Biocatalysis and Agricultural Biotechnology 12 (2017) 50–58

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Configuration modification of a submerged membrane reactor for enzymatic hydrolysis of cellulose

Thaothy Nguyenhuynh[a], Rajesh Nithyanandam[a,*], Chien Hwa Chong[a], Duduku Krishnaiah[b]

[a] School of Engineering, Taylor's University, Taylor's Lakeside Campus, No. 1 Jalan Taylor's, 47500 Subang Jaya, Selangor DE, Malaysia
[b] Faculty of Engineering, Universiti Malaysia Sabah, Kota Kinabalu, Sabah 88400, Malaysia

ARTICLE INFO

Keywords:
Submerged membrane reactors
Enzymatic hydrolysis
Intermittent product removal
Reusing enzyme
Ultrafiltration

ABSTRACT

In this study, a modified submerged membrane reactor was investigated as a new configuration for efficient enzymatic cellulose hydrolysis. From the results, effectiveness of ultrafiltration was increased due to the complete glucose permeation and enzyme retention up to 80%. Intermittent product removal at 50% volume replacement was better because it was able to regain the glucose concentration after ultrafiltration and minimised the over-dilution as occurred with the continuous product removal. Results obtained from response surface design showed the quadratic cellulose concentration (A^2), the enzyme to substrate ratio (B) and interaction (AC) of cellulose concentration with intermittent product removal were identified to be the significant factors to maximise the glucose concentration. The maximised glucose concentration (7.6 g/L) was obtained at the optimal conditions (10% cellulose concentration, 6% E/S ratio and 50% intermittent product removal). The retained enzyme could be reused to extend hydrolysis beyond 8 h as the glucose concentration was maintained at 7 g/L with an insignificant reduction. Thus, the modified submerged membrane reactor in this new configuration could be an alternative for the conventional batch reactor used in the enzymatic hydrolysis.

1. Introduction

Research and development in bioprocessing of agricultural residues available in abundance such as rice, wheat straws, sugar bagasse and corn stover with 30–50% of cellulose content are continuously improved and getting matured. Efficiency of the bioethanol fuel production mainly relies on the conversion of cellulose to glucose through enzymatic hydrolysis, followed by fermentation and purification of bioethanol product (Abels et al., 2013; Andric et al., 2010a).

The enzymatic hydrolysis of cellulose is a complex reaction system, which involves an interfacial heterogeneity of solid cellulose substrate, cellulase enzyme adsorption, inhibition of glucose and cellobiose on enzyme. Using batch reactors for hydrolysis has disadvantages of having severe product inhibition of the enzyme by glucose and cellobiose and high enzyme cost. These are known as the major challenges making the process far from being technologically and economically feasible. During hydrolysis, the released glucose is accumulated in the batch reactor and becomes an inhibitor of the enzyme (Andric et al., 2010a). Additionally, consumption of cellulase enzyme contributes significantly 20% of the total cost of the entire ethanol process and 50% of the hydrolysis step, but the enzyme is actually able to be recycled and reused for a longer period of time (Knutsen and Davis, 2002; Tu et al., 2007). To overcome these challenges, research related to the enzymatic hydrolysis has been progressed in improvement for the pretreatment step, simultaneous saccharification and fermentation (SSF) and developing new enzymes with higher tolerance for glucose inhibition. Integrating a membrane separation with the hydrolysis reactor is a better approach. The membrane integration approach with two configurations of membrane reactors, i.e. external loop and submerged membranes, has effectively solved two main issues (Malmali et al., 2015). First, ultrafiltration used in the membrane reactors allows an effective removal of glucose from the reactor to the permeate, hence minimises product inhibition. Secondly, enzyme is rejected by the membrane and retained inside the reactor for continuing hydrolysis of cellulose. However, these two configurations have some limitations such as a low glucose concentration in the permeate, occurrence of membrane fouling, operation at low cellulose concentrations from 2 to 5 w/v %, and enzyme recovery at liquid phase (Andric et al., 2010a; NguyenHuynh et al., 2017). Notably, research focused on solving these current limitations of the membrane reactor is rather scarce.

By redesigning the submerged membrane reactor, a new configuration was created and named as a modified submerged membrane reactor to carry out an efficient enzymatic cellulose hydrolysis (Nguyenhuynh and Nithyanandam, 2016). The arrangement of a sealed

* Corresponding author.
E-mail address: chemrajesh1982@gmail.com (R. Nithyanandam).

http://dx.doi.org/10.1016/j.bcab.2017.08.013
Received 21 March 2017; Received in revised form 28 August 2017; Accepted 28 August 2017
Available online 01 September 2017
1878-8181/

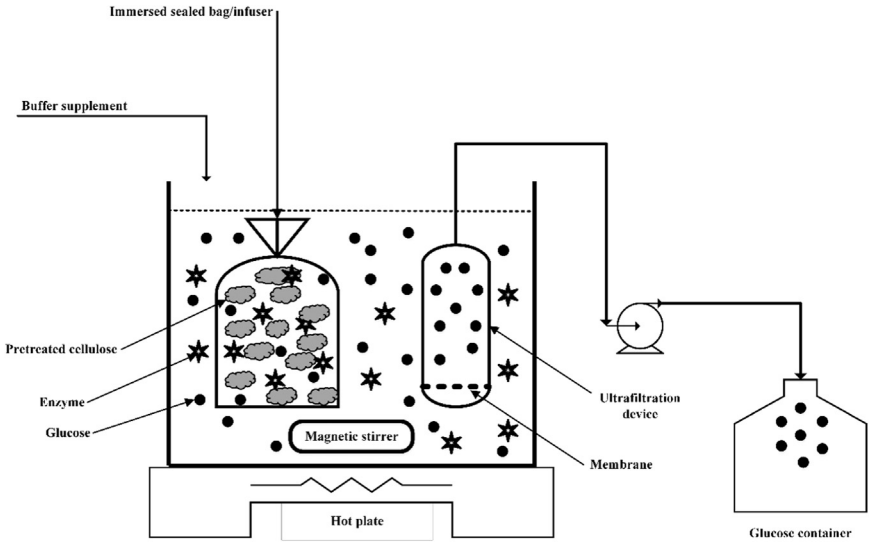

Fig. 1. A Schematic of the modified submerged membrane reactor.

bag/infuser and the ultrafiltration device (Fig. 1) both immersing in the hydrolysis reactor is novel. In some studies where a complete mixing of solid cellulose, enzyme and buffer was applied for the external loop and submerged membrane reactors, ultrafiltration was interrupted due to the deposit of cellulose on the surface of the membrane, known as membrane fouling, and consequently hydrolysis was affected (Gan et al., 2002; Lozano et al., 2014; Malmali et al., 2015). In addition, the factor of complete mixing for the content of hydrolysis reaction was reported to be insignificant in a wide range from 50 to 1200 rpm on the production of glucose (Gan et al., 2002; Liu et al., 2011). In a study of using dialysis, cellulose and enzyme were contained within the dialysis membrane, submerged in the liquid buffer while the agitator was at the other side of the membrane, the glucose recovery was 94%, glucose formation rate at 64 mg/L h, which were higher than that of 59% and 28 mg/L h compared to the use of ultrafiltration membrane reactor (Andric et al., 2010b). Hence, it proved that the mass transfer was not limited in this case. In the modified submerged membrane reactor as a new configuration in this study, the immersed infuser, which contains cellulose inside, would help to prevent the membrane fouling without affecting the contact and binding of enzyme with cellulose in hydrolysis to produce glucose. Thus, the cellulose-free hydrolysate was easily filtrated to remove glucose into the permeate, whereas retained enzyme inside the reactor by the ultrafiltration device.

It was hypothesised that the modified submerged membrane reactor not only can carry out hydrolysis of cellulose, but also increase effectiveness of ultrafiltration for product removal and enzyme recovery. Therefore, the objective of this research was to further investigate the technical feasibility of the modified submerged membrane reactor for efficient enzymatic cellulose hydrolysis. Experiments were conducted for glucose permeation and enzyme retention of ultrafiltration with 10 kDa cutoff. Reactor operation was studied at 2 modes of product removal (intermittent and continuous) together with a variation in cellulose concentrations at 10 w/v % and 20 w/v %. Response surface methodology was applied to study the influence of three factors namely cellulose concentration, enzyme to substrate ratio and volume of product removal on the enzymatic hydrolysis. With the retained enzyme in the submerged membrane reactor, potential of reusing enzyme was conducted to extend hydrolysis beyond 8 h.

2. Materials and methods

2.1. Materials

Cellic CTec2 enzyme, supplied by Novozymes (China) as free samples, was a complex blending of aggressive cellulases and high level of β-glucosidases (Novozymes, 2010). Sodium hydroxide (99.0% purity) was purchased from R & M Chemicals (Essex, UK). Citric acid (99.0% purity) was purchased from Bendosen Laboratory Chemicals (Selangor, Malaysia). Sodium citrate (99.0–100.5% purity) and anhydrous glucose were purchased from Systerm (Shah Alam, Malaysia). Cellobiose (99.9% purity) and microcrystalline cellulose (code: 435236) were obtained from Sigma Aldrich (St. Louis, MO, USA).

Polyethersulfone (PES) membrane type 146 was used as a filter for ultrafiltration with 10 kDa molecular weight cutoff, 47 mm diatmeter (Satorius Stedim Biotech GmbH, Germany). Each membrane filter was cut into four equal pieces with a smaller diameter of 23 mm to fit into the ultrafiltration device (Fig. 1).

2.2. Pretreatment of cellulose

Cellulose was weighted using an electronic balance (model: TX423L, Shimadzu, Japan). The weight of cellulose to be pretreated in this step was based on the cellulose concentration (weight/volume percentage concentration – w/v %, as shown in Eq. (1)), which was applied for the latter enzymatic hydrolysis.

$$w/v\% = \frac{weight of\ cellulose\,(g)}{volume\ of\ reaction\ mixture\,(mL)} \times 100 \quad (1)$$

The modified submerged membrane reactor could hold up at a fixed volume of 400 mL for the reaction mixture. The cellulose concentrations studied in the hydrolysis were at 10 and 20 w/v %. Therefore, the required weights of cellulose were 40 and 80 g respectively. Calculation were as followed.

$$Weight\ of\ cellulose\ at\ 10w/v\% = \frac{400 \times 10}{100} = 40g$$

$$Weight\ of\ cellulose\ at\ 20w/v\% = \frac{400 \times 20}{100} = 80g$$

The weighted amounts (40 g and 80 g) of cellulose were mixed with 0.5 M aqueous NaOH solution and made up the volume to 400 mL and 800 mL respectively in 1-l beakers. These beakers were then put in a Daniel electronic water bath at 100 °C with regular stirring for every 30 min. During the pretreatment, colour of the mixture changed from yellow to dark brown. After 3 h, the mixtures were cooled to room temperature (25 °C) and washed three times with distilled water to drain out the NaOH residue. The pretreated cellulose was preserved in distilled water at room temperature to be used for hydrolysis (Nguyenhuynh and Nithyanandam, 2016).