SUPPORTING INFORMATION

Membrane-Assisted Cell Retention for Intensified Continuous Bioethanol Production

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Experimental design

The experiments were designed to compare four operating modes: batch fermentation under anaerobic and aerobic conditions, continuous anaerobic fermentation without membrane, continuous anaerobic membrane bioreactor operation, and continuous aerobic membrane bioreactor operation (**Figure S1**). The main responses measured throughout the study were ethanol concentration, biomass concentration, and residual glucose concentration. These primary data were further processed into ethanol productivity, glucose conversion, apparent ethanol yield, and performance improvement factors.

The experimental design used glucose as the carbon source and *S. cerevisiae* as the fermenting microorganism. The continuous anaerobic non-membrane reactor was operated at a dilution rate of 0.10 h^{-1}. The continuous anaerobic membrane bioreactor was operated at dilution rates of 0.05 and 0.10 h^{-1}, while the continuous aerobic

membrane bioreactor was operated at a fixed dilution rate of 0.10 h^{-1} with aeration rates of 0.08, 0.20, and 0.30 vvm. These operating conditions follow the experimental scope of the original study, which compared the effect of membrane integration, dilution rate, and aeration rate on ethanol production performance.

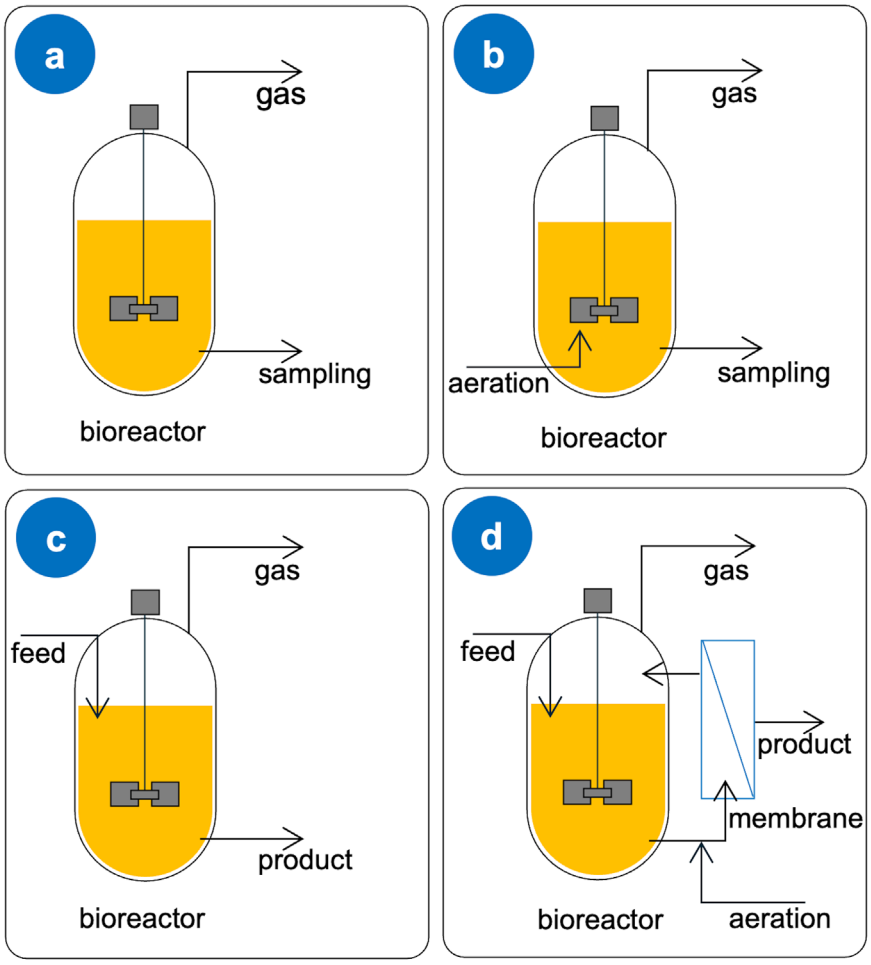

Figure S 1. Experimental set-up and bioreactor configurations. (a) Batch anaerobic bioreactor. (b) Batch aerobic bioreactor. (c) Continuous anaerobic bioreactor. (d) An-MBR and Ae-MBR.

Experimental Apparatus

The continuous fermentation system consisted of a stirred fermentor, feed reservoir, feed pump, membrane circulation loop, ultrafiltration membrane module,

retentate return line, and permeate outlet. The original report lists a continuous fermentor, a polysulfone ultrafiltration membrane, autoclave, spectrophotometer, gas chromatograph, cuvettes, glassware, shaker, microscope, counting chamber, pycnometer, thermometer, digital balance, and diaphragm pump as the main equipment. The membrane was a polysulfone ultrafiltration membrane (GDP Filter Indonesia) with a molecular weight cut-off of 100,000 Da and an effective membrane area of 0.25 m^2.

The membrane bioreactor configuration was operated as a cell-retention system. Fermentation broth from the bioreactor was circulated to the ultrafiltration module; yeast-rich retentate was returned to the fermentor, while the permeate was withdrawn as the product stream. In the anaerobic membrane configuration, membrane integration was placed on the outlet circulation line of the fermentor. In the aerobic membrane configuration, air was introduced into the outlet stream before the membrane module rather than through a conventional sparger. This configuration was intended to increase biomass concentration in the fermentor and help maintain permeate flux across the membrane.

Medium preparation and sterilization

Fermentation media were prepared by dissolving the required masses of glucose, peptone, yeast extract, urea, and $ZnSO_4 \cdot 7H_2O$ in demineralized water according to the compositions shown in **Table S1**. For agar slants, bacto agar was added to the medium before sterilization. Media and relevant glassware were sterilized prior to use. Sterile handling was applied during culture transfer and inoculation to reduce contamination risk. The original report provides flow diagrams for the preparation of liquid fermentation medium and agar slant medium.

Table S 1. Fermentation medium compositions used in this study

Medium	Glucose (g L^{-1})	Peptone (g L^{-1})	Yeast extract (g L^{-1})	Urea (g L^{-1})	$ZnSO_4.7H_2O$ (g L^{-1})	Bacto agar (g L^{-1})
Batch/inoculum medium	300	20	5	20	0.05	0

Continuous fermentation medium	300	15	5	20	0.05	0
Agar slant medium	20	10	5	0	0	20

For continuous operation, the feed medium was prepared separately and supplied to the fermentor through a feed pump. The glucose concentration in the continuous feed was 300 g L^{-1} based on the stated continuous medium composition. This value was used as the inlet substrate concentration for subsequent calculation of glucose conversion and ethanol yield.

Yeast culture preparation

For batch fermentation, *S. cerevisiae* culture was first developed through an inoculum preparation step before being transferred into the main fermentation medium. The original report describes this culture-development procedure schematically and states that the inoculum was transferred into the prepared main fermentation medium before batch fermentation. For continuous fermentation, commercial yeast was added directly into the fermenter without a separate inoculum-development step at an initial concentration of 3 g L^{-1}.

This distinction is important for interpreting the results. Batch experiments used a prepared yeast culture, whereas continuous experiments used direct yeast addition. Therefore, comparisons between batch and continuous fermentation should be interpreted primarily as process-configuration comparisons rather than strict biological comparisons under identical inoculum histories.

Analytical methods

The Gas Chromatography (GC) output was expressed as ethanol area percentage relative to water. This value was converted into ethanol concentration using a calibration curve. The area fraction was calculated as:

$$A_{EtOH}(\%) = \frac{A_{EtOH}}{A_{EtOH}+A_{H_2O}} \times 100 \qquad \text{(S1)}$$

where A_{EtOH} and A_{H_2O} are the GC peak areas of ethanol and water, respectively.

Residual glucose concentration was measured using a hand refractometer. The measured value was obtained in °Brix and converted into glucose concentration using a refractometer calibration curve. The calculated residual glucose concentration was expressed as:

$$S = DF \times f(B) \quad \text{(S2)}$$

where S is residual glucose concentration in g L^{-1}, DF is the dilution factor, B is the Brix reading (Brix), and $f(B)$ is the calibration function obtained from standard glucose solutions. When the calibration equation from the original dataset is used, this expression becomes:

$$S = DF(12.592B) \quad \text{(S3)}$$

Biomass concentration (S) was determined spectrophotometrically at a wavelength of 550 nm. The measured absorbance was converted into biomass concentration using a spectrophotometric calibration curve. The biomass concentration was calculated as:

$$X = DF(6.392A) \quad \text{(S4)}$$

where X is biomass concentration in g L^{-1}, DF is the dilution factor (-), and A is the measured absorbance at 550 nm.

Ethanol concentration, biomass concentration, and residual glucose concentration were processed to obtain ethanol productivity, glucose conversion, apparent ethanol yield, theoretical yield efficiency, and performance improvement factors. For continuous fermentation, volumetric ethanol productivity was calculated as:

$$P_{EtOH} = DC_{EtOH} \quad \text{(S5)}$$

where P_{EtOH} is the ethanol productivity in g L^{-1} h^{-1}, D is the dilution rate in h^{-1}, and C_{EtOH} is the ethanol concentration in g L^{-1}.

Glucose conversion was calculated as:

$$X_S = \frac{S_0 - S}{S_0} \quad \text{(S6)}$$

where X_S is glucose conversion (-), S_0 is the inlet glucose concentration (g L^{-1}), and S is the residual glucose concentration (g L^{-1}). For the continuous runs, S_0 was taken as 300 g L^{-1} based on the continuous fermentation medium composition.

The apparent ethanol yield was calculated as:

$$Y_{P/S} = \frac{C_{EtOH}}{S_0 - S} \quad \text{(S7)}$$

where $Y_{P/S}$ is the apparent ethanol yield in g ethanol per g glucose consumed.

The theoretical yield efficiency (-) was calculated using the theoretical ethanol yield from glucose fermentation:

$$\eta_Y = \frac{Y_{P/S}}{0.511} \quad \text{(S8)}$$

where 0.511 g g^{-1} is the theoretical ethanol yield from glucose.

The net ethanol formation rate ($r_{p,\,avg}$; g L^{-1} h^{-1}) was calculated from the increase in ethanol concentration:

$$r_{P,\text{avg}} = \frac{P_t - P_0}{t} \quad \text{(S9)}$$

Where P_t is ethanol concentration at t h, P_0 is ethanol concentration at 0 h, and *t*is fermentation time. The anaerobic batch gave an average ethanol formation rate of 0.86 g L^{-1} h^{-1}, whereas the aerobic batch gave 1.19 g L^{-1} h^{-1}. The interval-based rate analysis also showed faster ethanol formation under aerobic operation. The highest observed interval rate was 1.51 g L^{-1} h^{-1} for the anaerobic batch and 2.80 g L^{-1} h^{-1} for the aerobic batch, both occurring between 15 and 23 h.

The ethanol accumulation profiles were fitted using a modified Gompertz model with an initial ethanol offset:

$$P(t) = P_0 + A\exp\left\{-\exp\left[\frac{R_{\max} e}{A}(\lambda - t) + 1\right]\right\} \quad \text{(S10)}$$

where $P(t)$ is ethanol concentration (g L^{-1}) at fermentation time t (h), P_0 is the initial ethanol concentration, A is the ethanol production potential above the initial concentration, R_{max} is the maximum apparent ethanol formation rate (g L^{-1} h^{-1}), and λ is the apparent lag time (h). The modified Gompertz equation is commonly used for batch bioethanol production because it provides interpretable parameters for lag time, maximum production rate, and maximum potential ethanol concentration [1,2].

For a continuous system, ethanol productivity is:

$$P_{EtOH} = DC_P \quad \text{(S11)}$$

where C_P is the ethanol concentration in the product stream (g L^{-1}).

The apparent specific substrate uptake rate can be estimated from the steady-state substrate balance:

$$q_S = \frac{D(S_0 - S)}{X} \quad \text{(S12)}$$

where S_0 is the inlet glucose concentration (g L^{-1})., S is the residual glucose concentration (g L^{-1})., and $X$is biomass concentration (g L^{-1}).

The same interpretation is obtained from the apparent specific ethanol production rate (g L^{-1} h^{-1}):

$$q_P = \frac{DC_P}{X} \quad \text{(S13)}$$

Comparative performance of ethanol fermentations

Table S2 benchmarks the present membrane bioreactor results against selected ethanol fermentation systems using membrane-assisted or cell-retention strategies. The anaerobic UF-assisted MBR in this work achieved a relatively high ethanol concentration of >59 g L^{-1} with >75% glucose conversion at a low dilution rate, indicating effective substrate utilization and cell retention. Compared with previous membrane bioreactor studies, the productivity obtained here is within the lower-to-moderate range, while some optimized systems achieved higher productivity through very high cell density, ethanol-permselective membranes, or two-stage continuous operation. However, the present study provides a clear mechanistic comparison of

anaerobic and low-aeration UF-assisted cell-retention systems using the same glucose feed, highlighting the role of membrane-assisted biomass retention and aeration control in continuous ethanol fermentation.

Table S 2. Benchmark comparison with selected ethanol fermentation studies using membrane or cell-retention strategies

Microorganism	Configuration	Substrate / feed	Performances and findings	Ref.
S. cerevisiae	UF-assisted continuous cell-retention An-MBR	Glucose, 300 g L^{-1}	59.19 g L^{-1} ethanol at D = 0.05 h^{-1}; 2.96 g L^{-1} h^{-1}; 75.2% glucose conversion	This work
S. cerevisiae	UF-assisted continuous cell-retention Ae-MBR with low aeration	Glucose, 300 g L^{-1}	50.56 g L^{-1} ethanol; 5.06 g L^{-1} h^{-1} at 0.08 vvm	This work
S. cerevisiae	Cross-flow membrane bioreactor with retained cells	Sucrose medium with acetic acid stress	5.04–6.16 g L^{-1} h^{-1}; very high cell density up to about 200 g L^{-1}; stable operation at D = 0.5 h^{-1}	[3]
Baker's yeast	Immersed hollow-fiber MF membrane with cell retention	Synthetic glucose medium	62.4 g L^{-1} ethanol; 30 g L^{-1} biomass; at least 95% glucose conversion; 96 h operation	[4]
S. cerevisiae	Continuous membrane bioreactor with	Glucose, 100 g L^{-1}	Maximum productivity 6.49 g L^{-1} h^{-1} at D = 0.14 h^{-}	[5]

Microorganism	Configuration	Substrate / feed	Performances and findings	Ref.
Yeast-based continuous fermentation	ethanol-permselective PDMS membrane Innovative continuous two-stage configuration	Glucose	[1]; Moser kinetic model fitted better than Monod Productivity up to 41 g L^{-1} h^{-1} with complete glucose conversion	[6]

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