

# Experimental Study of Lactose Hydrolysis and Separation in Continuous Stirred Tank -Ultrafiltration Membrane Reactor

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## Abstract

In this study, lactose was hydrolyzed using  $\beta$ -galactosidase enzyme in a continuous stirred tank -Ultrafiltration (CSTR-UF) to produce galactose and glucose. The UF membranes of Molecular Weight Cut of 3 kDa of regenerated cellulose material were used to separate enzyme from products. Experiments were performed with 0.139 molar aqueous solution of lactose as feed. The effect of operating pressure ranging between 2 and 5 bar and time on rejection and permeate flux were studied. Results showed that the UF membrane rejects the enzyme completely. Also according to experimental data lactose concentration in permeate decrease with time due to concentration polarization and hydrolysis. It was found that rejection factor of lactose increases from 33 to 77%, with time from 5 to 85 min.

**Keywords:** Hydrolysis of lactose; Reactor membrane; Ultrafiltration

## Introduction

Whey disposal is one of the most important economical and environmental problems in dairy industry. This liquid produced when milk is changed in to cheese and approximately it consists of 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 standard for wastewater [1]. In addition, lactose as a disaccharide is scarcely digestible for some people due to lack of  $\beta$ -galactosidase enzyme in their body. Furthermore, high amount of lactose in dairy product like ice cream, condensed milk, etc., lead to undesirable grainy texture. Thus it is necessary to remove lactose from dairy product and waste streams through methods like hydrolysis or separation processes [1-3].

Lactose can be hydrolyzed by two principle method, 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.  $\beta$ -galactosidase is suitable enzyme to perform hydrolysis, that change lactose to glucose and galactose monosaccharide [4-6]. There are several mechanisms to describe this reaction in presence of water such as those presented by Segal and Cha [7]. Some researches consider in detail, using enzymatic lactose hydrolysis in batch CSTR reactor as well as fixed bed reactor. In the comparison, superior performance of CSTR reactor was clearly established [8-10].

A lot of researchers focus on methods, recover valuable components from dairy waste streams, like adsorption, NF, RO, UF membrane processes and chromatography [11-16]. In terms of lactose removal (342 Da) from dairy product and waste streams, 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 problem such as the effects of galactose formation on hydrolysis completion, and the high enzyme cost. One possibility to ensuring protection of purified enzyme is offered by using membrane processes [17]. So, UF system in combination with hydrolysis reactor might be preferred due to enzyme separation via membrane [18-19].

In present study, CSTR-UF system was used to hydrolyze lactose and separate  $\beta$ -galactosidase enzyme (116 kDa) from glucose

and galactose (180 Da). The UF membrane of MWCO of 3 kDa of regenerated cellulose (RC) material was used to separate enzyme from products. All experiments were carried out with 0.319 molar aqueous solution of synthesized lactose as feed. The effect of operating pressure ranging between 2 and 5 bar and time on rejection and permeate flux were studied.

## Materials and Methods

### Chemicals

$\beta$ -galactosidase (activity of the enzyme at 37°C = 3000 IU/cc) 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 CSTR-UF system is shown in Figure 1. The stirred cell was fed from 3 L reservoir which was pressurized using nitrogen gas cylinder. Feed pressure is monitored by pressure gauges and Permeate side was connected to the atmosphere so its pressure was assumed 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 sintered stainless still disc.

### Determination of lactose concentrations

Lactose concentration was determined by means of calibration plot

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of absorbance value. In order to plot standard absorbance curve, five sample of lactose were diluted to prepared aqueous solution of 0.01, 0.02, 0.03, 0.04 and 0.05 g.L<sup>-1</sup>. 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 absorbances of samples were measured via 490 nm UV-Vis, spectrometer (Perkin-Elmer, USA). By using absorbance data represented in Table 1, it is possible to determine lactose content in every permeate sample.

### Governing equation

The most frequent parameters used to characterize membranes are founded on its performance such as flux and rejection.

### 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 concentration in permeate and feed side, respectively.

### Volumetric flux

Permeate volumetric flux is expressed as volume of fluid permeated in certain time step. To meet this value equation (2) was used:

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

where  $J$ , volumetric flux (L.m<sup>-2</sup>.h<sup>-1</sup>),  $A$ , effective membrane area (m<sup>2</sup>),  $\Delta V$ , permeate volume (L) and  $\Delta t$  is sampling time (h).

## Result and Discussion

### Influence of pressure on permeate flux

In order to illustrate the effect of pressure on permeate flux, the

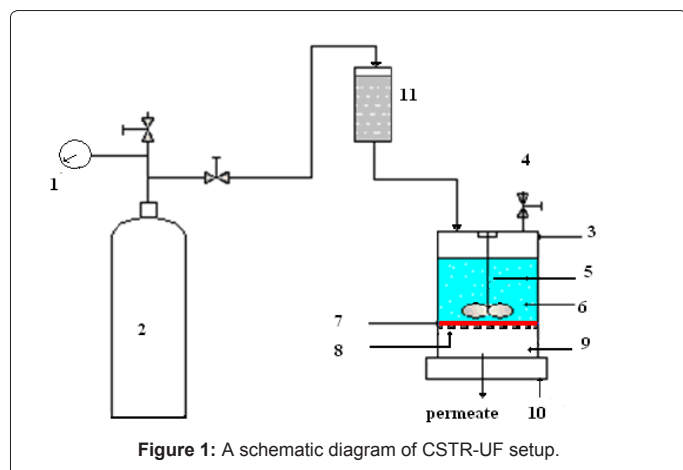


Figure 1: A schematic diagram of CSTR-UF setup.

Lactose concentration (g.L <sup>-1</sup> )	absorbance
0.01	0.130
0.02	0.155
0.03	0.175
0.04	0.190
0.05	0.207

Table 1: Calibration absorbance data.

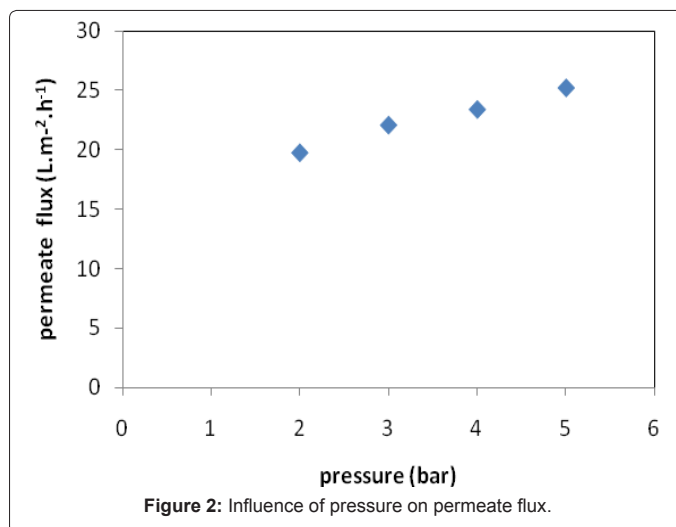


Figure 2: Influence of pressure on permeate flux.

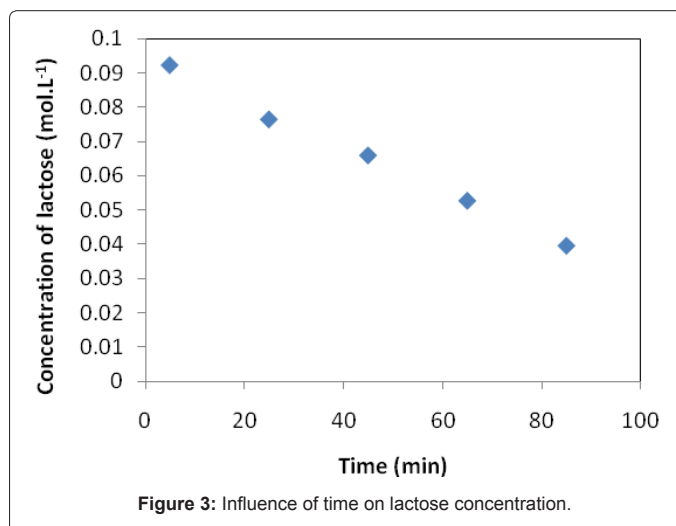


Figure 3: Influence of time on lactose concentration.

operating pressure was controlled at different value in the range of 2 to 5 bar. Permeate flux verses 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 be increased proportional with pressure at high level because of pore compressing. It should also be noticed that Figure 2 didn't show any compaction due to pressure effect [15,20].

### Influence of time on permeate concentration

To study the effect of time on lactose concentration at certain pressure, 2 bar, sample were collected from permeate at 5, 25, 45, 65 and 85 min. each sample were diluted thousand time and set for lactose amount determination. Test results are represented in Figure 3 and are shown that after 20 min lactose amount is decreased drastically from initial value of 0.139 mol.L<sup>-1</sup> to 0.078 mol.L<sup>-1</sup>, but further operation declined the value to 0.071 mol.L<sup>-1</sup>. This phenomenon is caused because of lactose consumption in hydrolysis reaction by time spending. On the other hand,  $\beta$ -galactosidas as a high molecular weight protein are accumulated near membrane surface which cause resistance against lactose permeation. This behavior is known as the concentration polarization which can lead to fouling and plugging of membrane pore

[1,15]. Although, stirring minimized this effect by good mixing of bulk feed solution and turbulent flow near membrane surface [20].

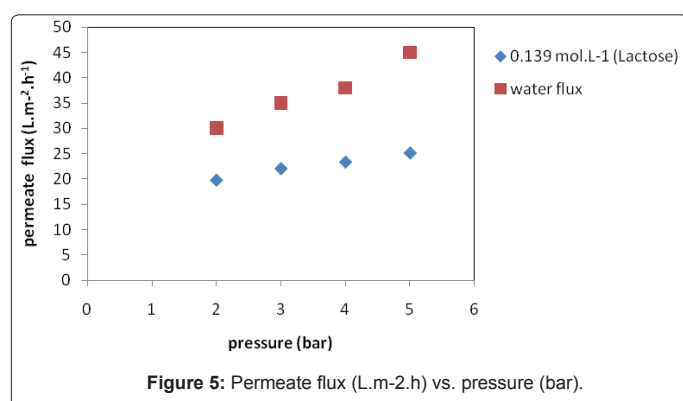
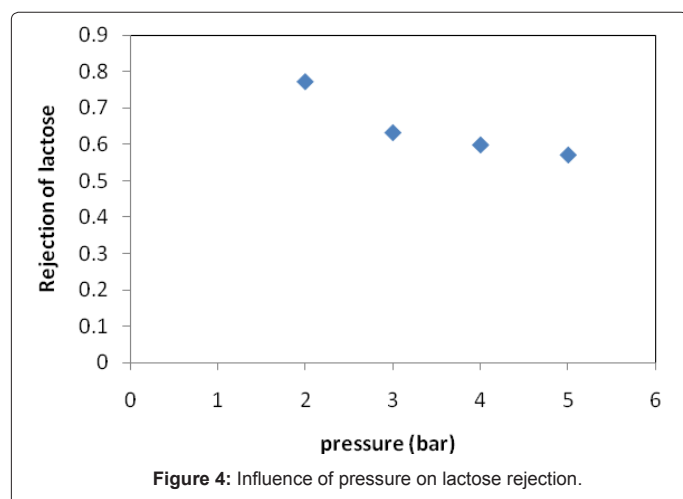
Based on equation 1, rejection is enhanced with time at constant lactose concentration in feed. It should be mentioned that in all cases, sample appearance reveal that the UF membrane rejects the enzyme completely.

### Influence of pressure on rejection of lactose

Effect of pressure rise between 2 and 5 bar on rejection of lactose is presented in Figure 4. Determinations of lactose amount on permeate side after 80 min showed that rejection is reduced from 0.77 to 0.57. This result suggest that increasing in permeate flux with pressure cause decrease of retention time of lactose in reaction zone. Thus lactose concentration on permeate side might be increased and rejection might be decreased. On the other hand, declining of rejection was slower at high pressure due to presence of enzyme molecules near membrane surface.

### Effect of feed concentration on permeate flux

Figure 5 shows permeate flux verses pressure for both pure water and 0.319 molar of lactose as feed. This figure indicates that as lactose and enzyme added to feed, permeate flux decreased at all pressure, considerably. Base on this result, one can conclude that lactose and enzyme have resistance role on water permeation by deposition on top surface and pore wall of membrane [20]. Although, flux reduction is for the most part due to surface fouling of membrane by existence of enzyme.



## Conclusion

Lactose hydrolysis has been investigated using  $\beta$ -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:

- Increase in operation pressure as driving force, enhance permeate volumetric flux.
- Unchanged color of permeate samples reveal that no enzyme present in permeate and the UF membrane rejects the enzyme completely.
- Lactose concentration in permeate decrease with time due to concentration polarization and hydrolysis.
- It was found that rejection factor of lactose increases from 33 to 77%, with time from 5 to 85 min due to drop lactose concentration.
- Fouling effect observed due to presence of lactose and enzyme in feed.

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