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Production of L-Phenylacetylcarbinol (L-PAC) by Encapsulated *Saccharomyces cerevisiae* Cells

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Abstract−In the present study, benzaldehyde was converted by both the free cells *Saccharomyces cerevisiae* (ATCC 834) and those immobilized in the calcium alginate liquid-core capsule into L-PAC during anaerobic fermentation in a medium containing benzaldehyde. In a free cells survey, skipping aerobic adaptation before anaerobic fermentation caused all of benzaldehyde to be converted by 220 g (wet weight) of cells in 100 mL of the medium even at a higher concentration of 8 g/L benzaldehyde. The yield of L-PAC based on the moles of converted benzaldehyde increased as the amount of benzaldehyde dose was increased. The encapsulation protected cells effectively from the toxicity of benzaldehyde. Even a small quantity, 1.1 g (dry weight), of encapsulated cells in 100 mL of the medium containing 0.6% benzaldehyde converted more than 95% of the benzaldehyde, and the corresponding yield of L-PAC was about 40%. The production of L-PAC by the encapsulated cells depended on the pH of the medium. The conversion of benzaldehyde decreased slightly, but yield of L-PAC increased as the pH of the broth solution was fixed at a lower value. Biotransformation in a small side reactor of the batch system caused higher yield of L-PAC than that in the batch reactor containing the same quantity of encapsulated cells during the first 4 hours of fermentation.

Key words: L-phenylacetylcarbinol, L-PAC, Benzaldehyde, *Saccharomyces cerevisiae*

INTRODUCTION

L-phenylacetylcarbinol (L-PAC) is used as a precursor of the L-epidrin, which is effective on congestion and asthma [Astrup et al., 1992a, b]. L-PAC is produced by the conventional extraction of *Ephedra* plants or by chemical reaction which also produces other isomers that cannot be used as pharmaceuticals. Benzaldehyde can be transformed to L-PAC by a reductive amination reaction which is catalyzed by enzymes from certain yeasts. Neuberg found out that live yeasts transformed benzaldehyde in the broth solution containing sugars to L-PAC by a fermentative metabolism [Neuberg and Lieberman, 1921]. The production of L-PAC is catalyzed by the enzyme pyruvate decarboxylase (PDC) and is competitively associated with the production of the by-product benzyl alcohol, which is catalyzed by the enzyme alcohol dehydrogenase (ADH). The conversion of benzaldehyde to L-PAC cannot reach 100% because of the oxidation of benzaldehyde to benzoic acid and the formation of the by-product benzyl alcohol [Agarwal et al., 1987]. L-PAC was produced in the fed-batch by using various yeasts [Culic et al., 1984; Mahmoud et al., 1990a; Vojtisek and Netrval, 1982] and the concentration of L-PAC reached 10-12 g/L [Culic et al., 1984; Mahmoud et al., 1990a; Vojtisek and Netrval, 1982]. The inhibition of resident benzaldehyde on PDC activity and the reduction of cell viability which was caused by direct contact with reactant benzaldehyde, by-product benzyl alcohol, and product L-PAC lowered the production yield of L-PAC [Agarwal et al., 1987]. The specific production rate of L-PAC showed optimal value when the dose of benzaldehyde was between 0.4 and 1.7 g/L. L-PAC was not produced at a higher value than 2.1 g/L of benzaldehyde dose [Agarwal et al., 1987]. Other papers reported that the optimal dose of benzaldehyde was 1.1 g/L [Tripathi et al., 1988] and the upper limit was 3 g/L [Rogers et al., 1997].

Cell immobilization method can be applied in order to protect microbial cells from the toxic reactant and products in the production of L-PAC. *Saccharomyces cerevisiae* cells entrapped in a bead were used to produce L-PAC in a semi-continuous culture [Mahmoud et al., 1990b; Seely et al., 1989]. Among various cell immobilization methods, entrapment in calcium alginate beads has commonly been used for immobilization of microbial cells. However, a bead entrapment method has a limit of 25% cell loading by volume because of the mechanical strength of the support [Buchholz, 1979]. An alternative immobilization method, encapsulation of cells in a liquid-core capsule which offers more space for cellular growth than entrapment [Kurosawa et al., 1989], is a good method for a high density culture. Chang and his colleagues developed a high cell loading encapsulation method which integrated immobilization and cell growth into one step and simplified preparatory steps for whole cell immobilization or whole cell fermentation systems [Cheong et al., 1993a]. *S. cerevisiae* cells were immobilized and cultured in calcium alginate capsules. Although the cell concentration of the cell-recycled continuous culture system [Lee and Yoo, 1994] was 130 g/L because of the rheological properties of the fermentation broth, the dry cell density reached 300 g/L on the basis of the space inside capsule, and ethanol fermentation was succcessfully carried out. Adding a small amount of surfactant to the calcium alginate membrane prevented capsule rupture caused by gaseous CO_2 produced during ethanol fermentation. Unlike the yeast cells entrapped in the bead, all the encapsulated cells stayed inside the capsules [Cheong et al., 1993a, b]. The specific invertase activity of the encapsulated *S. cerevisiae* cells of SEY 2102 harboring plasmid pRB58 with the SUC2 gene encoding invertase showed slightly higher ac-

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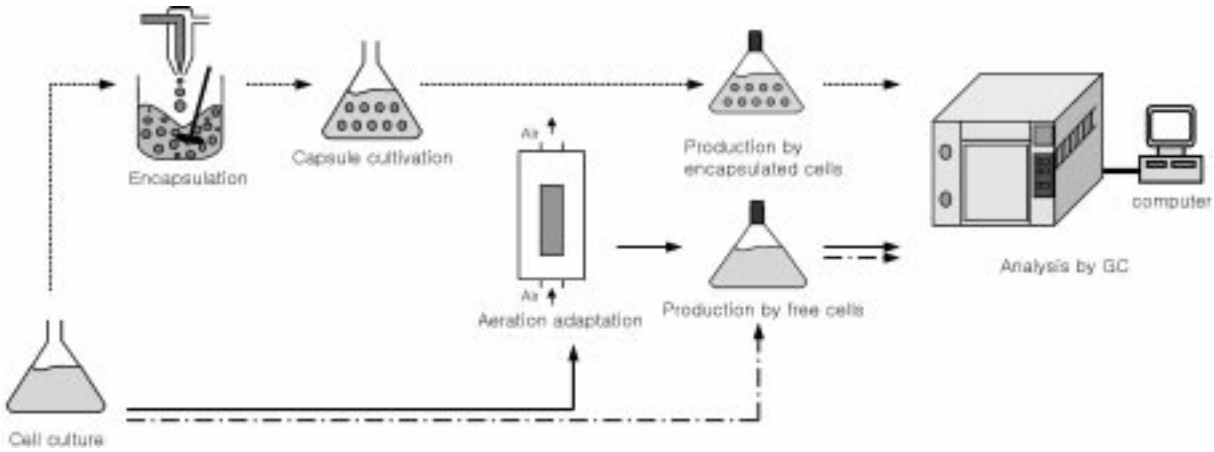

Fig. 1. Schematic of experimental procedure. Free cells with aerobic adaptation (——►). Free cells with aerobic adaptation (——►). Encapsulated cells without aerobic adaptation (·····►).

tivity than did the free cells in a batch culture [Chang et al., 1996]. The invertase activity of recombinant *S. cerevisiae* immobilized in the calcium alginate bead decreased 50% after 15 batches, but that of encapsulated cells retained the initial activity even after 25 batches [Chang et al., 1998]. The major aim of this work is to explore how the immobilization of the *S. cerevisiae* cells in the calcium alginate capsule affects the production of L-PAC.

EXPERIMENTAL

1. Microorganism and Culture Conditions

S. cereviasae (ATCC 834) was used to produce L-phenylacetyl carbinol (L-PAC). The yeast cultures employed were maintained on a medium that contained: yeast extract, 10 g/L; malt extract, 10 g/L; dextrose, 4 g/L; agar, 20 g/L. The medium used for growth of microorganism and production of L-PAC was composed of yeast extract, 6 g/L; $(NH_4)_2SO_4$, 4 g/L; $MgSO_4$, 0.6 g/L; KH_2PO_4, 1 g/L; glucose, 100 g/L; to which 5 g/L of $CaCl_2$ was added when the encapsulated cells were cultivated. The carbon sources and nitrogen sources were sterilized separately at 121 °C for 15 minutes in the autoclave. Initial pH of the growth medium was fixed at 6.2 for the free cell culture and was adjusted to be less than 5.4 for the encapsulated cell culture because when phosphate ions combined with calcium ions in the capsule membrane at the higher pH of the medium, they transformed to $CaH_4O_8P_2$. The free cells collected by centrifuging the broth solution were washed with distilled water and dried at 80 °C until their weight did not change in order to measure the dry density of the free cells. The difference between the dry weights of vacant capsule and cell occupied one was considered to be the dry cell weight of encapsulated cells.

2. Microencapsulation

Capsules whose core was packed densely with *S. cerevisiae* cells were prepared as described in the literature [Oh and Park, 1998]. The cells collected by centrifuging 10 mL of the broth solution were added to 100 mL of 1.3% (w/v) $CaCl_2$ (Duksan, Japan) solution containing 0.26% (w/v) xanthan gum (Sigma). This solution was added drop by drop into the swirling 0.6% (w/v) sodium alginate (Yakuri, Japan) solution containing a nonionic surfactant, nonoxynol [polyethyleneglycols mono(nonyl-phenyl) ether]. The adjusted air flow rate of 5 L/min at the annulus of the concentric double needles of the dispenser kept the capsule size constant at 2 mm. Cells inocculated in capsules were cultivated at 30 °C for 2 days in a shaking incubator.

3. Experimental Procedure

As shown in Fig. 1, *S. cerevisiae* cells were cultured in the growth medium whose pH was adjusted to 6.2 for one day at 30 °C and 200 rpm in a shaking flask incubator. There were three routes for the experimental procedure. The first was carried out by using free cells which were aerobically adapted before fermentation. The cells collected from 800 mL of the broth solution by centrifuging at 3,500 rpm for 10 minutes were moved into 100 mL of fresh medium in an air-lift reactor for aeration adaptation. After one hour aeration adaptation (air flow rate: 1,000 cc/min at 30 °C), the fermentation for L-PAC production was performed in an anaerobic state in a shaking flask incubator. The second was carried out by using free cells skipping an aerobic adaptation. The cells collected from 800 mL of the medium were directly moved into the production medium without an aeration adaptation. The third was performed by encapsulated cells without an aerobic adaptation. The encapsulated cells were prepared by the procedure described just before in the 'microencapsulation'. The encapsulated cells were moved into fresh medium and the anaerobic fermentation for L-PAC production was carried out in a shaking flask incubator. At the beginning of the fermentation, benzaldehyde was added to the medium as four doses of equal volume at 1-h intervals in order to lower the level of reactant and thus reduce its toxic effect on the live cells (for example: for the total amount of 6 g/L benzaldehyde, each 1.5 g/L of benzaldehyde was added to the production medium four times in three hours). A first sample of the product was obtained just after the fourth dose of benzaldehyde. Succeeding samples were obtained at 1-h or 2-h intervals during a 24-h fermentation.

4. Analysis

Glucose concentration was determined with PGO enzyme (Sigma No. 510-A). 0.5 mL of sample solution diluted 500 times with distilled water was added to 5 ml of combined enzyme-color reagent solution and kept at 37 °C for 30 minutes. OD of the reacted sample was measured at 450 nm with UV-VIS spectrometer (Shimadzu 1201) and compared with the standard curve of optical density for

glucose concentration.

L-PAC, benzaldehyde, and benzyl alcohol were determined by GC with a Hewlett-Packard model HP9890 series II gas chromatograph; the results were calculated on a model HP3396 series II integrator terminal. A Hewlett-Packard HP-1 column was used for analysis having a 0.11 μm crosslinked methyl silicon gum film and dimensions of 0.2 mm internal bore diameter by 25 m in length. Oven temperature was initially 80 °C, and increased at a rate of 2 °C per min to 100 °C and held at this temperature. The injection temperature was 250 °C and compounds were detected at a detector temperature of 250 °C. Aqueous L-PAC (a gift from Knoll, AG; Ludwigshafen, Germany), benzaldehyde (Sigma Chemical Co.), benzyl alcohol (Junsei Chemical Co.), and dodecane (Sigma Chemical Co.) were used as standards and 1 mL of sample solution, to which 0.2 mL of dodecane was added, was extracted with 9 mL of ether. 1 μL volumes were injected and helium was used as a carrier gas.

5. Cell Viability

The composition of solid medium used to check the cell viability was bacto peptone, 20 g/L, yeast extract, 10 g/L, glucose, 20 g/L, and agar, 20 g/L. The free cells collected by centrifuging 800 ml of broth solution obtained after 1 day cultivation were moved into the production medium and a given amount of benzaldehyde was added to the medium as described above. The cells carried out biotransformation of benzaldehyde for 5 and/or 10 hours after the last addition of benzaldehyde at 30 °C under anaerobic conditions. One mL of the production medium diluted 1,000 times with distilled water was scattered on the solid agar medium with a sterilized glass rod. The solid agar medium was cultured at 37 °C for 3 days in the incubator and the colonies of *S. cerevisiae* cells were counted.

6. Batch with Side Reactor System

In order to explore how the dense population of live cells in the reactor affects the production yield of L-PAC, we made the production medium circulate through a small reactor which was attached to the vessel containing the production medium containing benzaldehyde as shown in Fig. 2. The reactor was packed with 7.24 mL of calcium alginate capsules containing *S. cerevisiae* cells in the liquid core and the production medium was circulated at a rate of 2.06 mL per min.

RESULTS AND DISCUSSION

1. Conversion of Benzaldehyde by the Free Cells

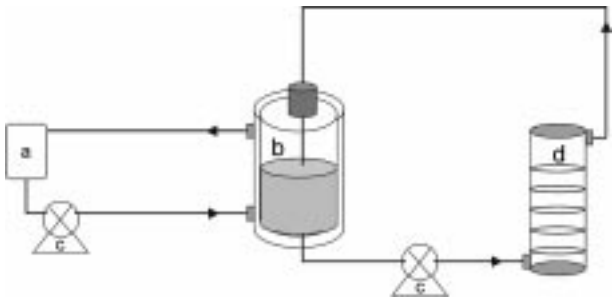

Fig. 2. Schematic representation of batch with a side packed reactor.

a. Cooling water bath
b. Medium storage
c. Peristaltic pump
d. Bioreactor

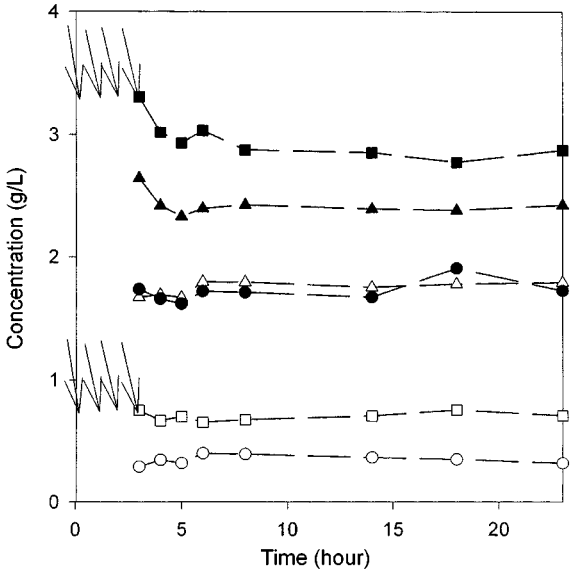

Fig. 3. Time course of the L-PAC, residual benzaldehyde, benzyl alcohol during biotransformation by free cells of *S. cerevisiae* ATCC 834 in a shaking incubator just after 1-h aerobic adaptation.

4 g/L benzaldehyde dose, 24 g wet cells/100 mL of medium: □ benzaldehyde, ○ L-PAC, △ benzyl alcohol; 8 g/L benzaldehyde dose, 21 g wet cells/100 mL of medium: ◆ benzaldehyde, ● L-PAC, ▲ benzyl alcohol; → benzaldehyde dose.

The conversion of benzaldehyde into L-PAC varied with the total amount of benzaldehyde dose. The time course of L-PAC, residual benzaldehyde, benzyl alcohol during biotransformation by free cells of *S. cerevisiae* in shaking incubator is shown in Fig. 3. Free cells in the reactor were adapted to the aeration for one hour before fermentation. Biotransformation was completed in 3 hours during benzaldehyde dosage. The fraction of converted benzaldehyde during biotransformation was 85.2 percent of the total dose of 4 g/L benzaldehyde and decreased as the dosage of benzaldehyde increased, although the mass of converted benzaldehyde increased. The concentration of benzaldehyde in the medium just after the last dose of 4 g/L benzaldehyde itself was as low as 0.7 g/L and as high as 3 g/L after the last dose of 8 g/L. Therefore, we can conclude that the toxicity of higher benzaldehyde concentration on the cell viability caused the decrease of benzaldehyde conversion. This phenomenon is partially coincident with other reports that the specific production rate of L-PAC was highest at a dose concentration of 1.7 g/L benzaldehyde [Agarwal et al., 1987; Wang et al., 1994]. There was no production of L-PAC by the free cells at a dosage concentration higher than 3 g/L [Rogers et al., 1997].

In this study, however, the production rate of L-PAC increased as the total amount of benzaldehyde dosage increased from 4 to 8 g/L. This may be due to the fact that the wet cell density in the fermentation medium used in this study was 220 g/L (dry basis: 48 g/L) and much higher than that in other studies. The yield of L-PAC represented as the mole ratio of product L-PAC to converted benzaldehyde increased from 7.7 to 23.9 percent as the total amount of benzaldehyde dose increased from 4 to 8 g/L. The yield of L-PAC and benzaldehyde conversion reported by Mahmoud et al. [1990a] for free cells were 16 and 78 percent, repectively, at the

Table 1. Conversion of benzaldehyde during anaerobic fermentation by 220 g (wet wt: dry basis, 48 g/L) of free cells, *Saccharomyces cerevisiae* (ATCC 834) per liter of medium

Aerobic adaptation before fermentation (hr)	Benzaldehyde dose (g/L)	Yield[1] (%)		Conversion of benzaldehyde (%)
		L-PAC	Benzyl alcohol	
none	4	7	57	99
	6	19	52	99
	8	25	47	99
1	4	8	53	83
	6	16	54	58
	8	24	47	64
3	6	1	64	35

[1]Yield: based on moles of converted benzaldehyde.

benzaldehyde dose of 6 g/L. However, the yield of benzyl alcohol decreased slightly as the total amount of benzaldehyde dosage increased as shown in Table 1. This result can be explained by another report that alcohol dehydrogenase which was used to produce benzyl alcohol from benzaldehyde was inhibited much more sensitively by the high concentration of benzaldehyde than pyruvate decarboxylase which was used to convert benzaldehyde to L-PAC [Long et al., 1989].

2. L-PAC Production by the Free Cells without Aerobic Adaptation

Skipping the aerobic adaptation of cells increased the fraction of converted benzaldehyde during anaerobic fermentation. All of the benzaldehyde injected into the medium at 1-h intervals in 3 hrs disappeared in 6 hrs of fermentation even when the total amount of benzaldehyde dosage was 8 g/L as shown in Fig. 4. Skipping aerobic adaptation made the cells produce much more L-PAC compared with that of the aerobically adapted cells, but the respective yields of L-PAC and benzyl alcohol on the moles of converted benzaldehyde were nearly the same as those of aerobically adapted cells as shown in Table 1. A longer aerobic adaptation was more harmful to cells and a 3-h aerobic adaptation made the free cells unable to produce L-PAC as shown in Table 1. These results indicate that aerobic adaptation of the cells stimulated respiratory chains, correspondingly inhibited fermentative biotransformation, and equally reduced the activations of enzymes such as pyruvate decarboxylase and alcohol dehydrogenase, although the longer 24-h aerobic

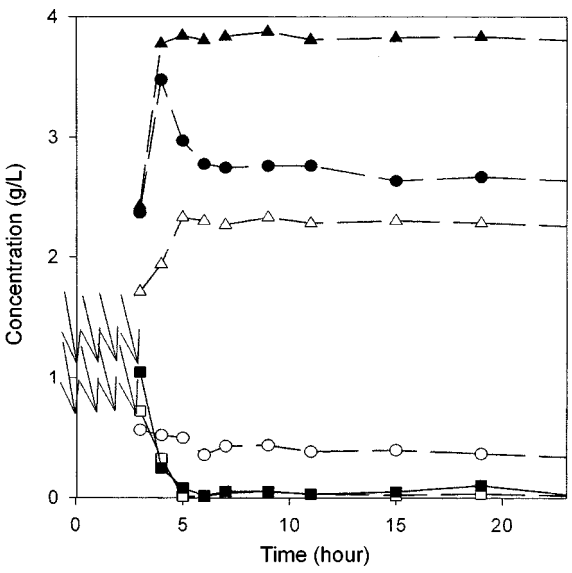

Fig. 4. Time course of the L-PAC, residual benzaldehyde, benzyl alcohol during biotransformation by free cells of *S. cerevisiae* ATCC 834 in a shaking incubator without aerobic adaptation.
4 g/L benzaldehyde dose, 21 g wet cells/100 mL of medium: □ benzaldehyde, ○ L-PAC, △ benzyl alcohol; 8 g/L benzaldehyde dose, 21 g wet cells/100 mL of medium: ◆ benzaldehyde, ● L-PAC, ▲ benzyl alcohol; → benzaldehyde dose.

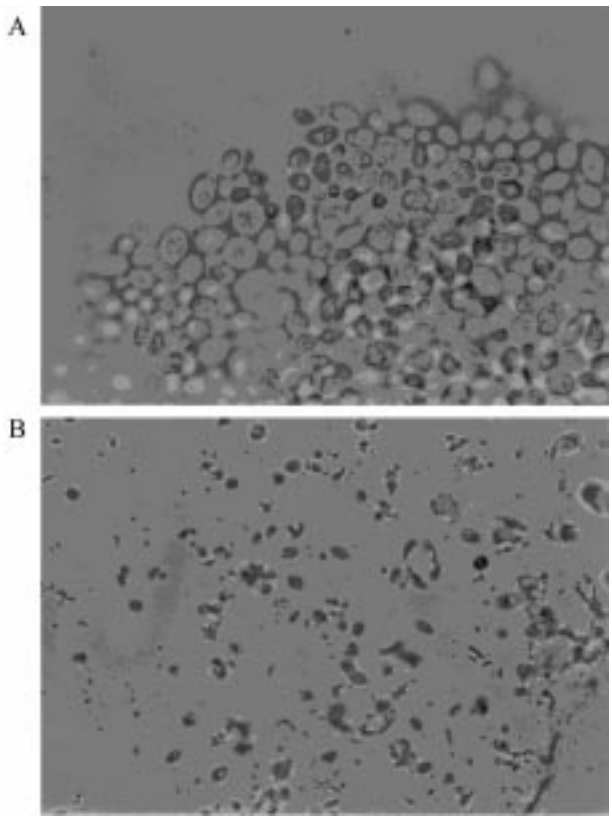

Fig. 5. The state of free cells of *S. cerevisiae* ATCC 834 after the fermentative biotransformation in the medium to which 8 g/L benzaldehyde was added.
A: 5-h, B: 10-h

Table 2. The viability of yeast cells after production of L-PAC in the medium to which 8 g/L benzaldehyde was added as four doses of equal volume at 1-h intervals without aerobic adaptation.

Fermentation time (hr)	Number of colony (/100 mL medium)	
	Dilution rate (1/1,000)	Dilution rate (1/10,000)
0	5.76×10^8	14.78×10^8
5	1.33×10^8	2.1×10^8
10	0	0

adaptation was essential for the entrapped cells to be used in the repeated cycles of fermentation [Mahmoud et al., 1990b]. The state of free cells used for 5 and 10 hrs, respectively, to convert benzaldehyde in the fermentation medium supplied with 8 g/L benzaldehyde as 4 doses of equal volume at 1-h intervals is shown in Fig. 5. Seventy five percent of the free cells' population died after 5-h fermentation and no viability of the cells was found after 10-h fermentation because of the interaction with benzaldehyde being more toxic than benzyl alcohol [Yeom and Yoo, 1997], as shown in Table 2.

3. Protection of Cells by Capsule Membrane

The dry density of encapsulated cells which had been cultured for 2 days in the growth medium reached 260 g/L on the basis of inner volume of the capsule. A respective 0.4-, 0.6-, and 0.8-g of benzaldehyde was added to 100 mL of liquid medium as four doses of equal volume after 5.6 mL of capsules were added. As shown in Fig. 6, all 4 g/L benzaldehyde was taken up by the encapsulated cells after 20 hrs of fermentation; L-PAC was detected in the medium after 10 hrs because of the mass transfer resistance through the capsule membrane. As reported earlier in the literature [Mahmoud et al., 1990a; Nikolova and Ward, 1994], immobilization of cells changed the yield of L-PAC based on the moles of converted benzaldehyde. In this study, encapsulation of the cells increased the yield of L-PAC 4- and 1.18-fold more than did free cells at the initial supply of 4- and 6-g/L benzaldehyde, respectively, even though the dry concentration of encapsulated cells was as low as 5.2 g per liter of medium and was only 2.5% of that of free cells. The increase of L-PAC production by encapsulated cells at the benzaldehyde dose of 4 g/L is much higher compared to that obtained by Mahmoud et al. [1990b] using cells immobilized in the bead, although the cell mass used for the capsule system was much less than for the bead system. These results may be caused by the fact that encapsulation protects cells more effectively from the toxicity of benzaldehyde than does immobilization in beads because there are no cells in the capsule membrane and the cells remain only in the capsule core. Moreover, cells immobilized in the bead smeared out from the bead and grew in the liquid medium, but the capsule membrane completely captured yeast cells during fermentation as observed by Cheong et al. [1993a]. However, as similar results obtained by Mahmoud et al. [1990b] for cells immobilized in the beads, the reduction of toxic or inhibitory effect of benzaldehyde on the encapsulated cells could not be found at the higher benzaldehyde dosage of 8 g/L, as shown in Table 3. Only 42.5% of benzaldehyde was taken up by the encapsulated cells during the 24 h of incubation; moreover, no L-PAC was produced. As mentioned in the literature [Mahmoud et al., 1990a], the lower benzaldehyde conversion rate at higher benzaldehyde concentration might be caused by the pellet formation of cells.

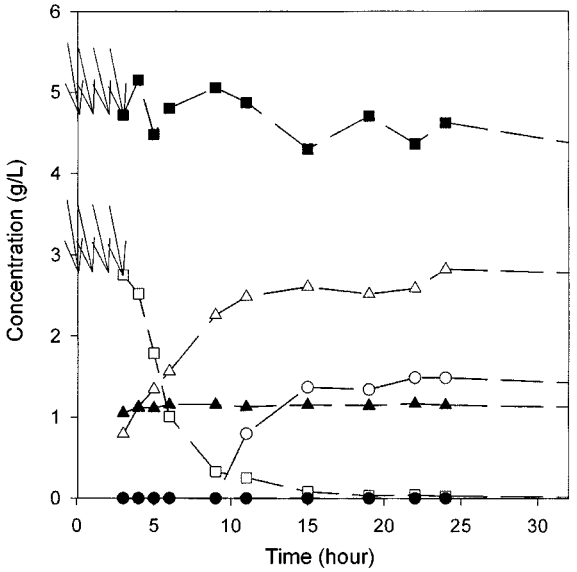

Fig. 6. The effect of specific doses of benzaldehyde based on the amount of cells on the production of L-PAC during biotransformation by the encapsulated cells of *S. cerevisiae* ATCC 834 in a shaking incubator.
4 g/L benzaldehyde dose, 5.2 g dry cells/L of medium: □ benzaldehyde, ○ L-PAC, △ benzyl alcohol; 8 g/L benzaldehyde dose, 5.2 g dry cells/L of medium: ◆ benzaldehyde, ● L-PAC, ▲ benzyl alcohol; → benzaldehyde dose.

4. Effect of Cell Loading on the Production of L-PAC by Encapsulated Cells

About 97% of benzaldehyde disappeared and yields of L-PAC

Table 3. Conversion of benzaldehyde during anaerobic fermentation by the encapsulated cells, *Saccharomyces cerevisiae* (ATCC 834)

Benzaldehyde dose (g/L)	Cell mass (g dry wt/L medium)	Yield[1] (%)		Conversion of benzaldehyde (%)
		L-PAC	Benzyl alcohol	
4	5.2	25	65	99
6	5.2	22	53	99
8	5.2	0	33	43
6	3.0	0	29	32
6	5.4	30	53	98
6	10.2	39	60	97

[1]Yield: based on moles of converted benzaldehyde.

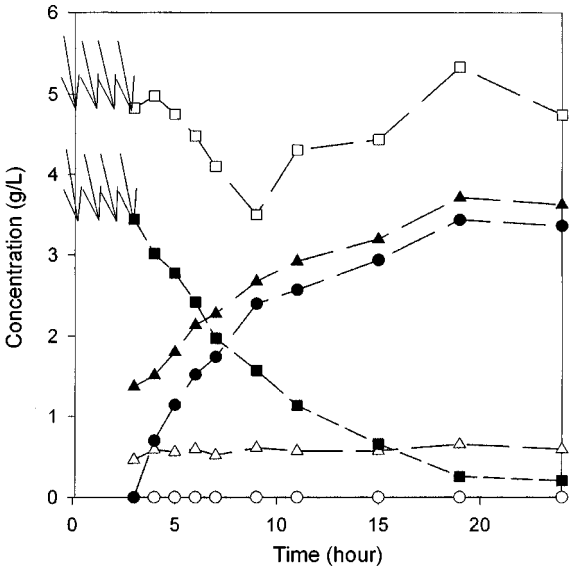

Fig. 7. The increase of cell loading in the medium improves the production of L-PAC and correspondingly decreases the toxicity of benzaldehyde on the encapsulated cells of *S. cerevisiae* ATCC 834 in a shaking incubator.
6 g/L benzaldehyde dose, 3.0 g dry cells/L of medium: □ benzaldehyde, ○ L-PAC, △ benzyl alcohol; 6 g/L benzaldehyde dose, 10.2 g dry cells/L of medium: ◆ benzaldehyde, ● L-PAC, ▲ benzyl alcohol; → benzaldehyde dose.

on moles of converted benzaldehyde were 39 and 30%, respectively, when 7.24 mL and 3.62 mL of capsules were added to 100 mL of the fermentation medium supplied with 6 g/L benzaldehyde, as shown in Table 3. Addition of 2.4 mL calpsules converted only 32.3% of the benzaldehyde and could not introduce any L-PAC production, as shown in Fig. 7. These results suggest that encapsulation of cells reduces the toxic or inhibitory effect of benzaldehyde and correspondingly increases the yield of L-PAC on benzaldehyde, but the yields of L-PAC production by encapsulated cells decrease inversely with the specific loading of benzaldehyde on cell mass. Using encapsulated cells increased the yield of L-PAC on the basis of moles of converted benzaldehyde, but the yield reached 39% as shown in Table 3 and not 90% as obtained by Mahmoud et al. [1990a] using cells immobilized in the bead. The yield of benzyl alcohol increased in accordance with L-PAC as the specific loading of benzaldehyde on cell mass decreased, and reached about 60% on the basis of moles of converted benzaldehyde.

5. Effect of pH on the Yield of L-PAC

In other studies for L-PAC production, the pH of the medium was fixed at 5.0 during incubation [Rogers et al., 1997] or adjusted to 5.0 at the beginning and not controlled during incubation [Mahmoud et al., 1990a]. In another report in which pyruvate was added together with benzaldehyde to the medium at the beginning of the incubation, the observed optimum pH was 4-4.5 [Long et al., 1989]. The variation of the pH of the medium during cultivation affected the growth of *E. coli* cells in the capsule [Oh and Park, 1998]. In this study, the pH of the medium was 5.0 at the beginning of fermentation of encapsulated *S. cerevisiae* cells and decreased to 2.8 after 20 h of incubation. The pH of the medium was controlled to be constant during fermentation by using an acid-base dosing method in expectation that the pH control affects the enzymatic activity of enzymes alcohol dehydrogenase and/or pyruvate decarboxylase and increases correspondingly the yield of L-PAC. As shown in Table 4, the uptake of benzaldehyde by the encapsulated *S. cerevisiae* cells slightly decreased from 99% to 95% in the medium supplied with 6 g/L benzaldehyde as the controlled pH of the medium was decreased from 4.5 to 3.5. However, the yield of L-PAC on the moles of converted benzaldehyde increased from 17% to 32% and was higher than that in the case of naturally decreasing pH, if pH of the medium was controlled to be lower than 4. In this study, change in pH of the medium did not affect the yield of benzyl alcohol, as shown in Table 4, although the benzyl alcohol produced during incubation might have been toxic to the cells as well as the substrate benzaldehyde [Long et al., 1989]. With these results, it can be suggested that the lower pH of the medium prevents the digestion of L-PAC by the cells and/or increases the activity of pyruvate decarboxylase, although the growth of cells was depressed at the lower pH.

6. Aerobic Adaptation of Encapsulated Cells

Aerobic adaptation of immobilized cells between anaerobic fermentation cycles can reactivate the cells. Without aerobic adaptation between anaerobic fermentation cycles, conversion of benzaldehyde by encapsulated yeast cells decreased as the 7-h fermentation cycle was repeated. The conversion of benzaldehyde in the 1 L medium containing 10 g of encapsulated cells decreased to 36% at the second cycle; yield of L-PAC also decreased from 32% to 15% at the second cycle. A 2-h aerobic adaptation in an air lift reactor activated encapsulated cells to convert more benzaldehyde in the second cycle compared to that without aerobic adaptation and also increased the yield of L-PAC in the second cycle to 41%, which is higher than that of the first cycle as shown in Table 5. The 24-h aerobic adaptation increased the conversion of benzaldehyde by the cells immobilized in the bead at the second trial [Mahmoud et al., 1990b], but the amount of beads was 100 mL in 400 mL of the medium supplied with 6 g/L benzaldehyde. The toxic effect of ben-

Table 4. The dependence of L-PAC production by encapsulated cells on the pH of the culture broth

Benzaldehyde dose (g/L)	Cell loading (g dry wt/L medium)	pH during fermentation	Yield[1)] (%)		Conversion of benzaldehyde (%)
			L-PAC	Benzyl alcohol	
6	5.2	3.5	32	55	95
6	5.2	4.0	25	57	98
6	5.2	4.5	17	57	99
6	5.2	5.0/2.8	22	53	99

[1)]Yield: based on moles of converted benzaldehyde.

Table 5. The effect of aerobic reactivation of encapsulated cells between anaerobic fermentation cycles on the production of L-PAC at the following cycle

Benzaldehyde dose (g/L)	Cell loading (g dry wt/L medium)	Aeration time (hr)	Fermentation cycle	Yield[1] (%)		Conversion of benzaldehyde (%)
				L-PAC	Benzyl alcohol	
6	2.6	none	First	32	58	66
			Second	16	49	36
6	2.6	2	First	33	54	76
			Second	42	58	49

[1]Yield: based on moles of converted benzaldehyde.

zaldehyde on the cells immobilized in the beads was not severe compared to that on the encapsulated cells because only 4.5 g cells were distributed in 100 ml of calcium alginate beads. This may indicate that the effect of aerobic adaptation on the L-PAC production by the encapsulated cells is not remarkable compared to that by the cells immobilized in the beads.

7. Batch with Side Reactor Packed with Encapsulated Cells

A higher cell density of encapsulated cells increased the conversion of benzaldehyde and yield of L-PAC, as shown in Table 3. A side reactor containing 1,000 capsules packed with 1.02 g of cells based on dry weight was attached to the tank containing 100 mL of medium, as shown in Fig. 2. The medium was recycled at a flow rate of 2.06 mL/min in expectation that the yield of L-PAC would be higher compared to that of the batch system because the cell density in the side reactor was higher. A total amount of 6 g of benzaldehyde was added to the medium tank as four doses of equal volume at 1-h intervals; no L-PAC was produced just after the last benzaldehyde dose to the batch system. However, L-PAC was found just after the last dose and the yield of L-PAC was about 20% in the side reactor system even though the conversion of benzaldehyde was nearly the same for both systems, as shown in Fig. 8. Use of encapsulated cells delayed the production of L-PAC compared to that of free cells as shown in Fig. 6 and 7. This result may be due to the mass transfer resistance through the capsule membrane. However, the fast flow of the medium through the side reactor increased the external mass transfer of the capsule and this made the early appearance of L-PAC in the medium of the side reactor system. There was no difference between the final conversions of benzaldehyde in both systems.

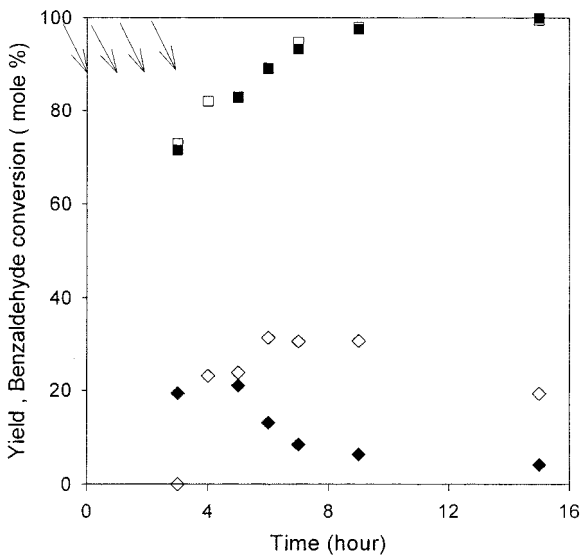

Fig. 8. Time course of the L-PAC yield (mole%), benzaldehyde conversion (mole%) during biotransformation by encapsulated cells of *S. cerevisiae* ATCC 834.
a batch system, 1.02 g dry weight of encapsulated cells/100 mL of medium. □ conversion of benzaldehyde, ◇ L-PAC yield; a batch with a side reactor system, flow rate 2.06 mL/min, 1.02 g dry weight of encapsulated cells, 100 mL of medium in a batch: ◆ conversion of benzaldehyde, ◆ L-PAC yield; → four doses of benzaldehyde at 1-h intervals during the first 3 h of incubation.

CONCLUSION

Encapsulation is a very effective means of protecting *S. cerevisiae* cells from the toxic effect of higher concentrations of benzaldehyde and correspondingly gives the encapsulated cells an opportunity to produce much L-PAC in the medium containing benzaldehyde. Higher loading of encapsulated cells in the liquid medium introduces a higher yield of L-PAC based on the moles of converted benzaldehyde and a higher conversion of benzaldehyde, but the encapsulation technique cannot effectively protect the cells from the toxicity of extremely higher concentrations of benzaldehyde if the specific benzaldehyde loading on the cells in the medium is very high. An aerobic adaptation between fermentation cycles refreshes encapsulated cells and improves the yield of L-PAC in the following cycle, although the aerobic adaptation at the beginning of anaerobic fermentation decreases the conversion of benzaldehyde by free cells. Shear stress on the capsules caused by the fast flow of the liquid medium and much loading of encapsulated cells in the side reactor may provide a high yield of L-PAC, protecting encapsulated cells from the toxicity of benzaldehyde because of the concentration gradient of benzaldehyde along the length of a side reactor.

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