

EXPERIMENTAL STUDY OF LACTOSE HYDROLYSIS AND SEPARATION IN CSTR-UF MEMBRANE REACTOR

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Abstract - In this study, the effect of processing conditions on the performance of continuous stirred tank - ultrafiltration (CSTR-UF) in dead-end mode was investigated. An UF membrane with a molecular weight cutoff of 3 kDa made of regenerated cellulose material was used to separate enzyme from products. The effect of operating pressure ranging between 2 and 5 bar and time on the performance of the CSTR-UF system was studied. The experiments were performed with a 0.139 molar aqueous solution of lactose as feed. According to the experimental data, the lactose concentration in the permeate decreased with time due to concentration polarization and hydrolysis. It was found that the rejection factor of lactose increases from 33 to 77% with time from 5 to 85 min. Permeation flux of the membrane was evaluated in terms of pure water flux (PWF) and lactose aqueous solution. Results showed that a high operating pressure led to a high permeation flux for both mentioned cases. Also, adding lactose and enzyme to pure water caused a reduction of the permeation flux due to concentration polarization.

Keywords: Hydrolysis of lactose; Reactor membrane; CSTR-UF; Whey; Ultrafiltration.

INTRODUCTION

Whey disposal is one of the most important economical and environmental problems in the dairy industry. This liquid is produced when milk is processed into cheese and consists of approximately 5% lactose, 0.7% protein and 93% water and salt. Lactose waste increases biochemical and chemical oxygen demand (BOD and COD) which is in contrast with legal standards for wastewater (Cuartas-Uribe *et al.*, 2009). In addition, lactose, as a disaccharide, is scarcely digestible for about 75% of adults worldwide due to lack of β -galactosidase enzyme in their body (Sieber *et al.*, 1997; Bulhões *et al.*, 2007). Furthermore, a high amount of lactose in dairy products like ice cream, condensed milk, etc., leads to an undesirable grainy texture. The objective of many researches is reduction of the lactose amount

in waste streams or dairy products through methods like hydrolysis of lactose or separation processes (Cuartas-Uribe *et al.*, 2009; Marwaha and Kennedy, 1988; Tragardh, 1991; Trevisan *et al.*, 1997). It should be mentioned that hydrolysis reduces lactose losses and converts it to beneficial products at the same time. Glucose and galactose, the hydrolysis products, are sweeter and more soluble than lactose and are widely used as sweetening agents for dairy products (Novain *et al.*, 2005).

Lactose can be hydrolyzed by two principal methods, using acid treatment at high temperature (above 150 °C) or enzymatic catalysis. The second one is preferred because of its milder operating temperature and pH. B-Galactosidase is a suitable enzyme to perform the hydrolysis, converting lactose to glucose and galactose monosaccharides (Illanes *et al.*, 1990; Bouhallab and Touze, 1995; Grosová

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et al., 2007; Freitas *et al.*, 2012). There are several mechanisms to describe this reaction in the presence of water such as those presented by Segal and Cha (Flashel *et al.*, 1982). Some researches consider in detail enzymatic lactose hydrolysis in a batch CSTR reactor as well as in a fixed bed reactor. In comparisons, the superior performance of the CSTR reactor was clearly established (Mehaia *et al.*, 1992; Prenosil and Hediger, 1988; Haider and Husain, 2009).

Several studies have focused on methods to recover valuable components from dairy waste streams, like adsorption, nanofiltration (NF), reverse osmosis (RO), ultrafiltration (UF) membrane processes and chromatography (Khider *et al.*, 2004; Hall, 1995; Barba and Beolchini, 2000; Barba *et al.*, 2001; Baker, 2005; Pouliot *et al.*, 1999). In terms of lactose removal (342 Da) from dairy product and waste streams, the RO and NF processes are more efficient, but need higher operating pressure and energy consumption than UF. On the other hand, enzymatic hydrolysis has some problems such as the effects of galactose formation on hydrolysis completion and the high enzyme cost. One possibility for ensuring protection of purified enzyme is offered by using membrane processes (Curcio *et al.*, 2006). Therefore, an UF system in combination with a hydrolysis reactor might be preferred due to enzyme separation via the membrane (Goulas *et al.*, 2003, Novain *et al.*, 2005).

In the present work, the performance of a CSTR-UF system was investigated for conversion of lactose in the presence of β -galactosidase enzyme. The UF membrane of MWCO of 3 kDa made of regenerated

cellulose material was utilized to separate the enzyme (molecular weight of 116 kDa) from glucose and galactose (molecular weight of 180 Da). All experiments were carried out in dead-end mode and under various operating pressures ranging between 2 and 5 bar. The experiments were conducted with a 0.319 molar aqueous solution of synthetic lactose as feed.

MATERIALS AND METHODS

Chemicals

β -Galactosidase (activity of the enzyme at 37 °C = 3000 U/ml) and lactose were purchased from Sigma-Aldrich Company. In order to analyze the concentration of lactose, sulfuric acid (98%) and phenol (89%) were obtained from Merck Company.

Membrane Module and Experimental Set-Up

Experiments were performed in a dead-end filtration setup. A schematic of the CSTR-UF system is shown in Figure 1. The stirred cell was fed from a 3 L reservoir, which was pressurized using a nitrogen gas cylinder. Feed pressure was monitored by pressure gauges and the permeate side was connected to the atmosphere so its pressure was assumed to be approximately equal to 0 bar (gauge pressure).

The RC ultrafiltration membrane of 3 kDa MWCO was placed in the 150 mL stirred cell and supported with a sintered stainless steel disc.

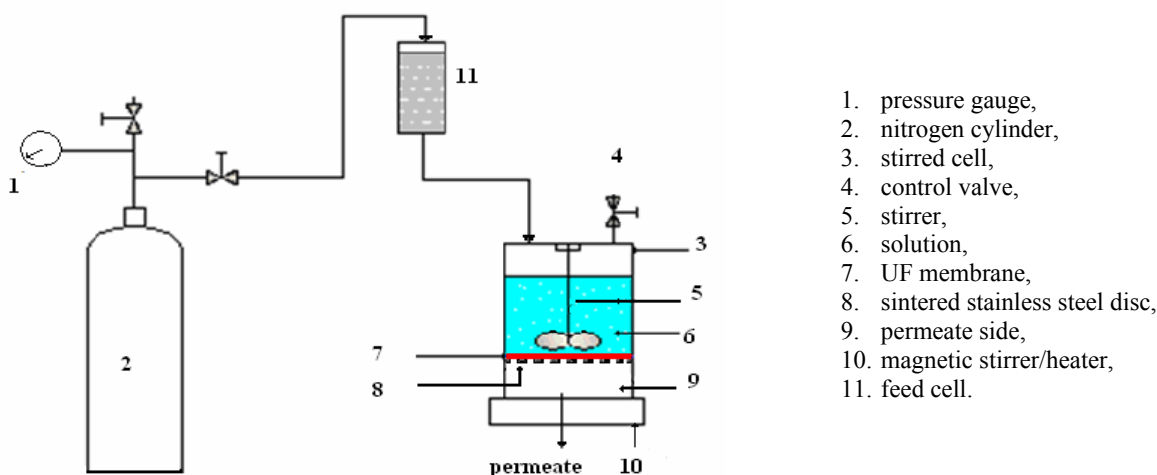


Figure 1: A schematic diagram of the CSTR-UF setup.

Determination of Lactose Concentrations

Lactose concentration was determined by means of calibration plot of absorbance as in the method of Chollangi and Hossain (2007). In order to plot the standard absorbance curve, five samples of lactose were diluted to prepared aqueous solutions of 0.01, 0.02, 0.03, 0.04 and 0.05 g.L⁻¹. Then 2 mL of each sample was mixed with 0.1 mL phenol solution and 6 mL sulfuric acid for 10 min at room temperature. The absorbance of each sample was measured at 490 nm in a UV-Vis spectrometer (Perkin-Elmer, USA). By using the absorbance data presented in Table 1, it is possible to determine the lactose content in every permeate sample.

Table 1: calibration absorbance data

Lactose concentration (g.L ⁻¹)	absorbance
0.01	0.103
0.02	0.155
0.03	0.175
0.04	0.190
0.05	0.207

GOVERNING EQUATIONS

The most frequent parameters used to characterize membranes are based on its performance, such as flux and rejection (Mulder, 1996).

Rejection

In order to evaluate membrane performance, rejection of lactose should be obtained. The observed rejection, R , was calculated using Equation (1):

$$R = 1 - \frac{C_p}{C_f} \quad (1)$$

where C_p and C_f are the solute concentrations on the permeate and feed sides, respectively.

Volumetric Flux

Permeate volumetric flux is expressed as the volume of fluid permeated in a certain time. To obtain this value, Equation (2) was used:

$$J = \frac{1}{A} \frac{\Delta V}{\Delta t} \quad (2)$$

where J is the volumetric flux (L.m⁻².h⁻¹), A the effective membrane area (m²), ΔV the permeate volume (L) and Δt is the sampling time (h).

RESULTS AND DISCUSSION

Influence of Pressure on Permeate Flux

In order to illustrate the effect of pressure on permeate flux, the operating pressure was controlled at different values in the range of 2 to 5 bar. Permeate flux versus pressure change is shown in Figure 2. It can be observed from the results that permeate flux increased with increasing operating pressure. Nevertheless, flux may not increased proportional to pressure at high levels because of pore compression. Figure 2 did not show any signs of compaction due to a pressure effect (Pouliot *et al.*, 1999; Chollangi and Hossain, 2007).

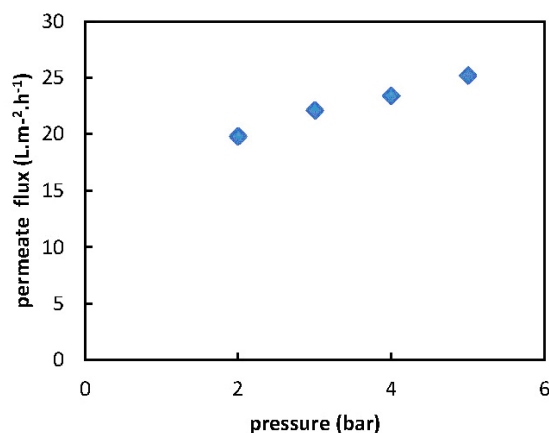


Figure 2: Influence of pressure on permeate flux

Influence of Time on Permeate Concentration

To study the effect of time on lactose concentration at a given pressure, 2 bar, samples were collected from the permeate at 5, 25, 45, 65 and 85 min. Each sample were diluted a thousand-fold and the lactose amount determined. Test results presented in Figure 3 show that, after 5 min, lactose decreased drastically from the initial value of 0.139 mol.L⁻¹ to 0.0921 mol.L⁻¹, while further operation decreased the value to 0.078 mol.L⁻¹ after 20 min. This was caused by lactose consumption via the hydrolysis reaction with time. On the other hand, β -galactosidase, as a high molecular weight protein, accumulated near the membrane surface, which can cause resistance against lactose permeation. This behavior is known as concentration polarization,

which can lead to fouling and plugging of membrane pores (Cuartas-Urbe *et al.*, 2009; Pouliot *et al.*, 1999). However, stirring minimized this effect due to good mixing of the bulk feed solution and turbulent flow near membrane surface (Mhurchú, 2008; Peinemann *et al.*, 2010).

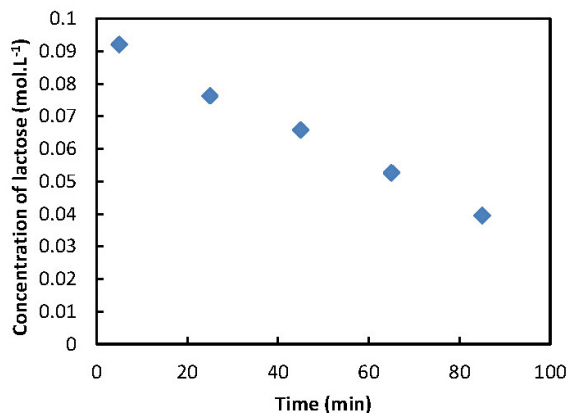


Figure 3: Influence of time on lactose concentration

Based on equation 1, rejection increased from 33 to 77% with increasing time from 5 to 85 min at constant lactose concentration in the feed. It should be mentioned that, in all cases, sample appearance revealed that the UF membrane rejected the enzyme completely.

Influence of Pressure on Rejection of Lactose

The effect of pressure between 2 and 5 bar on rejection of lactose is presented in Figure 4. Lactose concentration on the permeate side was analyzed for each pressure and the data used to determine rejection. Determinations of lactose on the permeate side after 80 min showed that rejection was reduced from 0.77 to 0.57. This result suggests that the increase in permeate flux with pressure caused a reduction of the retention time of lactose in the reaction zone. Thus, lactose concentration on the permeate side might be increased and rejection might be decreased. On the other hand, the decline of rejection was slower at high pressure due to the presence of enzyme molecules near the membrane surface and concentration polarization effects, which operate as a resistance against lactose transportation.

Effect of Feed Concentration on Permeate Flux

Figure 5 shows the permeate flux versus pressure for both pure water and 0.319 molar lactose as feed. This figure indicates that, when lactose and enzyme are added to feed, the permeate flux decreased

considerably at all pressures. Other researchers, in their works on whey ultrafiltration, observed the same behavior (Rektor and Vatai, 2004; Atra *et al.*, 2005; Butylina *et al.*, 2006; Baldasso *et al.*, 2011). Based on this result, one can conclude that lactose and enzyme increase resistance to water permeation by deposition on the top surface and pore wall of the membrane (Chollangi and Hossain, 2007).

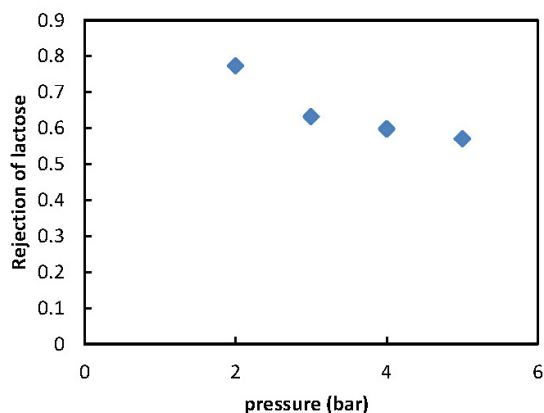


Figure 4: Influence of pressure on lactose rejection

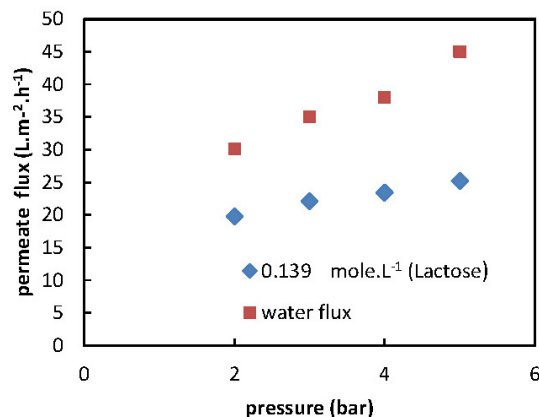


Figure 5: Permeate flux (l.m⁻².h) vs. pressure (bar)

CONCLUSIONS

Lactose hydrolysis has been investigated using β -galactosidase enzyme in a continuous stirred tank-ultrafiltration (CSTR-UF) to produce galactose and glucose. The major findings of the present study are summarized as follows:

- Increases in operating pressure, as the driving force, enhanced permeate volumetric flux;
- Lactose concentration in the permeate decreased with increasing time due to concentration polarization and hydrolysis;

- The rejection factor of lactose increased from 33 to 77% with increasing time from 5 to 85 min due to reduction of the lactose concentration;
- A fouling effect is observed due to the presence of lactose and enzyme in the feed.

NOMENCLATURE

A	effective membrane area	m^2
R	rejection	
C_f	solute concentration on the feed side	$g.L^{-1}$
C_p	solute concentration on the permeate side	$g.L^{-1}$
J	volumetric flux	$L.m^{-2}.h^{-1}$
Δt	sampling time	H
ΔV	permeate volume	L

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