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Adsorption of lactic acid from fermentation broth and aqueous solutions on Zeolite molecular sieves

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1. Introduction

The continuous decrease in crude oil resources makes the production of chemicals from renewable resources an eventual necessity. Limitations in available landfill space indicate the need for greater use of biodegradable materials in single-use products. The future of the plastic industry depends on its ability to synthesize biodegradable polymers of the required strength and durability.

The polymer material polylactic acid (PLA) meets both the needs for renewable resource and biodegradability. Dow Chemical and Cargill have the largest polylactide producing company with an annual capacity of 140,000 ton located in Blair, USA.

PLA is used extensively for the design of drug delivery systems for peptides and vaccines, for the manufacture of medical devices and wound dressings, as well as for fabricating scaffolds in tissue engineering (Langer and Vacanti, 1993). Moreover, the polymer can be formulated with a variety of desirable physical properties and degradation rates, making it extremely versatile. PLA is traditionally manufactured in a three-phase process: (1) fermentation by various strains of *Lactobacillus* to produce lactic acid; (2) recovery of lactic acid from the fermentation broth, and (3) polymerization of the lactic acid.

The cost of lactic acid (the raw material of PLA) is mainly due to the downstream processing costs rather than its synthesis costs. The major limitation of lactic acid fermentation is that lactic acid secretion by *Lactobacillus* is inhibited by both the presence of the lactic acid in the fermentation broth, and the associated drop in pH (Goksungur and Guvenc, 1997; Shimizu et al., 1996b). Undissociated lactic acid acts as an inhibitor for growth and lactic acid production by diffusing through the membrane and decreasing intracellular pH.

Solutions to these difficulties may be found in processes that use in situ removal of the acid product from the broth during fermentation (Sun et al., 1999). Methods investigated have included extraction, electrodialysis, ion exchange and adsorption on activated carbon, each with their own limitations.

Solvent extraction has been used for the purification of carboxylic acid such as lactic acid and succinic acid (Scholler et al., 1993; Hano et al., 1992–1993; Martin et al., 1992). But these solvents in situ are toxic as they rupture the cell membrane causing the metabolite to leak out. The toxicity of organic solvents was always a problem (Martak et al., 1997; Shimizu et al., 1996a). Although it can be overcome by immobilizing the cells (Yabannavar and Wang, 1991), it remains a problem if the cells are directly exposed to the extractant.

Electrodialysis has been used as a technique for the isolation and purification of lactic acid from fermentation media (Vonktaveesuk et al., 1994; Sato and Mijaki, 1988; Czytko et al., 1987). Since other anions also go with lactic acid and other broth components end up in the product stream by diffusion, further processing is necessary to remove these impurities. The cost of building an electrodialysis unit for large-scale operation is not economically feasible (Evangelista, 1994).

The sorption of carboxylic acids on ion exchange resins has also been studied (Tung, 1993; Kabawata et al., 1981). The major drawback of the ion exchange method is that the fermentation broth contains other anions (SO_4^{2-}, Cl^-) in addition to lactate, which compete with the acidic sites on the ion exchange material. Furthermore, these anions must be replenished, as some are necessary for the fermentation. This method also requires the use of additional chemicals (salt solutions) to recover the acid from the resin.

Ju and Chen (1998) examined the activated carbon in lactic acid fermentation from glucose using *Lactobacillus delbrueckii*. The cells were also rapidly adsorbed on activated carbon, which will limit its application in lactic acid fermentation. The problem originates from the structure of such materials, which has a wide pore size distribution. The interior is accessible by all sizes of molecules since the pore size ranges from micrometer to angstrom level. The competition by other heavier hydrocarbons in the broth for adsorption sites will reduce the selectivity and the capacity of the activated carbon.

An alternative method of recovery of lactic acid from fermentation broth, based on adsorption on Silicalite molecular sieves, has been investigated. Silicalite pellets have pore sizes suitable for adsorption of lactic acid, yet which can exclude larger size components of the fermentation broth, such as proteins. It also has low affinity for water and it is characterized by high thermal and hydrothermal stability.

2. Materials and methods

A YSI biochemistry analyzer YSI-2700 (Yellow Springs Inc., OH) was used in analyzing the lactic acid and glucose concentrations. The lactic acid used in all experimental work was a technical grade L(+) lactic acid (Aldrich Chemical Company, WI) with a label concentration of 85% w/w. This lactic acid solution was boiled overnight in a total reflux apparatus to hydrolyze any lactic acid polymer. Fermentation broth was obtained from the anaerobic culture of *L. rhamnosus* grown on MRS media (Difco Inc., MI) without pH control. Cells were removed from the fermentation broth via ultrafiltration. After about 5 days of culture, the fermentation broth contained 16.7 ± 1.0 g/L lactic acid and 5.7 ± 0.4 g/L glucose. The broth was supplemented with the refluxed 85% w/w lactic acid for some of the experiments. The glucose solution used in all experimental work was a sterilized D[+] glucose solution (Sigma Chemical Co., MO) with a concentration of 45% w/w. The adsorbent used in all the batch and column experiments were Silicalite pellets (UOP Molecular Sieves Co., IL).

Batch adsorption experiments were conducted by equilibrating 4 g Silicalite with 15 mL lactic acid solution or cell-free fermentation broth in 50 mL Erlenmeyer flasks. Flasks were kept well mixed at 24 ± 1 °C in a shaker bath overnight at various initial lactic acid concentrations. After the adsorption experiment was completed, batch desorption experiments were performed by diluting the solution in each flask with 15 mL of DI-water, and equilibrating the flasks at 24 ± 1 °C in a shaker bath. After 24 h, the supernatant was sampled and the lactic acid and glucose concentrations were measured.

Column adsorption experiments were conducted in an insulated brass fixed-bed column of 1.9 cm diameter and 25.5 cm length. The column was packed with 56.27 g of fresh Silicalite pellets. A peristaltic pump transported either broth or lactate solution to the column at an average flow rate of 3.95 mL/min. Glass wool was put on the top and bottom of the bed to obtain a good liquid distribution. The adsorption stepw as performed at 25 ± 1 °C, and then, the column was regenerated by preheating it to 95 ± 1 °C using heating tape, then passing steam through the bed at a flow rate of 6.5 g/L and a temperature of 138 ± 6 °C. Samples of the effluent condensate were collected and analyzed for lactate concentration.

Glucose breakthrough curves were measured in a similar manner, with 413 mL of fermentation broth with glucose concentration of 5.26 g/L fed to the adsorption column at a flow rate of 5.82 mL/min.

The effect of temperature on batch adsorption was studied by mixing 4.0 g of Silicalite with 15.0 mL of broth and allowing the flasks to equilibrate in a shaker bath for 24 h at 26, 52 and 66 ± 1 °C.

3. Results and discussion

Results from the batch adsorption and desorption experiments are shown in Tables 1 and 2 . The Silicalite adsorbed lactic acid upto 46 g/kg in aqueous solution and upto 37 g/kg

Table 1
Lactic acid adsorption and desorption isotherms at 24±1 °C from aqueous solution on Silicalite

Experiment number	Adsorption experiments		Desorption experiments	
	Equilibrium concentration (g/L)	Amount adsorbed (g/kg)	Equilibrium concentration (g/L)	Amount adsorbed (g/kg)
1	24.60	46.10	18.69	44.00
2	20.44	32.14	14.00	33.35
3	14.40	17.04	11.55	22.18
4	11.96	8.13	8.08	10.48
5	8.08	4.03	5.98	8.13
6	4.81	0.35	4.33	1.87

Table 2
Lactic acid adsorption and desorption isotherms at 24 ± 1 °C from fermentation broth on Silicalite

Experiment number	Adsorption experiments		Desorption experiments	
	Equilibrium concentration (g/L)	Amount adsorbed (g/kg)	Equilibrium concentration (g/L)	Amount adsorbed (g/kg)
1	34.75	37.26	17.60	37.77
2	27.28	32.23	14.16	30.10
3	21.87	18.72	11.25	17.76
4	15.42	12.13	7.78	12.58
5	12.20	7.69	6.32	6.85
6	8.45	3.93	4.37	3.39
7	4.55	0.411	2.25	0.90

in broth. The presence of extra chemical species in the broth caused Henry's constant to decrease from 2 ± 0.7 L/kg for the pure solution to 1 ± 0.5 L/kg for the broth. This adsorptive capacity is low compared to the amount of 210 g/kg reported for lactic acid adsorption on Amberlite IRA-35 (Kaufman et al., 1994). However, Amberlite's capacity quickly diminished 93% in subsequent loadings after three cycles because of the inability to regenerate the resin and the resins' instability at elevated temperature.

The equilibrium curves obtained from desorption from the Silicalite were lower than those obtained from adsorption. This hysteresis may be the result of the shape of the openings to the pores of the solid or of the complex phenomenon of the wetting of the solid by the adsorbate (Treybal, 1981). As shown in Table 2, the adsorption of lactic acid from fermentation broth is clearly irreversible and the hysteresis loop is even wider than that of the aqueous solution. This may be due to the presence of chemical species in the broth (nutrients necessary for bacterial growth such as yeast extract, meat extract, etc.) that alters the intermolecular forces.

Table 3 shows the breakthrough data of lactic acid from aqueous and broth solutions at 25 ± 1 °C. The difference between the data of the pure and broth solutions is consistent with the equilibrium adsorption curves obtained for the two solutions.

Table 3
Breakthrough data of lactic acid from aqueous and broth solutions

Aqueous solution		Fermentation broth	
Volume (mL)	Outlet acid concentration (g/L)	Volume (mL)	Outlet acid concentration (g/L)
1	0.14	1	0.20
23	0.14	24	0.16
45	0.74	48	2.77
68	2.88	72	8.10
91	4.86	96	10.57
122	7.00	120	12.13
154	8.53	148	13.16
186	9.79	182	13.32
217	10.76	225	14.51
254	11.93	263	15.23
290	12.62	311	15.59
326	13.18	373	15.64
371	13.97	411	16.11
403	14.27	415	15.70

An important characteristic of the breakthrough data is the break point that occurs after a collection of 50 mL of the solution. If the adsorption process were infinitely rapid, the breakthrough curve would be a straight vertical line after the break point and most of the bed capacity would be used at that break point (Geankoplis, 1993). Under the conditions of the experiment, a finite resistance to adsorption is present and a significant mass transfer occurred after the break point. The results also indicate that the length of the bed is not large compared to the length of the mass-transfer zone. It also shows that the adsorption column was almost saturated after the breakpoint in the case of the broth while significant mass transfer was still going after the break point in the case of the pure solution.

The overall mass transfer coefficient was obtained by fitting the experimental data to the model described in details elsewhere (Aljundi, 2001). The model consists of a mass balance equation on lactic acid derived from a shell balance on a volume element of differential thickness

$$\frac{\partial C}{\partial t} = -v\frac{\partial C}{\partial z} - \frac{(1-\varepsilon)}{\varepsilon}\rho_b \frac{\partial q}{\partial t},$$

$$\left\{ \begin{array}{l} C = 0,\ q = 0 \text{ at } t = 0 \text{ and } L > z > 0 \\ C = C_0 \text{ at } z = 0 \text{ and } t > 0 \end{array} \right\}. \quad (1)$$

A linear rate transfer equation, using the particle phase concentration difference as the driving force (LDFQ), was used to describe the rate of change in the adsorbed phase concentration. The LDFQ uses a lumped parameter, k, to account for the mass transfer resistances (Ruthven, 1984):

$$\frac{\partial q}{\partial t} = k(q^* - q), \quad \{q = 0 \text{ at } t = 0\}, \quad (2)$$

where q^*, the equilibrium value of q, can be represented by a linear isotherm of the form

$$q^* = KC. \tag{3}$$

The overall mass transfer coefficient will account for the resistance to diffuse through the fluid film around the Silicalite particle and the resistance to diffuse through the pores of Silicalite to internal adsorption sites. There are many empirical equations that correlate the mass transfer coefficient and Sherwood number to Reynolds number and Schmidt number. These recommended correlations have variation as high as ±50% (Geankoplis, 1993). An empirical equation for packed beds is (McCabe et al., 1993)

$$N_{\mathrm{Sh}} = 1.17 N_{\mathrm{Re}}^{0.585} N_{\mathrm{Sc}}^{1/3}. \tag{4}$$

The diffusivity of lactic acid is estimated from (Wilke and Chang, 1970):

$$D_v = 7.4 \times 10^{-8} \frac{(\varphi_B M_B)^{1/2} T}{\mu V_A^{0.6}}. \tag{5}$$

The fluid-phase mass transfer coefficient, k_e, calculated using Eq. (4) and the experimental conditions described previously was 2.99×10^{-3} cm/s. Letting $R_T = 1/ka$ (the overall mass transfer resistance), $R_e = 1/k_e a$ (the fluid-phase mass transfer resistance), and R_s (the solid-phase transfer resistance), we have $R_T = R_e + R_s$. The external surface area of the Silicalite particle, a ($\mathrm{cm}^2/\mathrm{cm}^3$), is given by $a = 6(1-\varepsilon)/D_P$. The calculated R_T for the broth solution was 99.0 s and R_e=20.9 s. Hence, external resistance is only 21% of the total resistance and the internal resistance (or the solid-phase resistance) is the dominant step in adsorption.

Lactic acid concentrations from the bed regeneration are shown in Table 4 . The average recovery of lactic acid from the amount retained in the column was 65.4% and 75%, for the broth and aqueous solutions, respectively.

It is believed that by choosing a high enough temperature, the speed of desorption front can be made faster than the speed of the thermal wave. In this case, desorbed species is accumulated at the front and a high concentration peak with less tail can be expected. On the other hand, when the temperature of desorption is not high enough, desorption gradually occurs and a long tailing will be expected (Suzuki, 1989).

Glucose, the carbon and energy source for cells is the major individual component in the fermentation broth other than lactic acid. Table 5 shows the breakthrough data of glucose at 25 ± 1 °C. The amount of glucose adsorbed was so small (94 mg adsorbed and 23 mg desorbed), indicating negligible adsorptive capacity of Silicalite for glucose.

The effect of glucose concentration on lactic acid adsorption was also determined as shown in Table 6. The breakthrough data did not change when the glucose concentration was increased five-fold, a clear sign that the presence of the glucose upto the studied concentration did not interfere with the adsorption of lactic acid.

Table 4
Desorption data of lactic acid from aqueous and broth solutions

Aqueous solution		Fermentation broth	
Volume (mL)	Outlet acid concentration (g/L)	Volume (mL)	Outlet acid concentration (g/L)
1	2.30	1	15.41
4	18.00	5	16.11
14	32.04	10	18.72
20.5	34.20	20	21.24
27	34.02	30	19.08
52	23.94	50	12.65
77	15.50	70	8.44
102	13.25	100	5.81
127	6.28	127	3.78
152	3.33	150	2.79
177	1.66	181	1.53
202	0.90	199	1.17
228	0.52	222	0.43
253	0.31	249	0.43
303	0.11	299	0.61
353	0.02	349	0.16
403	0.00	399	0.20

Table 5
Breakthrough data of glucose at 25±1 °C from fermentation broth solution

Volume (mL)	6	23	41	58	76	99	116	157	198	244	291	349
Outlet glucose concentration (mg/L)	0	0	23	150	209	250	265	285	282	290	292	289

Table 6
Breakthrough data of lactic acid with different glucose concentrations at 25 °C

Experiment A, glucose conc. = 5.3 g/L		Experiment B, glucose conc. = 26.3 g/L	
Volume (mL)	Outlet acid concentration (g/L)	Volume (mL)	Outlet acid concentration (g/L)
6	0.13	21	0.00
23	0.13	35	0.02
41	1.17	49	3.19
58	7.15	63	8.24
76	10.21	85	11.32
99	12.46	113	12.73
157	14.63	170	13.73
198	14.96	232	14.58
244	15.25	303	14.36
291	15.50	373	14.92
349	15.71	458	15.82
413	15.95	507	15.44

Similar behavior was also reported by Ju and Chen (1998), where they showed that glucose did not significantly affect lactic acid adsorption on activated carbon.

Adsorption is accompanied by evolution of heat since adsorbate molecules are more stabilized on the adsorbent

Table 7
Effect of temperature on lactic acid adsorption isotherm

$T = 26\,^{\circ}C$		$T = 52\,^{\circ}C$		$T = 66\,^{\circ}C$	
Aqueous phase concentration (g/L)	Solid phase concentration (mg/g)	Aqueous phase concentration (g/L)	Solid phase concentration (mg/g)	Aqueous phase concentration (g/L)	Solid phase concentration (mg/g)
14.16	10.51	15.78	4.66	16.08	3.59
10.64	4.64	11.53	1.45	11.90	0.14
6.63	2.93	6.92	1.88	7.19	0.93

surface than in the bulk phase. Since adsorption is an exothermic process, the concentration of the adsorbed material decreases with increased temperature at a given equilibrium concentration, as the lactic acid isotherms of Table 7 indicate. The temperature dependency of Henry's constant obeys the van't Hoff equation (Ruthven, 1984):

$$\frac{\mathrm{d}\ln K}{\mathrm{d}T} = \frac{\Delta H}{RT^2}. \tag{6}$$

Eq. (6) can be integrated to yield $\ln K = \ln K_o + (-\Delta H/RT)$. A plot of $\ln K$ versus $1/T$ will yield a straight line with a slope of $-\Delta H/R$. The heat of adsorption, calculated from the slope, was (29 ± 17) kJ/mol.

4. Conclusions

Silicalite adsorbed lactic acid upto 55 g/kg in aqueous solution and upto 37 g/kg in broth. Its capacity was maintained and was not diminished with repetitive use in the adsorption/desorption cycle. A linear isotherm was observed for the pure solution and broth with Henry's constant estimated as 1 ± 0.5 L/kg for the broth and 2 ± 0.7 L/kg for the aqueous solution. In addition, the adsorption isotherms showed a hysteresis loopwhen tested for reversibility where it was wider in the case of the fermentation broth. The kinetics of lactic acid adsorption on Silicalite was investigated in packed column studies. The amount adsorbed from the aqueous solution was approximately 50% higher than that from the broth under the same conditions. Adsorption in the column was controlled by the diffusion in the solid phase rather than the fluid. Glucose adsorbed to Silicalite to a negligible extent, and did not interfere with lactic acid adsorption. Regeneration of the bed using steam was successful in recovering 74% of the lactic acid in the pure solution, compared to 65% in the fermentation broth.

Although the Silicalite showed lower capacity for lactic acid than other polymeric adsorbents, this process still has the advantages of simplicity in operation. With in situ adsorption of lactic acid on Silicalite during fermentation, the number of process units can be reduced compared to traditional methods, and use of additional chemicals (other than water) can be eliminated.

Notation

a	external surface area of the Silicalite particle
C	lactic acid concentration in the fluid phase
D_P	diameter of the Silicalite particle
D_v	diffusivity
ΔH	heat of adsorption
k	mass transfer coefficient
K	Henry's constant
L	length of the adsorption column
M_B	molecular weight of solvent
N_{Re}	Reynold's number $(=\rho D_P v/\mu)$
N_{Sc}	Schmidt's number $(=\mu/\rho D_v)$
N_{Sh}	Sherwood's number $(=k_e D_P/D_v)$
q	adsorbed-phase concentration
q^*	equilibrium value of q
R	gas constant
R_e	fluid-phase mass transfer resistance
R_s	solid-phase transfer resistance
R_T	overall mass transfer resistance
t	time
T	temperature
v	interstitial velocity
V_A	molar volume of solute
z	height of the column

Greet letters

ε	porosity
μ	viscosity of the solution
ρ	solution density
ρ_b	bed density
φ_B	association constant for solvent

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