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Lactic acid Production with *in situ* Extraction in Membrane Bioreactor

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Abstract

Background and Objective: Lactic acid is widely used in the food, chemical and pharmaceutical industries. The major problems associated with lactic acid production are substrate and end-product inhibition, and by-product formation. Membrane technology represents one of the most effective processes for lactic acid production. The aim of this work is to increase cell density and lactic acid productivity due to reduced inhibition effect of substrate and product in membrane bioreactor.

Material and Methods: In this work, lactic acid was produced from lactose in membrane bioreactor. A laboratory scale membrane bioreactor was designed and fabricated. Five types of commercial membranes were tested at the same operating conditions (trans-membrane pressure: 500 KPa and temperature: 25°C). The effects of initial lactose concentration and dilution rate on biomass growth, lactic acid production and substrate utilization were evaluated

Results and Conclusion: The high lactose retention of 79% v v^{-1} and low lactic acid retention of 22% v v^{-1} were obtained with NF1 membrane; therefore, this membrane was selected for membrane bioreactor. The maximal productivity of 17.1 g l^{-1} h^{-1} was obtained with the lactic acid concentration of 71.5 g l^{-1} at the dilution rate of 0.24 h^{-1}. The maximum concentration of lactic acid was obtained at the dilution rate of 0.04 h^{-1}. The inhibiting effect of lactic acid was not observed at high initial lactose concentration. The critical lactose concentration at which the cell growth severely hampered was 150 g l^{-1}. This study proved that membrane bioreactor had great advantages such as elimination of substrate and product inhibition, high concentration of process substrate, high cell density, and high lactic acid productivity.

Conflict of interest: There is no conflict of interest.

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1. Introduction

Generally, dairy industries generate large quantities of effluents from milk processing in cheese production plants [1]. Whey consists of lactose, proteins, fats, vitamins, minerals and other essential micro-nutrients, which are suitable for microbial, and can be considered as a low-cost substrate for lactic acid production [2].

Lactic acid is widely used in the food, chemical, cosmetic and pharmaceutical industries [3]. It can be produced via chemical processes and microbial fermentation. In recent years, fermentation process has proved to be the most appropriate process for production of lactic acid, while demand for naturally producing lactic acid has increased [4,5]. Moreover, the cost for fermentation process may be lower than those for synthetic processes [6].

The major problems associated with lactic acid production are substrate inhibition, end-product (lactic acid) inhibition, and by-product formation [7]. Some papers showed that lactose initial concentration higher than 100 gl^{-1} in cheese whey reduced both the specific growth and the substrate utilization rates due to the substrate inhibition phenomenon [8]. Lactic acid production processes also suffer from end-product inhibition [9]. However, the mechanism of inhibition in production of lactic acid via fermentation is not fully understood. The most accepted mechanisms of inhibition by weak organic acids are related to the solubility of the non-dissociated form within the cytoplasm membrane, and the insolubility of the ionized acid form [10].

Another challenge in the conventional fermentation of lactic acid is downstream processing for lactic acid recovery; whereas the cost of recovery is proportional to

the amount of liquid to be handled and is inversely harmonized to the product concentration [11].

Due to the lactic acid properties, conventional continuous separation processes such as distillation have limitations to be used [12]. The lactate separation is usually done by precipitation, where the precipitated calcium lactate must be recovered employing a strong acid. This procedure implies a high chemical cost and waste generation. For this reason, other alternatives for lactate recovery such as solvent extraction, adsorption, direct distillation and membrane separation processes have been studied [4,13,14]. It has been estimated that the cost of recovery and concentration of lactate from the cultivation broth can be up to 80% of the total production cost; then research has been focused on developing alternatives for downstream processing [15]. Membrane based separation processes present some advantages like selective separation of lactate, being capable to operate aseptically, and basically generation of no by-products [16].

Membrane technology represents one of the most effective and energy saving processes for lactic acid production [17]. Coupling of membrane separation with biological process in one unit is very attractive configuration for the reactions where the continuous elimination of metabolites is necessary to maintain high productivity [18]. It causes to reduce substrate and product inhibitions and increase cell density and lactic acid productivity [18]. The combination of membrane and bioreactor certainly reduces downstream processing [19]. The major membrane processes are microfiltration, ultra-filtration, nano-filtration, reverse osmosis, and electro-dialysis. A number of researchers have investigated continuous lactic acid production by membrane bioreactor using different media, membranes and configurations [20-23]. Although lactic acid production has been well documented, improved production parameters that lead to reduced production costs are always of interest in industrial developments [24]. Despite its potential for large scale production and use in a wide variety of applications, cost-effective production of high purity lactic acid has remained a challenge for decades, mainly due to high downstream processing cost [16].

In the present study, the performance of membrane bioreactor for lactic acid production from whey lactose was investigated. The purpose was to reduce the inhibition effect of high concentration of substrate with membrane bioreactor (MBR). High lactic acid concentration, high cell density and high lactic acid productivity were obtained in this study.

2. Materials and Methods

2.1 Microorganism and Medium

The selected microorganism in this study was *Lactobacillus bulgaricus* (ATCC 8001). This strain was obtained from Iranian Research Organization Science and Technology (IROST). Man-Rogosa-Sharpe (MRS) medium was used for cultivation of lactobacilli bacteria. The composition of MRS medium is as follows: yeast extract, 5 g; meat extract, 5 g; peptone, 10 g; K_2HPO_4, 2 g; diammonium citrate, 5 g; glucose, 20 g; sodium acetate, 2 g; $MgSO_4.7H_2O$, 0.58 g; $MnSO_4.4H_2O$, 0.2 g, in 1 liter medium [25]. The medium was sterilized at 121ºC for 15 minutes before inoculation.

2.2 Growth medium

Sweet cheese whey was obtained from a dairy plant (Kalleh, Mazandaran, Iran). The whey was first filtrated in order to separate the coagulated proteins. Then lactose in the presence of dilute acid was hydrolyzed to galactose and glucose (1ml HCl in 100 ml whey). After 24 h, the whey was neutralized with 1M NaOH solution. The pH of pretreated whey was adjusted to 6.5. A 0.3% w v^{-1} yeast extract was added to the whey. Deproteinated and hydrolyzed whey was prepared as suitable medium or fermentation. The initial lactic acid concentration in the cheese whey was 2.9 g l^{-1}.

2.3 Membrane

The membranes used in the experiments are summarized in Table 1 with their particular properties as reported by the manufacturer. The membranes were selected due to their availability, price and performance in similar processes [16]. They were in flat sheet form and were tested for the enrichment of produced lactic acid in continuous fermentation. During the experiments, the pressure increased to 500 KPa.

Table 1. Characteristics of the ultra-filtration, nano-filtration and reverse osmosis membranes used in the current study.

Membrane	Manufacturer	MWCO*	NaCl rejection (%)
UF	CSM	15000	-
NF270	Filmtec	400-600	70
NF90	Filmtec	150-200	80
NF1	Sepro	150-200	90
RO	Filmtec	<100	99

* Molecular weight cut-off

The rejection factor of the examined components was determined to evaluate the performance of the membrane. Rejection (R) was calculated as percentage by comparing the concentrations of the component in the permeate (C_p) and in the feed (C_f) as follows:

$$R = (1 - \frac{C_p}{C_f}) \times 100 \quad \text{Eq. 1}$$

The permeate flux was monitored by measuring the permeate volume collected at a specific time described by Darcy's equation [26]:

$$J = \frac{1}{A_m}\frac{dV_p}{dt} \quad \text{Eq. 2}$$

Where, J is the permeate flux, A_m is the effective membrane area, V_p is the total volume of permeate, and t is the permeation time.

2.4 Experimental set-up

The continuous membrane bioreactor was fabricated from a cylindrical stainless steel column with an internal diameter of 114 mm and a total height of 250 mm. A temperature sensor (PT100, JUMO-Germany) was embedded inside the reactor. This sensor was connected to a proportional-integral-derivative controller (EMKO-Turkey), and the reactor temperature was adjusted via a heating jacket (AGS-CO-Iran) wrapped around the external surface area of the reactor. The fermentation experiments were conducted without pH control. The bioreactor was sterilized with sodium hypochlorite (200 mg $kg^{-1}$of NaOCl) for 60 min and washed with hot distilled water. The feeding solution was pumped into the bioreactor using a variable speed peristaltic pump (Longer-China). The experimental set up used for the continuous production of lactic acid is illustrated in Figure 1.The temperature of bioreactor was fixed at 42°C. The agitation rate for uniform mixing was 180 rpm and the initial pH was 6.5. The system was subsequently applied for *in situ* removal of lactic acid from the fermentation broth.

In continuous culture, after 12 h of incubation, when the cell concentration reached a stable condition, the fresh medium was fed into the bioreactor with defined flow rate. The system was allowed to come to a steady state condition. Samplings of the culture were taken in 6 h intervals a steady state was achieved. Steady state condition was achieved when the cell, lactose and lactic acid concentrations remained constant. As the steady state condition was identified, a new flow rate was introduced. The dilution rate of 0.04-0.32 h^{-1} was examined based on the working volume of the bioreactor, which was calculated from the fresh feed flow rate from 65-510 ml h^{-1} [27,28].

2.5 Analytical methods

Concentration of lactic acid and lactose was detected by HPLC (Shimadzu-Japan) equipped with a Shim-pack CLC-ODS column. The column, maintained at 75°C, was eluted with 4 mM H_2SO_4 at a flow rate of 0.4 $mlmin^{-1}$for 20 min. The retention time of lactic acid under these conditions was 18 min. The samples were centrifuged at 2795 ×g for 5 min and then filtered through 0.2 µm paper filter (Whatman). To obtain desired peak height, a clear solution with the sample size of 10 µl was injected into the HPLC [29].

Growth rates were monitored by measuring the absorbance at 620nm using a spectrophotometer (UNICO2100, USA). The dry cell weight was measured through the calibration curve of dry cell weight concentration versus optical density [30].

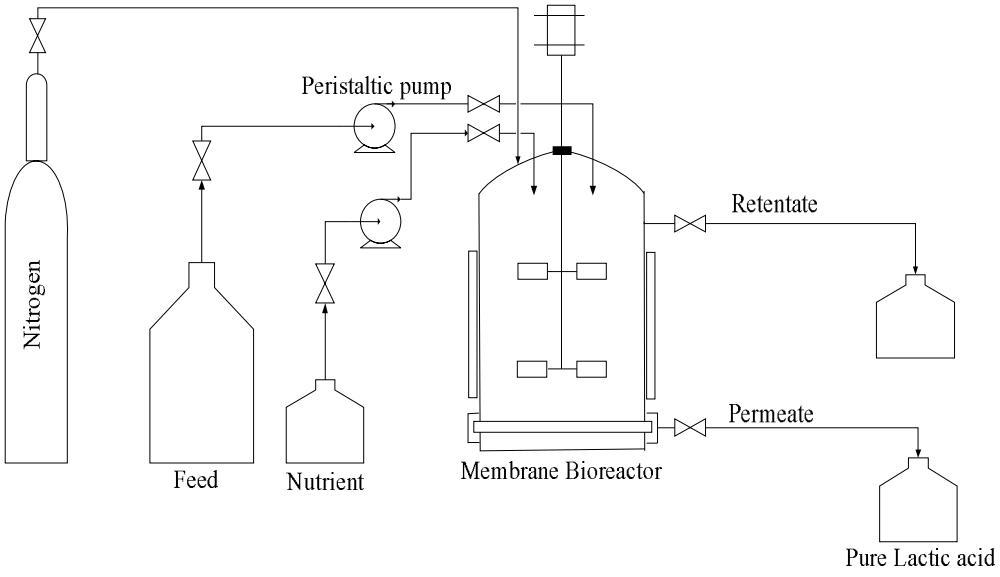

Figure 1. Schematic diagram of the laboratory-scale membrane bioreactor for lactic acid production.

3. Results and Discussion

3.1 Effect of different types of membrane

The effects of different membrane types on the lactic acid recovery from the fermented broth were investigated by taking feed and permeate samples at the end of the run. Five membranes, UF, NF70, NF90, NF1, and RO, were tested at the same operating conditions. Rejection factor and permeate flux are two significant properties of any membrane to be used for acid lactic production in membrane bioreactor. The rejection factors of lactose and lactic acid for all the membranes were determined, and the calculated values are shown in Figure 1. The results stated that the rejection factor obtained by using the NF membranes was significantly higher compared to the UF membrane. In the case of the NF membranes, NF1 showed the highest removal efficiency of lactose (about 79%). The most important membrane properties, which may affect the rejection, are permeability, MWCO, surface charge and hydrophobicity/hydrophilicity.

In general, the size exclusion is dominant mechanism for rejection of large components such as lactose. The rejection will generally increase with the molecular size. The UF membrane has the highest MWCO; therefore, it allows lactic acid to pass through the membrane. The lactose rejection of the UF membrane was about 30%. The NF membranes have lower pore size than UF membrane; hence, they allow less amount of lactose molecules to pass through the membrane, thereby increasing the lactose rejection. The lactose rejection of the NF70, NF90 and NF1 membrane was 41, 57, and 79%, respectively. Yebo Li et al. [23] reported that NF membranes with MWCO (0-400) can reject lactose in the range of 69-96%, but the lactic acid retention was also high (26-43%), which was not desirable. Simulated studies by Jeantet et al. [31] demonstrated the rejection of lactose and lactic acid as 97 and 12%, respectively. They used spiral wound module with NF membrane (MWCO = 400 Da) and pressure of 1000 KPa.

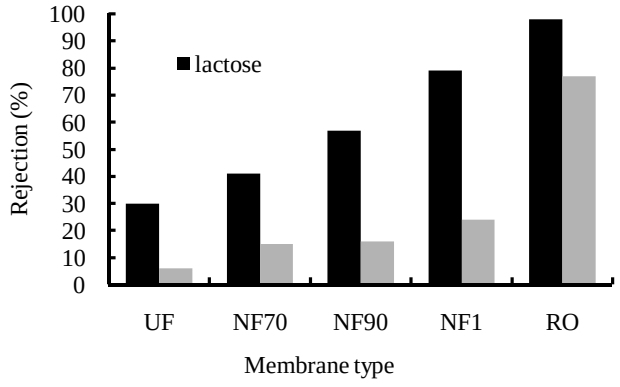

Figure 2 . Effect of membrane type on lactic acid and lactose rejection.

According to the experimental results, the lactose rejection of both the UF and NF70 membranes was low. The RO membrane performed high lactose and lactic acid retention due to lower molecular weight cut size which is not favored for lactic acid production process. In summary, in terms of the rejection factor of lactose and lactic acid, the NF1 and NF90 membranes presented a better performance than the other membranes.

The permeate flux of the all used membranes measured at room temperature and trans-membrane pressure (TMP) of 200 KPa for the UF membrane and 500 KPa for the NF membranes is represented in Figure 2. It can be observed that the permeate flux of the UF membrane is more than that of the others. Also NF70 showed a higher permeate flux compared to NF90 and NF1 at pressure of 500 KPa. There is no remarkable difference between the permeate flux of NF90 and NF1. Knowing that high lactose and low lactic acid rejection by the used membrane is essential for lactic acid production within a membrane bioreactor, based on the results obtained, the NF1 membrane was selected for lactic acid production in the membrane bioreactor.

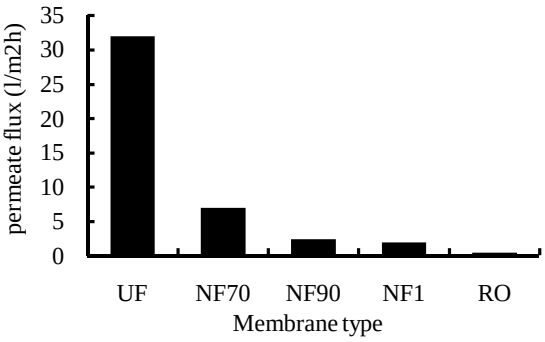

Figure 3. Effect of membrane type on permeate flux.

3.2 Continuous fermentation in membrane bioreactor

Continuous fermentation experiments were carried out in a well-designed membrane bioreactor. The performance of MBR with regards to lactic acid extraction at various dilution rates was investigated. In order to secure steady state condition, approximately 2 to 3 fermenter volumes of fresh medium were circulated through the bioreactor. The lactic acid concentration in the membrane bioreactor system was higher as compared to that in the conventional system because lactic acid extraction by the membrane causes to elimination of lactic acid inhibition. The cell concentration in MBR was higher than that in the conventional fermentation since broth was filtered by the membrane.

The effect of dilution rate on lactic acid production at various initial lactose concentrations in the conventional bioreactor and membrane bioreactor is shown in Figures 3 and 4, respectively. As it was expected, lactic acid concentration decreased with an increase in dilution rate.

At higher dilution rates or lower retention times, there was no sufficient time for cells to replicate; therefore, lactic acid production drastically decreased due to the wash out phenomenon.

In the membrane bioreactor, the maximum lactic acid concentration with the initial lactose concentration of 150 gl^{-1} and the dilution rate of 0.04 h^{-1} was 121.5 g l^{-1}. The cell concentration in the fermenter was proportional to lactic acid inhibition. Without removing the produced lactic acid from the fermenter broth, its accumulation may cause lactic acid inhibition. Thus, this fact that lactic acid concentration in the MBR was more than in the conventional fermentation implies that lactic acid inhibition has been removed.

Without extraction of lactic acid, when the concentration of lactose increased, the lactic acid production and yield decreased due to inhibition by high substrate and product concentrations.

In the conventional bioreactor, the maximum lactic acid concentration with the initial lactose concentration of 120 g l^{-1} was 43 g l^{-1}. At the high substrate concentration of 150 g l^{-1}, the concentration of lactic acid decreased.

Substrate consumption with respect of dilution rate in the conventional and membrane bioreactors is shown in Figures 6 and 7, respectively. It appears that the consumption of lactose at all initial lactose concentrations increased with an increase in dilution rate. This can be attributed to the less amount of biomass available in the system at higher dilution rates as well as insufficient time to consume the substrate. At low dilution rate, the substrate's residence time became longer and the biomass had adequate time to use lactose for its growth leading to decrease in lactose concentration. Due to the increase of cell density and the reduction of the lactic acid inhibition level, lactose consumption increased. All of these events were due to the positive response of the membrane bioreactor.

At the dilution rate of 0.04, for instance, the performance of MBR at higher initial substrate concentrations showed that lactose consumption was 94% while this value was lower in the conventional continuous fermentation.

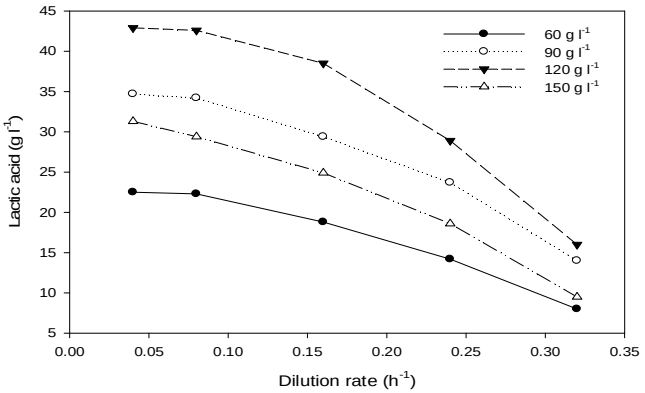

Figure 4. The effect of dilution rate on lactic acid production at various initial lactose concentrations in the conventional bioreactor.

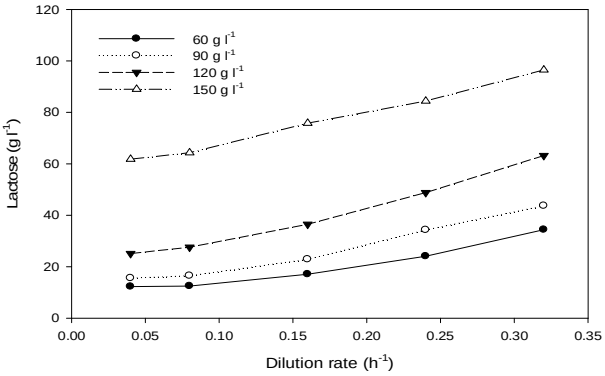

Figure 6. The effect of dilution rate on lactose utilization in the conventional bioreactor.

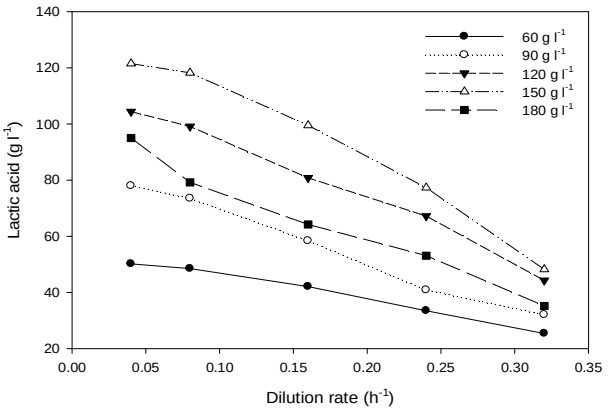

Figure 5. The effect of dilution rate on lactic acid production at various initial loctose concentrations in NF1 the membrane bioreactor.

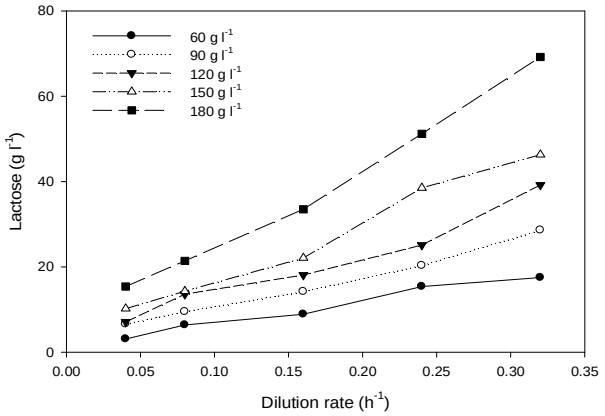

Figure 7. The effect of dilution rate on lactose utilization in the NF1 membrane bioreactor.

Figure 7 demonstrates the yield of lactic acid concentration based on substrate consumption versus dilution rate at various initial lactose concentrations in the membrane bioreactor. As shown, lactic acid yield increases with an increase of dilution rate for all initial lactose concentrations.

The maximum lactic acid yield of 96.2% was obtained with the initial lactose concentration of 90 g l^{-1}. This yield in the conventional fermentation was less than in the membrane bioreactor. The maximum yield of lactic acid production in the conventional bioreactor was 72% with the initial lactose concentration of 60 g l^{-1}, seemingly due to high lactose consumption and high cell concentration in the membrane bioreactor. The cell density tends to be zero at high dilution rates, and it seems that the fresh medium has washed out the entire cells existing in the fermentation broth.

Figure 7 also shows that the yield of lactic acid decreases with the increase of the initial lactose concentration. The yield of lactic acid significantly reduces when the lactose concentration reaches 180 g l^{-1}, and hence, the residual lactose starts to rise. It was further found that the critical lactose concentration at which the cell growth severely hampered was 150g l^{-1}.

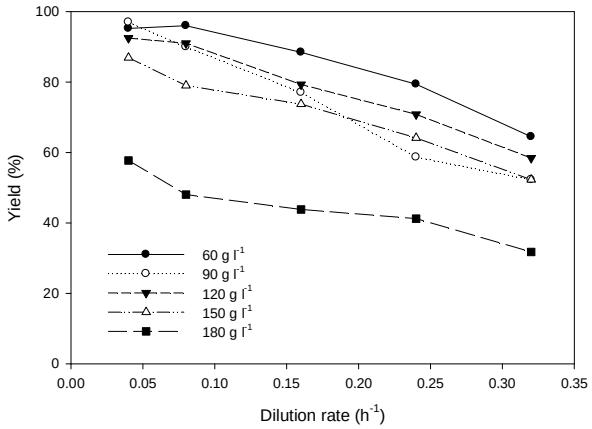

Figure 8. The effect of dilution rate on the yield of lactic acid in the NF1 membrane bioreactor.

The lactic acid productivity is the most important factor for the selection of suitable process. It is defined as the total lactic acid produced divided by the fermenter volume per unit time. When lactic acid is produced, the pH of the fermentation broth decreases and the fermentation is inhibited. Therefore, the recovery of lactic acid from the fermentation broth is very important for increasing its productivity.

Although the lactic acid concentration decreased with increasing dilution rate, lactic acid productivity actually increased up to the dilution rate of 0.24 as illustrated in Figure 8; it is because the productivity is, generally, related to the produced lactic acid concentration and the dilution rate. After that maximum point, it begins to decrease because of the cell wash-out phenomenon.

The maximum lactic acid productivity of the membrane bioreactor (17.1g $l^{-1}h^{-1}$) was obtained with the lactic acid concentration of 71.5 g l^{-1} and the dilution rate of 0.24 h^{-1}. Interestingly, maximum productivity does not necessarily occur at a dilution rate corresponding to the maximum conversion of substrate or the maximum lactic acid concentration. Therefore, optimization can be based on productivity, lactic acid concentration, residual substrate, etc. In this work, the optimum dilution rate of 0.24 was selected based on the maximum lactic acid productivity. The maximum lactic acid productivity of the conventional bioreactor (7.2 g $l^{-1}h^{-1}$) was obtained with the lactic acid concentration of 30 g l^{-1} and the dilution rate of 0.24 h^{-1}, which was much lower than that in the omembrane bioreactor under the same conditions.

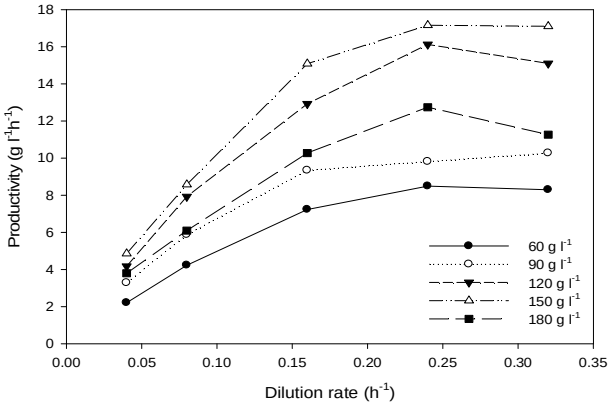

Figure 9. The effect of dilution rate on lactic acid productivity in the NF1 membrane bioreactor.

The maximum productivity obtained in this study was higher than those reported in several conventional fermentations by *Lactobacillus bulgaricus* using whey in the batch and continuous fermentations [4,32-34]. There are some published papers reporting higher productivities by using two-stage immobilized cells, retention cells or cell-recycling. For instance, Schepers et al. obtained the high LA productivity of 19-22 g $l^{-1}h^{-1}$ for immobilized *Lactobacillus helveticus* in a two-stage process [27]. Tejayadi and Cheryan reported a maximum lactic acid productivity of approximately 22 g $l^{-1}h^{-1}$ for *Lactobacillus bulgaricus* in fermentations involving cell-recycle [35].

4. Conclusion

The characteristics of continuous lactic acid production were studied in a membrane bioreactor. Different membranes and operating conditions were compared in terms of process characteristics and operational stability. The following were the main conclusions determined by this work:

NF1 well suits for continuous membrane lactic acid production. The high lactose retention of 79% and the low lactic acid retention of 22% were obtained with this membrane. The main disadvantage of this membrane was its slightly low flux due to low pressure.

The inhibiting effect of lactic acid on cell growth was not observed at high initial lactose concentrations. The critical lactose concentration at which the cell growth severely hampered was 150 g l^{-1}.

5. Acknowledgment

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6. Conflict of Interest

All the authors agree to submit the article in the Journal of Applied Food Biotechnology, and there is no conflict of interest.

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