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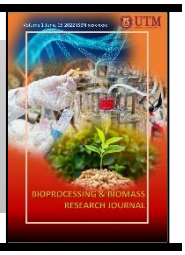

Research Article

Glucose Purification and Cellulase Recycling Using Enzyme Membrane Reactor

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ABSTRACT

The finding of a sustainable energy source leads to cellulosic ethanol as a potential alternative. Membrane filtration holds promise for purifying glucose, an important raw material, while saving production cost through enzyme recycling. This research focuses on the use of a cross-flow enzyme membrane reactor (EMR) to purify glucose from hydrolysed sodium carboxymethylcellulose solution while reusing cellulase for the next cycle through an ultrafiltration-dilution approach. The 30kDa polyethersulfone (PES) membrane would be used to separate the medium, and the EMR's efficiency was tested by the glucose yield, flux, enzyme rejection and glucose permeability. Although a high 26-fold initial dilution during retentate transfer caused an 84.60% ± 6.36% glucose yield decline in the second retentate cycle, the 6.93% ± 2.04% glucose yield in the second cycle indicates that EMR could recover and recycle cellulase without total denaturation if the volumetric dilution factor was optimised. The first cycle experienced stronger membrane fouling, with lower flux, higher enzyme rejection and lower glucose permeability, while the second retentate cycle had a lower separation stability due to membrane reuse. The result can provide useful insights for future commercialisation of EMR in upstream cellulosic ethanol production.

INTRODUCTION

The energy demand in 2024 rose by 2.2% globally, resulting in a 0.8% rise in total energy-related carbon dioxide emissions (IEA, 2025). This trend raises researchers' attention to finding sustainable alternatives, such as cellulosic ethanol produced from cellulosic or lignocellulosic waste (Huynh et al., 2017).

In the process, the hydrolysis of carbohydrates plays an important role in supplying glucose for downstream fermentation. Hydrolysis involves chemicals or enzymes, but enzymatic hydrolysis is preferred over chemical hydrolysis for its high selectivity, environmental friendliness and milder operating conditions (Abo et al., 2019). However, the high enzyme cost, which is approximated to half of the selling price of ethanol, remains a strong economic barrier for the commercialisation of this hydrolysis approach (Liu et al., 2015).

The complete enzymatic hydrolysis involves cellulase, the mixture of exoglucanases (CBH), endoglucanases (EG) and β-glucosidases (βG), to hydrolyse cellulose to cellobiose and finally to glucose (Sofia & Rodrigues, 2011). However, the final conversion of cellobiose to glucose has been found to limit the rate of hydrolysis due to the product inhibition where the rate of hydrolysis is lowered with the formation and accumulation of glucose in the reaction medium (Efrinalia et al., 2022; Tiwari et al., 2017).

To tackle these problems, enzyme membrane reactors (EMRs), which involve stirred tank reactors (STRs) and membrane filtration units, have been designed. Unlike the conventional STRs, the semi-permeable membrane used allowed the recycling and reuse of enzymes in the reaction medium while allowing simultaneous micromolecule product removal (Al-Mardeai et al., 2022).

Therefore, using EMR not only reduces the product inhibition by continuously removing glucose but also enhances enzyme reusability, with up to 98% enzyme retention reported (Acosta-Fernández et al., 2020; Lim and Ghazali, 2020). However, the EMR upscaling is hindered by the membrane fouling, which heavily reduces the

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membrane flux and separation efficiency due to blockage of membrane pores by the macromolecules (Padhan et al., 2023; Wang et al., 2025).

Hence, various types of EMR have been designed, such as dead-end, inverted dead-end, cross-flow, tubular and submerged EMRs, to reduce membrane fouling and increase the separation efficiency. Among them, cross-flow EMR offered a unique feature of using its tangential fluid flow with the membrane arrangement to generate shear stress to detach the loosely bound foulants on the membrane and reduce membrane fouling (Al-Mardeai et al., 2022). The high performance of cross-flow EMR was reported by Lozano et al. (2014), with a 95% microcrystalline cellulose hydrolysis rate, 98% enzyme retention rate and stable enzymatic activity up to 9 cycles

This study investigates the use of a cross-flow EMR to recover glucose from two cycles of 24-hour batch enzymatic hydrolysis of sodium carboxymethylcellulose (CMC). Using an ultrafiltration-dilution approach, fresh cellulase was used for the first cycle, while recycled cellulase from the retentate stream was diluted with fresh CMC solution and evaluated in the subsequent cycle. Although many studies of cellulose hydrolysis have been made, CMC as the substrate has not been investigated using EMR in a cross-flow mode. The separation efficiency of EMR will be assessed by permeate flux, glucose permeability and enzyme rejection rate. CMC was selected as the model substrate due to its water solubility and high enzyme accessibility without the need for pretreatment (Nguyen et al., 2015).

MATERIALS AND METHOD

Materials and chemicals

Cellulase from *Trichoderma reesei* and CMC powder were purchased from Sigma Aldrich (USA). Citrate buffer (0.5M) was prepared by mixing citric acid monohydrate, sodium citrate dihydrate and distilled water, and it served as the solvent for preparing the CMC solution. A 30kDa flat-sheet polyethersulfone (PES) membrane and the filtration cell, CF042, were purchased from Sterlitech Corporation (USA). The Bradford reagent and dinitrosalicylic acid (DNS) reagents were used to determine the samples' absorbance using a spectrophotometer, and the standard curves were plotted using D(+) glucose anhydrous and Bovine Serum Albumin (BSA) solution.

Enzymatic Hydrolysis

The batch hydrolysis was conducted in a Scott bottle (100 mL) using a 1 g/L CMC solution (50 mL) and cellulase (2 mL) at 50 °C, pH 6.0 and constant agitation for 24 hours, which was then used as the feed reservoir of EMR. The reaction conditions were advised by the materials supplier.

EMR Setup

A cross-flow filtration cell was connected to a peristaltic pump, and two pressure gauges via silicon tubing, with the retentate stream returned to the feed reservoir. The transmembrane pressure (TMP) was fixed at 1.375 bar, while the operating flow rate was fixed at 24.0 mL/min.

Pure Water and Blank CMC Solution Separation

Before the separation of hydrolysed CMC, the filtration unit was operated by feeding distilled water at a constant transmembrane pressure (TMP) and operating flow rate to determine the stable pure water flux (PWF). Subsequently, filtration was conducted using a non-hydrolysed CMC solution (50 mL) to determine the stable blank CMC solution flux and examine the flux resistance caused by the substrate alone.

Hydrolysed CMC Separation

After 24 hours of hydrolysis, the hydrolysate of the first batch cycle was fed into the EMR at a constant TMP and operating flow rate. Flux, enzyme, and glucose concentrations in the collected permeate were examined. To assess enzyme reusability using ultrafiltration-dilution approach, an aliquot of retentate (2 mL) was harvested from the feed reservoir at the end of the filtration process. Subsequently, the retentate was transferred and diluted with 1 g/L fresh CMC solution (50 mL) for another 24 hours of hydrolysis, and the separation was repeated.

Analytical Methods

The permeate flux, PWF and blank CMC solution flux were denoted as J and obtained by measuring the volume of permeate (V) collected over time (t) across the effective membrane surface area (A), shown as **Eq. (1)** (Lim & Ghazali, 2020). The flux decline between PWF and blank CMC solution flux was calculated using **Eq. (2)** and multiplied by 100%.

$$J\left(\frac{L}{m^2h}\right)=\frac{V\,(L)}{A(m^2)\times t(h)} \quad (1)$$

$$\text{Flux decline}(\%)=\left(1-\frac{\text{Blank CMC solution flux}\left(\frac{L}{m^2h}\right)}{\text{PWF}\left(\frac{L}{m^2h}\right)}\right) \quad (2)$$

The glucose and cellulase concentrations were quantified using the DNS and Bradford assays by measuring absorbance at 540 nm (OD_{540}) and 595 nm (OD_{595}), respectively, and comparing the results with the standard curves. Then, the glucose yield of the batch hydrolysis, enzyme-to-substrate ratio (E/S), glucose permeability, enzyme rejection, and the yield and E/S decline between two cycles were calculated using **Eq. (3)-(8)**. All equations, except **Eq. (5)**, needed to be multiplied by 100% (Lim & Ghazali, 2020; Liu et al., 2010). As a control measurement of the glucose yield in the second retentate cycle, the glucose yield was calculated by subtracting the initial glucose concentration, which was recorded right upon mixing the retentate and fresh CMC solution, from the final glucose concentration after 24 hours of hydrolysis.

$$\text{Glucose yield}(\%)=\frac{\text{Glucose concentration}_{\text{final-initial}}\,(g/L)}{\text{Fresh CMC concentration}\,(g/L)} \quad (3)$$

$$\text{Yield decline}(\%)=\left(1-\frac{\text{Glucose yield}_{\text{retentate}}(\%)}{\text{Glucose yield}_{\text{first cycle}}(\%)}\right) \quad (4)$$

$$E/S=\frac{\text{Enzyme concentration}\,(g/L)}{\text{CMC concentration}\,(g/L)} \quad (5)$$

$$E/S\text{ decline}(\%)=\left(1-\frac{E/S\text{ ratio}_{\text{retentate cycle}}}{E/S\text{ ratio}_{\text{first cycle}}}\right) \quad (6)$$

$$\text{Glucose permeability}(\%)=\frac{\text{Glucose}_{\text{permeate}}\,(g/L)}{\text{Glucose}_{\text{feed}}\,(g/L)} \quad (7)$$

$$\text{Cellulase rejection}(\%)=\left(1-\frac{\text{Cellulase}_{\text{permeate}}\left(\frac{g}{L}\right)}{\text{Cellulase}_{\text{feed}}\left(\frac{g}{L}\right)}\right) \quad (8)$$

All the experimental phases, including batch enzymatic hydrolysis and the cross-flow EMR operations, were performed in triplicate (n=3 independent trials). Quantitative data and performance metrics are expressed as mean ± standard deviation, calculated across the three independent experimental runs.

RESULTS AND DISCUSSION

Enzymatic Hydrolysis

Figure 1 shows the enzyme-to-substrate ratio (E/S) and glucose yield in the first and second retentate cycles, while **Table 1** shows the glucose and cellulase concentration in some stages of both cycles.

For the first cycle, the glucose yield was 46.20% ± 4.87%. For the second retentate cycle, the initial glucose concentration recorded right upon the retentate was diluted with fresh CMC solution, which was 0.07 g/L ± 0.01 g/L, while the final glucose concentration after 24 hours of hydrolysis was 0.14 g/L ± 0.02 g/L. This resulted in a glucose yield of 6.93% ± 2.04%, with a yield decline of 84.60% ± 6.36% when compared to the first cycle.

The huge glucose yield decline could be due to the massive dilution of the enzyme solution in the second cycle and potential cellulase inactivation. To initiate the hydrolysis in the second cycle, the retentate (2 mL) was diluted with fresh CMC solution (50 mL). This caused a 26-fold volumetric dilution, with an 89.47% ± 3.72% decline in cellulase concentration, from 0.72 g/L ± 0.25 g/L at the retentate stream in the first cycle to 0.11 g/L ± 0.04 g/L at the second cycle.

It led to a 95.50% ± 0.22% E/S decline when comparing the second cycle to the first cycle. At the first cycle, the E/S recorded was 1.54 ± 0.09, while the second retentate cycle had an E/S of 0.07 ± 0.0021. This huge E/S decline justified the low glucose yield in the second cycle, as less cellulase offered the active sites for enzymatic hydrolysis.

However, the glucose yield decline was still lower than both cellulase concentration decrement and E/S decline, indicating that the EMR could successfully recover functional cellulase without total denaturation. As the cellulase could be reused, EMR reduced the enzyme consumption and production cost if the volumetric dilution factor was optimised.

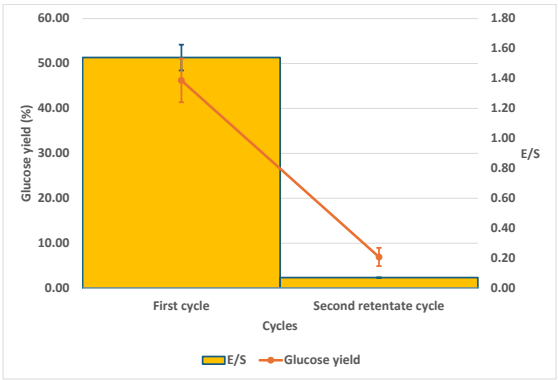

Figure 1 Enzyme-to-substrate ratio (E/S) and glucose yield of the first and second retentate cycles

Pure Water and Blank CMC Solution Separation

Figure 2 shows the stable pure water flux (PWF) and blank CMC solution flux. As discussed by Darcy's Law, flux is directly proportional to TMP, while inversely proportional to membrane resistance and viscosity. Hence, under laminar flow and negligible concentration polarisation (CP) difference, both fluxes shall remain constant across all sets since TMP and viscosity remained unchanged (Quezada et al., 2021).

However, experimental findings showed deviations in PWF, with an average of 15.30 L/m².h ± 2.30 L/m².h, and blank CMC solution flux, with an average of 11.00 L/m².h ± 1.36 L/m².h, which was likely due to membrane reuse, where residual irreversible fouling increased the initial membrane resistance despite cleaning protocols.

Furthermore, the flux decline recorded an average of 28.20% ± 2.75%. This finding illustrated that EMR effectively retained the macromolecular CMC substrate, causing severe concentration polarisation (CP) at the membrane surface. CP occurred due to the rapid generation of a high concentration gradient between the fluid layer near the membrane and the bulk solution due to the accumulation of rejected CMC on the membrane surface (Giacobbo et al., 2018).

A lower stable blank CMC solution flux was also explained by Dąbkowska-Susfał et al. (2024), where a high initial permeate flux decline was observed when a fresh membrane was used to separate polysaccharide solutions and subsequently became stabilised. In the initial phase of separation, the accumulation tendency of polysaccharides at the membrane boundary was higher than the surface renewal rate, causing a higher rate of CP building than the rate of CP shearing away from the membrane until the transient accumulation reached a dynamic equilibrium, resulting in a stable baseline equilibrium flux as the medium was filtered over time.

Due to this phenomenon, CP could be said to initiate and speed up the membrane fouling. As reported by Zhang and Hao (2022), the resistance caused by concentration polarisation had a linear relationship with the total fouling resistance of the membrane bioreactor (R^2 = 0.998).

Therefore, the flux decline between stable PWF and blank CMC solution flux indicated the membrane fouling caused by CMC molecules alone. Furthermore, as the unhydrolysed CMC molecules were retained in the fluid boundary of the membrane, it was expected to be carried to the second cycle with the retentate, reducing the E/S and glucose yield at the batch hydrolysis of the second retentate cycle.

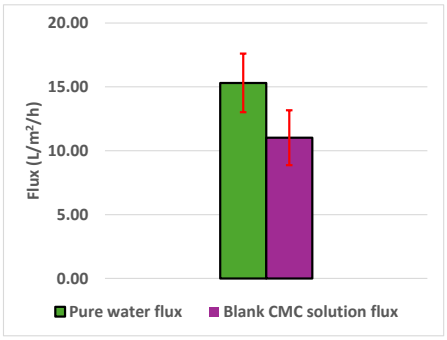

Figure 2 Stable pure water flux and blank CMC solution flux

Hydrolysed CMC separation

Figures 3 and **4** show the average flux, enzyme rejection, and glucose permeability for the first and second retentate cycles over time across trials, while **Table 1** reports their means and standard deviations.

As shown in the figures, both cycles showed that the average flux of all three trials decreased over time from 6.89 L/m².h to 5.47 L/m².h, indicating the membrane became more fouled over time until the system reached a stable fouling status. This could be described by the Collision-Attachment Theory reported by Liu et al. (2020), where the more frequent collisions of foulants and membrane surface over time increased their attachment to the membrane

surface, resulting in greater concentration polarisation (CP) and the deposition of a thicker fouling layer.

However, the first cycle showed a more severe membrane fouling than the second retentate cycle, with a lower flux, higher enzyme rejection and lower glucose permeability. This may be due to the high level of cellulase in the first cycle, which caused a more significant membrane pore constriction that hindered the solute passage.

According to Polyakov and Zydney (2013), proteins and enzymes, despite having a larger molecular size compared to the average pore radius, would absorb into the inner wall of the membrane pore and clog the pore. As a result, the membrane had a smaller effective pore radius and reduced both solutes' permeability through the membrane.

In contrast, the second retentate cycle had a higher level of partially hydrolysed or unhydrolysed CMC, as shown by its low glucose yield. As described by Sioutopoulos et al. (2019), polysaccharides deposited and fouled the membrane by forming a reversible cake on the membrane surface, dominating the membrane fouling in the second retentate cycle.

Hence, the reversible cake layer in the second cycle would be sheared off the membrane more easily by the tangential flow of cross-flow EMR if compared to the first cycle. Furthermore, the high level of CMC in the second cycle resulted in a high solution viscosity, which generated a high shear stress that destabilised the CP and fouling layer (Chalah et al., 2022; Luiz-Santos et al., 2020). Therefore, these factors may further accelerate foulant removal and reduce membrane fouling in the second retentate cycle.

Moreover, due to a more severe membrane fouling and lower flux observed in the first cycle, more cellulase and glucose could be retained and transferred to the second cycle with the retentate. Specifically, the high cellulase rejection of 97.6% ± 1.82% and relatively low glucose permeability of 38.5% ± 3.88% demonstrated that the retentate stream was rich in both components. Hence, in the second cycle, the cellulase could be successfully recovered, but the leftover glucose resulted in a lower glucose yield due to the product inhibition. This phenomenon, when combined with the 26-fold volumetric dilution, led to a massive 84.60% ± 6.36% decline in the second cycle's glucose yield.

In contrast, the second cycle had less severe membrane fouling, resulting in a higher cellulase and glucose permeability. Hence, while providing higher glucose recovery in the permeate stream, the higher enzyme leakage was less sustainable for further consecutive cellulase recycling.

Following this, a higher standard deviation across all parameters was observed in the second retentate cycle, leading to a lower hydraulic and separation stability compared to the initial fresh cycle. This may be due to the variation in the membrane's initial condition, as explained by Dąbkowska-Susfał et al. (2024), the reuse of membranes and recovered enzymes for consecutive cycles could lead to different EMR performance and fluid transport behaviour because of the irreversible residual membrane fouling and potential enzyme deactivation, leading to a higher inconsistency across all parameters recorded in the second retentate cycle.

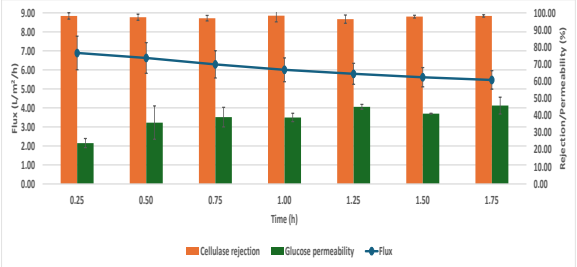

Figure 3 Flux, cellulase rejection and glucose permeability over time in the first cycle

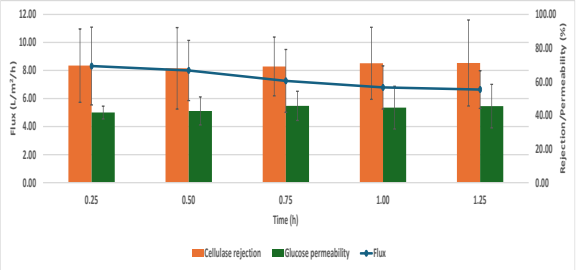

Figure 4 Flux, cellulase rejection and glucose permeability over time in the second retentate cycle

Table 1 Flux, cellulase rejection and glucose permeability in the first and second retentate cycles

Parameters	First cycle	Second retentate cycle
Flux (L/m².h)	6.10 ± 0.66	7.40 ± 2.01
Cellulase rejection (%)	97.6 ± 1.82	69.7 ± 22.1
Glucose permeability (%)	38.5 ± 3.88	44.0 ± 9.27

CONCLUSION

The research fulfilled its objectives, providing insights into the use of a cross-flow enzyme membrane reactor (EMR) to hydrolyse sodium carboxymethylcellulose (CMC) and to recycle the cellulase for a second cycle via an ultrafiltration-dilution approach. A low glucose yield was observed in the second retentate cycle, but this 84.60% ± 6.36% yield decline was due to cellulase dilution and potential inactivation. Furthermore, this research found that the 30 kDa polyethersulfone (PES) membrane retained the CMC macromolecules and cellulase. When separating the 24-hour hydrolysed CMC solution, the first cycle experienced a more severe membrane fouling, with a lower flux and solutes' permeate, while the second retentate cycle showed lower hydraulic and separation stability, with higher standard deviations recorded for all three parameters. Overall, the research provides valuable guidance for using cross-flow EMR for glucose production and recycling valuable enzymes for the next cycle through an ultrafiltration-dilution approach. Future work should focus on examining the enzymatic activity and stability, optimising the enzyme-to-substrate ratio and performing an economic study to enhance the process efficiency and economic viability.

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Conflicts of Interest

The author declares that there is no conflict of interest regarding the publication of this paper.

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