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Enhanced Bioethanol Production in Batch Fermentation by Pervaporation using a PDMS Membrane Bioreactor

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ABSTRACT

The integration of batch fermentation and membrane-based pervaporation process in a membrane bioreactor (MBR) was studied to enhance bioethanol production compared to conventional batch fermentation operated at optimum condition. For this purpose, a laboratory-scale MBR system was designed and fabricated. Dense hydrophobic polydimethylsiloxane (PDMS) membrane was used for pervaporation. For fermentation, pure stock culture of *Saccharomyces cerevisiae* as microorganism and glucose as substrate were used. In conventional batch fermentation, the yields of cell and ethanol concentration based on substrate consumption were 0.32 and 0.54 g/g, respectively. But in MBR, these values improved to 0.41 and 0.59 g/g, respectively. In addition, the ethanol productivity has increased at least by 26.83% over conventional bioreactor. Furthermore, ethanol concentration in permeated side was approximately 6 to 7 times higher than that of the broth. Based on Monod biological kinetic model, maximum specific growth rate was 0.966 h⁻¹ more than this value of 0.864 h⁻¹ in conventional system. Compared to conventional batch fermentation, the MBR resulted in increase of cell density, decreasing ethanol inhibition, improved productivity and yield, and resumption of clean and concentrated ethanol. These effects can be attributed to the presence of membrane as a selective separation barrier for removal of ethanol from fermentation broth.

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1. INTRODUCTION

Due to the rapid depletion of fossil fuel reservoirs and global warming caused by greenhouse gases, biomass has emerged as an alternative and renewable source of fuel and energy. Biomass can be converted to various biofuels such as ethanol that has widely been used as an alternative useful fuel and fuel additives [1-4]. Besides, ethanol is used in various processes including food industry, beverages and brewing process, medicinal and medical applications [5].

Conventional ethanol fermentation has some disadvantages such as low ethanol production because of ethanol inhibition, requirement of additional purification steps in downstream processing, low cell densities in the cultivated broth and incomplete use of nutrients [6, 7].

There are some processes to achieve a simultaneous separation of fermented ethanol as it is formed. The most important of them is membrane separation. Membrane technology demonstrates one of the most effective and energy saving separation processes. Combination of biological reaction and membrane separation in only one unit is a very attractive configuration for the reactions where the continuous removal of metabolites is required to maintain a good productivity [8]. Integration of reaction and selective separation reduces substrate and product inhibition and increases the productivity. Also, there is no need to downstream processing.

Pervaporation is claimed to be one of the most efficient and promising techniques for separation of ethanol/water mixture in biological processes because

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of the process being simple and does not required extra chemicals [9, 10]. Silicon containing polymers, especially polydimethylsiloxane (PDMS), have been widely used for the selective pervaporation of organics from organic/water mixtures [11]. It is hydrophobic, and has a good chemical stability and biocompatibility in long duration time compared to other polymeric and inorganic membranes such as poly[1-(trimethylsilyl)-1propyne] (PTMSP) and zeolite, respectively. It is suitable for constructing pervaporation membrane bioreactor which is applied for the bioconversion and separation of biological product [9, 12].

Membrane bioreactors (MBR) with different configurations have been implemented for production of metabolites, with simultaneous separation of fermented products using pervaporation technique. There are some reports in the literature discussing about continuous ethanol production by pervaporation using different cultures, membranes and configurations. Chen et al. [7] have investigated ethanol production by Saccharomyces cerevisiae in a continuous and closed-circulating fermentation (CCCF) system using a PDMS pervaporation membrane bioreactor. An ethanol volumetric productivity of 1.39 g/l.h was obtained in the third cycle, with an ethanol yield rate of 0.13 h⁻¹. Also, Ding et al. [13] have employed the similar configuration for ethanol fermentation with similar results. Nomura et al. [14] have studied the removal of ethanol from fermentation broth by silicalite zeolite membrane. They showed that separation efficiency of the silicalite membrane was disturbed during operation time. Little data is found in the literature regarding ethanol production in batch MBRs. Batch experiments would provide useful information in evaluating overall performance of MBR systems in a continuous process.

In this work, Bioethanol production by coupling of conventional fermentation with pervaporation in a batch membrane reactor was considered. For this purpose, a MBR was designed and fabricated. PDMS membrane was chosen as the selective separation barrier for removal of ethanol from fermentation broth. A comparison was made between conventional fermentation and integrated fermentation-pervaporation system (MBR) which shows a great improvement in bioethanol production in terms of ethanol yield and productivity.

2. MODELING OF GROWTH KINETICS FOR SACCHAROMYCES CEREVISIAE

In mathematical description of microbial growth kinetics the concepts of doubling time and exponential growth pattern are usually implemented. There is a premise that the growth rate of microorganism is proportional to the existing cell population, commonly known as cell density, and that the proportionality constant is a function of microorganism type. The simplest equation which describes such relation is given by Malthus law [9]:

$$\frac{dX}{dt} = \mu X \tag{1}$$

where X represents the cell density (g/l), μ is the specific growth rate (h⁻¹) and t denotes the fermentation time (h).

To describe the substrate utilization by the microorganism, a first order reaction kinetic in the following form might be used:

$$\frac{dS}{dt} = -k_s S \tag{2}$$

where S represents the substrate concentration (g/l) and k_s is the first order rate constant (h⁻¹).

2. 1. Monod Kinetic Model In order to relate the microbial growth rate of the microorganism to the concentration of limiting substrate, Monod equation is used in the following form:

$$\mu = \frac{\mu_{\max}S}{K_s + S} \tag{3}$$

where μ_{max} is defined as maximum specific growth rate (h⁻¹) and K_S is Monod half-saturation constant (g/l).

By substitution of Equations (1) and (2) into Equation (3) and performing integration, the following equation for the cell concentration was obtained:

$$X = X_0 \exp\left[-\frac{\mu_{\max}}{k_s} \ln\left(\frac{K_s + S_0 \exp(-k_s t)}{K_s + S_0}\right)\right]$$
(4)

where S_0 is the initial substrate concentration (g/l) and X_0 represents the cell concentration at the onset of fermentation process (g/l).

2. 2. Moser Kinetic Model One of the alternative useful kinetic equations is Moser model that represents inhibition- free substrate limitation kinetic such as Monod model as follows:

$$\mu = \frac{\mu_{\max} S^n}{K_s + S^n} \tag{5}$$

where n is the degree of substrate consumption which is determined by non-linear regression. In this case, such as Monod model, by substitution of Equations (1) and (2) into Equation (5) and performing integration, the equation for the biomass concentration versus time is obtained as below:

$$X = X_0 \exp\left[-\frac{\mu_{\max}}{nk_s} \ln\left(\frac{K_s + S_0^n \exp(-nk_s t)}{K_s + S_0^n}\right)\right]$$
(6)

2. 3. Logestic Kinetic Model Logistic kinetic model is a suitable model for prediction of lag, exponential and stationary phases of growth curve. The specific growth rate predicted by Logistic model is expressed as [15]:

$$\mu = \mu_{\max} \left(1 - \frac{X}{X_{\max}} \right) \tag{7}$$

where X_{max} is the maximum cell concentration (g/l). By substitution of Equation (1) into Equation (7) and integration, Equation (8) is obtained for determination of cell concentration:

$$X = \frac{X_0 \exp\left(\mu_{\max} t\right)}{1 - \left(\frac{X_0}{X_{\max}}\right) \left(1 - \exp\left(\mu_{\max} t\right)\right)}$$
(8)

In the present study, the above mentioned kinetic models (Equations 4, 6 and 8) were incorporated for the prediction of cell growth in the batch culture.

3. EXPERIMENTAL

3. 1. Design and Operation of the MBR System Figure 1a illustrates the design of a laboratory-scale MBR that was fabricated in such a manner that it could be readily assembled and disassembled between experiments for cleaning and sterilization. Glass column with 30 cm height, 10 cm I.D and 11 cm O.D was used as framework. The membrane was fixed at the bottom of the cell (8 cm I.D).

Fermentation performed along was with pervaporation process using the fabricated MBR with a fermentation chamber containing 1260 ml fermentation broth on the top and a pervaporation cell having 50.24 cm² effective permeation areas at the bottom of chamber. A schematic diagram of the batch fermentation-pervaporation system is presented in Figure 1b. The feed solution was well stirred to keep the concentration uniform. Temperature of the fermentation broth was kept constant at 32 °C by a temperature controller. The pressure on the top feed side was atmospheric, while the bottom of the membrane was evacuated with a vacuum pump (E2M2-Edwards) to keep the permeate-side pressure at a level lower than 3.5 Torr. Permeated vapor was condensed and collected in a cold trap containing liquid nitrogen at -196 °C. The permeation rate was determined from the weight of the collected samples at the certain period of time. Performance of the pervaporation unit in the fabricated MBR was evaluated by ethanol flux. Samples were taken every 2 h from the broth and cold trap.

3. 2. Membane In this study, asymmetric Polydimethylsiloxane (PDMS), a dense hydrophobic/organophilic and well-known ethanolpermselective membrane in flat sheet form was used for concentrating the produced ethanol through the pervaporation process. The membrane with an effective thickness of 3-5 μ m PDMS as top layer was supplied by Pervatech Company (Netherland). A support consisting of PET with thickness of 100 μ m as the sub layer to



1-Outlet of the lower compartment 2-Bottom main support 3-Porous metal disk 4-Porous glass support 5-Silicon gasket 6-Membrane 7-Silicon o ring 8-Top membrane support 9- Mixer 10-Top main support 11-Glass column $12-CO_2$ outlet 13-Feed inlet 14-DC motor 15-Sampleing port



5- Temperature controller 6-Heater 7-CO₂ outlet 8-Pirani gauge 9-N₂ cold trap 10-Vacuum pump 11-Temprature preservative box

(b)

Figure 1. (a) 3-Diminitonal design of the MBR for the integrated fermentation-pervaporation system, (b) schematic diagram of the MBR with pervaporation



Figure 2. SEM photograph of surface PDMS dense membrane

assure the mechanical strength of the membrane and an intermediate UF membrane polyimid (PI) as the first membrane layer with thickness of 150 μ m were used. As shown in Figure 2, PDMS is a dense homogeneous membrane and separation mechanism is not based on porosity. Difference in sorption and diffusion of components is the most important factor for its performance in selective separation of ethanol.

3. 3. Microorganism The pure stock culture of *Saccharomyces cerevisiae* was used for ethanol fermentation. The strain was originated from Persian Type Culture Collection (PTCC 24860), supplied by Iranian Research Organization for Science and Technology (IROST). The medium used for seed culture contained glucose, NH₄Cl and yeast extract with concentrations of 10, 0.45 and 1 g/l, respectively. The medium was autoclaved at 121 °C and 15 psig for 20 min. The sterilized medium was inoculated with 5% of pure seed culture of the microorganism and then the culture was cultivated in an incubator at 30 °C for 24 h.

3. 4. Growh Medium and Culture Conditions Batch experiments for ethanol production was carried out both in a conventional batch fermentation system and membrane bioreactor (MBR) by pervaporation at optimum condition [16]. The medium contained glucose, yeast extract and NH₄Cl with concentrations of 50, 3 and 5 g/l, respectively. A buffer solution for pH value of 5.2 was prepared by suitable proportion of potassium hydrogen phthalate (0.1 M) and sodium hydroxide (0.1 M). Experiments were carried out at 32 °C. Batch fermentation combined with pervaporation was performed in 1260 ml of designed MBR as mentioned earlier. Conventional batch fermentation was accomplished at the same condition except that the membrane was excluded from the system. Samples were periodically taken for every 2 h from the culture for

monitoring the cell optical density and determination of cell dry weight, glucose and produced ethanol concentration in both separated sets of experiments.

3.5. Analysis The optical density was measured by a spectrophotometer (Unico, USA) at the wave length of 620 nm. The cell dry weight was determined based on pre-developed calibration curve. For determination of glucose concentration, 2 ml samples were collected and cells were separated by centrifugation at 7000 g for 7 min by a centrifuge (Hermle, model: Z 233 M-2, Germany). The concentration of glucose in the supernatant was determined by color-metric method using DNS reagent [17]. The concentration of ethanol was measured using a Gas Chromatograph (Agilent, 7890A) equipped with a flame ionization detector (FID). The stainless steel packed column with 1.83 m length and 2.1 mm I.D and 80/100 mesh Porapak O (Supelco, USA) was used. The initial oven temperature was hold at 120 °C for 1 min. Then, the oven temperature was programmed to heat up at a rate of 40° C/min until reached to 185 °C and remained at this temperature for 8.5 min, while the detector temperature was 225 °C. Carrier gas was nitrogen with a flow rate of 30 ml/min. 2-propanol (Merck, Germany) solution was prepared as internal standard in 3% (v/v) and 10% (v/v) for conventional batch fermentation and MBR samples, respectively. For analyzing, exact 50 µl of internal standard was added to 0.5 ml of samples. Calibration curves for determination of cell dry weight, glucose concentration by DNS method and ethanol analysis by gas chromatograph are illustrated in Figures 3 and 4. It is mentioned that some samples for analysis was diluted.

4. RESULTS AND DISCUSSION

4. 1. Performance of Pervaporation Membrane At first, pervaporation performance using the PDMS membrane for the enrichment of ethanol from fermentation broth was separately tested. For this purpose, a set of preliminary experiments were conducted with ethanol/water mixture at concentration range of 0-20 %w/w. Figure 5 shows the plot of ethanol concentration in the permeate side versus ethanol concentration in the feed. It is clear that the permeate concentration is practically the same as the vapor determined by the vapor-liquid concentration equilibrium at 760 mm Hg, which is represented as the solid line in Figure 5. The obtained permeability data indicated the PDMS membrane, used in this study and supported by PET and PI, was ethanol-selective at low ethanol concentrations and therefore can be a useful separation barrier in combination with bioreactor for ethanol production.



Figure 3. Calibration curve for determination of (a) cell concentration (b) glucose concentration





Figure 4. Calibration curve for ethanol analysis by gas chromatograph with internal standard of (a) 3 v/v % (b) 10 v/v%



Figure 5. Pervaporation performance of PDMS membrane in terms of ethanol concentration in permeate versus feed concentration

Separation factor of ethanol/water varied between 6 to 7 when the concentration of ethanol was below 5 w/w%. The total permeate flux across the membrane was almost 0.460 kg/m².h and ethanol flux increased with increasing of ethanol concentration in the feed solution. Hydrophobicity is a very significant property of a dense membrane. If the membrane is not hydrophobic adequately, leakage of the feed solution through the pores occurs and causes lower permeate concentration. The result of these preliminary tests for characterization of pervaporation process confirmed the suitability of PDMS membrane for serving as a selective separation barrier in MBR system for removal of ethanol from fermentation broth.

4. 2. Conventional Batch Fermentation A set of conventional batch fermentation experiments was carried out with glucose concentration of 50 g/l, in order to entail the performance of the basic cultivation process. The results including glucose, ethanol and cell concentration changes versus time are illustrated in Figure 6 and summarized in TABLE 1.

In the stationary phase of growth which was achieved after 22 h, ethanol and cell concentrations were approximately constant at 22.12 g/l (2.23 w/w%) and 13.22 g/l, respectively, while glucose was almost completely consumed. The yield of cell concentration based on substrate consumption (Y_{x_s}) and the yield of

produced ethanol based on substrate consumption ($Y_{p_{s}}$) were calculated to be 0.32 and 0.54 g/g,

respectively and the productivity was 1.106 g/l.h.

4. 3. Batch Fermentation in MBR The integration of batch fermentation and membrane separation in the same unit to enhance the performance of bioreactor was investigated. Pervaporation of the fermentation broth was carried out at 32 °C as illustrated in Figure 1. The volume of the broth was 1260 ml. The results are shown in Figures 7 and 8 and listed in TABLE 1. As illustrated in Figure 7, glucose was totally consumed after 20 hours of fermentation. In the stationary phase of growth, the cell and ethanol concentrations in the broth were 15.35 g/l and 20.02 g/l (2.21 w/w%), respectively. The cell concentration in the broth was higher than that of the conventional batch



Figure 6. Profiles of glucose, ethanol and cell concentration during batch fermentation



Figure 7. Profiles of glucose, ethanol and cell concentration during batch fermentation with pervaporation

TABLE 1. Results of the two batch fermentation experiments

	Conventional fermentation	MBR fermentation
Glucose utilization (g/l)	50	50
Yield (g/g)		
Y _{x/s}	0.32	0.41
Y _{P/S}	0.54	0.59
Ethanol concentration		
Broth (w/w%)	2.23	2.21
Broth (g/l)	22.12	22.02
Permeate (w/w%)	-	13.80
Broth (g/l)	-	132.83
Cell density (g/l)	13.22	15.35
Productivity (g/l.h)	1.106	1.41

fermentation. But the ethanol concentration in fermenter was less than the conventional process. Such observation was probably due to the ethanol withdrawal from the fermentation chamber and elimination of ethanol inhibition which led to the enhancement of cell growth and reduction of ethanol concentration in broth. Both advantages are due to the integrating of batch fermentation with membrane separation process.

Figure 8 shows the results of batch membrane permeation system. As shown in Figure 8a, after about 16 h of fermentation, ethanol concentration in the broth reached to 2.21 w/w% and then reduced. The ethanol concentration at the permeate side increased along with

broth concentration to 13.80 w/w% and then decreased. The ethanol concentration in the permeate vapor was 6 to 7 times that of the broth during the fermentation. The yield of ethanol concentration based on substrate consumption $(Y_{P_{A}'})$ and the yield of biomass

concentration based on substrate consumption $(Y_{x/c})$

were 0.59 and 0.41 g/g, respectively. These values were more than conventional batch fermentation as listed in TABLE 1. The ethanol productivity was 1.41 g/l.h that showed an improvement of at least 26.83% over conventional batch fermentation.

Performance of the pervaporation system was evaluated by total flux and the separation factor. The separation factor (α) of the aqueous ethanol solution is defined as follows:

$$(\alpha) = \frac{\left[C_{Eth} / C_{H_2O}\right]_{Permeate}}{\left[C_{Eth} / C_{H_2O}\right]_{Feed}}$$
(9)

where C_{Eth} and C_{H_2O} are the weight fractions of ethanol and water, respectively.

As illustrated in Figures 8b and 8c, the separation factor and total flux across the membrane was constant during the batch fermentation at about 7 and 460 g/m².h, respectively. It was indicated that the PDMS membrane performance was not changed by adsorption of microorganisms in feed broth during 22 h. Therefore, PDMS membrane was found to be very durable, because there was a little tendency for the yeast cells to form a layer on the membrane surface. However, in long-term use of this system, the permeation properties through the membrane can be affected by the adsorption of microorganisms, inorganic salts that were added as nutrient and were not completely consumed and nonvolatile by-products which are not removed by pervaporation and accumulate in the broth-membrane interface. The ethanol permeate flux during the fermentation-pervaporation experiments increased to 64 g/m^2 .h and decreased after 16 h of operation due to the reduction of ethanol concentration in feed broth during the experiment.

To make a comparison between pervaporation and equilibrium distillation, ethanol concentration in the vapor phase was calculated based on vapor-liquid equilibrium considering broth concentration at 760 mmHg. The ethanol concentration in permeate was close to the vapor phase composition which indicates the performance of the PDMS membrane was somehow comparable to conventional distillation.

There is not sufficient data about ethanol batch fermentation by MBR using pervaporation process. Ikegami et al. [18] have investigated the performance of two kinds of silicalite membrane coated with silicon rubber in batch fermentation using MBR. They have demonstrated that separation factor of silicalite membrane was more than that of PDMS membrane. So, in this study, ethanol concentration in permeate side was almost 14 w/w% that this value was lower than reported data by silicalite membrane in range of 30-70 w/w%. But, total flux by PDMS is almost constant, whereas in silicalite membrane, it is drastically decreased during the experiments. This phenomenon is due to the high biological and chemical stability of the PDMS membrane in long duration time.

4. 4. Determination of Growth Kinetics Data obtained in conventional batch fermentation and MBR with pervaporation using *S. cerevisiae* in optimum condition were used to determine the cell growth kinetics.



Figure 8. Results of batch fermentation- pervaporation system (a) Ethanol concentration (b) Membrane separation factor (c) flux

The kinetic parameters were calculated by nonlinear least square analysis. Matlab software (V 7.1) was used to determine the Monod, Moser and Logistic growth kinetic parameters. In Moser kinetic model, considering similar values of glucose consumption in conventional batch fermentation and MBR with pervaporation, it was assumed that n=2 in both systems. The fitting results of cell concentration versus time for MBR and conventional batch fermentation using Equation (4), (6) and (8) are shown in Figure 9. Also, the kinetic parameters are listed in TABLE 2.

The maximum specific growth rate (μ_{max}) in MBR by pervaporation was higher than that of conventional batch fermentation as illustrated in TABLE 2. This could be due to the ethanol inhibition removal which increased the cell growth rate and improved substrate consumption. The experimental data were also in good agreement with Monod growth model. The higher coefficient of determination (R²) and lower root mean square error (RMSE) obtained for Monod kinetic model shows the high accuracy and capability of this model to interpret the experimental data in both batch bioreactors.

5. CONCLUSIONS

This research aimed to design and fabrication of a MBR consisting of a batch fermenter and a pervaporation system using PDMS membrane. The MBR used for ethanol fermentation by Saccharomyces cerevisiae. Ethanol concentration in permeate was 6 to 7 times higher than the broth. The ethanol productivity increased at least by 26.83% over conventional batch fermentation. In MBR, the yields of ethanol and cell concentration based on substrate consumption were 0.59 and 0.41 g/g, respectively. In addition, the ethanol productivity was 1.41 g/l.h. These values were more than conventional batch fermentation due to the selective extraction of ethanol, elimination of biological inhibition and increase of cell growth. This system also offers the advantage of no requirement for downstream processing and can perform at higher sugar concentrations. Studying the cell growth kinetics showed the suitability of Monod model to describe the kinetics of cell growth for ethanol production.

TABLE 2. Kinetic parameters for the ethanol production in conventional and MBR batch fermentation

Kinetic model	MBR fermentation			Conventional fermentation						
	μ _{max} (h ⁻¹)	Ks (g/l)	R^2	RMSE	μ _{max} (h ⁻¹)	Ks (g/l)	R^2	RMSE		
Monod	0.966	0.592	0.976	0.990	0.864	0.554	0.998	0.261		
Moser	0.832	0.493	0.951	1.407	0.742	0.457	0.992	0.479		
Logistic	0.853		0.959	1.227	0.769		0.995	0.367		



Figure 9. Kinetic models applied to the experimental data for conventional and MBR batch fermentation

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Keywords: Batch Fermentation Ethanol Inhibition Membrane Bioreactor Pervaporation Polydimethylsiloxane تلفیق فرآیند تخمیر ناپیوسته و جداسازی غشایی با استفاده از تراوش تبخیری در یک بیوراکتور غشایی به منظور افزایش تولید بیواتانول در مقایسه با فرآیند تخمیر سنتی در شرایط عملکرد بهینه، مورد مطالعه قرار گرفت. به این منظور، یک بیوراکتور غشایی در مقیاس آزمایشگاهی طراحی و ساخته شد. غشای متراکم آبگریز پلیدیمتیل سیلوکسان (PDMS) در فرآیند تراوش تبخیری استفاده شد. برای انجام فرآیند تخمیر از باکتری Saccharomyces cerevisiae به عنوان میکروارگانیسم و گلوکز به عنوان سوبسترا استفاده گردید. در فرآیند سنتی تخمیر ناپیوسته، راندمان غلظت سلولی و اتانول میکروارگانیسم و گلوکز به عنوان سوبسترا استفاده گردید. در فرآیند سنتی تخمیر ناپیوسته، راندمان غلظت سلولی و اتانول میکروارگانیسم و گلوکز به عنوان سوبسترا استفاده گردید. در فرآیند سنتی تخمیر ناپیوسته، راندمان غلظت سلولی و اتانول موجنین، غلظت اتانول در سمت تراوشی تقریباً 6 تا 7 برابر بیشتر از مقدار آن در محیط کشت داخل بیوراکتور بود. برمبنای مدل سیتیکی و بیولوژیکی مونود، حداکثر نرخ رشد ویژه برابر ¹⁰ 6 م9/90 بوده که آن بیشتر از مقدار آن بیشتر از مقدار آن بریوسته تا موراکتور سنتی بوده است. در مقایسه با فرآیند تخمیر سنتی ناپیوسته تا مقدار گرایش یافت. در بیوراکتور سنتی بوده است. در مقایسه با فرآیند تخمیر سنتی بیوراکتور غشایی باعث افزایش یافت. ماهمنین اتانول، بهبود بهره وری و راندمان و همچنین بازیافت اتانول تغلظ شده گردید. موارد فوق به دلیل حضور غشاء به عنوان یک جداساز گریش پذیر برای حذف اتانول از محیط کشت داخل بیوراکتور می باشد.

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چکیدہ