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SACCHARIFICATION OF NATIVE CASSAVA STARCH AT HIGH DRY SOLIDS IN AN ENZYMATIC MEMBRANE REACTOR

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Abstract

This study is aimed to develop a novel process scheme for hydrolysis of native cassava starch at high dry solids using an enzymatic membrane reactor (EMR). Firstly, liquefied cassava starch having solids content up to 50% by weight was prepared by three stage liquefactions in a conventional equipment using a commercially available heat stable α-amylase (Termamyl 120L). The liquefied cassava starch was further saccharified in an EMR using glucoamylase (AMG E). By using the developed process scheme, a highly clear hydrolysate with dextrose equivalent (DE) approximately 97 could be produced, provided the increase of solution viscosity during the liquefaction was precisely controlled. The excessive space time could result in reduction in conversion degree of starch. Moreover, a residence time distribution study confirmed that the EMR could be modelled as a simple continuous stirred tank reactor (CSTR). Using Lineweaver-Burk analysis, the apparent Michaelis-Menten constant (K_m) and glucose production rate constant (k_2) were 552 (g/l) and 4.04 (min^{-1}), respectively. Application of simple CSTR model with those kinetic parameters was quietly appropriate to predict the reactor's performance at low space time.

Key words: *cassava starch, enzymatic membrane reactor, hydrolysate, starch hydrolysis*

Abstrak

Tujuan penelitian ini adalah mengembangkan skema proses baru untuk hidrolisis tapioka asli pada konsentrasi substrat tinggi dengan menggunakan reaktor membran enzimatik. Mula-mula, tapioka dilikuifaksi tiga tahap di dalam sebuah reaktor konvensional sampai konsentrasinya sekitar 50% berat dengan menggunakan α-amilase yang tersedia di pasaran (Termamyl 120L). Larutan hasil likuifaksi disakarifikasi lebih lanjut di dalam reaktor membran enzimatik dengan menggunakan glukoamilase (AMG E). Dengan menggunakan skema proses yang dikembangkan ini, hidrolisat sangat jernih dengan kandungan dekstrosa ekivalen (DE) sekitar 97 dapat dihasilkan, asalkan peningkatan viskositas larutan selama likuifaksi benar-benar dikendalikan. Waktu reaksi yang berlebihan dapat menurunkan konversi. Selain itu, studi distribusi waktu tinggal menunjukkan bahwa reaktor membran enzimatik dapat dimodelkan sebagai reaktor tangki berpengaduk kontinu. Dengan menggunakan analisis Lineweaver-Burk, diperoleh konstanta Michaelis-Menten (K_m) dan konstanta laju reaksi produksi glukosa (k_2) masing-masing adalah 552 (g/l) dan 4,04 (mnt^{-1}). Model reaktor tangki berpengaduk kontinu bersama dengan parameter kinetika tersebut dapat digunakan untuk memprediksi kinerja reaktor membran enzimatik ini.

Kata kunci: *tapiok , reaktor membran enziamtik, hidrolisat, hidrolisis pati*

INTRODUCTION

Starch is one of the plentiful carbohydrate resources that consist of two polymeric compounds with very high molecular weight, amylose and amylopectin. Starch from all plant sources is in the granule form which differ markedly in size and physical characteristics each other. Cassava roots (*Manihot esculenta*) are a major source of starch in tropical countries such as Thailand, Vietnam, Brazil, and Indonesia. Compared to potato or maize starch,

cassava starch has lower temperature of gelatinization and higher amylose solubility (Patil, 1991). These properties are very important with respect to the enzymatic starch hydrolysis.

In enzymatic starch hydrolysis, both amylose and amylopectin are cleaved into glucose, maltose or other products depend on the enzymes used during the process. Generally, enzymatic hydrolysis of starch is carried out by two basic types of enzymes: (a) endoenzyme, which splits randomly one molecule of a substrate into two smaller molecules, and (b) exoenzyme, which peels off a monomer or a dimmer at the non reducing end of the substrate molecule (Fujii and Kawamura, 1985). In order to maximize the endoenzyme activity, the starch slurry is gelatinized to convert the granule form into viscous gelatine.

Starch hydrolysates are used in the production of glucose, high-fructose sweeteners, brewing syrups, and as fermentation substrates (Cheryan, 1998). In practice, it is generally desirable to obtain starch hydrolysate having the dry solid content as high as possible. However, it is customary practice to hydrolyze starch at a dry solid content not over 40% in a conventional batch reactor due to an attendant sharp increase in the viscosity (referred to as the viscosity peak) (Walon, 1980). The conventional production scheme has numerous inherent drawbacks, such as (i) high evaporation load due to low starch concentration, (ii) the complexity of both start-up and shut-down of the process, (iii) no enzyme recovery, and (iv) relatively low reactor productivity because of the residence time of 40–72 hours.

Enzymatic membrane reactor (EMR) is one of rational alternates to conventional batch reactor. A number reports involving the EMR in recycle configuration (Closset *et al*, 1974; Tachauer *et al*, 1974; Madgavkar *et al*, 1977; Darnoko *et al*, 1989; Nakajima *et al*, 1990; Sims and Cheryan, 1992; Lopez-Ulibarri and Hall, 1997) and in dead end configuration with immobilized enzyme (Widiasa and Wenten, 2003) for starch hydrolysis have shown the feasibility of such system. The use of soluble enzymes for the starch hydrolysis would minimize the mass transfer problem. However, those studies were most carried out with relatively low starch concentrations.

The objective of this study is to develop a novel continuous process scheme for cassava starch hydrolysis at high dry solid. As a consequence of the high dry solid, it is required a high enzyme concentration to reduce space time. Therefore, recycle of the enzyme in reactor system as long as possible has an important role. The application of EMR for such purpose is not only able to retain the enzyme in the reaction system, but also able to produce a better starch hydrolysate quality. Thereby, the EMR is expected to provide at least four advantages, such as (i) lower energy consumption, (ii) more enzyme-efficient utilization, (iii) shorter space time, and (iv) lower down-stream processing cost. All of them would give a positive impact on the reduction of production cost.

STEADY STATE KINETIC MODEL OF THE EMR

Hydrolysis of starch to glucose may be written as the following equation:

$$(C_6H_{10}O_5)_n + nH_2O \rightarrow nC_6H_{12}O_{12} \qquad (1)$$

According to the above rate equation, a water molecule is added to each glucose molecule produced by the hydrolysis reaction. This leads to an increase in the total solids concentration by a factor of 1.11. Complete conversion of starch to glucose will not be achieved due to the reversion reaction. The rapid formation of maltose and iso-maltose from glucose take place in parallel with the production of glucose (Shiraishi *et al*, 1985). Consequently, there is always a small quantity of residual substrate in the hydrolysis product. The concentration of the residual substrate is dependent on the activity (concentration) of the enzyme, the residence time of the substrate in the reactor, and the substrate-enzyme ratio (Sims and Cheryan, 1992).

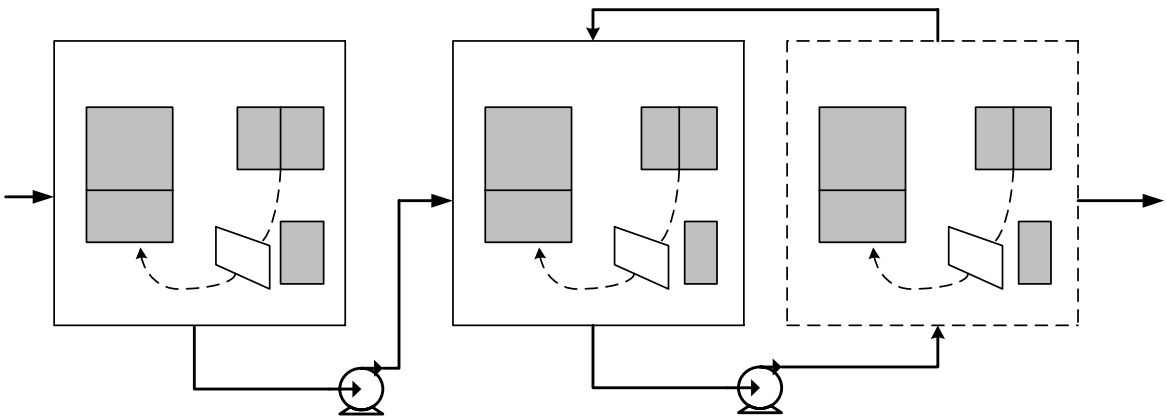

Figure 1. Illustration of sequence of reaction occurring during starch hydrolysis in an enzymatic membrane reactor (adapted from Deeslie and Cheryan, 1981; Sims and Cheryan, 1992). There is a little modification to take into account the presence of significant quantity of glucose in the liquefied cassava starch.

Figure 1 is a simplified illustration of the sequence of reactions that occur during the cassava starch conversion to glucose. In the liquefaction vessel, native cassava starch (S_0) is partially hydrolyzed with concomitant reduction in viscosity. The liquefied cassava starch entering the EMR consists of two fractions: the hydrolysable cassava starch (S*) and a small quantity of glucose (G*). Within the EMR, hydrolyzable liquefied cassava starch combines with the glucoamylase (E_G) to form an active enzyme-substrate complex. Then, this complex reacts to enzyme-product complex and breaks down to form glucose (P) and free enzyme. The enzyme is retained within the EMR system by the ultrafiltration membrane. The glucose (G) permeates the membrane and exits from the EMR system.

For simplicity the glucose fraction produced within the EMR was assumed entirely due to the glucoamylase activity. Hence, the glucose concentration in EMR effluent (G) is sum of the glucose formed during saccharification process (P) and the glucose arising from the prior liquefaction process (G*). The reactor effluent contains glucose and a small quantity of residual liquefied cassava starch (S). It should be emphasized that maltose and other higher saccharides are considered as the residual liquefied cassava starch.

A steady state kinetic model for the saccharification process in the EMR can be established by combining the mass balance in the reactor with enzyme kinetics (Sims and Cheryan, 1992; Lopez-Ulibarri and Hall, 1997). As the EMR could certainly be modelled as a ideal CSTR, the mass balance of the reactor can be expressed as:

$$r_G = \frac{S^* JX}{V} = \frac{S^* X}{\tau} \qquad (2)$$

where τ is the space time. Because the solutes composition in the reactor and the effluent is equal, it is possible to correlate the relationship between S_0, S*, G*, P, G, S, and the fractional conversion X. The fractional conversion may be defined as:

$$X = \frac{P}{1.11 S^*} \qquad (3)$$

From Figure 1, the following relationships can be established:

$$S^* = S_0 - \frac{G^*}{1.11} \qquad (4)$$

$$P = G - G^* \qquad (5)$$

$$S = S^* - \frac{P}{1.11} = S_0 - \frac{G}{1.11} \qquad (6)$$

The value of 1.11 is the correction factor as a result of the increase in total solids during the glucose production.

For this study, the following Michaelis-Menten equation was used for the reaction rate of glucose production:

$$r_G = \frac{V_{max} S}{K_m + S} = \frac{k_2 E_G S}{K_m + S} \qquad (7)$$

Combining Equation (2) and (7) gives

$$1.11X + \frac{1.11 K_m X}{\left(S_0 - \frac{G^*}{1.11}\right)(1 - X)} = k_2 \frac{E_G}{\left(S_0 - \frac{G^*}{1.11}\right)} \tau \qquad (8)$$

Equation (8) is a useful expression relating the fractional conversion in the EMR to the initial cassava starch concentration, the glucose concentration of the liquefied cassava starch, and space time.

EXPERIMENTAL

Experimental Set-up

Figure 2 shows the schematic diagram of the experimental set-up used to produce glucose syrup from native cassava starch. The system consisted of two stirred tank, a hollow fiber ultrafiltration module, a feed pump, and a recycle pump (Puricom UP-8000, maximum capacity 180 l/h, maximum pressure 80 psi, motor 48VDC/2A/50 hz). The system was also completed by pressure indicators (FTB, 0-60 psi), temperature indicators, a regulated water bath (HAAKE W13) and valve regulators. During the liquefaction process, the first stirred tank served as a liquefaction reactor to convert granular cassava starch to dextrin. The second stirred tank was coupled in semiclosed loop configuration to the ultrafiltration module. The system was referred to an EMR serving as a saccharification reactor to convert the dextrine to glucose. During the saccharification process, the first tank served as a container of the liquefied cassava starch (fresh substrate) to the EMR.

Membrane Characterization

As a prerequisite for successful operation when using EMR for starch hydrolysis is that the membrane must have high rejection of glucoamylase. The rejection, R, is given by:

$$R = \frac{C_f - C_p}{C_f} \qquad (9)$$

where C_f is the solute concentration in the feed and C_p is the solute concentration in the permeate.

Residence Time Distribution

Because of the information of mixing pattern and any deviations from ideal flow conditions of the EMR set-up is important to develop a performance equation, study on a residence time distribution was carried using glucose as a tracer at concentration 10 g/l.

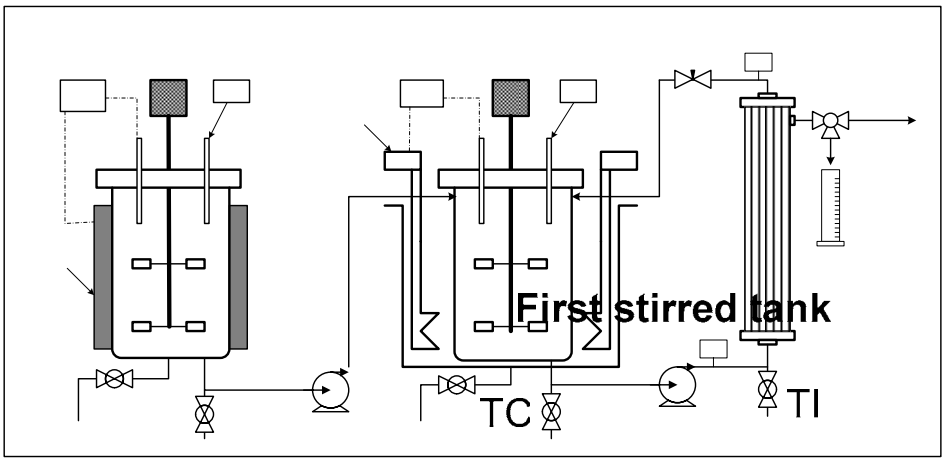

Figure 2. Schematic diagram of the experimental set-up used to convert native cassava starch to glucose syrup using an enzymatic membrane reactor (FP = feed pump; RP = recirculation pump; P = pressure indicator; TC = temperature controller; TI = temperature indicator)

The second vessel was initially filled with deionized water and the permeate flow rate was adjusted at 20 ml/min. After the system achieved steady condition, glucose solution was pumped from the first vessel. As the MWCO of the membrane was much higher than that of glucose molecule, the glucose molecule would be able to pass the membrane freely. Through-out the experiment, permeate sample were collected and their glucose concentrations were analyzed. For an ideal CSTR, residence time distribution can be calculated from the following equation (Levenspiel, 1999):

$$F(t) = \frac{C_t}{C_{in}} = 1 - e^{-t/\theta} \qquad (10)$$

Cassava Starch Liquefaction

Liquefied native cassava starch having solids content up to 50% by weight was prepared by conventional equipment using commercially available heat stable α-amylase (Termamyl 120L) from Novo Nordisk. Granular cassava starch supplied by PT. Raya Sugarindo Inti was added into an aqueous liquefied cassava starch produced from first step and second step at a temperature below the normal initial gelatinization temperature. Liquefaction of the mixture in first, second, and third steps were conducted at a normal condition, i.e., at temperature of 105±3°C for 5 minutes and then held at temperature of 90±3°C for 1–2 hours. Dry solid concentration of first, second, and third steps were 30%, 44%, and 50% w/w, respectively.

Saccharification of the Liquefied Starch

Further saccharification of the liquefied cassava starch was carried out in the EMR using glucoamylase (AMG E, from Novo Nordisk). Prior to be pumped to the EMR system, the liquefied cassava starch was prefiltered by a microfiltration membrane to remove its unhydrolyzable suspended solids. Furthermore, pH and temperature of the prefiltered liquefied cassava starch were adjusted to 4.6±0.1 and 57±3°C, respectively. The reaction temperature was continuously monitored and controlled within 57±3°C. The liquefied cassava starch was constantly stirred to assure its uniformity. Permeate was collected in a measuring cylinder for flow control and product composition analysis. Control of pH during the saccharification was not required.

Analysis

Glucose, maltose, and higher saccharides concentrations describing degree of polimerization were measured by high-performance liquid chromatography (Knauer HPLC) using metacarb column 67 C and refractive index detection. Viscosity of the cassava starch hydrolysate was measured with a Brockfield viscometer (LVT, serial 109549).

The industry of glucose syrup usually uses the expression dextrose equivalent (DE) to describe its products, i.e. the percentage hydrolysis of the glycosidic linkages present. Thus, pure glucose has a DE 100, pure maltose has a DE about 50, and starch has a DE efeectively zero. In practice, this may be determined analytically by the following expression:

$$DE = 100x\frac{S_{Re}}{C_T} \qquad (11)$$

where S_{Re} is reducing sugar expressed as glucose and C_T is total carbohydrate. The quantity of reducing sugar was measured by the Somogyi-Nelson method (Somogyi, 1952) assuming that one molecule has one reducing group. Hence, these values were the apparent number of molecule glucose.

Determination of Kinetic Parameters

Equation (7) may be inverted into the Lineweaver-Burk equation to determine kinetic parameters K_m and k_2:

$$\frac{1}{R_{G,0}} = \frac{K_m}{V_{max}}\frac{1}{S_0} + \frac{1}{V_{max}} \qquad (12)$$

The rate constant of the glucose production (k_2) is determined from the following equation:

$$k_2 = \frac{V_{max}}{E_G} \quad (13)$$

where E_G is the glucoamylase concentration in the reactor.

RESULTS AND DISCUSSION

Membrane Characteristics

Figure 3 shows the rejection data of glucoamylase through the membrane for total protein concentration 6 g/l at constant pressure of 0.4 bar and temperature of 55 ^oC. It can be seen that the membrane have rejection higher than 94%. Hence, it can be used as enzymatic membrane reactor for starch hydrolysis.

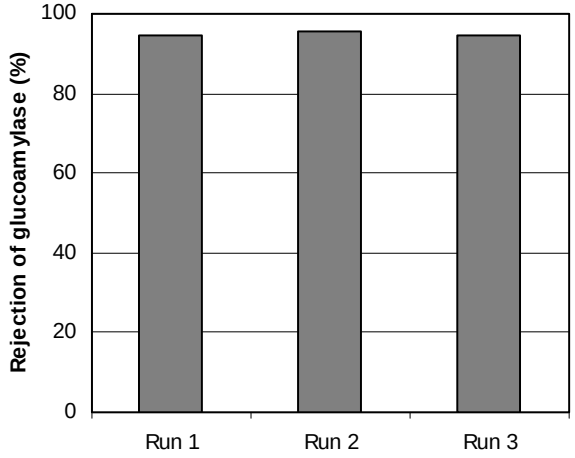

Figure 3. Rejection of glucoamylase through the membrane used in this study at temperature of 55 ^oC

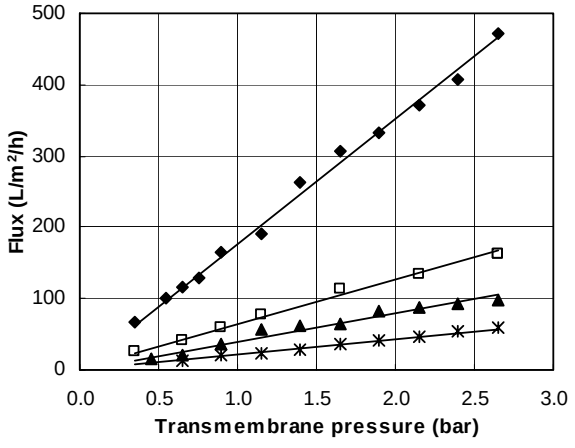

Figure 4. Dependence of permeate fluxes on glucose concentration at temperature of 55 ^oC (♦ deionized water; □ glucose concentration of 30 g/l; • glucose concentration of 40 g/l; Δ glucose concentration of 50 g/l)

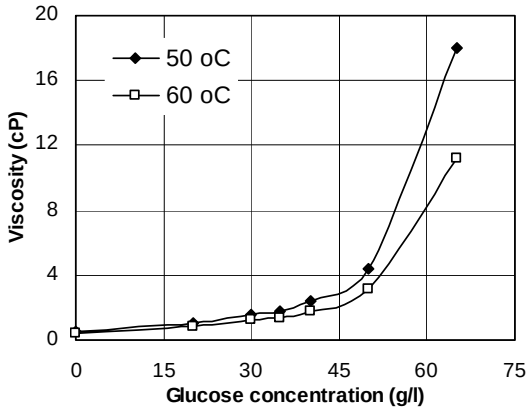

Figure 5. Dependence of glucose solution viscosity on its concentration and temperature.

Typical permeate flux-TMP relationships for glucose solutions (ca. 300 - 500 g/l) at constant temperature of 55^oC are shown in Figure 4. The flux data were collected after the filtration period of 4 hr. The data indicate that the increase of glucose concentration resulted in a significant decrease in permeate flux. This is presumably due to the increase in solution viscosity. As depicted in Figure 5, viscosity of the solutions increases with the higher of glucose concentration.

Residence Time Distribution

Results of the mean residence time distribution study for the EMR are shown in Figure 6. The solid line represents the ideal case for an ideal CSTR. As can be seen, the data points for glucose closely approximate that of the ideal CSTR, indicating that the EMR could be modelled as a simple CSTR. Similar results have been reported by Deeslie and Cheryan (Deeslie and Cheryan, 1981) using tracer tryptophenyl-*D,L*-tryptophan at a concentration 0.05 mg/ml and Sims and Cheryan (1992) using tracer consisted of 1% glucose, 1% maltose, and 0.5% maltotriose (w/v) in distilled water.

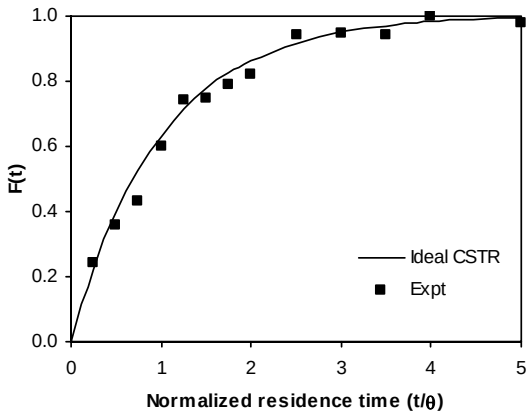

Figure 6. Residence time distribution in the EMR. Solid line was ideal CSTR determined by Equation (10) with θ = 1 hr, whereas points are experimentally measured data.

Saccharides Distribution of the Liquefied Cassava Starch

There are three stages in the conversion of native cassava starch to glucose: (i) gelatinization, involving the dissolution of the cassava starch granules to form a viscous suspension; (ii) liquefaction, involving the partial hydrolysis of the cassava starch with concomitant loss in viscosity; and (iii) saccharification, involving the production of glucose by further hydrolysis of liquefied cassava starch. Saccharides distribution produced from each liquefaction step is shown in Figure 7. As can be seen, saccharides distribution of the liquefied cassava starch is relatively different from step to step, in which the amount of DP1 – DP5 gradually decreased. However, the final liquefied cassava starch has dextrose equivalent (DE) value in the range of 8 – 20. It should be emphasized that the maximum DE is not over 40 because the prolonged treatment may lead to the formation of maltulose (4-α-*D*-glucopyranosyl-*D*-fructose), which is resistant to hydrolysis by glucoamylase and α-amylase.

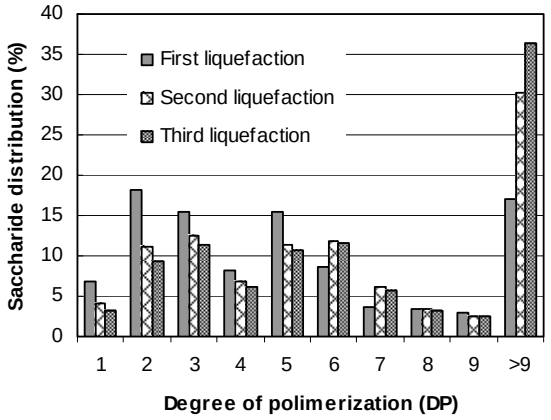

Figure 7. Saccharides distribution of the liquefied cassava starch produced from three steps liquefaction by a conventional equipment.

Viscosity of the Liquefied Cassava Starch

Viscosity is one of the important physical properties in industrial operation of starch hydrolysis. In addition to relating to the fluid flow, the liquefied cassava starch viscosity presumably affects the activity of glucoamylase. As reported by Özbek and Yüceer (2001) that increasing the viscosity of the process fluid led to reduction in enzyme activity. For this reason, the viscosity of the liquefied cassava starch produced was assessed. Figure 8 shows the increase of the viscosity of the produced liquefied cassava starch as a result of the higher dry solid content. The increase of dry solid from 30% to 50 w/w resulted in the increase of solution viscosity from 4.2 cP to 31.8 cP.

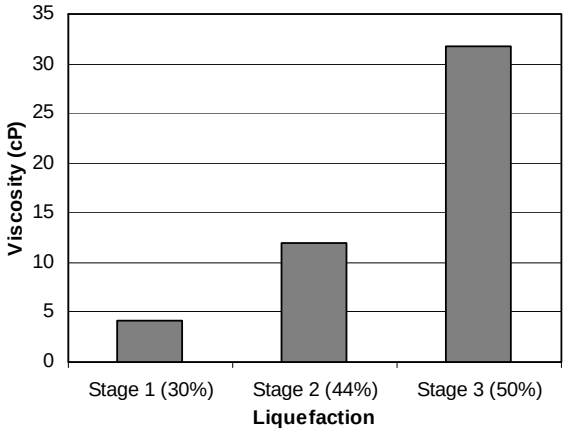

Figure 8. The increase of the liquefied cassava starch viscosity due to the higher dry solid concentration during the liquefaction process.

Characteristic of the Produced Hydrolysate

Figure 9 shows DE value of glucose syrup produced by continuous saccharification of liquefied cassava starch at high dry solid in an EMR. Generally, the EMR produced highly clear hydrolysates (permeate) having DE value approximately 97. This indicates that EMR allows not only to optimize enzyme utilization, but also to convert almost all of native cassava starch to clear glucose solution. Detailed sample analysis results of these experiments are presented in Table 1. However, the excessive space time could decrease the starch conversion due to the reverse reaction (Figure 10). This reverse reaction is accelerated at high concentrations of glucose (Lee *et al*, 1980). Kinetics of the reverse reaction has been well documented (Shiraishi *et al*, 1985; Nikolov *et al*, 1989).

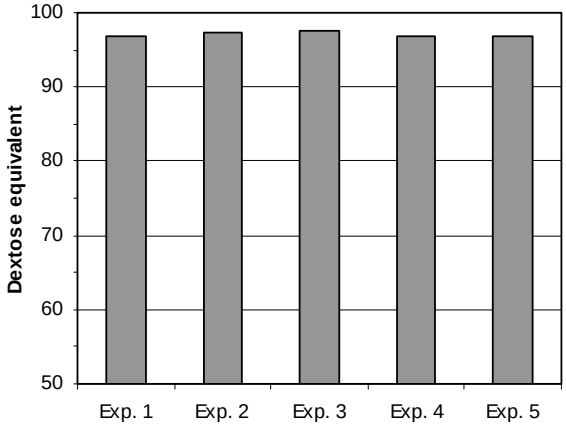

Figure 9. DE value of the EMR effluent for continuous saccharification of liquefied cassava starch having dry solid concentration 50% w/w at glucoamylase concentration 6 g/l, pH of 4.6±0.1, and temperature of 57±3^{o}C.

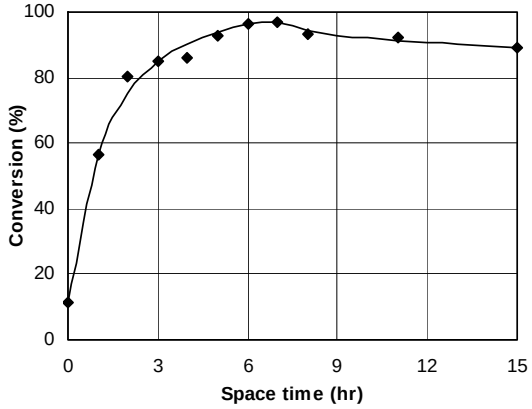

Figure 10. The effect of space time on conversion for continuous saccharification of liquefied cassava starch having dry solid concentration 50% w/w at glucoamylase concentration of 6 g/l, pH of 4.6±0.1, and temperature of 57±3°C.

Table 1. Saccharides composition of of the EMR effluent for continuous saccharification of liquefied cassava starch having dry solid concentration 50% w/w at glucoamylase concentration 6 g/l, pH 4.6±0.1, and temperature of 57±3°C.

Saccharides	Experiments no.				
	1	2	3	4	5
DP-1	94.96	96.63	97.13	95.79	95.48
DP-2	2.29	1.05	0.37	0.56	2.36
DP-3	1.75	ND	1.04	1.14	0.69
DP-4	0.35	ND	1.46	0.26	1.35
DP-5	0.40	ND	ND	0.25	0.12
DP-6	0.25	ND	ND	ND	ND
DP-7	ND	0.58	ND	ND	ND
DP-8	ND	ND	ND	ND	ND
DP-9	ND	ND	ND	ND	ND
> DP-9	ND	1.74	ND	ND	ND

Kinetic Parameters

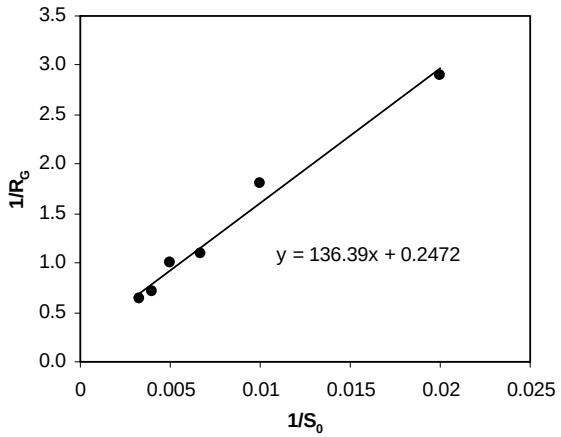

Figure 11. Plot of reciprocal initial rate of glucose production ($1/R_{G,0}$) verses reciprocal substrate concentration ($1/S_0$) at glucoamylase concentration (E_G) 1 g/l

A plot of $1/R_{G,0}$ against $1/S_0$ for the process is shown in Figure 11. The solid straight represents the prediction of Lineweaver-Burk equation. From the Lineweaver-Burk plot and Equation (13), the values of K_m and k_2 are 552 (g/l) and 4.04 (min^{-1}), respectively.

Evaluation of the Theoretical Model

As the triple stage liquefactions would result in glucose content of the liquefied cassava starch above 5%, it was taken into account in the established model. For this study, the average glucose content of the liquefied cassava starch entering the EMR was 11.30 ± 0.25%. The kinetic parameters previously determined by Lineweaver-Burk plot were used in Equation (12) to predict the EMR's performance. Figure (12) shows the degree of conversion (X_T) as a function of space time, τ for 50% w/w dry cassava starch at glucoamylase concentration of 6 g/l, pH of 4.6±0.1, and temperature of 57±3°C. The degree of conversion is defined as:

$$X_T = \frac{G}{1.11S_0} \text{ x } 100 \qquad (14)$$

The solid line represents the theoretical model predictions, where as points are experimental data obtained after the reactor had achieved steady state. As can be seen, application of simple CSTR model with those kinetic parameters is quietly appropriate to predict the reactor's performance at low space time.

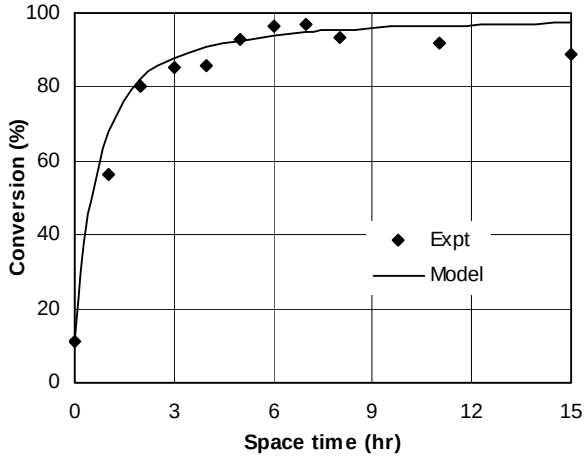

Figure 12. Comparison of theoretical and experimental results for hydrolysis of liquefied cassava starch at high dry solid in an EMR. Points are experimental data. Solid line represents the prediction curve calculated by Equation (11).

CONCLUSIONS

Hydrolysis of native cassava starch to glucose at high dry solid concentration using an enzymatic membrane reactor was studied both experimentally and theoretically. By using the developed process scheme, a highly clear hydrolysate with dextrose equivalent (DE) approximately 97 could be produced, provided the increase of solution viscosity during the liquefaction is precisely controlled. The excessive

space time could result in reduction in conversion degree of starch. Moreover, a residence time distribution study confirms that the EMR can be modelled as a simple continuous stirred tank reactor (CSTR). Using Lineweaver-Burk analysis, the apparent Michaelis-Menten constant (K_m) and glucose production rate constant (k_2) are 552 (g/l) and 4.04 (min^{-1}), respectively. Application of simple CSTR model with those kinetic parameters is quietly appropriate to predict the reactor's performance at low space time.

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