production due to its high stability to heat, in 2015, α-amylase produced from plant based sources e.g. rice, corn potato etc. accounted for about1683 ton useful in application in baking goods production. Globally, α-amylase has a market of about US$255 million which is expected to reach US$320 by 2024, this is characterized to it increasing use and markets in China, India, and the Middle East [http://www.grandviewresearch.com/industry-analysis/alpha-amylase-baking-enzyme-market)

Amylase Production

Amylase can be produced by either submerged fermentation (SmF) or solid-state fermentation (SSF) methods, both methods defend on the physicochemical factors or parameters.

Although SmF is considered traditionally useful in industrial production of important enzymes because of the ease to control parameters such as temperature, pH, aeration, oxygen transfer and moisture (Gangadharan *et al*., 2008). But In SSF process, the environmental conditions provided resembles that of the natural habitat of microorganism, therefore, SSF systems appear more promising due to the natural conditions its provides for microbial growth and therefore preferred choice for microorganisms to grow and produce useful value added products (Souza, 2010), in addition to high yield and high specificity, low moisture contents, which prevent bacterial contamination, lower levels of catabolite repression, and better product recovery in SSF than SmF (Shalini, 2014).

SSF is considered suitable for production of amylase from fungi while bacteria are considered unsuitable, nevertheless, several reports have shown that bacterial cultures can be manipulated and controlled in SSF processes to produce amylase (Pandey, 2003).

Moreover, better product recovery, lack of foam builds up, higher productivity, Easier and simpler techniques, lower capital investment, lower energy requirement and less water output are more advantages of SSF over SmF.

In Recent evaluation by researchers, SSF has been proven to be the most appropriate method of enzyme production for thermo liable products production and also the most suitable for developing countries compared to SmF, this is concluded because of the higher yields obtained in SSF than SmF (Tanyildizi, 2007; Shalini, 2014).

The usability of Amylase enzyme after extraction from microbial cells defends largely on a successful purification. The downstream process in purification depends strongly on the process cost, market value, quality of the final product as well as the type of technology available. Following crude isolation by precipitation and membrane separations, enzymes are purified using chromatography. For example, Amylase enzyme from *Aspergillus flavus var. columnaris* was purified using ammonium sulphate precipitation and gel filtration chromatography with a total fold of 5.6 and 9.6 respectively, this show a higher amount using the later (Prakash *et al*., 2009).

Characteristics/properties of Amylase

Physical and chemical parameters are key factors in growth of microorganisms for amylase production, that of bacteria and fungi have been extensively studied and described (Gupta *et al*., 2003; Shalini, 2014). Several physical and chemical parameters are important in the development of fermentation processes for the growth of microorganisms used in amylase production, this is because of their impact on the economy and practicability of the fermentation processes (Saini *et al*., 2017). Factors like pH, temperature, metal ions, carbon and nitrogen source, surface acting agents, phosphate and agitation have been studied for amylase production. This is because, several industrial processes require varying conditions of parameters as such must matched or favor its application. For example, ɑ-amylases used in detergent industry require high pH to be active and stable while that of starch need low pH values. Most notable among these are the composition of the growth medium, pH of the medium, phosphate concentration, inoculum age, temperature, aeration, carbon source and nitrogen source (Kirk, 2007; Sajedi *et al*., 2005; Saranraj and Stella 2013) and ion concentrations. Some of the most important parameters of pH and Temperature with their optimal values for different microorganisms are reported by Souza and Magalhães (2010); Saini *et al*., (2017) as shown in table 1**.**

Table 1: pH and Temperature Optimal values of various microorganisms for Amylase Production various Microorganisms

Microorganisms	pH optimal/stability	Temperature optimal/stability
Bacillus subtilis	7.0	37 °C
Bacillus amyloliquefaciens	7.0	33 °C
Bacillus sp.	4.5	70 °C
Aspergillus niger	5.5	70 °C
Aspergillus fumigatus	6.0	30 °C
Penicillium fellutanum	6.5	30 °C
Mucor sp.	5.0	60 °C
Malus pumila	6.8	37 °C
Penicillium olsonii	5.6,	30^{0}C

Souza and Magalhães (2010); Saini *et al*., (2017)

Industrial uses/Applications of Amylase

Amylase has been reported to be useful in many important industrial processes including but not limited to, food, brewing, chemicals, paper, pharmaceutical and textile. It importance has also been extensively studied in pharmaceutical industries specifically in digestive tonics processes. The most documented applications of ɑ-amylases are in the starch industry, where it is used un processes to converts starch to small units of sugar like fructose and glucose syrups (Nielsen and Borchert, 2000). Some of it most common documented uses are briefly discussed in this review.

1. **Production of Maltooligomer or oligosaccharides mixture**

Maltooligomer is a powdery substance that is highly hygroscopic, therefore, use in moisture regulation of food (Saini *et al.,* 2017). It does compose of Glucose, 2.2%; maltose, 37.5%; maltotriose, 46.4%; and maltotetrose and larger malto oligosaccharides, 14% as reported by Marc *et al*., (2002). The product (Oligosaccharides mixture or Maltooligomer mixture) is a product of corn starch digestion with alpha amylase, beta amylase and pullulanase.

Maltooligomer mixture is also use as a substitute for sucrose and other saccharides, it tastes less sweet than sucrose with a lower viscosity than corn syrup because of its low content of glucose, it also helps in the prevention of crystallization of foods that are of sucrose origin (Vander *et al*., 2002).

2. **Production of fructose and maltose syrup suspensions**

Suspensions containing fructose up to 42% are known as High fructose containing syrups (HFCS) 42 F, they are produced be enzymatic isomerization of glucose with glucose isomerase enzyme, in this process, starch is first converted to glucose by enzyme liquefaction and Saccharification and later to the fructose syrup (Saini *et al.,* 2017).

Maltose on the other hand occurs naturally as disaccharide, it is widely used as an intravenous sugar supplement and a sweetener also. It is a non-hygroscopic substance which low crystallization ability, therefore highly used by food industries (Sameh *et al*., 2011). More so, (Yakup *et al*., 2010) report that maltose sugar syrup has the chemical structure of 4-0-D-glucopyronosil- D-glucopyranose and this form most of it main component.

Plant base materials such as potato, sweet potato, Corn and cassava starches are used for maltose production (Uma *et al*., 2007). In the production of medial grade and food grade maltose, the concentration of starch slurry is adjusted to 10-20% and 20-40% respectively (Archana *et al*., 2011).

3. **Textiles Desizing**

Disizing refers to the Removal of starch sizer from textiles, In textiles processing, starch paste is generally applied to improve the strength of textiles during weaving, prevent loss of string friction etc. after the weaving the starch is removed before scouring and dying of the cloths. Alpha amylase are used extensively by textile industries to remove starch from textiles after weaving, the amylase enzyme is responsible for the hydrolysis and solublization of the starch on cloth which is later wash out to increase the stiffness of the final product, it's also used on grey cloth to remove starch before its further processing of bleaching and dying. Garments like the "jean" are also disized before mashing, before they are finally laundered and rinsed to obtain the final product (Saranraj and Stella, 2013 ; Saini *et al.,* 2017)

4. **Industrial Liquefaction**

Liquefaction is defined as the process by which insoluble starch granules are removed or dispersed from an aqueous solution followed by partial hydrolysis using thermostable enzymes. In many industrial processes, the viscosity is extremely high following gelatinization (Vander *et al*., 2002), Due to excess starch suspension of about 35% (w/v) (Damien *et al*., 2010). Therefore, a Thermo stable α-amylase enzyme is used as a thinning agent, which brings about decrease in viscosity and incomplete hydrolysis of starch.

5. **Bread Production**

For decades, enzymes have been used in baking industries, alpha amylases has been utilize for bread baking. The enzymes degrade starch into small dextrins making it easy for yeast to act upon and therefore work continuously during dough fermentation, proofing and the initial stages of baking. These activities result in increased bread volume and texture. Moreover, the sugars (e.g. glucose and maltose) produced by the enzymes enhance the Maillard reactions responsible for the browning of the crust and the development of flavor after baking (Lundkvist *et al*., 2007).

6. **Production of ethanol by direct starch fermentation**

In the production of ethyl alcohol, Starch containing products such as grain; potatoes etc. are used as a raw material. The starches in this product are first converted to fermentable sugars in the presence of amylases. Fungal amylases are used in alcohol production and brewing industries. They have the advantages of producing uniform enzyme action in mashes, increase rate of Saccharification, alcohol yield and yeast growth (Maria *et al*., 2011). The use of bacterial enzyme is slowly replacing malt in brewing industry, thus making the process more economically significant. Alpha amylases have also been reported to carry out reactions of alcoholysis by using methanol as a substrate (Saini *et al.,* 2017).

7. **Paper Production**

In the production of paper, Starch paste is used as a mounting adhesive together with additives such as protein glue or alum, this combination has been reported to cause damage to paper as a result of its embrittlement. Enzymes are been used to control the process, alpha amylase has the ability to disized starch, it's therefore applied as immersion or as a gel poultice to facilitate its removal. Use of Alpha amylase make it possible to disized raw starch and coat the paper instead of using the expensive chemically modified starch, starch is extensively used for some paper size press publications (Saini *et al.,* 2017).

8. Biofuel production

The world needs for biofuel is increasing day by day, this is due to the harmful effects caused by the pollutants produce by Fossil fuels with cause more harm than good to living life, as well as its high price or expensiveness. Biofuels have become the alternate for the world problem. Ethanol fuel is an important biofuel, its produced or generated from renewable resources such as agricultural waste and other byproducts. Alpha amylase and others like glucoamylase have the ability to ferment sugars and produce ethanol which is use as biofuel (Kirk *et al.*, 2002).

9. Removal of starchy stains

Saini *et al.*, (2017) reported that Amylase from apple in combination with commercial detergent has been used to remove starchy stains from fabrics, therefore useful in the removal of starchy stains. It has been well documented that industries that produce detergent the main users or consumers of enzymes (in volume and value). Enzymes make use in detergent formulations help in influencing the actions of detergents in removing tough stains and at the same time making the enzyme environmentally friendly. Several reports indicate that 90% of liquid detergents contain amylase, they are also among the most used in formulation of enzymatic detergents (Hmidet *et al.*, 2009; Mitidieri *et al.*, 2006). These enzymes have been proven effective in removing residues of starchy stains on dishes caused by foods like potatoes, chocolate, custard, etc., as well as laundry detergents (Mukherjee *et al.*, 2009). Obviously, detergents need to be stable and activity at lower temperatures and alkaline pH, this oxidative stability of amylase makes them an important part of detergent production (Chi *et al.*, 2009). Amylases from *Bacillus* or *Aspergillus* are the commonly used in industries (Mitidieri *et al.*, 2006).

CONCLUSION

Amylases have been in use for many decades, the use of microbial amylase in several industrial processes is becoming more promising due to its stable and easy way of production. This enzyme is produced in a large and considerable quantity by fungi and bacteria found in our environment, its easy method of production, lower capital investment, lower energy requirement and high yield during production are all factors identified that suggests it continual usage in industries.

Nevertheless, exploration of more microbes with enzyme production potentials from various environmental sources and waste to maximize it production and profitability as well as improved industrial Scale production for the betterment of the economy and improved industrial production of products is recommended.

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