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Publication details, including instructions for authors and subscription information:
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**To cite this Article** Chen, Chun-Chiang and Ju, Lu-Kwang(1998) 'Adsorption Characteristics of Polyvinylpyridine and Activated Carbon for Lactic Acid Recovery from Fermentation of *Lactobacillus delbrueckii*', Separation Science and Technology, 33: 10, 1423 — 1437
**To link to this Article: DOI:** 10.1080/01496399808545058
**URL:** http://dx.doi.org/10.1080/01496399808545058

SEPARATION SCIENCE AND TECHNOLOGY, 33(10), pp. 1423–1437, 1998

# Adsorption Characteristics of Polyvinylpyridine and Activated Carbon for Lactic Acid Recovery from Fermentation of *Lactobacillus delbrueckii*

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## ABSTRACT

Polyvinylpyridine (PVP) and activated carbon were examined for the adsorption characteristics pertinent to their application in lactic acid fermentation. For PVP the linear adsorption constant, $K_{ad}$, was between 0.7 and 1.0 for an equilibrium pH range of 3 to 9. The pH was adjusted by acid/base addition, similar to pH control in fermentation. The values of $K_{ad}$ in the pH-adjusted systems were much lower than that reported for pure lactic acid solutions, i.e., about 9.7. Furthermore, no clear effect of pH was observed. These are attributed mainly to the competition of anions ($Cl^-$ and lactate) for the adsorption sites of protonated pyridinal N. Its adsorption capacity was also found to decrease with the base regeneration (by about 14% each time) after being contacted with the culture broth. These limit its potential application in lactic acid fermentation. Activated carbon was much more effective in lactic acid/lactate adsorption than PVP. At pH 5.5 (optimal for fermentation), the value of $K_{ad}$ of activated carbon was about 7. The adsorption further favored lower pH under acid (HCl) addition. Activated carbon has been reported to adsorb glucose. However, the presence of glucose in 0–70 g/L was found in this study to have an insignificant effect on lactate adsorption. Cells of *Lactobacillus delbrueckii* also adsorbed rapidly on activated carbon. This cell adsorption had a negative effect on lactate adsorption.

## INTRODUCTION

Lactic acid has attracted increasing attention recently because of its application in therapeutic products, environmental plastics, and food additives. The

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biodegradability and biocompatibility of polylactic acid and copolymers are especially desirable for many industrial and biomedical applications. For example, polylactic acid has been recognized as a biodisposable plastic which incinerates well and poses no threat to the environment (1–3). Biomedically, polylactic acid and its copolymers have been used in resorbable prosthetic devices (4) and as degradable matrices for sustained release of medicinal ingredients (5).

About half of the market demand for lactic acid is met by production from fermentation; the rest by chemical synthesis (6). Lactic acid fermentation can be made with different microorganisms, either homofermentative (producing lactic acid only) or heterofermentative (producing lactic acid and other organic compounds). Only the former, from the genera *Lactobacillus*, *Streptococcus*, and *Pediococcus*, are of industrial importance for lactic acid manufacture (7). Among them, *Lactobacillus delbrueckii* is a common species used when glucose is the primary substrate in the fermentation (8, 9).



However, if accumulated in the medium, the lactic acid produced by the microorganisms inhibits both cell growth and further lactic acid synthesis in the fermentation (8). This occurs in two forms: if not neutralized, the lactic acid leads to a marked pH drop which, in turn, effects a general inhibition to cell function; upon neutralization, the accumulation of lactate ions gradually reduces the enzymatic activity by end-product inhibition. For *L. delbrueckii* the specific cell growth rate was found to drop from 0.43 to 0.03 $h^{-1}$ when 6 g/L of free (unneutralized) lactic acid was present (8). Similar inhibition was observed when the dissociated lactate ions accumulated to 50–60 g/L (8).

The product inhibition calls for its continuous removal from fermentation broth to enhance the productivity. Many methods have been tried, including calcium lactate precipitation (6, 10), amine extraction (8, 11), electrodialysis (12–14), and adsorption (15, 16).

With the precipitation method, calcium carbonate is added to react with the lactic acid produced, forming calcium lactate precipitate and preventing the lowering of pH (6). The lactic acid production was, however, found to slow down with the fermentation and was attributed, at least partly, to the negative effects of calcium ions (10). The lactic acid productivity of *L. delbrueckii* had been reported to decrease with increasing $Ca^{2+}$ concentration: only 60% of the productivity remained when 100 mM $Ca^{2+}$ was present in the medium (12). While $CaCO_3$ is practically insoluble in water, the solubility of calcium lactate is relatively high, i.e., about 50 g/L at 20°C (17). An increase in $Ca^{2+}$ concentration in the medium can therefore be expected to occur along with the lactic acid production.

A continuous removal of lactic acid can be achieved with electrodialysis (12–14). However, this may be complicated by the problems of membrane

fouling caused by the cells (12) and the removal of other essential ions from the medium during the process (12, 14). Amine extraction of lactic acid has also been studied (8, 11, 18, 19). Its inhibitory effects to cell growth, which compromise its application in situ, are well documented (8, 11). Adsorption has been reported for its capability to remove lactic acid from the fermentation broth without any direct negative effects on the cells (15, 16). The use of an ion-exchange resin (15) is, however, expected to remove anions other than lactate from the broth also. Other adsorbents that do not rely on ion exchange as the main mechanism for lactate removal deserve some more attention. Polyvinylpyridine (PVP) and activated carbon have been proposed to be used for this purpose (20, 21) but without detailed information on their pertinent adsorption characteristics.

In this work the adsorption characteristics of PVP and activated carbon are evaluated with emphases on their application to lactic acid fermentation.



## MATERIALS AND METHODS

### Microorganism and Medium

The microorganism used in this study for lactic acid fermentation was *Lactobacillus delbrueckii* (ATCC 9649). It was maintained with regular subculturing on tomato juice agar slants: tryptone (Difco 0123), 10 g/L; filtered tomato juice, 200 mL/L; and agar, 11 g/L. Inocula for the fermentor runs were prepared from the stock culture by incubation in shake flasks for 15 hours at 42°C. The medium used for inocula preparation and lactic acid fermentation contained glucose, 10 to 100 g/L, depending on the different study purposes; yeast extract, 1/10 of the glucose concentration; $(NH_4)_2HPO_4$, 1 g/L; and a mixed-salt solution, 5 mL/L. The mixed-salt solution had the following composition: $MgSO_4 \cdot 7H_2O$, 115.0 g/L; $FeSO_4 \cdot 7H_2O$, 6.8 g/L; and $MnSO_4 \cdot 2H_2O$, 24.0 g/L. A more detailed description about the experimental setup and procedures for lactic acid fermentation is available (10).

### Adsorbents and Adsorption Study

Two adsorbents, polyvinylpyridine (Reillex 425 from Reilly Industries, Indianapolis, IN) and activated carbon (Filtrasorb 100 from Calgon Carbon Corp., Pittsburgh, PA), were used in this study. The PVP resin is a weakly basic polymer (Fig. 1). It is insoluble in most solvents and has high stability for a broad pH range and temperatures up to 260°C (22).

Unless mentioned otherwise, the adsorption study was made by contacting the adsorbent with the medium at a ratio of 1 g to 5 mL for 24 hours at room temperature (22 ± 2°C). Dilute acid or base (0.1 N HCl or NaOH) was used to adjust the pH to the studied values. In experiments to generate complete

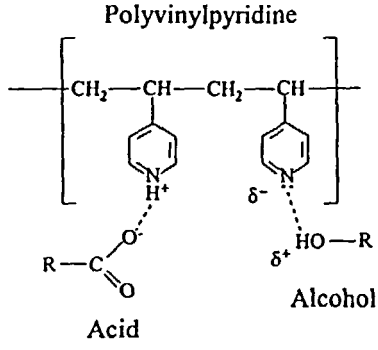


FIG. 1 Structure and proposed adsorption mechanisms of polyvinylpyridine.



isotherms, the initial aqueous-phase lactic acid concentration ranged from 5 to 60 g/L, the latter being the limiting concentration found to completely inhibit further lactic acid production in the fermentation (10). In many other experiments to examine the effects of pH and glucose on lactate adsorption, the initial lactic acid concentration was kept at 5 g/L. At this dilute range, the isotherm could be reasonably approximated with a linear dependency, as shown later. The effects of pH and glucose could therefore be examined by comparing the linear adsorption constant ($K_{ad}$), which is defined as the ratio between the loading on the adsorbent (in g of lactic acid per kg of adsorbent) and the remaining aqueous-phase concentration (in g of lactic acid per liter of aqueous solution) at equilibrium.

Regeneration of the adsorbents was done by contacting the adsorbents with 1 N NaOH at a weight-to-volume ratio of 1:5, followed by washing with distilled water.

Clearer presentations of the details of the individual adsorption studies are given in the Results Section.

## Analytical Methods

For fermentation samples, centrifugation was used to separate the cells from the supernatant. The supernatant was then analyzed for glucose and lactic acid concentrations. The cell pellet was washed once and resuspended in distilled water for determination of cell concentration.

### *Cell Concentration*

The optical density at 610 nm of the cell suspension was measured with a spectrophotometer and used to determine the cell concentration according

to a preestablished calibration curve. The cell dry-weight concentrations used for developing the calibration curve were obtained after drying the washed cell pellets from centrifugation to constant weights at 90°C for 48 hours.

### *Glucose Concentration*

Glucose analysis was done by the oxidase method (Sigma No. 510). Following the enzyme-catalyzed reaction, the color intensity of the solution was measured at 450 nm with a spectrophotometer. The glucose concentration was then determined according to a linear calibration curve established with three standard solutions of different glucose concentrations. The standard solutions were analyzed simultaneously with the samples.

### *Lactate Concentration*

Lactate concentration in the liquid sample was determined by an enzymatic method involving both lactate oxidase and peroxidase (Sigma No. 735). The lactate was first converted to pyruvate and $H_2O_2$ by lactate oxidase. Peroxidase then catalyzed the reaction of $H_2O_2$ and the chromogen precursors to produce a colored dye with a maximum absorbance at 540 nm. The optical density at 540 nm can therefore be used to determine the lactate concentration with the calibration curve established with three standard solutions analyzed simultaneously with the samples. Replicate assays were always performed. The precision of the analysis typically had standard deviations in the 1–3% range. The samples were analyzed again when the standard deviations of the replicate assays were larger than 5%.



## RESULTS AND DISCUSSION

### Effect of pH Swing on Cell Activity

Many potentially effective lactic acid removal techniques, such as reactive extraction using amines (8, 11, 18) and adsorption using adsorbents with basic functional groups (22), rely on the mechanism of acid–base interaction which strongly favors the medium pH lower than the $pK_a$ of lactic acid [i.e., 3.8 at 25°C (17)]. The effects on cells of lowering the broth pH for a brief period to achieve the desired lactate removal were therefore examined.

The adequate duration of the pH swing was first determined by examining the rate of lactic acid adsorption on PVP in a gently mixed vessel. Twenty milliliters of a 5 g/L lactic acid solution were contacted with 4 g of PVP and the pH followed. The pH was observed to stop changing after 15 minutes, indicating that equilibrium had been reached. The duration of pH swing was therefore chosen as 15 minutes in the following study.

The experiment of pH swing was conducted in four magnetically stirred 50-mL bottles. Each contained 30 mL of the fresh cultivation medium with 20 g/L of glucose. Three mL of the culture, harvested at the exponential growth phase, were inoculated into each bottle and incubated for 3 hours. The pH dropped to about 6.0, indicating active cell metabolism. A sterile HCl solution (0.1 N) was then added to three of the bottles in volumes of 0.5, 0.8, and 1.1 mL, respectively, to bring the pH of the corresponding bottle down to 3.6, 2.7, and 2.3. The fourth bottle served as the control (without being subjected to the pH swing). After 15 minutes the same volumes of a sterile 0.1 N NaOH were added into the three bottles to neutralize the acid added previously. The pH was then measured every 3 hours to monitor the recovery of cells from the pH swing. The premise was that lactic acid production by the recovered cells would lead to a continual decrease of pH.

The pH profiles obtained are shown in Fig. 2. For all the systems with a brief pH swing, the cells were found to recover their lactic acid production capability after a lag phase of about 3 to 5 hours. The experiments were repeated twice to cover a broader swing range of pH, i.e., from 1.7 to 4.4.



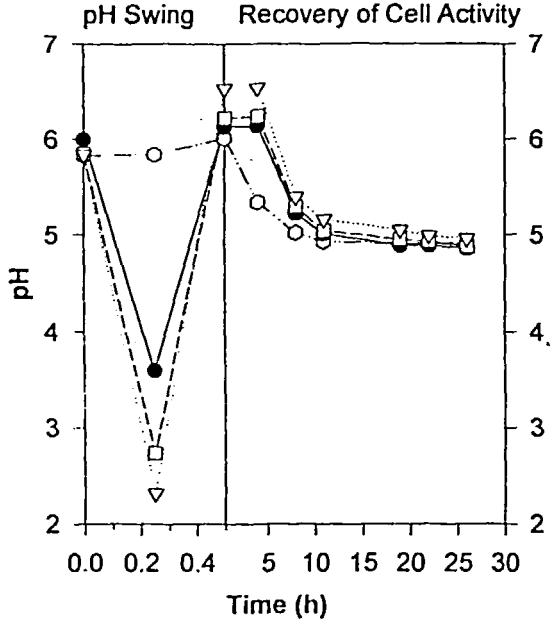


FIG. 2 Recovery of cell activity following pH swing. The cell activity was monitored by the drop of pH as a result of lactic acid production.

The same conclusion was obtained, although the duration of the lag phase varied with the extent of pH swing (results not shown). *L. delbrueckii* was therefore very acid-tolerant to allow for the incorporation of pH swing for lactic acid removal, if necessary. Nevertheless, the lag phase associated with the pH swing process, if performed frequently, could compromise the overall productivity of lactic acid fermentation.

## Adsorption Characteristics of Polyvinylpyridine

### *Effect of pH*

The adsorption isotherms of lactic acid on PVP were reported in the literature for two different temperatures, 25 and 90°C (23). The results can be well described by Langmuir equations (with $R^2 = 0.99$):

$$q = \frac{(392)y}{40.4 + y} \quad \text{at } 25°C$$

$$q = \frac{(196)y}{70.3 + y} \quad \text{at } 90°C$$

where $q$ refers to the loading on PVP (in g lactate/kg adsorbent) and $y$ refers to the equilibrium total lactate concentration in the aqueous solution (in g/L). Pure lactic acid solutions were used in the study, and the lowest equilibrium acid concentration involved was about 10 g/L. Consequently, the pH values of the systems (<1) were much lower than the $pK_a$ of lactic acid. Practically all lactic acid molecules were present in the free acid form. This was obviously far from the situation encountered in the fermentation.

Because pH swing was shown to incur an undesirable lag in the recovery of cells, the effect of pH on lactic acid adsorption was examined to see if pH swing would be necessary. Five bottles were used in the study. Each contained 20 mL of 5 g/L lactic acid solution that had been neutralized by 0.1 N NaOH to simulate the base addition for pH control in the fermentation. The pH values were then readjusted to 2.5–7.0 by addition of 0.1 N HCl. Four grams of PVP were added, i.e., in a 1:5 weight-to-volume ratio. After being contacted for 24 hours to ensure that equilibrium was reached, the supernatant and solids were separated. The remaining lactic acid concentrations in the supernatants were determined. Because of the low acid concentrations employed, linear adsorption isotherms were assumed and the equilibrium constants ($K_{ad}$) calculated. The results obtained at different pH values are summarized in Table 1 No clear trend of the effect of pH can be seen and the variation of $K_{ad}$ was not very significant (0.7 to 1.0) despite the wide range of equilibrium pH values (3 to 9) involved.

TABLE 1
Effect of pH on Lactic Acid Adsorption on Polyvinylpyridine

| pH | | Aqueous lactate concentration (g/L) | | |
|---|---|---|---|---|
| Initial | Equilibrium | Initial | Equilibrium | $K_{ad}$ |
| 6.88 | 8.76 | 5.0 | 4.2 | 1.0 |
| 4.34 | 5.33 | 5.0 | 4.4 | 0.7 |
| 3.38 | 3.98 | 5.0 | 4.3 | 0.8 |
| 3.06 | 3.61 | 5.0 | 4.2 | 1.0 |
| 2.48 | 3.17 | 5.0 | 4.4 | 0.7 |

PVP is a weakly basic polymer resin. The adsorption is supposed to depend on acid–base interaction and favors low pH ($< pK_a$). There are two possible reasons for the observed contradiction.

First, there are two functional groups in lactic acid, carboxylic acid and alcohol (Fig. 1). PVP has been reported to adsorb both organic acids [e.g., butyric acid and acetic acid (24, 25)] and alcohols [e.g., butanol (24)]. The adsorption of lactic acid on PVP may, therefore, occur through either the acid or the hydroxyl group. While the former is expected to be strongly pH-dependent, the latter is not. The selectivity of PVP to these two functional groups of lactic acid deserves further investigation.

Second, adsorption by acid–base interaction proceeds through protonation of the pyridinyl N. The protonated PVP may pick up the anions of other mineral acids, such as $Cl^-$, $SO_4^{2-}$, etc., rather than the lactate ions. In this adsorption experiment, higher $Cl^-$ concentrations were present in the systems of lower pH because HCl had been added for the initial pH adjustment. The competition of $Cl^-$ for the adsorption sites of PVP might have compromised the positive effect of low pH so that the adsorption of lactic acid appeared to be no specific function of pH.

The occurrence of the proposed compromise is supported by the much lower adsorption constants $K_{ad}$ obtained in this study than in pure lactic acid solutions. As shown in Table 1, the values of $K_{ad}$ obtained in this study are all no larger than 1. On the other hand, the value of $K_{ad}$ in pure lactic acid solutions is approximately 10, as indicated by the best-fit adsorption isotherm at 25°C. Protonated PVP appears to have higher affinity to $Cl^-$ than to lactate ions. The same phenomenon of anion competition has been observed in the extraction of lactic acid with tertiary amines which have functional structures similar to PVP (19, 26). More study is required to fully characterize the adsorption of acid and salt mixtures by PVP. Nevertheless, the results suggested that the pH swing was not beneficial.

### PVP Regeneration

A 3-L lactic acid fermentation was made in a bioreactor (BioFlo IIc, New Brunswick Scientific) coupled with an adsorption column (30 cm in height and 5 cm in diameter) loaded with 300 g of PVP. The pH in the fermentor was controlled at 5.5 with 8 N $NH_4OH$. After 40 hours of batch fermentation, a portion of the cell-containing fermentation broth (300 mL, metered by a calibrated peristaltic pump) was slowly circulated through the adsorption column and returned to the fermentor. Sterile air was passed through the column for about 10 minutes to facilitate drainage of broth from the column. A fresh 0.1 N NaOH solution (300 mL) was then pumped into the column to remove the adsorbed lactic acid and to regenerate the adsorbent. The equilibrated NaOH solution was collected in a harvest vessel. Samples were taken from both the fermentor and the NaOH harvest. The concentrations of lactic acid could then be used to estimate the values of $K_{ad}$ of the adsorbent after each base regeneration.



This adsorption/desorption cycle operation was repeated intermittently. With the intermittent lactic acid removal, the total amount of lactic acid produced reached about 300 g, much higher than that obtainable in a regular pH-controlled batch, i.e., 180 g (10). The lactic acid production eventually ceased at about 80 hours when the adsorption capacity of the regenerated PVP was no longer high enough to keep the lactate concentration in the fermentation broth lower than the inhibitory level. The decrease of adsorption efficiency of the PVP column with the number of regenerations, in terms of $K_{ad}$, is evident in Fig. 3.

This conclusion was further confirmed in a separately designed regeneration test, where each base (0.1 N NaOH) regeneration was followed by a thorough wash with distilled water. It was found that about 14% of the adsorption capacity was lost with each regeneration from contact with the culture broth. This could be attributed mainly to the irreversible adsorption of other organic materials in the complex fermentation broth on PVP.

While the approach of combined fermentation and adsorption to remove the inhibitory product was shown to be feasible, the cost of the adsorbent and its regeneration efficiency have to be carefully evaluated. For PVP, quoted at $75/kg for a 50-kg purchase, the significant loss of adsorption capacity upon regeneration represents a serious obstacle to its application in lactic acid fermentation. Of course, other methods such as the wash with organic solvents may be used to improve the regeneration efficiency. However, an operation to make the desorption process compatible with fermentation is likely to become more complicated. It requires the removal of extraction solvent by gas stripping/vaporization or by repeated water washes.

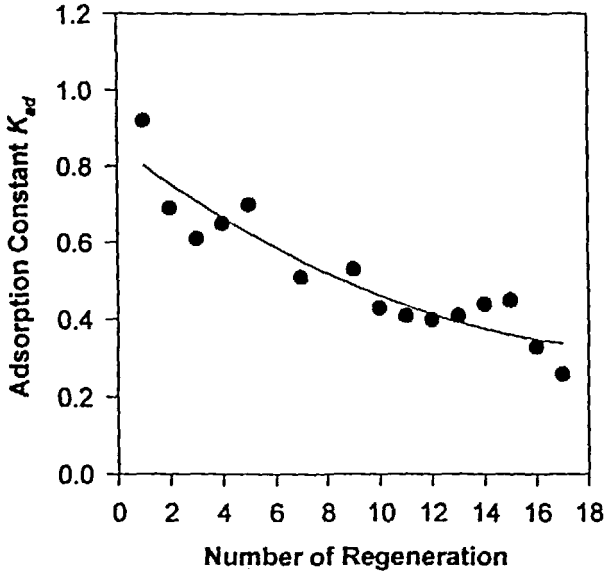


FIG. 3 The decrease of adsorption constant $K_{ad}$ of polyvinylpyridine with the regeneration by 0.1 N NaOH. The smooth curve is the result of a second-order regression.



## Adsorption Characteristics of Activated Carbon

### *Effect of pH*

For similar reasons and with a procedure similar to the study with PVP, the effect of pH on lactic acid adsorption was also examined with activated carbon as the adsorbent. The initial pH ranged from 3 to 13, adjusted with 0.1 N NaOH or HCl. The results are shown in Table 2. The adsorption constant ($K_{ad}$) of activated carbon increased with decreasing pH, indicating more effective adsorption of lactic acid molecules than of lactate ions. Note that at pH 5.5, which was optimal for lactic acid fermentation, the value of $K_{ad}$ of the activated carbon could be 7- to 8-fold higher than that of PVP.

### *Adsorption Isotherm at pH 5.5*

The adsorption isotherm at 22 ($\pm$ 0.5)°C was determined using a fresh fermentation media containing no glucose but various initial lactic acid concentrations, ranging from 5 to 60 g/L. The pH was adjusted to 5.5 by 0.1 N NaOH in all systems. These solutions were contacted with activated carbon in a fixed volume-to-weight ratio of 5:1. The pH values were measured

periodically and readjusted to 5.5. When the pH no longer changed, the remaining lactic acid concentrations in the aqueous solutions were analyzed and the loading on the solid phase was calculated from the material balance. The results are shown in Fig. 4. Within the examined range, the best-fit empirical Freundlich equation was

$$q = (14.6)y^{0.52}$$

with the standard error of $q$ being 5.1 ($R^2 = 0.97$).

### Effect of Glucose

Serving as the carbon and energy sources for cells, glucose is the major substrate in the medium. The adsorption of glucose on PVP was reported to be negligible (24). On the other hand, activated carbon was reported to adsorb more glucose than lactic acid (21). The effect of the glucose present in the medium on lactic acid adsorption was therefore evaluated in this study.

The experiments were conducted at pH 5.5 with an initial lactic acid concentration of 5.3 g/L and an initial glucose concentration ranging from 0 to about 70 g/L. The results are given in Table 3. Despite the high concentrations employed, glucose did not have a significant effect on lactic acid adsorption on activated carbon.

### Effect of Cell Adsorption

Experiments were made to investigate the adsorption of *L. delbrueckii* cells on activated carbon and the effects of the adsorbed cells on lactic acid adsorption.



TABLE 2
Effect of pH on Adsorption of Lactic Acid on Activated Carbon[a]

| Initial pH | Lactate concentration (g/L)[b] | $K_{ad}$ |
|---|---|---|
| 3.03 | 3.2 | 11 |
| 5.16 | 3.6 | 8 |
| 8.13 | 4.4 | 3 |
| 10.22 | 4.6 | 2 |
| 13.08 | 4.6 | 2 |

[a] The ratio of the two phases used in this study was 1 g of activated carbon to 20 mL of the aqueous solution.

[b] The lactate concentrations shown in the second column are those measured in the aqueous phase after the adsorption reached equilibrium. The initial aqueous-phase total lactate concentration was 5.0 g/L for all the systems studied.

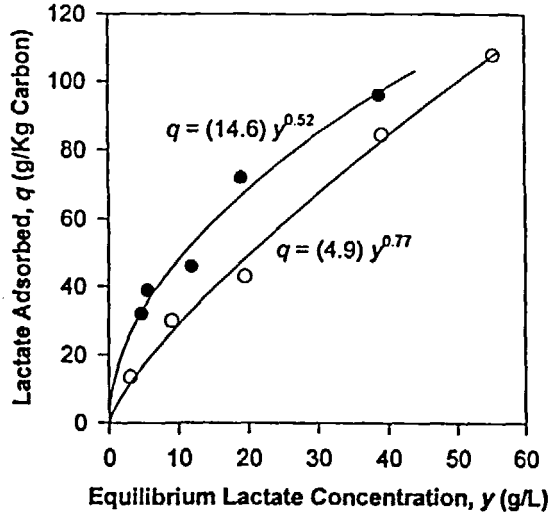


FIG. 4 The adsorption isotherms of lactate on activated carbon at 22°C. The fresh fermentation media, free of glucose and yeast extract, with (○) and without (●) cells were used as the aqueous phases in the study.



In the cell adsorption study, fermentation broth containing a high cell concentration was added to two test tubes. One of the test tubes contained activated carbon, the other did not. The cell concentration, in terms of $OD_{610}$, of each tube was followed periodically. The design was that if cell adsorption

TABLE 3
Effect of Glucose on Adsorption of Lactic Acid on Activated Carbon[a]

| Glucose concentration (g/L) | Lactate concentration (g/L)[b] | $K_{ad}$ |
|---|---|---|
| 0 | 4.0 | 7 |
| 33 | 4.0 | 7 |
| 53 | 3.9 | 7 |
| 72 | 3.9 | 7 |

[a] The ratio of the two phases used in this study was 1 g of activated carbon to 20 mL of the aqueous solution.

[b] The lactate concentrations shown in the second column are those measured in the aqueous phase after the adsorption reached equilibrium. The initial aqueous-phase lactate concentration, measured as 5.3 g/L, was the same for all the systems studied.

on activated carbon occurred, $OD_{610}$ in the carbon-containing system would drop in comparison with that in the carbon-free control. The fermentation broth used in the study was taken from an early stationary-phase culture, caused by lactate accumulation, so that no significant change in cell concentration was expected from growth or death of the cells during the cell adsorption study. Precautions had also been taken to minimize the possible concentration effect because of water absorption by the dry activated carbon. The dry activated carbon was therefore soaked in water and then filtered gently to get rid of excess interstitial water before being contacted with the fermentation broth. The experiment was run for 17 hours. The results clearly indicated a rapid adsorption of cells on activated carbon: $OD_{610}$ dropped from 0.83 to 0.63 in 1.5 hours, while that of the control (activated carbon-free system) remained around the initial value of 0.83 throughout the experiment.

The following experiment was made to examine if the adsorbed cells would affect lactic acid adsorption on the activated carbon. The cells were collected and washed by centrifugation from an early stationary-phase culture. They were then resuspended in a fresh medium, but without glucose for further lactic acid production. The cell suspension was added to five vials containing activated carbon presaturated with water, as described earlier. The ratio of the medium volume to the solid weight was 5 mL to 1 g. The vials then had lactic acid added to make different initial concentrations, and the pH was adjusted to 5.5. The pH of each vial was readjusted to 5.5 periodically until it no longer changed. The equilibrium total lactate concentrations were then measured. The results are shown in Fig. 4 and compared with the isotherm of the cell-free system. Cell adsorption is shown to have a negative effect on lactic acid adsorption. The best-fit isotherm for these cell-adsorbed systems was

$$q = (4.9)y^{0.77}$$

with the standard error of $q$ being 3.8 ($R^2 = 0.99$).

A more complete study is desirable to obtain a quantitative description and a fundamental understanding of the phenomena. The high adsorption affinity of the activated carbon to the cells of *L. delbrueckii* may render it an economical and effective support for cell immobilization. For this specific application its ability to adsorb lactic acid and lactate ions may serve to buffer the effects of product inhibition and allow for more process flexibility.

## REFERENCES

1. R. G. Sinclair, "Slow Release Pesticide System: Polymers of Lactic and Glycolic Acids as Ecologically Beneficial, Cost Effective Encapsulating Materials," *Environ. Sci. Technol.*, 7, 955–956 (1973).

2. E. S. Lipinsky, "Chemicals from Biomass: Petrochemical Substitution Options," *Science, 212*, 1465–1471 (1981).
3. R. H. Wehrenberg II, "Lactic Acid Polymer: Strong Degradable Thermoplastics," *Mater. Eng., 94*, 63–66 (1981).
4. R. K. Kulkarni, "Biodegradable Poly(Lactic Acid) Polymers;" *J. Biomed. Mater. Res., 5*, 161–181 (1971).
5. R. G. Sinclair and G. M. Gynn, *Preparation and Evaluation of Glycolic and Lactic Acid-Based Polymers for Implant Devices Used in Management of Maxillofacial Trauma*, US National Technical Information Service, No. 748410 (11 pp.) and No. 748411 (16 pp.), 1972.
6. T. B. Vickory, *Comprehensive Biotechnology: The Principles, Application and Regulation of Biotechnology in Industry, Agriculture and Medicine, Vol. 3*, Pergamon Press, New York, NY, 1985, Chap. 38.
7. R. Y. Stainer, E. A. Adleburg, and J. L. Ingraham, *The Microbial World*, Prentice-Hall, Englewood Cliffs, NJ, 1976.
8. V. M. Yabbannavar and D. I. C. Wang, "Bioreactor Systems with Solvent Extraction for Organic Acid Production," *Ann. N. Y. Acad. Sci., 506*, 523–535 (1987).
9. A. Demirci and A. L. Pometto III, "Enhanced Production of D-Levo Lactic Acid by Mutants of *Lactobacillus delbrueckii* ATCC 9649," *J. Ind. Microbiol., 11*, 23–28 (1992).
10. C.-C. Chen, "Combined Lactic Acid Fermentation and Adsorption," M.S. Thesis, The University of Akron, OH, 1995.
11. V. M. Yabannavar and D. I. C. Wang, "Extractive Fermentation for Lactic Acid Production," *Biotechnol. Bioeng., 37*, 1095–1100 (1991).
12. Y. Nomura, M. Iwahara, and M. Hongo, "Lactic Acid Production by Electrodialysis Fermentation Using Immobilized Growing Cells," *Ibid., 30*, 788–793 (1987).
13. A. Ishizaki and P. Vonktaveesuk, "Optimization of Substrate Feed for Continuous Production of Lactic Acid by *Lactococcus lactis* IO-1," *Biotechnol. Lett., 18*, 1113–1118 (1996).
14. P. X. Yao and K. Toda, "Lactic Acid Production in Electrodialysis Culture," *J. Gen. Appl. Microbiol., 36*, 111–126 (1990).
15. S. Aradhana, K. R. Pradip, and S. Vikram, "Extractive Lactic Acid Fermentation Using Ion-Exchange Resin," *Biotechnol. Bioeng., 39*, 607–613 (1992).
16. B. H. Davidson and E. T. James, "Simultaneous Fermentation and Separation of Lactic Acid in a Biparticle Fluidized-Bed Bioreactor," *Appl. Biochem. Biotechnol., 34/35*, 431–439 (1992).
17. C. H. Holten, A. Muller, and D. Rehbinder, *Properties and Chemistry of Lactic Acid and Derivatives*, International Research Association, Copenhagen, Denmark, 1971.
18. L.-K. Ju and A. Verma, "Characteristics of Lactic Acid Transport in Supported Liquid Membranes," *Sep. Sci. Technol., 29*, 2299–2315 (1994).
19. L.-K. Ju and A. Verma, "Extraction of Anions with Tertiary Amine from Aqueous Solutions of Mixed Acid and Salt," *Ind. Eng. Chem. Res., 34*, 4479–4485 (1995).
20. B. H. Davison and C. D. Scott, "A Proposed Biparticle Fluidized-Bed for Lactic Acid Fermentation and Simultaneous Adsorption," *Biotechnol. Bioeng., 39*, 365–368 (1992).
21. E. N. Kaufman, S. P. Cooper, and B. H. Davison, "Screening of Resins for Use in a Biparticle Fluidized-Bed Bioreactor for the Continuous Fermentation and Separation of Lactic Acid," *Appl. Biochem. Biotechnol., 45/46*, 545–554 (1994).
22. Product information for Reillex 425, Reilly Industries, Inc., Indianapolis, IN, 1995.
23. E. A. Ernst and D. W. McQuigg, *Adsorptive Purification of Carboxylic Acids*, Paper Presented at AIChE Annual Meeting, Miami Beach, Florida, November 1992.
24. X. Yang and G. T. Tsao, "Enhanced Acetone-Butanol Fermentation Using Repeated Fed-Batch Operation Coupled with Cell Recycle by Membrane and simultaneous Removal of Inhibitory Products by Adsorption," *Biotechnol. Bioeng., 47*, 444–450 (1995).

25. Y. Kuo, C. L. Munson, W. G. Rixey, A. A. Garcia, M. Frierman, and C. J. King, "Use of Adsorbents for Recovery of Acetic Acid from Aqueous Solutions," *Sep. Purif. Methods*, *16*, 31 (1987).
26. C. Scholler, J. B. Chaudhuri, and D. L. Pyle, "Emulsion Liquid Membrane Extraction of Lactic Acid from Aqueous Solutions and Fermentation Broth," *Biotechnol. Bioeng.*, *42*, 50–58 (1993).

*Received by editor June 5, 1997*
*Revision received October 1997*