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Research Note

Production of Lactic Acid from Whey by Immobilized Cells

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In this paper, the production of lactic acid from deproteinized whey, using immobilized *Lactobacillus casei* on wood chips is investigated. The immobilization process was considered on various supports like wood chips, brick particle and porous glass by adsorption and on eggshell with glutaraldehyde by covalent bonding. Wood chips demonstrated the highest adsorption among all the supports and, therefore, was chosen as the best support for production. Batch production was studied at four temperatures and three different pH after five days. The highest concentration of lactic acid (16 g/l) was observed at T = 28°C and pH = 5.5. The optimum condition of temperature and pH for the continuous system was chosen as 32°C and 5, respectively, based on the results obtained in the batch system. The continuous reactor was designed as a packed-bed of *L.casei* immobilized on wood chips.The highest concentration of lactic acid produced in this system (14.8 g/l) was obtained with $D = 0.2\ h^{-1}$ after five days.

INTRODUCTION

As a fundamental substance, lactic acid has a wide application in food, pharmaceutical and chemical industries, with a high annual production all over the world. About 50% of this acid is produced through fermentation, using homofermentative bacteria from sources which contain lactose.

Whey, a waste in the dairy industry, is a serious source of environmental pollution. However, this waste substance contains lactose, proteins, vitamins and mineral salts, having many applications; one of these applications is the use of lactose, as the raw material for production of lactic acid by fermentation.

Various methods have been considered for production of lactic acid using immobilized cell by fermentation. In most of these methods, different kinds of gel have been employed for immobilizing cells (Entrapment). Although the degree of cell adsorbance is high in these methods, they are costly [1-3]. Other methods such as adsorption on solid support have received less attention [4]; although, they have been used for immobilization of yeast cells on wood chips to produce ethanol from whey [5]. The aim of the present paper is batch and continuous production of lactic acid from whey using *L.casei* immobilized by adsorption onto solid supports. Wood chips are regarded suitable because they are cheap and have a lot of pore resulting in saving and reasonable adsorption of microorganisms.

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MATERIALS AND METHODS

Microorganism and Culture Condition

Lactobacillus casei (PTCC. No 1608) was propagated in 100 ml MRS broth (Merck, Cat. No. 10661) at 30°C for 24 h. Afterwards the broth containing 0.55 g of *L.casei* cells, was harvested through centrifugation at 10000 g for 20 min and the cells were resuspended in 100 ml fresh sterile MRS broth.

Immobilization of Cells

Batch and continuous methods were investigated for immobilization of *L.casei* on various supports.

Batch Method

1 g of support (wood chips or brick or porous glass) was placed in a 250-ml flask and sterilized at 124°C for 20 min. Then, 100 ml suspended cell was added

to that flask and was incubated at 30°C. This complex was shaken slowly every few hours.

The excess of suspended cells was discarded after 24 h of incubation and the supports were washed with 0.1 w/v sterile glucose solution to remove nonadsorbed cells. Meanwhile, the immobilized cells remained on the supports.

Continuous Method

The packed-bed reactor used for immobilization studies consisted of a glass column with height and i.d. of 16 and 1 cm, respectively. During immobilization, cell suspension (5 g/l) was circulated from a surge tank through the packing of supports in an upflow mode of operation.

The cell suspension was shaken continually in the surge tank. The column reactor, together with the support material and connecting tubes, were presterilized in an autoclave at 121°C for 30 min.

The cell suspension in the surge tank was pumped with a peristaltic pump at a constant dilution rate through a tube to the bottom of the packed column and upward through the column.

The effluent from the top of the packing was then recycled to the surge tank. The dilution rate should be less than the maximum specific growth rate (μ max) to avoid washout. The temperature of the column was also adjusted at 30°C. The immobilization circuit is, then, washed by the passage of 0.1 w/v glucose solution in a once-through mode after 24 h in order to remove the nonadsorbed cells from the column.

These two methods were based on adsorption but for eggshell, covalent bonding is used with glutaraldehyde. This immobilization is done as follows.

The eggshell was ground and sifted in a 40 mesh screen. Then, it was washed with distilled water several times and dried in an oven at 40°C. The 2% (w/v) solution of glutaraldehyde was obtained using citrate-phosphate buffer (pH = 5.8). Eggshell and glutaraldehyde were mixed with the proportion of 1:7 and shaken at 100 rpm for 30 min at 30°C. Then, the eggshell was washed with distilled water three times and was centrifuged to remove the excess of glutaraldehyde. The cell suspension, in a sterile decantor, was added to the eggshell in 10 ml of MRS which was shaken in a 250-ml flask at 50 rpm. Shaking was continued after the end of the cell suspension for 30 min until the process was completed.

This mixture was incubated at 30°C for 24 h. Then, the excess of cell suspension was discarded and the eggshell was washed to remove the nonadsorbed cells.

Preparation of Whey

The cheese whey was autoclaved at 124°C for 30 min in order to precipitate the protein. The precipitated protein was removed by filter paper after which a light green liquid was obtained. The pH of the whey (4.5) was adjusted to 5, 5.5 and 6 with 5 N, NaOH.

Subsequently, the deproteinized whey was supplemented with 2.5 g/l yeast extract and 0.03 g/l $MnSO_4$,H_2O [4]. The enriched whey was autoclaved at 124°C for 15 min.

Fermentation Condition

Batch Fermentation

Fermentation was carried out in a 250-ml flask, containing 100 ml of prepared whey and 1 g of support particle with immobilized cells of *L.casei*. Three of these selected 250-ml flasks with adjusted pH of 5, 5.5 and 6 were incubated at four temperatures (28°C, 30°C, 32°C and 35°C) under stationary conditions for 5 days.

The yield of fermentation and lactic acid concentration were measured after every 24 h.

Continuous Fermentation

Fermentation was carried out in a packed-bed reactor consisting of a glass column containing 1 g of support particles with immobilized cells of *L.casei*. The prepared whey in a store tank was pumped with a peristaltic pump through a tube to the bottom of the packed column and upward through the column.

The effluent from the top of the packing was stored in the production tank. D was set at 0.2 h^{-1}.

Temperature and pH of this process were adjusted by using the optimum condition from batch fermentation. The yield of fermentation and concentration of produced lactic acid were measured every 24 h, for 6 days.

Analytical Techniques

Initial lactose concentration of whey was determined through Somogi and Nelson spectroscopy method [6]. The concentration of free living and immobilized cells on various supports was measured via spectrophotometer method using standard curve of Abs. vs . cell dry weight at 600 nm [7].

Lactic acid concentration was estimated by HPLC using a SPHERISORB C18 column. The column was eluted with 0.2 M H_3PO_4 at a flow rate of 0.8 ml/min for 20 min. The retention time of lactic acid under these conditions was 3.68 min. The UV detector was adjusted at 210 nm. Each experiment was repeated

Table 1. Immobilization results for batch and continuous methods.

Type of Support	Immobilization System (as 1 g Support)	
	Batch (mg/l)	Continuous (mg/l)
Wood chips	150	200
Brick particle	20	50
Porous glass	80	100
Eggshell with glutaraldehyde	85	–

three times and the results were presented as an average of all repetitions.

RESULTS

Immobilization Results

The amount of adsorbed cells on various supports were determined at equal conditions and the results are presented in Table 1.

These results show that wood chips adsorbed the highest amount of cells and, therefore, was chosen here as the best support. Mulberry wood chips were used for immobilization because this type of wood has a lot of pore with suitable dimension for immobilizing lactobasillus. Brick particles also have a lot of pore, however, due to containing mineral ions, demonstrate unreasonable adsorbance. Porous glass is not as good as wood chips and glutaraldehyde, being poisonous, is not a good immobilization system.

Batch Fermentation

The yields of batch fermentation (the lactose convert percentage) from whey using immobilized cells of *L.casei* on wood chips have been presented in Table 2. The results demonstrate that the yield of batch fermentation approaches 80% after 5 days at T = 28°C and pH = 5.5. This is the highest yield among the various conditions studied.

However, it was found that yield of fermentation at T = 28°C and pH = 5.5 cannot be chosen as the optimum condition for continuous system as it takes a long time for *L.casei* to adapt to these conditions; while conditions under which steady state is approached as soon as possible is desirable.

According to these points and the results presented in Table 2 and shown in Figure 1a to 1d, continuous fermentation will be attained at T = 32°C and pH = 5.

Continuous Fermentation

The Continuous Packed-Bed Reactor (CPBR) used for fermentation contained wood chips particles with immobilized cells of *L.casei* under optimum conditions, which were determined based on batch fermentation results. The pH in the glass column was adjusted to 5. The dilution rate was chosen as 0.2 h^{-1} ($D < \mu max$). During the reactor operation, there were no cells in the

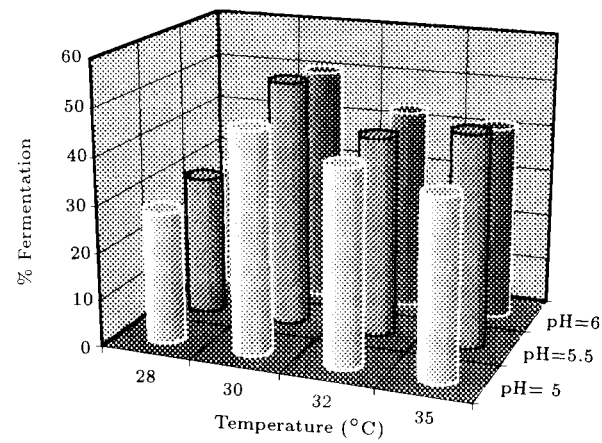

Figure 1a. Result of fermentation in batch reactor after 24 h.

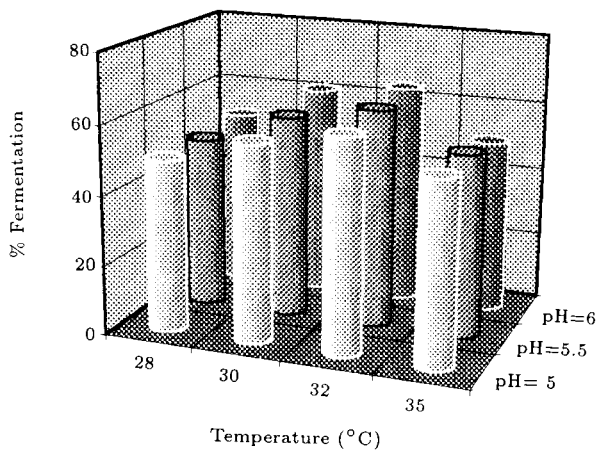

Figure 1b. Result of fermentation in batch reactor after 48 h.

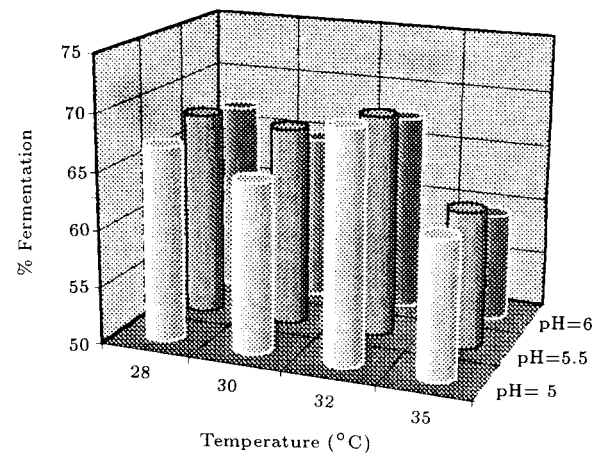

Figure 1c. Result of fermentation in batch reactor after 72 h.

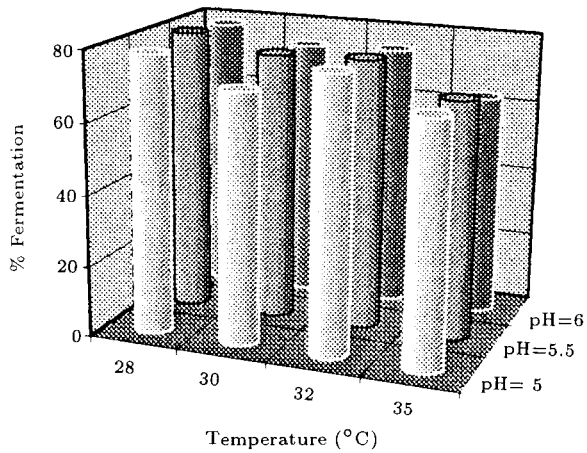

Figure 1d. Result of fermentation in batch reactor after 120 h.

Table 2. Yield of fermentation for batch reactor.

Time (h)	Temp. (°C)	pH = 5	pH = 5.5	pH = 6
24	28	27.9	29.7	27
	30	46.8	52.2	50.4
	32	41.4	42.3	43.2
	35	37.8	45	41.4
48	28	49.5	49.5	51.3
	30	56.8	58.5	61.25
	32	61.3	63	64
	35	53	53	50.4
72	28	67	68	67
	30	64.8	67.5	64.8
	32	70.2	69.4	67.5
	35	62	62	59.5
120	28	78.4	80	78.9
	30	70.3	75.7	73.9
	32	77.5	76.5	74.7
	35	68.5	67.5	63

Table 3. Continuous reactor results.

Time (h)	%Fermentation
24	45
48	70.26
72	71.16
96	72.06
120	72.96
144	73.89

effluent of the reactor. The results of this reactor are presented in Table 3, demonstrating that this system will attain steady state after 48 h (Figures 2 and 3).

CONCLUSION

The immobilization results presented in Table 1 illustrate that among the various supports studied, the

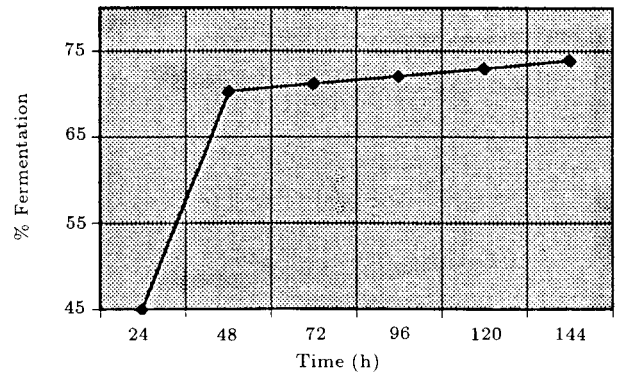

Figure 2. CPB fermentation at T = 32°C, pH = 5 and D = 0.2 (1/h).

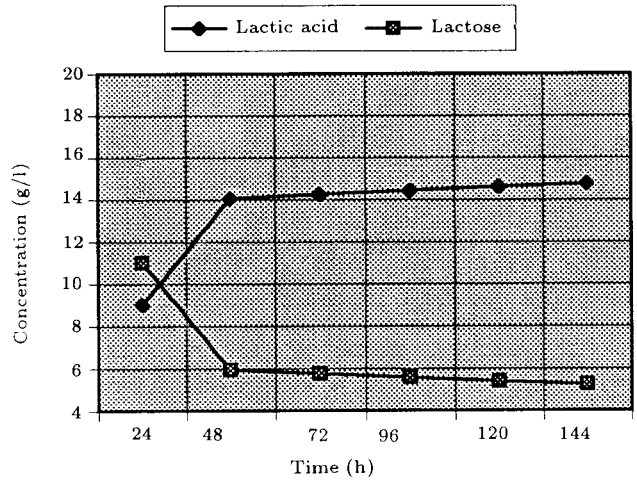

Figure 3. Production in CPBR.

highest amount of *L.casei* was adsorbed on wood chips. This was even higher than the covalent bonding method using eggshell and glutaraldehyde. These results were repeated with acetobacter for production of acetic acid and yeast cell for production of ethanol from whey, demonstrating that the wood chips are the best support for immobilization and highly suitable for these types of microorganisms. The highest production of lactic acid was observed in batch fermentation at T = 28°C and pH = 5.5 with 80% fermentation yield. This result is higher than fermentation yield (63%) obtained with free cells under equal conditions, showing that the immobilization process results in increase of yield because this process supports microorganisms against the environmental changes.

Another advantage of immobilized cell system is the ease of separation of microorganisms from the products in the reaction system, as a result of which, the microorganisms could be used in the reactor repeatedly or in the various types of continuous reactors.

In the continuous system, the mean yield of fermentation was 73%, which is 4% less compared to the batch system. This result can be explained by paying attention to the dimension of continuous reactor which

is smaller than that of the batch reactor. Furthermore, the continuous system is capable of converting more whey than the batch system, which can be shown by the fact that in the continuous system 240 ml of whey was converted but this amount was reduced to 50 ml for the batch system after 5 days. Considering these points, the productivity (g/l/h) of a continuous system will be more than the batch system after 5 days.

The highest concentration of lactic acid (16 g/l) in the batch fermentation was attained at 28°C and pH = 5.5 after 5 days. The highest concentration in continuous fermentation was obtained at 32°C and pH = 5 after 5 days.

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