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Short Communication

Citric acid production from beet molasses by cell recycle of *Aspergillus niger*

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SUMMARY

Production of citric acid from beet molasses at a varying pH profile using cell recycle of *Aspergillus niger* was investigated. Best results in terms of citric acid concentration, yield, productivity and specific citric acid productivity were obtained with a substrate pH of 3.0.

INTRODUCTION

Beet molasses is a suitable material for citric acid production because it is readily available and low priced. In recent years, several methods for citric acid production from beet and cane molasses have been proposed and various continuous or multi-stage processes have been patented [1,6]. Despite various advances in process development, the surface and submerged batch culture techniques are still being used for production of citric acid. However in the batch techniques a significant amount of productive biomass is discarded each time.

Cell recycling techniques have advantages over batch techniques because the biomass is conserved, batch downtime is mostly eliminated and productivity is often increased. These techniques make use of the fact that citric acid production occurs by cells which are not in the active stage of growth [3].

In this paper, we report on the use of cell recycle technique for surface fermentation of beet molasses to citric acid with a strain of *Aspergillus niger*.

MATERIALS AND METHODS

Microorganism. Aspergillus niger ATCC 9142 (American Type Culture Collection, MD) was used throughout the experiment and was maintained and cultivated as described previously [4].

Culture media. Beet molasses, from a Greek sugar factory (Platy, Thessaloniki), was diluted with distilled water to give a total sugar concentration of 14% (w/v). Three conical flasks (500 ml) each containing 100 ml of beet molasses, pH 6.0, were autoclaved at 121 °C for 15 min. The medium was treated while hot with potassium ferrocyanide to precipitate heavy metals [2] and then cooled to 30 °C.

Citric acid production and cell recycle. For the first cycle, the pH of the medium in three flasks was adjusted to 6.5 and inoculated with 0.5 ml of inoculum containing 5.4×10^7 spores/ml. The flasks were incubated at 30 °C as surface fermentation. When the fermentation was complete (after 18 days), the original medium was withdrawn from each flask and replaced with 100 ml of fresh sterile medium, the pH of which was already adjusted to 6.5, 4.5 or 3.0. For each of the above trials the fermentation was stopped when the highest concentration of citric acid was obtained. The fermented substrate was removed from each flask, replaced with fresh substrate at appropriate pH and a new cycle was started. Six cycles were carried out in total.

Analytical techniques. Citric acid, pH, biomass concentration and residual sugars were determined as described previously [4].

The reported data are the average values of three separate experiments.

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RESULTS AND DISCUSSION

Citric acid production and some of the characteristic fermentation parameters are shown in Tables 1 and 2. As mentioned previously, the initial substrate pH in the first cycle was 6.5 for all trials. For the second cycle of each trial, the pH of the substrate was initially adjusted to 6.5, 4.5 or 3.0. As can be seen from Table 1, the time needed for maximum citric acid concentration differed between the three trials and also between the cycles of the same trial. Also the quantity of citric acid produced was different. Maximum citric acid concentration using the batch culture technique, with a substrate pH of 6.5, was 65 g/l after 18 days of fermentation. By a single reuse of the biomass, the citric acid concentration was 45 g/l but the fermentation time needed was halved, and thus productivity was improved significantly. Better results in terms of productivity were achieved by adjusting the substrate pH to 4.5. In this trial, the productivity was higher in the second and third cycle and then declined. The best results in terms of citric acid concentration and productivity were obtained with a substrate pH of 3.0. The productivity was improved continuously until the fifth cycle and then declined but it remained at levels much higher (0.255 vs. 0.150 g/l per h) than that of the batch fermentation at pH 6.5.

Sugar utilization was better in the trial of pH 3.0 and citric acid yield (Yp/s) was superior to that of the other trials showing that citric acid formation was stimulated at low substrate pH values (Tables 1 and 2). These results are in agreement with those obtained by Vergnaud and Niquet [5] from the second stage of a dual-stage process during which additional sugar solution was added, while the pH was maintained at 3.0 or below, and good citric acid production occurred.

Total biomass formed during the six cycles of fermentation and also the biomass yield (Yx/s) and specific biomass production rate (q_x) were higher in the pH 6.5 and 4.5 trials than in the pH 3.0 but the specific citric acid productivity (q_p) was higher in the pH 3.0 trial, showing that despite the high biomass accumulation at pH 6.5 and 4.5 the specific productivity was strongly favored at low substrate pH (Tables 1 and 2).

In conclusion, the results demonstrate that the most significant advantages for citric acid production from beet molasses at pH 3.0 using the cell recycle technique are: (a) increase of citric acid production and productivity; (b) energy saving for the washing and re-sterilization of

TABLE 1

Effect of substrate pH on citric acid production by cell recycling technique

Cycle	Ferment. time (days)	Citric acid (g/l)		Citric acid product. (g/l per h)		Spec. cit. ac. prod. (q_p)[a]		Cit. ac. Yield (Yp/s)[b]	
		$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.
				$\times 10^3$		$\times 10^4$		$\times 10^3$	
1	18	65.0	1.0	150	2	71	1.0	506	7
2	9	45.0	1.4	208	6	42	0.7	380	15
3	9	28.0	0.7	130	3	16	0.8	230	12
4	9	20.0	1.0	92	4	8	0.4	156	7
5	9	7.5	0.5	35	2	2	0.2	55	2
6	9	7.5	0.7	25	3	1	0.2	50	6
1[c]	18	65.0	1.0	150	2	71	1.0	506	7
2	15	55.0	1.5	153	4	28	1.5	412	18
3	9	40.0	0.9	185	4	26	0.6	348	14
4	9	30.0	0.9	140	4	15	0.5	242	1
5	6	7.5	1.1	52	7	4	0.2	58	8
6	6	2.5	0.5	18	3	1	0.2	21	4
1[c]	18	65.0	1.0	150	2	71	1.0	506	7
2	18	75.0	1.5	174	3	40	0.5	616	10
3	15	70.0	1.8	194	5	33	1.6	525	31
4	12	65.0	2.4	225	8	33	2.0	506	19
5	9	62.5	1.0	289	4	35	1.5	484	25
6	9	55.0	1.9	255	8	26	1.8	440	22

[a] Specific citric acid productivity, q_p (g citric acid/g biomass dry wt. per h).

[b] Citric acid yield, Yp/s (g citric acid/g sugar utilized).

[c] For the first cycle the initial pH of the substrate was 6.5.

TABLE 2

Effect of substrate pH on biomass concentration during citric acid production by cell recycling technique

Cycle	Ferment. time (days)	Biomass dry wt. (g/l)		Biomass yield (Yx/s)[a]		Sp. Biom. prod. rate (q_x)[b]		Sugar util. (g/l)[c]		Final pH	
		$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.
				$\times 10^3$		$\times 10^5$					
1	18	21.0	0.62	163	4.9	30	1.1	128	0.50	1.5	0.05
2	9	28.0	1.32	236	9.1	100	4.1	118	2.87	2.0	0.02
3	9	29.2	1.32	240	6.6	110	3.0	122	3.96	2.5	0.00
4	9	36.7	1.80	285	8.8	130	4.0	128	2.53	2.9	0.03
5	9	30.0	0.88	222	8.6	100	3.9	135	3.96	3.0	0.00
6	9	27.6	1.68	251	15.8	110	7.3	110	1.32	3.2	0.00
1d	18	21.0	0.62	163	4.9	30	1.1	128	0.50	1.5	0.05
2	15	32.8	1.58	250	15.5	60	4.3	133	3.46	1.8	0.02
3	9	16.7	0.60	245	3.4	110	1.5	115	2.53	2.2	0.00
4	9	20.2	1.13	163	8.3	70	3.8	124	3.96	2.6	0.00
5	6	30.2	1.68	236	11.8	160	8.1	128	2.64	2.8	0.05
6	6	28.6	1.40	246	6.8	170	4.7	116	2.29	3.1	0.00
1d	18	21.0	0.62	163	4.9	30	1.1	128	0.50	1.5	0.05
2	18	23.4	0.88	192	3.5	40	0.8	122	2.29	1.1	0.00
3	15	13.2	0.45	99	1.2	20	0.3	133	4.54	1.3	0.05
4	12	9.0	0.62	69	3.2	20	1.1	129	3.00	1.3	0.04
5	9	16.0	1.74	124	11.0	50	5.0	129	4.67	1.2	0.05
6	9	14.4	0.75	115	3.5	50	1.6	125	3.12	1.3	0.00

[a] Biomass yield, Yx/s (g biomass dry wt./g sugar utilized).
[b] Specific biomass production rate, q_x (g biomass dry wt./g sugar utilized per h).
[c] Initial sugar concentration 140 g/l.
[d] For the first cycle the initial pH of the substrate was 6.5.

the equipment used; and (c) the repeated use of the culture up to six times could result in substantial cost savings.

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