Kor. J. Appl. Microbiol. Bioeng.
Vol. 11, No. 1, 33-38 (1983)

Optimization of Semi-Batch Process for Ethanol Production

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(Received December 7, 1982)

에타놀 생산을 위한 Semi-batch 발효 공정의 최적화

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(1982년 12월 7일 수리)

As flocculent strains are likely to have considerable potential for internal cell recycle, kinetic studies on glucose medium with flocculent *Saccharomyces uvarum* were carried out in batch and continuous culture. Using a mathematical model, the kinetic parameters at each temperature and pH were estimated in order to establish optimal conditions. It was found that an overall optimum temperature for growth and ethanol production in the range 33-35°C was desirable. With regard to the effect of pH, ethanol production by *S. vuarum* was found to be relatively insensitive to pH value between 4 and 6, with an optimum pH of around 5. At these optimal conditions a maximum ethanol productivity of 12 g/l/h was determined using semi-batch process together with *S. uvarum*.

With the increasing cost and scarcity of fossil fuels in recent years considerable research has been devoted to developing alternative energy sources. One area of particular interest has been the production of ethanol from biomass .Ethanol can be used as a motor fuel or combined with gasoline to make the fuel known as "gasohol". The mixture of 10% absolute ethanol and 90% gasoline has become standard gasohol in parts of the United States, although ethanol can be added to gasoline up to a proportion of 20% without major engine modifications being required.[1,2]

Some countries have developed extensive projects for the massive production of ethanol from biomass as a partial substitute for gasoline. The classical example is Brazil, where vast extension of sugar cane plantations represents an invaluable source of renewable energy.[1] Other countries such as Australia, Thailand, South Africa, Papua New Guinea and the Philippines have embarked on feasibility studies and R&D programmes.[3]

Since ethanol is relatively costly to produce and raw material costs account for 70-80% of production costs,[4,5] obtaining a high ethanol yield is important. Also important, however, is achieving a high ethanol concentration because the steam costs for distillation continue to fall as the ethanol content in the distillation stream rises up to about 10% (v/v).[6] Furthermore process improvements and high productivities have a substantial effect on the required fixed capital investment and will thus appreciably change the profitability of ethanol production via fermentation. These goals are to some extent incompatible and the attainment of an optimal process is dependent on the choice of a suitable microbial strain, on the establishment of an appropriate fermentation environment and on the selection of cost-effective technology of ethanol fermentation.

In the present study, a strain of the yeast *Saccharomyces uvarum*[7-9] isolated by Rose[10] was chosen for its registance to relatively high ethanol levels (100-120 g/l), its high sugar tolerance (up to 300 g/l) and flocculent characteristics. The present research is concerned with the kinetic properties of the flocculent strain and the development of a relatively high productivity process which exploits this characteristic.

Materials and Methods

Organism and materials

The strain of *S. uvarum* used in this study was ATCC 26602. The media composition and inoculum preparation

have been reported in an earlier publication[9] unless otherwise stated. All chemicals used were Reagent Grade.

A 1 *l* fermentor was used for kinetic studies such as batch and continuous culture.[9] For semi-batch fermentation, however, a fermentor with 2 *l* working volume incorporating pH and temperature control was used and agitation was provided with a flat-bladed turbine impeller (100 rpm)

Semi-batch fermentation

Semi-batch fermentation was carried out as described by Humphrey.[11] In this mode of operation the flocculent strain *S. uvarum* was grown as a batch culture and allowed to settle. A fraction of fermented broth (empting ratio of 0.7) was drawn off from an overflow device and the fermentor refilled with fresh medium and the operation repeated. After repeated fermentation in this manner for several cycles, it was found that a biomass concentration of 21 g/l was maintained for the given empting ratio.

The distinctive feature of the semi-batch process lies in internal cell recycle and therefore a flocculent strain is necessary for successful operation The empting ratio mentioned above is defined as follows:

$$R = \frac{V_f - V_i}{V_f} \quad (1)$$

where V_f is the total volume and V_i is the volume after removal of the fermented broth for each cycle. In this mode of operation it is necessary to calculate an ethanol productivity over the entire processing time, which includes not only the fermentation time t_f but also the time required to settle the flocculent strain t_s, to empty the fermentor t_e, and to charge fresh medium t_c. Therefore, the overall ethanol productivity (objective function) is described by:

$$J = \frac{R \cdot \int_0^{t_f} \frac{dp}{dt} \cdot dt}{t_c + t_f + t_s + t_e} \quad (2)$$

On the experimental results it was found that 15 minutes were required for servicing (each of 5 minutes for charging, settling and empting)

Analytical procedure

Dry weight of biomass were determined for 10 ml samples, washed with 10 ml of 0.85% NaCl solution and once with distilled water, and dried at 105°C for 20 h. The total residual glucose was estimated on the supernatant after centrifugation (4000 rpm, 10 min) using the dinitrosalicylic acid method.[12] For ethanol estimation, samples were analyzed using a Technicon Autoanalyzer and a procedure developed by Sawyer and Dixon.[13]

Results and Discussion

Development of mathematical model

It is well known that the accumulation of ethanol in a fermentation can inhibit the growth rate and ethanol production rate of yeast.[14-18] In batch culture therefore there is a progressive decrease in the specific growth rate and eventually the ethanol causes cessation of growth and subsequently ethanol production. In order to quantity some of the ethanol inhibition effects with *S. uvarum*, a mathematical model was developed for the ethanol fermentation:[9]

$$\frac{dX}{dt} = \mu_m\left(\frac{S}{S+K_s}\right)\left(1 - \frac{P}{P_m}\right)\cdot X, \frac{dX}{dt} = 0 \text{ for } P \geqq P_m \quad (3)$$

$$\frac{dS}{dt} = -\frac{1}{Y_{P/S}}\frac{dP}{dt} \quad (4)$$

$$\frac{dP}{dt} = \nu_m\left(\frac{S}{S+K_s'}\right)\left(1 - \frac{P}{P_m'}\right)\cdot X, \frac{dP}{dt} = \left|0 \text{ for } P \geqq P_m'\right. \quad (5)$$

On the basis of experimental results over a range of initial glucose concentrations from 150-300 g/l it was found that the substrate inhibition effects of glucose on growth rate and ethanol production for *S. uvarum* could be neglected For the simultaneous integration of the differential equations describing the model, a Nonlinear Simulation Package[19] which incorporates a fourth order Runge-Kutta method was used with a CYBER 72 digital computer together with a fixed step size of 0.125 h.

As illustrated by Fig. 1, which shows the computer curve and the experimental data at 33°C and pH 5.0 for example, good agreement was found between the model and the experimental results. For the kinetic analysis a value of $K_s = 0.5$ was assumed.[15,18,20] The accurate determination of this constant was not attempted in the present study. On the other hand, sensitivity analysis on K_s' gave a relatively high value of $K_s' = 15$. One possible reason of the relatively high value lies in the flocculent nature of *S. uvarum* an d the increased diffusional resistance associated with flocs of yeast. It was observed that flocculation was heaviest during the latter stages of fermentation when growth had nearly ceased. As implied in equation[5] this increased diffusional resistance towards the end of batch growth is likely to have a more pronounced effect on ethanol production than on growth rate. As suggested by Powe,,[21] the K_s' is really an apparent K_s viz.:

$$K_s'^{APP} = K_s' + K_d \quad (6)$$

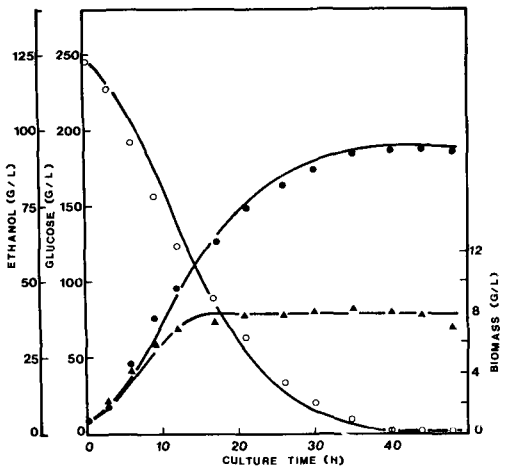

Fig. 1. Comparison of experimental data with mathematical model at 33°C and pH 5.0. Kinetic parameters are: μ_m=0.26, ν_m=1.33, P_m=60, P_m'=113, K_s=0.5, K_s'=15, $Y_{P/S}$=0.38.

where K_d is a function of size of floc and diffusivity. Although the exact mechanism of late fermentation flocculation is still not clear, it has been postulated that divalent ions, such as Ca^{++} Mg^{++} and Mn^{++}, act as bridges between negatively charged carboxyl groups on wall surfaces.[22-24] Fig. 2 shows a scanning electron (Cambridge S4 STEREOSCAN) micrograph of a *S. uvarum* floc taken from a fermentor.

Effect of temperature

In view of the interest in high productivity ethanol fermentations at increased temperatures, the effect of temperature on the kinetics of ethanol production by *S. uvarum* was investigated in the range 30-43°C. Using the mathematical model described above, the kinetic parametes at each temperature were estimated. In Table 1, the values of the kinetic parameters determined at each temperature are given. From the Table it is clear that the optimum temperature for growth is close to 33°C, while

Table 1. Estimation of kinetic parameters at pH 5.0.

Kinetic parameters	Temperature, °C				
	30	33	37	40	43
μm	0.23	0.26	0.23	0.21	0.16
νm	1.15	1.33	1.44	1.44	1.43
P_m	60	60	60	48	23
P_m'	113	113	113	90	68

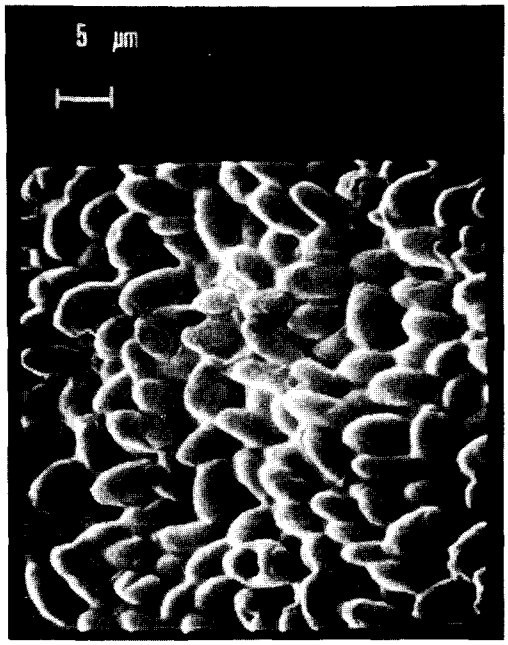

Fig. 2. Scanning electron micrograph of a *S. uvarum* floc.

the ethanol production rate reached a maximum in the range 37-43°C. It has been observed with yeast that the optimum temperature for fermentation is different to that for growth. The growth rate for a strain of *S. cerevisiae* was maximal at 30°C but fermentation proceeded most rapidly at 40°C.[25] The optimum temperature for specific rate of ethanol fermentation by *Saccharomyces* strains has been shown to be from 5 to 10°C higher than the optimum for growth.[3,26]

The values of of P_m and P_m' were constant up to 37°C and then decreased significantly, indicating a greater susceptibility to ethanol inhibition in *S. uvarum* at higher temperatures. From the relative decrease of P_m compared to P_m' it is evident that increasing concentrations of ethanol exert a greater degree of inhibition on growth rate than on the rate of ethanol production. As shown in Table 1, the maximum ethanol concentration at the end of batch culture was significantly affected by temperature. A similar effect of temperature on final ethanol concentration was also reported with yeast,[27,28] and it was found that ethanol inhibition increased at higher temperatures.

Effect of pH

The effect of pH on the kinetics of ethanol production by *S. uvarum* was determined by the same method as that used in the previous analysis of the temperature effect. The

experimental data on 250 g/l glucose medium at different pH values was analyzed with the mathematical model and the results are summarized in Table 2.

Table 2. Estimation of kinetic parameters at 35°C.

Kinetic parameters	pH 3.0	4.0	5.0	6.0
μm	0.12	0.20	0.26	0.20
νm	1.15	1.33	1.33	1.33
P_m	45	52	60	60
P_m'	90	113	113	113

As shown in Table 2, ethanol production by *S. uvarum* was found to be relatively insensitive to pH values between 4 and 6, only the growth rate was slightly affected. However, both growth rate and ethanol production rate were significantly affected at pH 3.0, compared with the kinetics at an optimum pH of around 5.0. Similar results have been reported by a number of workers. The rate of fermentation by yeast showed a rather broad optimum from pH 4.0 to 6.6. However, the yields of glycerol and acetic acid have been found to increase with increase of pH.[29] Aiyar and Luedeking[30] reported that where yeast growth was concerned an optimum value was obtained at pH 6.0, while for ethanol production the optimum rate was attained at pH 5.0 with *S. cerevisiae*. It is interesting to note that the absolute limits of pH for growth of most strains of *S. cerevisiae* have been reported to be 2.4 and 8.6 with an optimum growth at 4.5. The internal pH of *S. cerevisiae* has been found to be independent of external pH values ranging from pH 3 to 7, being controlled at a value between pH 5.8 to 6.3.[3]

Continuous culture kinetics

From the model developed from the batch culture kinetic data, predictions can be made for the continuous culture of *S. uvarum* growing under product limitation. The theoretical curves were derived from equations (3)-(5), and at steady-state were as follows:

$$\mu_m\left(\frac{S}{S+K_s}\right)\left(1-\frac{P}{P_m}\right)\cdot X-D\cdot X=0 \qquad (7)$$

$$D\cdot(S_0-S)-\frac{1}{Y_{P/S}}\cdot\nu_m\left(\frac{S}{S+K_s'}\right)\left(1-\frac{P}{P_m'}\right)\cdot X=0 \qquad (8)$$

$$\nu_m\left(\frac{S}{S+K_s'}\right)\left(1-\frac{P}{P_m'}\right)\cdot X-D\cdot P=0 \qquad (9)$$

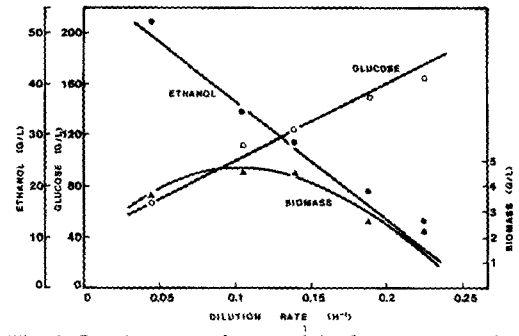

Fig. 3. Continuous culture with *S. uvarum* using 20% glucose feed at 33°C and pH 5.0.

The curves shown in Fig. 3 were calculated from the above equations, and it can be seen that the model is in good agreement with experimental results.

From the experimental results it is clear that a maximum ethanol productivity of 3.8 g/l/h was achieved at a dilution rate of D = 0.13 h^{-1}. The productivity using *S. uvarum* compares well the maximum productivity of 4.1 g/l/h reported by Ghose and Tyagi[18] using *S. cerevisiae*.

Optimization of semi-batch process

In order to determine a maximum ethanol productivity, the objective function J was calculated as a function of feed glucose concentrations by solving equations (3)-(5) together with equation (2) on the CYBER 72 computer with the nonlinear Simulation Package referred to previously. Fig. 4 shows the relationship between the objective function and the feed glucose concentration. As can be seen from Fig. 4, the maximum value of the objective function was determined to be 12 g/l/h for 15% glucose feed. As feed glucose concentration was increased from 15% the objective function decreased because more ethanol was produced at high glucose concentrations and ethanol inhibition increased.

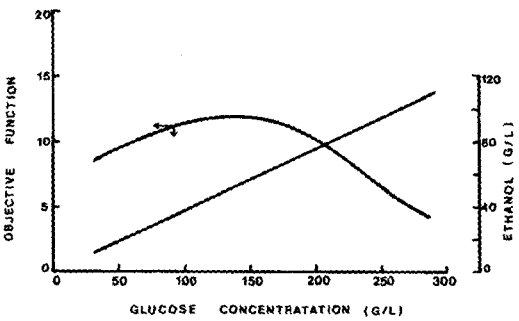

Fig. 4. Profiles of objective function and ethanol concentration against feed glucose concentration for semi-batch fermentation with *S. uvarum*.

At low glucose concentrations ethanol inhibition was decreased. However the servicing time controlled the objective function. Also shown in Fig. 4 is the final ethanol concentration as a function of feed glucose.

Although a maximum ethanol productivity could be achieved with 15% feed glucose, a semi-batch fermentation using 21% feed glucose was investigated as shown in Fig. 5 with the view to obtaining a high ethanol productivity together with a high ethanol concentration. As can be seen from Fig. 5, a final ethanol concentration of 80 g/l was

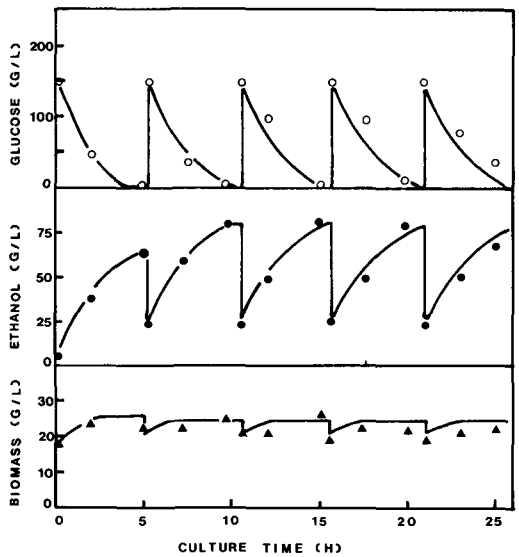

Fig. 5. Results of semi-batch fermentation with *S. uvarum* using 21% glucose feed at 33°C and pH 5.0.

maintained at the end of each cycle giving an overall ethanol productivity of 10 g/l/h. As the number of cycles was increased, the discrepancy between experimental data and the model was observed presumably due to deactivation of *S. uvarum*. Similar results have been reported for theMelle-Boinot process at high sugar concentrations due to prolonged exposure to high concentrations of ethanol.[31] However the semi-batch process together with yeast or *Zymomonas* using lower feed sugar concentrations resulted in more stable and reliable fermentations.[31,32] As reproducible cycles are essential for the semi-batch process, there is a limit in terms of feed glucose concentration. It appears that the semi-batch process using 15% feed glucose results in a higher ethanol productivity together with a high ethanol concentration. To gain some perspective on the improvement achieved with the semi-batch process, a comparison is provided in Table 3.

During semi-batch operation new cells form and a fraction of these cells are removed intermittently during the empting cycles. Some cell regeneration gives stability to the system and thus a strategy of computer sequence control could be readily adopted. Bearing in mind that the semi-batch process is similar to a conventional continuous fermentation system except that the semi-batch process is always operated under unsteady-state conditions then it is clear from Table 3 that the semi-batch process provides a means of achieving a high ethanol productivity together with a high ethanol concentration.

Table 3. Comparison of continuous culture and semi-batch process.

Systems	Strains	Ethanol Productivity (g/l)	(g/l/h)	References
Continuous	*S. uvarum*	29	3.8	This work
	S. cerevisiae	41	7.0	33
	S. cerevisiae	32	4.1	18
Semi-batch	*S. uvarum*	57	12*	This work
	Z. mobilis	73	50	32

Note: * Determined by computer simulation.

요 약

에타놀을 생산하는 Flocculent 균주는 균체순환을 위한 외부적인 장치 없이도 발효조 내부 자체에서 고농도의 균체를 유지시킬 수 있는 장점이 있다. 본 연구에서는 Flocculation 특성을 가지고 있는 *Saccharomyces uvarum*을 사용하여 Glucose 배지에서 회분식 및 연속식 발효특성을 고찰하였고 에타놀 발효의 수학적 모델을 만들었다. 이를 이용하여 여러 온도 및 pH에따른 발효특성을 비교 검토한 결과 균체성장과 에탄올 생산의 최적온도는 33~35℃ 이었고 pH에 대한 영향은 그리 크지 않았으나 pH5가 최적조건이었다.

이러한 환경적 최적 조건하에서 Semi-batch 발효에 의한 에타놀 최고 생산성은 12g/l/h이었다.

Nomenclature

D ; dilution rate ..... l/h

J ; objecive function ..... g/l/h

K_d ; function of cell flocs ..... g/l

K_s ; substrate limitation constant for growth ..... g/l

K_s' ; substrate limitation constant for ethanol production ..... g/l
P ; ethanol concentration ..... g/l
P_m ; maximum ethanol concentration above which cells do not grow ..... g/l
P_m' ; maximum ethanol concentration above which cells do not produce ethanol ..... g/l
R ; empting ratio ..... l/l
S ; substrate concentration ..... g/l
So ; feed substrate concentration ..... g/l
t_c ; charging time ..... h
t_e ; empting time ..... h
t_f ; fermentation time ..... h
t_s ; settling time ..... h
V_f ; semi-batch culture working volume l
V_i ; semi-batch residual volume after supernatant removal l
X ; biomass concentration ..... g/l
$Y_{p/s}$; ethanol yield ..... g/g
t ; time ..... h
μ_m ; maximum specific growth rate ..... l/h
ν_m ; maximum specific rate of ethanol production ..... g/g/h

Acknowledgements

This work was completed in the laboratory of Prof. P.L. Rogers, whose help and discussion I would like to acknoledge with gratitude. My thanks are also due to Dr. K.J. Lee for his guidance and suggestions throughout the course of this work.

References

1) Pimentel, L.S.: *Biotechnol. bioeng.*, **22,** 1989 (1980)
2) Scheller, W.A.: *Starch*, **33,** 1 (1981)
3) Jones, R.P., Pamment, N., Greenfield, P.F.: *Process Biochem.*, **15,** 42 (1981)
4) Cysewski, G.R., Wilke, C.R.: *Biotechnol. Bioeng.*, **20,** 1421 (1978)
5) Kosaric, N., Ng, D.C.M., Russel, I., Stewart, G.S.: *Adv. Appl. Microbiol.*, **26,** 147 (1980)
6) Leppanen, O., Denslow, J., Ronkainen, P.: Proc. 4th Int. Symp. on Alcohol Fuels Technology, p. 113 (1980)
7) del Rosario, E.J., Lee, K.J., Rogers, P.L.: *Biotechnol. Bioeng.*, **21,** 1477 (1979)
8) Kim, I.H., Kim, B.H., Mheen, T.I.: *Kor. J. Appl. Microbiol. Bioeng.*, **10,** 155 (1982)
9) Lee, J.H., Williamson, D., Rogers, P.L.: *Biotechnol. Lett.*, **2,** 141 (1980)
10) Rose, D.: *Process Biochem.*, **11,** 10 (1976)
11) Humphrey, A.E.: In Advances in Biotechnology, Vol. 1, p. 203, Pergamon Press.
12) Miller, G.L.: *Anal. Chem.*, **31,** 426 (1959)
13) Sawyer, R., Dixon, E.J.: *Analyst*, **93,** 680 (1968)
14) Holzberg, I., Finn, R.K., Steinkraus, K.H.: *Biotechnol. Bioeng.*, **9,** 413 (1967)
15) Aiba, S., Shoda, M., Nagatani, M.: *Biotechnol. Bioeng.*, **10,** 845 (1968)
16) Nagodawithana, T.W., Steinkraus, K.H.: *Appl. Environ. Microbiol.*, **31,** 158 (1976)
17) Bazua, C.D., Wilke, C.R.: *Biotechnol. Bioeng. Symp.*, **7,** 105 (1977)
18) Ghose, T.K., Tyagi, R.D.: *Biotechnol. Bioeng.*, **21,** 1387 (1979)
19) Dunn, A., Williamson, D., Payne, R.E.: In: A nonlinear Simulation Package, University of New South Wales.
20) Belaich, J.P., Senez, J.C., Murgier, M.: *J. Bacteriol.*, **95,** 1750 (1968)
21) Powell, E.O.: In: Microbial physiology and continuous culture, pp. 34-55, 3rd Int. Symp., HMSO, London.
22) Amri, M.A., Bonaly, R., Duteurtre, B., Moll, M.: *Eur. J. Appl. Biotechnol.*, **7,** 235 (1979)
23) Nishihara, H., Toraya, T., Fukui, S.: *J. Ferment. Technol.*, **54,** 351 (1976)
24) Johnston, J.R., Oberman, H.: *Prog. Ind. Microbiol.*, **15,** 51 (1979)
25) White, J., Munns, D.J.: *J. Inst. Brew.*, **57,** 280 (1951).
26) Stokes, J.L.: In: The yeasts, Vol. 2, pp. 119-134, Academic Press.
27) Gray, W.D.: *J. Bacteriol.*, **42,** 561 (1942)
28) Navarro, J.M., Durand, G.: *Ann. Microbiol.*, **129B,** 215 (1978)
29) Neish, A.C., Blackwood, A.C.: *Can. J. Technol.*, **29,** 123 (1951)
30) Aiyar, A.S., Luedeking, R.: *Chem. Eng. Prog. Symp.*, **62,** 55 (1966)
31) Espinosa, R., Cojulun, V., Marroquin, F.: *Biotechnol. Bioeng. Symp.*, **8,** 69 (1978)
32) Lee, J.H., Skotnicki, M.L., Rogers, P.L.: *Biotechnol. Lett.*, **4,** 615 (1982).
33) Cysewski, G.R., Wilke, C.R.: *Biotechnol. Bioeng.*, **18,** 1297 (1976)