0273-2289/90/2425—0613$02.60

Some Engineering Parameters for Propionic Acid Fermentation Coupled with Ultrafiltration

J. P. S. G. CRESPO, M. J. MOURA, AND M. J. T. CARRONDO*

Biochemical Engineering Laboratory, Department of Chemistry, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2825 Monte da Caparica, Portugal

ABSTRACT

The effect of circulation rate on permeate flux, the energy requirements for heating or cooling, the reactor homogeneity, and cell activity are discussed for a continuous culture system with cell recycle. The fermentation system was a continuous stirred-tank reactor with an ultrafiltration membrane unit for cell recycle. The membranes have a tubular configuration and are composed of a carbon support coated with zirconium oxide. The permeate flux obtained for a long-run fermentation with *Propionibacterium acidi-propionici* was higher when the circulation rate was increased: 5.13 L/m^2-h for a circulation rate of 0.810 m^3/h and 7.09 L/m^2-h for 1.104 m^3/h. The temperature rise inside the fermenter for different circulation rates was studied, allowing determination of the need of input or output of energy for temperature control. Residence time distribution studies showed that, with a circulation rate of 0.606 m^3/h, the dead volume was 9.2%, whereas at 1.104 m^3/h the reactor behavior was almost ideal. The influence of the circulation rate on loss of cell activity is also discussed, and rheological studies are suggested as an indirect indicator of cell viability.

Index Entries: Ultrafiltration coupled fermentation; Cell recycle bioreactor; Propionic acid production; Bioreactor hydrodynamics.

*Author to whom all correspondence and reprint requests should be addressed.

NOMENCLATURE

t	Time, h
A	Acetic acid concentration, g/L
P	Propionic acid concentration, g/L
G	Glucose concentration, g/L
X	Cell concentration, g/L
S	Substrate concentration, g/L
J	Permeate flux, $L/m^2 \cdot h$
u	Linear velocity, m/s
Δp_m	Average transmembranar pressure, bar
F	Feed rate, L/h
Q_r	Circulation rate, m^3/h
τ	Shear stress, Pa
j	Shear rate, s^{-1}

INTRODUCTION

Propionibacteria are used to produce Vitamin B_{12}, flavors, and starter cultures for the dairy industry, and have been suggested as potential producers of propionic acid from renewable resources. However, this fermentation is a slow process because of the low growth rate of these bacteria and strong product inhibition.

In continuous culture with cell recycle by ultrafiltration, the broth is pumped continuously through the ultrafilter, the microorganisms are recycled to the fermenter,and end products permeate out. Higher productivities are achieved because of the larger cell concentrations thus obtained and, if inhibitory products also permeate out, because of lower inhibitory effects (*1,2*). In particular, we have shown such an effect for propionic acid fermentation—*see* Table 1 and Fig. 1 (*3*).

When a cell suspension is pumped tangentially to a membrane, macrosolutes in the suspension rapidly form a thin layer immediately adjacent to the filter surface. This thin layer is called a concentration polarization layer, and it limits the permeate flow through the membrane. In fact, this layer becomes a secondary membrane itself.

For very low solute concentrations, the permeate flux is proportional to the transmembrane pressure. With increasing concentration, the flux gradually slows and can become independent of pressure at extreme conditions. Hydrodynamics strongly influence the system performance. High flow rates parallel to the membrane decrease the solute concentration at the membrane, lowering the effect of the concentration polarization layer on the permeate flux.

The purpose of this work was to study the influence of the circulation rate on permeate flux, heating/cooling energy requirements, reactor homogeneity, and cell activity for a continuous culture system with cell recycle.

Table 1
Performance Comparison in Continuous Fermentation Systems

		CSTR	CSTR, pH 6.0	ICR[a]	UFR[b]
Maximum volumetric productivity, g prod/L-h	Propionic	0.34	0.42	0.95	2.20
	Total	0.49	0.57	1.03	2.70
Corresponding acid concentration, g/L	Propionic	3.9	7.3	9.5	18.0
	Acetic	1.7	2.7	1.2	4.0
Yield for total acids, %, w/w		66	61	80	59
Percentage of theoretical maximum yield		86	79	104	77
P/A, mol ratio		1.9	2.2	6.5	3.5

[a]ICR = Immobilized cell reactor.
[b]UFR = Ultrafiltration reactor.

MATERIAL AND METHODS

The organism used was *Propionibacterium acidi-propionici* (ATCC 25562). The medium composition was: 0.87 g/L K_2HPO_4, 3.4 g/L KH_2PO_4, 7 g/L yeast extract, 7 g/L bactopeptone, 0.1 g/L $MgSO_4 \cdot 7H_2O$, 0.01 g/L $M_nSO_4H_2O$, 0.02 g/L $CoCl_2$, 1 mL/L Tween 80, 60 g/L glucose. The fermentation temperature was 37°C, and pH was controlled at 6.0 by the addition of 5*N* NH_4OH.

Cell concentration was estimated by dry weight measurement. Glucose concentration was measured by a glucose oxidase method using a YSI 27 from Yellow Springs Instrument Co. The rheology of fermentation broth was studied at 37°C using a concentric cylinder viscometer Rheomat 15T-FC (Contraves) for shear rates (j) ranging from 28.4 to 1350 s^{-1}. Samples of 20 mL volume were taken for the viscosity measurements.

The fermentation system used was a single continuous stirred-tank reactor with an ultrafiltration membrane unit for cell recycle. A 2-L glass fermenter (S.G.I. Setric) was used, and the total volume, including piping, was 4.275 L (fermentation volume, 3 L; permeate volume, 1.275 L). The tubular membranes (diameter, 0.6 cm; length, 0.64 m), manufactured by SFEC, are composed of carbon coated inside with zirconium oxide and have a total filtration surface of 0.1 m^2. The fermentation broth was circulated by a 3-lobe rotor volumetric pump equipped with a variable gear box.

RESULTS AND DISCUSSION

Hydraulic tests were first performed to determine the system response at different circulation rates, ranging between 0.606 m^3/h (pump at 50%)

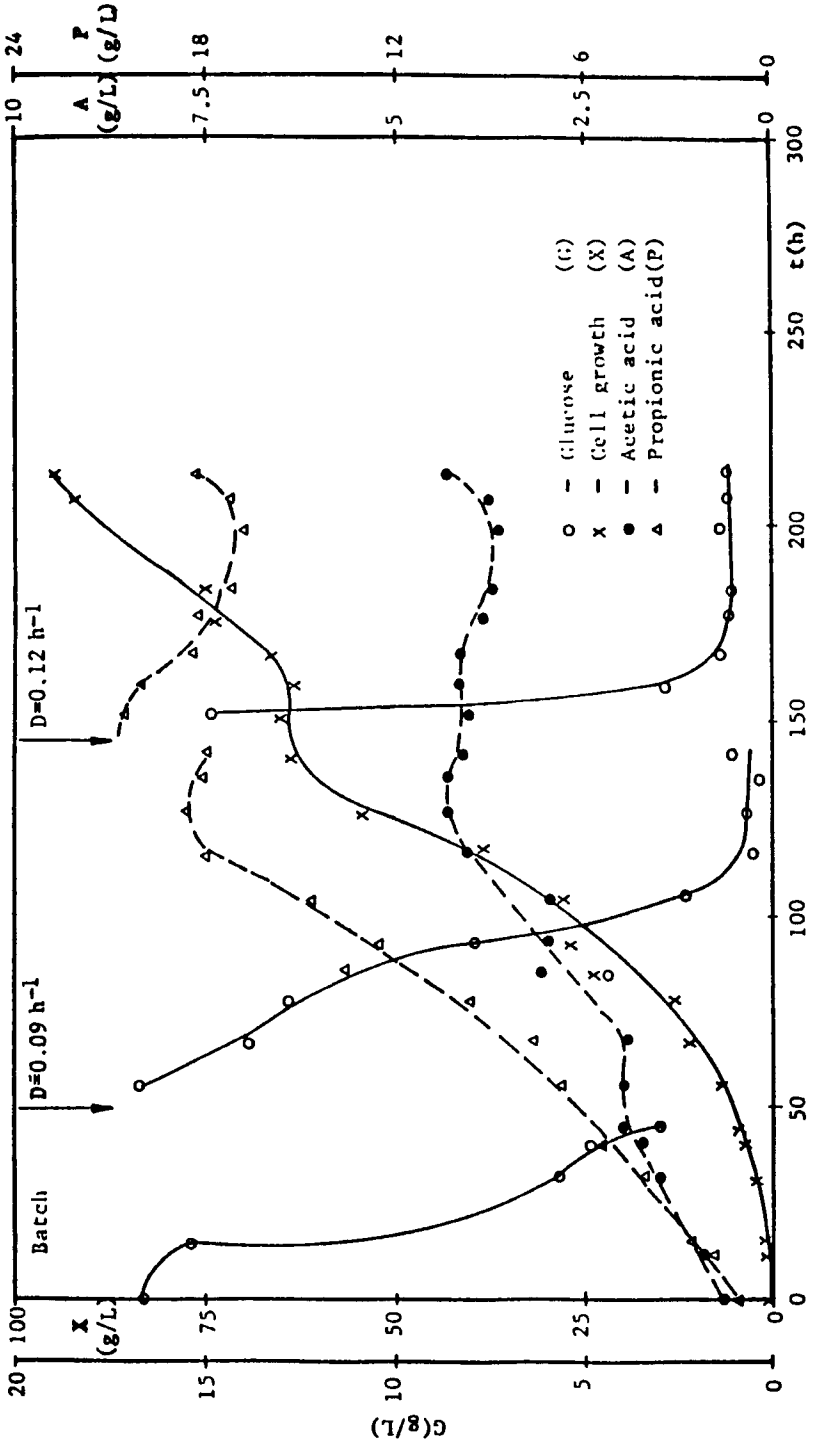

Fig. 1. Glucose consumption, cell growth, propionic, and acetic acids production in UF system.

and 1.290 m^3/h (pump at 100%). Figure 2 shows the variation of the permeate flux (J), the linear velocity inside the tubular membranes (u), and the average transmembranar pressure (Δp_m).

Permeate Flux

Figures 3 and 4 shows the permeate flux variation during fermentation with *Propionibacterium acidi-propionici,* using two different circulation rates. The experimental values suggest an empirical model of the type

$$J = A\, e^{-k_t t} + B \tag{1}$$

Parameters A, k_t, and B were determined for both cases using a nonlinear regression analysis. The results are summarized in Table 2. The functions obtained are drawn in Figs. 3 and 4 (continuous line).

It can be seen that the permeate flux obtained for a long-run fermentation is higher when the circulation rate is higher: 7.09 L/m^2-h for pump at 90% vs 5.13 L/m^2-h for pump at 70%. As the membrane filtration surface is 0.1 m^2 and the fermentation volume is 3.00 L, we can conclude that this system can only perform *P. acidi-propionici* fermentations at maximum dilution rates of 0.236 h^{-1} for pump position at 90% and 0.171 h^{-1} for pump position at 70%. These results confirm that the tubular-type modules, although allowing good flow conditions, are not the most desirable if high dilution rates are needed, since they have a low specific filtration surface.

Energy Input/Output

In an ultrafiltration coupled anaerobic fermentation system, the major energy inputs are for pumping and heating/cooling for fermenter temperature control. Figure 5 shows the temperature rise inside the fermenter for different circulation rates. This study permits the calculation of input or output of energy (and respective costs) for a selected circulation rate and a required fermentation temperature. For instance, for the previously selected circulation rate of 0.97 m^3/h and a fermentation temperature of 37°C, there are no requirements for energy for temperature control.

The ΔT values were obtained after stabilization, when the heat loss to the ambient equals the heat generated by fluid shear and mechanical dissipation at the pump head. A heat balance can be written as

$$m\, C_p dT = q\, dt - C_T (T_{uf} - T_{amb})\, dt \tag{2}$$

where q is the heat generated by shear and C_T is the relationshp between the heat loss and the corresponding driving force. For boundary conditions

$$t = t_0 \qquad T_{uf} = T_{amb} \qquad q = m\, C_p\, (dT/dt)\, t = 0 \tag{3}$$

$$t = t_f \qquad dT = 0 \qquad C_T = q / (T_{uf} - T_{amb})_f \tag{4}$$

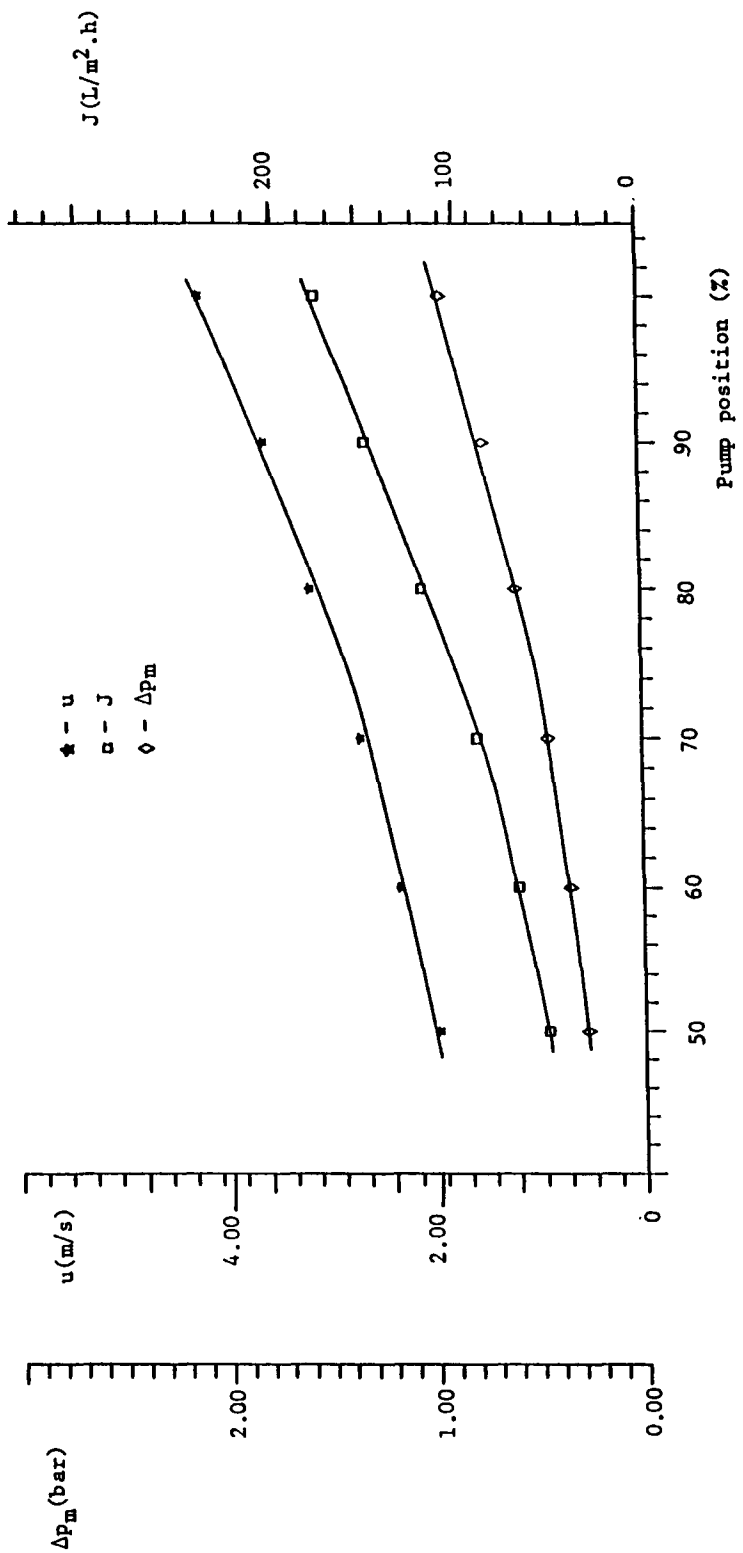

Fig. 2. Variation of permeate flux (J), linear velocity (u), and average transmembranar pressure (Δp_m) with different pump regimes.

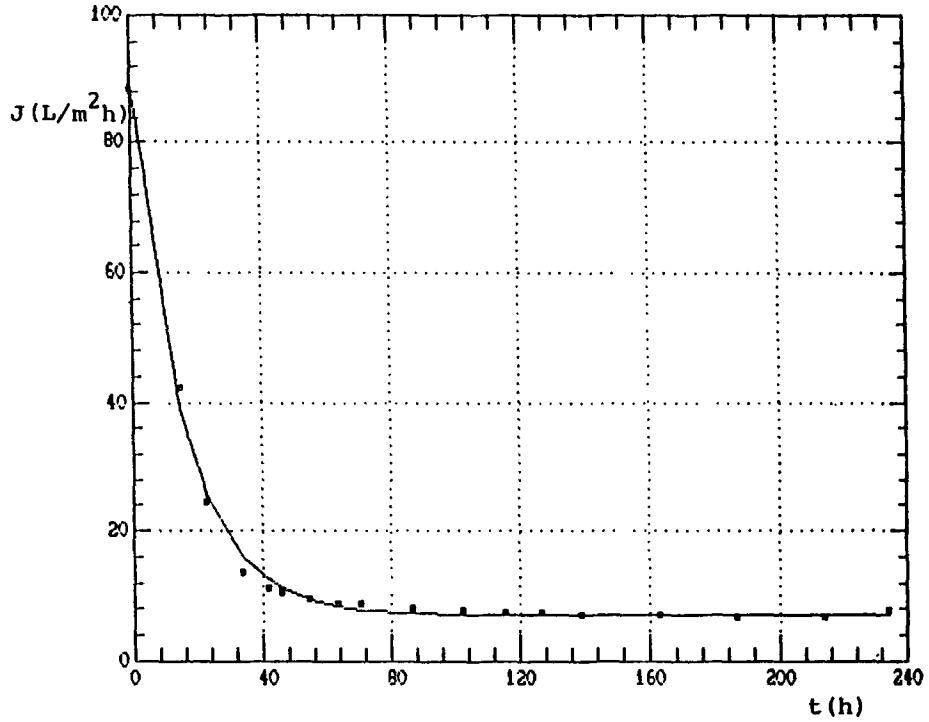

Fig. 3. Permeate flux during fermentation with *P. acidi-propionici*. $D = 0.1$ h^{-1}; Pump—90%; $Q_r = 1.104$ m^3/h.

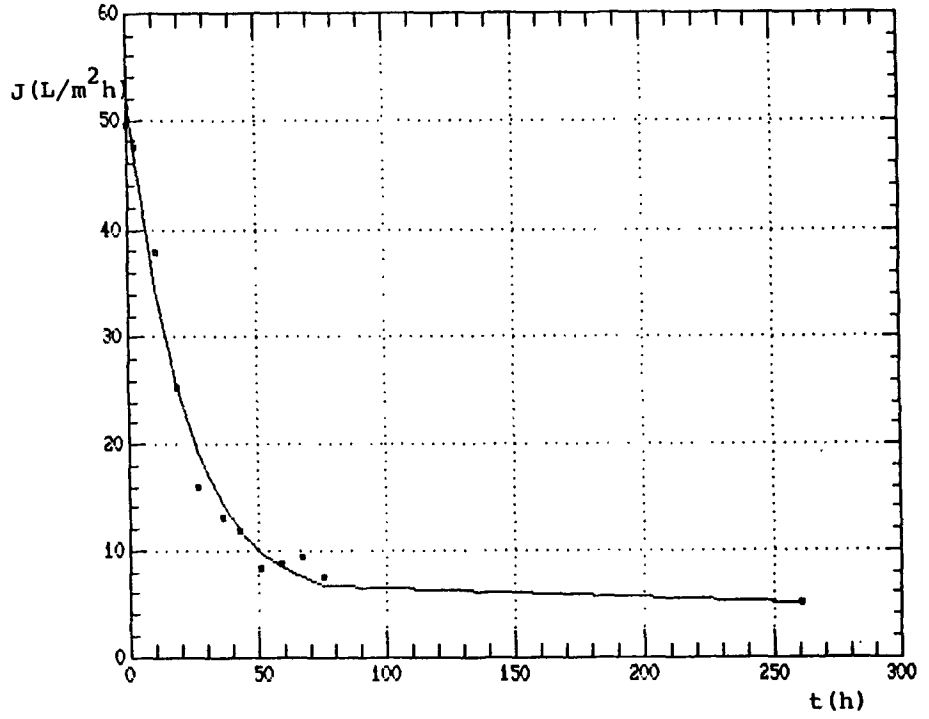

Fig. 4. Permeate flux during fermentation with *P. acidi-propionici*. $D = 0.1$ h^{-1}; Pump—70%; $Q_r = 0.810$ m^3/h.

Table 2
Estimated Flux Model Parameters for Two Different Circulation Rates

Circulation pump	A	k_t	B
90%	81.83±1.26	0.065±0.002	7.09±0.37
70%	46.89±1.94	0.045±0.005	5.13±1.39

Table 3
Temperature Rise and Estimated Model Paramerer for Different Circulation Rates

Pump, %	50	60	70	80	90
Q_r, m^3/h	0.606	0.714	0.810	0.972	1.104
q, J/s	27.04	42.35	62.40	87.47	111.15
C_T, $J/°C\text{-}s$	4.46	5.13	5.67	6.20	5.91
ΔT_f, °C	6.2	8.25	11.0	14.1	18.8

These parameters have been determined for each circulation rate (Table 3). Figure 6 represents the experimental temperature rise and the predicted curves using the estimated parameter for different circulation rates.

Residence Time Distribution Studies

The effect of the circulation rate on reactor ideality has also been studied. The reactor was filled with a 100 g/L glucose solution and fed with water at a known rate. Samples were taken periodically.

A reactor mass balance can be written as

$$-(dS/dt) = DS \tag{5}$$

which, upon integration, yields

$$t = 0 \qquad S = S_0 \qquad \ln S = \ln S_0 - Dt \tag{6}$$

Figures 7 and 8 show ln$S$ vs time. The results are summarized in Table 4. These results show that, with a low circulation rate of 0.606 m^3/h, a reasonable volume fraction (9.2%) is dead reactor volume. By increasing the circulation rate to 1.104 m^3/h, almost ideal reactor behavior is achieved in the whole system.

Cell Activity

In order to reduce the concentration polarization effect, high linear velocities have to be applied to the membrane. Therefore, high shear rates will occur, especially near the membrane and pipe walls. Cell deactivation has been reported during ultrafiltration (4) and pumping.

It has also been found that the total recycle of microorganisms can contribute to the accumulation of intracellular compounds from cell lysis.

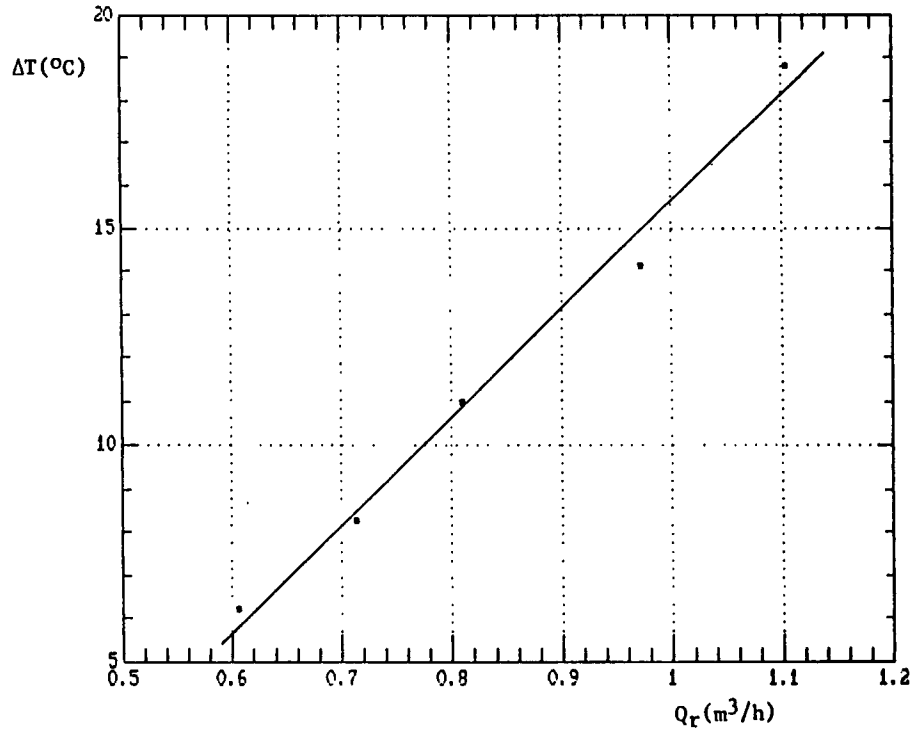

Fig. 5. Temperature variation inside the fermenter for different circulation rates. Ambient temperature—$T_{amb} = 22.5\,°C$.

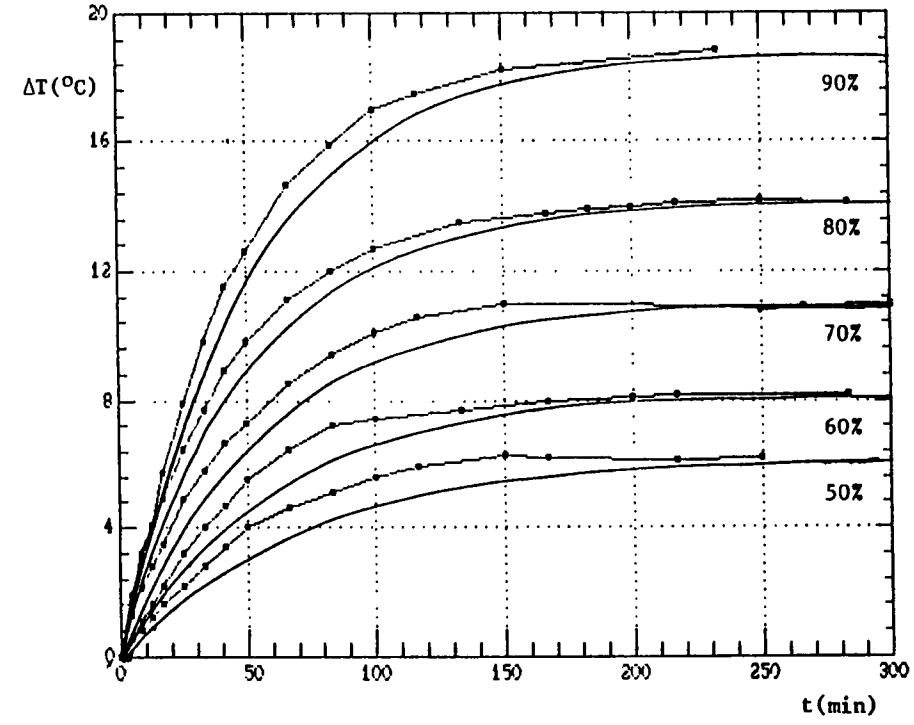

Fig. 6. Experimental temperature rise and predicted curves for different circulation rates. Ambient temperature—$T_{amb} = 22.5\,°C$.

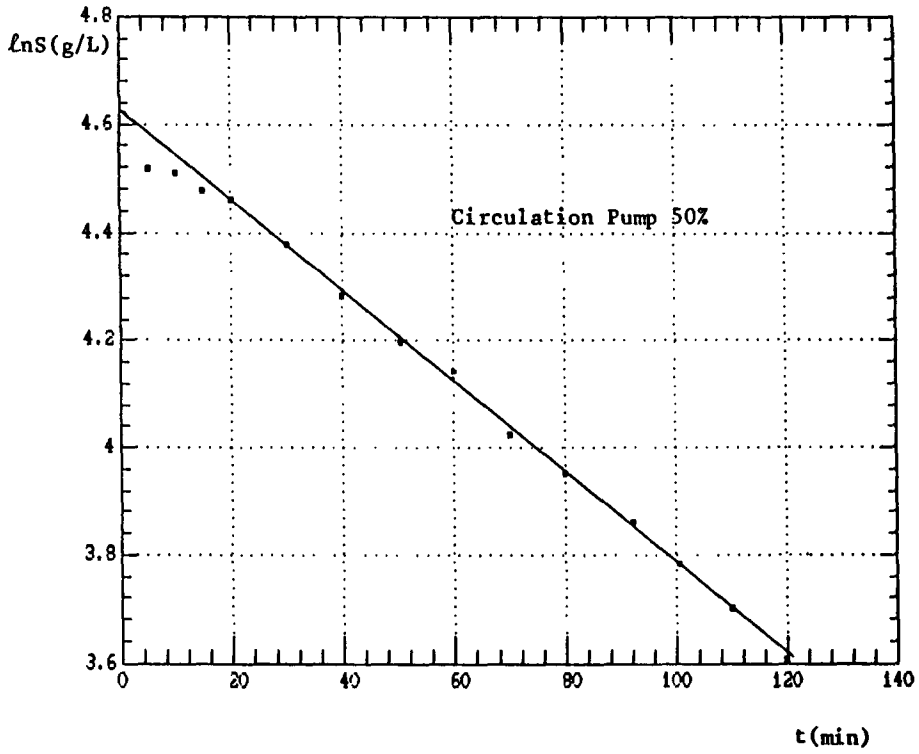

Fig. 7. Residence time distribution study at $Qr = 0.606$ m^3/h.

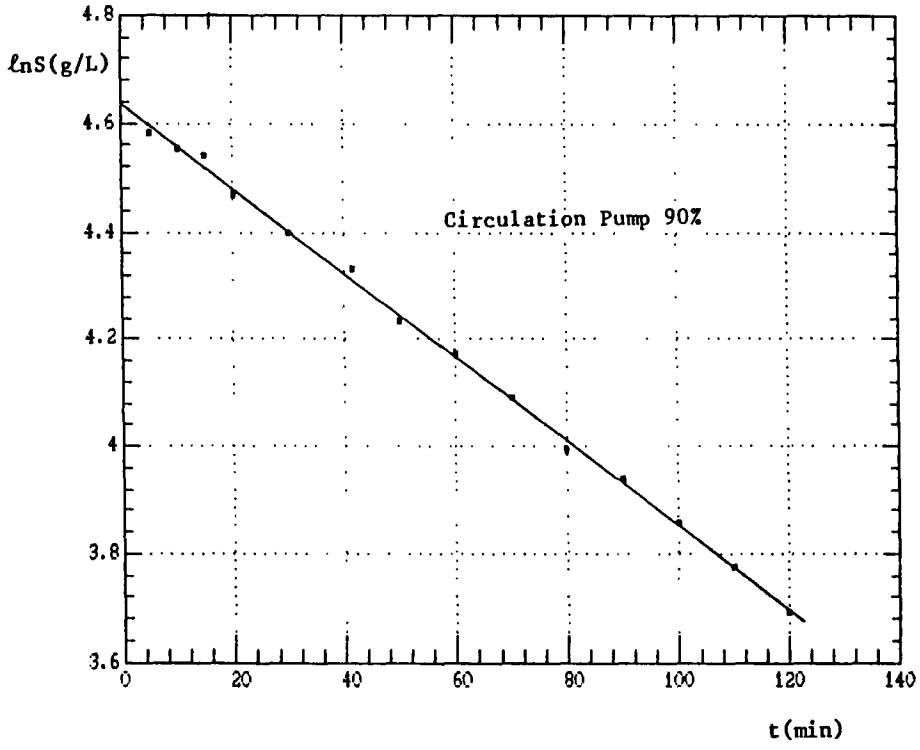

Fig. 8. Residence time distribution study at $Qr = 1.104$ m^3/h.

Table 4
Results of Residence Time Distribution Studies at Two Different Circulation Rates

	S_0, g/L	D, h^{-1}	V, L	% V_{dead}
Theoretical	100	0.4604	4.275	-
Pump 50%	102.4	0.5068	3.883	9.2
Pump 90%	103.1	0.4685	4.201	1.7

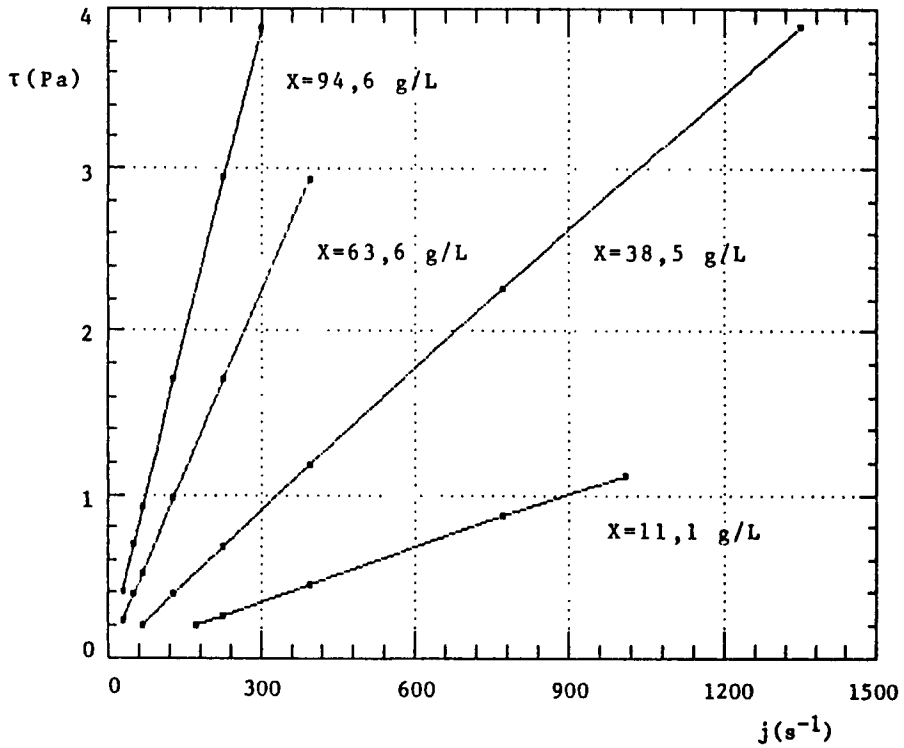

Fig. 9. Shear stress vs shear rate for different cell concentrations.

Malinowski et al. (*5*) reported, in a high cell concentration continuous culture of *S. cerevisiae,* a loss of cell viability during fermentation, observing simultaneously a progressive change on the rheological behavior of the culture (Newtonian to pseudoplastic). That effect was attributed to cell lysis products accumulating in the broth. The results obtained show that a Newtonian behavior occurred throughout the whole fermentation, even at high cell concentrations (Fig. 9).

The rheological analysis is only an indirect indicator of cell viability. We are in the process of correlating these two parameters with the objective of optimizing cell purge from the system. It is hypothesized that the pumping rate required for a given steady-state condition will be a function of cell growth in the autocatalytic regime and cell death mainly resulting

from mechanical shear. This will constrain circulation rate differently depending on the biology used in the fermentation, but for reasonably resilient cells, it should be possible to use these two process parameters—circulation and microorganism purge rate—to optimize the fermentation.

CONCLUSIONS

Most of the literature studies on continuous culture systems with cell recycle assume the dilution rate (D) and the cell bleed rate (B) as the main operational parameters. Less importance has been given to the circulation rate or the broth linear velocity inside the ultrafiltration modules. Usually this velocity is maintained between 3 and 5 m/s, but its selection has not previously been reported. This work reports the effect of the circulation rate and linear velocity on membrane permeability, reactor homogeneity, energy requirements, and cell viability. The following conclusions can be drawn:

1. Circulation rate has a strong influence on membrane permeability. For lower circulation rates, membrane permeability stabilized at lower values, thus affecting the possibility of working at extreme dilution rates.
2. The effect of circulation rate on reactor homogeneity is less marked; still, dropping the recirculation rate from 1104 m^3/h to 810 m^3/h changes the reactor behavior from ideal to almost 10% dead volume—as much as is often encountered in impeller-agitated fermenters.
3. Since pumping and turbulence on the membrane warm the liquid broth, an increase in circulation rate might change the fermentation system from energy-dependent for heating to energy-dependent for cooling.
4. For a given cell biology, an increase in circulation rate increases cell damage and lysis. This can be indirectly observed through the rheological behavior of the fermentation broth. Changes in cell viability are now being assessed by a kinetic method.

ACKNOWLEDGMENTS

We acknowledge the financial support of Junta Nacional de Investigação Cientitífica e Tecnológica, Lisboa (JNICT) under contract No. 720.85.74; US Program in Science and Technology Cooperation, Washington, DC, Grant No. 936-5542-G-SS-4003-00; as well as the sponsorship of Fundação Calouste Gulbenkian, of IPE, SA, and Portucel, E. P. M. J. Moura acknowledges a research grant awarded by JNICT.

We hereby express our recognition for the ongoing collaborations with James Gaddy (University of Arkansas) and Gerard Goma (INSA, Toulouse, France).

Originally presented at the Tenth Symposium on Biotechnology for Fuels and Chemicals, May 16–20, 1988, Gatlinburg, TN.

REFERENCES

1. Blanc, P. and Goma, G. (1987), *Bioprocess Eng.* **2,** 137–139.
2. Mota, M., Lafforgue, C., Strehaiano, P., and Goma, G. (1987), *Bioprocess Eng.* **2,** 65–68.
3. Carrondo, M. J. T., Crespo, J. P. S. G., and Moura, M. J. (1988), *App. Biochem. and Biotechnol,* **17,** 295–312.
4. Mota, M. J. (1985), Docteur Ingenieur thesis, INSA, Toulouse.
5. Malinowski, J. J., Lafforgue, C., and Goma, G. (1987), *J. Ferment. Technol.* **65**(3), 319–323.