Critical Review of Membrane Bioreactor System Used for Continuous Production of Hydrolyzed Starch

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Recent developments of the starch hydrolysis have concentrated on continuous production of hydrolyzed starch, combination with the secondary processes that requires hydrolyzed starch as the raw material, and newly developed materials such as thermo-stable enzymes, and membrane applications as well. In continuous recycle membrane bioreactor systems (CRMR), thermo-stable α-amylase enzymes have to be used if simultaneous hydrolysis to be applicable. The CRMRs also require a pre-hydrolysis stage, and also the starch milk mass fraction is limited to about w = 10 %, thus reducing viscosity and avoiding overloading which causes heavy fouling to the membrane. High temperatures are also restricted to the used of membrane materials. The other problems are accumulation of higher molecular mass of oligosaccharides in the recirculation system, and inactivated enzymes. In this paper, these problems have been discussed for process flexibilities and system improvements for certain applications are given.

Keywords:
Membrane reactor, starch hydrolysis, enzyme immobilization, immobilization and Fouling.

Introduction

There are number of continuous membrane reactor systems that can be applied to the starch hydrolysis: they are; a) continuous recycle membrane reactors (CRMR),[1,13] b) continuous fluidized bed membrane reactors (CFBMR) where immobilized enzyme is fluidized, c) continuous packed bed membrane reactors (CPBMR) where immobilized enzyme particles are used as a packed bed, d) enzyme membrane reactors (EMR)[23,33] where enzymes are immobilized onto the membrane surface, and e) enzyme emulsion liquid membrane reactors (EELMR).[22] All of these reactors have been used for variety of applications.[1–7,13,14,21–26] The applications of CFBMRs and CPBMRs have not yet been studied for the starch hydrolysis. However, these two types of reactors have been used in the study of other applications such as waste treatments and fermentations.[24–26]

Starch hydrolysis and developments

There are two methods the starch hydrolysis can be; the acid and the biocatalyst reaction. Acid hydrolysis of starch has had widely spreading in the past. Acid hydrolysis that cleaves randomly the polymers of anhydroglucopyranose units (AGUs) was an ancient method discovered by a German's chemist, *Gottlied Sigmund Constantin Kirchoff* in 1811. Nowadays, biocatalytic hydrolysis using amylolytic enzymes or debranching enzymes, which were firstly extracted by French chemists, *Payen* and *Persoz* in 1833, have vastly applied to replace the acid method. Amyollytic enzymes, isolated from active friction of malts and contain the mixture of α and β-amylases, react specific to certain bonding, thus have made possible to control and design required products. The heat-stable bacterial α-amylase produced from *Bacillus subtilis*, that are active up to about 90 °C, was introduced in 1960s. The developed thermo-stable enzyme has realized the simultaneous reaction of gelatinization and saccharification to be applicable. However, the enzyme won't penetrate into the industry due to the fact that it is insufficiently stable at high temperatures required to the complete gelatinization of starches. Whereas acid liquefactions require the starch be heated to approximately 140 °C for rapid gelatinization. In 1973, the thermo-stable α-amylase was introduced, derived from *Basillus licheniformis*, which is active at a higher temperature of 110 °C. The temperature is high enough for complete gelatinization making, both, high temperature stages and second α-amylase additions of the prior enzymatic liquefaction process unnecessary.[38]

Since 1811 onwards, the study of starch hydrolysis is still ongoing. In early 1990s, research trends of starch hydrolysis are in newly developed materials, such as thermo-stable enzymes, membranes,

Table 1 – *Summary of enzymes used in starch hydrolysis*

Enzyme	EC number	Source	Action
		Bacillus amyloliquefaciens	Only α-1,4-oligosaccharide links are cleaved to give α-dextrins and predominantly maltose (DP2), DP3, DP6 and DP7 oligosaccharides
α-Amylase	3.2.1.1	*B. licheniformis*	Only α-1,4-oligosaccharide links are cleaved to give α-dextrins and predominantly maltose, DP3, DP4 and DP5 oligosaccharides
		Aspergillus oryzae, *A. niger*	Only α-1,4 oligosaccharide links are cleaved to give α-dextrins and predominantly maltose and DP3 oligosaccharides
Saccharifying α-amylase	3.2.1.1	*B. subtilis* (*amylosaccharitic*us)	Only α-1,4-oligosaccharide links are cleaved to give α-dextrins with maltose, DP3, DP4 and up to 50 % w glucose
β-Amylase	3.2.1.2	Malted barley	Only α-1,4-links are cleaved, from non-reducing ends, to give limit dextrins and β-maltose
Glucoamylase	3.2.1.3	*A. niger*	α-1,4 and α-1,6-links are cleaved, from the nonreducing ends, to give β-glucose
Pullulanase	3.2.1.41	*B. acidopullulyticus*	Only α-1,6-links are cleaved to give straight-chain maltodextrins

(Adopted from C. Martin, 2003 [46])

and combination processes, that require hydrolyzed starch as raw materials.[39]

Enzymes nomenclature of the starch hydrolysis used commercially for starch hydrolysis is summarized (table 1). Each enzyme is given the nomenclature of the EC number, although they are somewhat confusing which sometimes lump together enzymes with subtly different activities.

The α-amylases (1,4-α-D-glucan glucanohydrolases) are endohydrolases which cleave 1,4-α-D-glucosidic bonds and can bypass but cannot hydrolyse 1,6-α-D-glucosidic branch-points. Commercial enzymes used for the industrial hydrolysis of starch are produced by *Bacillus amyloliquefaciens* (supplied by various manufacturers) and by *B. licheniformis* (supplied by Novo Industri A/S as Termamyl). They differ principally in their tolerance of high temperatures, Termamyl retaining more activity at up to 110 °C, in the presence of starch, than the *B. amyloliquefaciens* α-amylase. The maximum DE obtainable, using bacterial α-amylases is around 40 but prolonged treatment leads to the formation of maltulose (4-α-D-glucopyranosyl-D-fructose), which is resistant to hydrolysis by glucoamylase and α-amylases. DE values of 8–12 are used in most commercial processes where further saccharification is to occur. The principal requirement for liquefaction to this extent is to reduce the viscosity of the gelatinised starch to ease subsequent processing.[30]

Enzyme activity

It is sometimes unlikely of many publications that the glossary's terms of the inhibition and the deactivation seem to be ill defined, thus the discussion and the explanation of the enzyme activity is unclear. These have resulted in misunderstandings, and in many cases of study, no efforts have been taken in preventing or reducing the enzyme inactivation. Therefore, basic explanations of the enzyme inactivation are discussed. The deactivation and the inhibition are different mechanisms, but the result is the same, inactivation of enzymes, thus contributing to reduction of an overall activity of the enzyme.

The enzyme deactivation can be defined as a reaction where active enzymes molecules undergo chemical changes into the inactivated form, which is also known as decay. Active enzymes turn to be inactivated under certain denaturing conditions, which is influenced by many factors, i.e. pH, temperature, viscosity, and mechanical agitation.[20] The denaturing of enzyme reactions can be reversible or irreversible; depending on the load of denaturing conditions has been experienced. For the purpose of assaying enzyme deactivation, data is usually obtained by exposing an enzyme to a denaturing condition for certain time interval without the substrate, then making an initial rate activity assay thereafter returning the deactivated enzyme solution to the standard condition and adding the substrate.

There are three types of inhibit behaviors that can be found during enzyme-catalyzed reactions, they are competitive uncompetitive, and non-competitive. The inhibition can be defined as enzyme--substrate reaction(s) is/are impaired by the inhibitor. Inhibitors could be chemical substances: inert which came along with feeding materials (substrates, enzymes, and water), or/and by-products have been formed while enzyme-substrate reactions,

or/and the products itself, or/and the enzyme(s) itself. The loss of activity can either be reversible, which may be restored by removal of inhibitor(s), or irreversible where the loss of activity in time dependents could not be restored during the time scale of interest. If the inhibited enzyme has been totally inactivated, irreversible inhibition behaves as the time dependent loss of the enzyme concentration (i.e lower γ_{max}), in other cases, involving incomplete inactivation, they may be time-dependent changes in both γ_{max} and K_m.

In case of the enzymatic hydrolysis of starch, inhibitor substances can be as follows:- many alcohols, ascorbic acid, lactose, oxalate, phosphates, sucrose, dextrin, maltose and glucose, and their inhibition effect can be non-competitive, uncompetitive or competitive.[9,16,17,28,34] Other inhibitors that might be introduced with the feeding materials, also known as inert substances, are heavy-metal ions (e.g. mercury and lead). Heavy metal ions are the most crucial inhibitor as they usually cause an irreversible reaction by binding strongly to the amino acid backbone. However, some inorganic ions such as Ca^{2+}, Mg^{2+}, Sr^{2+}, and Zn^{2+} are required to act as coenzymes, thus enhance the enzyme stability, although the concentration required varies according to the source of the enzyme. *L.H. Lim* et al.[9] have reported that Ca^{2+} ion helps in stabilizing the tertiary structures of α-amylase. Since enzymes are highly charged proteins, the use of soluble enzymes might also give similar effects as suggested by *Belma Ozbek* et al.,[20] i.e. some sort of enzyme-enzyme inhibition resulting in ionic disturbances between a pair of enzyme molecules that reduces the ability of active sites to bind the starch particles effectively.

Belma Ozbek et al.,[20] have investigated the effects of pH, the temperature, the viscosity, the amount of enzyme preparations added, the impeller speed, the quantity of hydrolysate, and the processing time. The optimum conditions are given as a temperature of 60 °C, an impeller rate of 300rpm, and pH 6.5. At this point, the α-amylase enzyme has lost 48 % of the initial activity, and the degree of wheat starch hydrolysis was only 40 % in 30 min of operations. *D. Paolucci-Jeanjean* et al.,[1,7] have studied enzyme activities of the termamyl in the retentate of a CRMR. They have found that the enzyme activity has obeyed a law of exponential decay as below;

$$a = a_0 e^{-\phi/\tau_{av}} \qquad (5)$$

where a and a_0 are running and initial activity in the retentate (g cm^{-3} min^{-1}), τ_{av} is an average space time while Ø (see table 2) is a dimensionless constant which is much similar to e and is effectively linked to the loss of the enzyme activity in their model. They also have indicated that loss of enzyme activities have underlined similarity between the production of small oligosaccharides in a batch reactor and a continuous membrane reactor.

Table 2 – *Value of ε and ϕ for different experiments[a] (adopted from Paolucci-Jeanjean et al.[7])*

σ_E, cm^{3}dm^{-3}	1.8	2.7	3.7	5.5
γ_E, g dm^{-3}	0.32	0.30	0.31	0.29
Ø			0.29	0.28

[a] θ = 80 °C, pH 5.8, γ_{oS} = 100 g dm^{-3}, V_R = 6 dm^3

From results presented, both, in batch and continuous operations, loss of enzyme activities are similar from which it can be concluded that, in continuous operations, addition of fresh enzymes is required to replace inactivated enzymes, hence the process would be maintained at a high performance. Accumulation of inactivated or non-productive enzymes in the re-circulation system might also be subjectolyses to form small derivative compounds, and although unlikely, it could also impair the operation by dept fouling, and inhibition thus the system requires shut-down for cleanings, and replacing the reactor contents with the fresh substrate and enzymes.

Processes for production of hydrolyzed starch

Industrially, various manufacturers use different approaches to starch liquefaction but the principles are the same. Granular starch is slurried at w = 30–40 % with cold water containing w = 20–80 · 10^{-6} ppm Ca^{2+} (which stabilises and activates the enzyme), at pH 6.0–6.5, and the enzyme is added (via a metering pump). The α-amylase is usually supplied at high activities so that the enzyme dose is 0.5–0.6 kg t^{-1} (about 1500 U kg^{-1} dry matter) of starch. When Termamyl is used, the slurry of starch plus enzyme is pumped continuously through a jet cooker, which is heated to 105 °C using live steam. Gelatinisation occurs very rapidly and the enzymatic activity, combined with the significant shear forces, begins the hydrolysis. The residence time in the jet cooker is very brief. The partly gelatinised starch is passed into a series of holding tubes maintained at 100–105 °C and held for 5 min to complete the gelatinisation process. Hydrolysis to the required DE is completed in holding tanks at 90–100 °C for 1 to 2 h. These tanks contain baffles to discourage backmixing. Similar processes may be used with *B. amyloliquefaciens* a-amylase but

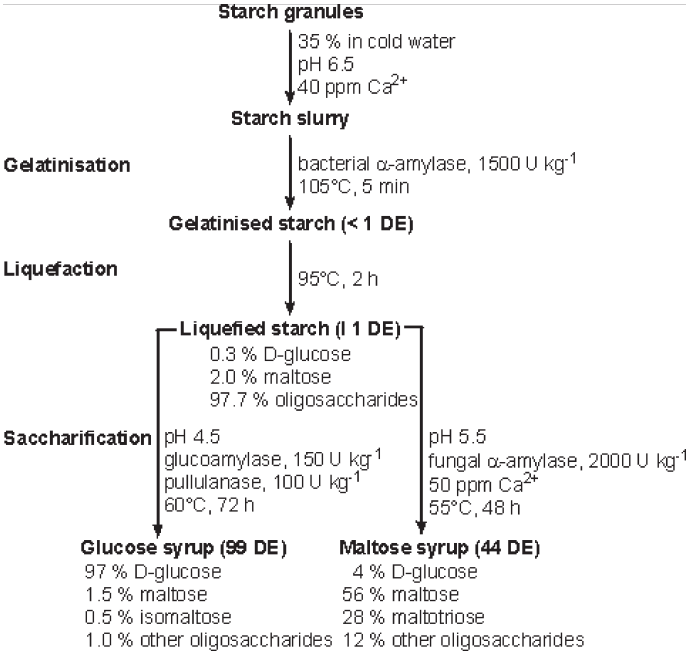

Fig. 1 – *Conversional processing of starch (Adopted from C. Martin, 2003)*[46]

the maximum temperature of 95 °C must not be exceeded. This has the drawback that a final 'cooking' stage must be introduced when the required DE has been attained in order to gelatinise the recalcitrant starch grains present in some types of starch, which would otherwise cause cloudiness in solutions of the final product.[46]

A summary of various method processes of different products used for the starch hydrolysis is given in figure 2. The processes (a), (b), (c), and (d), all require rapid gelatinization reactions at higher temperatures of more than 80 °C in which the granular starch is cooked and then followed by liquefaction and saccharification reactions. A newly invented process, as indicated in process (e), is simultaneous acting of the liquefaction and the saccharification reactions carried out at low temperature ranges from 40–60 °C. This process allow slow gelatinization reactions, and becomes the limiting factor in which the reaction rate should be a bit slower or equal to the liquefaction rate carried out in simultaneous processes at optimum low temperatures. Kinetics modeling, verifications, and the process development of a membrane bioreactor system is discussed elsewhere. Preliminary assessments have shown that when an optimum temperature of saccharification was used; results have followed the assumption of "gelatinization becomes the limiting factor". Temperature selections are taking considering a higher rate of the slower reaction involved in an optimum simultaneous reaction, and flexibilities increased of the used of membranes. Viscosity observations have shown that they do not increase throughout a batch hydrolysis of the tapioca starch.

The CRMR suggested by *Paolucci-Jeanjean* et. al. uses the (d) process, except no further saccharification process was involved. A high temperature process within the system of 80 ^{0}C requires a pre-hydrolysis step and a low starch milk mass concentration of about w = 10 % to reduce viscosity as well as a method of preventing fouling.[7] At the use of ceramic membranes it is also necessary to sustain a continuous high operating temperature and pressures. The thermostable enzyme, isolated from genetically modified bacteria used for the simulta-

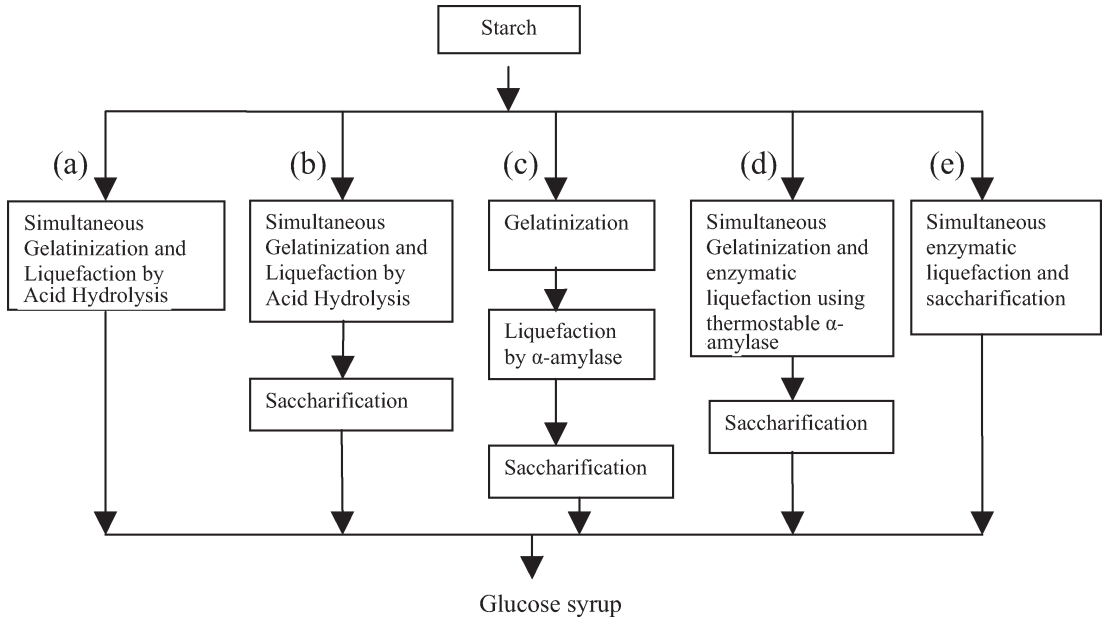

Fig. 2 – *Various processes alternative of the starch hydrolysis from the starch*

neous liquefying hydrolysis must also be applicable. Thus, this is an example of a modified enzyme created to sustain high temperature processes. Whereas in animal cells, the energy predominantly derives from glucose, produced is by carbohydrates conversion by several enzymes with certain requirements and is necessary throughout the digesting system carried out at a body temperature. The nature of this example has generated ideas and could be potentially adopted in bioprocesses engineering especially when involving biocatalysts. Therefore, the processes would be designed to follow the enzyme nature conditions. As result a low temperature and mixed enzymes behavior is suggested and now under investigation as indicated in fig 2 as (e) process. In fact, enzyme(s) reacts specific to the particular substrate(s) simultaneously, or consecutively or alternately in a mixture, or segregated.

Kinetics of starch hydrolysis

The kinetics of the starch hydrolysates production of the starch hydrolysis would be determined by the enzymes types (table 3) and the starch origins. Many of the enzymatic reactions use the classical Michaelis-Menten equation;

$$\Gamma = \frac{\Gamma_{max}\gamma_S}{K_m + \gamma_S} = \frac{k\gamma_E\gamma_S}{K_m + \gamma_S} \qquad (2)$$

where

Γ = the production rate, g dm^{-3}h^{-1}

γ_E = enzyme mass concentration, g dm^{-3}

γ_S = substrate mass concentration, g dm^{-3}

Γ_{max} = mass reaction rate, dm^{-3} h^{-1}

K_m = aquilibrium constant, g dm^{-3}

k = the kinetic coefficient cm^3 g^{-1} h^{-1}

The best fitting values of Γ_{max}, K_m and k are obtained from the batch or continuous reaction experiments. However in many cases, the basic Michaelis-Menten model needs modifications to consider correctly more complex reactions. The complexity of starch reactions models could be due to the substrates origins, inert components, deactivations, inhibitions, and the physical properties of substrates and enzymes, as well as experimental designs.

Inhibitions consideration

In order to take into account products inhibition, the following equation is used,[42,44]

$$\Gamma_{app} = \frac{\Gamma_{app\,max}\gamma_S}{K_m^{app}(1+\gamma_p/K_i)+\gamma_S} \qquad (3)$$

γ_P = the product concentration, g dm^{-3}

K_i = an inhibition constant, g dm^{-3}

Γ_{app} = appearance rate of reaction, g dm^{-3} h^{-1}

K_m^{app} = appearance Michaelis-Menten constant, g dm^{-3}

With the model, kinetic constants, both, for batch and membrane reactor systems[42] and pack bed immobilized enzyme reactors[44] were identified. The kinetic model actually has suggested that considered mixed inhibitions, which appear in the starch hydrolysis, have to be observed.[9, 16, 17, 28, 34]

Enzyme decay

Houng et al.[42] have proposed the following equation (3) used for the starch hydrolysis into maltose in a CRMR as;.

$$\Gamma_{app} = \frac{\Gamma_{max}^{app} e^{(-\alpha t+\beta t^2)\gamma_S}}{K_m(1+\gamma_p/K_i)+\gamma_S} \qquad (4)$$

where α (h^{-1}) and β (h^{-2}) are two new constants. The equation could be good to describe the enzyme decay as the enzyme deactivation is linked with the time, and constants should vary for different processes (batch or continuous), rigs and experimental designs. As shown in equation (1) *Paolucci-Jeanjean* et al.,[1,7] have proposed an enzyme activity decay of the termamyl in the retentate of a CRMR. Combining equation (1) and equation 4, hence;

$$\Gamma_{app} = \frac{\Gamma_{max}^{app} e^{-\phi t/\tau_{av}}\gamma_S}{K_m(1+\gamma_p/K_i)+\gamma_S} \qquad (5)$$

Temperature effect

The rate of chemical reactions increases with temperature. This is described by the Arrhenius relationship, which can be written in the form:

$$\ln\frac{k_2}{k_1} = \frac{\mu}{R}\left[\frac{1}{T_1} - \frac{1}{T_2}\right] \qquad (6)$$

where T_1 and T_2 are the absolute temperatures corresponding to coefficients of reaction rate $k_1$and k_2, R is the gas constant, and μ is the *critical thermal increment*: a coefficient characterising the particular reaction. When this relationship holds, a more friendly measure can be calculated: $Q_{10} = k_{t+10}/k_t$, where k_t is the coefficient rate at temperature t, and γ_{So} is the coefficient rate at 10 °C higher. γ_{So} Q_{10} simply tells us by what factor a rate increases for a 10 °C rise in temperature.

In biochemical processes, often composed of a complex pathway of many intermediate reactions, μ (and Q_{10}) is only a constant over a limited temperature range, which might be smaller than that of the phenomenon under study. A change in temperature

can determine which of the steps in the pathway is the rate-limiting one, resulting in a sharp change in μ and Q_{10} at particular temperatures. In practice, the interaction of several potentially rate-limiting processes (physical as well as chemical) can lead to gradual, rather than sharp, changes in μ with temperature. Even for a single biochemical reaction, the rate increase with temperature falls off as temperature increases, presumably because of the destruction of enzymes on which they depend.[47] Nevertheless, the Arrhenius relationship holds for many biological phenomena under temperature ranges of interest, and is even reflected in behaviours. The slope of the linear relationship between the log of the rate of most biological reactions and the reciprocal of absolute temperature is the Arrhenius μ divided by approximately 4.6, with μ defined by the limiting step.[48] For thermochemical (enzymatic) reactions, Q_{10} is typically somewhere between 2 and 3 : they often go about twice as fast for every 10 °C rise in temperature.

The temperature effect to the starch hydrolysis reaction of the SLGS reactions will be studied. It includes the temperature effect to enzyme decay and the reaction rates.

pH Effect

pH effect might not be not considered in this case as the simultaneous reaction is maintained at an optimum pH throughout the processes.

The constant evaluations of the hydrolysis

Evaluations of hydrolysates of oligosaccharides are categories into non hydrolysable oligosaccharides (NHO) of DP 1–3 and intermediately oligosaccharides (IO) of DP 4–7. The concentration of NHO with respect to time is given as;[41]

$$P(t) = \frac{a\,t^2}{b^2 + t^2} \tag{9}$$

where a and b is constants.

Gaouar et al (1997)[45] have proposed a similar equation for maltose and glucose production starting from liquefied starch using Maltogenase® for saccharification;

$$P(t) = P_0 + \frac{a'\,t^2}{b'^2 + t^2} \tag{10}$$

where *Po* is the initial product concentration (g dm^{-3}), *a'* (g dm^{-3}) and *b'* (min) two constants.

As suggested by *Paolucci-Jeanjean* et al. (2000),[41] IO concentration cannot, however, be fitted as a simple equation. After an initial latency, concentration increases and then decreases until a constant value is reached. The new equation may be written as;

$$P(t) = \frac{a\,t^2}{b^2 + t^2} - \frac{c\,t^4}{d^4 + t^4} \tag{11}$$

where c (g dm^{-3}), and *d'(h)* two new constants

Tapioca starch hydrolysis kinetics with the termamyl enzyme at 80 °C in a batch and CRMR, has been investigated, and a semi-empirically equation is proposed.[1,41] An empirical model has been describing oligosaccharides polymerization (DP) concentrations ranging from 1 to 5 throughout the cassava starch hydrolysis, both for a batch and a continuous mode, and may be calculated as [1];

$$P_{\mathrm{n}} = k_{\mathrm{n}}\left(\sum_{j=m}^{\infty}\gamma_i - \sum_{j=m}^{\infty}\gamma_i\ \mathrm{lim}\right)\gamma_{\mathrm{E}} \tag{12}$$

where P_n is the production of oligosaccharide (g dm^3 h^{-1}), γ_i the concentration of the oligosaccharide with a DP equal to i (in the retentate for CRMR) (g dm^{-3}), γ_i lim the limit concentration of the oligosaccharide with a DP equal to i (in the retentate for the CRMR) (g dm^{-3}), m_n corresponds to the DP of the smallest oligosaccharide leading to the production of a sugar with a DP equal to n, E_{act} the concentration of active enzymes (cm^3 dm^{-3}), always equal to the initial concentration in the batch reactor or continuously decreasing according to $\gamma_{\mathrm{E_{act}}} = \gamma_{\mathrm{E}}\mathrm{e}^{-\varepsilon t/\tau_{\mathrm{av}}}$ in the CRMR, k_n a constant (dm^3 cm^{-3} h^{-1}). The values of k_n are similar in both reactors, whereas limit concentrations are different due to retention of high molecular mass products by the membrane and limited reaction yield in the CRMR.

Membrane selections and fouling

In the contact of this study, membranes are referred to synthetic selective barriers used in separation of sugar after/within the hydrolysis process of ultrafiltration or nanofiltration in cross flow filtration membrane systems or membrane reactors. The retention time and the operational conditions would define the filtration unit(s) act as filters or reactors. When the reaction does mostly in membrane areas, they are referred to as membrane bioreactors; otherwise membranes act as the filter. Enzymes used in the starch hydrolysis have been reported to be inhibited by products such as dextrin, maltose and glucose[9,16,17,28,34] whereby continuous and rapid removal of products become vital thus reducing concentration and contact-time with enzymes. The used of membrane has offered lots of benefits including sterilization, purification, and reused enzymes, pro-

viding the fouling is assayed with proper membrane selections and process designs.

In starch hydrolysis processes, mixture of enzymes and starch are introduced to be in contact with the membrane. Some of low degree of equivalents (DE) of oligosaccharides are permitted to pass through the membrane, so called "permeate", while enzymes and higher molecular mass compounds which are retained (rejected) in the circulation system are called "retentate". Therefore, selections of membrane required considerations of;

1. The reactions involved (main reactions, side reactions and unwanted reactions).

2. Physical and chemical properties of reactants, products, intermediate products, by products, and enzymes. (In case of ultrafiltration, sieving mechanisms, pore sizes, and practical diameters or MWCO (molecular weight cut off), determine the separation characteristics.

3. The trans-membrane pressure and the operational temperatures. Many hollow fiber polymer membranes would sustain to about 2–3 bars pressures and 50–60 °C continuously applied the temperature. A hollow fiber membrane can be operated at slightly higher pressures by flowing the retentate outside the lumen. For most ceramic membranes, they are more durable to high pressures, high temperatures, and chemicals.

4. Inactive enzymes or non productive enzymes and lyses enzymes.

Since the membrane used for the starch hydrolysis involving micro molecules (sugar) and macro molecules (enzymes, higher molecular mass, and unreacted starch), sugars that have molecular mass less than 400 and diameters range from 0.8–1 nm[40], would be easily passing the nano or the ultra membrane, while active amylase enzymes which have molecular mass varying from 10 kD to 210 kD[37] would be retained in the circulation without major troubles. The fragmentations of enzymes suppose to be smaller than enzymes itself. Thus, the cut off value of the membrane should be based on the lower cut off value of molecular mass introduced into the membrane. Nevertheless, the selection criteria should not be used as a definite criterion, but most likely as a guide as the manufactured membrane pore volumes are unevenly distributed, and they use statistic estimations, and pure component tests of producing the value.[31] In addition, during the operation run, the membrane properties could also change, whereby try and error are necessary. Computations may also help to predict the result and the membrane used, provided there are enough data. Other factors that should be taken into considerations are maintenance, costs, and cleaning or regeneration.

The leading fouling factor of starch hydrolysis in the CRMR is accumulation of higher molecular weights.[7] Since ill defined of the fouling mechanisms given, explanations will be discussed from theoretical views. However, the best results might be obtainable from SEM (scanning electron microscope) analysis, before and after runs. The membrane fouling occurs by interactions between the membrane surface and the component in the reactor. They can be adsorption of compounds onto the membrane surface, pore blocking and depth fouling (by particulates getting trapped in the porous structure), concentration polarizations retained in membrane surfaces, fouling layer (gel/cake), and osmotic pressure. In many cases, fouling layers determine the cut-off of separation instead of the membrane itself.[18]

Membrane bioreactor systems

Investigations have been undertaken using membrane bioreactor system for the production of low molecular mass syrups.[1–7,13,14,35] The results show advantages of continuous mode productions rather than batch modes. Moreover, quantitative comparison results have shown advantages in terms of the enzyme usage and activity, and the reaction times.

In general, problems of using membrane bioreactor systems (both hollow and flat sheet membrane configuration) are; fouling, accumulation of higher molecular mass products in the bulk system, and enzymes inactivation. These have impaired the steady state operation,[1–7,13,14,35] thus CRMRs would no longer be operated at continuous mode, but most likely to be semi-continuous operations with continuous removal of formed product(s), sequential additions of enzyme(s), and accumulations of higher degree of polymers (DP)s in the bulk system that has been limiting the operational time. Thus the system is to be dependent upon the enzyme(s) inactivation reactions, and the membrane fouling. The kinetics reaction(s) could be an exponential phase during start-up, followed by a quasi-steady state for certain period of times with slight decrease of formed products due to enzyme inactivation, and an exponential decrease when complete fouling has taking place.

Continuous recycle membrane reactors

A CRMR has been proposed by *Paolucci-Jeanjean* et at.,[1,2] to be used for the starch hydrolysis of tapioca starch using the thermo-stable α-amy-

lase isolated from *Basillus licheniformis* sp. and supplied by NOVO industry with Termamyl as the synonym. A ceramic membrane with 50 kD MWCO was used in the CRMR, which according to them the membrane is able to reject enzymes and non-hydrolyzed starches, as well as tolerate operational conditions of high viscosity, high temperatures, and high pressures. The system also uses conventional pressurized membrane at less than 1 bar. In order to balance the products passing through the membrane, level control was used by pumping in the starch milk when level was dropped. Unfortunately, un-reacted starches and high DPs were allowed in contact with the membrane. Continuously adding the starch milk could also induce an overloading of the membrane. Therefore, rather than solely control of the level, it is suggested the un-reacted starch and the accumulation of higher DPs.

As shown in Fig. 1, the permeate concentrations of oligosaccharides with DP 1–7 and higher, oligosaccharides of DP 6, DP 7 and above, are kept increasing from beginning until 6 hrs hydrolysis, while DP1 to DP5 are slightly decreased. This is common phenomenon as the α-amylase (EC3.2.1.1) derived from *Basillus licheniformi* was used that cleaves α –1, 4 oligosaccharides to produce α-dextrins, predominantly maltose, DP3, DP4, and DP5.[46] Since the excessive substrate was supplied, the main reason of the product decreased could be due to enzymes inactivation.

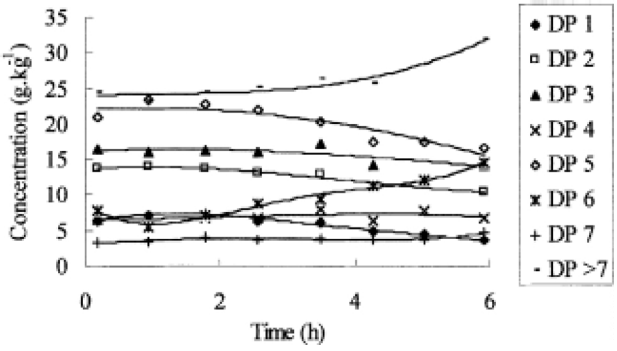

Fig. 3 – *Permeate concentrations of oligosaccharides with a DP 1–7 and higher than 7 during starch hydrolysis in the CRMR (adopted from Paolucci-Jeanjean et al. 2000)*[7]

Suggestions of system improvements

Rapid gelatinization at higher temperature than 80 °C has produced highly viscous solute, thus reducing better intimate of enzymes and substrates, and also consumed much energy for agitations and pumping, as results to low efficiency. The thermo-stable enzyme isolated from genetically modified bacteria must be used for simultaneous hydrolysis at high temperatures are to be applicable. This meant the CRMR always requires a pre-hydrolysis stage, and a low milk starch mass fraction of about w = 10 % was used to control the viscosity, and to avoid overloading and heavy fouling to the membrane. High temperatures have also restricted the use of ceramic membranes that sustain high temperatures, as well as increase enzymes inactivation. The other problems are soluble enzymes might also increase enzyme-enzyme inhibitions, while deactivation is due to the operating system (i.e. agitation, and pumping). The other consequent problems are the system might be saturated by the unreacted starches and the ultimate higher DPs of oligosaccharides as the system was continuously fed with the starch milk Nevertheless, in order to improve process flexibility, such as utilizations of other membranes (such as hollow fiber), and also to discourage accumulation of gelatinized starch in the system, the simultaneous low temperature process is suggested for production of glucose or other related products. This process would allow gelatinization to become the limiting factor for liquefaction and saccharification reactions. Starch milk concentration can be increased by adding a settling tank, therefore retain solids and higher molecular weight components and thus preventing them from contacting with the membrane, and increase the retention time for simultaneous reactions as well.

Advanced system improvement

Fouling prevention

Fouling phenomenon preventions have been vastly studied in many fields in membrane applications.[8,9,11,18,21,31,36] *T.R Noordman* et al.,[21] have suggested using particles such as glasses and steels at different sizes thus promoting turbulence stream in the fluidized bed. As a result, it has improved the flux and the rejection in a membrane ultra-filtration system. In their findings, particles fluidization was the best method used for higher viscosity liquids; otherwise the membrane was subjected to the potential risk of damage. The other limitations are: increasing the cut off size and the density of particle could risk the damage of membrane and increase the energy consumption. They claim that the best particles should be as lighter and small as possible, provided an acceptable flux, low energy consumptions and minimal risks of membrane damage. Although there is no discussion on how the fouling has been reduced, the reported results show a flux improvement and rejection characteristics. Therefore, the study could be useful to the success of the particle immobilizations.

Q. Gan et al.,[31] have proposed an integrated membrane reactor for the enzymatic cellulose hy-

drolysis that is a bit modified from the continuous stir tank reactor (CSTR). They have used a bench scale reactor in their study which was fabricated from a modified Amicon dead end and a filtration cell (Amicon PM10) with maximum holding volume of 2.5 l. A flat sheet polysulfone ultrafiltration membrane with the molecular weight cut-off (MWCO) value of 10kD is used as the disc with diameter of 150 mm and the surface area of 0.0177 m^2, it has been installed at the base of the filtration cell. The separation characteristics of selected membrane have permitted total rejection of the cellulose enzymes and zero rejection of the reducing sugar during combination reaction in an ultrafiltration process. The design has considered reuse of the enzyme, increasing of the reaction rate, and products inhibition. An extra feature of the design is an electrical cathode and anode that allows the reactor to be controlled periodically by the electrical back-pulse that prevents accumulation of enzyme molecules and substrate particles at the membrane surface. Because of the arrangement of the negative and positive electrodes, and molecules and substrates are negatively charged at the reaction pH, they are likely to be rejected from the membrane surface by controlling the intermittent electrical back-pulsing. As a result, the pseudo-steady state flux after initial rapid decline is reached in the first 5 h in the typical of 100 h of continuous operation. The 20 s electrical back-pulse at 300 V normally produces an instantaneous flux increase up to six times immediately after the impulse. However, the high flux level increased unsustainably even for a short period, and would normally have fallen back to the original pseudo-steady state level within 120 s after the electrical back-pulse. Drop of the flux level could be resulted by accumulation of higher molecular weights onto the membrane surface as the cross flow filtration is used. Thus, the author feels the system has dimmer views on applications of integrated reactor–membrane systems for cellulose hydrolysis.[31] Nevertheless, the electrical anode and cathode might be considerable techniques to be used in the CRMR, where simultaneous fouling layers cleaning is offered. However, the design and costs should be taken into account and should be compared to other suggestions which have been given.

Enzyme immobilizations

Enzyme immobilizations are classified as physical adsorptions, ionic bonding on the ion exchange resin, physical entrapment such as an inclusion in micro-porous gels or fibers, or by micro-encapsulations, cross-linking of enzymes, and covalent bonding. Enzyme immobilizations used for starch hydrolysis processes have been tested.[9,13,19,27]

Despite many advantages of immobilized enzymes, several problems need attentions prior to immobilizing enzyme utilization. Depending on immobilization methods and use, losses of enzyme activities of 10–90 % have been reported.[32] Steric hindrance, enzyme-substrate orientation, and diffusional restriction problems may occur that affect activities and specificities, especially with macromolecular or colloidal substrates.[32] Nevertheless, a mass transfer problem could be reduced if the diameter of particles used for immobilizations is smaller rather than the dimension of soluble enzymes, thus there could be negligible increased difficulties in attaching enzymes to the substrate particles.[9] Study on the barley α-amylase immobilization was reported that at 45 °C, the lifetime of immobilized enzymes has three times improved rather than without immobilizations, and also the immobilized enzymes have degraded at the rate constant of three times lower than the soluble enzyme. Moreover, the thermostability has also been increased. The immobilized technique could be practical to immobilize other enzymes such as the α-amylase and amyloglocosidase. Despite the advantages, immobilized enzymes are reported not to be significantly stabilized in respect to the inhibitory effect of the sugar products.[9]

Another technique, that could give promising results in term of cost effectives and simplifies the α-amylase immobilization method is by an entrapping with a UV-curing coating.[19] The technique has retained an adequate enzymatic activity, and the immobilized enzyme can be reused for more than 50 times under certain experimental conditions. Although some of immobilization techniques have reported unable to stabilize enzymes from products inhibition, the suggested techniques could be useful as to be the alternatives to stabilize enzymes from deactivations as well as to prolong the enzyme lifetime. Many publications have also give evidence that immobilization is able to stabilize the enzyme from deactivation, and permit reuse as well.[10,19,27,28,30]

Advanced membrane reactor designs

Advanced explorations of enzyme stabilizations, other than the natural stabilization of low temperature operation, and reducing of the enzyme stress from the heavy mechanical contact, are by enzyme immobilizations. Two way immobilizations could be applied in the CRMR, attachments into the membrane (flat sheet or hollow), or by fixing/entrapping onto bigger particles, then they can be packed or fluidized in a membrane reactor. A theoretical analysis of the transport phenomena in a hollow fiber membrane reactor with immobilized biocatalyst have extensively been reviewed by *V.*

Calabro et al.[33] Attachment of an enzyme into the membrane has been experimented by *L.Giorno* et al.[23] by entrapping it in an asymmetric capillary of fumarase enzyme. The stability has shown no activity decay during more than 2 weeks of continuous operations with 80 % product conversion. Fortunate for the fumarase, reactions are not inhibited by the product. The other advantages are surface immobilizations what might help to reduce the polarization whereas the reaction occurs in membrane surfaces. Thus the membrane reactor designed by *L. Giorno* et al., has last for two weeks. Despite promising results, disadvantages of the system are the immobilization cost and the chances substrate in contact with the enzyme [2]. Other consequents are back-flushing could scrap off immobilized enzymes, and membrane cleaning using acid and base, and higher temperatures could cause enzymes are denatured.

Packed bed reactor would be easier to control; however pressure drops across the bed could become a cut off to be chosen. The choices between re-circulating and fluidization might need further consideration of membrane designs and arrangements, and later on experimental results will reveal the facts. Although re-circulating systems might be a potentially applicable in reactor designs, neither the benefits nor limitations, of the highly flux re-circulations and the trans-membrane pressure, should be optimized. A highly flux could help to scrap-off the fouling layer and could provide the good mixing of enzymes and substrates; however system might also potentially increase enzymes inactivation. The fluidization would offer good mixings between the enzymes and the substrates, immobilized enzymes are retained within the vessel, low pressures process, and as the immobilized enzyme stays in within the reactor vessel, thus there is no mechanical contact of enzymes and mechanical equipments. The limitations could be polarizations and cakes fouling as the process have to be operated at a fluidized velocity. Thus, there could be a time limit in which back flushing or back pulse is necessary.[31] The settling tank has been suggested to retain higher molecular mass in the membrane reactor system; therefore the CFBMR could be greatly potential.

Conclusion

As mentioned, the main problems of starch hydrolysis carried out in the recycles membrane bioreactor system are inactivation enzymes, highly viscous solute produced by rapid gelatinization, and an accumulation of higher DPs. We conclude: the system might be improved by operating at the low temperature, adding the settling tank, and further advanced to utilize the immobilization and/or fluidization, if necessary. Proper choice of the techniques and the conditions, the immobilisation might offer a great potential opportunity to prevent fouling, improves enzyme stabilization, and thus prolong the operation time.

Nomenclatures

τ_{av} – average space time, h^{-1}
$Ø$ and ε – dimensionless constant
v – the production rate, g $dm^{-3}h^{-1}$
γ_E – enzyme mass concentration, g dm^{-3}
γ_S – substrate mass concentration, g dm^{-3}
Γ_{max} – mass reaction rate, g dm^{-3} h^{-1}
K_m – Michaelis-Menten constant, g dm^{-3}
k – the kinetic constants, g $cm^{-3}h^{-1}$
γ_P – the product mass concentration, g dm^{-3}
K_i – an inhibition constant, g dm^{-3}
Γ_{app} – appearance rate of reaction, g $dm^{-3}h^{-1}$
K_m^{app} – appearance Michaelis-Menten constant, g dm^{-3}
α (h^{-1}) and β (h^{-2}) – constants
R – the gas constant,
μ – the *critical thermal increment*: a coefficient characterising the particular reaction.
k_{t+10} – the rate at 10 °C higher
Q_{10} – factor a rate increases for a 10 °C rise in temperature
$\Gamma_{P(t)}$ – product mass concentration rate, g dm^{-3} h^{-1}
P_n – production of oligosaccharide, g dm^3 h^{-1}
γ_i – the concentration with DP equal to i (in the retentate for CRMR), g dm^{-3}
γ_i lim– the limit concentration with DP equal to i (in the retentate for the CRMR), g dm^{-3}
m_n – the smallest oligosaccharide with DP equal to n,
$\sigma_{E_{act}}$ – the volume concentration of active enzymes, cm^3 dm^{-3}
E – enzyme concentration
k_n – coefficient, dm^3 cm^{-3} h^{-1}
w – mass fraction, %
σ – volume concentration, cm^3 dm^{-3}
V_R – volume reactor, dm^3

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