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Enhancement of Characteristics and Potential Applications of Amylases: A Brief Review

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ABSTRACT

Starch is the major storage carbohydrate of plant products. Amylases are the group of enzymes hydrolyzes starch and related polymers to smaller oligosaccharides and less amount of monosaccharide. Microbes are the major sources of amylases, exploited for large scale production in different industries. Recently, protein engineering has been applied to improve the structural and physicochemical properties of the enzyme for its potential applications. Amylases are mostly used for liquefaction of starch in the purpose of glucose, maltose, and high fructose containing syrup preparation, malto-oligosaccharides production, desizing, production of bio-fuel, detergent preparation, waste management, and preparation of digestive aids.

Keywords: α-Amylases, solid-state fermentation, protein engineering, applications, and Enhancement.

INTRODUCTION

Starch is the major storage plant polysaccharide, cleaved by a group of hydrolytic enzymes known as amylases. Amylolytic enzymes are widely distributed in microbial, plant and animal kingdoms (Saini *et al.,* 2017). Several enzymes like α-amylase, pullulanase, isoamylase, CGTase (Cyclodextrin glycosyl transferase), branching enzymes, α-glucosidase, amylopullulanase, and neopullulanase belong to amylase family. The production of amylases was carried out by submerged fermentation or solid state fermentation; however, later has several potential benefits including cost effectiveness, easy to operate, and better for downstream processing (Sharif *et al*., 2019). Use of agricultural products in solid sate fermentation reduces the pollution levels as they are commonly destroyed by open burning. Protein engineering is the most powerful approach to increase the physicochemical properties of the enzymes. Enzyme engineering technique is applied for preparation of high efficiency, oxidation insensitive, and chelator resistance enzyme for industrial uses (Uddin *et al*., 2017). Starch-degrading amylolytic enzymes have great significance in biotechnological applications in different sectors. The commercial application of amylase was started from 1894, when Japanese scientist Jokichi Takamine prepared 'Taka Diastase' from *Aspergillus oryzae*. The era of bacterial amylases was stared form the year 1917 when α-amylase was isolated from *Bacillus subtilis* by the French scientists Boidin and Effront (Md *et al*., 2014).

The demand of microbial enzymes for industrial applications is increasing day-by-day. Among the different enzymes, amylases itself have 25% share of the world's enzyme market. Additionally, about 90% of all liquid detergents contain α-amylase, which make them more efficient for automatic dish-washing (Abdulaal, 2018). In the industrial sectors, microbial amylases are potentially used in different purposes

like liquefaction of starch, food preparation, baking, brewing, detergent preparation, desizing, paper making, bio-fuel production, and digestive medicine preparation (Yan and Wu, 2016).

Types of amylases

Initially, the term amylase was used to describe the enzyme hydrolyses α-1,4 glycosidic bond of amylose, amylopectin, glycogen and related products. However, microbial amylases are commonly classified in different groups.

1. Exo-Acting Amylases

a. **Glucoamylases:** Hydrolyze terminal α-1,4 glucosidic linkages from non-reducing ends of the polysaccharide chain and release beta-D-glucose.

b. **β-Amylases:** Cleave α-1,4 glucosidic bond from the non-reducing ends of the polysaccharide chains. They produce maltose from amylose, and maltose and β-limit dextrin from amylopectin and glycogen.

c. **α-glucosidase:** Cleaves α-1,4 glucosidic linkage of terminal glucose residue at non-reducing end. It gives single glucose molecule in alpha configuration.

d. **Maltogenic amylase:** Cleaves α-1,4 glucosidic linkages in the substrates containing only two glucose units at the non-reducing end and releases maltose.

e. **Maltotetraose forming amylase**: Hydrolyzes starch from its non-reducing end releases maltotetraose.

2. Endo-Acting Amylase: α-Amylase: Randomly hydrolyzes α-1,4 glucosidic linkages in polysaccharides containing three or more α-1,4 linked D-glucose units. It produces several maltooligomers, branched dextrins, and maltose. All the products contain reducing group in the alpha-configuration.

3. Debranching Enzymes: Cleave α-1,6 glucosidic linkages and give certain maltooligomers. Pullulanase, isoamylase and other debranching enzymes belong in this class.

4. Cyclodextrin-Producing Enzyme (Cyclodextrin glycosyl transferase): Form α-1,4 glucosidic linkages. It can catalyze different reactions: cyclization, coupling and hydrolysis. The product is cycldextrins and few maltooligomers.

5. **Transferases (branching enzyme):** Form α-1,6 glucosidic linkages. It transfers a segment of α-1,4 D-glucan chain to the primary hydroxyl group in a similar glucan chain.

Commonly, amylases are classified into three main groups α-amylases (EC 3.2.1.1), β-amylase (EC 3.2.1.2) and γ-amylase (EC 3.2.1.3) (Rani *et al*., 2015; Oluwadamilare *et al*., 2019).

Production of amylases

Several biotech industries are using large amount of various microbial enzymes like amylase, cellulose, pectinases, protease, lipase, and others. Among these, amylases are maximally used for owing their multipurpose applications. Different microbes are the good sources of amylases (**Table 1**). Large-scale production of amylases by using microbes is advantageous for their high yielding capacity that minimizes cost/production ratio. At the industrial level, amylase production is carried out in both solid state fermentation (SSF) and submerged fermentation (SmF). Several parameters including nutrient supplementation, pH of the medium, temperature, water activity, and aeration are being considered for optimum growth of the microorganisms as well as enzyme production (Shahen *et al*., 2019).

Among the different factors, pH and temperature have greater impact on microbial growth and enzyme production. Fungi are commonly grown in slightly acidic medium; whereas bacteria needs neutral to slight alkaline medium for their growth and enzyme production. Most of the organism can grow in particular temperature. Mesophilic organisms are able to grow in wide range of temperature 30-45 °C, but give thermostable enzymes. Supply of nutrients like carbohydrate, protein, ammonium salts, vitamins (B_1, B_2, B_6, biotin, folic acid, etc.) and minerals (calcium, magnesium, zinc, copper, sulfate, phosphate, etc.) provide energy for cellular growth, supply essential components for protoplasm formation, and create

favorable conditions for enzyme synthesis (Samanta *et al.,* 2014). Surface area is most important to provide much amount of oxygen. In SSF, surface area is greatly increased, which influences the rate of oxygen transfer, aerobic metabolism, and enzyme production. Initially, SmF was more popular for its easy handling and optimization. Later, SSF has gained more importance for large-scale production of enzymes in view of several economical and engineering advantages.

Table 1: Sources of microbial α-amylase.

Origin	Source	Reference
Bacteria	*Bacillu licheniformis*, *B. stearothermophilus*, *B. amyloliquefaciens*, *B. subtilis* KIBGE, *B subtilis* D19, *B megaterium* VUMB109, *B. coagulans*, *B. polymyxa*, *B. mesentericus*, *B. vulgaris*, *B. megaterium*, *B. cereus*, *B. halodurans*, *B acidicola, Bacillus sp.* WPD616, *Bacillus* sp. NRC22017, *Geobacillus caldoxylosilyticus* TK4, *G. thermoleovorans, Pseudomonas stutzeri, Streptococcus brevis Clostridium acetobutylicum.*	Mojsov, 2012; Sundarram and Murthy, 2014; Gopinath *et al*. 2017; Balkan and Ertan, 2007; Jana *et al*. 2013; Rao and Satyanarayana, 2007; Prakasham *et al*. 2007; Sindhu *et al*. 2009; De Souza and Magalhaes, 2010; Mehta and Satyanarayana, 2016; John, 2017; Elmansy *et al.* 2018; Abdulaal, 2018; and Almanaa *et al*. 2020.
Fungus	*Aspergillus oryzae, A. niger, A. awamori, A. fumigatus, A. kawachii, A. flavus, Penicillium brunneum, P. fellutanum, P. expansum, P. chrysogenum, P. roqueforti, P. janthinellum, P. camemberti, P. olsonii, Streptomyces rimosus, Thermomyces lanuginosus, Pycnoporus sanguineus, Cryptococcus flavus, Thermomonospora curvata,* and *Mucor* sp., *Trichoderma pseudokoningii.*	

Table 2: Nutrients present in different agricultural substrates used in SSF.

Nutrients (Wt % dry)	Wheat bran	Rice bran	Cassava root	Wheat straw	Rice straw
Cellulose	-	-	-	37.8	36.0
Hemicellulose	-	-	-	26.5	24.0
Lignin	3.8	1.5	1.7	17.5	15.6
Carbohydrate without starch	45-50	18-23	5-8	-	-
Starch	15-18	22-30	80.4	-	-
Crude protein	17.5	12.5	3.0	4.3	4.2
Lipid	4.5	17.5	0.2	1.5	-
Dry matter	87.0	90.0	87.5	91.0	93.0
Calcium (g/kg)	1.4	0.6	1.7	4.8	3.0
Magnesium (g/kg)	4.5	6.1	1.0	1.2	1.9
Phosphorus (g/kg)	11.1	13.9	1.1	0.7	0.9

SSF is the useful technique for production of enzymes, pharmaceutical components, bio-bleaching agents, food ingredients (Soccol *et al.,* 2017). Most of the time, synthetic or semi-synthetic medium was used in SmF. However, moist agricultural polymeric substrates such as wheat, rice bran, rice husk, cassava, sunflower meal, cottonseed meal, soybean meal, and pearl millet are used in SSF process (Soccol *et al.*, 2017; Abdulaal, 2018). These substances provide solid support and nutrients (polysaccharides - cellulose, hemicellulose, pectin, starch; crude protein; lipids, ions - calcium, magnesium, phosphate) for enzyme synthesis (**Table 2**).

Applications of agricultural wastes decrease production cost as well as pollution rate. Supplementation of protein, vitamins (B_2, B_6 biotin,

folic acid), and minerals (magnesium, calcium, zinc, copper, phosphorus, sulfur) can increase the rate of enzyme production many folds. Thus, SSF has several advantageous over the SmF (**Table 3**).

The usual method of process-optimization was alteration of one variable at a time (OVAT). OVAT is a time-consuming, less predictable and inconvenient process as the independent variables are separately optimized. Later, Taguchi method was adopted in this purpose, which was based on computerized statistical calculation. The observed data were feed to the software that gives the indication of optimum production levels by reducing the variance.

Table 3: Comparative features between submerged fermentation (SmF) and solid state fermentation (SSF).

Parameters	Submerged fermentation (SmF)	Solid state fermentation (SSF)
Habitat	Artificial process	Similar as natural system
Instrumentation	Properly required	Minimum required
Medium/substrate	Mostly chemical medium, contains ingredients for growth and enzyme synthesis	Use of solid agro-industrial wastes as substrate
Sterilization	Extensive sterilization required	Minimum sterilization required due low water activity
Controlling capacity	Monitoring of pH, dissolved oxygen, temperature, concentration of water soluble components, antifoaming agents, agitation, mixing, bubble formation are possible	Except moisture, extensive monitoring of factors similar to SmF is not required.
Catabolite repression and end product inhibition,	Most of the time decreases production yields. Feedback inhibition by product or other metabolites also possible	Minimum possibilities of inhibition
Energy expenditure	High	Very low
Contamination rate	Highly susceptible to contamination	Less susceptible due low water activity
Productivity	Less efficient than SSF	Highly productive and very minimum cost/production ratio
Downstream processing	Extensive purification is necessary	Easier to product isolation
Toxicity	Chances of organic solvent toxicity during extraction	Minimum chances of solvent toxicity
Waste water	Release large amount of waste water	Waste water production is very minimum
Cost	Expensive, high capital investment and operating costs	Low capital investment and operating costs
Ecological importance	Chances of environmental hazards	Eco-friendly and less chance of pollution
Microorganism	Bacteria are more efficient	Fungus are more efficient
Industrial application	Used for production of biomass, bio-fuels and others	Used for large scale production industrially important enzymes like amylase, cellulase, pectinase, protease and others

The response surface methodology (RSM) was the next approach for process-optimization. Originally, Box and Wilson developed this RSM method in 1951. It is applicable for designing of chemical, physical and biological experiments. In microbiology, this technique is useful in optimization of process to assess the impact of individual factor on overall efficacy (Mohandas *et al.,* 2010). RSM simplifies the task of optimization process of enzyme production in industrial scale. RSM is a combination of mathematical and statistical techniques develops an empirical model in which internal statistical calculation determines the interaction among the variables. The objective of RSM is to determine the

response (output variable) that is influenced by the impact of various independent and cumulative interactive factors (input variables) on the system response. Finally this system provides an indication of optimal operating region. The design of experiments (DOE) is the fundamental part of RSM, which indicates the points where the response should be evaluated.

Table 4: Advantages and disadvantages of RSM and ANN.

Advantages of RSM ➢ It deals with the multiple response problems and explores the relationships between several explanatory variables and one or more response variables. ➢ This model is easy to estimate overall response and also be applicable even when little is known about the process. ➢ It is applied to maximize the production of a special substance by optimization of operational factors. ➢ It is a first-degree polynomial model which depends on factorial experiment or a fractional factorial design. This model has sufficient capacity to determine effective variables which controls the response variable(s) of interest. ➢ This model identifies the significant explanatory variables which are used for development of central composite design as well as second-degree polynomial model.
Disadvantages of RSM ➢ Design of experiment is necessary to obtain an optimal response. ➢ The response generated through this model on the basis of approximation. ➢ It estimates minimum variance which is related to un-correlation. ➢ The moments of the distribution of the design points are constant. ➢ Multiple response variables in RSM create some problem because what is optimal for one response may not be optimal for other responses. ➢ Practitioners must be aware about its approximation to reality as it is fully based no statistical methods. ➢ There is chance of variation between estimated optimum point and optimum in reality due to errors of the estimates and inadequacies of the model.
Advantages of ANN ➢ It can be implemented in any application and applicable for complex linear, non-linear and complex relationships. ➢ One or more descriptors and/or response variables can be used to develop the model. It is applicable for categorical and continuous data. ➢ The model is less sensitive to noise than statistical regression models. ➢ Preparation of model is possible in case of incomplete information. The processing of information occurs in a highly parallel way and can continue without any problem when an element of the neural network fails. ➢ It can infer unseen relationships and predict on unseen data from initial inputs. ➢ It does not impose any restrictions on the input variables. Fixed relationship in the data is not necessary. ➢ This model is better than any other model where data is heteroskedastic with high volatility and non-constant variance relationships. ➢ It has ability to store the information of the entire network in the form of working memory or associative memory.
Disadvantages of ANN ➢ The model is computer dependent and needs training to operate. It requires high processing time for large neural networks. ➢ It is not possible to explain the total behavior of the network. ➢ Some difficulties arise to determine the proper network structure. ➢ It is very difficult to screening the problem present in the network. This model does not give any meaningful way how the results are calculated. ➢ To optimize the parameters, many parameters to be set in a neural which is challenging, due to overtraining.

Two types of plot i) three-dimensional space, ii) contour plots are used to visualize the shape of the response in graphical system. Analysis of variance (ANOVA) is applicable to determine the lack of significance of experimental data fitting in RSM (Most *et al*., 2018). RSM provides information about the optimum response where each variable interacts among them for maximum yield (Gu *et al*., 2005; Rao and Satyanarayana, 2007).

Artificial neural network (ANN) is the recent technique for process-optimization. Basically, it is a mathematical model having an extensive range of applications from biological system to computer engineering. ANN has three layers of connections. First layer is inputs of the independent variables (neuron), second layer is hidden neurons, and third layer represents output neurons. The neurons of the first layer are connected to hidden neurons where several calculations have been done to achieve output response. The algorithm was made on the basis of influencing input neurons and their relations to the output result. The results of ANN are also plotted through contour plots. In case of nonlinear data, ANN methodology is better than RSM as ANN does not require a design to obtain predictive models (Cheok *et al*., 2012). The advantages and disadvantages of RSM and ANN are given in **Table 4**.

Protein engineering and approach to modification of amylases

Protein engineering is the step to improve the functional characteristics and stability of the protein. It is mostly applied in fewer proteins (enzyme) those are industrially important. The protein engineering is primarily carried out by site-directed mutagenesis which makes a precise change in the sequence of encoded genes on DNA to alter the amino acid sequence of the desire enzymes. Site-directed mutagenesis technique simplifies DNA manipulations as it lowers the hazards of crude mutagenesis of cells or organisms and easy to isolate the desire mutants from thousands or millions of offspring.

Site-directed mutagenesis has several importances. It gives idea about structural-functional relationships of gene, helps to develop a mutant protein with novel properties. There are several methods for site-directed mutagenesis. The first method was primer extension (the single-primer method). It is based on *in vitro* DNA synthesis for preparation of oligonucleotide (7-20 nucleotides long) that carries a base mismatch with the complementary sequence (Uddin *et al*., 2016). This process is easy to perform in single stranded vector (e.g. M13).

Table 5: Representation of limitations of single-primer method of site directed mutagenesis and their expected remedial measures.

Problem	Expected solution
The newly formed double-stranded heteroduplex molecules are contaminated with either single-stranded non-mutant template DNA or partially double-stranded molecules. This contamination decreases the number of mutant progeny.	They can be removed by sucrose gradient centrifugation or by Agarose gel electrophoresis.
In *in vivo* condition, during DNA synthesis a mixed population of mutant and non-mutant progeny has been developed.	Mutant progeny have to be purified away from the wild type molecules.
In vivo DNA repair system *favors* the mismatch repair of non-methylated mutant DNA and thereby eliminating a mutation which lower the yield of mutant progeny.	This problem is solved by preventing the methyl-directed mismatches repair system. It is done by using host strains carrying the *mut*L, *mut*S, or *mut*H mutations.
Both mutant and non-mutant progeny will be grownup after upon replication.	Suppression of the growth of non-mutants is essential.
Always require a single-stranded template.	In PCR-based mutagenesis the template can be single-stranded or double-stranded, circular or linear.

Instead of its simplicity, there are numerous difficulties in single-primer method (**Table 5**) and the yields of mutants depend upon several factors. Two or three adjacent nucleotides are possible to change by using site-directed mutagenesis which generates an amino acid substitution at the site of interest. However, several oligonucleotides substitutions are time consuming and laborious. Another process for alteration of one amino acid is cassette mutagenesis, a fragment of the gene is replaced by another fragments containing the desired codon. Application of doped oligonucleotides is the alternative approach for desire mutagenesis (Neylon, 2004).

The polymerase chain reaction (PCR) can efficiently be used for site-directed mutagenesis. Single base mismatched between the amplification primer and the template can include a mutation in amplified DNA. Alternatively, incorporation of mutant base is also possible in any parts of PCR-product. This method is more complicated as it requires four primers for two sets of overlapping DNA and three PCRs (two for two sets of DNA and one to fuse the overlapping segments). Using the commercial kit, PCR mediated mutagenesis is much easier. In this purpose, two methods are adopted. According to Exsite method, desire DNA is present in both strands of vector and amplified through PCR while one of the primers carries the desired mutation. After amplification, linear duplexes carry the mutated gene along with original circular template DNA which can be cleaved by using *Dpn*I endonuclease when the template DNA is derived from *E. coli*. The second method is Gene Tailoring in which the target DNA is methylated before mutagenesis and the overlapping primers carry the desire mutation. Linear amplicons are produced along with methylated template DNA. After transformation, linear mutated DNA is circularized but host cell *Mcr*BC endonuclease digests the methylated template.

To improve the characteristics of a protein, it is better to mutate the gene at random instead of site-directed mutagenesis. Normally, PCR is error prone due to low fidelity of *Taq* polymerase and there is high probability of base alteration in amplicons. The random mutation is possible by using error-prone PCR system. This is achieved by different ways: i) introduce a small amount of Mn^{2+} instead of Mg^{2+}, ii) to include an excess of dGTP and dTTP relative to other two nucleoside phosphates and iii) by using nucleoside analogs. First two methods have possibilities to achieve error rates of one nucleotide per kilobase. Error-prone PCR can randomly change the one amino acid to another. For example, a single point mutation in a valine codon can change it to others encoding phenylalanine, leucine, isoleucine, alanine, aspartate, or glycine. Random insertion/ deletion (RID) process is used for random incorporation or elimination of amino acids and this technique is mediated by ligating an insertion or deletion of a cassette at nearly random locations within the desire gene (Murakami *et al*., 2003).

Engineering of amylase improves the physicochemical characteristics like pH profile, thermostability, chelator insensitivity, and oxidation resistance. Altered enzymes are exploited in food, textile, paper, detergent industry and clinical system. The complete three dimensional structures, active site residues, and the position of functional amino acid residues have been determined by using bioinformatics through various software's (DSMODELER; Geno3D, and Qsite Finder). Several strategies have been applied to make the better quality of enzymes. Exchange of some oxidation prone amino acids (methionine, tryptophan, cysteine, and histidine) by other amino acids (not affected by oxidizing agents) increases oxidation resistance capacity.

Introduction of disulphide bonds by inserting cysteine makes chelator resistance. Insertion of proline increases structural stability. Substitution of deaminating amino acid (asparagines) by Lys or Asp enhances the possibilities of ionic interaction with solvent to improve the stability of the enzyme. Introduction of histidine increases the chance of hydrogen bonds and salt bridge formation for better stability. Moreover, helix capping, removal of deamidating residues, and cavity-filling improve the pH profile and thermostability (Khemakhem *et al.*, 2009; Chi *et al*., 2010; Kumari *et al*., 2012; Yang *et al*., 2013; Mehta and Satyanarayana, 2016).

Application of amylases

Amylases are more useful for the industrial and biotechnological applications. α-amylase, pullulanase, isoamylase, CGTase, branching enzymes, α-glucosidase, amylopullulanase, neopullulanase, maltogenic amylase, maltotetraose forming amylase, maltohexaose forming amylase, β-amylase, and glucoamylase are used in starch based industry. These enzymes act either endo-acting or exo-acting fashion. α-amylase mostly cleaves α-1,4 glycosidic linkages. Pullulanase, neopullulanase, and isoamylase can break α-1, 6 glycosidic linkages. Pullulanase, isoamylase, α-glucosidase, β-amylase, and glucoamylase are used for saccharification of partially hydrolyzed starch slurry. Maltogenic amylase, maltotetraose forming amylase, and maltohexaose forming amylase produce maltose, maltotetraose, and maltohexaose respectively from partially hydrolyzed starch. CGTase helps to form cyclodextrin. All these amylases are used in food and pharmaceutical industry, brewing process, ethanol and biofuel production, detergent preparation, animal feed preparation, and waste management (Yan and Wu 2016; Saini *et al.* 2017; John, 2017). Liquefaction of starch is most important for preparation of glucose and fructose/glucose syrups. Enzymatic treatment produces maltose from starch. Maltose is widely used as sweetener, used in food industries due to its low tendency of crystallization and relatively nonhygroscopic nature.

Table 6: Comparative features of different types of desizing process.

Process of desizing	Agents	Temperature	Controlling factors	Advantages	Disadvantages
Enzymatic	thermostable α-amylase	High temperature (70- 90 ºC	pH, temperature, water hardness, electrolytes, surfactant	No use of aggressive chemicals; No harmful effects on the fibre; Wide variety of application process; Increases brightness; High bio-degradability and eco-friendly	No effect on tapioca starch; Other impurities are present.
Oxidative	Potassium/sodium persulfate; sodium bromite	Normal temperature	–	Supplementary cleaning effects; Effective against all types of starch	Risk of damage of cellulosic fibre is very high
Acid wash	Cold solution of dilute sulfuric (H_2SO_4) or hydrochloric acid (HCl)	Low temperature	–	Effective against all types of starch	Risk of damage of cellulosic fibre of cotton fabrics
Fermentative	Using Generally Regarded as Safe (GRAS) grade microorganisms	Maintenance of growth temperature	Control of pH, temperature, water activity	Cheaper, economically favorable; Low resources consumption and emission than enzymatic method; Eco-friendly in nature	Removal of microbes is necessary; Long time treatment may able to damage the fibre
Removal of water soluble sizes	Hot water with surfactant and mild alkali	High temperature (~90 ºC)	Maintenance of high temperature	Less harmful to the fabrics.	Process is less efficient to remove total amount of starch

Malto-oligosaccharides mixture (maltooligomer mix) is obtained from corn starch after its amylolytic digestion. Maltooligomer mix is less sweet than sucrose and low viscous than corn syrup. It is mainly used as substitute of sucrose. Maltotetraose syrup is the ingredient of geriatric and infant foods. It is used as replacement of sucrose without altering the taste and flavour of the food. This application also controls the freezing points of the frozen foods. Amylases are also used for the clarification of beer and fruit juices, which improve the quality and clarity of the products (Kumari *et al*., 2012; Dey and Banerjee, 2014). Application of α-amylase in desizing process is one of the oldest uses of amylase in industrial sector. Starch paste is used as sizer during weaving and enzymatic treatment removes starch sizer from cotton fabrics. Although, there are several processes of desizing, but use of thermostable α-amylase in this purpose is more convenient (**Table 6**).

Alcohol and biofuel production is carried out by using α-amylase and saccharifing amylases which hydrolyze the starch into smaller saccharides followed by alcoholic fermentation. Starchy wastes promote microbial growth and environmental pollution. Amylolytic treatment of starchy wastes can produce valuable products such as microbial biomass, animal feed, and biofuel (Kumar *et al*., 2014).

Table 7: Composition of enzyme based different digestive medicines.

Brand name	Formulation	Composition	Manufacturers
Neopopeptine	Syrup	Alpha amylase 100 mg; Papain 50 mg/5ml	RAPTAKOS BRETT
	Capsule	Alpha amylase 100 mg; Papain 50 mg/5ml; Simethicone 30 mg	
Zymosaf	Syrup	Alpha amylase 100 mg; Papain 50 mg/5ml	SAF FERMION
	Capsule	Alpha amylase 20 mg; Alpha-galactosidase 100 mg; Bile constituents 25 mg; Cellulase 25 mg; Charcoal 50 mg; Dimethicone 25 mg; Invertase 10 mg; Lactase 10 mg; Lipase 50 mg; Protease 50 mg	
Digeplex	Syrup	Alpha amylase 62.5 mg; Pepsin 20 mg	PIRAMAL HEALTHCARE
Aristoztme	Syrup	Fungal diastase 50 mg; Pepsin 10 mg/5ml	ARISTO PHARMACEUTICALS PVT LTD
	Capsule	Fungal diastase 50 mg; Pepsin 10 mg	
Unienzyme	Syrup	Fungal diastase 50 mg; Pepsin 10 mg/5ml	TORRENT PHARMACEUTICALS LTD
	Capsule	Fungal diastase 100 mg; Papain 60 mg; Charcoal 75 mg	
Vitazyme	Syrup	Caraway oil 500 µg; Cardamom 500 µg; Cinnamon oil 250 µg; Fungal diastase 40 mg	EAST INDIA PHARMA
	Capsule	Fungal diastase 20 mg; Lactobacillus 150 million spores	
Enzar Forte	Capsule	Alpha amylase 15000 IU; Lipase 4000 IU; Protease 15000 IU; Sodium tauroglycocholate 65 mg	TORRENT PHARMACEUTICALS LTD
Biozyme	Syrup	Diastase 50 mg; Pepsine 10 mg	MECOSON LABS.
Lupizyme	Syrup	Fungal diastase 18.75 mg; Pepsin 12.5 mg	LUPIN LABORATORIES LTD.
	Capsule	Nicotinamide 15 mg; Cyanocobalamin 1 µg; Pyridoxine 1.5 mg; Riboflavin 1 mg; Thiamine 2 mg; D-Panthenol 1.5 mg; Pepsin 125 mg; Fungal Diastase 18.75 mg	

Amylases, especially prepared through protein engineering bear chelator resistance, alkalophilic and oxidant insensitive characteristics are potentially used in enzyme based detergent formulation (Sundarram and Murthy, 2014; Kumari *et al*., 2012). The fungal α-amylase from *Aspergillus oryzae* is used as digestive aids for the treatment of indigestion. The liquid and capsule formulations of the digestive medicines are available in the market (**Table 7**).

CONCLUSION

In the era of biotechnology, microbes are most useful for mankind. They are exploited for antibiotic production, enzyme preparation, biomass and single cell protein synthesis, production of medicinal components (amino acids, vitamins, steroid), and waste management (Rahman *et al*., 2019). Microbial enzymes have multidimensional uses starting from food industry to pollution control. Among the microbial enzymes, α-amylase has promising market value. The demand of microbial α-amylases is increasing day-by-day. Several agro-industrial residues are competently used in SSF to reduce production cost for large scale enzyme preparation. This step is an eco-friendly process and a great advancement towards clean world. An approach to protein engineering makes the enzyme more thermostable, oxidant resistant, chelator insensitive along with better pH profile. All these factors increase the potential uses of amylases in different sectors. Amylases, particularly α-amylase are efficiently used in food, pharmaceutical, textile, detergent, paper, and brewing industry. Thus, amylases are fairly excellent industrial enzymes and the demand would always be high in near future.

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CONFLICT OF INTEREST

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