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A REVIEW ON USING MEMBRANE REACTORS IN ENZYMATIC HYDROLYSIS OF CELLULOSE

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Abstract

Enzymatic hydrolysis of cellulose in the conventional batch reactors is severely affected by product inhibition due to the accumulation of glucose inside. The approach of using ultrafiltration membranes has created an effective way for simultaneously glucose removal and enzyme recovery with two main configurations of membrane reactors developed by many researchers. This short review aims at examining the main features of external loop and submerged membrane reactors, i.e. its operation conditions such as substrate concentration, enzyme to substrate ratio, and mixing which affect the performance of hydrolysis in term of conversion, glucose formation, concentration polarisation and fouling. Challenges of membrane reactors were realised as low output glucose concentration, unsuitable to run at high cellulose concentrations, and the total recovery of enzymes adsorbed in the solid cellulose and liquid hydrolysate. Besides that, other two new configurations (dialysis and the modified membrane reactor) are highlighted as very potential methods to not only effectively remove glucose for minimising product inhibition and recover enzymes but also able to handle higher cellulose concentrations form 10% or higher. Further research should focus on the degree of glucose removal from hydrolysis reaction membrane reactor systems, and operational feasibility in disposal of unconverted substrate after hydrolysis.

Keywords: Membrane reactors, Ultrafiltration, Inhibition, Glucose removal, Enzymatic hydrolysis.

1. Introduction

1.1. Bioethanol process from cellulose

In the effort of searching for alternates to fossil fuel, the priority of selection should be given to renewable energy sources, which is carbon-neutral and

environmental-friendly. Bioethanol fuel technology has been attracting increasing interests in research and development in the past few decades as a potential alternate, especially from cellulose-based feedstock which can be found abundantly in agricultural residues such as wheat/rice straws, palm empty fruit bunches or sugar bagasse. Depending on the sources, cellulose content is from 30% to 50% [1-3].The cellulosic polysaccharide comprises of a linear arrangement of glucose monomers which can be liberated via some treatment processes and converted to bioethanol fuel.

The process designed for producing bioethanol fuel from cellulose consists of four main stages, i.e. pretreatment, hydrolysis, fermentation and purification (Fig. 1) [4]. One of the major challenges in the cellulosic bioethanol process is enzymatic hydrolysis to efficiently release fermentable hydrolysate which is rich in glucose as the first prerequisite [5, 6]. In comparison with acid hydrolysis, enzymatic hydrolysis offers more advantages to overcome the higher energy input due to milder operating conditions (temperature 40-50ºC, pH 4-5), avoid the formation of byproducts like furan and furfural, result in higher sugar yield, and hence improve the economics of the overall process [1, 7].

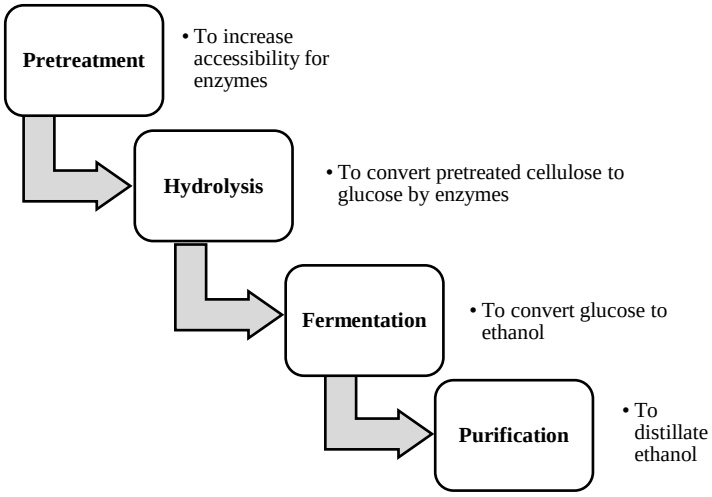

Fig. 1. Fuel ethanol process from cellulose biomass.

In order to be practical realisation, it is important for the enzymatic hydrolysis to be economically and technically feasible by reducing the enzyme cost, minimising product inhibition and designing an ideal hydrolysis reactor. First, the high cost of cellulase enzymes used in hydrolysis is identified as one of the most significant production expenses as it accounts for approximately 50% of the cost for hydrolysis and 20% of the total cost of the entire cellulosic bioethanol process [8, 9]. Secondly, inhibition of products (glucose and cellobiose) on cellulase enzymes severely retards hydrolysis or exacerbates the low reaction rate and consequently reduces glucose yield [10-12]. Mechanism of product inhibition on enzyme cellulases is showed in Fig 2. Cellulases are a synergy of three different enzymes which consists of endo-glucanases, exo-glucanases to convert pretreated cellulose to intermediate cellobiose which is then further hydrolysed to glucose by

β-glucosidases. However, cellobiose and glucose turn to inhibit its enzymes, consequently slow down the reaction and reduce conversion of cellulose [13]. Thirdly a lack of an ideal reactor system which is able to handle the complexity of interfacial heterogeneous hydrolysis involving solid cellulose substrate, cellulase adsorption and desorption, inhibition of cellulases by cellobiose and glucose, is another major difficulty in the enzymatic hydrolysis to reach its full potential [14-17]. Up to date, there are two strategies to tackle these three challenges, i.e. increase accessibility of enzymes by improving the pretreatment method and reducing product inhibition by glucose removal and enzyme recovery [5].

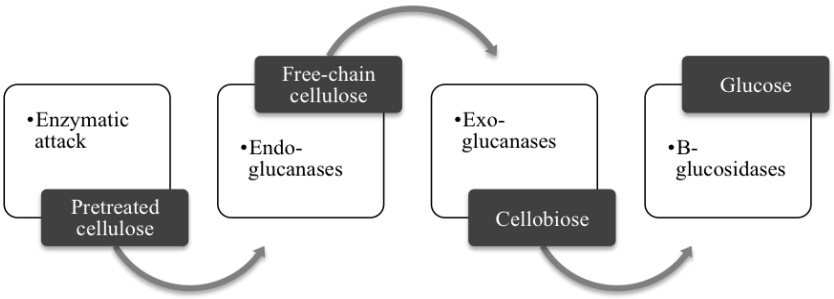

Fig. 2. Product inhibition in enzymatic hydrolysis.

1.2. Potentials of applying membranes in hydrolysis

Over the last ten years, membrane technology has been progressively explored in biorefinery with a rapid increase in the number of publications for journals and patents from 35 to nearly 200 [18]. For cellulose-based bioethanol process especially in enzymatic hydrolysis step, the application of membranes offers a solution to product inhibition by removing glucose from the reaction. The conventional methods of glucose removal by evaporation, solvent extraction, overliming, active charcoal adsorption and ion exchange actually result in an extra processing cost, increase the complexity of the overall process due to the generation of waste by-products such as gypsum, require a long processing time and cause the loss of sugar product [19-22].

However, a significant amount of enzymes which still remains active after the cellulose hydrolysis, is used only at once under the conventional batch mode [23, 24]. Therefore a membrane filtration can simultaneously remove glucose from hydrolysis for minimising product inhibition, and retain enzymes in the reactor for using in a longer period of time, hence reduce the operational cost [25]. With a suitable molecular weight cutoff, the use of a membrane would effectively retain large molecular weight enzymes, whereas allow permeation of the glucose.

Up to date, numerous research work has been performed to test with different configurations of the membrane filtration integrated with the hydrolysis reactor at various conditions to evaluate its effectiveness in technical as well as operational feasibilities, the economic viability of recycling enzymes, and separation of

glucose for subsequent fermentation stage. The obtained results were proved its suitability and technical feasibility. The use of membrane bioreactor becomes a key role for the second strategy which makes it possible to recover and reuse enzymes, has an excellent fractionation of glucose with low chemical consumption and reduces energy requirement [14, 26-28].

With the supportive literature, the purpose of this review is to appraise the continuous improvement in applying membrane reactors in enzymatic hydrolysis of cellulose regarding configurations of membrane reactors, to discuss the current challenges as well as future research perspective in this field.

2. Application of Filtration Membranes in Enzymatic Hydrolysis

2.1. Effect of product inhibition and limitations of batch reactors

The presence of cellobiose and glucose affects the performance of the enzymatic hydrolysis of cellulose. To minimise product inhibition, the excessive addition or supplement of β-glucosidases extracted from fungus *Aspergillus niger* is made to quickly convert the intermediate cellobiose to glucose [13]. However, still the conversion of cellulose in enzymatic hydrolysis is interfered by the inhibition of glucose on enzymes.

Based on theory, the inhibition on enzymes follows the non-competitive product inhibition [6]. From Eq. (1), as the reaction progresses with increasing glucose formation (*P* increases), the reaction rate *dG/dt* will decrease consequently. Graphical expressions of the reaction rate versus yield, and the reaction rate versus glucose concentration modelled by [13] show a reduction of the reaction rate occurring at the initial stage of hydrolysis to a lesser extent but significantly decrease and approach retardation as glucose concentration increases.

$$\frac{dG}{dt} = \frac{n\, k_{cat}\, E_o\, S}{(K_M+S)\left(1+\frac{P}{K_{IP}}\right)} \quad (1)$$

where *P*, product inhibitor concentration (mM); K_{IP}, disassociation constant for enzyme inhibitor complex (mM); K_M , Michaelis-Menten constant (mM); k_{cat}, turn over number (h^{-1}); E_0, initial enzyme concentration (mM); and *S*, substrate concentration (mM).

Other experimental work has reported the profound effect of product inhibition on decreasing the rate of reaction especially under high cellulose substrate concentrations [6, 13, 29-31]. The decrease in the reaction rate varies from 10% to nearly 100% depending on experimental setups, and reaction conditions such as enzyme to substrate ratio (*E/S*), substrate concentration. It is found that the occurrence of product inhibition is decided by the ratio of glucose inhibitor to enzymes, whereas glucose concentration plays a key role in the extent of inhibition by decreasing the reaction rate [13].

Conventional batch reactors with the charge of materials at the beginning and discharge of products as well as by-products at the end of the reaction actually exacerbate the product inhibition in hydrolysis due to the accumulation of glucose inside reactors. A research on the hydrolysis of cellulose at substrate concentrations from low to high level (2%-40%) in a batch reactor by Andric et al. [13] concluded that product inhibition occurred at all substrate levels.

Although the consumption of cellulose substrate constituted to the decrease in the reaction rate to a lesser extent, the main contribution to this reduction was mainly the inhibitory effect of glucose on enzymes. Particularly the severity of product inhibition was observed at high substrate levels [32]. With batch hydrolysis, enzymes instead of being recycled and reused for new batches, can be used at once only and an addition of new enzymes is needed for a new batch of hydrolysis. Therefore, the use of batch reactors for enzymatic hydrolysis of cellulose brings some limitations in term of the low extent of cellulosic conversion to glucose, a rapid reduction in the reaction rate, enzyme loss without being recycled and product inhibition caused by the accumulation of glucose inside the batch reactor [21].

Due to the presence of glucose causing product inhibition, it is necessary to separate glucose from the reaction. In the review of Andric et al. [13], models of continuous reactors, i.e. continuous stirred tank reactors (CSTR) and plug flow reactor (PFR) were investigated its effectiveness to replace batch reactors. As a result, a correlation was found between conversion and volume of the reactor. Basically, in order to have a continuous conversion of cellulose, it required to design a continuous reactor at a very large size with low yield and low glucose output concentration. In fact, for a CSTR and a PFR, the reactor size increases profoundly with the increase of product concentration for a higher conversion.

This is the case of product inhibition exclusion. If product inhibition is taken into account, an increase from 2 to 10 times for CSTR or 1.5 to 6 times for PFR is required for cellulose conversion of 15% and 80% respectively. A CSTR requires a volume of 293 m^3 and volume for PFR is 75 m^3 with glucose inhibition compared to without glucose inhibition at 31 m^3, and 12.8 m^3 respectively for obtaining a low glucose output concentration of nearly 9 g/l.

Another similar study based on simulation of a membrane continuous reactor for hydrolysis of cellulose also concluded conversion of cellulose to glucose largely depends on the reactor volume [22]. In the study, simulated results reveal for a conversion of 50% at a very low substrate loading of 4.6% the required volume for a CSTR attached with a membrane was 125 m^3 for glucose concentration of 13%. In both studies, the demand for a very large reactor to facilitate the enzymatic hydrolysis was agreed to be economically unfeasible as this would induce a large capital investment for the construction of huge reactors while the low concentration of output glucose is not satisfied. Therefore, for batch and continuous processes in which product inhibition is sensitive, the extent of conversion is significantly affected or even retard the hydrolysis reaction rate.

A better approach of performing the enzymatic hydrolysis is indeed very important to consider for achieving a higher conversion of cellulose, minimizing product inhibition, operating at a higher substrate concentration, removing glucose and recovering enzymes. The strategy of product removal from the reaction is believed to be the best alternative for minimizing inhibition of product by integrating a membrane separation unit with the reactor [13].

2.2. Membrane reactors for hydrolysis

The concept of applying membrane reactors for the hydrolysis of cellulose via enzymatic hydrolysis has been appealing interest in many studies and research to

work on different aspects of the process. Membrane reactors provide a unique advantage of simultaneous enzyme recovery and glucose removal which are lacked in the conventional batch reactors and continuous reactors like CSTR and PFR.

The mechanism of the membrane separation integrating with a hydrolysis reactor is mainly about the selectivity of components based on their molecular weight (1 g/mol = 1 Dalton or 1 Da). In most studies, ultrafiltration membranes are widely used in the filtration process to selectively fractionate glucose and separate enzymes cellulases as well as cellulose because the molecular weight cutoff of ultrafiltration is in the range from 5 to 50 kDa [8, 9]. Particles with small molecular weight such as glucose (180 Da) and cellobiose (300 Da) can permeate through the ultrafiltration membrane, whereas particles with large molecular weight like cellulose (about 10,000 Da or 10 kDa) and enzyme cellulases (5 kDa to 90 kDa) are retained at the retantate of the membrane. Table 1 shows the molecular weight of each component in the membrane filtration. The suitability of the membrane filtration integrating in the enzymatic hydrolysis process has been confirmed in many research work using various enzyme systems with different cellulose substrates and reached a conclusion that the separation by using ultrafiltration membranes shows a complete rejection cellulose and enzyme cellulases, and zero rejection of glucose [5, 13, 14].

Table 1. Molecular weight of some components in hydrolysis [6, 13, 18].

Components	Molecular weight
Glucose	180 Da
Cellobiose	300 Da
Enzyme cellulases	5-90 kDa
Cellulose	> 10 kDa

The effect of product removal via membrane filtration on the performance of the enzymatic hydrolysis reaction was studied intensively to compare with hydrolysis in batch mode. It is concluded in some studies that the removal of glucose by using membrane reactors results in a higher conversion of cellulose than that in batch reactors as can be seen in Fig. 3 [15, 33-35]. In addition, by using aspen wood and wheat straw as a source of cellulose substrate, hydrolysis yield of glucose increased substantially by 35% and 31% respectively after 24 hours when performing enzymatic hydrolysis with intermittent product removal in comparison with continuous hydrolysis without interruption in 48 hours [36].

A study on a system consisting of a reactor coupled with ultrafiltration running at high substrate concentration of 100 g/L and enzyme to substrate (*E/S*) ratio of 0.04 (w/w) reported that product inhibition was not encountered whereas the batch enzymatic hydrolysis with a lower substrate concentration of 40 g/L and *E/S* of 0.1 (w/w) encountered the inhibition of product glucose on enzymes [37]. An increase by six folds was obtained in the total reducing sugar including cellobiose and glucose under fed-batch or semi-continuous hydrolysis in comparison with batch hydrolysis [38].

In overall, the problem of inhibition by the inhibitory products such as glucose is encompassed by the membrane reactors, which results in a higher extent of cellulosic conversion than the conversion achieved in batch hydrolysis. Especially

the conversion in hydrolysis of cellulose could reach from 70% to 90% [13]. Variation in the achieved conversion from different studies is mainly due to the reaction conditions with respect to the types of cellulose substrates, substrate concentration, the enzyme system, enzyme to substrate ratio, reaction time, the molecular weight cutoff of ultrafiltration membranes. On the other hand, low conversion of less than 70% was due to the accumulation or build-up of cellulose substrate and enzymes on the surface of the membrane causing interruption in hydrolysis in the reactor and affecting glucose removal [14].

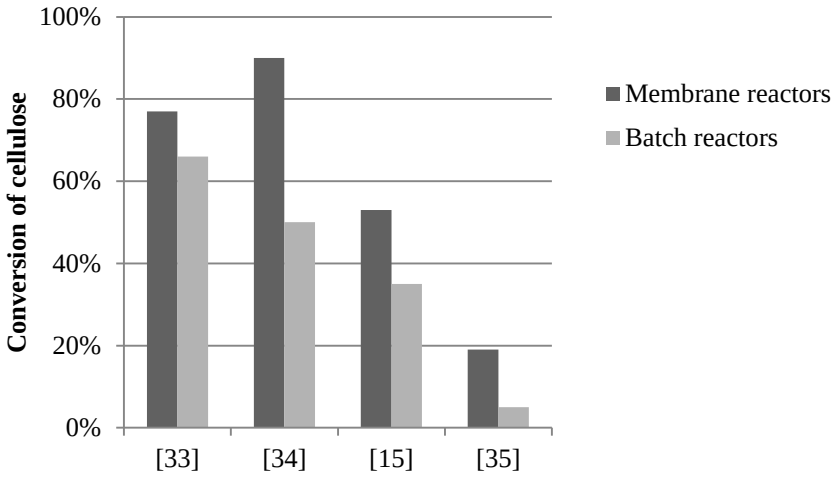

Fig. 3. A comparison of cellulose conversion between membrane reactors and batch reactors in some studies.

The employment of an integrating system between a reactor with a membrane separation unit offers many advantages for an effective enzymatic hydrolysis of cellulose, which is heavily limited under the conventional batch or continuous reactors. The major advantages of product removal via membrane reactors during the enzymatic hydrolysis are summarized as followed [13].

- Allow the enzyme cellulases to be utilized in a longer period of time due to the retention of enzymes in the reactor by the membrane separation.
- Achieve a higher conversion of cellulose as product inhibition is minimized by the removal of glucose from the reacting system.
- Obtain a stream of glucose product collected at the permeate of the membrane free from impurities such as unconverted cellulose, enzymes which can negatively affect the subsequent fermentation process.
- Maintain the product stream at a constant concentration without adding enzymes for further hydrolysis.

The major concern of product inhibition has been proved to be technically solved and research is ongoing to make more improvement with respect to optimization in reaction conditions, testing with new configuration and experimental setups for the membrane reactors used in the enzymatic hydrolysis.

2.3. Configurations of membrane reactors

A membrane reactor system consists of a reactor and a membrane separation unit which can be arranged into two main configurations as shown in Fig. 4. For external loop membrane reactors, transportation of the reaction media via pumping from the reactor to the membrane unit is needed for filtration in which glucose permeates through the membrane while cellulose and enzymes are retained and recycled back to the reactor. The external membrane filtration unit can be arranged in various setups such as a reactor coupled with ultrafiltration unit [10, 23, 37], reactor coupled with an adsorption column [38], reactor coupled with a flat sheet membrane unit or tubular membrane unit [39], reactor coupled with a series of ultrafiltration, nanofiltration and electro-dialysis [5]. For submerged membrane reactors, the membrane filtration unit is located inside the reactor without a pumping effort. Flat sheet membrane with dead-end flow is widely used for the setups of submerged membrane reactors [15, 35, 40, 41]. A side membrane reactor was used in the work of Al-Zuhair et al. [42] whereas tubular membrane was tested by Belafi-Bako et al. [14]. Due to the in-situ removal of glucose in membrane reactors, supplement of fresh buffer which may contain additional enzymes or cellulose is required in order to maintain a constant volume of the reaction [13].

Under these two configurations, various experimental set ups were studied to investigate its suitability in applying for enzymatic hydrolysis of cellulose biomass. Direct comparison of the results is difficult due to the differences in operation conditions such as types of substrate and enzymes, enzyme to substrate ratio , substrate concentrations, degree of mixing, types of membranes and modes of operation [6, 13].

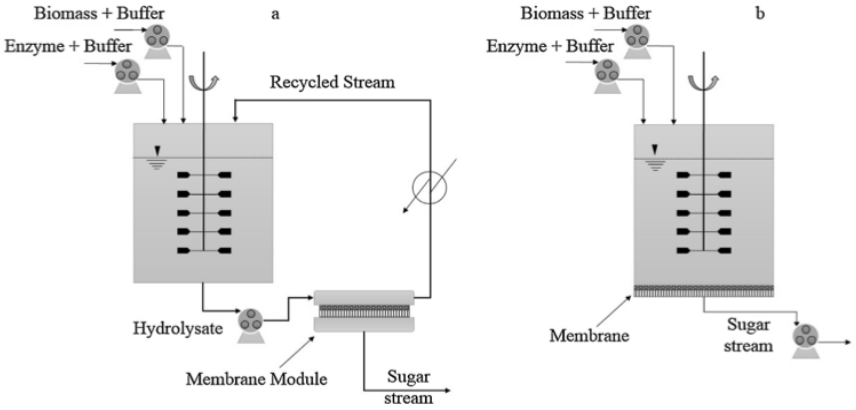

Fig. 4. Schematic diagrams of (a) an external loop membrane and (b) a submerged membrane reactor [40].

2.3.1. Substrate concentrations

Membrane reactors used for the enzymatic hydrolysis are restricted to operate at a low substrate concentration up to 5% (w/v) in most experimental setups as listed in Tables 2 and 3 regardless of configurations or arrangements of

membrane such as flat sheet, at side or in tubular forms. In case of the external loop membranes, running at a low substrate concentration allows an easier transportation of the reaction mixture containing substrate, enzymes and glucose to the external filtration unit, whereas a high substrate concentration can cause a difficulty in pumping due to the high viscosity of the slurry reaction mixture [40]. Another reason is to minimise the effect of membrane fouling caused by the accumulation of solid cellulose on the membrane surface [13]. At a higher substrate concentration from 10% to 15% (w/v), fouling on the membranes were reported in the studies of Malmali et al. [40] and Gan et al. [15].

2.3.2. Enzyme to substrate ratios

The complete rejection of cellulase enzymes by ultrafiltration provides a flexibility for membrane reactors to run in a wide range of the enzyme to substrate (*E/S*) ratios without enzyme loss from 0.4% to more than 100% (w/w) as found in Tables 2 and 3. At a low *E/S* ratio of 0.4% (w/w), glucose concentration was the highest at 10 g/l. In contrast at a higher *E/S* ratio of 2% (w/w), enzymatic productivity increased significantly by three folds when substrate concentration was fixed at 2.5% (w/v) in hydrolysis of alpha-cellulose fibre [15].

Enzyme concentration is one of the factors that affects the formation of glucose because of their direct proportional relationship [15]. Changes in the enzyme concentration result in changes in the ratio of enzyme to substrate. On the one hand, low *E/S* ratios in the range of 2.5%-10% result in a cellulosic conversion at approximately 50%. On the other hand, at enzyme to substrate ratios of higher than 10%, conversion can be achieved up to 90% as shown in Tables 2 and 3. In the study on optimization of the hydrolysis process using the membrane reactor, conversion of cellulose derivative CMC reached a maximum of 90% at *E/S* ratio of 25% (w/w) [41]. A similar result is obtained with 91% conversion of Solke-Floc cellulose in an external loop membrane (hollow fibre cartridge) coupled with a CSTR at *E/S* ratio of 171% (w/w) [34]. Moreover, under semi-continuous and continuous product removal at a low *E/S* ratio of 0.4% (w/w), conversions at 51% and 53% respectively were obtained in hydrolysis of alpha cellulose fibre from hard wood pulp powder [15]. Beside the advantage of being able to run at high *E/S* ratios leading to a higher conversion, concentration polarization of enzymes entrapping on the membrane surface was observed. This caused a reduction in enzyme concentration inside the reactor and a resultant decrease in the reaction rate, especially at an elevated flow rate of product removal [15].

2.3.3. Modes of operation, types of membranes and setups

A variation in modes of operation and types of membranes arranged in different setups has been studied extensively by different researchers. The accumulation of the inhibitory glucose inside the batch reactor is effectively solved by the use of membrane reactors for hydrolysis in which glucose is removed in two modes, i.e. continuously and non-continuously (intermittently). A majority of the research focuses on hydrolysis in membrane reactors with continuous product removal to make comparison with the hydrolysis in batch reactors [10, 14, 41,

43]. Cellulose conversion obtained from hydrolysis in membrane reactors is significantly higher than that from hydrolysis in batch reactors as shown in Figure 3. However, the modes of product removal in the membrane reactors whether continuous or intermittent shows an insignificant difference in conversion of cellulose.

A study on the saccharification of alpha-cellulose extracted from hard wood via an enzymatic hydrolysis reactor with a submerged flat sheet membrane unit reported the cellulosic conversions of 51% under intermittent and 53% under continuous product removal modes [15]. Similarly the cellulosic conversion was insignificantly different between batch feeding and continuous feeding of the substrate [39]. A very high conversion of 90% was achieved within one hours and nearly 100% after four hours in the study of the reactor coupled with an external flat sheet membrane unit operating with batch feeding. While the continuous feeding of fresh cellulose resulted in 95% conversion [39]. However, the continuous substrate feeding mode can cause an increase in the cellulose substrate concentration in hydrolysis reactor when the rate of cellulose consumption for hydrolysis reaction is less than the rate of continuous cellulose addition.

Three types of membranes, i.e., ultrafiltration, nanofiltration and microfiltration were tested. For the simultaneous enzyme recovery and product removal, ultrafiltration with molecular weight cutoff of 10 kDa is widely used in hydrolysis because of its total rejection of enzyme cellulases and complete permeability of glucose [23, 37, 39]. The addition of nanofiltration is to increase the concentration of glucose before proceeding to fermentation. Enzyme loss to the permeate side of the membrane was reported when using microfiltration due to its larger molecular weight cutoff which enzyme molecules can pass through [40].

Experimental set-ups were varied for the hydrolysis reaction to facilitate the enzyme recovery and product removal. All these setups can be categorized into two configurations, i.e. submerged membrane or an external loop membrane reactor. Tables 2 and 3 summarise some important studies on the membrane reactors used for hydrolysis, focus on substrate concentrations, enzyme to substrate ratios, mode of product removal, types of membranes, its cutoff and results with conversion of substrate. The integration of membrane filtration into hydrolysis reactors offers an advantage of simultaneous hydrolysis and separation. However, the disadvantage of this integration is membrane fouling which occurs when testing with these setups, due to the blockage of solid cellulose on the surface of membranes during separation. This leads to a restriction for both configurations to operate at low substrate concentrations of less than 5% in order to minimise fouling [13].

Therefore, the presence of cellulose on the one hand needs for hydrolysis, on the other hand becomes a limiting factor for separation. Improvement on the current configurations should be considered for not only simultaneous hydrolysis with separation but also minimising fouling.

Table 2. Studies on the external membrane reactors.

[S] %	[E/S] %	Product removal	Membranes		Results		Ref.
			Type	Cutoff (kDa)	Conversion %	Others	
0.045	171	Continuous	UF	50	90	Lower conversion (40-70%) in the batch reactors.	[34]
5	6	-	UF	10,30, 50,100	-	Glucose rejection less than 100% for all membranes. 100% enzyme rejection for 30 kDa membrane.	[23]
10	4	Continuous	UF	5	40	Product inhibition was encountered in batch mode but not in membrane reactors.	[37]
2	26	Continuous	Dialysis	1	-	Product inhibition was encountered in batch reactors. In-situ product removal results in a substantial decrease, followed by a regain by 50% at 24 mg/l.h.	[10]
10	60	Continuous	Adsorption column		-	Glucose formation in fed batch was 6t times higher than in batch mode.	[38]
2	3.08 ml/g	Non-Continuous	UF	5, 10	90 >95	For flat-sheet membrane reactor. For ceramic membrane reactor.	[39]
1	5	Continuous	UF	10	60	Glucose concentration obtained was at 6 g/L.	[5]

Notes: [*S*]: substrate concentration, [*E/S*]: enzyme to substrate ratio, UF: ultrafiltration, NF: nanofiltration.

Table 3. Studies on the submerged membrane reactors.

[*S*] %	[*E/S*] %	Product removal	Membranes		Results		Ref.
			Type	Cutoff (kDa)	Conversion %	Others	
2.5	0.4-2	Continuous & non-continuous	UF	10	Batch (35%), non-continuous (51%) and continuous (53%).		[15]
10, 15	5	Continuous	MF	-	-	Reaction rate in batch was lower than that in continuous mode.	[40]
0.0103	32	Continuous	UF	10	-	The highest glucose concentration was 0.09 g/L. Space velocity and conversion are related.	[44]
3.1	-	Continuous	UF	10	-	A transient behaviour was observed when an increase in reaction rate followed by a steady state increase in flowrate, and caused low glucose concentration.	[43]
0.15	12.5-100	Continuous	UF	10	90	Optimisation of the hydrolysis in membrane reactors was conducted.	[41]
4.6	2	Continuous	UF	10	50	From the simulation results, a required volume of 125 m^3 to achieve 50% conversion was not economical.	[42]
2.5	5	Continuous	UF	30	Conversions of 48% for flat sheet and 70% tubular membrane reactors.		[14]
2.5	0.42	Non-continuous	UF	2	Low conversion of 5% for batch reactor, while 15% conversion for membrane reactor as enzyme productivity increased from 0.53 to 2.37		[35]

Notes: [*S*]: substrate concentration, [*E/S*]: enzyme to substrate ratio, UF: ultrafiltration, NF: nanofiltration.

2.3.4. Effects of mixing on hydrolysis

Beside the effects of some key process parameters (substrate concentration, enzyme to substrate ratio, modes of operation) on the cellulosic conversion and the glucose concentration during the membrane filtration as discussed earlier, the influence of other parameters such as stirring speeds was studied. In the two configurations of the membrane reactors used for the hydrolysis reaction, mixing on the one hand helps to improve the homogeneity of the reaction mixture inside the reactor, on the other hand ensures a sufficient contact between enzymes and cellulose. Studies on the influence of mixing on the performance of the enzymatic hydrolysis reactors reveals inconclusive results which contradict between authors in a review done by Van Dyk and Pletschke [1].

The intensity of mixing in the membrane reactors contributes a significant impact on the hydrolysis reaction under a certain range as reported in some studies. It is stated that having a stirring speed above 200 rpm lowered the activity of enzyme and thus affected the enzymatic hydrolysis reaction, while a maximal stirring speed of 400 rpm was recommended [45]. Especially at a high substrate concentration, an increase in the intensive mixing could help to ensure a good mixing, hence allow the binding of cellulases on cellulose [40, 46]. In one experiment which handles at a high substrate concentration of 100 g/l (10% w/v) under stirring speeds from low (100 rpm) to high (600 rpm) in a batch hydrolysis, a higher glucose concentration of 50 g/l was obtained at a high stirring speed of 600 rpm in comparison with the lower glucose concentration of 30 g/l at 100 rpm [40].

In contrast, an insignificant effect of the mixing intensity on the conversion as well as glucose formation rate in hydrolysis was found in some other research [15, 36, 41]. In an attempt of recovering the enzyme cellulases remained in the liquid hydrolysate by two methods of adsorption which were substrate plug, i.e. pouring the hydrolysate into a column of new cellulose substrate, and vigorous shaking in the reaction container. In Fig. 5, at 6% substrate concentration, the adsorption of enzymes was insignificantly different since the percentage yield are 11.7% for the substrate plug and 9.7% for the vigorous shaking [36].

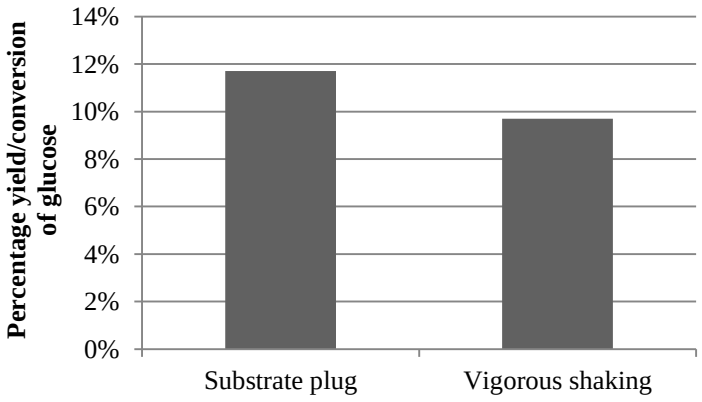

Fig. 5. Adsorption of enzymes by two methods [36].

Other results were found in the enzymatic hydrolysis using a submerged membrane reactor [15, 41]. By operation at two stirring speeds of 50 rpm and 90 rpm, the final concentration of glucose at 50 rpm is 7 g/l which was insignificantly different with the concentration of glucose of 11 g/l at 90 rpm under 1% (w/v) cellulose substrate concentration as shown in Fig. 6 [15].

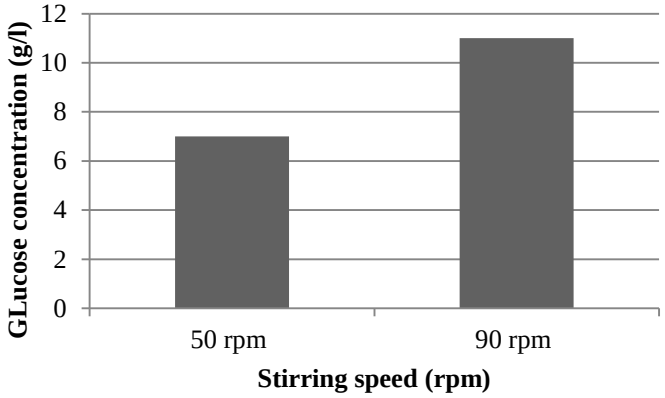

Fig. 6. Study on the effect of stirring speed on glucose concentration [15].

With a wider range of stirring speed between 300 rpm and 1200 rpm at 1.5% (w/v) substrate concentration and 12.5% (w/w) enzyme to substrate ratio, the conversion of cellulose only increased gradually as the stirring speed increased by 4 folds from 300 rpm to 1200 rpm [41]. Therefore, it is concluded that the intensity of the stirring has an insignificant effect on the yield of sugar converted from cellulose and the resultant glucose concentration in the enzymatic hydrolysis.

Unlike the complete mixing of the reaction media containing solid cellulose, enzymes, and buffer in the current design of the membrane reactors, the homogeneity required for the reaction mixture inside the hydrolysis reactor becomes less important in the case of in situ product removal via dialysis [10]. The working mechanism of dialysis as a separation technique is based on concentration gradient of solute on both sides of the dialysis membrane with a molecular weight cutoff of 1 kDa in the study which allows simultaneously permeation of glucose molecules through the dialysis membrane and retention of enzymes and cellulose within the membrane [10]. In Fig. 7, cellulose substrate and enzymes were actually contained within the dialysis membrane while the agitation is in the other side of the membrane. Thus, it is obvious that the intensity of mixing for the reaction media in the enzymatic hydrolysis seems not to be very important. In comparison with product removal in the membrane reactors under the same reaction conditions of 350 rpm and 2% (w/v) substrate concentration, the recovery of the product glucose in the permeate side of dialysis membrane was 94% which is higher than that of 59% obtained from the hydrolysis in the ultrafiltration membrane reactor. Furthermore, the rate of glucose formation or reaction rate at 64 mg/l.h obtained in dialysis was considerably higher than that at 28 mg/l.h in the membrane reactor [10].

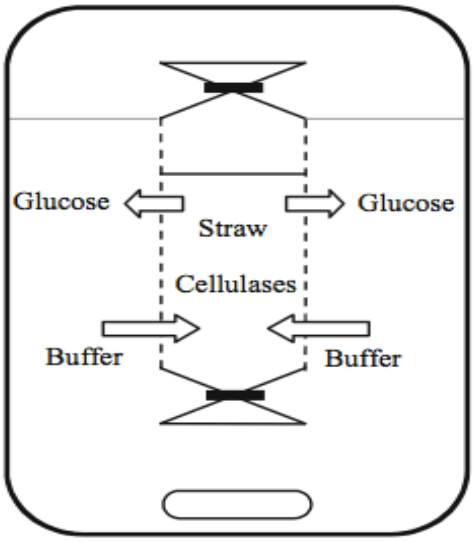

Fig. 7. A schematic diagram of in situ product removal by dialysis [10].

Throughout these studies on the effect of mixing on the enzymatic hydrolysis of cellulose under the different configurations (batch, membrane reactors and dialysis), the effect of the stirring speed in round per minute (rpm) on the performance of hydrolysis in term of the conversion, and reaction rate can be said to be inconclusive whether significant or insignificant.

2.3.5. Glucose removal

The employment of membrane reactors in hydrolysis results another critical process parameter, i.e. the degree of product removal from the reactor. Because it plays a role in the cellulosic conversion, glucose concentration at permeate, product inhibition and concentration polarization on the membrane. The studies on the degree of product removal in hydrolysis of cellulose are only available in a limited number of research work. The degree of product removal is expressed in various terms, for example space velocity [44], tangential velocity [37], mass transfer coefficient [42] and removal rate [23]. Due to the accumulation of the glucose product inside the hydrolysis reactor causing inhibition on the cellulolytic enzymes (cellulases), the removal of glucose is necessary to minimise the inhibitory effect. However, inclusive results were reported regarding the degree of glucose should be removed.

The level of the glucose inhibitor should be kept at low as possible in the reactor [13]. But maintaining a low glucose concentration in the reactor requires an increase in the removal rate. Correspondingly the dilution rate of the fresh buffer must increase to keep a constant reaction volume. As a consequence, glucose in the permeate stream is over diluted or have a low concentration, which leads to either an additional concentrating equipment to gain the glucose concentration sufficiently for the fermentation stage or bear in more cost in the distillation process of ethanol [13].

In the simulation work of a membrane bioreactor for hydrolysis, the conversion of cellulose was related closely to mass transfer coefficient. As a significant increase in the mass transfer coefficient from 100 to 1500 m3/h corresponded to an increase in the conversion of glucose from 40% to 50%. An explanation was that more glucose removed by the increase of mass transfer coefficient left a less amount of glucose in the reaction media. Hence the product inhibition was reduced via the selectivity of the type and porosity of the membranes [42].

However, the effect of the tangential velocity on the yield or conversion of cellulose was obtained differently as the tangential velocity varied from 0.3 m/s to 1.1 m/s [37]. At the highest velocity of 1.1 m/s, the yield (%) and corresponding instantaneous productivity (g/g) reached the highest at more than 40 % and above 30 g/g respectively in comparison with the lower velocities of 0.3 and 0.6 m/s. But the concentration polarization on the membrane surface where the enzyme concentration was higher than that in bulk was observed. This is because there was a declined in permeate flux at the velocity of 1.1 m/s in the beginning followed by a regain. However constant permeate flux profiles during the entire hydrolysis course were obtained at the lower velocities.

In a study on kinetics of enzyme hydrolysis of cellulose in a submerged flat-membrane reactor, an introduction of a term 'space velocity' - a quotient of the flow rate to the reactor volume was brought up another finding on the relationship between the conversion, maximum glucose concentration under the control of space velocity [43, 44]. The maximum concentration of glucose in the enzymatic hydrolysis was obtained when the glucose formation rate is equal to the product removal rate, i.e. literally the amount of glucose liberated from cellulose was totally removed. Thus the inhibitory glucose concentration would be as low as possible and the amount of time needed to reach the maximum glucose concentration depends on the space time which is a reciprocal of space velocity. A lower space time resulted in the faster the maximum glucose concentration to reach.

A further study on the effect of space velocity from low to medium and high (0.168 to 0.460 h^{-1}) on the cellulosic conversion shows that the conversion increased as the space velocity increased from low to medium values. An increase in the space velocity to higher values led to an decrease in the conversion due to the concentration polarization of enzymes on membrane surface and the resultant reduction in the glucose concentration in the permeate [44]. This effect was confirmed again by the same authors in 1983 in the study on the extension of a transient behaviour as the glucose concentration reduced after reaching a maximum in the elevated space velocity [43]. The degree of the extended transient behaviour is dependent on the space time. Therefore, the continuous increase in the flow rate of product removal in enzymatic hydrolysis actually results in the negative effect on a decline in conversion due to concentration polarization, and the time required to reach the maximum glucose concentration. The space velocity was recommended to be maintained at low to medium values [43].

In the previous study on the enzymatic hydrolysis of cellulose conducted in a modified membrane reactor, a similar result which agrees with the obtained result from Alfani et al. [43] shows that the concentration of released glucose is dependent on the flow rate of glucose removal from the membrane reactor [47]. At 3% enzyme to substrate (*E/S*) ratio, an increase in flow rate from 3 to 15 ml/min led to a remarkably increase in glucose concentration from around 1

ml/min to nearly 9 ml/min. In comparison between different *E/S* ratios and flow rates, although with a lower *E/S* ratio of 3% and 15 ml/min flow rate, concentration of glucose is actually 2.5 times significantly higher than that at a higher *E/S* ratio of 6%, 30 ml/min (Fig. 8). Therefore a selection of a suitable flow rate of product removal in the range from low to medium and the corresponding the concentration of the inhibitory glucose in the reactor should be further studied at various enzyme concentrations and resident time [43].

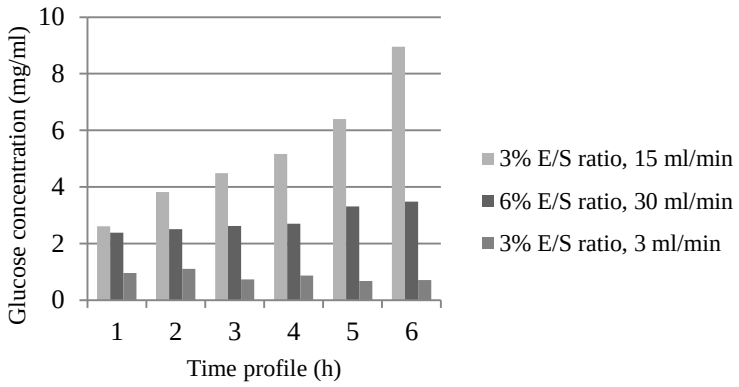

Fig. 8. Hydrolysis of cellulose at various *E/S* ratio, and flow rate of glucose removal [47].

Surprisingly there is a very limited number of research work studying on the glucose removal for the membrane reactor in hydrolysis. Arising questions are how much the final glucose product should be removed from the reaction system and whether the rate of glucose removal is constant or changed during hydrolysis as glucose concentration increases. Therefore, more in-depth studies should be conducted to determine the degree of product removal sufficiently for not only having a higher conversion but also avoid concentration polarisation of enzymes in enzymatic hydrolysis.

2.3.6. Enzyme recovery

The recovery of enzymes by ultrafiltration membranes is limited at the amount of enzymes present in the liquid phase of the reaction media, while the amount of enzymes adsorbed on the solid cellulose has not been studied sufficiently except few papers [48-49]. A rapid enzyme adsorption onto the solid cellulose was observed at the initial stage of hydrolysis due to the significant reduction of the enzyme concentration in the liquid phase. The amount of enzyme adsorbed by cellulose substrate was approximately 50% of the total enzymes added into the reactor and remained to be bound on the solid residue even the hydrolysis was completed while the rest 50% of the added enzymes were free in the liquid phase [48, 50]. Therefore, the use of ultrafiltration membranes for hydrolysis is obviously only for retaining free enzyme in the liquid phase, whereas the bound enzymes in the solid phase would be discharged with the unconverted substrate in the end of hydrolysis.

With respect to the recovery of enzymes in the liquid phase, a total rejection of the enzyme cellulases in the ultrafiltration membranes was achieved. Under the external loop membrane configuration, the enzyme retention capacity of ultrafiltration membranes (5 kDa and 10 kDa cutoff) shows 98% of enzymes being retained, plus the retained enzymes were able to continuously run up to 9 cycles in the extended hydrolysis [39]. Quantification of the enzyme activity in the permeate of the ultrafiltration 5 kDa cutoff was only 1.5% which indicates a complete rejection of the large cellulase molecules [37].

With respect to the recovery of enzymes in the solid phase, an additional desorption process is able to extract the bound enzymes back into liquid phase at an optimal condition of 44.4ºC, pH 5.3, surfactant concentration 0.5% Tween 80. With the recovered enzymes from the desorption, the yield obtained in the subsequent round of hydrolysis increased by 25% [51].

2.4. Two new configurations of membrane reactors

The concept of glucose removal and enzyme recovery by membrane filtration in hydrolysis is also applied in other two configurations which are different from the external loop and submerged membrane reactors. In the work of Andric et al. [10], dialysis as a separation technique is based on concentration gradient of solute on both sides of the dialysis membrane with a molecular weight cutoff of 1 kDa (Fig. 7). It allows simultaneously permeation of glucose molecules through the dialysis membrane and retention of enzymes and cellulose within the membrane. As can be seen in Fig. 7, cellulose substrate and enzymes were actually contained within the dialysis membrane while the agitation is in the other side of the dialysis.

A modification on the design of the submerged membrane reactor was made to create a new but simple configuration which is not only able to handle a higher substrate concentration at 10% (w/v), minimise fouling, but also ensure the effective removal of glucose for minimising the product inhibition [47] as shown in Fig. 9. The modified submerged membrane reactor is based on two main changes. First is from the completed mixing of the reaction media to the use of a suspended mesh ball/infuser. This mesh ball contains cellulose, and at the same time infuses glucose into the liquid hydrolysate. Second is from the submerged membrane and external loop membrane reactors to the ultrafiltration membrane device in a smaller size submerged in the hydrolysis reactor (Fig. 10). The enzymatic hydrolysis of cellulose was carried out at a high substrate concentration of 10% (w/v) with various enzyme to substrate ratios from 1.5% to 6% (w/w) and different flow rates of product removal in the range from 3 ml/min to 30 ml/min. As a result, it shows that the glucose concentration obtained from the hydrolysis conducted in the modified configuration of the membrane reactor was remarkably higher than that in the batch reactor due to the effective product removal. Moreover, the new configuration was able to run at a low enzyme to substrate ratio of 3% (w/w) which produced the highest glucose concentration at almost 9 g/l in 6 hours in comparison with the rest of other enzyme to substrate ratios of 1.5% and 6%.

Unlike the submerged membrane and external loop membrane reactors which requires a good mixing of the reaction content for hydrolysis, mixing becomes

less important for the two new configurations (dialysis and the modified configuration of membrane reactor) developed by Andric et al. [10] and Nguyen Huynh and Nithyanandam [47] because cellulose is actually contained either in the dialysis membrane (Fig. 7) or in the mesh ball (Fig. 9) which is submerged in the reactor. Product removal by dialysis and the modified configuration of membrane reactor are promising to be effective methods of conducting hydrolysis of cellulose using enzymes by offering the reactor to handle at higher substrate concentrations from 10% and higher, minimisation of product inhibition and enzyme recovery.

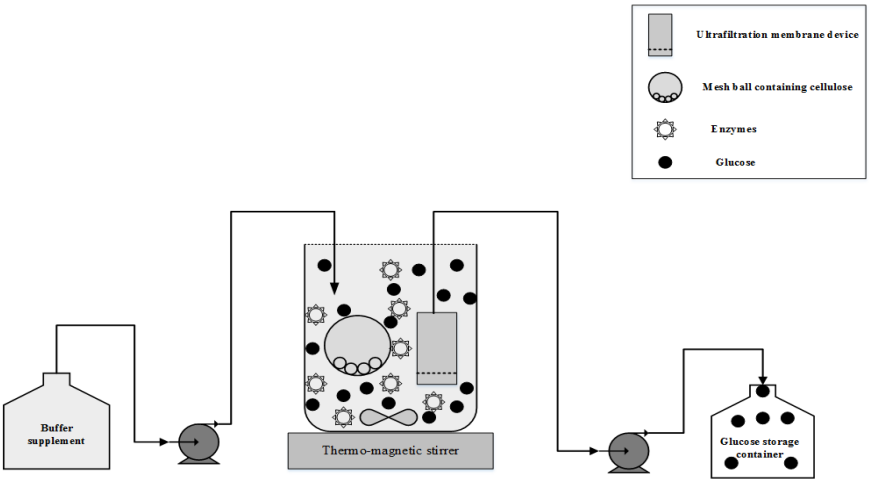

Fig. 9. The modified configuration of membrane reactor for hydrolysis [47].

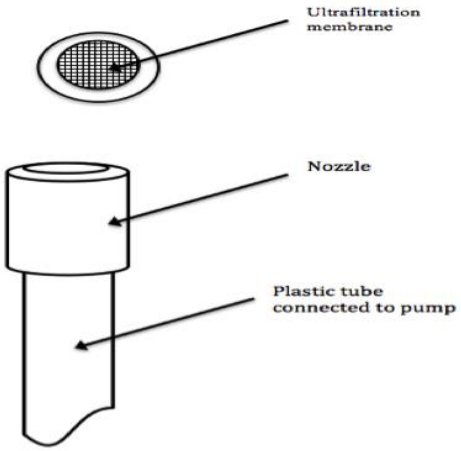

Fig. 10. Submerged ultrafiltration membrane device [47].

2.5. Challenges of membrane reactors in enzymatic hydrolysis of cellulose

To further enhance the enzymatic hydrolysis of cellulose in the membrane reactor, the challenges relating to the output concentration and operational feasibility are very important to be addressed.

The first challenge is the low concentration of glucose at less than 1% at the output of the membrane filtration which will eventually lead to a low ethanol concentration in fermentation and possibly add an extra cost in the purification stage [13]. The low glucose concentration is due to the fast product removal from the reactor by increasing the flow rate of the effluent with the purpose of minimising product inhibition.

The second challenge lies in the operational feasibility of the current membrane reactors, which is unsuitable to run at high substrate concentrations. An increase in the severity of the membrane fouling would result from the increase of the substrate concentration to 10% or higher. The insoluble cellulose substrate suspended in the reaction mixture will accumulate on the surface of the membrane causing fouling or blockage and resultant reduction in the membrane flux [13, 15]. So far some efforts have been made to fix the problem of membrane fouling such as vigorous stirring in the retentate side of the membrane or increasing the rate of glucose removal or transmembrane pressure for higher permeate flux, which unfortunately results in concentration polarisation of enzymes near the membrane surface [14]. Other solution of applying in situ electro-kinetic membrane cleaning was proposed but not really effective since the effect was only temporary and soon fouling reoccurred [15].

Thirdly, only the unbound enzymes which is free in the liquid phase were recovered by the current use of ultrafiltration in the membrane reactors whereas the amount of bound enzymes accounting for approximately 50% of the total added enzymes on the solid phase have not recovered yet except few studies [48, 51]. On the other hand, in the case of ultrafiltration retaining enzymes which is well-mixed with the substrate inside the reactor, the tasks such as disposal of unconverted substrate after hydrolysis and the subsequent refill of new substrate into the membrane reactors could probably create new issues on the operational feasibility of the membrane reactor. Therefore, a modification on the current membrane reactors used for the enzymatic hydrolysis is of paramount importance to enhance it for producing a higher glucose concentration, handling at higher solid loading, reducing the effect of fouling and facilitating the discharge, refilling of the fresh substrate and allowing recovery of enzymes on both solid and liquid phases.

Lastly, the degree of product removal expressed in term of space velocity, tangential velocity, removal rate, mass transfer coefficient has not further explored to determine a suitable level of liquid hydrolysate without causing the concentration polarisation and fouling on membranes, and over-dilution of glucose solution obtained at permeate.

3. Conclusion

The application of ultrafiltration membrane in enzymatic hydrolysis with two configurations (external loop and submerged membranes) has proved to be

effectively perform glucose removal to minimise product inhibition and recover enzyme in the liquid hydrolysate. Operating conditions of a membrane reactor should be involved the critical process parameters such as substrate concentration, enzyme to substrate ratio, mixing and glucose removal due to their effect on the performance of hydrolysis in term of conversion, final glucose concentration, concentration polarisation and fouling.

The current limitations of the membrane reactor for hydrolysis highlighted earlier in some review work such as low glucose concentration at the output of membrane filtration, unsuitability for operating at high substrate concentrations and recovery of enzymes adsorbed in the solid cellulose substrate. In this review, two additional challenges should be considered for further research. One is the degree of glucose removal from hydrolysis reactor to determine an optimal rate for not only minimising product inhibition, but also avoiding over dilution of glucose solution. Other is the operation feasibility relating to disposal of unconverted substrate which adsorbs cellulose enzymes and the refill of fresh substrate.

It is very promising for the two new configurations, i.e. product removal by dialysis and the modified configuration of membrane reactor as effective methods to conduct enzymatic hydrolysis due to its advantage of not only minimising product inhibition, recovering enzyme but also being able to handle at higher substrate concentrations.

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