APPLICATIONS OF MICROBIAL ALPHA AMYLASE IN SUGAR, BAKING, TEXTILE AND OTHER INDUSTRIES

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ABSTRACT

Amylases are the most major significant enzymes which are used in industry. Amylases have many substantial applications, as nutrition, yeast and therapeutic industries. α -amylases can be gained from flowers, beasts, germs and a solitary family. For the manufacture of α -amylase, the important step is to convert the simple starch molecules into oligosaccharides. Variations in microbial α -amylases are used in altered business sectors i-e nutrition, cloth, daily and cleansing agent industries. The synthesis of α -amylase has been carried out by using immersed fermentation. The features of α -amylase involve thermo stability, pH assortment, pH firmness and Calcium-independency; which play a strategic role in the process of fermentation. . By the use of α -amylase in starch hydrolysis, applied for industrial uses, as washing and ceramic cleansing agent or as anti-staling managers in roasting. In this review article we engrossed on the industrial applications of microbial α -amylase.

Keywords: Amylase, sugar, baking, textile, fuel, paper, detergent industry

1. Introduction

α -Amylases (E.C.3.2.1.1) are those which cleave the hydrolysis of interior alpha-1, 4-glycosidic connections in starch polymer which are in little molecular mass products, such as glucose, maltose and malt triose parts (Gupta *et al*., 2003). Amylases can be attained from numerous sources, such as shrubberies, creatures and microbes. Currently a large quantity of microbial amylases is created from microorganisms and is commercially accessible and these enzymes are lastly substituted by chemical cleavage in starch treating industry. The microbial alpha-amylase have a wide-ranging spectrum of manufacturing applications as they are more stable as compared to those amylases that are equipped from vegetable and bodily sources (Tanyildizi*et al*., 2005). The principal improvement of consuming bugs for the invention of amylases is that it is economically beneficial and the additional factor is that microbes can easily be used to obtain the features of those enzymes which we looked-for. α- Amylase has been acquired from some altered species of toadstools, molds and germs. Consequently, enzymes from fungal and bacterial species have subjugated several applications in industries (Gupta *et al*., 2003). α -Amylases have various submissions in a number of trade developments such as nourishment, yeast, clothing, broadsheet, laundry detergent and therapeutic industries. Nevertheless, with the progression of bioengineering, the solicitations of amylase has extended in many fields such as scientific, homeopathic and diagnostic chemistry and also included in starch saccharification, fabric, foodstuff, fermenting and purifying industries (Pandey*et al*., 2000). α- amylases are one of the supreme well-known and essential systems of industrial amylases.

2. Manufacturing of Infectious and Archaeal α-Amylases

The assembly of α-amylases has been investigated enthusiastically in water-logged fermentations or compact state of fermentations. Origination of α-amylase has been extracted from thermophiles and hyper-thermophiles bacteria and Achaea like *Pyrococcus*, *Thermococcus*, and *Sulfolobus*species, *G.thermoleovorans*(Sunna*et al*., 1997). Miscellaneous Carbon sources such as starch, fructose, glucose, and rice flour are utilized for the creating enzymes. Nitrogenbasis is a foremost aspect that affects the invention of alpha-amylase. Several steel ions like Ca^{2+}, Fe^{2+}, Mg^{2+} and K^{+} are auxiliary to the medium for enzyme assembly (Sunna*et al*., 1997). Phosphate is an imperative necessity for viruses as it normalizes the synthesis of principal and tributary metabolites. Worse and multifaceted levels of phosphate in the standards expressively affect the

progress and waged of enzymes (Hillier *et al*., 1997). The fabrication of infective amylases is substantially affected by corporal and organic constraints (Gigras*et al*., 2002).

3.α- Amylase Construction

The invention of α-amylase by waterlogged fermentation or (SMF) and rock-hard state of fermentation or (SSF) has been investigated and depends on a diversity of physicochemical aspects. SMF has been conventionally used for the formation of industrially important enzymes for the comfort of control of different constraints such as pH, hotness, aeration, oxygen allocation and humidity (Couto*et al*., 2006). Rock-solid state of fermentation exhibits the natural habitat for bugs and is consequently, the healthier choice for bugs to grow and yield useful and valuable goods. Underwater fermentation can be used in contradiction with ordinary environment of bacteria, especially of mildews (Singhania*et al.*, 2009). Mushrooms and molds were measured as appropriate microorganisms for SSF bestowing to the hypothetical concept of water motion, even though germs are measured in appropriate for solid state fermentation.

There are additional many advantages of SSF over SMF, comprising of greater output, humbler techniques, inferior wealth investment, subsidiary energy obligation and fewer aquatic harvest, better merchandise retrieval and lack of spray figure up, also it is stated to be the most appropriate process for unindustrialized countries. Freshly investigators considered that SSF is the unsurpassed organization for fabricating enzymes. Readings display that SSF is a clear-cut process for the construction of enzymes and other thermo labile foodstuffs, expressly when sophisticated profits can be attained when associated it to SMF (Tanyildizi*et al*., 2007).

The protagonist of innumerable features, including pH, heat, silver ions, carbon and nitrogen foundation, shallow substitute, phosphate and distress have been premeditated for alpha-amylase invention. The belongings of α-amylases such as thermo stability, pH silhouette, pH firmness and calcium-independency can be allied to its solicitation as conferred in Table 1. For illustration, α-amylases canister be used in thickener diligence are of dynamic and are firm at truncated pH, but relentless at extraordinary pH principles in the cleansing negotiator industry.

Table 1:

Properties of Bacteriological and Fungal α- Amylases

Microorganisms	Fermentation	pH Optimal/ Stability	Temperature Optimal/ Stability	Molecular Weight (kDa)	Inhibitors
Bacteria					
Bacillus Amylo liquefaciens	SMF	7.0	33	72	
Chromo halobacter sp. TVSP 101		7.0-7.9	65		
Caldimonas Taiwanensis sp. nov.	SSF	7.0	55		Galactose, Malate, Malonate, Sucroses
*Halo Bacillus sp*MA-2		7.5-8.5	50		Cd^{2+},Cu^{2+}
Haloarcula Hispanic	SMF	6.5	50	43.3	EDTA

(Tanyildizi*et al.*, 2007)

4. Depiction of Bacteriological and Archaeal α-Amylases

α-amylase displays a wide variety of substrate degradation. They destroy amylose, amylopectin, cyclodextrins, glycogen and dextrin nonetheless, they hold utmost specificity near to starch (Antranikian*et al*., 1990). α-amylase is a steel galvanized enzyme and has high affinity towards Ca^{2+}. The metal ion, which constrains α-amylase activity, includes Hg^{2+} ions (Mamo*et al*., 1999). The inhibition by Hg^{2+} ions shows the incidence of COOH clusters in enzyme glimmer (Dey*et al*., 2002). Additionally, an mercuric ion is also acknowledged to liquefy in dole disc and to interdepend with pungent ring present in tryptophan (Zhang *et al.*, 2007).

5. Decontamination of α- Amylase

The marketable practice of α-amylase does not necessitate distillation of the enzyme, but enzyme solicitations in curative and irrefutable areas entail great photograph amylases. Diverse approaches for refinement of enzymes obligate to be considered, abusing explicit individualities

of the mark biomolecules. Inquiry of workroom gage refinement for α-amylase embraces innumerable mixtures of ion confrontation; gel sanitization; hydrophobicity associations and reverses phase chromatography. Otherwise, α-amylase abstraction protocols using organic diluents such as ethanol, acetone and ammonium sulfate drizzle (Hamilton *et al.*, 1999) and ultrafiltration process have been assumed (Moraes*et al.*, 1999). Molten–fluid withdrawal is the allocation of convinced constituents as of single segment to alternative point when immiscible or moderately fathomable gooey segments come together in exchange through every point. That progression is generally secondhand in the biochemical industry due to its affluence of admittance, low-slung charges and ease of route. Sanitization of biomolecules by molten–liquefied abstraction has been efficiently passed out on an outsized scale for additional time span. This organization has various benefits including subordinate breadth, inferior charge of elements and dumpier slice departure time (Mazzola*et al.*, 2008).

6. Industrial Applications of α- amylase

α-amylases can be used at industrial level in sugar, textile, baking, paper, and brewing industries as shown in Fig 1.

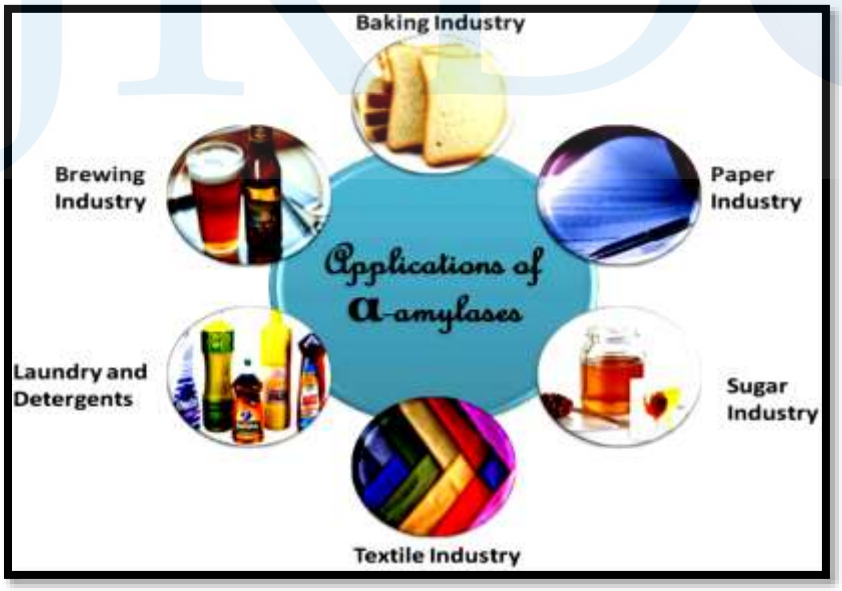

Fig. 1: Applications of α- amylases in different industries (Guzman-Maldonadao and Paredes-Lopez, 1995)

Every application requires a α-amylase with specific characteristics. A bar graph is plotted using the range of temperature and pH requirements of a-amylases to be used for different industrial applications that shown in Fig 2.

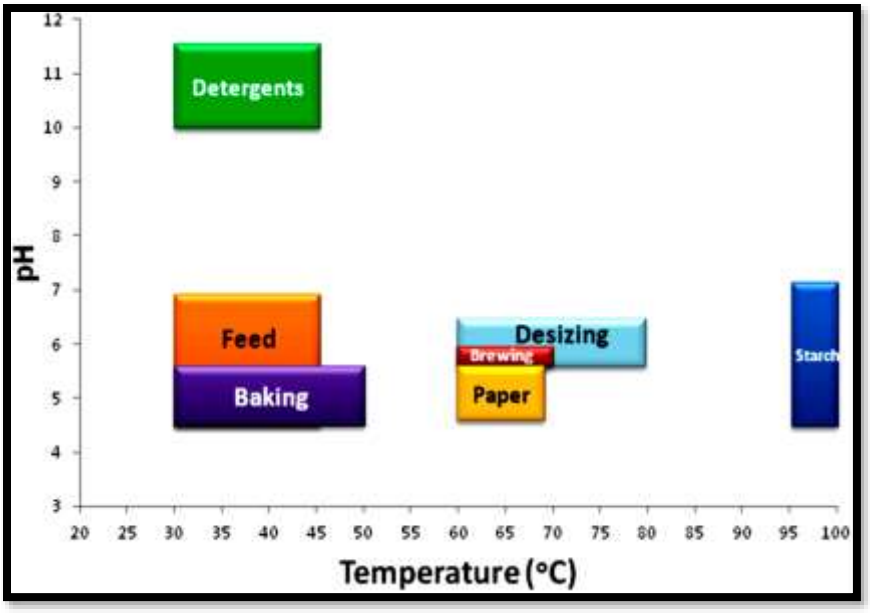

Fig. 2: Characteristics of alpha- amylases for definite applications

Different commercially available a-amylases with varying properties are used for different applications are shown in Fig 2.

Table 2: Commercially available different bacterial a- amylases

Commercial name of a- amylase	Manufacturer	Producer microorganism	Application
AmzymeTX	Parchem7	*Bacillus amyloliquifaciens*	Foods and feeds
Aquazym120l	Novo Nordisk, Denmark5	–	Desizing of textiles
AquazymUltra250l	Novo Nordisk, Denmark5	–	Desizing of textiles
BANTM	Novozymes	*B. amyloliquifaciens*	Foods and feeds, paper industry
Enzymex(Cocktail),	Exotic Biosolutions Pvt. Ltd. 4	*B. amyloliquifaciens*	Foods and feeds
Fructamyl® FHT	ERBSLOEH3	–	Starch saccharification

Liquozyme® SC DC	Novozymes6	Genetically engineered from *B. licheniformis*	Starch saccharification
Natalase®	Novozymes6	–	Detergent industry
Stainzyme® plus	Novozymes6	Genetically engineered	Detergent industry
Thermamyl®, Takaterm	Novo Nordisk, Denmark5	*B. licheniformis*	Detergent industry, paper industry
ValidaseBAA	DSMValleyResearch2	*B.amyloliquifaciens*	Food industry
VERON® XTENDER	ABenzymes1	–	Baking industry

(Pritchard, 1992)

6.1. Sugar industry

In the mid of 1900s the large scale of starch dealing industry has been appeared (Bergthaller*et al*., 1999). In the start, glucose syrups were made by hydrolyzing starch by using acid treatment. In 1811, sweet tasting syrup was gain when starch water suspension was treated with dilute acid that found by Kirchhoff. A commercial process for the production of glucose from starch was show by Newkirk in 1921. In starch industry for the formation of high corn syrups is the primary use of α-amylases, which is used in beverage industry as sweeteners for soft drinks depending on the degree of hydrolysis that is required (Nielsen and Borchert, 2000). The enzymatic conversion of glucose into high glucose syrup including following steps: Liquefaction, Saccharification, Hydrolysis**.**

6.1.1. Liquefaction

During liquefaction, starch granules gelatinized, forming a viscous suspension which involves the dissolution of starch granules. The gelatinization takes place in a jet cooker where temperature is kept 105-110°C for 2-3 hours and pH is adjusted 5.8-6.5 (Vieille and Zeikus, 2001).

6.1.2. Saccharification

The saccharification processes produced glucose and maltose through further saccharification (Jorgensen *et al*., 1997). The saccharification is done using exo-acting glucoamylase which have optimum pH 4.2 and 60°C a stable temperature (Gupta *et al*., 2003). Depending on the specification of the final product, saccharification process is performed for 12-96 hour and temperature is kept 60-62°C (Goto*et al*., 1998).

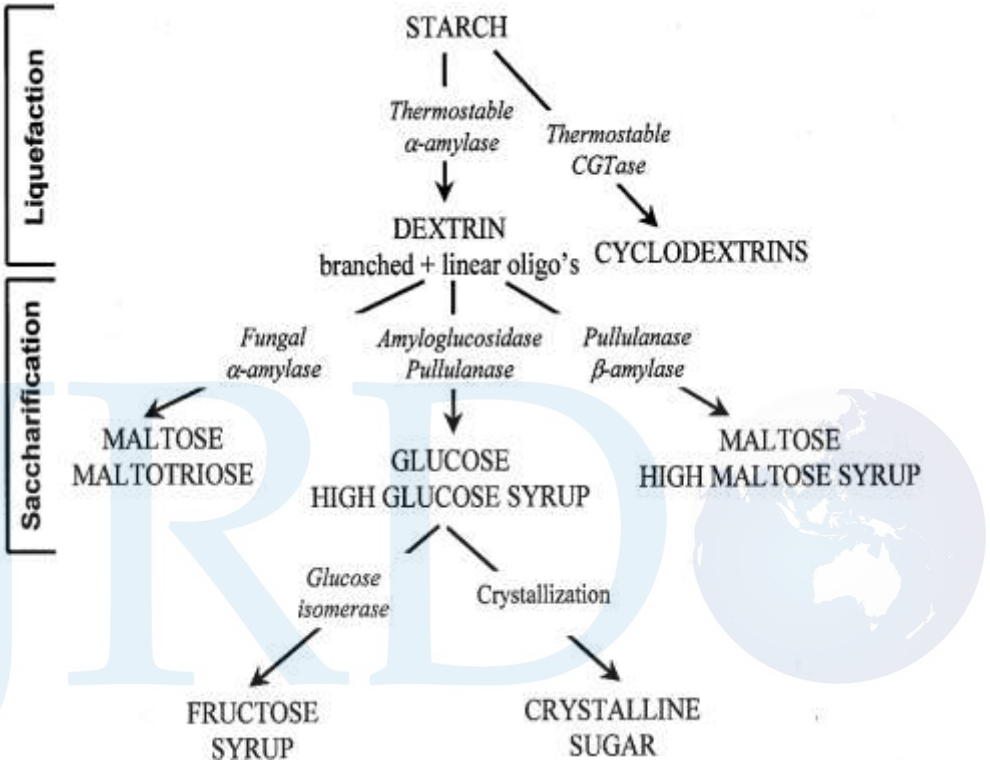

Fig.3. Overview of the industrial processing of starch into cyclodextrins, maltodextrins, glucose or fructose syrups and crystalline sugar.

6.1.3. Hydrolysis

The conversion of high glucose syrup into high fructose syrup is the third step which is hydrolysis. Fructose is almost twice as sweet as glucose and is an isomer of glucose. First refine the high glucose syrup, then carbon filtered, concentrated to over 40% dry solids and adjusted to pH 7-8 (Jorgensen *et al*., 1997). Bacillus species enzymes have special significance for large-scale biotechnological processes because of their significant thermostability. And an effective expression systems for these enzymes are available (Prakash and Jaiswal, 2009).

6.2. Baking industry

In processed-food industry identical to roasting, fermenting, making cakes, preparation of gastric utilities, fruit juices and starch sauces, amylases are widely employed. Baking industry is the tender of α- amylase. The bread is complemented with some flavors to stop the staling of bread and other arid things and also mend its shelf-life and consistency (Pritchard, 1992). The bacterial maltogenica-amylases taking thermostability turn as anti-staling negotiators and lessen the bit steadfastness by making malto-oligosaccharides through stowing and letting the yeast to achieve naturally through primary steps of baking and bread fermentation.

Supplementation of barren from α-amylase from *G.thermoleovorans* progresses the crumb grain, savor, consistency, size and shelf life of the dough. For supplementation of bread, the bread which is ready with α-amylase of *B. acidicola* took an advanced wetness content, solvable protein and reducing sugars as an alternative of that dough completed by viable enzyme. The shelf-life of this bread took three days at room temperature that increase smoothness and surface (Sharma and Satyanarayana, 2010). α-amylases also used in alcoholic drink creating, fruit juices, in the animal foods for digestibility, in the grounds of washing and dishwashing cleaners.

6.2.1. Bakery and anti-staling

Baking industry is a huge customer of starch and starch-modifying enzymes. Bread baking initiates with dough training by accumulating water, flour, yeast, salt and extra conceivable flavors. Flour entails starch, gluten, lipids and non-starch polysaccharides. Subsequently bread training, the yeast activates just before uproar the assessable sugars into carbon dioxide and alcohols that base dough mounting. Amylases once supplementary into dough, it damages the injured starch in the flour into smaller dextrin's which are provoked by yeast. After growing, the dough is baked. A sequence of variations pledge when bread is uninvolved from the oven and it hints failure in the excellence. Such variations contain rise of morsel in flexibility, reduction in dampness content of the crump, loss of freshness of the crust and damage of bread zest.

Completely undesirable fluctuations that do occur upon stowage jointly are termed staling. In staling, Retrogradation of the starch segment in the bread is actual significant. Largely the grade of amylopectin retrogradation relates with the stiffening amount of dough (Champenois*et al*., 1999). Baking industry has a northworthy significance by staling as it confines the time period of bakery yields. Many extracts may be used in bread baking to slow

down staling, to grow well surface, zest of bakery foodstuffs. These entail of trivial sugars, enzymes, compounds or mixture of these. Gluten, emulsifiers, milk concentrate, gritty fat, oxidant, cysteine, sugars and salts are well-known extracts (Spendler and Jorgensen, 1997). Numerals of enzymes have been recommended to develop as bread or bread reformers by adjusting single of the chief bread mechanism.

Hemicellulase, lipase, glucose oxidase, protease and xylanase are instances. Enzymes that energetic on starch act as anti-staling mediator i.e. α-amylases, branching and debranching enzymes, maltogenic amylases, β-amylases and amylo-glucosidases. α-amylases once added through dough preparation, it harvests fermentable compounds. Inspite of production fermentable components, this enzyme also have an anti-staling stuff in dough blazing that improves the resistance retaining of baked foodstuffs. Overuse of α-amylase consequences in gluey bread, so the usage of α-amylases as anti-staling agent is not communal. Afterward three to four days, help full possessions of suspended staling are planned (Olesen, 1991). The healthier gummyness of α-amylase preserved bread is connected with the assemblage of branched maltodextrins of DP20-100. Debranching enzymes lower the difficulties allied with the use of α-amylases as anti-staling agents in case of baking. An α-amylase and a thermostable pullulanase are used cooperatively.

The complex related with α-amylase pickled bakery produce eradicates by pullulanase and this composite accountable for the stickiness. Branching enzyme rise loiter size and shelf-life of baked goods. Such possessions are gained by educating the starch stock in the dough through baking. When branching enzyme is applied with extra enzymes like α-amylase, cyclodextrin glycosyltransferase, β-amylase, maltogenic amylase, cellulose, oxidase and lipase formerly well class of baked goods is attained. Loiter size of the baked imports is amplified by the usage of cyclodextrin glycosyltransferase as bread stabilizer. Such enzymes stay bread staling by lessening the understanding the amylopectin complex to reversing in the bakery foodstuffs. Synergetic use of α-amylase and β-amylase increase the shelf life of baked goods (Van Eijk, 1991). Though α-amylases base thickness of baked goods, mainly once overdid.

Such enzymes are known as maltogenic amylases, produce undeviating oligosaccharides of 2-6 glucose remainder. Maltogenic amylases creating maltotriose, maltose and maltotetrose improve the shelf-life by postponing retrogradation of the starch composite. Newly, a

thermostable maltogenic amylase of *Bacillus stearo thermophilus*is recycled in the bakery industry commercially. However this enzyme takes internal activity (Christophersen*et al*., 1998). It also was liability as an external acting enzyme in case of blazing, by adjusting starch at that heat where utmost of the starch initiates to gelatining stage.

6.3. Fuel alcohol production

The maximum applied biofuel is ethanol. Starch is supreme frequently used as substrate for the ethanol. Construction, due to it little value and effortlessly available rare material in furthermost zone of the world (Chi *et al*., 2009). Starch has to be melted in this manufacture and then succumbed to two enzymatic phases for gaining fermentable sugars. Liquefaction and saccharification comprises bioconversion of starch into ethanol. Starch is distorted into sugar by consuming α-amylase, and sugar is changed into ethanol via yeast *Saccharomyces cerevisiae (*Moraes *et al.*, 1999*)*. Among bacteria, α-amylase learnt from *Bacillus licheniformisor (*engineered strains of *Escherichia coli or Bacillus subtilis)* is cast-off throughout the chief stage of hydrolysis of starch suspension.

6.4. Fabric industry

In textile industry for desizing process amylases are used. Make sure a fast and safe weaving process, sizing agents such as starch is added to yarn before fabric production. Starch is very attractive, easily available in the world, and it can be eliminated easily. In textile finishing industry, in case of a soaked method starch is detached soon from the woven fabric. Desizing consist of starch elimination from the fabric that act as the establishment mediator to stop flouting of the wrap thread in weaving process (Feitkenhauer, 2003). The α-amylases eliminate the size selectively and do not assault the fibers. From *Bacillus* strain amylase derived that was used in textile industries for a long time.

6.5. Detergent industry

Primary consumers of enzymes in term of volume and value are the detergent industries. In detergents formulation, enzyme utilization improves the detergent ability to eliminate stiff stains and forming the detergent safe environmentally. In detergent formulation amylases are the second kinds of enzymes that are utilized and all liquid detergents 90% consist of such enzymes (Hmidet*et al*., 2009). Amylases are used in detergents for laundary and dishwashing to break the

remains of starchy foods such as gravies, custard, chocolate, potatoes, etc into dextrins and many other shorter oligosaccharides (Mukherjee *et al*., 2009). Amylases show its activity well at low temperature and alkaline PH., to maintain the stability under suitable situation. The most important criteria for amylases in case of detergents are the oxidative stability from where washing environment is much oxidizing (Chi *et al*., 2009). Starch removal from the exterior is also essential in whiteness profit. Amylases utilized in the detergent industry are derivatives of *Bacillus* or *Aspergillus.*

6.6. Paper and pulp industry

For the modification of starch of coated paper α-amylases are used i.e. for low viscosity generation, high molecular weight starch in the pulp and paper industry (Gupta *et al*., 2003). Coating method makes the paper surface strong and smooth, improves the writing value of the paper. For paper sizing the viscosity of the natural starch is much higher and it can be changed partially by breaking the polymer with α-amylases in a batch process. Starch as a sizing agent improves the value and erasebility, relatively than a good coating for the paper (Bruinenberg*et al*., 1996). This size improves the strength and stiffness in paper. There are different amylases acquired from microorganisms used in paper industry.

Conclusion

The α-amylase family can be separated into two subgroups: starch-hydrolyzing enzymes and the starch-modifying enzymes. This enzyme has been detected by the starch-processing industry as an acid hydrolysis replacement. α-amylase is frequently working for the elimination of starch in fruit juices, beer, from clothes and porcelain. These enzymes are actuality working in certain industries for a range of applications. For precise industrial applications, cloning, expression, structural educations, and protein engineering of a-amylases from diverse archaeal and bacterial sources have been approved for emerging enzymes with the chosen structures.

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