ELSEVIER

PII: S0144-8617(97)00048-9

Carbohydrate Polymers 33 (1997) 101–108
© 1997 Elsevier Science Ltd
All rights reserved. Printed in Great Britain
0144-8617/97/$17.00 + .00

Effect of mixing on enzymatic liquefaction of sago starch

Arbakariya B. Ariff[a], **B. A. Asbi**[b], **M. N. Azudin**[b] **and J. F. Kennedy**[c]*

[a]*Department of Biotechnology, Faculty of Food Science and Biotechnology, Universiti Pertanian Malaysia, 43400 UPM Serdang, Selangor DE, Malaysia*
[b]*Department of Food Technology, Faculty of Food Science and Biotechnology, Universiti Pertanian Malaysia, 43400 UPM Serdang, Selangor DE, Malaysia*
[c]*Birmingham Carbohydrate and Protein Technology Group, School of Chemistry, The University of Birmingham, Birmingham B15 2TT, UK*

(Received 16 September 1996; accepted 6 December 1996)

The effect of mixing as a function of agitation speed and impeller diameter on the rate and degree of enzymatic liquefaction of sago starch was carried out using a stirred tank reactor with a single Rushton turbine impeller. The performance of the reactor as a mixing device was first examined using different concentrations of carboxymethylcellulose, which exhibited pseudoplastic behaviour similar to that of the solution during the sago starch liquefaction process. A correlation between mixing time (t_m) and Reynolds number (Re) in the form of $t_m = b\mathrm{Re}^c$ is presented; the constants for the correlation depended on viscosity of the fluid. For the two ratios of impeller diameter (D_i) to tank diameter (D_t) used, 0.407 and 0.542, agitation speed gave significant influence on both overall rate and degree of liquefaction of sago starch. Mixing time (t_m) was independent of impeller diameter used, and correlated well with the overall rate of liquefaction (P) (calculated as the reducing sugar produced divided by time of liquefaction) and expressed as $P = 1.95t_m^{-0.362}$. © 1997 Elsevier Science Ltd

INTRODUCTION

In Malaysia, the use of sago starch has increased and it is presently being used for the production of glucose. Glucose obtained from sago starch is used as a substrate for the fermentation industries (Rashid, 1990) and also for the production of high fructose syrup (Yan, 1991). However, it has not been fully utilized by the food, textile and paper industries because of its poor and varying quality.

Starch can be converted to glucose through two enzymatic steps, which are commonly known as the liquefaction and saccharification steps. The liquefaction step involves very viscous raw and gelatinized starch solutions. Continuous enzymatic liquefaction processes have been widely used on an industrial scale (Svanholm, 1985). In this technique, raw starch slurry containing thermophilic α-amylase is pumped through a holding cell in which the temperature is controlled at 105°C using live steam injection. Since sago starch exhibits higher viscosity (Arbakariya *et al.*, 1990) and gelatinization temperature (Sim *et al.*, 1991) as compared to other starches, continuous liquefaction is not suitable for sago starch due to the reduction in both the rate and degree of liquefaction. A higher fouling rate due to poor quality is another factor that limits the use of continuous processes for liquefaction of sago starch (Hassan *et al.*, 1996). Although much work has been done on the conversion of sago starch, the data on the stability of sago starch as a substrate for oligosaccharides and glucose syrup production are lacking.

One of the problems in batch enzymatic liquefaction of starch is to design and operate a mixer that can maximize the rate of conversion. Generally, a complete mixed batch stirred tank reactor is used for a liquid-phase homogeneous catalytic reaction. A well mixed reactor system assists in the initial dissolution of the enzymes used (i.e. increase mass transfer rate)

---

*To whom correspondence should be addressed.

and moderates any concentration, temperature and pH variations within the reactor. In the enzymatic hydrolysis of various polysaccharides using glucoamylase, shaking the flask at 150 rpm significantly improves the hydrolysis rate as compared to a static flask (Miranda and Murado, 1991). Since the liquid-phase during the liquefaction of sago starch is very viscous and its rheological properties change with reaction time (Arbakariya *et al.*, 1990), the design of a well mixed reactor for this process becomes a problem.

In this work, the effect of mixing as a function of the impeller size and speed on the rate and degree of enzymatic hydrolysis of sago starch in batch process is presented.

MATERIALS AND METHODS

Starch and enzyme

Sago starch purchased from Nee Seng Lee Co., Sarawak, was used in all experiments. The chemical composition of this starch was estimated by using standard methods (Official Methods of Analysis, 1980) and were as follows: moisture (10.3%), ash (0.23%), fibre (0.32%), fat (0.3%) and protein (0.57%). The enzyme α-amylase (Termamyl 120L) was obtained courtesy of NOVO, Malaysia. The α-amylase is thermostable, produced by *Bacillus subtilis*. This enzyme has an optimum activity at pH 6.5 and temperature of 105°C.

Equipment

The stirred tank reactor with a working volume of 1.5 litre was used throughout the study. The reactor consisted of a double jacketed borosilicate glass vessel and a stainless steel top plate with several opening ports for pH and temperature electrodes. The internal diameter and the height of the vessel were 118 mm and 137 mm, respectively. For agitation, two different sizes of four-bladed Ruston turbine impeller (48 mm and 64 mm diameter) were used.

The agitation was controlled at the required speed by the motor and speed controller. The agitation speed was measured by using a transmitted light barrier in which the output sequence reached the speed controller via a frequency/voltage converter. The pH of the reaction mixture was measured by using an Ingold pH electrode and was controlled by the pH control module (LH Fermentation, UK) either by adding 1 N NaOH or 1 N HCl. Two platinum electrodes placed at two different locations were used to measure the temperature profile during the process and the value was recorded using a recorder (Kipp and Zonen, The Netherlands).

Mixing time

In order to evaluate the performance of the reactor as a mixing device, the mixing time was determined at different agitation speeds and using different impeller sizes. The method proposed by Blakebrough and Sambhamurthy (1966) was used for the determination of mixing time. In this study the mixing time of the reactor at different operating variables was estimated using a simulation medium of carboxymethylcellulose (CMC) with different concentrations. The reactor vessel was filled with 1.5 litres CMC solution and 7.5 ml of 2% phenolthalein was added as an indicator. Once the agitation was started at the required speed, 0.1 N NaOH was added drop by drop until the solution became just pink, followed by 0.2 N HCl until the pink colour just disappeared. After this step, 3 ml of the alkali was added and the content was agitated for a sufficient time to establish a dynamic equilibrium. A chemically equivalent quantity of acid was instantaneously introduced through a stainless steel tubing (1 mm internal diameter) placed just below the impeller. The time between the injection of the acid and the moment at which the last wisp of pink colour disappeared from the surface was taken as the mixing time. The mixing time taken for defined operating variables was an average of five determinations.

Rheology

The Brookfield viscometer (model LVT-5X) was used to determine the viscosity and shear rate of the simulation media and reaction mixture during the liquefaction process. For measurement of the rheological properties of the solution during the liquefaction process, the concentric cylinder containing the sample was placed in a water bath at a temperature similar to the temperature of the solution in the reactor at the time the sample was taken. From the apparent viscosities measured at different shear rates, the rheological property of the liquid was determined using the power law equation, which is given as

$$\tau = K\lambda^n \tag{1}$$

where

$$\mu_{\mathrm{app}} = K\lambda^{(n-1)}$$

$$\log\mu_{\mathrm{app}} = \log K + (n-1)\log\lambda$$

where K is the fluid consistency coefficient (N s m^{-2}), n is the flow behaviour index (dimensionless), λ is the shear rate (s^{-1}), τ is the shear stress (N m^{-2}) and μ_{app} is the apparent viscosity (N s m^{-2}).

Liquefaction

The reactor was filled with 1.5 litres of 30% (w/v) of starch slurry and the pH was adjusted to 6.5. The agitation was set at the required speed and the reaction mixture was then heated by pumping boiling water (100°C) through the jacketed vessel. When the temperature of the starch slurry reached 30°C, 0.0245% (w/v) of Termamyl 120L was added and the time was taken as 0 h of the liquefaction process. The pH of the reaction mixture was controlled at 6.5±0.1 throughout the liquefaction process either by adding 1 N NaOH or 1 N HCl. The temperature profile during the process was monitored and recorded. The samples were taken at time intervals and analysed for viscosity and reducing sugar concentration. The portion of the sample used for reducing sugar determination was immediately adjusted to pH 3 with 0.2 N HCl in order to inactivate the α-amylase and then neutralized with 0.2 N NaOH. The concentration of reducing sugar was determined using the dinitrosalicylic (DNS) method (Miller, 1959) with glucose as a standard.

RESULTS AND DISCUSSION

Performance of reactor as a mixing device

In this study, different concentrations of CMC ranging from 0.8 to 2% were employed to produce some of the rheological properties observed in solution of the liquefaction process of sago starch, enabling mixing efficiency to be studied in conjunction with impeller design, size and agitation speed. The parameters *K* and *n* calculated from the power law equation were used to characterize the rheological characteristics of different concentrations of CMC and this is shown in Table 1. The parameter *K*, which is a direct measure of viscosity, increased with increasing concentration of CMC. For all concentrations of CMC, the values of *n* were about the same and less than 1, indicating that the solutions have pseudoplastic characteristics. According to the previous report (Arbakariya *et al.*, 1990; Sim *et al.*, 1991), the solution during the enzymatic liquefaction of starch is also pseudoplastic in behaviour.

Mixing is obtained by creating turbulence through the agitation of mechanical stirring. The effectiveness of mixing can be expressed in terms of a mixing time (t_m), which is the time taken to disperse a liquid droplet completely into liquid of the same physical properties. The effect of impeller diameter and speed on mixing time for different concentrations of CMC is shown in Fig. 1. As can be seen, the mixing time increased with increasing concentration of CMC or viscosity of the solution. At different agitation speeds,

Table 1. Rheological properties of carboxymethylcellulose solutions

Carboxymethylcellulose concentration (% w/v)	K (N s m^{-2}×10^{-3})	n
0.8	2.50	0.599
1.2	2.72	0.570
2.0	3.16	0.46

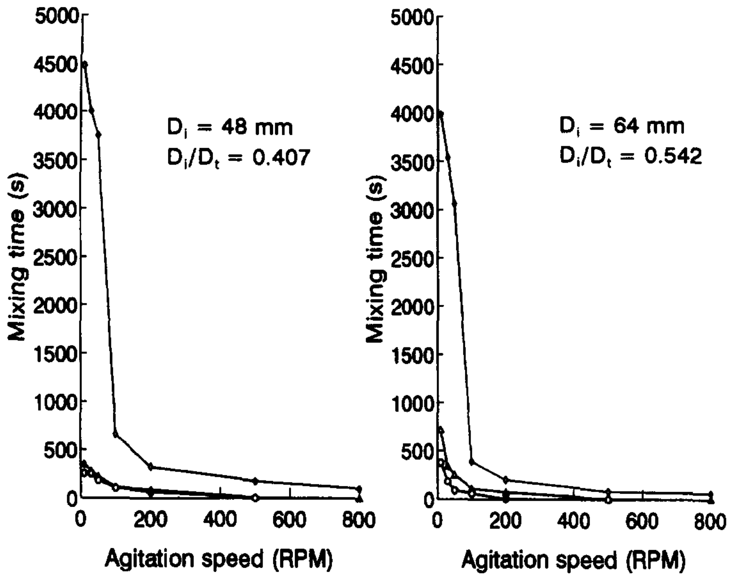

Fig. 1. Mixing time at different agitation speeds for various concentrations of carboxymethylcellulose (CMC) solution. Symbols represent: (○) 0.8% (w/v); (△) 1.2% (w/v) CMC; (◇) 2% (w/v) CMC.

mixing times were not significantly different for CMC concentration ranging from 0.8% to 1.2%. However, a significantly large difference in mixing time at different agitation speeds was observed when 2% CMC was used. This is because the higher CMC concentration produced high K values. For both sizes of impeller used, mixing time decreased with increasing agitation speed but the difference was very small for an agitation speed above 400 rpm.

At the same agitation speed, mixing time was also reduced when larger impeller diameters was used. For example, at the agitation speed of 200 rpm the mixing times for impellers with diameters of 48 mm and 64 mm were 200 s and 672 s, respectively. This means that the use of an impeller with a diameter of 64 mm reduced the mixing time by about 3.36 times as compared to an impeller diameter of 48 mm. The ratio of impeller diameter to tank diameter (D_i/D_t) used in this study was lower than the value of 0.69 as suggested by Steel and Maxon (1962), which was found to be the most suitable for complete mixing. It is also interesting to note that for low viscosity solutions (low concentration of CMC), the mixing time for large and small impellers was not significantly different, especially at high impeller speeds. It can be concluded that t_m is a function of agitation speed, D_i/D_t and the viscosity of the fluid, and this is in agreement with a previous report (Bilikina *et al.*, 1973).

Mixing time is normally correlated with the degree of turbulence in the liquid which is indicated by the Reynolds number (Norwood and Metzner, 1960). In a mechanically agitated stirred tank the Reynolds number Re is defined as

$$\mathrm{Re} = \rho N D_i 2/K \tag{2}$$

where ρ is the density of the liquid (kg/m^3), N is the agitation speed (s^{-1}) and D_i is the diameter of the impeller (m). Figure 2 shows the data for mixing time and Re for two different sizes of impeller and three different concentrations of CMC plotted on a logarithmic scale to examine the fit of an equation of the form $t_m = b(\mathrm{Re})^c$. It was found that the mixing time depended on the concentration of CMC used or the viscosity of the liquid and did not depend on the diameter of the impeller. The coefficients b and c for different concentrations of CMC were determined from regression analysis and the results are as follows:

For 0.8% – 1.2% CMC ($K = 2.5 - 2.7 \times 10^{-3}\,\mathrm{N\,s\,m^{-2}}$),

$$t_m = 4.95\mathrm{Re}^{-0.98}. \tag{3}$$

For 2.0% CMC ($K = 3.16 \times 10^{-3}\,\mathrm{N\,s\,m^{-2}}$),

$$t_m = 5.69\mathrm{Re}^{-0.98}. \tag{4}$$

The correlation between t_m and Re at different fluid viscosities is very useful for the estimation of agitation speed (N) and impeller diameter (D_i) to be used in a well mixed enzyme reactor. A short mixing time is required to minimize differences throughout the entire volume of the liquid for reduction of diffusional limitation and also for development of an efficient pH and temperature control (Blanch and Bhavaraju, 1976). Norwood and Metzner (1960) defined a mixing factor (Φ_m), which for geometrically similar tanks was related to the mixing time. The correlation is expressed as $\Phi_m = [(\text{constant} \times t_m N^{2/3})/D_i^{1/6}]$. The mixing factor is varied with Re in a manner very similar to that of the power number. This type of correlation is useful for scale-up purposes.

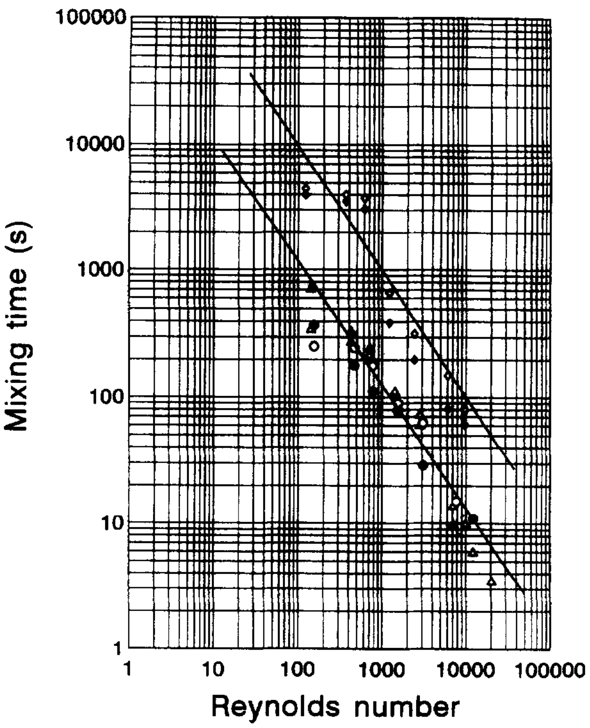

Fig. 2. Relationship between mixing time and Reynolds number for different concentrations of carboxymethylcellulose (CMC). Symbols represent: (○) 0.8% (w/v); (△) 1.2% (w/v) CMC; (◇) 2% (w/v) CMC. Open and close symbols are for 48 mm and 64 mm diameter impellers, respectively.

Effect of impeller speed and diameter on the degree and rate of liquefaction

The profiles of temperature during the liquefaction process using two different sizes of impeller and agitated at different speeds are shown in Fig. 3. In all cases, the temperature of the solution increased with time and reached maximum values after about 2 h. However, the rate of increase in temperature depended on the speed of agitation and the impeller diameter. In other words, the rate of increase in temperature depended on mixing. For both sizes of impeller used, the rate of increase in temperature increased with

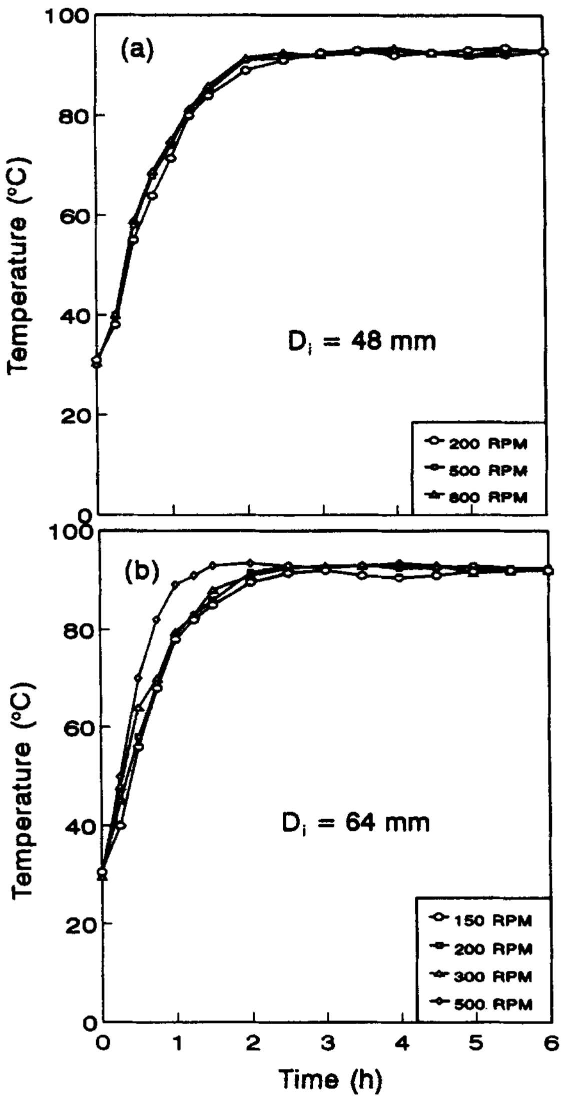

Fig. 3. Temperature profiles during the liquefaction process at different agitation speeds. (a) impeller diameter of 48 mm; (b) impeller diameter of 64 mm.

agitation speed. When the impeller of 64 mm diameter was used, the rate of increase in temperature for agitation at 500 rpm was significantly higher than for agitations at 300 rpm and below. It is important to note that when the temperature became steady (after 2 h) the values were not significantly different for the different degrees of agitation investigated. In all cases, after 2 h the temperature was maintained at values of around 90–93°C, indicating that the liquefaction rate after 2 h of the process was not influenced by the temperature. It can be suggested that the variation in the rate of liquefaction was solely dependent on the rate of mixing.

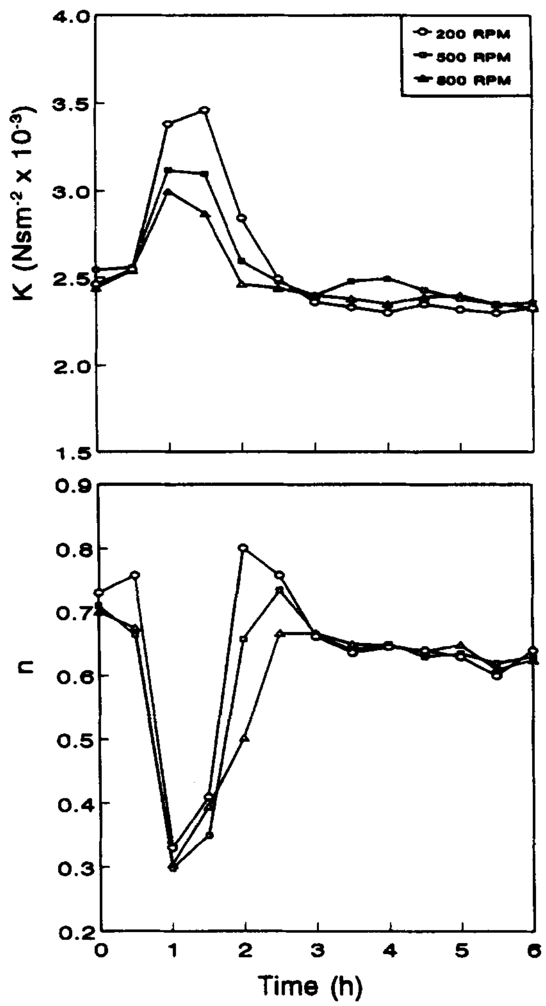

Fig. 4. The changes in rheological properties during the liquefaction process using an impeller with 48 mm diameter at different agitation speeds.

Figure 4 shows the changes in the rheological properties of the solution during the liquefaction proceses of sago starch using an impeller with 48 mm diameter and agitation at different speeds. In all cases the values of *K* increased drastically with time and reached a maximum value after about 1 h, at which time the temperature of the solution attained 70°C. At temperatures between 71–74°C the sago starch slurry gelatinized (Sim *et al.*, 1991; Rasip, 1989) and became very viscous as shown by the high value of *K*. The value of *K* during the gelatinization phase varied with the degree of agitation. High agitation speed produced a solution that exhibited higher *K* values and this was due to the greater swelling capacity and solubility (Sim *et al.*, 1991). After gelatinization, starch molecules dispersed into solution and α-amylase started to attack the swollen starch and hydrolysed them to reducing sugar (Svanholm, 1985). This resulted in decreasing *K* values or viscosity and increased the stability against retrogradation. On the other hand the values of *n* decreased with liquefaction time and reached minimum values at 1 to 1.5 h. This was the point where the starch gelatinized. During the liquefaction process, the value of *n* was less than 1, indicating that the solution had non-Newtonian pseudoplastic behaviour. The liquid with this type of behaviour showed a decrease in μ_{app} with increasing shear rate or agitation speed.

For all agitation speeds investigated, after 2 h of the liquefaction process (at which point the temperature was maintained at maximum value) the values of K and n remained almost constant at $2.4 \, N s sm^{-2}$ and 0.65, respectively. It is important to note that the values of K and n for starch slurry before gelatinization were only slightly higher than the values for solution after gelatinization. The solution was only very viscous for a very short time (about 1 h) during the gelatinization phase.

The effect of impeller speed and diameter on the degree and rate of liquefaction of sago starch is shown in Fig. 5. In all cases, the rate of liquefaction as indicated by the production of reducing sugar increased with time. Enzymatic liquefaction of starch is a complex of reactions. The α-amylase is an endo-splitting enzyme which attacks the 1→4 glycosidic linkages of starch molecules and hydrolyses them randomly to soluble limit dextrins and oligosaccharides (Fullbrook, 1984). During the initial stages of the liquefaction process (0–1.5 h), an increase in reducing sugar concentration was observed even though the starch was not yet gelatinized. This observation suggests that the α-amylase used in this study was capable of hydrolysing raw or ungelatinized starch. However, the rate of hydrolysis for ungelatinized starch was slower compared to the rate for gelatinized starch. The rate of starch hydrolysis decreased with time and levelled off towards the end of the process. In all cases the degree of liquefaction became maximum after 5 to 6 h.

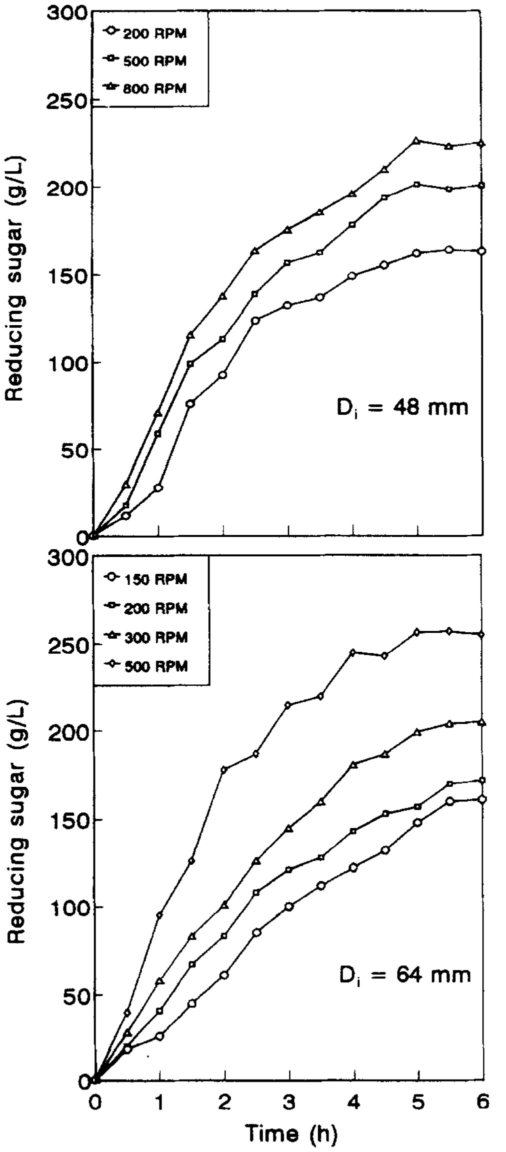

Fig. 5. Effect of agitation speed on the rate and degree of liquefaction for a process carried out using two different impeller speeds. (a) 48 mm diameter; (b) 64 mm diameter.

The maximum concentration of reducing sugar obtained after 5 h for liquefaction using different impeller diameters and speed is shown in Table 2, which also includes the overall rate of liquefaction. For both sizes of impeller used, it was very obvious that an increase in agitation speed resulted in a significant increase in the rate and degree of liquefaction. This is in agreement with the previous report on the production of reducing sugar from hydrolysis of starch by glucoamylase (Miranda and Murado, 1991). An agitated flask produced reducing sugar at about twice the rate that a static flask did. This suggests that the enzymatic hydrolysis of starch by α-amylase was controlled by the mass transfer rate, which is a function of mixing efficiency. Since variations in pH and temperature of the reaction at different experiments were not significant, it can be suggested that complete mixing reduced the diffusional limitation and hence increased the action of the α-amylase on the linkages of the starch molecules.

Relationship between mixing time and degree of liquefaction

A high value of K during liquefaction process occured only for a very short time and remained almost constant at an average value of $2.4 \times 10^{-3} N s m^{-2}$ throughout the process (refer to Fig. 3). The density of the solution (ρ) was also almost constant at an average value of $995 \, kg/m^3$. Since the values of K and ρ were almost constant throughout the liquefaction process, the Reynolds number and hence the mixing time can be estimated according to eqns (2) and (3), respectively. Log–log plots of t_m against the concentration of reducing sugar produced (R_5) after 5 h of liquefaction process and against overall rate of liquefaction (P) are shown in Fig. 6. As can be seen, the relationships are linear and from the regression analysis, which showed that the coefficient of determination is more than 90%, the correlations are as follows:

Table 2. Comparison of rate and degree of liquefaction of sago starch after 5 h of processing using two different impeller diameters and agitation at different speeds

Agitation speed (rpm)	Impeller diameter = 48 mm R_5 (g/litre)	Impeller diameter = 48 mm P (g/litre per h)	Impeller diameter = 64 mm R_5 (g/litre)	Impeller diameter = 64 mm P (g/litre per h)
150	—	—	160.0	29.1
200	164.2	29.8	170.1	30.9
300	198.6	36.1	204.5	37.1
500	—	—	257.1	46.7
800	223.0	45.5	—	—

R_5 is the reducing sugar concentration after 5 h liquefaction.
P is the overall rate of liquefaction (reducing sugar produced per time of liquefaction = $R_5/5$).

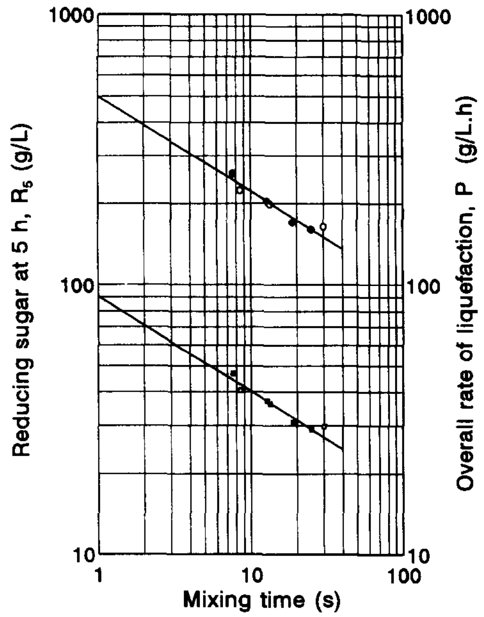

Fig. 6. Relationship bestween mixing time and degree of liquefaction, and mixing time and overall rate of liquefaction. Symbols represent: (o) reducing sugar produced; (□) overall rate of liquefaction. Open and closed symbols are for 64 mm and 48 mm diameter impellers respectively.

$$R_5 = 2.69t_m^{-0.362} \quad (6)$$

$$P = 1.95t_m^{-0.362}. \quad (6)$$

A simple relationship between t_m and the rate and degree of liquefaction as obtained in this study can be used for reactor design purposes, scale-up and also for optimization of the reactor operation for the sago starch liquefaction process. A variety of impeller designs, such as paddle, gate and leaf, have been developed to increase mixing and to reduce the possibility of stagnant regions (Godfrey, 1985). Although larger impeller diameters gave better mixing, the energy consumption is considerably higher than for smaller impellers. Thus, the choice of impeller speed and diameter should be compromised with the minimum mixing efficieny for an effective cost of operation and maximum performance of the reactor.

For a given impeller diameter, the agitation speed to be used to liquefy starch to a required degree can also be estimated using the above-mentioned relationship. If the rheological properties of the reaction mixture could be estimated continuously, the agitation speed could be manipulated in order to get minimum complete mixing without wasting power due to excessive speed of agitation.

ACKNOWLEDGEMENTS

The authors are grateful to the Ministry of Science, Technology and Environment, Malaysia, and Universiti Pertanian Malaysia for their financial support towards this research project.

REFERENCES

Arbakariya, A., Ali Asbi, B. and Norjehan, R. (1990) Rheological behaviour of sago starch during liquefaction and saccharification. In *Enzyme Engineering 10*, ed. H. Okada, A. Tanaka and H.W. Blanch, Vol. 613, pp. 610–613. Annals New York Academy of Sciences.

Bilikina, E.S., Ruban, E.A. and Nikitina, S.T. (1973) Advances in microbial engineering. In *Biotechnol. and Bioeng. Symp.*, ed., B. Sikyta, A. Prokop and M. Novak, Vol. 4, pp. 331–356. John Wiley, New York.

Blakebrough, N. and Sambhamurthy, K. (1966) *Biotechnol. and Bioeng.* **8,** 25–42.

Blanch, H.W. and Bhavaraju, S.M. (1976) *Biotechnol. and Bioeng.* **18,** 745–790.

Fullbrook, P.D. (1984) The enzyme production of glucose. In *Glucose Syrup—Science and Technology*, ed. S.Z. Dzeidzic and M.W. Kearsley, pp. 124–145. Elsevier Applied Science, London.

Godfrey, J.C. (1985) Mixing of high-viscosity fluids. In *Mixing in the Process Industry*, ed. N. Harnby, M. F. Edwards and A. W. Nienow, pp. 86–97. Butterworths, London.

Hassan, M.A., Ghani, B.A., Rajab, N., Azudin, M.N., Ariff, A.B. and Mohd Dahan, M.M. (1996) *ASEAN Food Journal*, **11,** 65–69.

Miller, G.L. (1959) *Anal. Chem.* **31,** 426–428.

Miranda, M. and Murado, M.A. (1991) *Enzyme. Microb. Technol.* **13,** 142–147.

Norwood, K.W. and Metzner, A.B. (1960) *A.I.Ch.E. Journal* **61,** 432–437.

Official Methods of Analysis (1980) 13th edn. Assoc. Office. Anal. Chem., Washington.

Rashid, K. (1990) Personal communication. Ajinomoto Sdn. Bhd., Kuala Lumpur.

Rasip, N. (1989) MSc thesis, Universiti Pertanian Malaysia, Malaysia.

Sim, S.L., Oates, C.G. and Wang, H.A. (1991) *Starch/Stärke* **43,** 459–466.

Steel, R. and Maxon, W.D. (1962) *Biotechnol. and Bioeng.* **4,** 231–240.

Svanholm, H. (1985) *Production of High Fructose Syrup.* NOVO Co., Kuala Lumpur.

Yan, V.P.K. (1991) Personal communication. Jamanis Sdn. Bhd., Penang.