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Membrane Fermentation of Lactic Acid

Levente L. Diosady* and Taya Puzanov

*Department of Chemical Engineering and Applied Chemistry,
University of Toronto,
200 College St., Toronto, Ontario, Canada M5S 3E5*

Abstract: In the continuous membrane fermentation of *Lactobacillus rhamnosus* at biomass concentrations above 100 g/L the viscosity of the system increased sharply resulting in decreased permeate flux. At cell densities higher than 108 g/L a slight deviation from Newtonian behaviour was observed. Steady state was achieved and main kinetic characteristics remained constant for around 100 hours when the biomass concentration was maintained at a constant level by continuous bleeding.

Keywords: biomass bleeding; membrane fermentation; *Lactobacillus rhamnosus*; viscosity; rheology.

1. Introduction

Lactic acid is traditionally used in the food, pharmaceutical, and chemical industries, and recently its potential for producing biocompatible and biodegradable plastics has been actively pursued (Goncalves et al. 1991; Jeantet et al. 1996). Approximately half of the world's supply of lactate is produced by fermentation. Although batch processes are currently used, a number of more advanced techniques have been investigated in order to improve the process efficiency. Promising results have been achieved using tangential flow filtration in continuous fermentation systems (Vick Roy et al. 1983; Mehaia & Cheryan 1985; Aeschlimann & Stockar 1991; Zhang & Cheryan 1993; Crespo et al. 1992; Ye, Jin, Shimizu 1996).

One of the main advantages of tangential flow filtration is that higher biomass concentrations are possible because cells are continuously recycled back to the fermentor. A dense population of cells not only accelerates production of lactic acid but also minimizes contamination by foreign microorganisms. Thus high cell density continuous systems could increase the efficiency of lactic acid production.

Usually, at low cell concentrations, bacterial fermentation systems display Newtonian rheology. However, when the biomass concentration increases above a critical value, specific for each bacterial strain, the rheological behaviour of cell suspensions change to non-Newtonian. Crespo and Xavier (1992) observed that for *L.plantarum* and *P.acidi-propionici* at high cell concentrations (60 g/L and 90 g/L respectively) large amounts of foam were produced and the trans-membrane pressure increased.

Lactobacillus delbrueckii is the most common strain used for lactic acid production. Although it does not form filaments or clumps, which are the cause of dramatic rheological changes in other microbial systems, it is

* Corresponding author: e-mail: diosady@chem-eng.utoronto.ca *Accepted for Publication: April 22, 2005*

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known to produce cell wall, capsular polysaccharides (Xavier et al. 1995; Wicken et al. 1983) and bacteriocins (Bhugaloo-Vial et al. 1997). Bhugaloo-Vial et al. observed low transmission of the bacteriocin, divercin, through ultrafiltration and microfiltration membranes, and suggested that this could be due to formation of high molecular weight complexes by aggregation of bacteriocin molecules or their binding to cellular components. The increased aggregation in the broth might lead to increased viscosity and consequently, to decrease of the permeate flux. As a result of flux decline, previously reported attempts to maintain a long-term steady state operation failed. The situation could be improved by removing part of the broth by bleeding. Crespo et al. (1992) showed that bleeding of biomass could reduce the viscosity in the continuous membrane fermentation system and improve permeate flux.

This study was undertaken to investigate the rheological changes in the fermentation broth of *Lactobacillus rhamnosus* during the course of membrane fermentation and the system performance at high cell densities in terms of permeate flux. The effect of continuous bleeding on the main kinetic parameters and on the long-term stability of the system was also studied.

2. Materials and methods

2.1. Microorganism

The organism used was *Lactobacillus rhamnosus* NRRL B445 (formerly *Lactobacillus delbrueckii*), a facultative anaerobe, Gram-positive, homofermentative, mainly L(+) lactic acid producer. It was obtained from ATCC (USA) in lyophilized form. The starter culture was diluted 1:1 (w/v) with 20% glycerol and stored at -20°C.

2.2. Growth medium

The culture medium had the following composition: yeast extract (Difco) - 15 g/L; K_2HPO_4 - 0.2 g/L; KH_2PO_4 - 0.2 g/L; $MgSO_4 \cdot 7H_2O$ - 0.1 g/L; $MnSO_4 \cdot H_2O$ - 0.03 g/L; Tween-80 - 0.1% (v/v). The amount of glucose was varied depending on the experiment.

The medium was sterilized at 121°C and 225 kPa for 30 min. Glucose was sterilized separately (to avoid caramelization) and combined aseptically with the rest of the nutrients after cooling to room temperature.

2.3. Experimental equipment

The fermentor (1) (Multigen, USA) consisted of a 2 L glass vessel provided with temperature control and agitation (Figure 1). The temperature in the fermentor was maintained at 42°C. The microfiltration module (2) was connected to the bioreactor with silicon tubing. An Amicon polysulfone hollow fibre cartridge (H1MP01-43) with pore size of 0.1 μm and total surface area of 0.03 m^2 was used through these experiments. Cell-free permeate was collected in the reservoir (3). Fresh medium was added at a desired dilution rate from reservoir (4). The pH was maintained at 6.2 by the addition of 5M NaOH solution with an automatic pH-stat (5) (Metrohm system, Brinkmann Instruments, Canada). Peristaltic pumps (6) and (7) were used for feed and permeate flow control, respectively. Cell bleeding was performed using another peristaltic pump (8). The total liquid volume of the system was 1L (0.9 L in the fermentor and 0.1 L in the recycling loop).

After each experiment the membrane was washed with 5% enzyme detergent (Terg-A-zyme®, Alconox, Inc.), 0.1N NaOH, and 10^{-2} molar HNO_3 solutions respectively. The fermentation vessel and tubing were sterilized in an autoclave at 121°C and 225 kPa for 30 min. The membrane was sterilized by contact with a 200-ppm solution of NaOCl followed by rinsing with 15 L of sterile water.

The preculture consisted of two successive

inoculations. First the growth medium was inoculated with 1% (v/v) of the starter culture in a 5-mL vial and was allowed to grow for 16-17 h at 42°C without agitation or pH control. For the second inoculation the fermentation broth from this vial was transferred and diluted with 19 volumes of the sterile medium and incubated for a further 24 h under the same conditions. Then inoculation into the bioreactor was made at 7% (v/v) level.

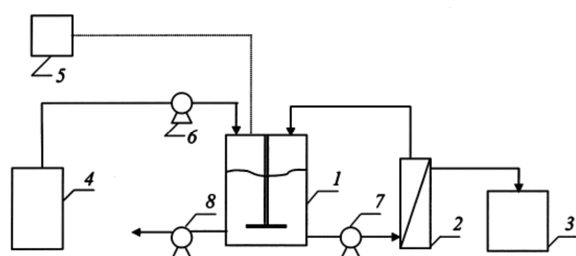


Figure1. Schematic diagram of the continuous fermentation system with cell bleeding (1-fermentor, 2-microfiltration module, 3-permeate reservoir, 4-fresh medium reservoir, 5-pH-stat, 6, 7, 8-peristaltic pumps).

2.4. Analytical methods

Dry cell weight per unit volume was determined by measuring the optical density at 610 nm using a Beckman DU-7 Spectrophotometer (Beckman Instruments Inc., USA) and calculating the results using gravimetric calibration data. Glucose concentrations were determined by the dinitrosalicylic acid method (Sumner 1925). Lactic acid concentrations were determined enzymatically using Boehringer Test Kits (Boehringer Mannheim GmbH, USA).

2.5. Rheological analysis

The viscosity of the fermentation broth was measured at 42°C using a Brookfield rotational viscometer, model LV (Brookfield Engineering Lab. Inc., USA).

3. Results and discussions

The rheological characteristics of the system during the course of fermentation were evaluated by measuring the viscosity of the fermentation broth at four shear rates (7.3 s^{-1}, 14.7 s^{-1}, 36.7 s^{-1}, and 73.4 s^{-1}). The range of biomass concentrations obtained during the experimental run varied from 11 g/L through 132 g/L. After 73 hours of the continuous fermentation, feeding was stopped and the fermentation broth was concentrated by means of microfiltration in order to accelerate the increase of cell densitiy. Glucose concentration in the feed solution was 100 g/L. The results are presented on a log-log plot in Figure 2 to fit the equation:

$$\log_{10} \eta_{app} = \log_{10} k + (n-1)\log_{10} \gamma$$

As indicated by the horizontal lines in the graph, no dramatic changes in the rheological regime could be observed within the range of biomass concentrations analyzed. The viscosity remained independent of shear rate ($n \approx 1$) in most of the samples tested. A slight deviation from the Newtonian behaviour (n=0.92) could be observed at biomass concentrations above 108 g/L.

The changes in the viscosity were more distinctive. Figure 3 illustrates the increase of the broth viscosity with the increase of the biomass concentration. It can be seen from the graph that the viscosity remained low (in the range of 1.2 – 2.8 mPa·s) up to 46 g/L of cell concentration. A sharp increase in viscosity was observed after the system reached cell densities of around 100 g/L concurrently with a shift of the rheological behaviour from Newtonian toward pseudo plastic ($n < 1$).

During the first 73 hours of continuous fermentation a biomass concentration of 70 g/L was reached. The viscosity increased very slowly during this period from 1.2 mPa·s at biomass concentration of 11 g/L to 7.9 mPa·s at 70 g/L.

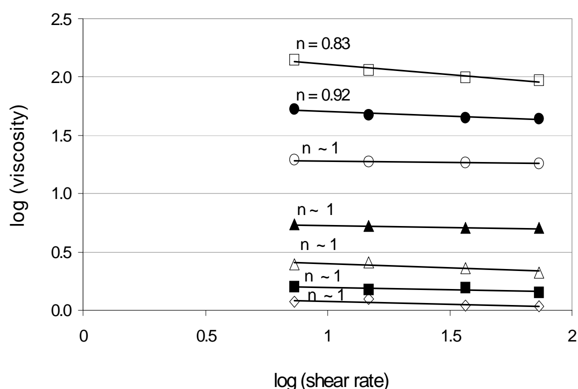


Figure 2. Rheological behaviour of the fermentation broth at different biomass concentrations (biomass concentrations: ◇ 11 g/l, ■ 23 g/l, △ 36 g/l, ▲ 65 g/l, ○ 101 g/l, ● 109 g/l, □ 132 g/l).

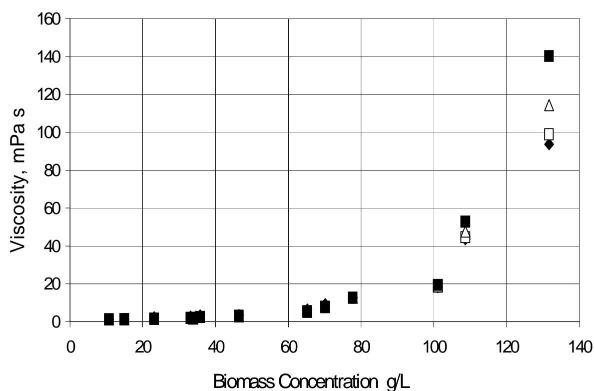
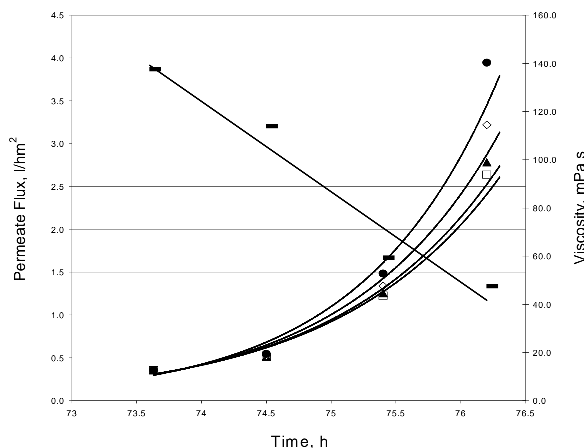


Figure 3. Viscosity vs. biomass concentration during the fermentation process with *L.rhamnosus* (the viscosity of each sample was measured at four shear rates: ◆ 73.4 s^{-1}, □ 36.7 s^{-1}, △ 14.7 s^{-1}, ■ 7.3 s^{-1}).

The permeate flux during this period was affected more by concentration polarization than by viscosity. At the 73rd hour the feeding was stopped while permeate continued to be removed by microfiltration. As a result, during the next 3 hours the biomass concentration increased to 132 g/L. The changes in viscosity and permeate flux during this 3 hour period is shown in Figure 4. The steep increase of the broth viscosity resulted in a fast drop of permeate flux to 1.3 L/(m^2·h), which indicates that in membrane fermentation the cell concentration should be kept well below this level.



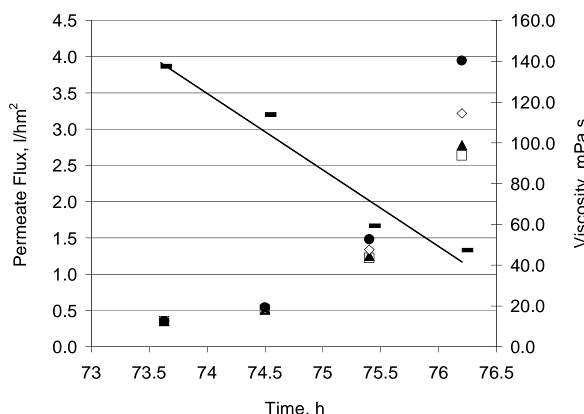


Figure 4. Permeate flux decay at high viscosities in the membrane filtration of *L.rhamnosus* experiments (▬ flux; viscosity: ● at 7.3 s^{-1}, ◇ at 14.7 s^{-1}, ▲ at 36.7 s^{-1}, □ at 73.4 s^{-1}).

There could be several reasons for the rheological changes during the course of fermentation. Although it is known from the literature (Cheryan 1986; Crespo et al. 1992) that viscosity increases with increased biomass concentration, broth age and composi-

tion could also affect the rheological characteristics of the broth. Products of cell lysis and metabolism, which do not permeate through the membrane, accumulate in the system with time. These compounds could be deposited on the membrane surface, changing the retention characteristics of the membrane and resulting in ever faster accumulation of total solids in the system. Accordingly, bleeding of some broth might be advantageous during a long-term operation, as this could reduce the accumulation of cell debris and allow optimal broth composition and constant biomass concentration.

The bleeding rate was selected to maintain a steady state in terms of biomass concentration:

$$\frac{dX}{dt} = \mu X - BX$$

At steady state $\frac{dX}{dt}$ and $B = \mu$.

It would be uneconomical to remove biomass during the exponential phase since bacterial growth rate is high and biomass densities are low during this period. The preliminary studies of the system showed that the biomass growth rate slowed down to approximate 0.01 h^{-1} after some 24 hours of operation. By that time the level of nutrients was greatly reduced resulting in very low specific growth rates. The experimental run with bleeding was performed at the best conditions determined as a result of a 3×2 full factorial experimental design (Puzanov 1999): 50 g/L of initial glucose concentration, 0.1 h^{-1} dilution rate, and 400 rpm agitation rate. Bleeding was initiated at 47th hour when the biomass concentration approached 30 g/L. The results of the run are illustrated in Figure 5.

As shown by horizontal lines in the graph steady state was achieved and maintained during the course of fermentation for some 100 hours and the biomass, glucose and lactic acid concentrations remained essentially constant. The flux stabilized at 5 $L/(m^2 \cdot h)$, which allowed a long and stable operation. The fact that the lactic acid concentration remained constant during the whole course of fermentation indicated that despite the deficiency of glucose, due to a high substrate utilization (99%), the amount of lactic acid did not decrease in favour of by-product formation (a phenomenon observed by a number of researchers in cell recycle systems (Major & Bull 1989; Puzanov 1999)). This suggests that opening the system by continuous bleeding could also improve the product purity – definitely, an economic advantage considering the high purification costs.

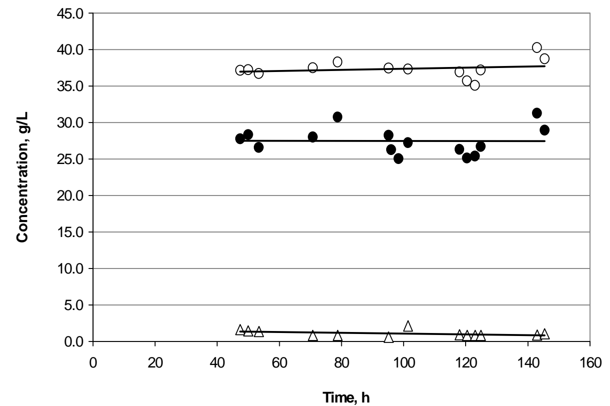


Figure 5. Effect of continuous bleeding on the fermentation kinetics of *L.rhamnosus* (● biomass, △ glucose, ○ lactic acid).

4. Conclusions

Rheological studies of the fermentation broth of *Lactobacillus rhamnosus* showed that the fermentation system remained in the same rheological regime over a wide range of cell concentrations. The viscosity of the fermentation broth remained low at biomass concentration as high as 70 g/L. Thus high cell density systems could be developed using membrane bioreactors in order to increase product concentration and productivity.

Steady state was achieved in the membrane system by continuous bleeding of cells. The main process characteristics, such as product concentration, substrate conversion and per-

meate flux, remained constant during the period of fermentation when excess biomass was removed by bleeding at a constant rate. Thus continuous cell bleeding can be used to maintain the fermentor at steady state for extended periods, and may be the basis of efficient commercial fermentation systems.

5. Symbols and units

B bleeding rate (h^{-1})
k consistency index ($Pa \cdot s^n$)
n dimensionless flow behaviour index
t time (h)
X biomass concentration (g/L)
γ shear rate (s^{-1})
η_{app} apparent viscosity of the fluid (Pa·s)
μ specific growth rate (h^{-1})

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