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Effect of operating parameters on amylase efficiency in a selected Sugar refinery

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Abstract

Amylase is a significant industrial enzyme that is used in a variety of industries, including scarification of starchy materials, pharmaceuticals, food, textiles and detergents. This research work is concerned with the optimization increase enzyme efficiency of dissolution unit from Etihad Food Industries Company (Sugar plant), Babylon, Iraq. Effects of operating parameters such as amylase concentration (0-50 ppm), time (0-15 min) and temperature (25-85 C◦) on the starch's removal efficiency were investigated. Also, the results indicated that the temperature has the main effect on the amylase efficiency. Under optimized operating conditions of initial temperature =85 C, amylase concentration =25 ppm, and time for reaction=15 min the removal efficiency of starch was found to be 60% which is relatively higher than the previous works.

Keywords: Melting sugar, Amylases, Starch removal, Temperature

1. Introduction

To make edible sugar, the sugar industry processes sugar beets and sugar cane. The latter accounts for more than 60% of global sugar production, with sugar beets accounting for the remainder. Sugar canes are typically washed before their juice is extracted. For removing, the juice is clarified, then evaporated to make syrup, crystallized for separating the liquor, also centrifuged for separating the molasses from crystals [1]. Sugar crystals are dried and refined before being packaged for shipment. In a few places (for instance, South Africa), juice is extracted using a diffusion process, which allows for high extraction rates while using less energy and lowering maintenance and operating costs. Only the washing, extraction and preparation processes differ when processing sugar beets (75% water, 17% sugar) [1].

After being washed, the beets are sliced and placed in a slowly-rotating diffuser, in which a water's countercurrent flow is applied for removing sugar from slices. Per metric ton of beet processed, water of 15 m3 and energy of 28kWh are consumed. Impurities are removed and the sugar is decolonized during the refining process. Affinitions (centrifugation and mingling), clarification, melting, evaporation, decolonization, finishing and crystallization are the unit operations that follow. Granular activated carbon, ion exchange resins and powdered activated carbon are used in decolonization approaches[2]. Sugar is content of starch and rice content it also [3]. Enzymes, acids, or a combination of the two can hydrolyze starch to produce molecular fragments ranging in size from large molecular weights to small oligosaccharides and D-glucose. High fructose corn syrup and corn syrup, two commercially significant sweeteners, are made from starch hydrolysis and, in the case of HFCS, partial isomerization. Starch is one of the most significant components of human diet and is processed enzymatically and chemically into many products in food industry, including glucose syrups, starch hydrolysates, malto-dextrin derivatives, fructose, and cyclo-dextrins. Furthermore, the sugars that have been produced might be fermented in order to yield ethanol.

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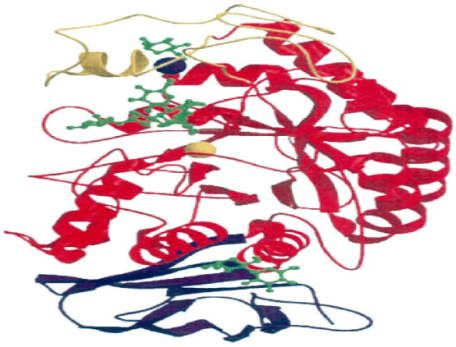

Fig.1 α-amylase structure. Domain A has been represented in red, domain B in the yellow and domain C in the purple. In catalytic center, ion of calcium has been represented as blue sphere and chloride ion in yellow sphere. Green structures have been bound to active site and to sites of surface binding [4].

Despite the fact that there are many plants that can produce starch, just a few are significant for industrial starch processing. Maize, tapioca, wheat and potato, are the most common industrial sources, yet limitations like thermal resistance, low shear resistance, thermal decompositions, and high tendency to retrograde limit its uses in certain industrial food applications [5],[6]. Because of its utility in a variety of food products, starch is gaining popularity among carbohydrate polymers. Starch is commonly utilized in food as well as industrial applications as colloidal stabilizer, thickener, bulking agent, gelling agent, and water retention agent, also it contributes significantly to textural characteristics regarding various foods[7] . Starch is a glucose polymer that is linked to another by a glyosidic bonding. Amylopectin and amylose are 2 glucose polymer types found in starch (fig.2). Amylopectin and amylose are structurally and functionally distinct. Amylose can be defined as linear polymer that has been made up of about 6,000 units of glucose that are linked together by α1,4 glyosidic bonds. Short α1,4 linked to the linear chains that include 10–60 units of glucose and α1,6 linked to the side chains of 15–45 units of glucose make up amylopectin. Granule bound starch synthase has been thought to be responsible for amylose synthesis by elongating malto-oligosaccharides for forming amylose. The synthesis of the unit chains of the amylopectin has been thought to be carried out by soluble starch synthase. α1,4 glycosidic bonds in inner amylose or the amylopectin chain part can be cleaved by α -amylase[8], [9], [10]&[5]. The basic chemical formula of the starch molecule is (C6H10O5) n can see it in fig.2.

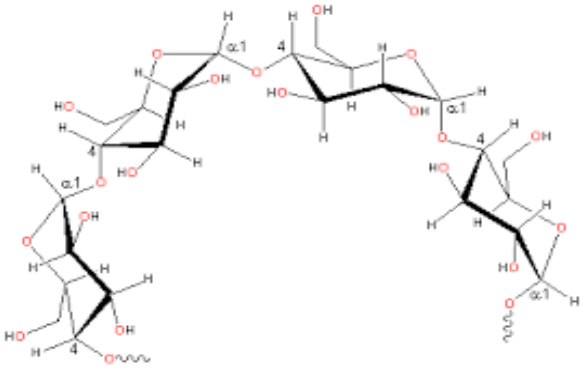

Fig.2 starch structure

The main amylase application, starch scarification, had totally replaced chemical utilizations with the hydrolysis of the amylase enzyme. Scarification takes place at a high temperature, and thermophilic micro-organisms may be the optimal candidates for production of the amylase since they create thermo-stable amylase. Therefore, the search for new microbial strains for the purpose of meeting industrial enzyme demand continues. Furthermore, as a result of the high alkaline pH stabilities that are needed for industries, amylase has been supplemented in local detergents [3].

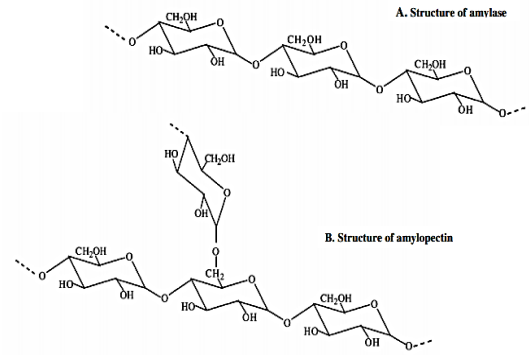

Figure 3. Two glucose polymer types that have been present in the starch: amylose (A) represents linear polymer that consists of about 6,000 units of glucose with α1,4 glyosidic, amylopectin (B) includes short α1,4 linked to the linear chains of 10–60 units of glucose and α1,6 that is linked to the side chains that have 15–45 units of the glucose [8].α-amylase, a major commercial enzyme process, hydrolyzes starch into small oligosaccharides.

Amylases are utilized in a variety of industrial processes, which include detergents, food, paper industry and textiles, to hydrolyze starch [11], [12]&[13]. The composition of saccharides acquired

following starch hydrolysis depends heavily on hydrolysis conditions, temperature, and enzyme origin. The enzymes' specificity, pH response and thermos ability, are all important characteristics for industrial use[14]. Amylase includes a 3D structure that allows it for binding to substrate and promote glycoside link breakage through the action regarding highly-specific catalytic groups [15]. Human α-amylase is considered as a Ca-containing enzyme with 512 amino acids in a single. Furthermore, thermophilic amylase is needed to produce sweeteners from starch as well as the starch's scarification for biochemical production[3]. Micro- and microorganisms can produce them[13]. The major goal of this research was to find the most efficient enzyme for degrading sugar starch.Amylases represent enzymes which break down glycogen and starch. Amylases might be found in a variety of places, including microbes, animals and plants. The main benefit of utilizing microorganisms to make amylases is cost-effective bulk production capacity, as well as ease with which microbes can be manipulated for obtaining enzymes with the required properties [16]. Now, many different microbial amylases have been commercially-available, and they nearly totally substituted chemical starch hydrolysis in the industry of processing starch. Microorganisms' amylases have a wider range of industrial applications that have been compared with the animal and plant α-amylases because they are more stable. α-amylases can be considered as the most commonly used in starch industries, where they're utilized for starch hydrolysis in process of starch liquefaction, converting starch to glucose and fructose syrups[17] . Gelatinization, which is involving starch granules' dissolutions and the formation of a viscous suspension; liquefaction, including loss of viscosity and partial hydrolysis; and scarification, involving glucose and maltose production through further hydrolysis[18] . Those enzymes have been utilized in automatic dish-washing machines and laundry detergents to break down starchy food residues, like gravies, potatoes, chocolate, custard, and other oligosaccharides into dextrin and other small oligo-saccharides[19]. Amylases have activities at low temperatures and alkaline pH, allowing them to keep the required stability in detergents. Amylases' oxidative stability is a major criterion for their utilization in the detergents with a highly oxidizing washing environment. Because starch has been considered as attractant for various particulate soils, the removal of the starch from the surfaces is significant as well in presenting a whiteness benefit. The most commonly utilized liquid bio-fuel is the ethanol. The starch represents the major commonly deployed substrate for producing the ethanol due to its inexpensiveness and availability in the majority of the parts worldwide [20]&[21]. For the purpose of obtaining fermentable sugar types, starch has to be solubilized first and after that subjected to 2 enzymatic steps, which are scarification and liquidification, where the starch is converted into sugar with the use of amylolysis micro-organism or enzymes like α-amylase, are followed via fermentation, in which sugars are converted to ethanol with the use of ethanol fermenting microorganism like yeast. Saccharomyces cerevisiae is a type of yeast. Amylases are utilized in the desiring process in the textile industry. For ensuring secure and fast weaving process, sizing agents like the starch have been utilized to yarn before the production of the fabric. Starch is an appealing size since it is inexpensive, widely available in the majority of world parts, and easily removed. In textile finishing industry, the starch is after that removed from the woven fabric using wet-process. De-sizing is the process of removing starch from a fabric, acting like a strengthening agent for preventing warp thread from breaking throughout the process of weaving. The α-amylases remove the size selectively while avoiding the fibers[22] . Amylase that has been derived from Bacillus stain was long utilized in the textile industry.

2. Experimental work:

The sugar content starch samples were provided by Etihad food industries Comp. Samples was take from buffer tank melting unit. Characterization of this sample is shown in Table 1.

Table 1. Characteristics of the raw sugar in Etihad Food Industries Comp...

Test	Buffer tank sample
Starch	450-500 ppm
Dextran	50-70 ppm
Color	900-1000 Icumsa
PH	6.2-6.3

All these samples kept the pH is not more 7 because the pH influence on amylase activity. The activity of the amylase has been high at pH 7, and it decreased slightly as the pH has been increased (in other words, in the case where the solution has been made more alkaline). In the case where a higher acidity pH (pH 5) has been utilized, the activity has been considerably reduced in comparison to when pH 7 was used [23].

3. Results and discussion

Plants, microorganisms, and higher organisms all contain α-amylase (α-1,4glucan-4-glucanohydrolase) [24]. α-amylase is a member of endo-amylase family, which catalyzes initial hydrolysis related to starch to shorter oligo-saccharides through the cleaving of the α-D-(1-4) glyosidic bonds. α-amylase can't cleave either terminal glucose residues or α1,6-linkages [25]. α-amylase action produces oligo-saccharides of variable length with α-configuration and α-limit dextrin that represent a mix of the maltose,

maltotriose, and branched oligosaccharides of 6–8 units of the glucose with α-1,6 as well as α-1,4 linkages. Other amylolysis enzymes are involved in starch breakdown process, yet, contribution regarding α-amylase has been considered as most significant for start of the process[26]. Amylase has 3D structure that allows it to bind to substrate and promote glycoside link breakage through the action related to highly-specific catalytic groups. Human α-amylase represents Ca-containing enzyme with 512 amino acids in a single. A, B, and C are the three domains that make up the protein, as seen in Figure 3. The largest domain is A, which has a typical barrel-shaped (β/α)8 superstructure. B domain has been inserted between C and A domains and is disulphide-bonded to A domain. C domain appears to be an independent domain with unknown function, with a beta-sheet structure that is linked to A domain via a simple poly-peptide chain. The active site (substrate-binding) of α-amylase is located between carboxyl end of the A and B domains in a long cleft. Ca2+ is located between B and A domains and might act as an allosteric activator as well as a 3D structure stabilizer. Asp206, Glu230, and Asp297 bind substrate analogs, implying that they are involved in catalysis. Catalytic site is located at sub-site 3 of the substrate-binding site, which has 5 subsites. Substrate may be binding to the first residue of the glucose in subsites 1 or 2, causing cleavage between the 1st and 2nd or 2nd and 3rd glucose residues, respectively. The analysis several laboratory experiments were conducted to determine the efficiency of the enzyme in various conditions, like the temperature and reaction duration it results are as following:

A normal experiment was conducted by adding an enzyme concentration (50 ppm) at the laboratory temperature in the quality lab in Comp., which is about 25 degrees Celsius, and without time reaction, and the result was that the efficiency of the enzyme was only about 22%. The same first experiment was conducted, but by adding two parameters, temperature of 65 degrees Celsius and a reaction time of 15 minutes, which are conditions similar to the conditions of the juice from melting it until it reaches the first carbonation, and the result was that the efficiency of the enzyme became (41%). And the starch concentration was measured for a sample of raw sugar after providing conditions of a temperature of 65 degrees Celsius and a reaction time of (15 minutes) and a concentration of (25 ppm), and the result was that the efficiency was (32%), and the starch was measured for a simultaneous sample with the crude sample from the buffer tank, and it was the result is that the efficiency is (24%), and this may be explained by the fact that there is a difference in temperature and reaction time between the laboratory experiment and the reality of work in dissolution. A fourth experiment was conducted by taking different temperatures, which are (65° C) and (85° C), while stabilizing the reaction time and concentration (25 ppm) The results were that at high temperature the results of the enzyme were better, and the same fourth experiment was repeated several times for the purpose of verifying the results, and the results were similar. Another experiment was conducted, assuming that the place of adding the enzyme to the circulating line could be changed upon dissolution.The conditions were taken that the temperature would rise to close to 85 degrees Celsius for about two minutes and 13 minutes at 65 degrees Celsius, which is approximately the temperature of the juice in the evaporating tank the removal approximately (57%). In the end, an experiment was conducted by taking different concentrations of the enzyme, the purpose of which is determining optimal concentration of added enzyme for obtaining a good starch removal and to know the extent of benefit from raising the concentration of the enzyme. These experiments were done at a temperature of 75 degrees Celsius for two minutes and 65 degrees Celsius for (13 min).

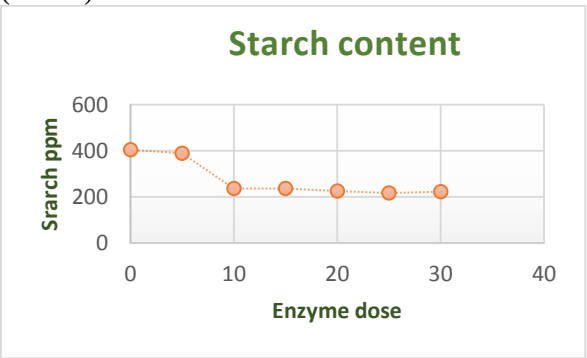

Fig.4 Starch content with Enzyme dose

Table.2 Experimental Results for starch Removal

Run	Starch content (ppm)	Amylase added (ppm)	Temperature (°C)	Time (min)	Starch remaining(ppm)	Starch removal efficiency
1	445	50	25	Without time	350	22
2	453	50	65	15	269	41
3	453	25	65	15	310	32
4	431	25	65	15	245	43
5	431	25	85	15	169	60
6	419	25	65	15	231	44
7	440	25	85	15	148	65

An experiment was conducted by taking different concentrations of the enzyme and the purpose of which is determining the optimal concentration of added enzyme to obtain to remove good starch and know the benefit of raising the concentration of the enzyme. These experiments have been performed at a temperature of 75 degrees Celsius for two minutes and 65 Celsius for 13 minutes. As follows:

Table.3 Experimental data for optimum Enzyme concentration

Enzyme Dose (ppm)	Starch Content (ppm)	% Removal
0	406	Nil
10	236	42
15	236	42
20	225	44
25	216	46.5
30	222	45

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The present research focused on investigating the effect of many operating parameters such as starch content, removal efficiency, temperature and time on activity for amylase in the Etihad food industries comp. we can see that there is a direct relationship between heat and the enzyme. That is, the higher the temperature, the better the work and efficiency of the enzyme. And this is something It is also shown in the file explaining the efficiency of the enzyme, which shows that the best temperatures at which the enzyme works are between (90-95) degrees' percentage. Increasing the concentrations of the enzyme has very little benefit in terms of removing starch, and it was found that adding a concentration of 10 ppm of the enzyme would be enough. Most of the removal of starch found in raw sugar. If this modification is applied and works as in the laboratory results, it will have clear results on improving the performance of the filters first. It is also reflected in the reduction of viscosity by cooking waste.

4. Conclusions

The present research focused on investigating the effect of many operating parameters such as temperature, amylase concentration, and time on the starch removal in the melting of Al-Etihad Food Industries Comp. The optimum condition was temperature between (80-85 °C), time (15 min) and concentration of amylase (25 ppm). Results show that the temperature has the main effect on starch removal in the present work because when decreased the temperature to (60° C) the efficiency of enzyme decreased down (25-20%).

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