Continuous Ethanol Fermentation in Immersed, Cross-Flow Microfiltration Membrane Bioreactor with Cell Retention

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Abstract: The key objective of this study was to devise a continuous ZeeWeed® membrane-based, immersed, microfiltration (MF) laboratory scale fermentation system for ethanol production with cell retention to achieve effective ethanol productivity, flux rates and sugar utilization. The new bioreactor was compared to the fermentation kinetics' of the ultrafiltration unit. A synthetic glucose based medium was fermented by fresh, baker's yeast to produce ethanol. The cells were not recycled; the medium was continuously withdrawn by filtration through an internal, immersed hollow-fiber cartridge. In this way, the inside of the membrane was exposed to the ethanolic solution, while broth with viable yeast cells remained outside the membrane. This design, with a cell retention system, provided much less membrane fouling (loss of about 76% of the original water flux after 96 hours of filtration) than while using the ultrafiltration (UF) external hollow-fiber membrane with cell recycling (loss of 97% of the original water flux after 2-3 hours of operation). Both modules converted at least 95% of glucose with biomass concentration of 30 g/L, and the final ethanol concentration of 62 g/L. However, the UF membrane became plugged after only 2 hrs of operation. The ZeeWeed® membrane operated successfully for 96 hrs with a final flux of 4 L/h m^2 with ethanol concentration of 62.4 g/l, biomass yield 0.34 g/g and cell viability of 95.3%. This concept could be successfully used for biofuel production. A very strong positive correlation was observed between the biomass and EtOH concentration ($R=0.98$; at $p<0.05$).

Keywords: Continuous ethanol fermentation, hollow fiber, cross-flow microfiltration, membrane bioreactor, cell retention, *Saccharomyces cerevisiae.*

INTRODUCTION

Fermentation process is employed in the conversion of agricultural waste into alcohol, which can be used as a biofuel. To meet the needs of today's industry and increasing demand for environmental protection and sustainability, the conversion of various types of pretreated, inexpensive carbohydrate based waste sources, such as oil press cakes, coffee husks, etc. into useful products, such as ethanol and cell biomass, could be of great importance [1, 2].

Until recently almost all of the industrial ethanol produced by yeast fermentation using *Saccharomyces cerevisiae* was carried out by batch and fed-batch methods. However, this has several disadvantages: low productivity, high equipment and labor costs, and batch-to-batch variation in the product. Membrane bioreactors, using cross-flow MF or UF hollow fiber modules with cell recycle that continuously remove the fermented product, have received much attention [3]. Cross-flow (tangential) MF and UF have emerged as effective and economical filtration choices in the food industry for uses such as wine filtration, protein concentration, purification of natural products, and microorganisms removal from liquid eggs to name a few [4-6].

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The main drawback to using membrane technology has been the permeate flux decay and membrane fouling as a function of operation time due to the high concentration of soluble and insoluble solids in the raw material. The fouling phenomenon occurs due to specific solute-solute and solute-membrane physical and chemical interactions that are independent of changes in operating conditions. In this case, permeate flux recovery is possible after chemical cleaning of the membrane, as one of the available methods, depending on the membrane type [7].

Traditionally, ethanol production has been conducted almost exclusively by *S. cerevisiae* because of its high fermentation rate and ethanol tolerance. The inhibitory effect of ethanol on *S. cerevisiae* produced during fermentation is complex and is the main reason for slow and incomplete fermentations. Still, yeast cell viability after fermentation remains in the range of 80-90% (2). During the last decade several systems were investigated for continuous ethanol fermentation including membrane reactors, removal of ethanol by pervaporation [8] and immobilized yeast for beer production [9].

Ethanol production from glucose in a hollow fiber bioreactor using *Saccharomyces cerevisiae*, was introduced in the early eighties. Mehia and Cheryan [10] performed continuous fermentation without cell recycling and obtained fairly high ethanol productivity

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(40 g/L/h) with low glucose utilization (30%) using glucose solution as a feed. In another report Cheryan and Mehia [11] applied much higher cell concentrations with cell recycle and obtained similar results: higher glucose concentration (200 g/L) resulted in an increase in ethanol concentration (65 g/L) and productivity (130 g/L/h), but lowered the substrate conversion (70%). Both projects reported membrane fouling and decreasing permeate flux as major problems.

For more than a decade, Zenon Membrane Solutions, currently part of GE Water & Process Technologies, has been developing ultrafiltration membrane bioreactor (MBR) systems. The ZeeWeed® MBR uses immersed hollow-fiber membranes to carry out ultrafiltration, mainly of waste water systems to produce high-quality permeates. Immersed hollow-fiber MF was used for removal of toxic substances from seawater [12] and for graywater treatment [13]. Ethanol fermentation using cross-flow microporous membranes to ferment sugars, i.e. glucose in pretreated organic waste into ethanol or vinegar, is a potential commercial application of the same technology as it will result in decreased membrane fouling compared to other membrane systems.

To the best of our knowledge, there is no published study on a continuous ethanol fermentation bioreactor with immersed hollow-fiber MF membrane. The objective of this research was to devise a continuous ethanol fermentation bioreactor using an immersed microporous membrane with cell retention while providing effective ethanol production, glucose utilization and cell viability for at least 3 days (72 hrs) without process disruption for membrane cleaning. Also, the objective was to compare this new membrane with traditional UF membrane in terms of flux that is directly related to the design of the system.

MATERIALS AND METHODS

Materials

Saccharomyces cerevisiae (fresh, commercial, baker's compressed yeast: Fleischmann Ltd., Mississauga, ON, Canada) was used in this study. A complete synthetic glucose medium was prepared according to the following formulation: 100 g glucose; 10 g Yeast extract (BD-Difco, Sparks, MD, USA); 6 g $(NH_4)_2SO_4$; 5 g NH_4Cl; 3 g $Na_2HPO_4 \cdot 7\ H_2O$; 2 g K_2HPO_4 ; 1 g $MgSO_4 \cdot 7H_2O$; 0.5 g $CaCl_2 \cdot 2\ H_2O$; 0.1 g $MnSO_4 \cdot H_2O$; 4 g citric acid; 2 mg calcium pantothenate; 0.1 mL Antifoam in 1.0 L distilled water. All chemicals were of analytical grade (Sigma Chemical Co., St. Louis, MO, USA). The medium was filtered through a 0.45 μm membrane filter (Millipore, Billerica, MA, USA), and its pH was adjusted to 4 with H_2SO_4 prior to fermentation and it was maintained with a potassium hydrogen phthalate buffer.

Methods

Yeast cell viability was determined by the methylene blue staining method as described previously [14]. The stained cells were examined as a wet mount with an 8x eyepiece and 10x lens, using an electronic microscope (Olympus, Tokyo).

Biomass concentration (yeast cell dry weight per unit volume, g/L) was determined gravimetrically [15]. Five mL of fermentation broth was withdrawn into a centrifuge tube and 0.5 mL of 32% formaldehyde was added to stop the cell growth [16]. Before chemical analysis of the samples, cells were removed by centrifugation (at 2000 rpm for 5 min.) immediately after collection and cell dry weight was determined. One mL of the supernatant was diluted 50 times with distilled water and it was filtered through a 0.45 μm membrane filter. The supernatant was stored at -20°C to avoid further fermentation until ethanol and glucose analysis could be performed. Ethanol concentration was measured by gas chromatography using 0.1% n-propanol as an internal standard, according to a modified 984.14 AOAC Official Methods of Analysis [17]. A Hewlett Packard 5890 gas Chromatograph (6 ft.x2 mm glass column, packed with 60/80 Carbopack B/5% Carbowax 20M, Supelco, Bellafonte, USA) with a flame ionization detector was used. Both injector and detector were kept at 150°C and the column oven at 85°C. Helium was used as carrier gas at a flow rate of 20 mL/min, at an operating pressure of 150 kPa. The detector flame used a mixture of hydrogen and nitrogen in air. One μL of a solution, formed by mixing an equal volume of the 0.1% n-propanol solution with the sample was injected. Ethanol concentration calculation was performed based on a standard calibration curve.

Glucose concentration was determined by the 3,5-dinitrosalicylic acid (DNS) method measuring absorbance at 580 nm, against water blank [18], using a Beckman DU-7 Spectrophotometer (Beckman Coulter, Fullerton, CA, USA). The absorbance was converted to glucose concentration by means of a calibration curve prepared with known concentrations of glucose.

Filtration Systems

Short-term, continuous ethanol fermentation with cell recycle using a hollow-fiber UF unit was tested first. The membrane bioreactor operated as a continuous stirred tank reactor (1 L volume) with cell recycle connected to the membrane module (hollow fiber). A medium volume level was kept steady by matching the inflow and outflow rates, using fresh medium. The hollow-fiber polysulphone membrane used in the UF unit was made by Amicon Division (Beverly, MA. USA), Type H1P100-43, trans-membrane pressure 25 psi; temp. 50^{o}C; molecular cutoff 100,000 daltons). Each of the 55 fibers had 1 mm i.d. and was 203 mm long. The total hollow-fiber surface area was 350 cm^{2}. A cartridge was provided with a process (feed) inlet, process outlet, and a pair of permeate outlets on either end of the cartridge. The retentate outlet was coupled with the fermentor in order to recycle cells.

Four experiments were performed with fresh yeast concentrations of 30, 50, 70 and 90 g/L. The initial medium consisted of 100 g/L of glucose. The initial pH was 3.7 and impeller speed of 300 rpm. Neither fermentation vessel nor medium was sterilized.

After three hours of fermentation, when most of the glucose was consumed, the ultrafiltration unit with a peristaltic pump was started. For the next three hours, continuous glucose fermentation and cell recycling was performed. Fresh medium (100 g/L) was added by gravity and the permeate was collected in a storage tank, while the retentate (yeast cells) was recycled to the fermentor. A schematic diagram of the UF membrane recycle fermentor system is shown in Figure **1**.

The second set of experiments investigated the effect of glucose concentration on ethanol production. Fresh yeast (70 g/L) was used and the medium consisted of 100, 125, 150 and 175 g/L of glucose. The initial pH value was 4.0. The ultrafiltration unit started after two hours of fermentation; the final pH was between 2.8 and 3.1.

Dilution rate (D, h^{-1}) was calculated according to the following equation [19]:

$$D=F/V \quad (h^{-1}) \qquad (1)$$

where: F-the flow rate of the medium feed (L/h); V-volume in reactor (L).

New membrane concept

In order to eliminate membrane plugging problems and to increase the permeate flux, a novel membrane configuration was developed by Zenon Environmental Inc. The key structural feature of this membrane is that it is “inside out”, compared to conventional membranes [20], the filter membrane is on the outside of the fiber, and the flow is from the outside bulk liquid into the inside of the fibers.

The membrane support is inside the tubes, as illustrated in Figure **2** (a modified illustration based on the Romicon Inc. drawing). The tubes were immersed

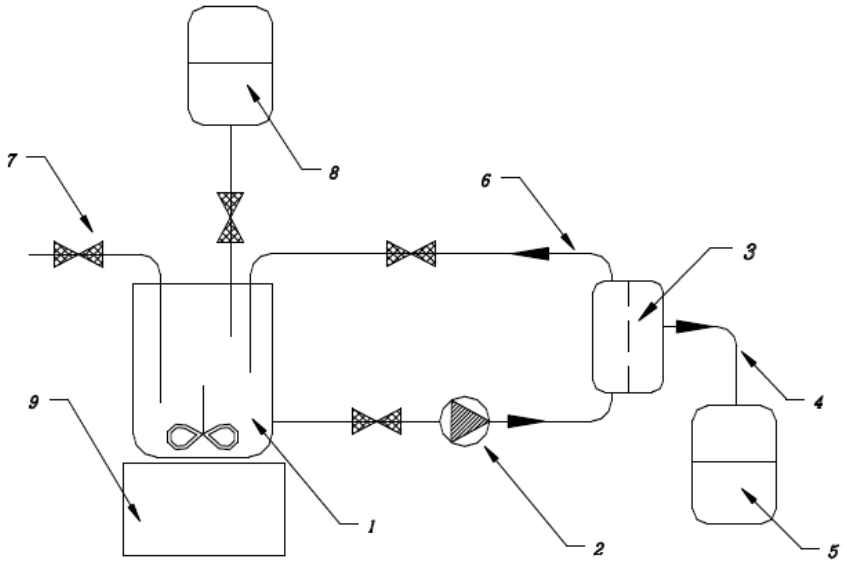

Figure 1: Cell recycle apparatus for continuous fermentation using UF external membrane: 1 - Fermentor; 2 - Peristaltic pump; 3 - External hollow-fiber module; 4 - Permeate outlet; 5 - Filtrate chamber; 6 - Retentate outlet; 7 - Sampling port; 8 - Fresh medium tank; 9 - Magnetic stirrer device.

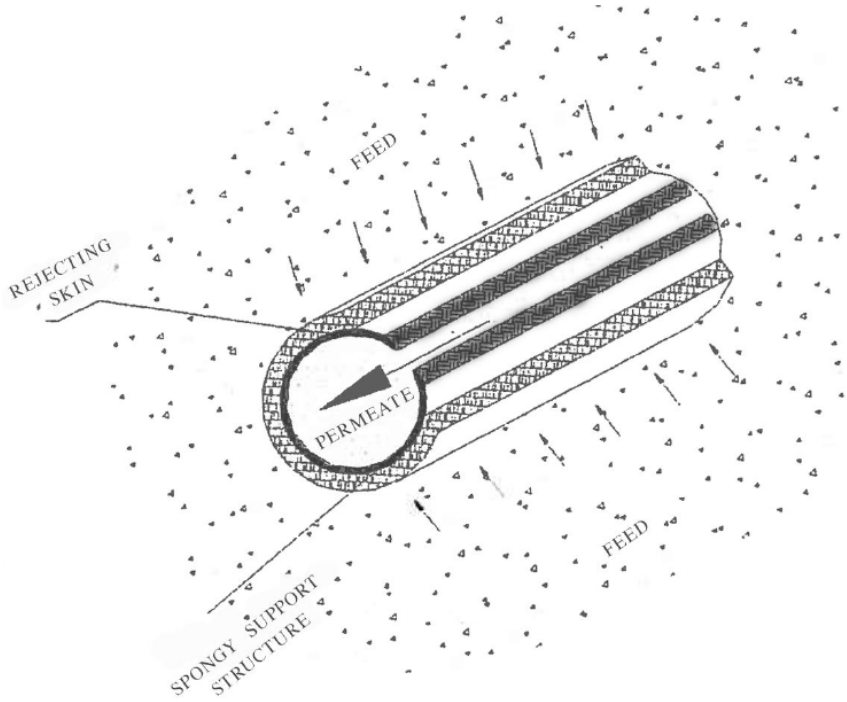

Figure 2: Schematic of the Zenon's® hollow fiber MF process (Feed is consisting of low and high molecular weight solute).

in the bioreactor; fermentation broth remained in the vessel, while permeate was pumped through the membrane module to the receiving reservoir under a slight vacuum.

The Zenon's (GE Water & Process Technologies, Zenon Membrane Solutions, Oakville, Ontario, Canada; formerly Zenon Environmental Inc, Burlington, Ontario, Canada) prepared a membrane module based on the proprietary ZeeWeed® hollow fibers for our experiments. The apparatus is shown schematically in Figure **3**.

The membrane was made of a hydrophilic polymer, slightly negatively charged, which can be pasteurized (temperature up to 100^{o}C) and withstand a pressure of up to 10 psi (0.689x10^{5} Pa). The outside fiber diameter was 1.85 mm, and pore size was in the range of 0.08-0.1 μm. Module characteristics include: a material of construction that was CPVC, polyurethane formulation potting; fiber length was 0.14 m; number of fibers was 19 giving a total surface area of 0.0155 m^{2}. Pure water permeates flow at 20^{o}C and 0.3 bar vacuum was 24.7 mL/min and flux of 95.6 Lm^{2}/h. Temperature range was 0-100^{o}C. A membrane module, provided with a process outlet, was placed in a bioreactor (Figure **3**). The fermentation broth remained in the vessel, only ethanol and by-products in the exhausted medium were removed. Although previous set of experiments suggested the need for stronger pumps in order to have higher inflow and outflow rates, they were not available for this experiment.

Long-term fermentation was performed with the fresh yeast as a 10 g/L (2.9 g/L of dry mass). The initial medium consisted of 40 g/L of glucose, and the

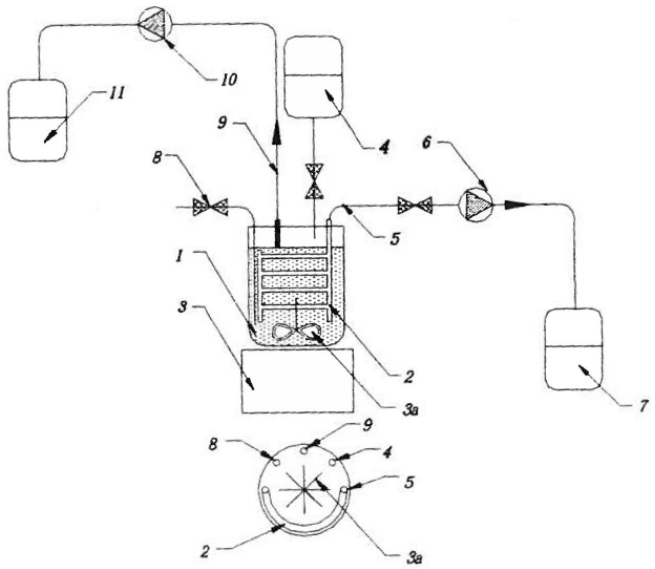

Figure 3: Continuous fermentation system based on the immersed ZeeWeed® microporous membrane with cell retention: (1) Bioreactor; (2) Membrane module; (3) Magnetic stirrer and (3a) Stirrer; (4) Feed reservoir; (5) Permeate outlet; (6) Reversible peristaltic pump; (7) Chamber for cell-free filtrate; (8) Sampling port; (9) Level control; (10) Peristaltic pump; (11) Reservoir for bioreactor effluent containing biomass.

glucose concentrations were changed as described later. The initial and final pH was 4 and impeller speed was maintained at 200 rpm. The whole system was pasteurized to ensure that no contamination could occur and disturb the fermentation process. Fresh medium was added by gravity [4] into a bioreactor [2] (Figure **3**). A pump (Perista-mini pump, model SJ-1211, Japan) was used to ensure a constant level of the fermentation broth, at one liter, so the extra liquid was removed to the small reservoir [11]. The peristaltic pump (Perista-mini pump, model SJ-1220, ATTO, Japan) was adapted to be time-controlled in order to pump the permeate into a receiving vessel [7] for 10 minutes; then the membrane was back-flushed in the opposite direction for one minute. This was necessary for the proper functioning and cleaning of the membrane.

The samples were withdrawn two times per day (every 12 hours) and analyzed according to described procedures. Ultrafiltration membrane (polysulfone), equipment and medium were not sterilized and operated at room temperature. When the ZeeWeed® microporous membrane was tested, all of equipment was pasteurized at 100°C.

Membrane Cleaning

After each use, the UF filter was cleaned with the 0.5% of enzyme detergent (TERG-A-ZYME, Alconox, Inc., New York, USA) for half an hour, and then rinsed with 4 L of distilled water. The cartridge was kept in 1% formaldehyde solution. For the MF membrane, the following procedure was applied: after rinsing with distilled water, the MF membrane soaked for 2 hours in the bioreactor in a solution of 10 mg/L NaOCl at 40°C; afterwards it was soaked and stored in a 10 mg/L NaOCl solution at room temperature. Before use, both cartridges were soaked in distilled water overnight at room temperature and rinsed thoroughly.

Statistical Analyses

Analytical measurements were conducted in triplicate for all oils and results were recorded as means ±SD (standard deviation). Statistical significance between the means (pairwise comparisons) was determined using Minitab software (State College, PA, U.S.A.) (Version 16) and the Tukey's test at $p < 0.05$. The Pearson's product-moment correlation coefficient (r) was used to analyze the correlation between chemical analyses of obtained samples.

RESULTS AND DISCUSSION

Studies in this set of experiments have confirmed the feasibility of a continuous fermentation process with cell recycle, specifically the effects of the yeast cell and glucose concentrations on ethanol production in a UF membrane bioreactor. The pH value decreased during the course of fermentation to: pH 2.5 (30 g/L of yeast); pH 2.8 (50 g/L of yeast); pH 3.1 (70 g/L of yeast) and pH 3.4 (90 g/L of yeast). The pH drop could be explained by the formation and presence of inhibitors, such as lactic and acetic acid. Bakers' yeast, when

Table 1: Fermentation Kinetics in UF Membrane Fermenter at Various Yeast Concentration Levels

Run	1	2	3	4
Initial wet cell conc. (g/L)	30	50	70	90
Overall dilution rate (h^{-1})	0.31	0.48	0.53	0.42
Initial glucose conc. (g/L)	100	100	100	100
Conversion of glucose (%)	97.6^{d}	99.2^{b}	98.9^{c}	99.4^{a}
Initial biomass dry weight (g/L)	8.6^{d}	14.5^{c}	20.2^{b}	26.0^{a}
Biomass conc. (dry weight, g/L)	14.7^{d}	29.5^{c}	30.6^{b}	31.7^{a}
Biomass yield (g of cells per g of glucose utilized)	0.15^{d}	0.30^{c}	0.31^{b}	0.32^{a}
EtOH yield (g EtOH/ g glucose consumed)	0.63^{c}	0.8^{b}	0.8^{b}	0.81^{a}
Maximum EtOH productivity (g/L h^{-1})	13.3^{*d}	20.1^{*c}	22.5^{**b}	30.5^{**a}
Overall EtOH productivity 6 hrs (g/L h)	10.3^{d}	13.3^{b}	13.2^{c}	13.4^{a}
Overall inflow rate of fresh medium (mL/h)	306.7^{d}	373.3^{c}	411.7^{b}	413.3^{a}
Overall outflow rate(mL/h) of product in exhausted medium	286.7^{d}	426.7^{b}	463.3^{a}	396.6^{c}

*after 3 hours; **after 2 hours.
Values are means ± SD; n=3. Means in the same row followed by different superscript letters are significantly different ($p < 0.05$).

Table 2: Fermentation Kinetics in UF Membrane Bioreactor at Different Glucose Concentration Levels

Run	1	2	3	4
Initial glucose conc. (g/L)	100	125	150	175
Overall dilution rate (h^{-1})	0.53	0.34	0.34	0.23
Final glucose conc. (g/L) after 6 h	1.0^{d}	0.9^{c}	4.1^{b}	7.4^{a}
Conversion of glucose (%)	98.9^{b}	99.3^{a}	97.2^{c}	95.7^{d}
Initial biomass dry weight (g/L)	20.2^{a}	18.7^{d}	18.8^{c}	18.9^{b}
Biomass conc. (dry weight, g/L) continued	30.6^{a}	29.1^{b}	26.1^{d}	27.0^{c}
Biomass yield (g of cells per g of glucose utilized)	0.31^{a}	0.23^{b}	0.18^{c}	0.16^{d}
Final EtOH conc. (g/L)	79.5^{d}	96.1^{c}	102.6^{b}	105.0^{a}
EtOH yield (g EtOH/ g glucose consumed)	0.8^{a}	0.77^{b}	0.7^{c}	0.6^{d}
Overall EtOH productivity 6 hrs (g/L h)	13.2^{d}	16.0^{c}	17.1^{b}	17.5^{a}
Overall inflow rate of fresh medium (mL/h)	411.7^{a}	300.0^{c}	308.7^{b}	236.2^{d}
Overall outflow rate(mL/h) of product in exhausted medium	463.3^{a}	308.7^{c}	315.0^{b}	210.0^{d}

Values are means ± SD; n=3. Means in the same row followed by different superscript letters are significantly different ($p < 0.05$).

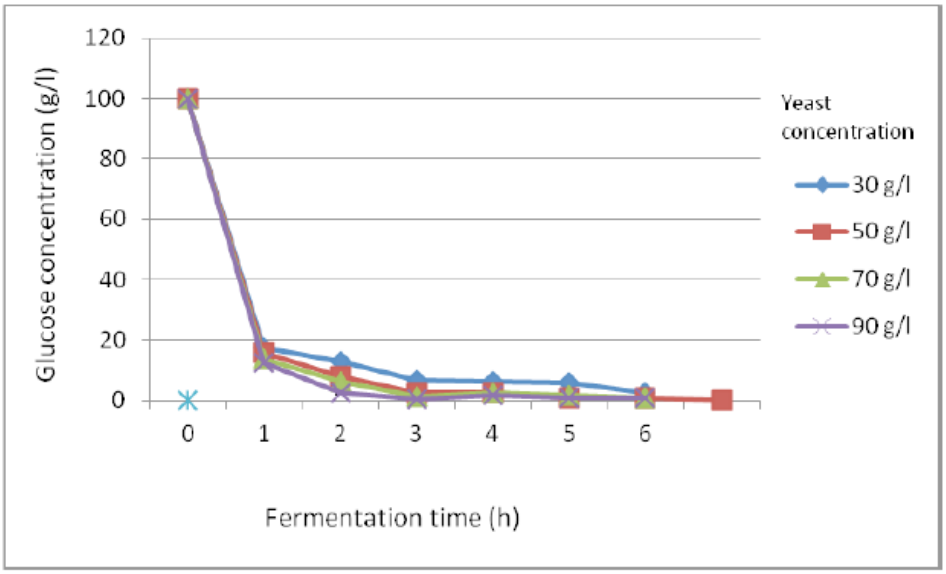

Figure 4: Effect of yeast concentration on glucose concentration (g/L) in the UF membrane bioreactor. *Error bars* represent standard deviation (not shown as < ±1).

grown aerobically with excess glucose present, excretes copious amounts of acetate as the availability of substrate exceeds the oxidative capacity of the bacterial cells [21].

The continuous fermentation process with cell recycle offers useful ethanol productivity and biomass yield (Tables **1** and **2**). In all cases, glucose was almost completely utilized after 2 hours of fermentation with >90% efficiency (except of run 1, where 87% was achieved), when broth withdrawal with the UF unit was started (Figure **4**).

The best results, in terms of ethanol concentration, were obtained using 7 or 9% of yeast (Figure **5**).

The effect of starting glucose concentration on glucose utilization was observed. The best results were obtained using 100 g/L of glucose, followed by 125 g/L, 150 and 175 g/L (Table **2**). In all cases ~ 95% glucose was utilized. The critical point in the fermentation was the second hour, when maximum ethanol productivity was achieved in all cases (Figure **6**).

The highest overall ethanol productivity was obtained using 175 g/L of glucose, obviously because

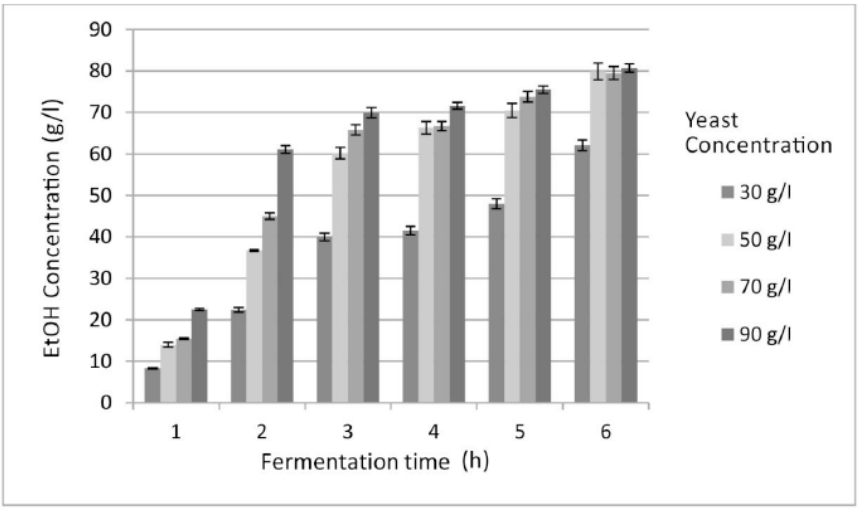

Figure 5: Effect of yeast concentration on ethanol production in the UF membrane bioreactor. *Error bars* represent standard deviation.

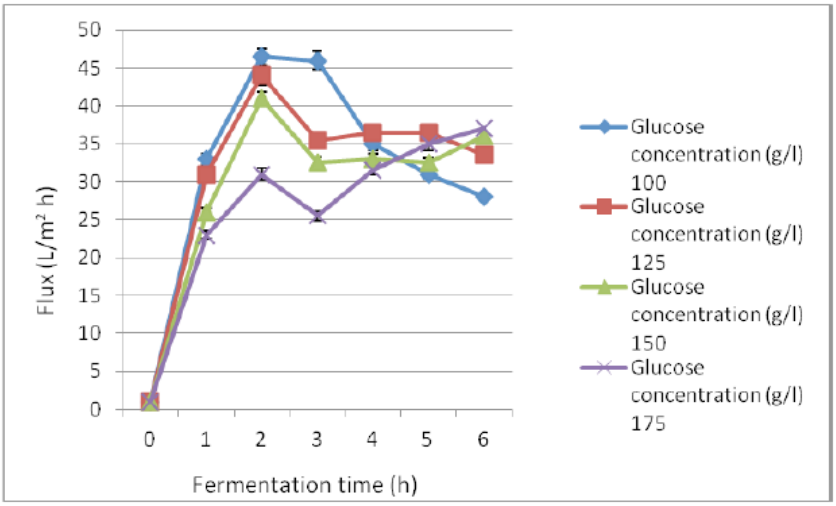

Figure 6: Ethanol production rate vs. fermentation time depending on the glucose concentrations in the UF membrane bioreactor. *Error bars* represent standard deviation.

more glucose was fermented. In this run, besides the maximum overall productivity, the highest ethanol concentration was achieved (105 g/L). This is contrary to the fact that the maximum product concentration at which fermentation activity is completely halted is 87.5 g/L [22]. These results are lower than reported elsewhere [23]. They obtained ethanol productivity of 27 g/L/h with 85 g/L of cell concentration, using 150 g/L of glucose, and concluded that this system might be available for the primary product formation at high dilution rates (0.6 h^{-1}) and high cell concentrations, where our dilution rate was 0.34 h^{-1}.

Similarly, other researchers [24, 25] concluded that a cell reuse system with the MF module is applicable whenever high productivity in ethanol fermentation is required, but noticed that at higher cell concentrations, membrane fouling can be severe and an appropriate membrane module must be used to minimize the membrane-related problems.

Cross-flow filtration of yeast broth cultivated in molasses was examined by others [26] and this mechanism was clarified by analysis of the change in the flux with time and observations of the filter surface with scanning electron microscopy. They back-flushed the membrane after 10-min periods of permeation, and observed the increase in flux after backwashing. The restored flux depended on the pore size of the membrane. When the nominal pore size of the membrane was 0.8 µm or smaller, the flux decreased as in continuous cross-flow filtration. However, when pore size was 3 to 5 µm, the flux was almost completely restored by back-flushing. The decrease in

flux in the module with smaller pores was mostly due to plugging of the pores by yeast cells which were easily removed by back-flushing.

In our experiments, fermentation was very intensive and maximum values for all parameters were found just after two hours of fermentation. In spite of employing very high yeast concentrations, biomass productivities were high, probably because fermentation products were removed and the cells had better environmental conditions for growth and multiplication. Similar results were obtained using either 70 or 90 g/L of fresh yeast and 100 g/L of glucose when the effects of yeast concentration were investigated. Ethanol concentration of 80 g/L and overall productivity of 13.3 (maximum of 22.5 and 30.5, for 70 and 90 g/L of yeast, respectively) were achieved (Table **1**).

When the effect of glucose concentration was evaluated, the best result was obtained using 100 g/L of glucose. This was likely due to the fact that the fermentation broth had the lowest viscosity at this glucose concentration. The pH value decreased during the course of fermentation, however the acidity decreased with increased yeast concentration: pH 2.5 with 30 g/L of yeast; pH 2.8 with 50 g/L of yeast; pH 3.1 with 70 g/L of yeast and pH 3.4 with 90 g/L of yeast. The maximum overall ethanol productivity (17.5 g/L h^{-1}) was observed when 175 g/L of glucose and 70 g/L of fresh yeast were used (Table **2**).

However the membrane fouling occurred in both experiments after only one hour of running the UF unit (Figure **7**). The permeate flux declined drastically regardless of yeast or glucose concentrations. Permeate flux decreased drastically in each run (between 76-94%) after only 2-3 hours of operation and had to be stopped, as it was plugged.

When the ZeeWeed® proprietary, low fouling microporous membrane (resilient polyvinylidene fluoride PVDF was tested without cell recycle, steady increase in ethanol productivity was obtained with the gradual increase in glucose concentration, as more sugar was fed (Table **3**). The maximum ethanol concentration was 62.4 g/L and had a productivity of 10.3 g/L h^{-1}. Ethanol yield was 0.63 g EtOH/ g glucose consumed and much higher than obtained by others [27] using extractive continuous fermentation (0.41 g/g).

The data indicated that the yeast cells had a good fermentation environment, transforming at least 96% of glucose into ethanol (Figure **8**). In all phases of the fermentation process, yeast cell viability was between 89-95%, in the absence of a cell bleed, which is in good agreement with the published data (*2*).

Although the permeate had high ethanol concentrations (max. 62.4±5.4 g/L), the values for ethanol productivities were small (Figure **9**) when

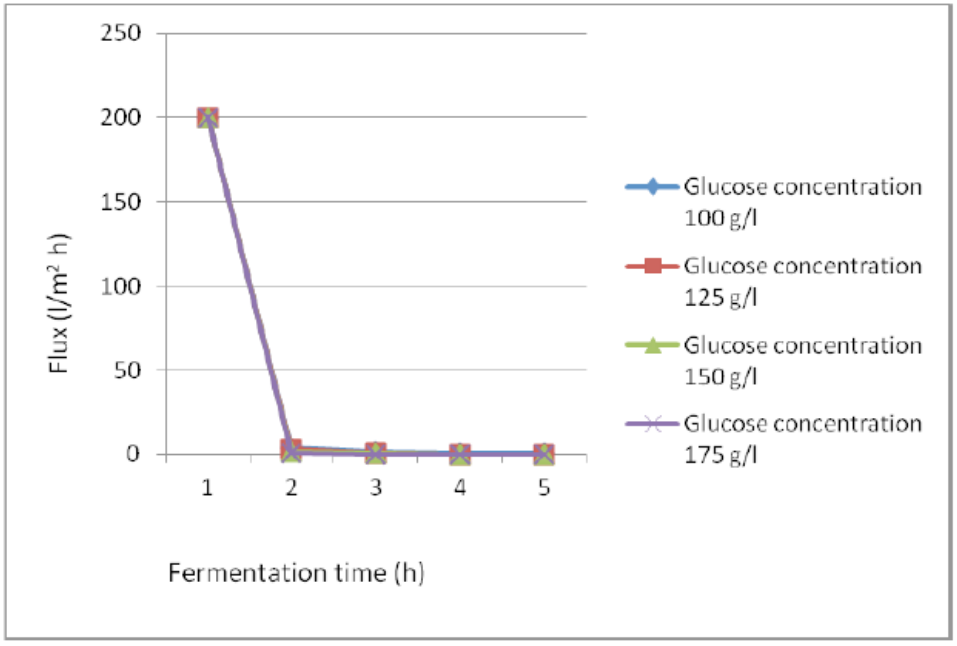

Figure 7: Flux decline with time in the UF membrane bioreactor (10 g/L yeast concentration). *Error bars* represent standard deviation (not shown as < ±1).

Table 3: Fermentation Kinetics in a ZeeWeed® Membrane Bioreactor

Run	1	2	3	4
Fermentation time (hours)	1-24	25-48	49-72	73-96
Overall dilution rate (h^{-1})	0.10	0.07	0.07	0.06
Initial glucose conc. (g/L)	40	60	80	100
Conversion of glucose (%)	98.8^{b}	96.0^{c}	98.8^{b}	98.9^{a}
Biomass yield (g/g)	0.24^{b}	0.20^{c}	0.24^{b}	0.34^{a}
EtOH yield (g EtOH/ g glucose consumed)	0.76^{a}	0.52^{d}	0.59^{c}	0.63^{b}
Maximum EtOH productivity (g/L h)	4.9^{d} (24 hours)	5.7^{c} (48 hours)	8.3^{b} (72 hours)	10.3^{a} (96 hours)

Values are means ± SD; n=3. Means in the same row followed by different superscript letters are significantly different ($p < 0.05$).

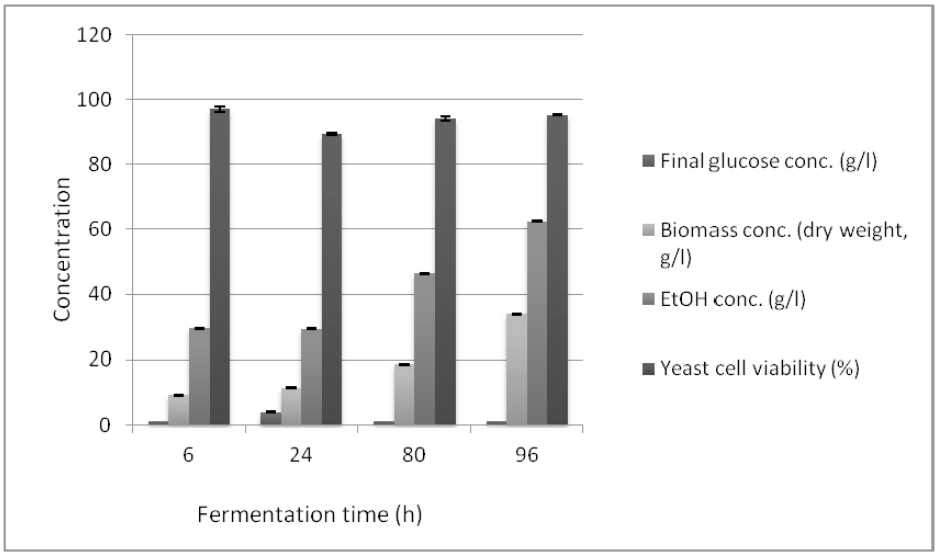

Figure 8: Fermentation kinetics in the ZeeWeed® membrane bioreactor. *Error bars* represent standard deviation.

considering the continuous membrane process, due to inadequate membrane surface.

The higher ethanol concentration was obtained possibly because glycogen from yeast cells fermented and provided extra ethanol. Other authors [28] investigated increased yields of ethanol (higher than theoretical) during the rapid batch fermentation of sugar-cane blackstrap molasses, where three assumptions were examined as possible causes of higher yields: 1. temporary ethanol accumulation within the yeast cells; 2. variation of the dry matter content and/or of the microorganism density during the fermentation; 3. transformation of sugars into undetectable extra-cellular fermentable compounds at the initial stages of the process. Based on the experimental results presented in their study, supported by the previous results, they explained the observed increase in the ethanol yield confirming the third assumption. Namely, the transformation of sugars into fermentable compounds (undetectable by the adopted analytical methods) that temporarily accumulated in the medium and were later fermented producing ethanol, could explain the observed yield variation. This is supported by the fact that yeast cells produce more alcohol when reproducing than when resting. Our results indicated high levels of viable yeast cells during the entire fermentation process, which could be explained by the high rate of their multiplication and therefore higher ethanol concentrations.

The permeate flux decreased by about 43%, probably because the fermentation medium became more viscous, and there was no cell bleed. In addition, the membrane was probably slightly plugged with the yeast cells, which is expected in every filtration process. Clearly the membrane filtration area was not

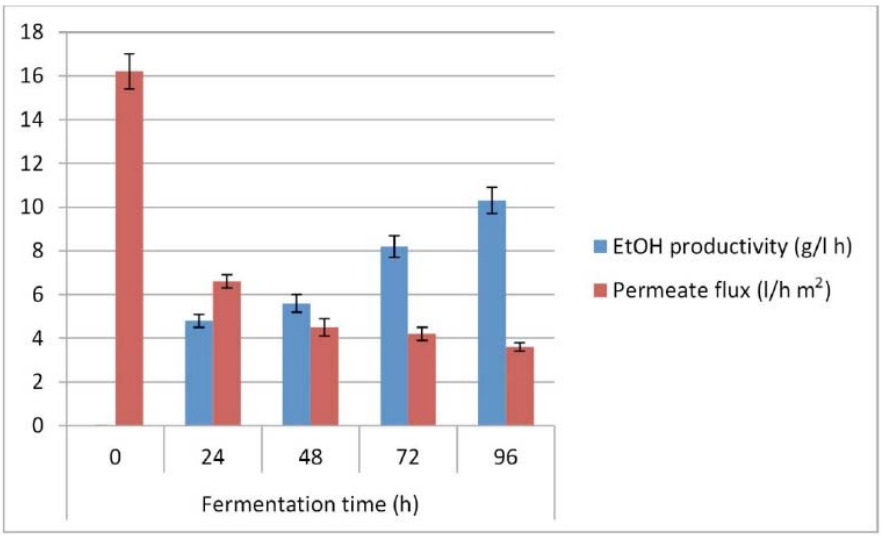

Figure 9: Flux decline and ethanol productivity in the membrane bioreactor ZeeWeed® membrane bioreactor. *Error bars* represent standard deviation

sufficient to efficiently operate the fermentation. Despite the low surface area, much smaller decrease in permeate flux was observed in this continuous system than that observed in the UF module.

CONCLUSIONS

Continuous fermentation with or without cell recycle can yield an appreciable ethanol and biomass productivity than the standard batch system. The hollow fiber UF module was unsuitable for solid/liquid separation, as it plugged very quickly (after 2-3 hrs of operation). The Zeeweed® based MF membrane had very good characteristics in terms of maintaining filtrate flux for a long time (96 hrs), compared to the UF system.

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