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Enzymatic hydrolysis of molasses

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Abstract

Kinetic studies of the enzymatic hydrolysis of molasses were conducted using glucoamylase. Central Sugar Refinery SDN BHD contains 13–20% glucose. The molasses was diluted and the kinetic experiments were conducted at 67 °C with 100–1000 mg/l of glucoamylase. The glucose contents of the molasses were enhanced after hydrolysis of molasses solution with 1000 mg/l glucoamylase. A Lineweaver–Burk plot was obtained based on enzyme kinetic data. The rate constant, K_m and maximum reaction rate, V_{max} for 500 mg/l of glucoamylase were 100 mmol/l (18 g/l) and 5 mmol/l min (0.9 g/l min), respectively. The maximum reaction rate, V_{max} for 1000 mg/l of glucoamylase was doubled, to 100 mmol/l (18 g/l) and the rate constant, K_m was the same for 500 mg/l of glucoamylase. The substrate inhibition model was noncompetitive based on the resulting Lineweaver–Burk plot for enzyme concentration of 500 and 1000 mg/l.

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Keywords: Enzyme kinetics; Enzymatic hydrolysis; Glucoamylase; Molasses

1. Introduction

Amylolytic enzymes are classified into α -amylase and glucoamylase which are involved in the complete conversion of starch to sugar. α -Amylase (1,4- α -glucan-glucanohydrolases) is an extracellular enzyme which hydrolyzes starch into maltose, glucose and maltotriose by attacking the α -1,4-glycosidic bonds in starch and related substrates. Glucoamylase (α -1,4-glucan-glucohydrolases) acts on starch by splitting glucose units from the nonreducing ends. Amylolytic enzymes can be produced from a variety of sources, including fungi (Najafpour et al., 1999), and bacteria. The α -amylases are widely used for liquefaction and saccharification of starchy substances in the food, confectionary and beverage industries (Bolton et al., 1997). A primary use is in the production of sweetener and reduction of dough viscosity to improve the texture and appearance of bread. It is also present in pharmacologically active digestive aids, used in the process of recovering sugar from scrap candy and is involved in the manufacture of syrups in chocolate. *Saccharomycopsis fibuligera* has been

used to ferment 20% solid starchy waste from poultry processing waste to single cell protein (Najafpour et al., 1994). Also *Saccharomycopsis fibuligera* has been used to ferment premalted 20% wheat starch (Gogoi et al., 1987) and α -amylases from other microorganisms have been used to convert cassava starchy waste and in the saccharification of sugar beet pulp (Ejiofor et al., 1996; Micard et al., 1996).

Molasses is the by-product of the sugar refinery process. It is the most economical source of carbohydrate for ethanol and citric acid fermentation. It contains reduced polymeric sugars that can further react to form fermentable sugar during enzymatic hydrolysis. A normal cane molasses usually has a water content of 17–25%, a sugar content (sucrose, glucose, fructose) of 45–50% and polysaccharides (dextrin, pentosans, polyuronic acids) containing 2–5%. The purpose of the research presented in this paper is to upgrade the quality of molasses for higher sugar content, for use as feed stocks in fermentation processes. Also presented is a preliminary kinetic results of enzymatic hydrolysis of molasses using glucoamylase enzyme for upgrading the fermentable sugar content of the Malaysian export molasses and potentially to enhance the yield of productivity used in ethanol production and other fermentation processes.

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2. Methods

The molasses for the experiments was obtained from Central Sugar Refinery SDN BHD, Malaysia. Two types of molasses were provided, ‘export’ and ‘local’ samples. The ‘export’ sample is more concentrated than the ‘local’ sample. The characteristics of the molasses are summarized in Table 1. The type of molasses used in this research studies was the export brand from Central Sugar Refinery Ltd., Malaysia, which was enriched of higher dimmers, trimmers of sugar monomers and polysaccharides.

The dinitrosalicylic acid (DNS) method was used to measure liberated sugar during enzymatic hydrolysis. Total reducing sugars were determined as maltose equivalents by a colorimetric method (Summers, 1924). The reagent was prepared by dissolving 10 g of DNS [3,5-(NO₂)₂C₆H₁₂-2OH-COONaH₂] in the 2 M sodium hydroxide solution mixed with 1 M solution of sodium-potassium tartrate [KNaC₄H₄O₆ · H₂O] (Fluka Sigma—Aldrich, Buchs, Switzerland). The resultant liquid yielded a clear orange color, which was used to measure total reducing sugars. The desired range of total sugar concentrations were from 400 to 1600 mg/l. The intensity of the created color was detected using a Metertek model SP-85 spectrophotometer (Turner Barnstead Thermolyne Corp. Japan) at a wavelength of 540 nm.

The polysaccharides and starch concentrations were measured using a 0.1 molar iodine solution, also a colorimetric method using the spectrophotometer at a wavelength of 620 nm (Thomas and Chamberlin, 1980). The starch molecules formed a dark blue complex with the iodine molecule with the intensity of the generated color being directly proportional to the amount of starch present in the solution.

The glucoamylase enzyme used in this study originated from *Rhizopus* mold and was obtained from Sigma Chemicals (St. Louis, MO, USA). It contained approximately 35% protein, determined by Biuret method (Thomas and Chamberlin, 1980), with the balance primarily diatomaceous starch and sugar. The enzymatic hydrolysis of molasses was studied by mixing a fixed concentration of molasses solution with different

volumes of glucoamylase solutions and distilled water to form 5 ml samples with different glucoamylase concentrations. The test tubes containing the samples were heated and shaken in a hot bath of for 2 h for complete hydrolysis. When the hydrolysis reaction was completed, the samples were centrifuged to separate the enzyme and liquid. The clear liquid was analyzed by the DNS method for the liberated sugar.

The data presented in the Figs. 1, 4 and 5 were replicated three times. The data in Figs. 2 and 3 were carried out for a single run. The mean values for the

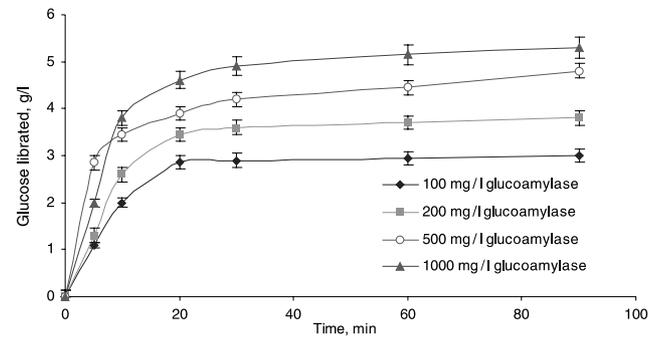


Fig. 1. Enzymatic hydrolysis of 10 g/l molasses at 67 °C with various concentration of glucoamylase.

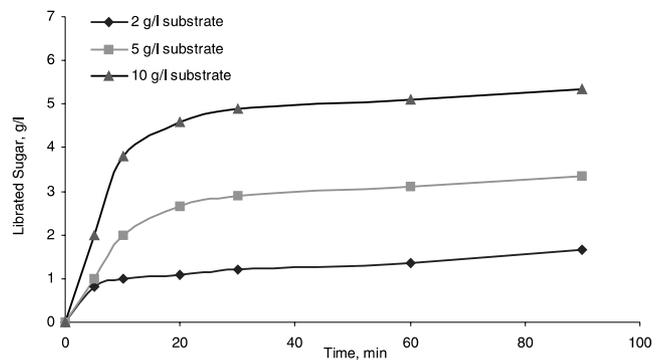


Fig. 2. Enzymatic hydrolysis of molasses (2–10 g/l) with fixed concentration of enzyme (1000 mg/l) at 67 °C.

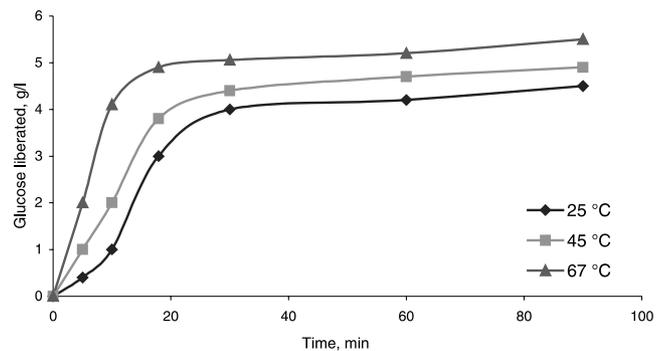


Fig. 3. Effect of temperature on enzymatic hydrolysis using glucoamylase (1000 mg/l) and 10 g/l of molasses.

Table 1
Summary of the molasses characteristics

| Characteristic | Local | Export |
|----------------------------------|------------------|-------------------|
| Color | Brown | Dark brown |
| Viscosity (cp) | 22.1 (at 10 RPM) | 30.6 (at 7.0 RPM) |
| Density, g/ml | 1.441 | 1.354 |
| Water content, % | 47 | 35 |
| Suspended solid and others, % | 38 | 58.6 |
| Initial sugar concentration, g/l | 18.7 | 194 |
| Final sugar concentration, g/l | 600 | 611 |

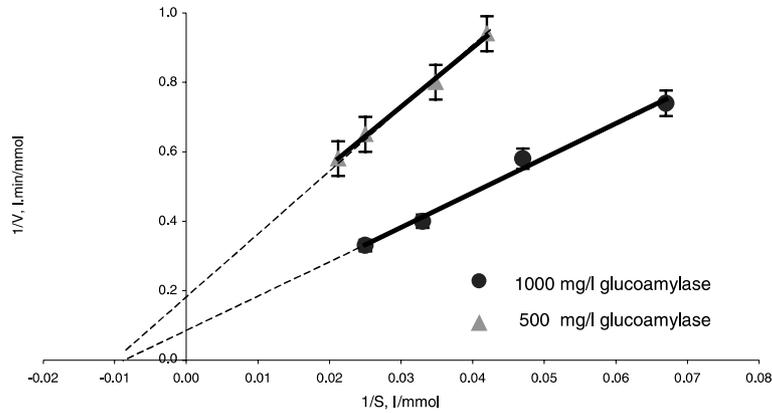


Fig. 4. Lineweaver–Burk plot of the enzymatic hydrolysis of 10 g/l molasses solution at 67 °C with 500 and 1000 mg/l glucoamylase.

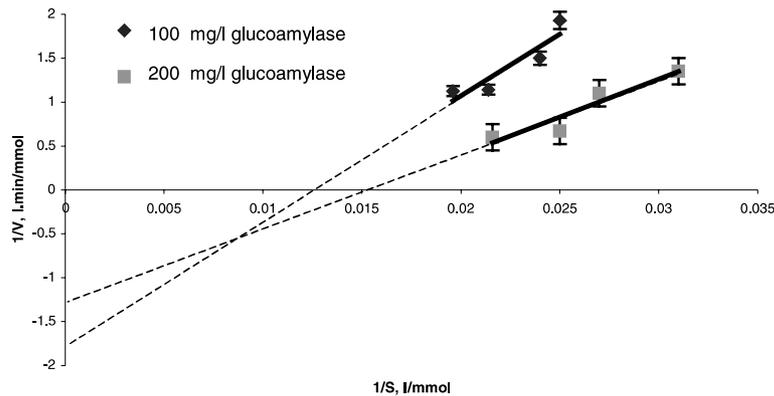


Fig. 5. Lineweaver–Burk plot of the enzymatic hydrolysis of 10 g/l molasses solution at 67 °C with 100 and 200 mg/l glucoamylase.

replicated data are presented in the graphs. The standard deviation for each collected data in Fig. 1 was approximately 5%. There was no error analysis for Figs. 2 and 3. In Fig. 4 the standard deviation for 500 and 1000 mg/l of glucoamylase was approximately 10% and 5% respectively. The standard deviation for low enzyme concentration (100 and 200 mg/l of glucoamylase) in Fig. 5 was approximately 5%.

3. Results and discussion

The objective of this paper was to increase the fermentable sugar content of Malaysian export brand molasses is used as feedstock for fermentation processes. Molasses are enriched with invert sugars, natural polymer of carbohydrates, unable to be crystallized in sugar refineries. Enzymatic hydrolysis is the method of choice to enhance the potential, to increase the amount of fermentable sugar for ethanol production. The upgraded molasses mostly with six carbon sugar is used in fermentation processes that may enhance the yield of productivity of the process.

Experimental results were obtained in monitoring the effect of temperature, substrate and concentration of glucoamylase on the enzymatic hydrolysis of molasses. The enzymatic hydrolysis of molasses at 67 °C with 100–1000 mg/l of glucoamylase is shown in Fig. 1. The concentration of glucose liberated from molasses in the enzymatic hydrolysis was doubled as the enzyme concentration increased from 100 to 1000 mg/l.

The results obtained from enzymatic hydrolysis of molasses, substrate concentration of 2–10 g/l, with fixed concentration of enzyme (1000 mg/l), at 67 °C is shown in Fig. 2. The amount of glucose liberated was proportional with initial substrate concentration. At low substrate concentration of 2 g/l molasses the conversion was at 83% but the conversion gradually dropped to 55% as the concentration of molasses increased to 10 g/l.

Enzymatic hydrolysis of 10 g/l molasses was carried out with 1000 mg/l of glucoamylase at three temperatures 25, 45 and 67 °C. The effect of temperature on enzymatic hydrolysis is shown in Fig. 3. Increasing temperature may increase the rate constant, which enhances the enzyme activation energy. However, comparing the experimental results obtained at 25, 45 and

67 °C exhibit minimal changes in the amount of liberated sugar from the hydrolyzed solution.

The enzymatic hydrolysis reaction rates increased linearly at the initial part of the reaction and then became constant. The reaction was first order at low substrate concentrations and zero order at high concentrations of molasses as the substrate. The enzymatic hydrolysis reaction rate increased proportionally with the amount of glucoamylase present. Several sets of enzyme rate experiments were conducted to obtain the glucoamylase hydrolysis kinetic data on molasses. Glucoamylase with a concentration of 100–1000 mg/l was reacted with molasses solution at 67 °C. The results for the kinetic studies are illustrated on a Lineweaver–Burk plot shown in Fig. 4 for 500 and 1000 mg/l glucoamylase solutions. The intercepts at the y -axis, $1/V_{\max}$ are 0.2 and 0.1 lmin/mmol for 500 and 1000 mg/l glucoamylase, respectively. The intercept at x -axis, $1/K_m$ for both 500 and 1000 mg/l glucoamylase is 0.01 l/mmol. The values for maximum reaction rates were proportional to enzyme concentrations. The corresponding values for the maximum reaction rates or limiting velocities, V_{\max} for 500 and 1000 mg/l glucoamylase were 5 and 10 mmol/lmin (0.9 and 1.8 g/lmin), respectively. The Michealis constant, K_m for both enzyme concentrations was 100 mmol/l (18 g/l).

The slope of the Lineweaver–Burk plot increased as the concentration of enzyme decreased indicating substrate inhibition in the kinetic model. For diluted molasses solutions noncompetitive substrate inhibition was noticed based on obtained kinetic data generated from enzyme concentrations of 500 and 1000 mg/l. Once the enzyme concentration was lower than 500 mg/l the substrate inhibition was greater and the kinetic behavior changed to mixed mode inhibitors, which caused the data to be scattered. The data presented in Fig. 5 show that mixed inhibitions of competitive and noncompetitive affected the rate model. The values for K_m were changed as the concentration of enzyme doubled and the V_{\max} did not follow the same pattern as V_{\max} increased with the enzyme concentration as shown in Fig. 4.

4. Conclusions

The enzyme-catalyzed reaction was initially first order and became zero order slowly as the substrate concentration was increased. The reaction rate and amount of glucose liberated increased proportionally with the

amount of glucoamylase. Higher temperatures accelerate the enzymatic reaction. However, there is an optimum temperature; beyond the optimum temperature, denaturation of enzyme may occur. The maximum reaction rate, V_{\max} and Michealis constant, K_m were defined based on experimental results for defined enzyme and molasses concentrations. The concentration of 1000 mg/l glucoamylase resulted in a high yield of enzymatic hydrolysis of molasses. The fermentable sugar content of molasses by enzymatic hydrolysis was increased from 194 to 611 g/l. The upgraded molasses enriched in sugar yielded highly fermented product such as ethanol. The obtained sugar enriched molasses represents a better quality of feed stock for fermentation industries.

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