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ORIGINAL PAPER

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Coupled lactic acid fermentation and adsorption

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Abstract Polyvinylpyridine (PVP) and activated carbon were evaluated for coupled lactic acid fermentation and adsorption, to prevent the product concentration from reaching inhibitory levels. The lactic acid production doubled as a result of periodical circulation of the fermentation broth through a PVP adsorption column. The adsorbent was then regenerated and the adsorbed lactate harvested, by passing 0.1 N NaOH through the column. However, each adsorption–regeneration cycle caused about 14% loss of the adsorption capacity, thus limiting the practical use of this rather expensive adsorbent. Activated carbon was found much more effective than PVP in lactic acid and lactate adsorption. The cells of *Lactobacillus delbrueckii* subsp. *delbrueckii* (LDD) also had strong tendency to adsorb on the carbon. A study was therefore conducted using an activated carbon column for simultaneous cell immobilization and lactate adsorption, in a semi-batch process with periodical medium replacement. The process produced lactate steadily at about 1.3 g l^{-1} h^{-1} when the replacement medium contained at least 2 g l^{-1} of yeast extract. The production, however, stopped after switching to a medium without yeast extract. Active lactic acid production by LDD appeared to require yeast extract above a certain critical level (<2 g l^{-1}).

Introduction

Lactic acid production has attracted increasing attention, primarily because of the desirable biocompatibility and biodegradability of its polymer and copolymers (Drumright et al. 2000; Wehrenberg 1981). Approximately half of the lactic acid consumed is derived from fermentation, the other half from chemical synthesis (Vickory 1985). Many attempts have been made to improve the fermentation productivity, which suffers from serious product inhibition. $CaCO_3$ is commonly added in industrial lactic acid fermentation, to minimize the product inhibition by calcium lactate [$Ca(La)_2$] precipitation. Lower productivity, however, has been observed due to the accumulation of calcium ions (Nomura et al. 1987). Many investigators studied the extraction of lactic acid into an immiscible solvent phase (Brink and Tramper 1985; Yabannavar and Wang 1991b). While lactic acid can be removed rather effectively, a major concern remains with the potential inhibition/toxicity to cells caused by the solvent and/or the extractants (such as amines) added to enhance the extraction capacity (Yabannavar and Wang 1987, 1991a). Electrodialysis has also been used to remove the lactic acid produced (Ishizaki and Vonktaveesuk 1996; Nomura et al. 1987; Xuemei et al. 1999; Yao and Toda 1990). While it may increase the fermentation rate by up to 60% (Freidman and Gaden 1970), the approach faces the problems of membrane fouling, deionization of the fermentation broth, and a higher operating cost (Wang et al. 1988).

Many adsorbents have been examined for lactic acid removal from fermentation (Aradhana et al. 1992; Davidson and James 1992; Moldes et al. 2001; Ye et al. 1996). Compared with extraction, adsorption offers the advantage of low or no negative effects to cells (Aradhana et al. 1992; Davidson and James 1992). Adsorption is also potentially simpler and cheaper than electrodialysis. However, ion-exchange resins also remove essential anions other than lactate from the broth. Non-ion-exchange adsorbents deserve more attention. Previously, we reported the adsorption characteristics of lactic acid and lactate on two such adsorbents, polyvinylpyridine (PVP) and activated carbon (Chen and Ju 1998). These adsorbents were further evaluated in this work for coupled fermentation and adsorption.

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Materials and methods

Microorganism, media, and adsorbents

Lactobacillus delbreuckii subsp. *delbreuckii* (LDD; ATCC 9649) was used in this study. The stock culture was maintained at 4 °C on tomato juice agar slants and subcultured monthly. Active cells from the stock culture, after about 24 h incubation at 42 °C in 10-ml medium (composition given below), were inoculated into 200 ml of medium. After incubation for 15 h at 42 °C in a shaker, the liquid culture was used as the inoculum for the fermentor.

The medium used for stock-culture agar slants contained (per liter): 10 g of tryptone (Difco 0123), 200 ml of filtered tomato juice (pH 7.0), and 11 g of agar. The liquid medium for inoculum preparation and lactic acid production contained glucose (concentration varied for different studies), yeast extract at 10% of the glucose concentration unless specified otherwise, 1 g of $(NH_4)_2HPO_4$ l^{-1}, and 5 ml of a mixed-salt solution l^{-1}. The mixed-salt solution had the following composition (per liter): 115.0 g $MgSO_4 \cdot 7H_2O$, 24 g $MnSO_4 \cdot 2H_2O$, and 6.8 g $FeSO_4 \cdot 7H_2O$.

Two adsorbents, PVP (Reillex 425, Reilly Industries, Indianapolis, Ind.) and activated carbon (Filtrasorb 100, Calgon Carbon Corp., Pittsburgh, Pa.) were used in this study. The PVP resin is a weakly basic polymer with high stability for a broad pH range and temperature up to 260 °C; and it is insoluble in most solvents. Adsorbents were typically regenerated with a 5-fold volume of 1 N NaOH for 24 h.

Experimental setup and procedures

For comparison, fermentations were first made without pH control and with pH control by NH_4OH or $CaCO_3$. The fermentation temperature was always kept at 42 °C. The run without pH control was carried out in a shake flask at ~150 rpm. The pH-controlled runs were made in a 6-l fermentor (BioFlo IIc, New Brunswick Scientific, Edison, N.J.), slowly agitated with two two-blade turbines at 110 rpm for adequate mixing but minimal oxygen transfer. The pH was controlled at 5.5±0.1 by the addition of 8 N NH_4OH, except in the $CaCO_3$-added system where pH was self-regulated by precipitation of the lactic acid produced and by the buffering ability of the carbonate/bicarbonate formed in the reaction. In the latter system, the $CaCO_3$ powders were sterilized by dry heating at 160 °C for 6 h and added (at 0.5 M) to the medium before inoculation.

For the coupled fermentation and PVP adsorption, a portion of the broth (ca. 300 ml) was passed from the BioFlo IIc fermentor, through a PVP column (30 cm in height, 5 cm in diameter, without temperature control), and back to the fermentor. A 0.1 N NaOH solution was then pumped through the column to remove the adsorbed lactic acid and to regenerate the adsorbent. This adsorption–regeneration cycle was intermittently conducted, starting from 40 h. Samples were taken periodically from the fermentor and the harvest vessel.

A similar setup was used for the fermentation with cell immobilization in an activated carbon column. However, instead of the BioFlo IIc fermentor, a 500-ml magnetically stirred flask was used. pH and temperature were again controlled at 5.5 and 42 °C, respectively. The column temperature was controlled at 37 °C, using a heating tape. After being grown for 40 h in the flask containing 400 ml of medium, the broth was continuously circulated at 10 ml min^{-1} between the flask and the column. Part of the medium was harvested and replaced by fresh medium periodically.

Analytical methods

Samples taken from the fermentations were centrifuged (at 6,000 *g*) to separate the cells from the supernatants. The former were resuspended in distilled water and measured for their optical density values at 610 nm (OD_{610}), which were converted to cell-dry-weight concentrations using an established calibration. For samples from fermentation with $CaCO_3$ addition, the OD_{610} decreased rapidly initially and then reached relatively steady readings, because the precipitates of $CaCO_3$ and $Ca(La)_2$ settled much faster than cells. The steady readings were used for estimation of cell concentrations. The supernatants were analyzed for glucose and lactate concentrations. Glucose concentrations were measured by oxidase methods (No. 510, Sigma). The total lactate concentrations, including both the free acid and the dissociated lactate ions, were also determined enzymatically, using lactate oxidase and peroxidase (No. 735, Sigma). For samples from the system with $CaCO_3$ addition, H_2SO_4 was added to react with the $Ca(La)_2$ precipitate (forming lactic acid and calcium sulfate) before centrifugation to collect the supernatant for lactate analysis.

Results

Fermentation without and with pH control

The results of typical lactic acid fermentation without pH control are shown in Fig. 1A. The lactic acid production lasted only 12 h, with a maximum lactic acid concentration of about 9 g l^{-1}. While the product yield from glucose was very good (>95% at 12 h), less than 10 g of glucose l^{-1} was converted. The results from the fermentation with pH control by NH_4OH addition are shown in Fig. 1B. Active lactic acid production paralleled cell growth, reaching 50 g l^{-1} at 15 h. The culture then entered the stationary phase and the product concentration increased very slowly to 60 g l^{-1} at 40 h. Evidently, pH control alone is not enough for optimal lactic acid production. A mechanism to prevent the accumulation of lactate ions has to be included.

Fermentation with $CaCO_3$

Conventional lactic acid fermentations are carried out with an initial addition of excess $CaCO_3$. It reacts with lactic acid to form $Ca(La)_2$ precipitate and the carbonate neutralizes the hydrogen ions:

$$CaCO_3 + 2HLa \Leftrightarrow Ca(La)_2 + H_2O + CO_2 \quad (1)$$

Thus, the inhibition caused by both free acid and lactate ions may be alleviated.

The results obtained are shown in Fig. 2. $CaCO_3$ prevented the pH from dropping below 5.0. However, the lactic acid production was much slower than that in the fermentation with pH control by NH_4OH addition. Nonetheless, the precipitation of $Ca(La)_2$ reduced the concentration of inhibitory lactate ions. Production continued until the end of the fermentation (Fig. 2), while the production leveled off very early in the NH_4OH-controlled fermentation (Fig. 1B). The results confirmed the need for combining pH control with a better method for lactate removal.

Fermentation coupled with PVP adsorption

The time profiles observed are shown in Fig. 3 where, for easy comparison, the lactic acid concentration was calcu-

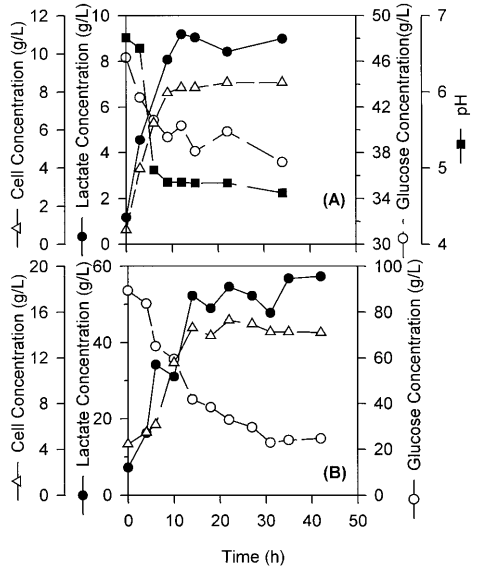

Fig. 1A, B Time profiles of lactic acid fermentation. **A** Without pH control. **B** With pH control by NH_4OH addition. *L* Litre

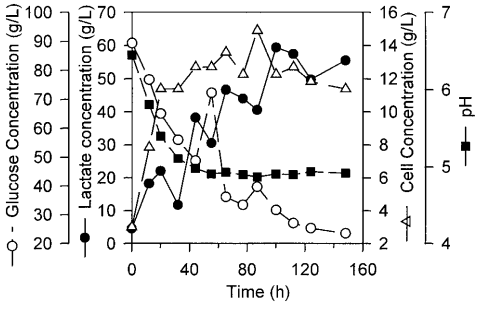

Fig. 2 Profiles of cell growth, lactate production, glucose consumption, and pH obtained in lactic acid fermentation with $CaCO_3$

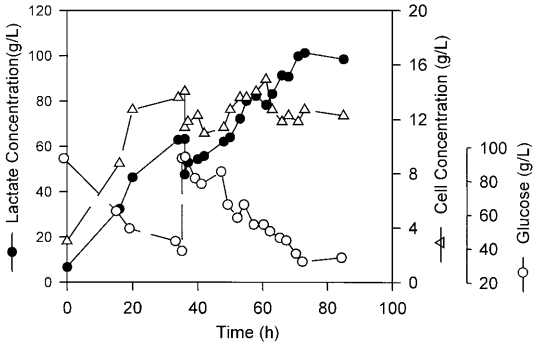

Fig. 3 Profiles of lactate production, glucose consumption, and cell growth in coupled lactic acid fermentation and polyvinylpyridine adsorption

lated as if all the lactic acid produced (including that harvested in the NaOH solution) was contained in the fermentation broth. The results of the first 40 h were similar to those obtained in the fermentation with pH control by NH_4OH addition (Fig. 1B): the total lactate concentration reached the inhibitory level of about 60 g l^{-1} and cell growth stopped. At 40 h, a concentrated medium (600 ml) containing 200 g of glucose, 2 g of $(NH_4)_2HPO_4$ and 20 g of yeast extract was added to prevent any nutrient limitation. With intermittent removal of lactic acid by adsorption, the glucose concentration dropped again and the lactic acid production continued. The total amount of lactic acid produced was almost double that obtained in the fermentation with pH control alone (Fig. 1B). The production, however, lasted only about 80 h. Afterwards, the adsorption capacity of the repeatedly regenerated PVP was no longer high enough to keep the broth lactate concentration lower than the inhibitory level.

Fermentation with cell immobilization and adsorption in activated carbon column

Taking advantage of the carbon's high affinity to LDD cells, we conducted an experiment with cells immobilized in a 570-ml column by adsorption and/or deep-bed filtration. The experiment involved five stages. In stage 1 (0–40 h), cells were grown in the 500-ml flask to a high concentration. The broth was then continuously circulated at 10 ml min^{-1} between the flask and the column (stage 2, 40–87 h). Lactic acid was removed and cells were immobilized in the column. Half of the spent medium was replaced daily with fresh medium of the following composition (per liter): 150 g of glucose, 1 g of $(NH_4)_2HPO_4$, 14.5 g of yeast extract, and 5 ml of the mixed salt solution. The operation in stage 3 (87–183 h) was the same as in Stage 2, except that the fresh medium for replacement contained 100 g of glucose l^{-1}, instead of 150 g of glucose l^{-1}. In stage 4 (183–318 h), the yeast extract concentration in the fresh medium was decreased to 2 g l^{-1}, to study its effect and the possibility of lowering the medium cost. Finally, in stage 5 (318–506 h), the medium was replaced every other day with pure glucose solution (100 g l^{-1}) to see whether lactic acid production could be maintained without yeast extract.

The lactate, glucose, and cell concentrations in the samples taken from the circulating medium are shown in Fig. 4A. The total quantities of lactate produced (including that harvested in the spent medium) and glucose consumed are given in Fig. 4B. The lactate concentration reached about 60 g l^{-1} in 40 h. Throughout stages 2–4, it stayed around that inhibitory level, except for a brief drop when the medium replacement was added early in stage 2. The high lactate concentration caused a rather low but steady lactate production rate, 1.25 g l^{-1} h^{-1}. Higher production rates can be expected with more frequent medium replacement to minimize the duration of inhibition due to high lactate concentrations.

The steady lactate production during stages 2–4 also suggested that the yeast extract concentration might be decreased (to 2 g l^{-1} in stage 4) without negative effects. However, when the medium was replaced by pure glucose solution in stage 5, the lactate concentration in the circulating medium increased only slightly during the

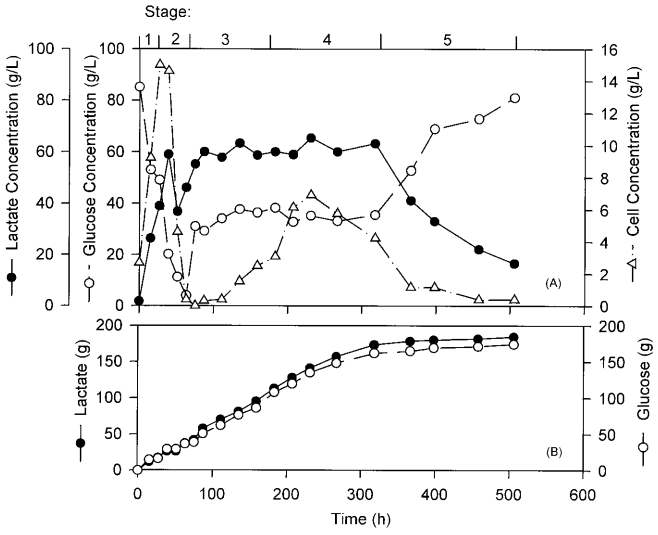

Fig. 4A, B Lactic acid fermentation with cell immobilization and lactate adsorption by activated carbon column. **A** Concentration profiles of lactate, glucose, and cells in the fermentation flask. **B** Amounts of total lactate produced and glucose consumed

first 2 days and then decreased rapidly, due to the dilution effect of medium replacement in the following 6 days (Fig. 4A). The initial lactate production could have been supported by the residual yeast extract remaining in the spent medium and that desorbed from the adsorbent. After the cells depleted the residual yeast extract, the lactate production dropped to a trivial rate of 0.062 g l^{-1} h^{-1} (Fig. 4B). A certain critical level (<2 g l^{-1}) of yeast extract was therefore necessary for maintaining active lactate production.

As for the cell concentration in the circulating broth, it decreased to about zero during stage 2 as the cells were immobilized into the activated carbon column (Fig. 4A). The cell concentration then began to increase during stage 3 as the further cell growth exceeded the immobilization capacity of the column. The cell concentration did not change significantly during stage 4, implying that the cell growth rate under the low yeast extract concentration (2 g l^{-1} in fresh medium) approximately compensated for cell removal by the daily medium replacement. In stage 5, no cell growth was possible from the pure glucose solution without a nitrogen source. As the medium replacement removed the cells present initially in the circulating broth, the cell concentration dropped rapidly to zero as expected (Fig. 4A).

Although not optimized, the results obtained in this study demonstrated the feasibility of using an activated carbon column as a simple and practical method of cell immobilization for continuous lactic acid production.

Discussion

In the fermentation without pH control, the short duration of microbial activity was apparently caused by the rapid decrease of pH accompanying the lactic acid accumulation. The observation agreed well with literature reports: the specific cell growth rate was reported to drop from 0.43 h^{-1} to 0.03 h^{-1} when 6 g l^{-1} of free (not neutralized) lactic acid was present (Yabannavar and Wang 1987). In the fermentation with pH control by NH_4OH addition, the performance improved because of the conversion of lactic acid into the much less inhibitory lactate ions. However, lactate ions also caused inhibition when accumulated to high concentrations. The critical concentration was found to be about 50 g l^{-1} for the LDD strain used in this study.

The slower lactic acid production in the fermentation with $CaCO_3$ was partly attributed to the negative effects of Ca^{2+}. The lactic acid productivity of LDD was reported to decrease with increasing Ca^{2+} concentration: only 60% of the productivity remained at 100 mM Ca^{2+} (Nomura et al. 1987). Since $CaCO_3$ is practically insoluble in water, the initial Ca^{2+} concentration in the fresh medium would be low and without deleterious effects. However, $Ca(La)_2$ formed with the production of lactic acid. The solubility of $Ca(La)_2$ is relatively high, i.e., about 5% at room temperature (Holten et al. 1971). Ca^{2+} concentration was therefore expected to increase along the lactic acid production.

Another factor contributing to the slower lactic acid production was pH. The optimal pH for lactic acid production by LDD was 5.5–6.0 and the productivity dropped by 22% at pH 5.0 (Yao and Toda 1990). The specific LDD strain used in this study appeared to be even more sensitive to low pH. As shown in the run without pH control (Fig. 1A), the cell growth and lactic acid production ceased when pH dropped to about 4.8. In the fermentation with $CaCO_3$ addition, pH was maintained by the buffer effect of $CaCO_3$ at about 5.0 after 30 h (Fig. 2). The cell activity afterwards could be significantly lower because of the unfavorable pH.

In the fermentation coupled with PVP adsorption, the PVP adsorption constant K_{ad} (=q/y, where q is the adsorbed concentration on PVP, in g kg^{-1}, y is the aqueous-phase concentration, in g $^{-1}$l) decreased from 0.95 to 0.25 in 17 cycles of regeneration (detailed data not shown). The incomplete regeneration was confirmed in a separately designed experiment (Chen and Ju 1998): after each contact with the fermentation broth and the subsequent regeneration using 0.1 N NaOH and a wash with distilled water, about 14% of the adsorption capacity was lost. The strong adsorption of some materials in the complex fermentation broth was likely responsible.

While the combined fermentation and adsorption was shown feasible for removing the inhibitory product, the cost of the adsorbent and its regeneration efficiency has to be carefully evaluated. PVP, quoted at U.S. $ 75 kg^{-1} for a 50-kg purchase, appears to be too expensive for lactic acid production, especially when considering the significant loss of adsorption capacity upon regeneration. Other methods, such as washing with strong organic solvents, may improve the regeneration efficiency but require additional step(s), e.g., solvent removal by gas stripping/vaporization or repeated water-washing, to ensure compatibility with the fermentation.

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