MODELLING A CELL RECYCLE FERMENTER USING *ZYMOMONAS MOBILIS* : STEADY STATE ANALYSIS

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Abstract

A mathematical model of a cell recycle bioreactor utilising Zymomonas mobilis was developed using an unstructed model. The equations of the model system were solved using Newton's technique where the matrix should be non-singular or has an inverse. Experimental evidence from literature survey proved that the steady state analysis can predict the behaviour of the fermentation processes.

Introduction

A great attention has been given to research for the production of alternative fuel from renewable resources such as ethanol. The present work is focused on the effect of partial cell recycle towards the production of ethanol.

The term recycle used in this paper describes a continuous and automatic process whereby microbial cells are recovered from effluent and recycled back into the fermenter. Pirt and Kurowski (1970) described an experimental study and outlined the theory for the production of yeast cells in a chemostat. Other studies, such as by Cysewki and Wilke (1977), have demonstrated the use of recycle for the production of the metabolic by-product of ethanol from yeast. A brief mathematical description of the partial cell recycle process and some of its implications will be presented, followed by experimental evidence from literature survey to support the theory.

The rate of product generation in fermentation can be related to the rate of formation, the concentration and physiological history of the biomass. The biomass provides the enzymes and the physiological conditions that control the conversion of substrates into product. Any change of operating conditions in a fermenter that improves the rate, concentration, or history of the biomass (cells) will generally result in improved product formation, although the relationship is complex.

The productivity of a simple continuous fermenter is limited by ethanol inhibition and low cell concentration. The reason for this low cell density is due to the dilution rate (D) must be less than the maximum specific growth rate (Uo). This problem can be solved by utilising cell recycle. When much of the microbial biomass is returned to the fermenter an extremely high cell concentration may be maintained. To retain cell viability, a fraction of biomass is removed on a continuous basis. For this type of system the density of the cell is as high as 83 g/L can be maintained in the fermenter (Del Rosario *et al.*, (1979)).

The organism used in this study is *Zymomonas mobilis* which use Etner-Doudorof pathway to produce ethanol from glucose. The advantage of using this organism compared to yeast is higher specific ethanol production rate and higher glucose or substrate uptake rate which is around two to three times that of yeast.

The steady state performance of a partial cell recycle is studied in this work by doing a simulation study on the theoretical modelling on the effect of cell recycle ratio.

Model Development

The fermenter could be modelled as an ideal continuous stirred tank reactor plus recycle. As shown in Figure 1, a material balance can be written for the system for any component as:

$$\text{Accumulation} = \text{Flow in} - \text{Flow out} + \text{Generation} \tag{1}$$

If we apply the above balance to Figure 1, the cell (X), substrate (S) and product formation (P) will be:

$$V\frac{dX}{dt} = FoXo - BX + Vrx \tag{2}$$

$$V\frac{dS}{dt} = FoSo - FS + Vrs \tag{3}$$

$$V\frac{dP}{dt} = FoPo - FP + Vrp \tag{4}$$

Further modification on the equations by corporating product, biomass and substrate inhibition the mass balance of X, P and S become:

$$\frac{dX}{dt} = UX - D(1 - R)X \tag{5}$$

where:
$$U = Umax \frac{S}{Ks + S}(1-(P/Pm)^2)(1-X/Xmax)^2\,(Ki/(Ki+(S-Si)) \tag{6}$$

$$\frac{dP}{dt} = rp - DRP \tag{7}$$

where:
$$rp = qpmax \frac{S}{Ks'+S}(1-(P/Pm')^2)\,(Ki'/(Ki'+(S-Si'))\,X \tag{8}$$

$$\frac{dS}{dt} = rs + D(So - RS) \tag{9}$$

where:
$$rs = -\frac{1}{Yp/s}\,rp \tag{10}$$

Equations (5) and (7) represent a system of simultaneous differential equations which could solved for the variables X, and P using R, D and So as operating parameters and proper biological constants. The third variable, S, is then linearly dependent on P. The values of the biological constants are listed in Table 1.

Table 1. The constant values used in Equation [5] to [10]

SYMBOLS	DESCRIPTIONS	VALUE	REFERENCE
Umax	Maximum specific growth rate	$0.5\ h^{-1}$	(a)
Ks	Saturation constant for U	$0.27\ gl^{-1}$	(a)
Pm	Maximum ethanol concentration for cell growth.	$46\ gl^{-1}$	(b)
Xmax	Maximum cell growth	$80\ gl^{-1}$	(b)
q_{pmax}	Maximum specific ethanol production rate	$5\ gg^{-1}h^{-1}$	(a)
Pm'	Maximum ethanol concentration for ethanol production.	$127\ gl^{-1}$	(a)
Ks'	Saturation constant for q_p	$0.5\ h^{-1}$	(a)
Yp/s	Ethanol yield	0.48	(a)
Ki	Substrate inhibition constant for growth.	$220\ gl^{-1}$	(a)
Ki'	Substrate inhibition constant for ethanol production.	$500\ gl^{-1}$	(a)
Si	Threshold substrate concentration for cell growth.	$100\ gl^{-1}$	(c)
Si'	Threshold substrate concentration for ethanol production.	$100\ gl^{-1}$	(c)

Note:

(a) Lee and Rogers (1983)
(b) Kamarul 'Asri (1989)
(c) Huang and Chen (1988)

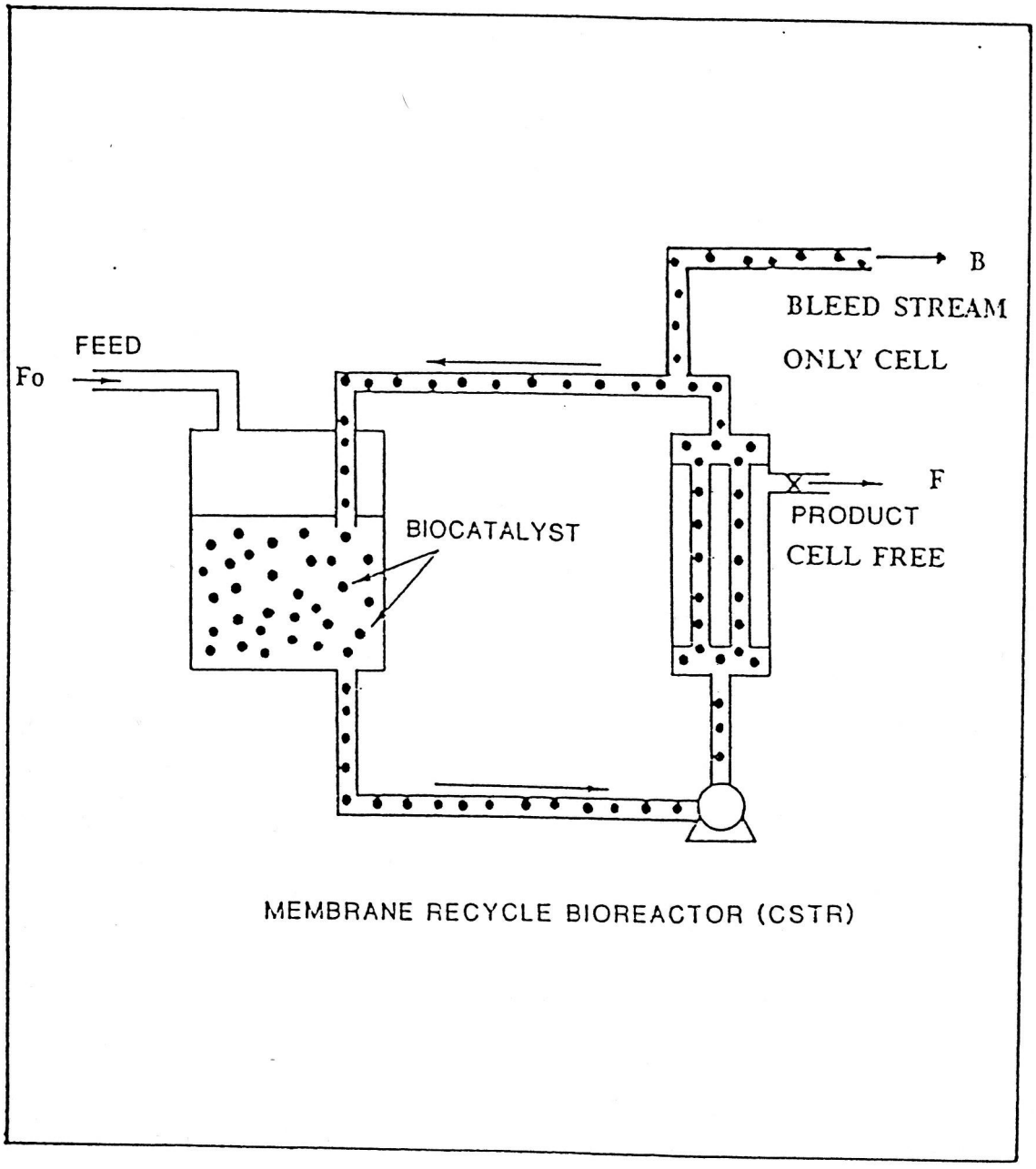

Fig. 1. Schematic diagram of a membrane cell recycle bioreactor.

Results and Discussions

To characterise the steady state of the system illustrated in Figure 1, S, P and DRP (r_p) were calculated. The parameters used in the calculation were D, (1 - R) and So.

In the simulation, So and D(1 - R) were held constant. D was varied from 0.1 to 3 $hour^{-1}$, (1 - R) therefore varied from 0.01 to 0.1. Figure 2 shows the experimental work done by Rogers *et al.* (1982) for a steady state operation of a continuous cell recycle system utilising *Zymomonas mobilis*. The dotted line for qp indicates its maximum value in earlier continuous culture without cell recycle while the dotted line for cell concentration shows the minimum concentration required for complete conversion of glucose to ethanol based on the value of qp (it was assumed that no loss of cell viability occurred at high cell concentration). If one looks at Figures (3) to (5), there is a close resemblance of the modelling job to the experimental work done by Rogers *et al.* (1982). The modelling value of glucose concentration is a bit higher compared to the experimental results. This goes for the specific ethanol productivity (r_p), which in the range of 4.8 to 5 g/g/h compared to around 2 to 4 g/g/h from the experimental result. Since there is a close agreement between the result from the model and experimental values, it is justified to extend the simulation beyond the mentioned dilution rate in order to find the optimum condition for producing ethanol.

Conclusions

This article has attempted to model the fundamental aspects of a bioreactor. The generalised mass balances provide a starting point for any studies in this area. While the application of the concepts has been illustrated for cell recycle bioreactor system, the fundamental equations should always be considered when modelling a new system.

References

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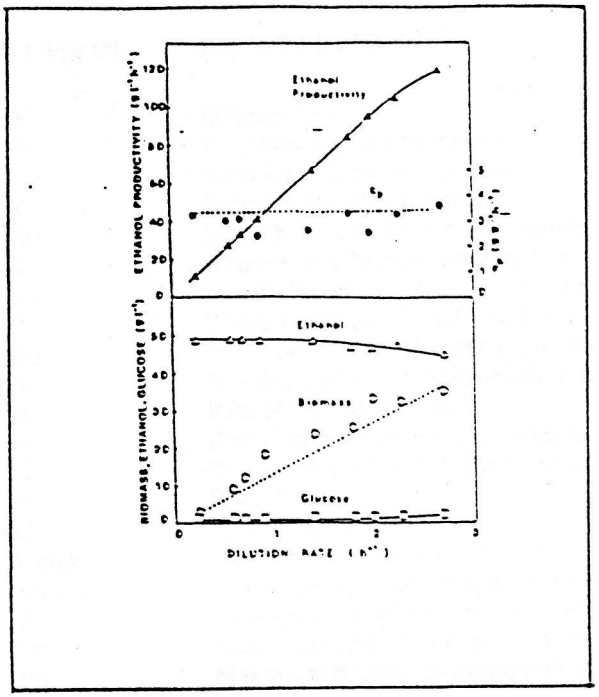

Figure 2: Effect of dilution rate on continuous cell recycle system with *Zymomonas mobilis* using 100 g/L glucose medium. (Experimental work done by Rogers *et al.* (1982))

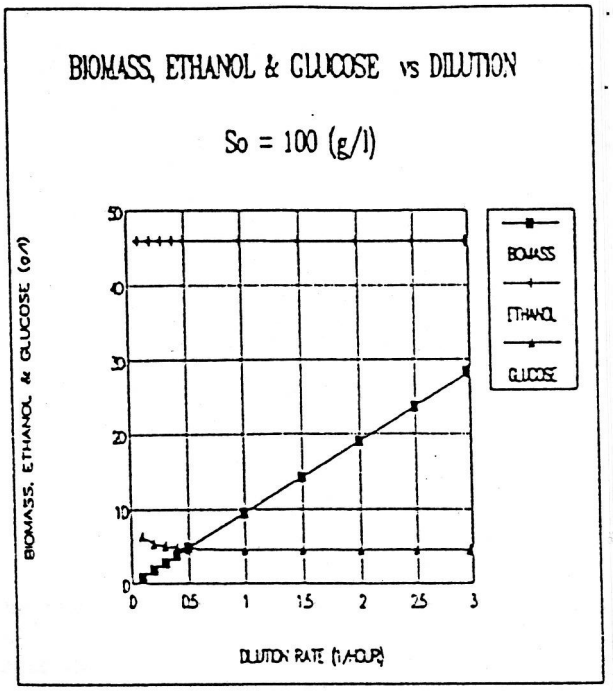

Figure 3: Graph of biomass (X), ethanol (P) and glucose (S) concentration versus dilution rate.

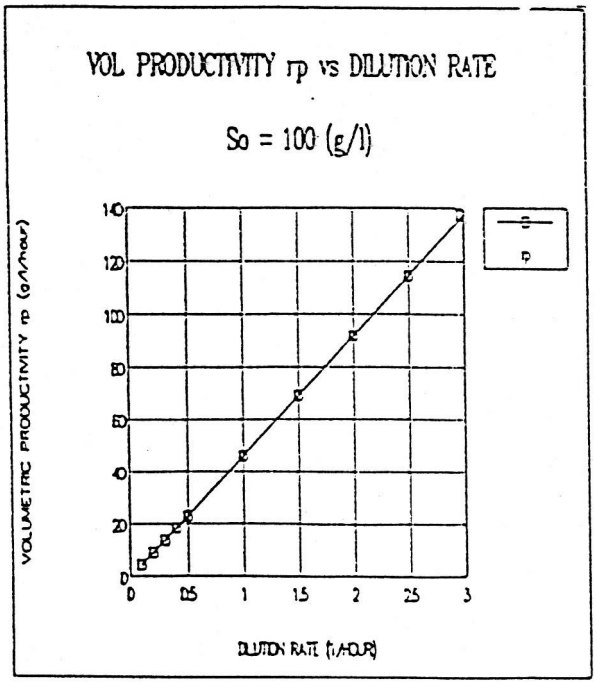

Figure 4: Graph of volumetric productivity (rp) versus dilution rate.

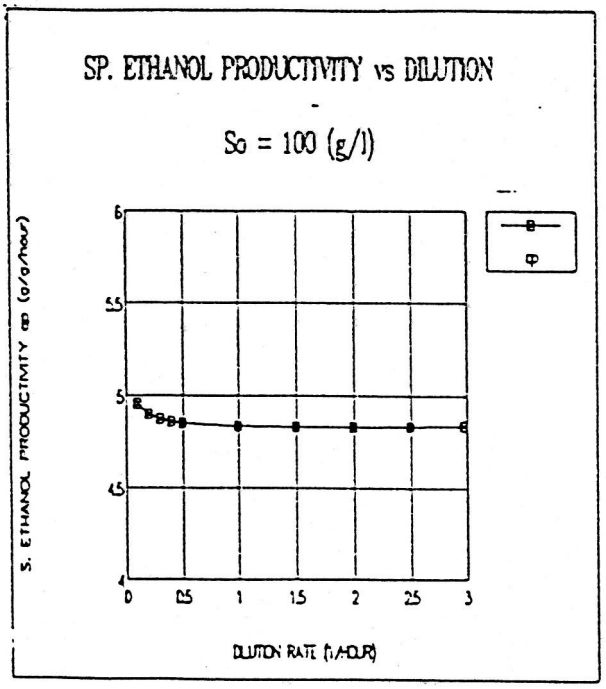

Figure 5: Graph of specific ethanol productivity (qp) versus dilution rate.

NOMENCLATURE

SYMBOL	DESCRIPTIONS
D	Dilution rate
Fo	Feed flowrate into fermenter
V	Working volume of fermenter
X	Biomass or cell concentration
Xo	Initial biomass or cell concentration
S	Glucose or substrate concentration
So	Initial glucose or substrate concentration
P	Product or ethanol concentration
Po	Initial product or ethanol concentration
B	Bleed stream of biomass or cell concentration
rx	Rate of biomass produced
rp	Rate of ethanol or product produced
rs	Rate of glucose or substrate utilisation
R	Recycle ratio
U	Specific growth rate
Umax	Maximum specific growth rate
Ks	Saturation constant for specific growth rate
Ks'	Saturation constant for specific ethanol production rate
Pm	Maximum ethanol concentration for cell growth
Pm'	Maximum ethanol concentration for ethanol production
Xmax	Maximum cell growth
qpmax	Maximum specific ethanol production rate
Yp/s	Ethanol yield
Ki	Substrate inhibition constant for growth
Ki'	Substrate inhibition constant for ethanol production
Si	Threshold substrate concentration for cell growth
Si'	Threshold substrate concentration for ethanol production