General Introduction

The food processing and paper industry produces different starchy waste which causes the pollution of environment. It is a major problem of our modern society. Amylase can degrade starchy material in waste water, released from food processing industries and purifies the effluent (Bergeman et al., 1988; Kingspohn et al., 1993). Amylases are also used in different digestive aids (Beazell, 1942). Another social emerging issue is to take different junk food throughout the day by a good number of people and a majority of them cannot digest properly so they also suffer from indigestion and acidity problem. To relief from these indigestion problem people takes different antacid tablet but these tablet are not always fruitful. In recent day's different enzyme containing digestive syrup are used. In this regard use of microbial amylase (starch digesting enzyme) is predominant. The acidophilus amylase is mostly used in digestive syrup due to its ability to sustain at stomach pH. Enzymes are biological catalyst which plays an important role in biological reaction. Enzymes are required in metabolic pathways, respiration, digestion, destroying toxins, muscle building and it can also maintain the homeostasis of body. The lacking or deformation of enzyme due to genetic mutation can cause disease like phenylketonuria etc.

Conventionally different chemical catalysts have been used in different biological and chemical reaction processes for many years but there was disadvantages like reactions need high temperature and high pressure for catalysis. These restrictions were solved by use of enzymes. Enzymes are specific and catalyze reactions faster than chemical catalyst (Berg et al., 2002). Now a days enzyme are used in different industries, like amylases are used in detergent, pharmaceutical, baking, textile, fructose syrup producing industry; lipase are used in textile, detergent, food, cosmetics industries; proteases are used in silver recovery, leather, baking industries; chitinases are used in waste management, pharmaceutical industries; tannases are used in chemical, food, leather industries etc. (Li et al., 2012; Halder et al., 2016).

At the present time researchers have been also concentrated on the production of amylases from different microorganisms, the many reasons are: i) Enhanced production of enzymes

by microbes are possible due to faster growth of microorganisms. ii) The production is cost effective.

Table G1. Source of amylases

Name of amylase	Origin	Source
α- amylase	Human	Saliva (Date, 2005); pancreas (Danilewsky, 1862; Mandel, 2012)
	Animal	Porceine pancreas, rat pancreas (David et al., 1970; Gopal and Muralikrishna 2009)
	Plant	Wheat malt, rice malt (Anto et al., 2006)
	Bacterial	*Bacillus amyloliquefaciens* (Bezbaruah et al., 1991; Tigue et al., 1994; Hiller et al., 1996; Syu and Chen, 1997; Gupta et al., 2003; Gangadharan et al., 2006); *Bacillus cereus* (Aiyer et al., 2004; Anto et al., 2006; Singh et al., 2010); *Bacillus licheniformis* (Krishnan et al., 1983; Lin et al., 1998; Hamilton et al., 1999; Khajeh et al., 2006; Divakaran et al., 2011; Natasha et al., 2011);*Bacillus stearothermophillus* (Lynn et al., 1999; Chakraborty et al., 2000; Riaz et al., 2007); *Bacillus acidocaldarius* (Thippeswamy et al., 2006; Hashemi et al., 2012); *Bacillus megaterium* (Jana et al., 1997); *Bacillus subtilis* (Konsula et al., 2004; Mishra et al., 2005; Ahlawat et al., 2009; Yandri et al., 2012; Jadhav et al., 2013; Dash et al., 2015); *Clostridium acidobutyricum* (Souza and Magalhaes, 2010); *Sulfitobacter brevis* (Mojsov, 2012); *Lactobacillus sp* (Giraud et al., 1993; Hussain et al., 2013); *Bacillus polymixa* (Sundaram and Krishna Murthy, 2014); *Escherechia coli* (Hassan et al., 2018)

	Fungal	*Aspergillus oryzae* (Abe et al., 1988); *Aspergillus fumigatus* (Goto et al., 1998; Ahmed et al., 2015); *Aspergillus tamarii* (Moreira, 1999); *Aspergillus flavus* (Viswanathan et al., 2001); *Aspergillus niger* (Pandey et al., 1994; Gupta et al., 2003; Monga et al., 2011; Asrat and Girma 2018); *Penicillium brunneum* (Gupta et al., 2003); *Saccharomyces kluyveri* (Moller et al., 2004); *Saccharomyces cereviseae* (Knox et al., 2004); *Thermomyces lanuginosus* (Nguyen et al., 2002; Kunamneni et al., 2005); *Aspergillus awamori* (Prakasham et al., 2006); *Penicillium fellutanum* (Kathiresan and Manivannan, 2006); *Penicillium chrysogenum* (Balkan and Ertan, 2007); *Penicillium expansum* (Erdel anf Taskin, 2010); *Mucor sp* (Souza and Magalhaes, 2010); *Streptomyces rimosus* (Hussain et al., 2013); *Thermomonospora curvata* (Sundaram and Krishna Murthy, 2014); *Penicillium camemberti* (Nouadri et al., 2010)
	Archaebacteria	*Pyrococcus woesei* (Koch et al.,1991)
β- amylase	Plant	soybean (Hirata et al., 2004); Sweet potato (Ramakrishnan and Rathnasamy, 2016)
Amylo glucosidase	Plant	Rice (Pestana et al., 1985); corn (Pavezzi et al., 2008)
	Fungal	*Aspergillus awaamorii* (Pestana et al., 1985 Prakasham et al., 2006); *Aspergillus niger* (Ramadas et al.,1995); *Saccharomyces diastaticus* (Searle and Tubb 1981)

Types of amylase

On the basis of anomeric sugars produced by enzymes, amylase are classified as; α , β and γ amylases (Kuhn, 1924; Ohlsson, 1930) and on the basis of action of amylase, enzymes are classified into endo-amylases and exo-amylases (Myrback and Neumuller, 1950). Endo-amylases catalyze the hydrolysis of starch molecule at interior sites. Whereas, exo-amylases are hydrolyze at non-reducing end.

Classification of amylases on the basis of pattern of hydrolysis:

A. Exoamylases:- Hydrolyse α-1,4 or α-1,6 bonds of the external glucose residues of amylose or amylopectin and produce α- or β- anomeric products

a. **Amylogucosidases (glucoamylases)** and **α-glucosidase:-** Hydrolyse α-1,4 and α-1,6 linkages and produce glucose from starch.

b. **β-Amylases:-** Hydrolyse both α-1,4 bonds and α-1,6 linkages in amylopectin and glycogen. They produce maltose from amylose, maltose and β-limit dextrin from amylopectin and glycogen**.**

c. **Cyclodextrin-Producing Enzyme:-** Hydrolyse starch to a homologous series of cyclic, D-glucosyl polymers called cyclodextrins.

d. **Other exo-acting enzymes:-** Hydrolyse α-1,4 bonds and cannot bypass α-1,6 linkages and produce end-products other than maltose from starch.

B. Endoamylases:- These enzymes catalyze α-1,4 bonds present in the inner part of the amylose or amylopectin chain.

C. Debranching Enzymes:- Hydrolyze only α-1,6 bonds of long linear polysaccharides.

D. **Transferases**: The α-1, 4 glycosidic bond of the donor molecule is hydrolyzed by this tranferase enzyme and the segment of donor is transferred to a glycosidic acceptor therefore, a new glycosidic bond is formed.

Classification on the basis of anomeric sugar production

α- amylase- Kirchhoff first discovered α- amylase (EC 3.2.1.1) in 1811. Starch is polysaccharide which consists of amylose and amylopectin. Amylopectin is made up of glucose linked with α- 1,4 bond with α- 1,6 bonds in every 24 to 30 glucose units. Alpha

amylase catalyze the hydrolysis of α- 1, 4 linkages in starch to produce glucose and maltose (Raghu and Rajeshwar, 2015). Alpha amylases are important because of hydrolytic activity on starch and it can produce glucose and fructose syrup from starch. These are endo acting amylase because it hydrolyses α- 1, 4 bonds and by pass α- 1, 6 linkages. This can act on the interior of the substrate molecule.

β- amylase- It (EC 3.2.1.2) is an exo-hydrolase, can hydrolyse α- 1,4 but cannot bypass α- 1,6 linkages. It acts on the non-reducing end of the polysaccharide chain by hydrolysis of α- 1, 4 linkages to yield maltose units. During ripening of fruits β- amylases produce maltose from starch and increase the sweetness of fruits. It can be used in brewing and distilling industries.

γ- amylase- γ- amylase (EC 3.2.1.3) is exo-hydrolase enzyme cleaves α-1,6 glycosidic linkages and α- 1,4 glycosidic linkages at the non-reducing end of amylase and amylopectin.

Starch is the major source of carbohydrate in human diet and it is also the storage components of different crops like rice, potato, wheat, maize etc. The internal glycosidic linkage of starch is hydrolyzed by ptyalin (an amylase) which was described by Erhard Friedrich Leuchs in 1831. After that French chemists Anselme Payen and Jean Francois Persoz in 1833 invent the production of amylase from germinating barley. And this new amylase was named diastase. Salivary amylase deactivated in stomach due to its low pH. But in pancreas pancreatic amylase is also secreted and continues the hydrolysis process. The separation of pancreatic amylase from trypsin was carried out by Alexander Jakulowitsch Danilewsky in 1862. Pancreatic amylase hydrolyses starch into maltose and maltotriose and some oligosaccharides.

Alpha amylases are produced by different microorganisms like bacteria, fungi and genetically modified microbes. Most commonly used bacteria for the commercial production of α- amylase is *Bacillus spp.* Among them the species like *Bacillus amyloliquifacience* and *Bacillus licheniformis* are noteworthy. In different industries like food, fermentation, textiles and paper industries α- amylase is used which is produced from *Bacillus licheniformis, Bacillus amyloliquifacience, Bacillus stearothermophillus* (Kalllio et al., 1987; Haq et al., 2005; Sivaramakrishnan et al., 2006; Bozic et al., 2011). There are

many microorganisms which can grow under acidic pH ranges between 1.0- 4.0 and they can produce acidophilic amylase. They maintain their cytoplasmic pH near neutrality for adaptation under acidic conditions. Therefore, very few microorganisms can adapt to this extreme condition. The Gram positive *Alicyclobacillus acidocaldarius* produce acidophilic amylase was studied by Matzke et al. (1997). Fungal sources of α- amylase is found from different places, mostly *Aspergillus sp* (Varalakhsmi et al., 2009). Thermostable amylases production by *Acremonium sporosulcatum* was studied by Varaparla (2010). In the commercial production of α- amylase mostly used fungal strain are *Aspergillus spp.* and among the *Aspergillus* species most commonly used species are *Aspergillus niger* (Sundar et al., 2012), *Aspergillus oryzae* (Sivaramakrishnan et al., 2007), *Aspergillus fumigatus* (Pervez et al., 2014; Singh et al., 2014), *Aspergillus awamoris* (Negi and Banerjee, 2006). Other fungi from legume seeds like *Fusarium oxysporum, Penicillium canescens, Penicillium waksmanii, Penicillium chrysogenum, Alterneria tunnuissima, Rhizoctonia solani* can also produce α- amylases was studied by Saleem and Ebrahim (2014). Most of the thermostable α- amylase was produced by *Bacillus subtilis* (Swain and Ray, 2007), *Bacillus stearothermophilus* (Srivastava and Mathur, 2008), *Bacillus amyloliquefaciens* (Kalllio et al., 1987). The importance of thermostable amylase performed the saccharification of starch at high temperature (100-110 ^{o}C). Different types of valuable products like glucose, crystalline dextrose, maltose, dextrose syrup are produced by thermostable amylase. Many genetically modified organisms are also used in α- amylase production. Improvement of enzyme production was made by the manipulation of microorganisms at genetic level.

Production of amylase

There are two major methods for large scale production of α- amylase: (a) solid state fermentation (SSF) and (b) submerge fermentation (SmF) (Akpan et al., 1999; Soni et al., 2003).

In SmF (submerge fermentation) for bacterial amylase production mainly synthetic medium is used (Singh et al., 2014). The cost of different synthetic media is very high therefore these media are replaced with low cost agricultural by products to minimize the cost of production medium (Solange and Jose, 2010).

The solid substrate gives only support and nutrition to the fermentation medium (Hashemi et al., 2012). The solid state fermentation by *Aspergillus oryzae* were mostly reported where *wheat bran* used as the substrate and fermentation environment was at 30 ^{o}C temperature, pH 5.0, where it produces 14967 IU/gds (gram dry substrate) enzyme (Sivaramakrishnan et al., 2007). Suganthi et al. (2011) studied that the alpha amylase production by *Aspergillus niger* in solid state fermentation by using different agro industrial waste.

SSF is preferred over the SmF because of simple process, low capital investment, lower levels of end product inhibition and better product recovery (Okolo et al., 2000; Singhania et al., 2009). Many other substrates are also used in SSF like rice husk, soybean meal, cotton seed meal, rice bran etc. for the production of amylase by *Bacillus* sp (Maryam et al., 2010). The maximum amylase production of 2311.1 U/g by *Bacillus* sp was showed with pH-7, when SSF was carried out at 37 ^{o}C for 48 h using a substrate with 75% moisture content (Saxena and Singh, 2011). The amylase production by *Aspergillus* sp in SSF where wheat bran, rice bran, coconut oil cake, groundnut oil cake, corn cob are used as substrate, was studied by Alva et al. (2007). Mathew et al. (2016) also showed that the amylase production by *Aspergillus niger* in solid state fermentation by using cassava peels, jackfruit seeds, coffee husks etc. In presence of soluble starch the amylase activity increases to 1% in respect of glucose and sucrose (Rameshkumar and Sivasudha, 2011). The maximum alpha amylase production was found at 80 h of incubation, 55 ^{o}C temperature, 1:1 substrate moisture ratio, pH 5.0, 10% inoculums level and 0.5g/lit glucose. On the above study glucose was the best carbon source but the presence of nitrogen source also reduced the production of amylase (Singh et al., 2010).

SSF technique is generally confined to the process involving fungi (Saxena and Singh, 2011). In SSF mainly agro industrial residues are used due to their potential advantage for filamentous fungi which are capable of penetrating the substrate by turgor pressure present at the tip of the mycelium (Maryam et al., 2010). The production of amylase from *Penicillium expansum* in solid state fermentation using waste loquat kernels was studied by Erdal and Taskin (2010). Production of fructo oligosaccharide from agro industrial residues through solid state fermentation by *Aspergillus japonicas* was reported by Solange

and Jose, (2010). Production of α- amylase and gluco-amylases by *Bacillus* sp and *Aspergillus* sp in solid state fermentation where wheat bran was used as substrate was (Soni et al., 2003).

Mode of action

Van der Maarel et al. (2002) reported that α- amylase act through α- retaining double displacement mechanism. The active site is located between the domain A and domain B. The substrate binding site can take four to ten glucose units of starch. The catalytic site contains two aspartic acids and one glutamic acid. Glutamic acid and aspartic acid act as acid/base catalyst and nucleophile respectively during the formation of the intermediate. When the substrate (starch) binds to the active site glutamic acid donates proton to the glucosidic oxygen and aspertate creates nucleophilic attack to the C (1) of glucose at subsite -1. After that the glycosidic bond was finally splited. When the protonated glucose molecule of (+1) subsite moves away from the active site then the water molecule enters into the active site. The hydroxyl group at C1 was formed by oxygen when glutamate pulls off the proton from water. Another aspartate plays also an important role in formation of bondings substrate and OH (2) and OH (3) (Uitdehaag et al., 1999). Other conserved residues like histidine, arginine and tyrosine help in present the substrate to the active site in proper orientation and also correct the direction of the nucleophile (van der Maarel et al., 2002).

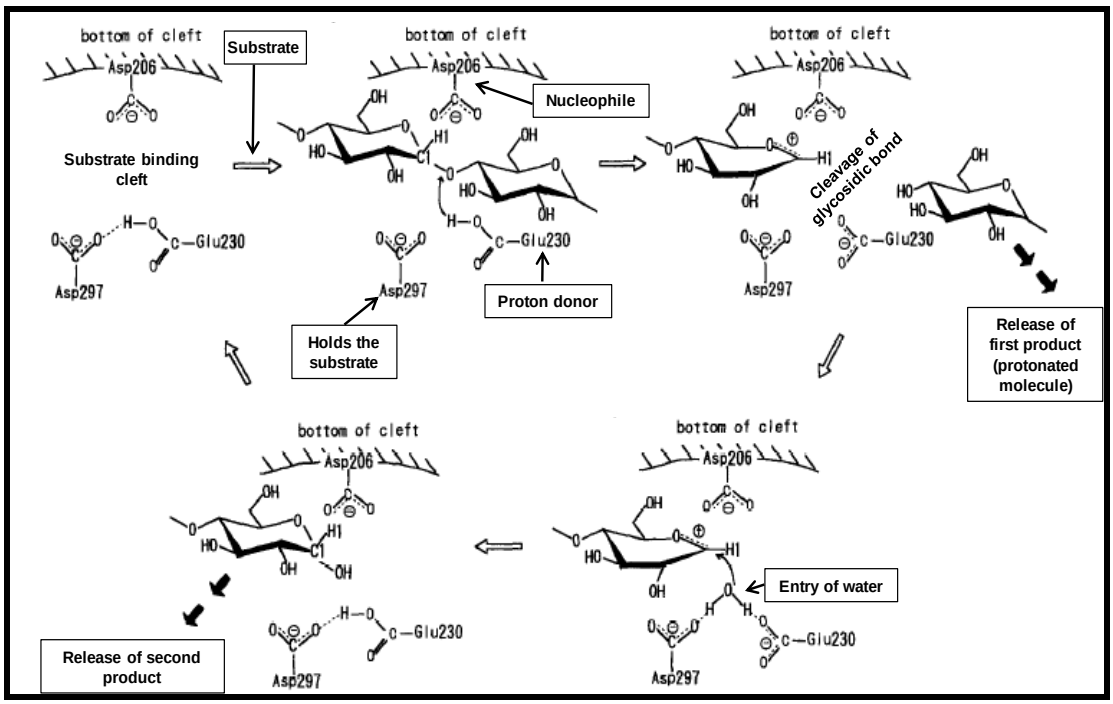

Fig G1. The α-retaining double displacement mechanism of α-amylase catalytic reaction system (adapted from Kapeko et al., 1998).

Purification and kinetics of amylase

The some commercial use of α- amylases does not require purification of the enzyme, but enzyme applications in pharmaceutical and clinical sectors require high purity amylases. Purified α- amylases are used in studies of biochemical properties and structure- function relationships (Gupta et al., 2003). The purification of enzyme was carried out by precipitation, chromatography and liquid extraction process. After followed the purification process the obtained enzyme is highly purified. The level of purity depends on the number of purification steps (Gupta et al., 2003; Gangadharan et al., 2006). After filtration and centrifugation the crude enzyme was acquired from fermented mass. In case of intracellular enzyme raw corn starch may be added by filtration and subsequent steps are followed. The crude amylase enzyme can be precipitated and concentrated by ammonium sulphate precipitation or organic solvent. The precipitated sample can be subjected to dialysis against water or a buffer for further concentration (Shih and Labbe, 1995). This

can be followed by any of the chromatographic technique like ion exchange, gel filtration and affinity chromatography for further separation and purification of enzyme. In case of thermotolerant amylases produced by *Thermotoga meritima* MSB 8, the cell extract obtained after centrifugation which is free of cell debris; can be subjected to high temperature in order to denature thermolabile proteins, and then the purification of amylase was carried out by anion exchange chromatography. The purity is then analyzed by SDS-PAGE (Liebl et al., 1997). The purity also measured by size exclusion chromatography where the molecular weight of the purified protein can be determined. The kinetic parameters like *Km* and *Vmax* are studied. *Km* is the substrate concentration that is required for the reaction to occur at 1/2*Vmax*.

Characterization of amylase

Characterization of enzyme was carried out after its purification. The purified enzyme samples along with molecular markers like BSA (67 kDa) and ovalbumin (43 kDa), are run on gel. The resulting bands were seen through Coomassie Brilliant Blue staining (Paquet et al., 1991) and silver nitrate (Mukesh et al., 2012). The electrophoresis separation also indicates the purity and homogenicity of the obtained enzyme. In previous study Mukesh et al. (2012) and Karim et al. (2014) showed that α- amylases produced respectively by *Bacillus* MNJ 23 and *Aspergillus flavus* strain the molecular mass of enzymes were characterized by SDS-PAGE (12% acrylamide gel).

Table G2. List of different commercially available amylases

Company	**Trade name**
The Standard Chemical Company, Ohio, USA	Forbes liquor diastase
Carolina Biological supply, North Carolina, USA	Diastase (malt)
AmericanMasterTech, California, USA	Diastase (source of α-amylase)
Alpha Chemika, Mumbai,	Diastase (Fungal)

Maharashtra, India	
Merck , Germany	Diastase
Sigma- Aldrich, USA	Diastase (from *Aspergillus oryzae*)

Review of literatures on α- amylase

Series of events that leads to understanding the production and properties microbial α-amylase is represented below.

Table G3. Significance contribution of knowledge on the production and application of α-amylase

Inventor of amylase	**Title of reports**	**Reference**
Lonsane and Ramesh, (1990)	Production of bacterial thermostable α- amylases by solid state fermentation: A potential tool for achieving economy in enzyme production and starch hydrolysis.	Adv. Appl. Microbiol. 35:1-56
Jensen and Olsen, (1992)	Physicochemical properties of purified α- amylases from thermophilic fungus *Thermomyces lanuginosus.*	Enzyme. Microbiol. Technol. 14: 112- 116
Eriksen et al. (1998)	Effect of glycosylation on secretion, activity and stability of amylase from *Aspergillus oryzae.*	Currt Microbiol, 37: 117-122
Shih and Labbe, (1995)	Purification and characterization of an extracellular α- amylase	Appl. Environ. Microbiol. 61:1776-1779

	from *Clostridium perfringens* Type A.	
Chadha et al. (1997)	Shake culture studies for the production of amylases by *Thermomyces lanuginosus*.	Acta Microbiol Immunol, 44: 181-185
Jana et al. (1997)	Thermostable, high salt tolerant amylase from *Bacillus megaterium* VUMB- 109	Acta. Microbiol. et . Immunol. Hungarica. 44: 281- 289
Raimbult (1998).	General and microbiological aspects of solid state fermentation.	Electron J Biotechnol. 1: 1-20
Moraes et al. (1999)	Purification and some properties of an amylase glucoamylase fusion protein from *Saccharomyces cerevisiae*.	World J Microbiol Biotechnol, 15: 561-564
Akpan et al. (1999)	Production of amylase by *Aspergillus niger* using rice bran and agricultural. material.	Tropical Sci, 39: 77-79
Nguyen et al. (2000)	Optimization of composition of media for the production of amylolytic enzymes by *Thermomyces lanuginosus* ATCC 34626.	Food Technol. Biotechnol. 38: 229- 234
Carlsen et al. (2000)	Morphology and physiology of an α- amylase producing strain of *Aspergillus oryzae*	Biotechnol. Bioeng, 49: 266–276

	during batch cultivations.	
Demirjian et al. (2001)	Enzymes from extremophiles.	Curr Opin. Chem. Biol., 5: 144-151
Ulger and Curakoglu (2001)	α- amylases production by *Bacillus subtilis* `and *Bacillus amyloliquefaciense* in different PEG solutions.	World J. Microbiol. Biotechnol. 17: 93- 94
Van der Marrel et al. (2002)	Properties and application of starch converting enzymes of the α- amylase family.	J. Biotechnol., 94: 137-155
Saboury, (2002)	Stability, activity and binding properties study of α-amylase upon interaction with $Ca^{2+}$and Co^{2+}.	Biologia—Section Cellular Mol Biol, 57: 221–228
EI-Safety and Ammar, (2004)	Purification and characterization of -amylase isolated from *Aspergillus flavus.*	Environ Res; 7: 93- 100
Francis et al. (2003)	Use of response surface methodology for optimizing process parameters for the production of α-amylase by *Aspergillus oryzae*.	Biochem Eng J, 15: 107–115
Abu et al. (2005)	Raw starch degrading amylase production by mixed culture of *Aspergillus niger* and *Saccharomyces cerevisae* grown on sorghum	Afr. J. Biotechnol., 4: 785-790

	pomace.	
Ayer (2005)	Amylase and their applications.	Afri J Biotechnol, 4: 1525-1529
Djekrif-Dakhmouche et al. (2005)	Application of a statistical design to the optimization of culture medium for α-amylase production by *Aspergillus niger* ATCC 16404 grown on orange waste powder.	J Food Process Eng, 73: 190–197
Deng et al. (2005)	Production of a bioflocculant by *Aspergillus parasiticus* and its application in dye removal, Colloids and Surfaces.	Biointerfaces, 44: 179–186
Sodhi et al. (2005)	Production of a thermostable α- amylases from *Bacillus* sp. PS-7 by solid state fermentation and its synergistic use in the hydrolysis of malt starch for alcohol production.	Process Biochem., 40: 525-534
Hernández et al. (2006)	Amylase production by *Aspergillus niger* in submerged cultivation on two wastes from food industries.	J Food Process Eng, 73: 93–100
Hussein et al. (2006)	Identification of Bacitracin produced by local isolate of *Bacillus licheniformis*	Afr. J. Biotechnol., 18: 1600-1601

	thermophilic microorganisms and life at high temperatures.	
Norouzian et al. (2006)	Fungal glucoamylases	Res. Rev. Paper, 24: 80-85
Prakasham et al. (2006)	Enhancement of acid amylase production by an isolated *Aspergillus awamori*.	J Appl Microbiol, 102: 204-211
Kathiresan and Manivannan, (2006)	α- amylase production by *Penicillium fellutanum* isolated from mangrove rhizosphere soil	Afr. J. Biotechnol. 5: 829-832
Bilal and Figen, (2007)	Production and Properties of alpha amylase from *Penicillium chrysogenum* and its Application in Starch Hydrolysis.	Prep Biochem Biotechnol, 35: 169–178
Bhargav et al. (2008)	Solid state fermentation : An overview	Chem. Biochem. Eng. 22: 49-70.
Kammoun et al. (2008)	Application of a statistical design to the optimization of parameters and culture medium for alpha-amylase production by *Aspergillus oryzae* CBS 819.72 grown on gruel (wheat grinding by-product).	Bioresour Technol, 99, 5602-5609
Varalakshmi et al. (2009)	Production and	Pol J Microbiol, 58: 29-36

	characterization of α-amylase from *Aspergillus niger* JGI 24 isolated in Bangalore.	
Upadhyay and Pandey, (2009)	Production and optimization of α- amylase from *Aspergillus niger* MTCC 281 under solid state fermentation.	J Plant Sci Res, 25: 49-52
Singhania et al. (2009)	Recent advances in solid state fermentation	Biochem. Eng. J, 44: 13-18
Valaparla, (2010)	Purification and properties of a thermostable α-amylases by *Acremonium sporosulcatum*	Int. J. Biotechnol. Biochem., 6: 25- 34
Castro et al. (2010)	Economic analysis of the production of amylases and other hydrolases by *Aspergillus awamori* in solid state fermentation of babassu cake	Enzyme Res. 1: 1-9
Maryam et al. (2010)	Development of solid state fermentation process for production of an alpha amylase with potentially interesting properties	J. Biosci. Bioeng, 110: 333-337
Rameshkumar and Sivasudha (2011)	Optimization of nutritional constitute of enhance α-amylase production using by solid state fermentation	Int J of microbiol Res, 2: 143-148

	technology	
Maity et al. (2011)	Isozymes of α- amylases from newly isolated *Bacillus thuringiensis* CKB19: Production from immobilized cells	Biotechnol Bioprocess Eng.,16: 312-319
Dehkordi and Javan, (2012)	Application of α- amylases in biotechnology	J. Biol. Today's World, 1:15-20
Senthilkumar et al. (2012)	Amylase production by *Bacillus* sp. Using cassava as substrate	Int. J. Pharm. Biol. Sci., 3:274-280
Alariya et al. (2013)	Amylase activity of starch degrading bacteria isolated from soil	Arch Appl Sci Res., 5:15-24
Aygan et al. (2014)	Production and characterization of alkaliphilic α- amylases from *Bacillus subtilis* A10 isolated from soils of Kahramanmaras, Turkey	Afr J. Microbiol., 8: 2168-2173
Sundaram and Krishnamurthy, (2014)	α- amylases production and application	J Appl Environ microbiol., 2: 166-175
Raul et al. (2014)	Production and Partial Purification of α- amylase from *Bacillus subtilis* (MTCC 121) Using Solid State Fermentation	Biochem Res Int, 2014: 1-5
Singh et al. (2014)	Production of fungal amylase using cheap, readily available agro residues, for	Biomed Res Int, 2014: 1-9

	potential application in textile industry	
Sethi and Gupta, (2015)	Isolation, characterization and optimization of culture conditions for amylase production from fungi	J Global Biosciences, 4: 3356-3363
Singh et al. (2016)	Amylase: A note on current applications	Int Res J Biol Sci, 5: 27-32
Saini et al. (2017)	Amylase: Characteristics and Industrial application	J Pharmacologn Phytochem, 6: 1865-1871
Gopinath et al. (2017)	Biotechnological processes in microbial amylase production	BioMed Res Int, 2017: 1-9
Simair et al. (2017)	Production and partial characterization of α-amylase enzyme from *Bacillus* sp. BCC 01-50 and potential application	BioMed Res Int., 2017: 1-9, ID 9173040
Malik et al. (2017)	Isolation and screening of amylase producing fungi	Int J Curr Microbiol App Sci, 6: 783-788
Mankar and Barate, (2018)	Isolation and identification of amylase producing bacteria from soil receiving kitchen and agricultural waste	Int J Recent Sci Res., 9: 23408-23411

Table G4. Patent on amylase

Title	Patent number	Owner/ Year
Maltogenic amylase enzyme, preparation and use	USP 4604355A	Outtrup H., 1986

thereof		
α- amylases mixture for starch liquefaction	EP0252730A2	Carrol O. J., Swanson R.T., Trackman C.P., 1988
Detemination of amylase	EP0309256A2	Batchelor J.M., Williums C.S., Green J.M., 1990
α- amylases of a new type	EP0258050A2	Melasniemi H., Korhola M., 1990
Oxidatively stable α-amylase	WO 9418314	Antrim R.L., Barnett C.C., Mitchinson C., Power S.D., Requadt C.A., Solheim L.P., 1998
Reagent for assay of amylase isozyme activity	EP0937778A2	Keiichi M., Shigeki A., Yoshihisa K., 2002
Cloning of a yeast α-amylase promoter and its regulated heterologous expression	US6541622B1	Johnway G., Rodney S.S., Brian S.H., Daniel B.A., 2003
A process for the extraction of amylase rich fraction from malted cereals	Indian Patent 222585	Gudipati M., Mavila N., 2005
Fungamyl – like α- amylase variants	US20070128313A1	Frantzen H.B., Pederson S.S.A., 2008
α- amylase combinatorial variants	US20180163191A1	Cuevas W., Sharma V., Wildes E.D., Lee S., Finan D., 2008
Geobacillus stearothermophillus α-amylase variants with improved properties	US8084240B2	Cuevas W.A., Lee S., Sandra W.R., Andrew S., Amr R.T., David E.E., Sura H.H., 2011

Liquid washing or cleaning agent containing protease and amylase	USPT 08752102	Mussmann N., Eiting T., Bastigkeit T., Bonda C., Hellmuth H., 2017
α- amylase variants having improved performance and stability	US20180142224A1	Andersen C., 2018
α- amylase variant with altered properties	US9920307B2	Andersen C., Oedtdal H., Skagerlind J.P., 2018
Composition of high stability α- amylases variants	US9896673B2	Svendsen A., Johansen A.H., Bjoernvad M., Rasmuseen W.F., Michael S., Larsen E.S., Oebro J., Kaasgaard S., Beier L., 2018

Application of amylase

Enzymes have many promising and broad applications on the industrial sectors (Saini et al., 2017). The chemical hydrolysis process was replaced by enzymatic hydrolysis process to make environment pollution free. The history of the industrial production of amylase dates back to the time when Dr. Jhokichi Takamine began the production of digestive enzyme preparation from wheat bran koji culture of *Aspergillus oryzae* in 1894. Dextrose powder and dextrose crystals were first produced industrially in the year of 1959 from starch by using α- amylase and glucoamylase. Subsequently amylases are used for several purposes. Conversion of starch into sugar, syrup and dextrins are the major part of starch processing industries. The starch hydrolysates are used as sweeteners for the manufacture of food products and beverages. Hydrolysis of starch into specific products like glucose, maltose, malto oligomers etc. is brought about by controlled degradation (Norman 1978; Ayer 2005).

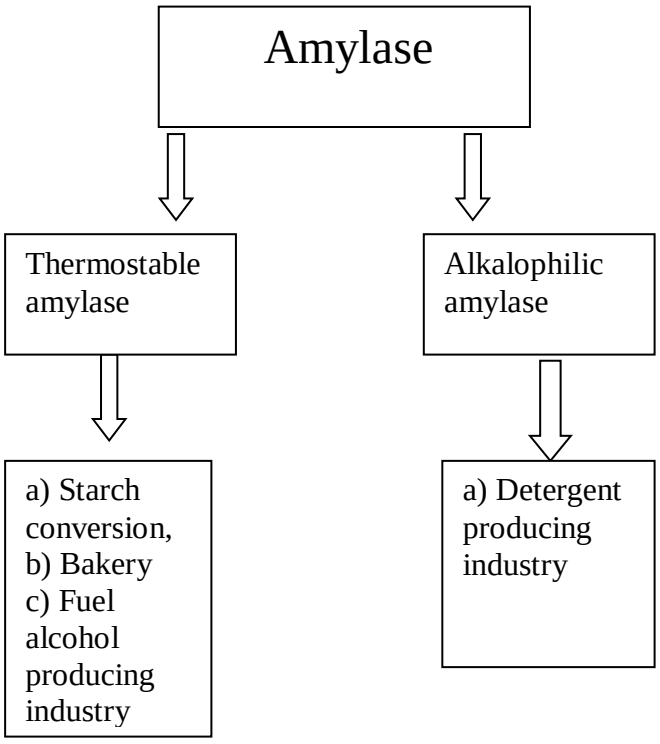

Fig G2. Schematic representation of application of amylase.

Glucose and fructose industry

Alpha amylase is used in many industries for the production of glucose and fructose. This hydrolysis process occurred in three steps the gelatinization, liquefaction, and saccharification. Gelatinization involves in the dissolving of starch granules in water to form a starch suspension. Liquefaction of starch is the partial hydrolysis of starch suspension into small chain dextrins by alpha amylase. Saccharification is carried out by the production of glucose and fructose syrup by further hydrolysis (Haq et al., 2005). Most of the α- amylases are produced by *Bacillus amyloliquefaciens*, now which is replaced by *Bacillus stearothermophillus* (Van der Marrel et al., 2002) and *Bacillus licheniformis* (Van

der Marrel et al., 2002; Haq et al., 2003). Amylases from *Chromobacter sp.* have special interest in the industrial process (Prakash et al., 2009). *Aspergillus niger* and *Aspergillus oryzae* also used in this glucose and fructose industry (Souza and Magalhaes, 2010).

Bakery industry

In bakery industry α- amylase plays an important role in improvement of aroma, taste and porosity of product. Addition of this enzyme hydrolyzes the starch into small dextrins which are then fermented by yeast. Starch hydrolysis also decreases the viscosity of the dough therefore improving its texture. This enzyme affects the anti salting in baking of bread, moreover the softness of bread also raised by this enzyme (Gupta et al., 2003).

Detergent industry

The uses of enzyme in detergent also improve the quality of detergent. Enzymes are also environmentally safe and work at mild conditions. Alpha amylases digest food particles into smaller water soluble oligosaccharides. The stability of α- amylases at basic pH and low temperature contribute to its extensive use in detergent. Amylase is the second type of enzyme used in the production of different enzymatic detergent and most of the liquid detergent contains this enzyme (Gupta et al., 2003). These enzymes are also used as dishwashing detergent and can degrade starchy foods like custard, chocolate, potatoes etc (Haq et al., 2005).

Fuel alcohol production

Now a days ethanol is used as biofuel. Starches from grain, potatoes are mainly used for the production of ethanol. Production of ethanol from starch required in two steps, first step is the liquefaction and saccharification where starch is converted into sugar by amylase and the next step is microbial fermentation where sugar is converted into ethanol by species of *Saccharomyces kluyveri* (Moller et al., 2004) and *Aspergillus fumigatus* (Pervez et al., 2014)

Textile desizing

Alpha amylases are used in most of the textile industries. These enzymes act as desizing agent by hydrolyzing starch (sizing material) into water soluble components. Sizing agents are mainly used to strengthen the thread by forming a layer on the thread and it can be removed after fabric is woven. Amylase acts only on the starch molecule but not on the fibers (Gupta et al., 2003; Ahlawat et al., 2009). Amylase from *Bacillus* is mostly used in the textile industries for long time. Singh et al. (2014) reported that *Aspergillus fumigatus* had immense effect on textile industries.

Paper Industry

In paper industry starch is used as sizing agent to increase the strength, paper quality, smoothness of the paper. Sizing agents also protect papers from mechanical forces during processing. Alpha amylase hydrolyses the sizing agent into glucose and fructose (van der Marrel et al., 2002; Gupta et al., 2003). In paper industry there are many amylases are used like Termamyl, Fungamyl (Novozymes, Denmark), Amizyme (PMP Fermentation products, Peoria, USA).

Aim and objectives of the present study

Several studies had clearly indicated that the fungal amylase is mainly used in digestive syrup production as compared to bacterial amylase. Fungi are eukaryotic organism, so, it has best adaptability to the human stomach. The fungal amylase mostly accepted enzyme because it is Generally Recognized as Safe (GRAS) (Gupta, 2003). Fungal acidophilus amylase was produced by *Aspergillus awamori*, at pH 3.5- 5.5 (Prakasham et al., 2007) *Aspergillus niger*, *Rhizopus sp, Fusarium sp*, *Aspergillus flavus,* at pH 5.5- 6.5 (Pathak and Narula, 2013).

However sustainability of fungal amylase in stomach at highly acidic condition and ability to digest consumable starchy materials and heterogeneous food are yet not properly explored in previous literature. Therefore, in this present treatise, an account of acidophilic amylase, including its array, production and purification have been considered from newly isolated soil fungal strain.

Hence for this study the following objectives were taken into consideration

1. Isolation and identification of potent acidophilic amylase producing fungal strain from starchy waste.
2. Utilization of starchy waste for amylase production by isolated fungal strain.
3. Optimization of physico-chemical process parameters for maximum acidophilus amylase production by the isolated fungi through solid state and submerged fermentation using potato peel as substrate.
4. Purification and kinetic characterization of the acidophilus amylase.
5. Application of the enzyme for saccharification of natural raw starch, different food stuff, waste materials and its cytotoxicity study.
6. In Silico Study on molecular adaptation of acid α-amylase: with special reference to *Aspergillus niger*.