Continuous Starch Liquefaction Through In-line Static Mixer Reactor

S. Chamsart¹, ², A. Sriprasit¹, S. Thungkal¹, ³ and Y. Waiprib¹, ⁴
¹IBBERG Bangsaen Biochemical Engineering Research Group: http://bberg.buu.ac.th
²Department of Biology, ³Department of Microbiology, ⁴Department of Food Science
Faculty of Science, Burapha University, Chon Buri 20131, Thailand
Email:bchemeng@hotmail.com, Tel. 05 08 62624

Abstract

Hydrolysis of cassava starch using α-amylase for liquefaction step through an in-house design and construction of In-Line Static Mixer Reactor (ISMR) can save cost and process time when compared to starch hydrolysis in a stirred tank reactor. Starch at different concentrations was hydrolysed by α-amylase at 90 ºC through the ISMR at different flow rates from 15 mL/min to 50 mL/min and different tube lengths up to 12.6 m. Lysis at a flow rate of 15 mL/min with the maximum tube length of 12.6 m gave superb result as this condition possessed the longest reaction residence time of 65.94 min. The concentrations of reducing sugars of 20.81 mg/mL with a Dextrose Equivalent (DE) value of 18.99 and 55.46 mg/mL with a DE of 44.75 were obtained from liquefaction of starch at the concentrations of 25% and 32 % w/v, respectively. Viscosity of starch and sugar dextrin solution during liquefaction reduced as a function of hydrolysis distance, residence time, and a low flow rate. The flow regime of starch solution was very laminar with a Reynolds number (Re) value of <15 during liquefaction through the ISMR. This ISMR was essentially shown an excellent performance with scaleable potential for the commercial production of sugar from starch hydrolysis using residence time as a key parameter.

Key words: Cassava starch, Liquefaction, α-amylase, Static mixer, Continuous starch hydrolysis

Introduction

The production of sugar solution i.e. glucose syrup from hydrolysis of starch is one of the world’s most popular methods. Liquefaction of starch is the first important step to liquefy starch solution by reducing the degree of polymerisation of starch chains using an amylase before further saccharification step. The liquefaction step catalysed by alpha-amylase at a high temperature normally around 95 ºC. It is normally done in batch using an expensive stirred tank with a process time of 2 h. (Rojanaritpichet et al. (2003), 3 h (Sawangwon, 2004) and 4 h. (Srirot et al., 1982). Other methods for starch hydrolysis other than using stirred tank have been studied, for examples, Grafelman and Meagher (1995) gelatinised starch using single-screw extruder and liquefied starch using static mixer, Govindasamy et al. (1997) hydrolysed sago starch by enzymes using a twin-screw extruder give an DE value of <10 and Ulibarri and Hall (1997) used hollow-fibre membrane reactor for enzymatic hydrolysis of cassava starch. However, no novel efficient continuous method for starch liquefaction by enzyme has been reported so far. That is why our group much made an attempt to invent a novel process and equipment with the aims (i) to reduce liquefaction time (ii) to save liquefaction and equipment costs (iii) to enable continuous liquefaction operation and (iv) to enable integrating (i) to (iii) with further saccharification step which also will be operated continuously and simultaneously. Simultaneous continuous liquefaction and saccharification has been being done successfully (not reported now). Only continuous saccharification was reported in the next paper. The In-Line Static Mixer Reactors have been design and fabricated for the study of continuous starch liquefaction here.

Materials and methods

Design and fabrication of the ISMR

A stack of 6.3 m ISMR is composed of three rows (layers) and another stack of 12.6 m ISMR is composed of six rows. Each row 2.1 m in length is comprised of seven segments, each a 10 mm inside diameter glass tube, 0.3 m in length. Segments were connected by 10 mm diameter connectors. Inside each glass tube is a static mixer, 24-static element Kenic type 0.24 m in length, 10 mm diameter and made of PVC. Feeding lines, sampling ports, other components are illustrated in Fig. 1.
Starch liquefaction

Study of appropriate feed rates

A Saccharification of cassava starch using alpha-amylase (Thermamyl 120, Novo Nordisk) was done at the optimum conditions of our group by Sawngwan (2004). Cassava flour was suspended at a concentration of 25% w/v in a pH 6.5 buffer solution with 10 µg of CaCl₂ per 1 kg of flour. The mixture was then dispersed with alpha-amylase at a concentration of 0.04 % volume per flour weight. Then the mixture was pumped, using a peristaltic pump, through the ISMR which was immersed in 90 °C water in a water bath, at the flow rates of 15, 30, 43 and 50 mL/min. Starch solution from flour suspension mixture was simultaneously gelatinised and hydrolysed continuously by alpha-amylase during flowing through the 6.3 m ISMR with the residence times at each flow rate of 32.97, 16.49, 11.50, and 9.89 min, respectively. The sugar dextrin samples were withdrawn from different lengths of the ISMR i.e. 2.1, 4.2, 6.3, m. A 5 mL of hydrolysis reaction of the sugar dextrin samples was ceased by the addition of 5 mL of 0.4 mmol HgCl₂ solution before further sugar concentration assay.

Lengthening the ISMR

As starch liquefaction through ISM has not been finished within 6.3 m in length, known from still increasing reducing sugar concentrations and DE values with steep gradients, thus a longer ISMR unit of 12.6 m in length was fabricated for increasing the efficiency of starch liquefaction. Again starch was liquefied by alpha-amylase as the same conditions and flow rates above, but through 12.6 m ISMR. With this length, the residence reaction times for feeding flour suspension mixture at the rates of 15, 30, 43 and 50 mL/min were 65.94, 32.97, 23.00, and 19.78 min, respectively. The sugar dextrin samples were withdrawn from different lengths of the ISMR i.e. 2.1, 4.2, 6.3, 8.4, 10.5, and 12.6 m. The hydrolysis reaction of the sugar dextrin samples was ceased as above.

Liquefaction of starch at a higher concentration

Whether starch liquefaction at a higher concentration through the 12.6 m ISMR could be carried out, thus flour starch at a concentration of 32 %w/v was hydrolysed by alpha amylase with the same conditions, flow rates and tube lengths above.

Reducing sugar and DE analysis

Concentration of reducing sugars of the samples was determined using DNS assay method and using a rang of glucose concentration as a standard and the DE was determined as a fraction of the reducing sugar concentration to the concentration of total solid of the sugar dextrose sample.

Results and discussion

Feed rates for liquefaction through the ISMR

Cassava flour suspension mixture at a concentration of 25 % w/v was liquefied by alpha-amylase through the 6.3 m ISMR at the flow rates of of 15, 30, 43 and 50 mL/min. The sugar dextrin samples were withdrawn from different lengths of the ISMR i.e. 2.1, 4.2, and 6.3, m. Result in Figures 2 show that at all feed rates concentrations of reducing sugars increased as the tube lengths and residence reaction times increased. The concentrations of reducing sugars and DE values obtained from lysis at low feed rates were higher than those of higher feed rates because the low feed rates generated longer residence times. Reducing sugars, at the end of lysis through 6.3 m ISMR, obtained from those feed rates were 13.31, 11.25, 10.45, and 8.57 mg/mL with the DE values of 12.85, 9.84, 9.48, and 7.64, respectively. Reducing sugar concentrations and DE values in Figures 2 were still increasing though reached the end of ISMR of 6.3 m and the DE values of all feed rates were fairly
low i.e. <20. These indicate that maximum liquefaction has not been achieved with those conditions i.e. feed rates, residence times or lysis distance so a new ISMR set of 12.6 m in length was constructed to study the increase in efficiency of enzymatic hydrolysis of cassava starch.

**Extending the distance for liquefaction through the ISMR**

To hydrolyse starch with longer residence times, an ISMR of 12.6 m in length was used. Cassava flour suspension mixture at a concentration of 25 % w/v was liquefied by alpha-amylase through the 12.6 m ISMR at the flow rates of 15, 30, 43 and 50 mL/min. The sugar dextrin samples were withdrawn from different lengths of the ISMR i.e. 2.1, 4.2, 6.3, 8.4, 10.5, and 12.6 m. Figures 3 show that as a result of extending the length of the ISMR from 6.3 m to 12.6 m, reducing sugar concentrations and DE values increased as a function of tube length due to longer enzymatic hydrolysis or residence reaction time occurred. Again the concentrations of reducing sugars and DE values obtained from lysis at low feed rates were higher than those of higher feed rates because of the same reason addressed above. Reducing sugars, at the end of lysis through 12.6 m ISMR, obtained from those feed rates were 20.81, 18.38, 16.23 and 13.31 mg/mL with the DE values of 18.99, 14.90, 13.66, and 12.46, respectively. Different feed rates gave significantly different results and they showed significant difference of those values within the same feed rate.

**Liquefaction of a higher concentration of starch**

Cassava starch at a higher concentration of 32 % w/v was also hydrolysed by alpha-amylase through the 12.6 m ISMR at the same conditions and flow rates as above. Figures 4 show that higher reducing sugar concentrations and DE values were obtained from hydrolysis at low feed rates. At all feed rates the concentrations of reducing sugars and DE values of the samples from the distance of ≤4 m were rather low because starch has not been completely gelatinized resulting in lagged liquefaction. This explanation was supported by turbidity of those samples especially at high concentration of starch and feed rate (Pictures not shown) and by high viscosity of the samples (Figure 5). The reducing sugar concentrations and DE values gradually increased as the hydrolysis distance from 6.3 m increased to 12.6 m. Reducing sugars, at the end of lysis through 12.6 m ISMR, obtained from those feed rates were 55.46, 44.23, 39.35 and 33.97 mg/mL with the DE values of 44.75, 30.54, 27.82, and 24.03, respectively. Different feed rates gave significantly different results, but they showed no significant difference of those values from 8.4 m to 12.6 m within the same feed rate. This implies that complete liquefaction may have been done after 8.4 m. with residence reaction time between 13.19 and 43.96 min depending on feed rates.

**Viscosity**

Viscosity of sugar solutions from different tube lengths during liquefaction of starch at concentrations of 25 and 32 % w/v and at flow rates of 15 and 30 mL/min was measured by a viscometer (LvDvI-13, Brookfield USA) using a spindle sc4-18 and a cylinder 13 RP. The viscosity of samples was measured at a shear rate range from 20 to 300 s⁻¹. Peak viscosity of the samples from each condition was plotted in Figure 5. The viscosity of sugar dextrin solution during liquefaction of starch solution at a concentration 25 % w/v and feeding at a feed rate of 15 mL/min was lower than that at a higher concentration of 32 % w/v and feeding at a higher rate of 30 mL/min. The viscosity values of the samples from all conditions were very high with a range between 50 and 1,000 mPas within 2 m of hydrolysis through the ISMR because within this distance with the residence reaction times of 5:30 or 11:00 min starch has not been completely gelatinised so alpha-amylase has not worked well yet. As a result it was essentially difficult for highly viscous starch solution to flow through the tube inserted with static element i.e. ISMR. This bottle neck will be over come by improving the design of a new ISMR set (later). However, after 2 m the viscosities of all runs reduced as a function of liquefaction distance and residence reaction time due to the reduction of degree of polymerisation of starch molecules with a value of ~ 10 mPas at 10.5 m. This value is very usual for dextrin solution to be subjected to further saccharification process.
**Flow regime during liquefaction and a proposed scale-up strategy**

Flow regime during liquefaction in this static mixer type was calculated from the Reynolds equation, \( \text{Re} = 3157Q_kS/\mu D \) (Cole-Parmer, 2003) where \( \text{Re} \) is the Reynolds number, \( Q_k \) is the fluid flowrate (gallons/min), \( S \) is specific gravity, \( \mu \), fluid viscosity (mPa s), and \( D \), the inside pipe diameter (inches). Figure 6 shows laminar flow (\( \text{Re} < 980 \)) during starch hydrolysis of all conditions i.e. two feed rates, two feed stock concentrations, and all distances. The flow regime of all hydrolysis condition was very laminar especially within liquefaction distance of \( \leq 2 \) m. because of very high viscosity of starch solution mentioned above causing resistance to flow through such the tube inserted with static element as ISMR. As all was laminar, it was difficult to make relation between the reducing sugar yield or DE and the Re, whilst the residence reaction time (controlled by the tube length and feed rate) controlled the reducing sugar productivity and DE. This clearly indicates that a scale-up strategy must involve residence time as a key parameter. Residence time, \( T_R \) (s) can be calculated from \( L/u \) where \( L \) is tube length (m) and \( u \), flow velocity (m/s), calculated from \( u = Q/A \) where \( Q \) is flow rate (m³/s) and \( A \), cross-sectional area of the tube. Thus, a scale-up model can be obtained as:

\[
\frac{1}{1/2} \approx \text{constant}
\]

![Fig.2](image1.png)  **Reducing sugar concentration and DE from liquefaction of starch at a concentration of 25% w/v through 6.3 m ISMR at different feed rates**

![Fig.3](image2.png)  **Reducing sugar concentration and DE from liquefaction of starch at a concentration of 25% w/v through 12.6 m ISMR at different feed rates**
The 3rd Conference of Starch Technology

Conclusion
The state-of-the-art In-Line Static Mixer Reactors (ISMNR) of 6.3 and 12.6 m in length were invented for enzymatic hydrolysis of cassava starch continuously and efficiently by alpha-amylase “Thermamyl” for liquefaction process. It was used for continuous liquefaction of starch solutions at the concentrations of 25 % and 32% w/v with the feed rates of 15, 30, 43, and 50 mL/min. The degree of reducing sugar concentration and DE value was obtained as a linear function of the tube length or lysis distance and as a reverse function of feed rate. Both lysis distance and feed rates controlled the residence reaction time which was the key factor for continuous starch liquefaction through the ISMR.

The best reducing sugar concentrations of 20.81 mg/mL with a DE value of 18.99 and of 55.46 mg/mL with a DE of 44.75 were obtained from liquefaction of starch solutions at the concentrations of 25% and 32 % w/v, respectively at the lowest feed rate of 15 mL/min with the maximum tube length of 12.6 m. With this length, the residence reaction time for feeding flour suspension mixture at a rate of 15 mL/min was 65.94 min. The viscosity of sugar dextrin solutions of all conditions was very high.

**Fig. 4** Reducing sugar concentration and DE from liquefaction of starch at a concentration of 32 % w/v through 12.6 m ISMR at different feed rates

**Fig. 5** Viscosity of sugar dextrin solutions during liquefaction of starch at a concentration of 32% w/v through 12.6 m ISMR

**Fig. 6** Reynolds number of sugar dextrin solutions during liquefaction of starch at a concentration of 23 % w/v through 12.6 m ISMR
with a value between 50 and 1,000 mPas during the early stage of liquefaction i.e. ≤2 m after that it reduced as the hydrolysis distance and residence time increased with a value of ~10 mPas at 10.5 m. The flow regime of starch and dextrin solution during flowing through 12.6 m ISMR of all conditions was very laminar with a Reynolds number of <15 especially at the distance of ≤2 m. with a value of ≤2.5. However, this ISMR showed an excellent performance with scaleable potential using residence reaction time as a key parameter for the commercial production of sugars from starch hydrolysis.

References