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Original Research Article

Membrane-Assisted Cell Retention for Intensified Continuous Bioethanol Production

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Abstract

Continuous ethanol fermentation can achieve higher volumetric productivity than batch operation, but its performance is often limited by yeast washout, incomplete glucose conversion, and reduced fermentative activity under high-substrate and ethanol-stress conditions. This study evaluated an ultrafiltration membrane bioreactor for continuous ethanol fermentation using cell retention and controlled aeration. Glucose fermentation by *Saccharomyces cerevisiae* was evaluated in batch mode, conventional continuous anaerobic operation, anaerobic membrane bioreactor operation at dilution rates of 0.05 and 0.10 h^{-1}, and aerobic membrane bioreactor operation at 0.10 h^{-1} with aeration rates of 0.08–0.30 vvm. Batch fermentation provided a reference for interpreting the continuous experiments: aerobic operation increased the final ethanol concentration from 65.7 to 89.5 $g.L^{-1}$ and the apparent maximum ethanol formation rate from 1.17 to 2.60 $g.L^{-1}.h^{-1}$, based on modified Gompertz fitting. In continuous operation, membrane-assisted cell retention reduced washout and increased biomass retention, glucose conversion, ethanol concentration, and volumetric productivity. At the same dilution rate of 0.10 h^{-1}, the anaerobic membrane bioreactor increased ethanol concentration from 2.8 ± 0.8 $g.L^{-1}$ to 43.4 ± 2.1 $g.L^{-1}$ and productivity from 0.3 ± 0.1 $g.L^{-1}$ h^{-1} to 4.3 ± 0.2 $g.L^{-1}.h^{-1}$ relative to the non-membrane reactor. Lower dilution rate favored ethanol accumulation, whereas higher dilution rate favored volumetric productivity. Controlled aeration further enhanced productivity, reaching 5 $g.L^{-1}.h^{-1}$ at 0.08 vvm, but excessive aeration increased biomass accumulation while reducing ethanol yield and selectivity.

Keywords: Bioethanol; Fermentation; Reactor integration; Ultrafiltration; Operation mode; Dilution rate

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1. Introduction

Liquid biofuels remain an important part of the low-carbon fuel portfolio because they can be deployed in existing fuel-distribution systems and can partially substitute petroleum-derived transport fuels. Bioethanol is one of the most established renewable liquid fuels because it can be produced through microbial fermentation of carbohydrate-rich feedstocks and blended with gasoline using existing fuel infrastructure. The International Energy Agency projects that biofuels will increase from 5.6% of total liquid transport fuel demand in 2023 to 6.4% by 2030, reaching approximately 215 billion $L.year^{-1}$ in its main case. This growth is expected to be concentrated in the United States, Europe, Brazil, Indonesia, and India, reflecting the combined effects of fuel mandates, greenhouse-gas reduction targets, and policy incentives [1]. Under the main-case renewable energy outlook, global road biofuel demand is projected to reach approximately 205 billion L by 2030, with additional growth expected in the aviation and maritime sectors [2,3]. This projected demand

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increases the need for bioethanol production processes with higher productivity, lower energy consumption, and greater suitability for continuous operation.

Ethanol fermentation using *Saccharomyces cerevisiae* is widely applied, but its performance is limited by physiological stress and reactor-operating constraints. The theoretical ethanol yield from glucose is 0.5 $g.g^{-1}$, but practical yields and productivities are reduced by ethanol inhibition, osmotic stress, substrate inhibition, by-product formation, and loss of yeast viability under high-gravity conditions. Recent reviews identify limited yeast tolerance to ethanol, osmotic stress, and inhibitory compounds as major barriers to improving industrial bioethanol production [4,5]. High-gravity and very-high-gravity fermentation can increase ethanol titer and reduce water use, but these conditions intensify osmotic and ethanol stresses, which can slow fermentation and leave residual sugars. Improving bioethanol production therefore requires strain and feedstock optimization together with reactor configurations that maintain a high concentration of active cells and favor ethanol formation [6].

Continuous fermentation is attractive because it can increase volumetric productivity and reduce downtime compared with batch operation [7]. However, in conventional continuous fermentation, hydraulic residence time and cell residence time are coupled, which restricts biomass retention [8]. At a high dilution rate, yeast cells may leave the reactor faster than they are replenished by growth, resulting in washout, low biomass concentration, incomplete glucose utilization, and low ethanol productivity [9,10]. This limitation has motivated the development of cell-recycle and membrane-assisted fermentation systems [11]. Earlier and more recent studies have shown that membrane modules can retain yeast biomass within the bioreactor while allowing cell-free permeate containing fermentation products and residual sugars to leave the system [12,13]. In these systems, the membrane retains cells and allows the cell residence time to exceed the hydraulic residence time.

A clear distinction is needed between ultrafiltration membranes used for cell retention and selective membrane processes used for ethanol recovery [14]. Much of the membrane literature for bioethanol focuses on pervaporation [15,16], membrane distillation [17,18], or integrated separation schemes for ethanol recovery [19], where the membrane directly contributes to ethanol enrichment or product removal. In contrast, an ultrafiltration membrane bioreactor for ethanol fermentation acts primarily by retaining yeast cells and suppressing washout. This distinction is important because, in a cell-retention MBR, productivity is governed by dilution rate, retained biomass concentration, glucose conversion, and the metabolic state of the yeast. Previous membrane bioreactor studies have demonstrated effective yeast retention, but the relationships among dilution rate, specific ethanol-producing activity, and carbon allocation between ethanol and biomass remain insufficiently clarified [13,20].

Aeration also affects the balance between yeast viability, biomass formation, and ethanol production. Although ethanol formation by *S. cerevisiae* is associated with fermentative metabolism, limited oxygen availability can support yeast viability by enabling sterol and unsaturated fatty acid synthesis, which are important for membrane integrity and ethanol tolerance. Controlled aeration can improve yeast viability and ethanol productivity during very-high-gravity fermentation. Redox-potential-based aeration has also been proposed to supply limited oxygen for maintaining biomass activity and viability under severe osmotic and ethanol stress [21–23]. However, excessive aeration can shift substrate utilization toward biomass formation and respiratory metabolism, reducing ethanol selectivity. Thus, in a membrane bioreactor with high cell retention, aeration should be controlled according to its effects on both cell growth and ethanol metabolism.

The present study evaluated a membrane bioreactor for continuous ethanol fermentation. The experiments included anaerobic and aerobic batch fermentation, conventional continuous anaerobic fermentation, anaerobic membrane bioreactor operation at dilution rates of 0.05 and 0.10 h^{-1}, and aerobic membrane bioreactor operation at 0.10 h^{-1} with aeration rates of 0.08, 0.20, and 0.30 vvm. Ethanol concentration, biomass concentration, and residual glucose concentration were measured, and the data were used to calculate productivity, glucose conversion, ethanol yield, apparent specific rates, and carbon-partitioning indicators. In this study, ultrafiltration was used to retain the biocatalyst and decouple cell residence time from hydraulic residence time, while aeration was varied to assess its effect on continuous ethanol production.

2. Materials and Methods

2.1. Materials

The materials used were *Saccharomyces cerevisiae* R-58 culture, commercial baker's yeast (Fermipan), demineralized water, glucose (Merck KGa), $ZnSO_4 \cdot 7H_2O$ (EMSURE®), urea (Merck, Sigma-Aldrich), peptone (Millipore, Merck KGa), yeast extract (Millipore, Merck KGa), NaOH (Merck KGaA, ≥99 %), HCl (Merck KGaA, 37 %),

ethanol (Millipore, Merck KGa), acetone (EMSURE®), and KOH (EMSURE®). Commercial baker's yeast (Fermipan) was used directly in the continuous fermentation experiments. Commercial yeast was selected to evaluate reactor-level performance rather than strain optimization.

The batch fermentation and inoculum media were prepared using a modified YPD formulation containing glucose, peptone, yeast extract, urea, and $ZnSO_4 \cdot 7H_2O$. The batch medium contained 300 g.L^{-1} glucose, 20 g.L^{-1} peptone, 5 g.L^{-1} yeast extract, 20 g.L^{-1} urea, and 0.05 g.L^{-1} $ZnSO_4 \cdot 7H_2O$. The continuous fermentation medium contained 300 g.L^{-1} glucose, 15 g.L^{-1} peptone, 5 g.L^{-1} yeast extract, 20 g.L^{-1} urea, and 0.05 g.L^{-1} $ZnSO_4.7H_2O$. The agar slant medium contained 20 g.L^{-1} glucose, 10 g.L^{-1} peptone, 5 g.L^{-1} yeast extract, and 20 g.L^{-1} bacto agar.

2.2. Batch Fermentation Experiments

The fermentation medium (Table S1) was prepared and sterilized as described in the Supporting Information. The yeast culture was prepared according to the procedure described in the Supporting Information. Batch fermentation was conducted to compare ethanol production under anaerobic and aerobic conditions. The experiments were conducted in 1 L Erlenmeyer flasks with a working volume of 500 mL. The fermentation temperature was maintained at 30 °C and the initial pH of the medium was 4.5. No pH control was applied during fermentation. The batch experiments were operated for 71 h. Samples were first collected after 15 h and subsequently every 8 h. For the aerobic batch condition, the flask was placed on a shaker operated at 110 rpm. For the anaerobic batch condition, the flask was placed in a closed cabinet without agitation. Ethanol concentration was used as the primary response variable for comparing the two batch conditions.

2.3. Continuous Anaerobic Fermentation without Membrane

Continuous anaerobic fermentation without membrane was performed in a 10 L fermentor with a working volume of 5 L. Commercial yeast was inoculated directly at an initial concentration of 3 g.L^{-1}. The fermentation temperature was maintained at 30 °C, and the initial medium pH was 4.5. Antifoam was added as required to control foaming. No pH control was applied during the run. Agitation was set at 300 rpm.

The reactor was first operated in batch mode for 24 h without feed addition to establish sufficient biomass. During start-up, air was supplied to promote biomass growth. After 24 h, the air supply from the sparger was stopped to establish anaerobic conditions. The feed and outlet streams were then opened to initiate continuous operation. Glucose solution was fed at a flow rate corresponding to a dilution rate of 0.10 h^{-1}. Continuous operation was conducted for 60 h. Samples were collected every 10 h from 0 to 40 h and every 5 h from 40 to 60 h.

The dilution rate was defined as:

$$D = F/V \quad (1)$$

where D is the dilution rate in h^{-1}, F is the feed flow rate in L.h^{-1}, and V is the working volume of the fermenter in L.

2.4. Continuous Anaerobic Membrane Bioreactor (An-MBR) Operation

Continuous anaerobic membrane fermentation was conducted under the same operating conditions as the non-membrane system, except that an ultrafiltration membrane module was installed in the outlet circulation loop. The membrane system consisted of a feed reservoir, feed pump, fermenter, circulation pump, ultrafiltration module, retentate recycle line, and permeate outlet. The retentate was returned to the fermenter to retain yeast cells, while the permeate was collected as the cell-free product stream. Two dilution rates were evaluated: 0.05 and 0.10 h^{-1}.

This configuration enabled the cell residence time to exceed the hydraulic residence time. Yeast cells were retained by the 100,000 Da ultrafiltration membrane, while soluble fermentation products and residual glucose passed through the permeate stream. The anaerobic membrane bioreactor therefore functioned as a cell-retention bioreactor rather than an ethanol-selective separation unit.

2.5. Continuous Aerobic Membrane Bioreactor (Ae-MBR) Operation

Continuous aerobic membrane fermentation was performed using the same membrane configuration, with air introduced into the outlet stream before the membrane module. Air was introduced into the circulation loop rather than through a conventional sparger inside the fermenter. This configuration was selected to increase biomass concentration while helping maintain membrane permeate flux.

The dilution rate was fixed at 0.10 h^{-1}, corresponding to a feed flow rate of 0.50 L.h^{-1} for a 5 L working volume. Three aeration rates (Q_{air}) were evaluated: 0.08, 0.20, and 0.30 *vvm*. The corresponding volumetric air flow rates for a 5 L working volume (V) were calculated from the vvm definition as follows:

$$Q_{air} = vvm \times V \quad (2)$$

2.6. Analytical Methods

Ethanol concentration was determined by gas chromatography (Shimadzu 2014, Japan). Before GC analysis, 10 mL of fermentation sample was diluted with 5 mL demineralized water. NaOH solution (0.1 M) was added to neutralize organic acids prior to distillation. The mixture was distilled to separate ethanol from non-volatile salts and other broth components. A 10 mL distillate sample was then analyzed by GC. Gas chromatography was performed using a thermal conductivity detector equipped with a Porapak-Q column (Shimadzu). The column, injector, and detector temperatures were 150, 180, and 200 °C, respectively. Ethanol concentration was calculated using equation S1 and procedure reported in supplementary information.

3. Results and Discussion

3.1. Batch Fermentation: Anaerobic vs Aerobic

Batch fermentation was first evaluated to provide a reference for interpreting the continuous MBR. Ethanol concentration increased with fermentation time in both batch systems, but the aerobic batch produced ethanol more rapidly and reached a higher final concentration than the anaerobic batch (Figure 1). Under anaerobic conditions, ethanol concentration increased from 4.9 g.L^{-1} at 0 h to 65.7 g.L^{-1} at 71 h. Under aerobic conditions, ethanol concentration increased from the same initial value to 89.5 g.L^{-1} at 71 h.

The net ethanol formation rate was calculated from the increase in ethanol concentration (equation S9). These results indicate that limited oxygen availability enhanced ethanol production during batch fermentation. Although ethanol production is generally associated with fermentative metabolism, limited oxygen can improve yeast physiological robustness. However, *S. cerevisiae* requires oxygen for the biosynthesis of key membrane lipids, especially sterols and unsaturated fatty acids, which are essential for membrane integrity, nutrient uptake, stress resistance, and ethanol tolerance. Recent literature on yeast lipid physiology shows that sterols are central to plasma membrane structure and yeast development, and ergosterol is produced under aerobic conditions [24]. Under anaerobic or hypoxic fermentation, *S. cerevisiae* depends strongly on lipid availability to maintain membrane adaptation under stress [25]. Therefore, the higher ethanol accumulation under aerobic conditions is attributed to improved physiological robustness rather than oxidative metabolism.

The ethanol accumulation profiles were fitted using a modified Gompertz model with an initial ethanol offset (equation S10) [26,27]. For the present dataset, P_0 was fixed at the measured initial ethanol concentration of 4.89 g.L^{-1} for both batch systems. Nonlinear least-squares fitting gave high goodness of fit for both conditions, with $R^2 = 0.984$ for the anaerobic batch and $R^2 = 0.993$ for the aerobic batch. The fitted parameters are summarized in Table 1.

The fitted model also indicates a higher ethanol production rate under aerobic conditions. The fitted ethanol production potential increased

Table 1. Kinetic parameters from modified Gompertz fitting for batch operation.

Batch condition	P_0 (g.L^{-1})	A (g.L^{-1})	R_{max} (g.L^{-1}.h^{-1})	λ (h)	R^2
Anaerobic batch	4.89	67.79	1.17	2.06	0.984
Aerobic batch	4.89	85.62	2.60	5.39	0.993

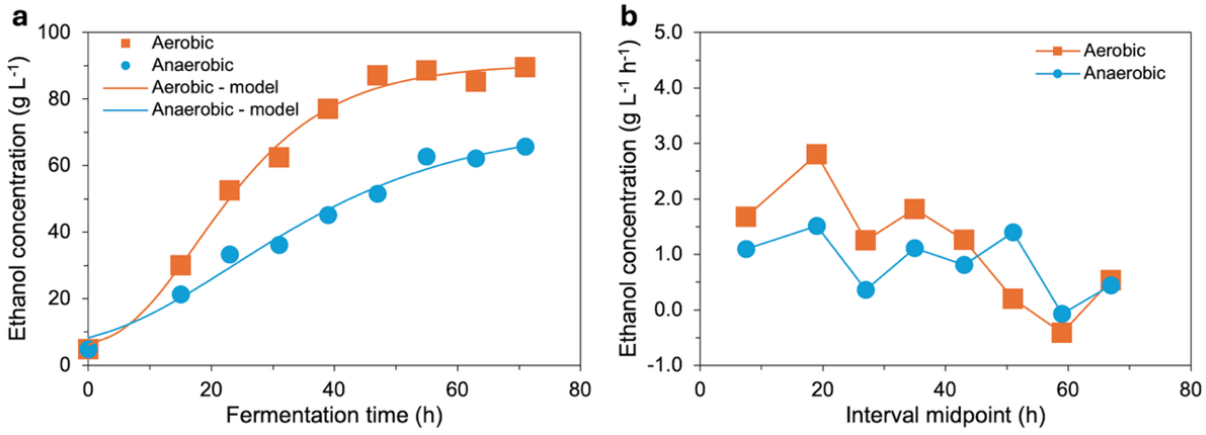

Figure 1. Batch fermentation performances. (a) Ethanol profile of batch fermentation and fitted model. (b) Ethanol formation rate.

from 67.8 g.L^{-1} under anaerobic conditions to 85.6 g.L^{-1} under aerobic conditions. More importantly, the fitted maximum apparent ethanol formation rate increased from 1.2 to 2.6 g.L^{-1}.h^{-1}. This 2.2-fold increase in R_{max} is consistent with the interval-rate analysis and supports the conclusion that oxygen availability accelerated ethanol accumulation during the active production phase.

The higher ethanol concentration and R*max* suggest that limited oxygen improved yeast physiological performance during high-glucose fermentation. This does not imply that ethanol was produced through aerobic respiration. Rather, oxygen likely supported cellular components needed to maintain fermentative activity under stress. Ethanol accumulation causes membrane perturbation, affects protein function, and reduces yeast viability at sufficiently high concentration; recent reviews identify ethanol tolerance as a major physiological constraint in *S. cerevisiae* fermentation [5]. Membrane lipid composition is one of the key determinants of ethanol tolerance, and changes in yeast membrane lipids are associated with adaptation to ethanol stress [28].

3.2. Membrane-Assisted Cell Retention Suppresses Washout During Continuous Fermentation of Anaerobic Condition

The apparent contrast between the batch and continuous results reflects the different controlling mechanisms in the two operating modes. In batch fermentation, yeast cells remain in the vessel throughout the run. The higher ethanol concentration under aerobic batch operation therefore reflects the physiological benefit of limited oxygen availability, especially its role in supporting membrane lipid synthesis and ethanol tolerance. In continuous fermentation, the key limitation is different. Cells can leave the reactor with the outlet stream, and ethanol production collapses when biomass retention is insufficient. The strong improvement observed in the An-MBR therefore arose mainly from suppression of washout and decoupling of cell residence time from hydraulic residence time. Thus, aerobic batch operation improved yeast robustness, while anaerobic MBR operation improved continuous reactor performance by retaining the biocatalyst.

The effect of membrane-assisted cell retention is most clearly demonstrated by comparing the continuous anaerobic bioreactor (without membrane) and the An-MBR at the same dilution rate of 0.10 h^{-1} (Figure 2, Table 2). X and S in Figure 2 were calculated by using equations S2 – S4. Under non-membrane continuous operation, ethanol concentration was only 2.8 ± 0.8 g.L^{-1} and the corresponding volumetric productivity was 0.3 ± 0.1 g.L^{-1}.h^{-1}. In contrast, the An-MBR produced 43.4 ± 2.1 g.L^{-1} ethanol and reached a productivity of 4.3 ± 0.2 g.L^{-1}.h^{-1}. Thus, membrane integration increased ethanol concentration by approximately more than 15-fold and productivity by approximately more than 15-fold at identical hydraulic dilution rate. This large improvement demonstrates that membrane-assisted cell retention substantially improved continuous reactor performance.

The improvement is further supported by the biomass and residual glucose data. In the anaerobic bioreactor, the steady-state biomass concentration was only 6.9 g.L^{-1}, while residual glucose remained high at 193 g.L^{-1}. This means that the reactor still contained abundant substrate but lacked sufficient retained active biomass to convert it into ethanol. By contrast,

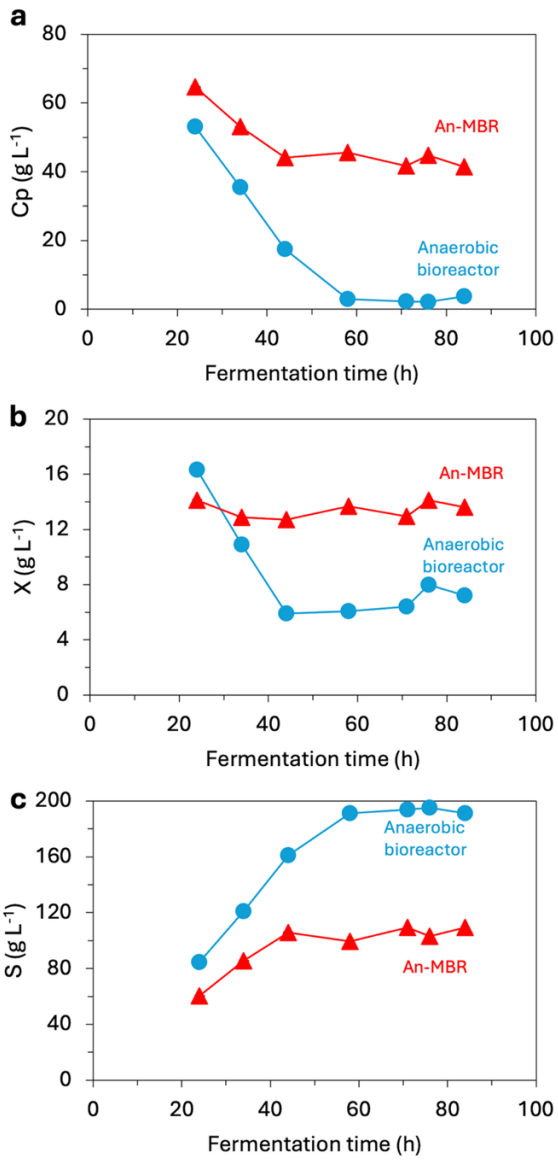

Figure 2. Ethanol production in anaerobic bioreactor and An-MBR. (a) Ethanol concentration (CP), (b) Biomass concentration (X), and (c) Residual glucose (S).

the An-MBR maintained 13.4 g.L^{-1} biomass and reduced residual glucose to 105.5 g.L^{-1}. Glucose conversion increased from 35.7% in the anaerobic bioreactor to 64.8% in the An-MBR.

This behavior is characteristic of washout limitation in conventional continuous fermentation. In a suspended-cell continuous stirred tank reactor, the hydraulic dilution rate determines both the residence time of the liquid phase and the residence time of the cells. When cells are withdrawn together with the effluent, the reactor can only maintain biomass if the specific growth rate is sufficient to compensate for dilution. If this condition is not met, the biomass concentration decreases, glucose remains unconsumed, and ethanol productivity collapses. The non-membrane continuous reactor in this study showed exactly this pattern: low biomass, high residual glucose, and very low ethanol productivity.

In An-MBR, broth is circulated through an ultrafiltration module, where retentate is returned to the fermentor, and permeate is withdrawn as the product stream. This design converts the fermentor from a conventional suspended-cell continuous reactor into a cell-retention bioreactor. In previous study, Ylitervo et al. reported continuous ethanol fermentation using an external cross-flow membrane module in which *S. cerevisiae* cells were retained inside the bioreactor, while cell-free permeate containing fermentation products and unfermented sugars was removed through the membrane [13]. Kargupta et al. analyzed a continuous membrane bioreactor with cell recycling during ethanol fermentation and specifically examined how cell recycling affects ethanol productivity [29]. Earlier work also showed that using membranes in continuously operated stirred bioreactors can increase cell concentration and ethanol productivity [12]. These findings are consistent with previous reports showing that cell retention transforms continuous ethanol fermentation into a high-cell-density process.

The improvement is also reflected in ethanol productivity P_{EtOH} (calculated by equation S11). At the same D = 0.01 h^{-1}, the productivity increases from 0.3 to 4.3 g.L^{-1}.h^{-1} (Table 2) cannot be attributed to a change in hydraulic throughput, because the dilution rate was identical. It must therefore originate from the higher ethanol concentration generated inside the reactor. This higher ethanol concentration, in turn, is linked to increased retained biomass and improved glucose conversion.

The apparent specific substrate uptake rate can be estimated from the steady-state substrate balance, q_S (equation S12). The apparent specific glucose uptake rates are similar, but the membrane bioreactor retained nearly twice as much biomass (Table 3). This indicates that the major benefit of the membrane was not necessarily a higher specific glucose uptake rate per unit biomass, but a larger retained biocatalyst inventory. In other words, the membrane increased the volumetric reaction capacity by increasing X, not by fundamentally changing q_S.

The same interpretation is obtained from the apparent specific ethanol production rate q_P (equation S13). Unlike q_S, the apparent qP increased strongly after membrane integration (Table 2). This suggests that the retained biomass in the An-MBR was not only more abundant, but also more effectively directed toward ethanol formation. The anaerobic bioreactor consumed part of the available glucose but generated little

Table 2. Performance of Anaerobic bioreactor and An-MBR.

Parameter	Anaerobic bioreactor	An-MBR	Improvement by membrane
D (h^{-1})	0.1	0.1	-
C_P (g.L^{-1})	2.8 ± 0.8	43.4 ± 2.1	-
X (g.L^{-1})	6.9 ± 0.9	13.6 ± 0.5	-
S (g L^{-1})	193.0 ± 1.9	105.5 ± 5.0	-
P_{EtOH} (g.L^{-1}.h^{-1})	0.3 ± 0.1	4.3 ± 0.2	15.8
X_S (-)	0.36 ± 0.01	0.65 ± 0.02	1.8
$Y_{P/S}$ (g.g^{-1})	0.03 ± 0.01	0.22 ± 0.01	8.7
η_Y (-)	0.05 ± 0.01	0.44 ± 0.01	8.7
q_S (g glucose.g^{-1} biomass.h^{-1})	1.6 ± 0.2	1.4 ± 0.0	0.9
q_P (g ethanol.g^{-1} biomass.h^{-1})	0.04 ± 0.01	0.32 ± 0.01	7.9

Table 3. Performance of An-MBR at different dilution rate D.

D (h^{-1})	HRT (h)	C_P (g.L^{-1})	P_{EtOH} (g.L^{-1}.h^{-1})	X (g.L^{-1})	S (g.L^{-1})
0.05	20	59.5 ± 1.4	3.0 ± 0.1	14.8 ± 0.5	73.3 ± 1.2
0.10	10	43.4 ± 2.1	4.3 ± 0.2	13.6 ± 0.5	105.5 ± 5.0

ethanol, implying poor carbon conversion to product. Possible reasons include biomass instability, physiological stress, by-product formation, and low effective residence time for active yeast.

It is important to distinguish the role of the ultrafiltration membrane in this study from ethanol-selective membrane processes. Pervaporation and membrane distillation can remove ethanol from fermentation broth and reduce product inhibition by selectively transferring ethanol or ethanol-rich vapor across a membrane [15,18]. Here, the membrane function is as a cell-retention and bioreactor-intensification element. This also explains why the membrane effect is most visible in continuous operation. In batch fermentation, cells remain in the vessel by design, so there is no hydraulic washout. In continuous fermentation, cell retention becomes essential because liquid withdrawal can otherwise remove the biocatalyst.

Future optimization should focus on membrane operation. Since the membrane primarily retains cells, performance will depend on membrane area, crossflow velocity, shear environment, cell deposition, fouling, cleaning strategy, and long-term flux stability. Ylitervo et al. showed that membrane bioreactors can maintain high yeast cell density during continuous ethanol fermentation by retaining cells in the bioreactor, but such operation must be managed together with membrane performance and inhibitor effects [13]. Similarly, the present system demonstrates the biological benefit of cell retention, but longer-term experiments are needed to determine whether the membrane can maintain stable flux, cell viability, and productivity under extended operation.

3.3. Dilution-Rate-Dependent Trade-off in An-MBR

The effect of dilution rate (D) was evaluated in the An-MBR by comparing operation at D = 0.05 h^{-1} and D = 0.10 h^{-1}. Both systems used the same membrane-assisted cell-retention concept but differed in hydraulic residence time. At D = 0.05 h^{-1}, the hydraulic residence time was approximately 20 h, whereas at D = 0.10 h^{-1}, it decreased to 10 h.

The results show a clear dilution-rate trade-off (Table 3). Decreasing the dilution rate from 0.10 to 0.05 h^{-1} increased ethanol concentration from 43.54 to 59.19 $g.L^{-1}$. Glucose conversion also increased from 64.8 to 75.2%, while residual glucose decreased from 105.5 to 74.5 $g.L^{-1}$. These trends indicate that the lower dilution rate provided longer residence time for glucose uptake and ethanol accumulation. However, volumetric ethanol productivity decreased from 4.35 to 2.96 $g.L^{-1}.h^{-1}$ because productivity is directly proportional to dilution rate.

These observations are consistent with the expected behavior of continuous fermentation systems. Increasing dilution rate increases medium throughput but decreases residence time. If the decrease in residence time prevents complete glucose conversion, ethanol concentration and yield decline. Nevertheless, volumetric productivity can still increase if the higher flow rate compensates for the lower ethanol concentration. Thus, D = 0.05 h^{-1} favored ethanol titer and conversion, whereas D = 0.10 h^{-1} favored reactor productivity.

Similar trade-offs have been reported in continuous ethanol fermentation with cell recycle. Wang et al. showed that high cell-recycling ratios allowed continuous ethanol fermentation to operate at high dilution rates, but productivity depended strongly on feed concentration, cell-recycle intensity, and dilution rate. Their reported productivity range of 6.9–7.5 $g.L^{-1}.h^{-1}$ at feed concentrations of 80–200 $g.L^{-1}$ provides a useful benchmark for high-throughput cell-recycle operation [10]. Brandberg et al. also reported that cross-flow-filtration-based cell recirculation increased sugar conversion in continuous fermentation at D = 0.10 h^{-1}, demonstrating the importance of cell retention in maintaining conversion at practical dilution rates [30]. Compared with these studies, the present anaerobic MBR achieved a moderate productivity of 4.3 $g.L^{-1}.h^{-1}$ at D = 0.10 h^{-1}, but with a relatively high ethanol concentration of 43.5 $g.L^{-1}$. The lower productivity relative to optimized cell-recycle systems is not surprising because the present configuration used a simple single-stage MBR.

Apparent specific rates were calculated to further interpret reactor performance (Figure 3; all parameters in the figure were calculated by using equations S6-S13). The increase in q_S and q_P at higher dilution rate suggests that the retained yeast population operated at a higher apparent metabolic flux when exposed to faster medium turnover. However, the higher specific activity did not translate into higher ethanol concentration because the liquid residence time was shorter. In other words, the cells processed substrate faster per unit biomass, but the reactor provided less time for conversion before the broth exited the system.

The biomass yield, $Y_{X/S}$, remained nearly unchanged between the two dilution rates. These results indicate that dilution rate primarily affected residence time rather than biomass formation. Instead, the dominant effect was hydraulic: shorter residence time reduced conversion and ethanol accumulation. The ethanol-to-biomass selectivity index, $SI_{P/X}$, decreased from 3.9 to 3.2 when the dilution rate increased from 0.05 to 0.10 h^{-1}, indicating a

modest reduction in ethanol selectivity relative to biomass formation.

This behavior agrees with the modeling literature on continuous ethanol fermentation. Recent control and modeling studies continue to treat dilution rate as a central manipulated variable because it controls residence time, substrate availability, biomass retention, and product washout in continuous ethanol processes. Qin *et al.* studied continuous *S. cerevisiae* fermentation and emphasized the importance of regulating input and output flow rates, which are directly related to dilution rate, for stabilizing ethanol fermentation performance [7]. More recently, Oliveira *et al.* modeled an industrial multistage continuous alcoholic fermentation process with cell recycling and variable volumetric flow rates and substrate concentrations, reinforcing that dilution rate and cell recycling remain key design and control variables in industrial ethanol fermentation [31].

The preferred dilution rate depends on the process target. If the target is maximum ethanol concentration and higher glucose conversion, D = 0.05h^{-1} is preferable because it produced 59.5 ± 1.4 g.L^{-1} ethanol and 75.2% glucose conversion. This condition is useful when downstream separation energy is a major concern. If the target is maximum volumetric productivity, D = 0.10 h^{-1} is preferable because it increased productivity to 4.3 ± 0.2 g.L^{-1}.h^{-1} by increasing throughput. For productivity-oriented continuous production, the best condition in this study was the Ae-MBR at D = 0.10 h^{-1} and 0.08 vvm, which reached 5.1 ± 0.2 g.L^{-1}.h^{-1}.

3.4. Aeration-Dependent Metabolic Shift in Ae-MBR

Aeration had a pronounced but non-linear effect on the continuous Ae-MBR (see Table 4; Figure 4; all parameters in Figure 4 were calculated by equations S6-S13). At a fixed

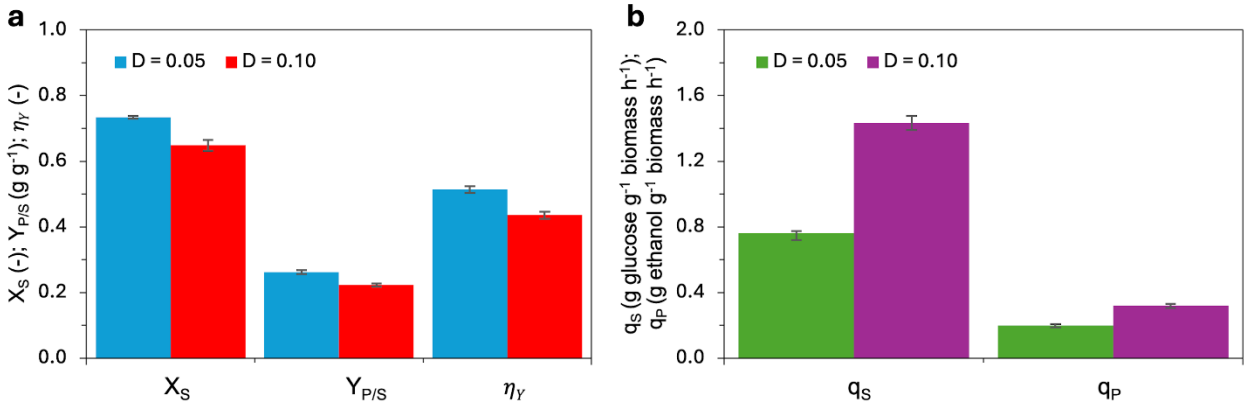

Figure 3. Effect of dilution rate on An-MBR performance. (a) Glucose conversion (X_S), ethanol yield ($Y_{P/S}$), and theoretical yield efficiency (η_Y). (b) Apparent specific substrate uptake rate (q_S) and apparent specific ethanol production rate (q_P).

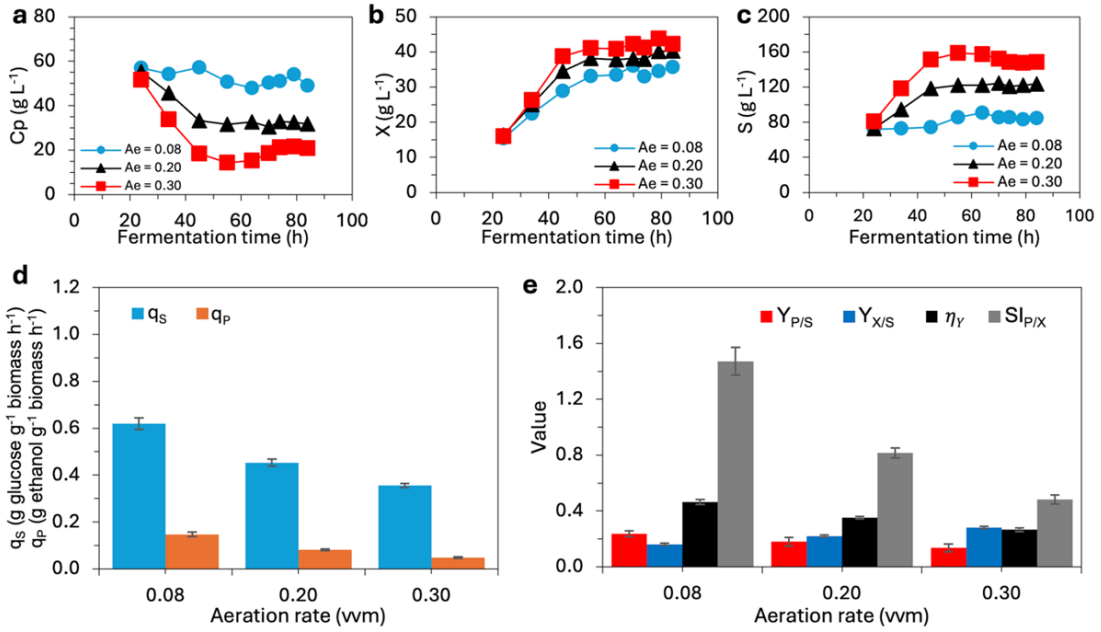

Figure 4. Ae-MBR at various aeration rate. (a) Ethanol concentration, (b) biomass concentration, (c) residual glucose, (d) kinetic parameters, and (e) yield, efficiency, and selectivity.

dilution rate of 0.10 h^{-1}, the lowest aeration rate tested, 0.08 vvm, gave the best ethanol-producing performance among the Ae-MBR runs. Ethanol concentration reached 50.6 $g.L^{-1}$ and volumetric productivity reached 5.1 $g.L^{-1}.h^{-1}$. This productivity was higher than that of the An-MBR operated at the same dilution rate, which produced 43.5 $g.L^{-1}$ ethanol with a productivity of 4.3 $g.L^{-1}.h^{-1}$. Thus, introducing limited aeration improved ethanol productivity by approximately 16.3% relative to the anaerobic membrane bioreactor at the same hydraulic residence time.

The improved performance at low aeration is consistent with known yeast physiology. Although ethanol formation by *S. cerevisiae* is commonly associated with fermentative metabolism, oxygen is still important for maintaining cell viability and fermentative capacity. Molecular oxygen is required for the synthesis of sterols and unsaturated fatty acids, which influence yeast ethanol tolerance, membrane integrity, and fermentation performance [32]. Under very-high-gravity conditions, controlled oxygen supply has also been reported to support biomass accumulation and cell viability under osmotic and ethanol stress, improving productivity and yield [33,34]. Therefore, the improved productivity at 0.08 vvm is best interpreted as a physiological support effect: limited oxygen helped maintain active yeast cells while the membrane retained them inside the reactor.

Increasing the aeration rate above 0.08 vvm did not further improve ethanol production. Instead, ethanol concentration decreased from 51.2 $g.L^{-1}$ at 0.08 vvm to 31.9 $g.L^{-1}$ at 0.20 vvm and 20.5 $g.L^{-1}$ at 0.30 vvm. The corresponding ethanol productivity decreased from 5.06 to 3.20 and 1.86 $g.L^{-1}.h^{-1}$. Glucose conversion also decreased from 71.4% to 59.1% and 49.3%, while ethanol yield decreased from 0.236 to 0.180 and 0.126 g ethanol.g^{-1} glucose consumed.

This decline occurred despite an increase in biomass concentration. Biomass increased from 34.30 $g.L^{-1}$ at 0.08 vvm to 38.75 $g.L^{-1}$ at 0.20 vvm and 41.92 $g.L^{-1}$ at 0.30 vvm. Therefore, higher aeration generated more retained biomass, but this additional biomass did not translate into higher ethanol productivity. Carbon utilization shifted from ethanol production toward biomass formation.

This result shows that ethanol productivity was not controlled by biomass concentration alone. If total biomass were the dominant determinant, the highest aeration condition should have given the highest productivity. The opposite occurred. This shows that the relevant variable is not simply retained biomass but retained fermentatively active biomass. At excessive aeration, a larger fraction of substrate was apparently directed toward biomass formation, maintenance, and respiratory metabolism rather than ethanol formation. This trend is consistent with previous studies on ethanol fermentation. Controlled aeration can improve yeast health, but excessive oxygen can shift carbon distribution toward growth and away from ethanol. Recent work on redox-potential-driven aeration emphasizes that oxygen addition must be regulated, especially under high-gravity conditions, because oxygen is needed for yeast viability but can also alter metabolic balance [34]. The present data are aligned with that concept: a small oxygen input improved productivity, whereas higher oxygen input reduced ethanol selectivity.

To distinguish biomass accumulation from ethanol-forming activity, apparent specific rates were calculated from steady-state balances (Figure 4d). At 0.08 vvm, the apparent specific glucose uptake rate was 0.62 g glucose.g^{-1} biomass.h^{-1} and the apparent specific ethanol production rate was 0.15 g ethanol.g^{-1} biomass.h^{-1}. At 0.30 vvm, these values decreased to 0.35 and 0.04 $g.g^{-1}.h^{-1}$, respectively. Thus, the highest aeration condition produced the highest biomass concentration but the lowest ethanol-forming activity per unit biomass. The selectivity index decreased sharply from 1.48 at 0.08 vvm to 0.82 at 0.20 vvm and 0.44 at 0.30 vvm (Figure 4e). This trend confirms that increasing aeration shifted glucose utilization away from ethanol and toward biomass formation. The decrease in q_P also indicates that the retained cell population became less ethanol-productive on a cell-mass basis.

Cell-retention systems are often designed to increase biomass concentration, and higher biomass is generally expected to improve volumetric productivity. However, the present results show that this assumption is valid only when the retained biomass remains metabolically directed toward the desired product. In ethanol fermentation, excessive aeration can increase biomass concentration

Table 4. Effect of aeration on steady-state performance of continuous aerobic membrane fermentation.

Aeration (vvm)	C_P ($g.L^{-1}$)	P_{EtOH} ($g.L^{-1}.h^{-1}$)	X ($g.L^{-1}$)	S ($g.L^{-1}$)
0.08	51.2 ± 2.1	5.1 ± 0.2	34.8 ± 1.4	84.7 ± 1.2
0.20	31.9 ± 1.1	3.2 ± 0.1	39.1 ± 1.2	122.8 ± 1.6
0.30	20.5 ± 1.3	2.0 ± 0.1	42.4 ± 1.1	149.2 ± 2.2

while reducing ethanol selectivity. The productivity obtained in the present study remains lower than that reported for optimized membrane cell-recycle systems. Melzoch *et al.* reported continuous fermentations of *S. cerevisiae* in a membrane recycle bioreactor where an ultrafiltration module retained biomass and allowed continuous removal of fermentation products in a cell-free permeate [20]. Ylitervo *et al*. later showed that external cross-flow MBRs can be useful for continuous ethanol fermentation with inhibitory lignocellulosic media, although they emphasized membrane fouling as a key obstacle [13]. The present system follows the same cell-retention principle, but the productivity is moderate compared with highly optimized cell-recycle fermentations. This difference likely reflects differences in substrate type, cell density, dilution rate, membrane area, hydraulic configuration, strain adaptation, and operating control.

Compared with pervaporation-based ethanol recovery systems, the present ultrafiltration MBR should be interpreted differently. Pervaporation studies use the membrane to remove ethanol from the fermentation broth, thereby reducing product inhibition and enriching ethanol in the permeate. For example, early MBR work integrated fermentation with pervaporation for continuous ethanol extraction [35]. In the present system, the ultrafiltration membrane does not selectively extract ethanol; it retains yeast cells. The improvement at 0.08 vvm therefore comes from the combined effect of cell retention and oxygen-controlled yeast physiology, not ethanol removal.

Recent yeast technology reviews continue to emphasize that improving ethanol production requires both strain-level and process-level strategies. A review on *S. cerevisiae* applications highlighted continuing interest in yeast performance in bioethanol production [36]. More recent work on ethanol-tolerant *S. cerevisiae* also shows that adaptation to increasing ethanol concentrations involves changes in physiological response during continuous exposure to ethanol stress [37]. These studies reinforce the interpretation that the retained biomass in an MBR must be evaluated not only quantitatively, by mass concentration, but also qualitatively, by its physiological state and ethanol tolerance.

The combined data indicate the existence of an optimum aeration window. At zero aeration under anaerobic membrane operation, ethanol productivity was high but lower than at 0.08 vvm. Low aeration likely supported sterol and unsaturated fatty acid synthesis, improved membrane integrity, and helped maintain cell viability under high-glucose and ethanol-stress conditions. At higher aeration, the balance shifted toward biomass formation and reduced ethanol selectivity.

For future process development, aeration should be controlled together with dilution rate, membrane flux, pH, and dissolved oxygen. Redox-potential-driven aeration is particularly relevant because it can supply oxygen when needed for yeast viability, while avoiding excessive respiratory carbon loss. Prior work under very-high-gravity fermentation showed that oxygen control through redox potential can benefit biomass accumulation and viability under osmotic and ethanol stress [21,33]. Applied to the present membrane bioreactor, such a control strategy could help maintain an ethanol-productive retained biomass population.

3.5. Comparative Performance of Fermentation Configuration

Figure 5 summarizes the processed steady-state performance indicators obtained from the continuous fermentation experiments. The conventional anaerobic bioreactor showed the weakest performance, including ethanol concentration, ethanol productivity, and glucose conversion. The residual glucose concentration remained high, indicating poor substrate utilization. This behavior is consistent with the expected limitation of conventional continuous fermentation. When biomass is not retained, the culture can be partially washed out, reducing the active biocatalyst concentration and leaving glucose incompletely converted.

Membrane integration substantially improved the performance of continuous ethanol fermentation. The membrane-assisted operation increased ethanol concentration by approximately 15.8-fold and productivity by approximately 15.5-fold relative to the conventional anaerobic bioreactor. This result supports the central hypothesis that the ultrafiltration membrane intensified fermentation by retaining yeast cells and suppressing washout.

The comparison between batch and continuous configurations reveals a clear process trade-off. The aerobic batch system reached the highest ethanol titer. In contrast, the best continuous MBR condition, aerobic MBR at 0.08 vvm, reached a lower ethanol concentration but a much higher productivity. Therefore, batch operation favored ethanol accumulation, whereas continuous MBR operation favored production intensity. Therefore, the membrane bioreactor should not be framed as a system that necessarily maximizes ethanol titer. Its main advantage is that it increases volumetric productivity by combining continuous throughput with cell retention. This is particularly relevant for industrial fermentation, where productivity strongly affects reactor volume, capital cost, and process throughput. Similar logic has been

reported in previous high-cell-density yeast bioprocesses, where retaining or immobilizing high concentrations of productive cells can greatly improve volumetric productivity compared with conventional suspended-cell operation [38].

The best-performing configuration depends on the criterion used. If the target is ethanol titer, batch aerobic fermentation was superior, followed by anaerobic MBR at $D = 0.05\ h^{-1}$. If the target is continuous productivity, aerobic MBR at 0.08 vvm was the best condition (Figure 5). If the target is glucose conversion among continuous systems, anaerobic MBR at $D = 0.05\ h^{-1}$ gave the highest conversion. If the target is biomass accumulation, aerobic MBR at 0.30 vvm gave the highest biomass concentration, but this did not translate into the highest ethanol productivity.

Thus, the most useful design criterion is not biomass concentration alone, but the balance among biomass retention, glucose conversion, ethanol yield, and volumetric productivity. The aerobic MBR at 0.08 vvm appears to provide the best compromise in the present dataset. It retained high biomass, maintained relatively high glucose conversion, and achieved the highest continuous ethanol productivity. By contrast, higher aeration increased biomass at the expense of ethanol selectivity.

The comparison indicates several conclusions. First, continuous fermentation without cell retention is vulnerable to washout or low-biomass operation under the tested conditions. Second, ultrafiltration membrane integration improves continuous ethanol fermentation primarily by retaining yeast cells, not by selectively recovering ethanol. Third, aeration has a non-monotonic effect: low aeration enhances productivity, while excessive aeration shifts the system toward biomass formation and lower ethanol yield. These conclusions are consistent with the broader literature on membrane bioreactors and high-cell-density fermentation [13,39].

The present membrane bioreactor substantially outperformed the conventional anaerobic continuous reactor, increasing ethanol productivity from 0.3 to 5.1 $g.L^{-1}.h^{-1}$ under the best operating condition while maintaining effective biomass retention. Compared with previous membrane-assisted ethanol fermentations, its performance falls within the lower-to-moderate range reported for single-stage cell-retention systems. For example, Ylitervo et al. reported ethanol productivities of 5.0–6.2 $g.L^{-1}.h^{-1}$ using a cross-flow membrane bioreactor operated at a substantially higher dilution rate and much higher cell density, whereas other optimized cell-recycle configurations achieved even greater productivities through reactor staging and

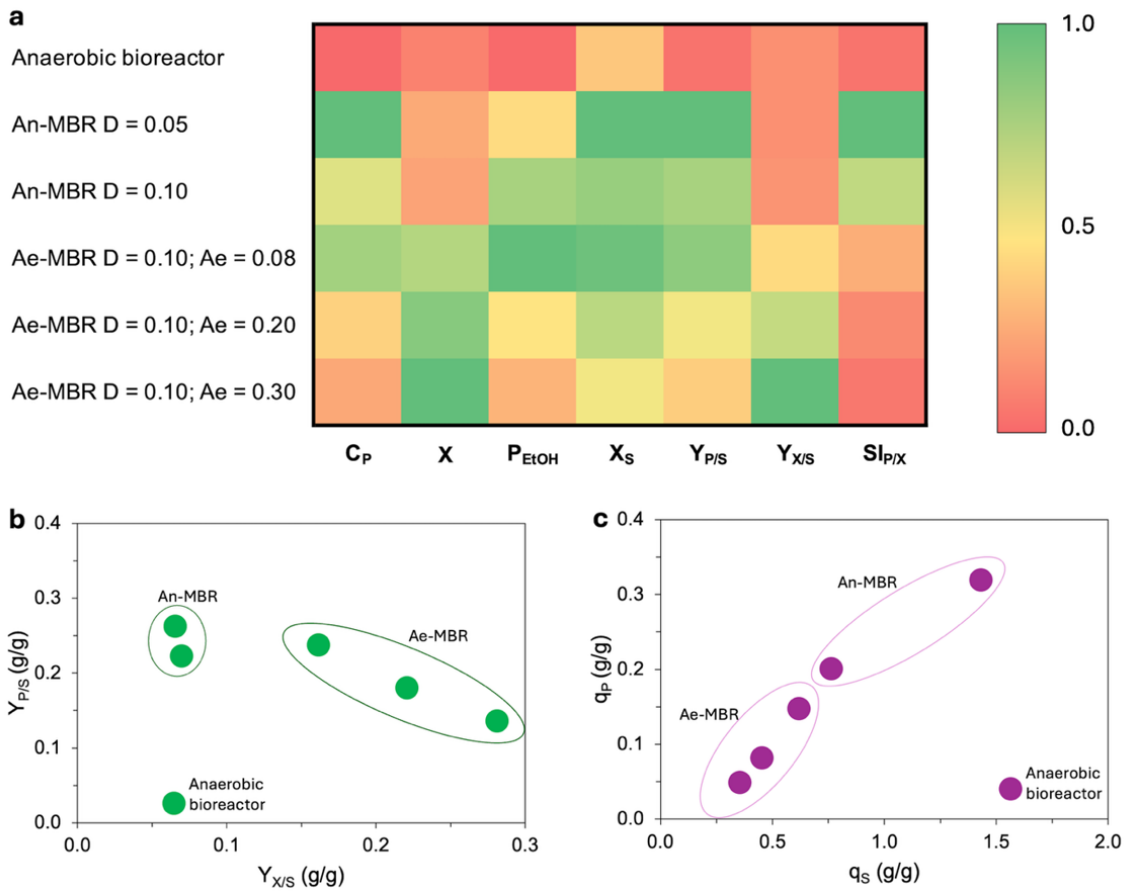

Figure 5. Comparison of bioreactor configuration. (a) Performance heat map. (b) $Y_{P/X}$ vs $Y_{S/X}$. (c) q_S vs q_P.

intensified biomass retention [13,40,41]. These differences largely reflect variations in reactor configuration, cell density, dilution rate, substrate composition, and process optimization. Therefore, the principal contribution of the present work is not the highest productivity reported to date, but the systematic demonstration of how ultrafiltration-assisted cell retention and controlled aeration improve continuous ethanol fermentation relative to a conventional continuous reactor operated under comparable conditions. The comparison highlights the specific strength and limitation of the present system. Its strength is the clear demonstration of how ultrafiltration-assisted cell retention improves continuous fermentation relative to a non-membrane control under matched dilution rate. Its limitation is that the productivity and conversion remain below the best reported optimized cell-retention systems.

The productivity obtained in the present Ae-MBR at 0.08 vvm, 5.1 $g.L^{-1}.h^{-1}$, is markedly higher than that of the conventional anaerobic bioreactor tested under the same dilution rate, 0.3 $g.L^{-1}.h^{-1}$. This improvement confirms the benefit of UF-assisted cell retention for suppressing washout and maintaining an active yeast inventory. Compared with previous membrane-assisted ethanol fermentations, the present productivity falls within the lower-to-moderate range of reported cell-retention MBR systems. Ylitervo et al. reported 5.0 to 6.2 $g.L^{-1}.h^{-1}$ in a cross-flow MBR operated at higher dilution rate and very high cell density, while two-stage cell-recycle systems have reached much higher productivities when cell density, residence time, and reactor staging were optimized [13]. Therefore, the present system performs better than the non-membrane control and is comparable to some single-stage MBR systems, but it remains below highly optimized multi-stage or high-cell-density processes. The main contribution of this work is the controlled comparison between non-membrane continuous fermentation, anaerobic UF-assisted cell retention, and low-aeration UF-assisted cell retention under the same glucose feed basis.

4. Conclusions

This study demonstrates that UF-assisted cell retention can intensify continuous ethanol fermentation by suppressing yeast washout and decoupling cell residence time from hydraulic residence time. At the same dilution rate of 0.10 h^{-1}, the An-MBR increased ethanol concentration from 2.8 ± 0.8 to 43.4 ± 2.1 $g.L^{-1}$ and increased productivity from 0.3 ± 0.1 to 4.3 ± 0.2 $g.L^{-1}.h^{-1}$ compared with the conventional anaerobic bioreactor. This result confirms that the UF membrane functioned primarily as a biocatalyst-retention unit rather than as an ethanol-selective separation membrane. Dilution rate controlled the balance between ethanol accumulation and reactor productivity. A lower dilution rate of 0.05 h^{-1} favored ethanol concentration and glucose conversion, while 0.10 h^{-1} favored volumetric productivity. Controlled aeration provided an additional process-control variable. Low aeration at 0.08 vvm gave the highest productivity, 5.1 ± 0.2 $g.L^{-1}.h^{-1}$, but higher aeration shifted substrate use toward biomass formation and reduced ethanol yield and selectivity. The results show that continuous bioethanol production should be optimized not only by increasing biomass concentration, but also by maintaining an ethanol-directed metabolic state through coordinated control of dilution rate, cell retention, and aeration.

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CRedit Author Statement

Author Contributions: Tri Partono Adhi: Writing original draft, Writing review and editing. Handika Prasetya Dwiyasni: Investigation, Data curation, Formal analysis. Mirani Susiloputri: Investigation, Data curation, Formal analysis. Reynard Reynard: Writing original draft. Khoiruddin Khoiruddin: Writing original draft. I Gede Wenten: Conceptualization, Supervision, Resources, Writing review and editing. All authors have read and agreed to the published version of the manuscript.

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