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PRODUCTION OF ACETIC ACID BY A REPEATED BATCH CULTURE WITH CELL RECYCLE OF *ACETOBACTER ACETI*

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SUMMARY

A comparison of volumetric production rates of acetic acid in *Acetobacter aceti* M23 was conducted for repeated batch (RB), cell-recycling repeated batch (CRB) and continuous (C) cultures. Best result was obtained with CRB culture. The magnification of productivity was 1.7 (to RB culture) and 3.3 (to C culture) for aiming final acetic acid concentration of 60 g/l and 42 g/l, respectively.

INTRODUCTION

We have been investigating the enhancement of acetic acid production rate in a high cell-density culture of *Acetobacter aceti* using a completely mixed flow reactor (Park *et al.*, 1989a). The cells in the reactor were subjected to high acetic acid concentration higher than 40 g/l throughout the operation time (Park *et al..*, 1989b). It was difficult to maintain high production rate in a continuous culture at high acetic acid concentration even though the cell density was elevated by membrane filtration. If the high cell-density culture was performed in a batch process, the extent of the product inhibition must be reduced considerably. In this investigation, we attempted to increase the

production rate of acetic acid at a high concentration attainable in a batch culture by repeating the batch culture with cell recycle.

MATERIALS AND METHODS

Acetobacter aceti M23 was provided from Nakano Vinegar Co. Ltd. (Aichi, Japan). The composition of basal medium was (g/l distilled water) glucose, 1; peptone (Kyokuto Pharmaceutical Co., Tokyo), 2; yeast extract (Difco, Co., Detroit), 5; acetic acid, 10; ethanol, 47.4. Acetic acid and ethanol were added to the basal medium sterilized with steam for 15 min at 121 ^{o}C.

The cells of *Acetobacter aceti* were cultivated in a jar fermenter with a working volume of 1500 ml (Mituwa KMJ-5a, Osaka, Japan) at a constant impeller rotational speed of 900 rpm. The air flow rate was controlled at 0.1~0.3 vvm not to deplete dissolved oxygen concentration. The hollow fiber membrane filter module was equipped to the fermenter to separate the cells from the culture broth and to recycle back to the fermenter. The membrane in filter (model PMP-103, Asahi, Kasei Co., Ltd., Tokyo) was characterized by 0.7 mm *i.d.* and total surface area of 0.2 m^2 and nominal pore size of 0.1 μm. The cartridge of module was made of a polysulphone cylinder, with 4.2 cm *i.d.* and 28.5 cm long and contained 400 fibers.

When the acetic acid concentration of the culture increased to a desired level, a part of the cell broth was withdrawn and the same volume of fresh medium was fed to the culture. Subsequent batch cultures were conducted in a similar manner. When cells were recycled back to the fermenter in a repeated batch culture a defined fraction of the culture was filtered out at the end of each run: the remaining culture was supplemented with the fresh medium to an initial liquid volume. The desired acetic acid concentration of the culture was 60 g/l for the repeated batch culture without cell recycle; 60, 40 and 30 g/l for the culture with cell recycle.

The methods of measuring concentrations of ethanol, acetic acid, total cells and viable cells have been described previously (Park *et al.*, 1989a).

RESULTS AND DISCUSSION

A repeated batch culture of *A. aceti* without cell recycle was carried out 4 times. The changes in concentrations of ethanol, acetic acid and cells is shown in Fig. 1. When acetic acid concentration reached about 60 g/l two-thirds volume of the culture broth was withdrawn and a same volume of fresh medium was fed (feed exchange ratio = 0.67). At every 13 h from starting repeated batch culture, ethanol concentration decreased from 30 g/l

to 5; viable cell concentration increased from about 0.1 g/l to 0.46 g/l at the end of each run. Acetic acid production rate was calculated as 2.89 g/l/h on the average.

A repeated batch culture with cell recycle to attain the acetic acid concentration of 60 g/l was carried out. When acetic acid concentration

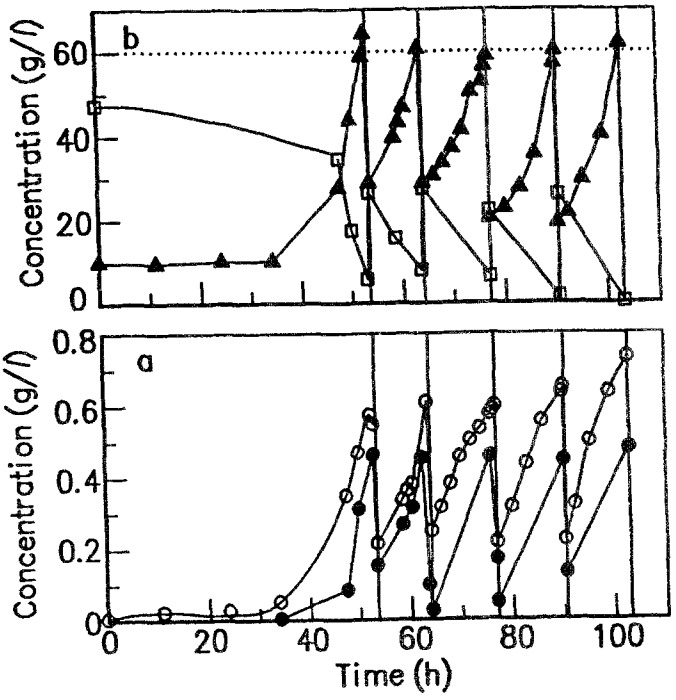

Figure 1. Concentrations of total and viable cells (a), acetic acid and ethanol (b) in repeated batch culture without cell recycle. Symbols: ○ , total cells; ● , viable cells; ▲ , acetic acid; □ , ethanol. The dotted line indicates the desired final acetic acid concentration.

reached 60 g/l culture broth of 1 liter was filtered by using a hollow fiber membrane filter module and cells were recycled back to the fermenter. A fresh medium, the same volume as the filtrate was fed to the fermenter. In a similar manner the batch culture was repeated 6 times. The result is shown in Fig. 2. The initial and final concentrations of viable cells at each experimental runs were virtually constant about 0.25 g/l. On the other hand total cell concentration increased with the culture time. It took the culture time of 6.3 h to increase acetic acid concentration from 27.7 g/l to 60. This time reduction contributed to improve the acetic acid production rate

1.7 times as high as that without cell recycle. The acetic acid production rate was 5.03 g/l/h on the average. It is considered that the improvement in acetic acid production rate was due to the increased concentration of viable cells at the start of each run. However the viable cell concentration (in Fig. 2) did not increase during the repeated batch culture. This is explained by acetic acid inhibition. Therefore we lowered the final acetic acid concentration from 60

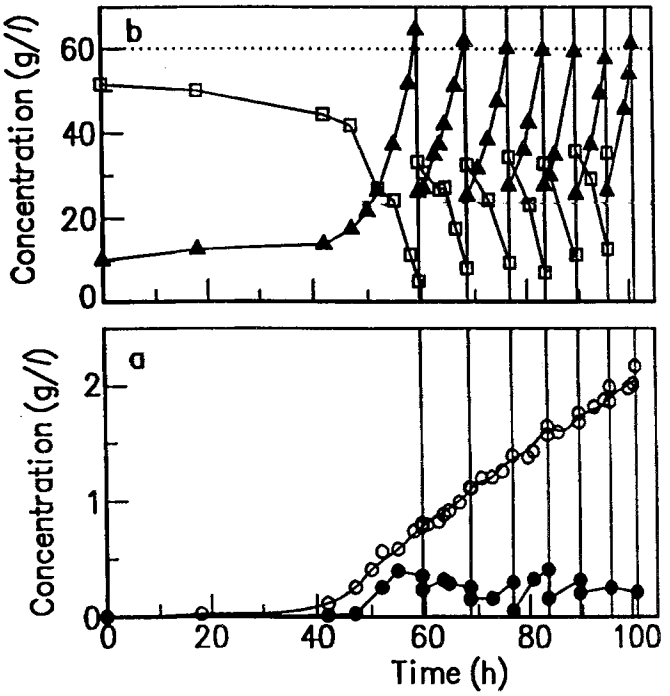

Figure 2. Concentrations of total and viable cells (**a**), acetic acid and ethanol (**b**) in repeated batch culture with cell recycle. Symbols are the same as those in Fig. 1.

g/l to about 30 and 40 g/l, and investigated the acetic acid production rate. The results are shown as Experiments 1 and 2 in Table 1.

The final viable cell concentration after several repeated batch runs approached to a quasy-steady state value, which was higher than that in a batch culture of which final acetic acid concentration was set at about 60 g/l. The acetic acid production rates of 10.25 and 14.3 g/l/h were obtained for obtaining the final acetic acid concentrations of 30 and 40 g/l, respectively.

Table 1. Results of repeated batch culture of A. aceti with cell recycle.

	Experiment 1					Experiment 2				
Run	P_i[a]	P_f	X_{vf}[b]	Δt[c]	PD[d]	P_i	P_f	X_{vf}	Δt	PD
1	16.4	31.5	0.05	5.5	2.75	25.5	37.4	$5x10^{-4}$	5.0	2.38
2	16.4	32.7	0.36	2.5	6.52	22.2	40.4	0.61	4.5	4.04
3	22.2	31.0	0.47	1.0	8.80	23.4	41.2	0.95	2.5	7.12
4	21.0	29.5	0.75	1.0	8.50	26.3	44.4	1.07	2.0	9.05
5	20.9	31.5	0.95	1.0	10.60	26.9	40.6	1.31	1.2	11.40
6	22.8	32.7	1.05	1.0	9.90	25.1	45.0	1.34	1.5	13.30
7	-	-	-	-	-	26.5	44.4	1.55	1.3	13.80
8	-	-	-	-	-	25.7	39.7	1.54	1.0	14.00
9	-	-	-	-	-	25.7	40.9	1.51	1.0	15.20
Ave.	21.9	32.1	1.00	1.0	10.25	26.0	41.7	1.53	1.0	14.30
	±1.3	±0.8	±0.07		±0.49	±0.5	±2.4	±0.02		±0.80

Superscripts i, f and v denote initial, final and viable cells, respectively.
[a]acetic acid concentration (g/L).
[b]cell concentration (g/L).
[c]culture time, $t_f - t_i$ (h).
[d]acetic acid production rate, $(P_f-P_i)/\Delta t$ (g/L/h).
Ave. average of runs 5-6 for experiment 1 and runs 7-9 for experiment 2.

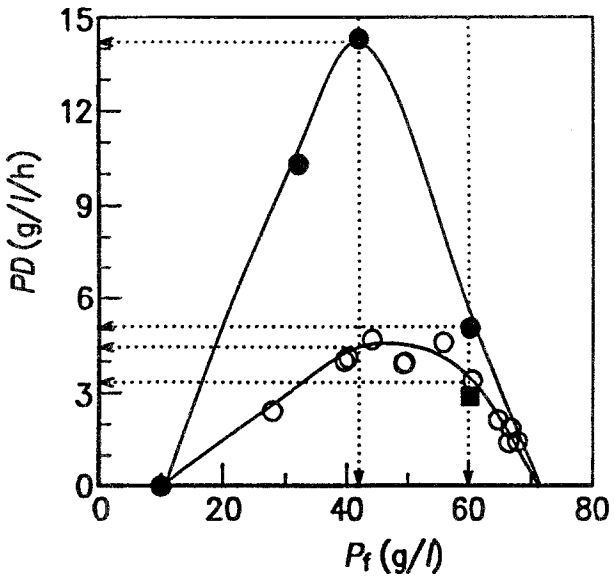

Figure 3. Effect of final acetic acid concentrations on acetic acid production rate in continuous and in repeated batch cultures with and without cell recycle. Symbols: ○, continuous culture; ■, repeated batch culture without cell recycle; ●, repeated batch culture with cell recycle.

A comparison of the results of repeated batch culture to those of continuous culture is shown in Fig. 3 in terms of effect of final acetic acid concentration on acetic acid production rate. When the final acetic acid concentration is 60 g/l, which is very inhibitory to growth and production, the obtained acetic acid production rates were 5.03 g/l/h, 2.89 and 3.2 in the repeated batch culture with and without cell recycle, and the continuous culture (Park *et al.*, 1989c), respectively. When the final acetic acid concentration is set at 42 g/l, the acetic acid production rate was 14.3 g/l/h, which is 3.3 times higher than that of the continuous culture.

In this study it was found that the batch culture with cell recycle can improve the acetic acid production rate, especially in the presence of inhibitory metabolite. And it is also very important to set the final value of acetic acid concentration appropriately. As far as *Acetobacter aceti* M23 is used, it is recommended to select final acetic acid concentration in repeated batch culture at around 42 g/l (see Fig. 3). The acetic acid concentration of 42 g/l is that of standard vinegar in Japan. If it is intended to manufacture the vinegar with higher acidity such as 60 g/l, it is expected that the acetic acid production rate increased to about 1.7 times higher than that of the continuous culture.

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