AENSI Journals

JOURNAL OF APPLIED SCIENCE AND AGRICULTURE

ISSN 1816-9112

Journal home page: www.aensiweb.com/JASA

Resistance in Series Model for Ultrafiltration Xylose Reductase from Product Mixtures

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ARTICLE INFO

Article history:
Received 11 October 2014
Received in revised form 21 November 2014
Accepted 25 December 2014
Available online 14 January 2015

Keywords:
Please insert 5 keyword

ABSTRACT

This study investigates the fouling mechanism in ultrafiltration membrane during separation of xylose reductase from product mixtures. The Resistance – In – Series Model was used in order to identify the responsible hydraulic resistance. The resistance against the flux was assumed to be comprised as membrane hydraulic, adsorption, pore pugging and fouling resistance. The profile of total resistance and corresponding flux decline were calculated and compared with the experimental data. The result showed that adsorption resistance (Rad) was the main contributed the rate of flux decline. Moreover the significant organic fouling that contribute during xylose reductase separation revealed that the fouling potential was Rad > Rpp > RF. The measure flux recovery of filtration xylose reductase was 93%.

To Cite This Article: B. Noor Suzana and A.M. Mimi Sakinah., Resistance in Series Model for Ultrafiltration Xylose Reductase from Product Mixtures. ***J. Appl. Sci. & Agric.,*** *10(5): 222-227, 2015*

INTRODUCTION

Xylose reductase (XR) is an intracellular enzyme commonly found in yeast and filamentous fungi, often in several isozyme forms in the same species. This enzyme occurs in the cytoplasm of microorganisms, where it catalyzes the first step of D- xylose metabolism by reducing xylose to xylitol with the concomitant oxidation of NAD (P) H to NAD (P) + (Ronzon *et al*., 2012 and Zhao *et al*., 2009). The obtainment of the enzyme xylose reductase is an essential step in the development of the enzymatic process. This enzyme present in different species of yeasts and is responsible for the process of oxidation of xylose into xylitol by these microorganisms (Yokoyama *et al*., 1995). This enzyme has the potential applications in the biotechnological production of xylitol, sorbitol, and ethanol from xylose, which make the enzyme a focus of interest. The applications of enzyme have demanded an efficient and large scale enzyme separation technique.

Membrane technology has been applied in biotechnology industries since last two decades. It is gradually emerging as a powerful bioseparation process for purification, fractionation, separation and concentration of bioproducts such as therapeutic drugs, enzymes, hormones, antibodies, fruit juice and cheese. Ultrafiltration (UF) is a powerful separation technique based on the sieving mechanism of retentive or partly permeable membranes. UF is widely used in biotechnology for the concentration of macromolecules, e.g. enzymes in aqueous solutions. Therefore, in this study, ultrafiltration membrane can be alternative method used in order to separate XR from the product mixtures (Choi *et al*., 2005). Despite the fact that there are numerous points of interest offered by membrane, the application of membrane technology is still limited. This is due to the fouling problem which reduces the membrane performance. In addition, membrane fouling reduces the production rate and increases complexity of membrane operations. Different models were proposed to investigate and foresee the flux behavior during filtration of macromolecular solution. This can be classified as osmotic pressure controller, gel layer controlled and resistance – in- series models (Rai *et al*., 2006).

The reason that membrane process is not utilized on much large scale is the flux decline during the separation process (Sakinah *et al*., 2009). Flux decline cause the several phenomena in, on and near the membrane (Derradji *et al*., 2005). The flux decline during separation by UF is the cumulative effect of several mechanisms which is adsorption of solutes on the membrane surface, pore plugging (Sakinah *et al*., 2007) and concentration polarization (Ko and Pellegrino, 1998). Adsorption of membrane

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surface by solute particles was determined by membrane-solute interaction (Choi *et al*., 2005; Sakinah *et al*., 2008). Moreover, pore plugging was mainly governed by relative size of the solute and membrane pore as well as the operating conditions. Concentration polarization is the accumulation of solutes particles over the membrane surface. Framing a developing gel layer or expanding the osmotic pressure at the membrane solution interface cause decreasing the effective driving force (Guo *et al*., 2012). By the proper membrane cleaning procedure, the type of fouling is reversible in nature and the permeability can be determined. The purpose of this study is to investigate the fouling mechanism in ultrafiltration membrane during separation of xylose reductase from product mixtures and to identify the responsible hydraulic resistance using The Resistance – In – Series Model.

MATERIALS AND METHODS

Experiment system:

The cross flow membrane system was shown in Fig 1 and the part of each membrane was illustrated in Table 1. The cross flow membrane comprises with membrane module unit, pump, feed outlet, permeate outlet and feed pressure gauge. The area of ultrafiltration membrane cassette was 0.01m2.

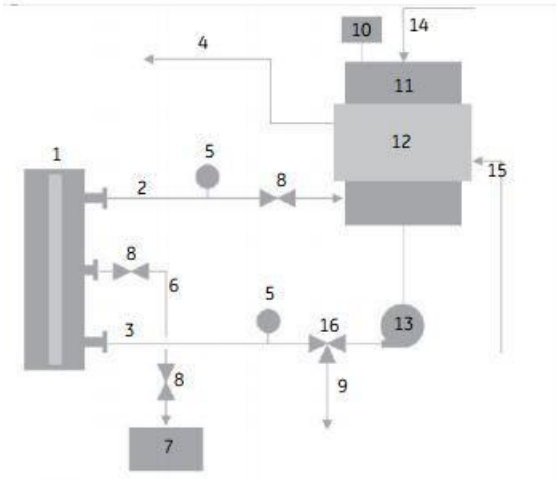

Fig. 1: Kvick Lab cross flow system flow diagram.

Table 1: Parts in Figure 1.

Part	Description
1	Kvick Lab cassette holder
2	Retentate line
3	Feed line
4	Cooling/Heating connection for jacket
5	Pressure gauge
6	Permeate line
7	Collection vessel
8	Valve
9	Drain line
10	Vent part
11	Feed reservoir
12	Cooling/heating jacket
13	Pump
14	Diafiltration (solution) feed line
15	Cooling/Heating water
16	Double valve

Separation process:

The mixtures of the feed for the membrane were been formulated according enzymatic process of xylitol by Rafiqul, 2012. It was applied in order to ensure that the feed of the membrane have equally concentration of mixtures. Concentration of xylitol, xylose, glucose, arabinose, acetic acid and XR was 16.28 g/L, 2.52 g/L, 4.64 g/L, 2.55 g/L, 3.2 g/L and 0.94g/L respectively. After that, the mixtures were continuously subjected into the filtration process using an ultrafiltration cassette membrane (Figure 1). Filtration process was run at 1.2 bar and 1.06cm/s. The pure water flux (Jpwp) was measured by taking the volume of permeates in every 10 minutes during the preliminary filtration stage. After that, DI water was replaced with the mixtures from the formulated and the volume of permeates was collected and

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measured every 10 minutes until the flux reached a steady state

Resistance- in – series- model:

In order to determine the hydraulic resistance of membrane separation, Resistance- in – series model was used based on Darcy's law. There are four parameters of resistance – in –series model which were used to quantify their influence on flux decline. The equations that describing the cross flow filtration process are as following:

$$J = \frac{\Delta P}{\mu(Rm + Rpp + Rf + Rad)} \quad (1)$$

where J is flux through the membrane (m/s), ΔP is the transmembrane pressure (Pa), μ is the dynamic viscosity (Pa.s), Rm is the membrane hydraulic resistance, Rpp is the pore plugging resistance, Rf is the fouling resistance and Rad is the adsorption resistance (all resistance are in m -1).

RESULTS AND DISCUSSION

Determine of hydraulic resistance:

The amount of the hydraulic resistance can be converted into the ratio of the hydraulic resistant to the amount of the total hydraulic resistance as shown in Fig. 2 and Table 2. Based on the result, the membrane hydraulic resistance was the 57% of the total hydraulic resistance because of intrinsic property of the membrane. However, the adsorption resistance, pore plugging resistance and fouling resistance exhibited about 21 %, 14% and 8% of the total hydraulic resistance.

Flux decline in the UF filtration membrane:

Membrane fouling was the main reason that cause flux decline in the cross flow membrane. In the beginning of separation process, particles from the reaction mixtures were blocked the smallest pores of the membrane. The bigger pores of the inner membrane surface were covered, and then some particles entered the membrane and covered the other arrived particles, while the others were directly blocked some of the pores. Finally, the cake layer began to develop (Behnaz *et al*., 2012).

Fig. 3 shows the declination of the flux in the ultrafiltration cross flow membrane. Based on the hydraulic resistance from the membrane, the flux of membrane was first obtained due to the property of the membrane itself. The declination of flux was obtained as the increasing amount of total resistance by using Darcy's Law. Moreover, once the pressured was released, the flux was increased due to the vanishing of the concentration polarization. Furthermore, after membrane was cleaned with clean water, the gel layer was moved out from the membrane surface which impacts an expanding of membrane's flux. The flux of the membrane discovered to be increment after chemical cleaning. It was presumed to be due to dynamic balance between adsorption and description of the soluble organic matter into the matrix of membrane.

Table 2: Percentage of hydraulic resistance of the UF membrane in separation of xylose reductase.

Type of resistances	Percentage (%)
Rm	57
Rad	21
Rpp	14
Rf	8

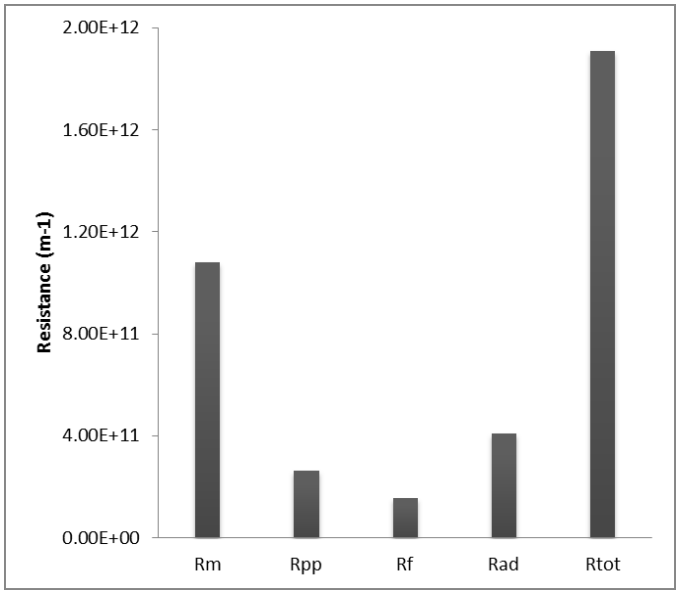

Fig. 2: Amount of hydraulic resistance of the UF membrane.

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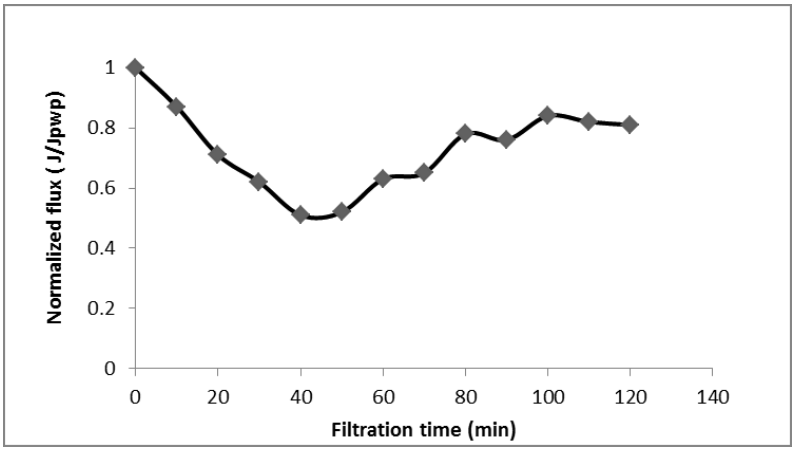

Fig. 3(a-d): Flux decline in UF cross flow filtration (a) Flux declined during filtration, (b) Pressure released, (c) Water cleaning (d) chemical cleaning.

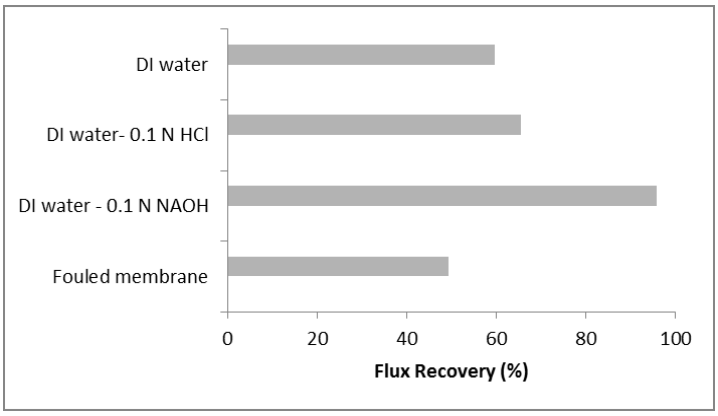

Fig. 4: Percentage of flux recovery of membrane cleaning during XR separation.

Flux recovery during xylose reductase separation:

The flux of the cleaned membrane was measured through a filtration with DI water after each membrane cleaning step. The ratio of the flux (LMH) of the cleaned membrane to the new membrane flux (LMH) was used to evaluate the flux recovery (Mo and Huang, 2003) and the results are shown in Fig. 4 and Fig. 5. Fig. 4and 5 show that cleaning with DI water could recover the membrane flux by about 59.7%. The hydraulic cleaning was found capable of cleaning the outmost and loose fouling layer of the fouled membrane surface.

Furthermore, the flux recovery for the cleaned membrane by using alkaline and acidic solution was about 93 % and 65.6% respectively. Alkaline cleaning, compared to acidic cleaning, had and obvious effect on the recovery of the membrane flux. Moreover, alkaline cleaning resulted in an additional recovery of 5% of the membrane flux. As reported by other researchers, the alkaline solution was more effective in removing the organic foulants both on the exterior and inner surfaces of the membrane. In this study, the feed was rich in organic solutes (xylose reductase, xylitol, xylose, glucose etc.), and thus this finding supports that alkaline cleaning more suitable in gaining the initial flux.

Conclusion:

A resistance in series model was proposed to quantify the flux decline during UF of xylose reductase separation. A systematic method was outlined to quantify the time variation of each constituent resistance, namely, the membrane resistance, adsorption, pore plugging and reversible fouling resistance. The results obtained from this research were:

- The major fouling mechanism was that reversible fouling which was about 21 % of the total hydraulic resistance
- The irreversible fouling mechanism could be elevated by chemical cleaning which is 42% from total hydraulic resistance.
- The maximum achievable flux recovery for xylose reductase separation was 93% by cleaning with alkaline solution.

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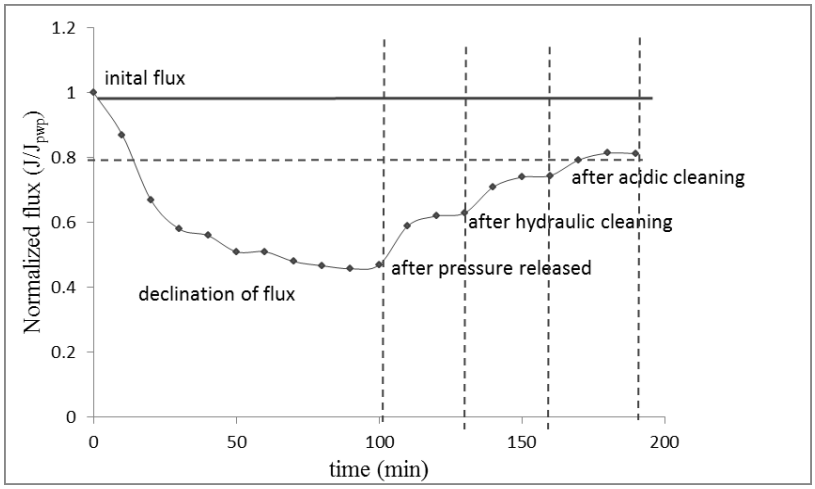
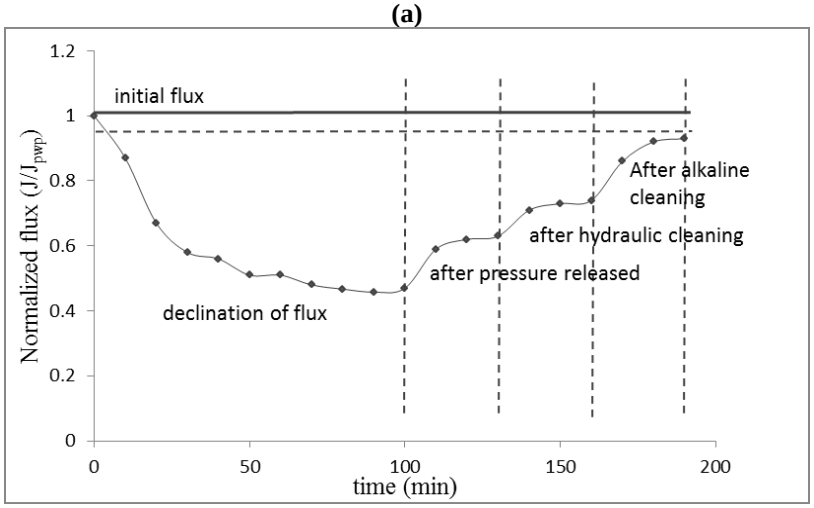


(a)



(b)

Fig. 5: Flux profile of PES membrane by using (a) acidic cleaning (b) alkaline cleaning.

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