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A Fluidized-Bed Continuous Bioreactor for Lactic Acid Production

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ABSTRACT

A laboratory bioreactor consists of a fluidized bed of monosized activated carbon coated with a biofilm of the homolactic fermentative organism *Streptococcus thermophilus*. Biofilm growth moves the carbon beads through the bed, and adsorption of substrate and product at the bottom and top of the bed, respectively, reduces their inhibitory effects on the organism. Theory shows that high reactor productivity and rapid recirculation of carbon through the bed require a biofilm thickness of 25–45% of the carbon particle radius on particles fed into the base of the bed. This could not be achieved in practice owing to the fragility of the biofilm. A productivity of 12 gm/L h was achieved without any pH control and the outlet product concentration was higher than the completely-inhibiting concentration (found from batch experiments) at the outlet pH. This is possible because it is the undissociated form of the acid that both inhibits metabolism and adsorbs on activated carbon.

Index Entries: Fluidized-bed; activated carbon; biofilm; lactic acid.

NOMENCLATURE

b	Langmuir affinity constant for adsorption on activated carbon
B	Density ratio (biofilm-liquid)/(support particle-liquid)
C_a	Concentration at carbon/biofilm interface
D	Diffusivity of lactic acid in biofilm
e	Exponent in drag coefficient correlation
H	Bed height

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p Lactic acid concentration in liquid
$\bar{p}$ Lactic acid concentration that completely inhibits product formation
$\bar{p}_{un}$ Concentration of undissociated lactic acid that completely inhibits product formation
q Mass adsorbed per unit volume of carbon
$\bar{q}_p$ Maximum specific product formation rate
Q Maximum mass that can adsorb per unit volume of carbon
R Radius of activated carbon particle
S Substrate concentration in liquid
T Volumetric flow rate of carbon per unit area of bed
u Superficial liquid velocity
U_t Settling velocity of particle
x (volume of biofilm)/(volume of support particle)
X Cell volume fraction in liquid
Y Cell yield
η Effectiveness factor defined by Eq. (2)
ρ Biofilm density; dry weight per unit volume
γ Dimensionless radius (Thiele modulus) defined by Eq. (3)
$\bar{\mu}$ Maximum specific growth rate of biomass

SUBSCRIPTS

c Particles with no biofilm
i Inlet
o Outlet
p Product
s Substrate

INTRODUCTION

Consider the ideal bioreactor for producing lactic acid, a fermentation recently reviewed by Vickroy (*1*). Product separation is a major expense in this process and it is complicated by the presence of unfermented sugars. It follows that the ideal reactor would give a high product concentration and complete substrate conversion while being as small as possible (i.e., having the highest possible volumetric productivity). Since the product inhibits the metabolism of the microorganisms that produce it, these three requirements are conflicting and achieving them calls for efficient reactor design. The main features of this ideal reactor are obvious from first principles. It should be continuous, have minimal mass transfer resistances, and contain the highest possible concentration of microorganisms. Also, the liquid should move in plug flow and there should be some continuous removal of product from the reactor in order to minimize it's inhibitory effects. Existing reactors have some but not all of these features. High cell concentrations have been achieved in a continuous

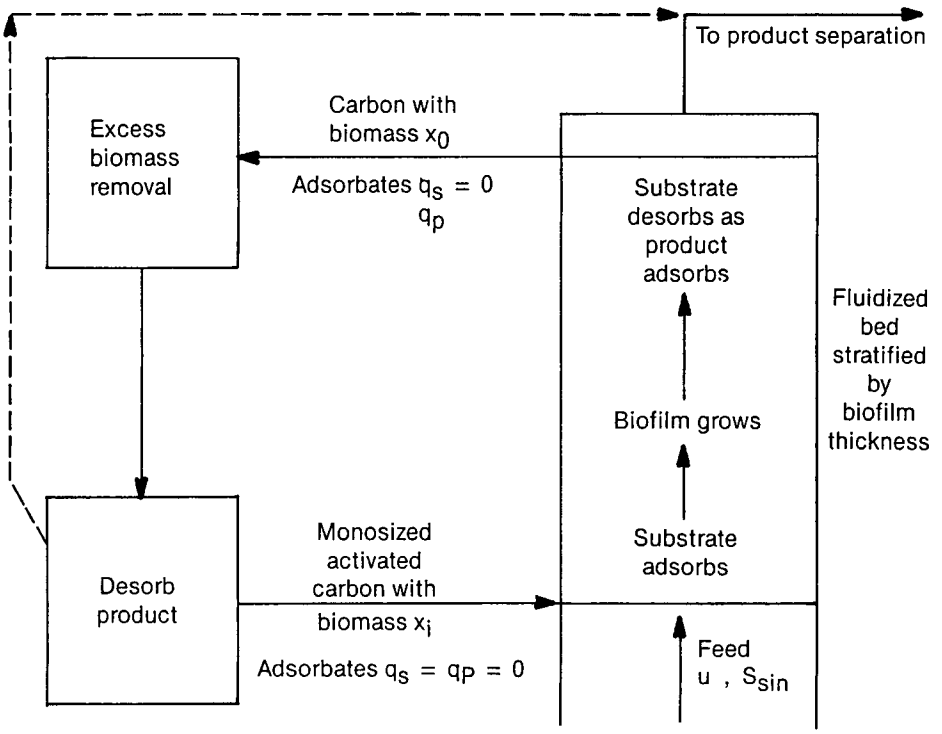

Fig. 1. The proposed reactor.

stirred tank reactor by cell recycle (2). Continuous product removal by dialysis has been demonstrated (3). Plug flow can be achieved in a packed bed of gel-immobilized cells (4). The objective of this work was to provide a preliminary laboratory demonstration of a reactor design that incorporates all of them.

The reactor (Fig. 1) consists of a fluidized bed of Purasiv® activated carbon supplied by the Linde Division of the Union Carbide Corporation. This is a beaded carbon that is almost monosized with an average diameter of 700 μm and a standard deviation of 60 μm. Carbon particles coated with biofilm are injected into the base of the bed. Substrate diffuses through the biofilm and adsorbs in the carbon, thus reducing the substrate concentration in the liquid and any substrate inhibition effects. The biofilm then grows, reducing the settling velocity of the particle, and because liquid-fluidized beds tend to stratify based on the particle settling velocity, this reduction moves the particle up the bed. This orderly particle flow is counteracted by the tendency of particles in the bed to move around randomly, so the question of solids stratification vs solids mixing is one that must be resolved by experiment. As the biofilm gets thicker and the particle moves into regions where the substrate/product ratio is lower, the substrate naturally tends to desorb from the carbon whereas product tends to adsorb. This increases the substrate/product ratio near the base of the biofilm (compared with a film on a nonadsorbent surface) and thus

reduces the effect of the biofilm's resistance to diffusion on the metabolic rate of the organisms there. Eventually the activated carbon is saturated with product. It is removed from the top of the bed, excess biofilm is removed, and the carbon is regenerated before being recycled to the base of the bed.

The reactor had been demonstrated previously for ethanol production using a flocculant strain of *Zymomonas mobilis* (*5*). These tests were unsuccessful first because the CO_2 bubbles produced caused slugging of the bed and tended to strip the biofilm from the activated carbon, and second because it was difficult to desorb the ethanol from the carbon without killing the remaining biofilm. It seemed more appropriate for lactic (or acetic) acid production. The homolactic fermentation produces no gas, and the adsorption of lactic acid on activated carbon is pH dependent, so desorption can be accomplished by immersing the particles in a mild base. Also, some additional benefits could be expected because it is the undissociated form of the acid that is the principal microbial inhibitor (*1*), and it is the undissociated form that is selectively adsorbed by activated carbon.

THEORY

The designer of one of these reactors must specify three main quantities. They are the amount of activated carbon put in the bed, the height the bed is allowed to reach as the biofilm develops, and the amount of biomass on the carbon returned to the base of the bed. A detailed mathematical model capable of specifying these quantities has been published (*6*) and is summarized here to illustrate the main design requirements and to set up an "ideal reactor" against which the performance of the laboratory reactor can be judged. The amount of biofilm is described by a variable x, defined as the ratio of biofilm volume to carbon particle volume.

Stratification and x_o

The reactor will work as described only if the growth of the biofilm reduces the particle settling velocity. Otherwise, the heavily coated particles would sink to the base of the bed instead of rising to the top. The biofilm affects the settling velocity in two ways; it increases the particle diameter, which tends to increase U_t, but also decreases the average particle density, which tends to decrease U_t. If a drag coefficient correlation of the form $C_D = f/R_{et}^e$ is assumed the net result is (*6*)

$$\frac{U_t}{U_{tc}} = \frac{(1 + Bx)^{1/(2-e)}}{(1 + x)^{1/3}} \tag{1}$$

This is plotted in Fig. 2 for $e = 0.85$, which is appropriate for Purasiv activated carbon in water. For very light support particles ($B \rightarrow 1$) the diameter effect is dominant and adding biofilm increases the settling

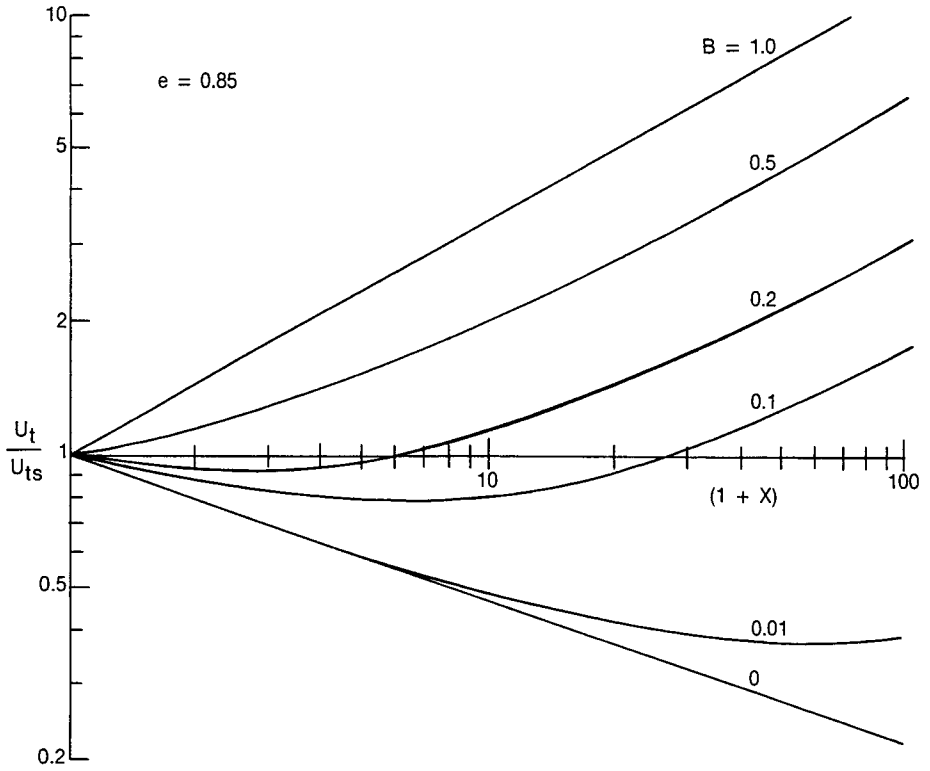

Fig. 2. Variation of settling velocity with film volume.

velocity. For very dense support particles (B→0) the density effect is dominant and the settling velocity is reduced. Purasiv carbon soaked in water has a density of 1.58 g/cm^3 so B~0.05 and, from Fig. 2, a reduction of settling velocity can be expected up to $x=8$. This sets an upper limit of $x_o \leq 8$ on the value that can be allowed at the top of the bed.

Reactor Productivity and x_i

It has been shown by Andrews and Przezdiecki (*7*) that the local volumetric productivity in any fluidized bed fermentor is proportional to an effectiveness factor defined by

$$\eta = \frac{\text{Product formation rate in particle}}{\left(\begin{array}{c}\text{Product formation rate if whole particles were}\\ \text{biomass exposed to liquid-phase conditions}\end{array}\right)} \quad (2)$$

This is plotted vs the amount of biofilm (x) in Fig. 3 for the case of linear product inhibition kinetics and negligible mass transfer resistance in the liquid and adsorbent phases (*6*). The first parameter on this graph is a dimensionless carbon particle radius

$$\gamma = R\left(\frac{\bar{q}_p \rho}{D\bar{p}}\right)^{1/2} \quad (3)$$

The diffusivity of lactic acid in biomass, D, was taken to be one-half the value in water (1.05×10^{-5} cm^2/s). Batch culture experiments in the range $3 < pH < 6$ with the organism used in the reactor tests gave values for the maximum specific productivity $\bar{q} = 2$ g/g h, the cell yield on product $Y_p = 0.15$ g/g, the product yield from substrate $Y_{ps} = 0.98$ g/g and the biomass density $\rho = 80$ mg dry wt/cm^3 (*9*). The results agreed with the hypothesis that inhibition is owing mainly to the undissociated form of the acid, and that metabolism is completely inhibited by a concentration $\bar{p}_{un} = 0.82$ gm/L. This agrees with early work on Streptococcus lactis (*8,10, 11*) and implies that the inhibitory total acid concentration, $\bar{p}$, decreases rapidly as the pH drops and the dissociation equilibrium

$$LA \rightleftharpoons LA^- + H^+ \qquad pK = 3.86 \tag{4}$$

shifts to the left. The two values $\gamma = 0.35$ and $\gamma = 1.5$ shown in Fig. 3 correspond to pH = 5.9 and 4.5, respectively.

The other parameter on this graph is related to the concentrations of product in the liquid, P, and adsorbed in the carbon. When there is no adsorbed product, the equilibrium concentration at the film/carbon interface $C_{pa} = 0$, the parameter is ≥ 1 and the effectiveness factor is much larger than it would be for a nonadsorbing support particle of the same size. (It may even be greater than one.) At the other extreme, when the carbon contains a lot of product so that $C_{pa} = \bar{p}$ the effectiveness factor is much lower.

Although these calculations are approximate, two important results follow from Fig. 3. First, whatever the values of pH and C_{pa} it is impossible to achieve a high effectiveness factor with $x < 1$. So, if the particles returned to the bed have $x_i < 1$ there will be a region at the base of the bed where the product formation rate and film growth rate will be low. This sets a lower limit on the value of x_i. Second, at the conditions near the base of the bed (small x, high pH) there is little mass transfer resistance in the biofilm. This is shown by the fact that the line for nonadsorbing supports with $\gamma = 0.35$ is very close to both the line for $C_{pa} = p$ (i.e., no product concentration gradient in the film), and to the curve $x/(1+x)$ (the fraction of the particle that is biomass), which would be the solution for a particle with no mass transfer limitation. This implies that diffusion through the film is inherently more rapid than metabolism, which means that substrate will be able to diffuse through the film and saturate the carbon bead before it moves up the bed. More detailed calculations (*6*) have confirmed that this adsorption happens almost instantaneously (on the time scale of the carbon residence time in the bed) even with quite thick biofilms (x_i up to 2).

Carbon Residence Time and Adsorbed Product

A mass balance on cells, substrate, and product in the liquid and solid phases in the bed gives

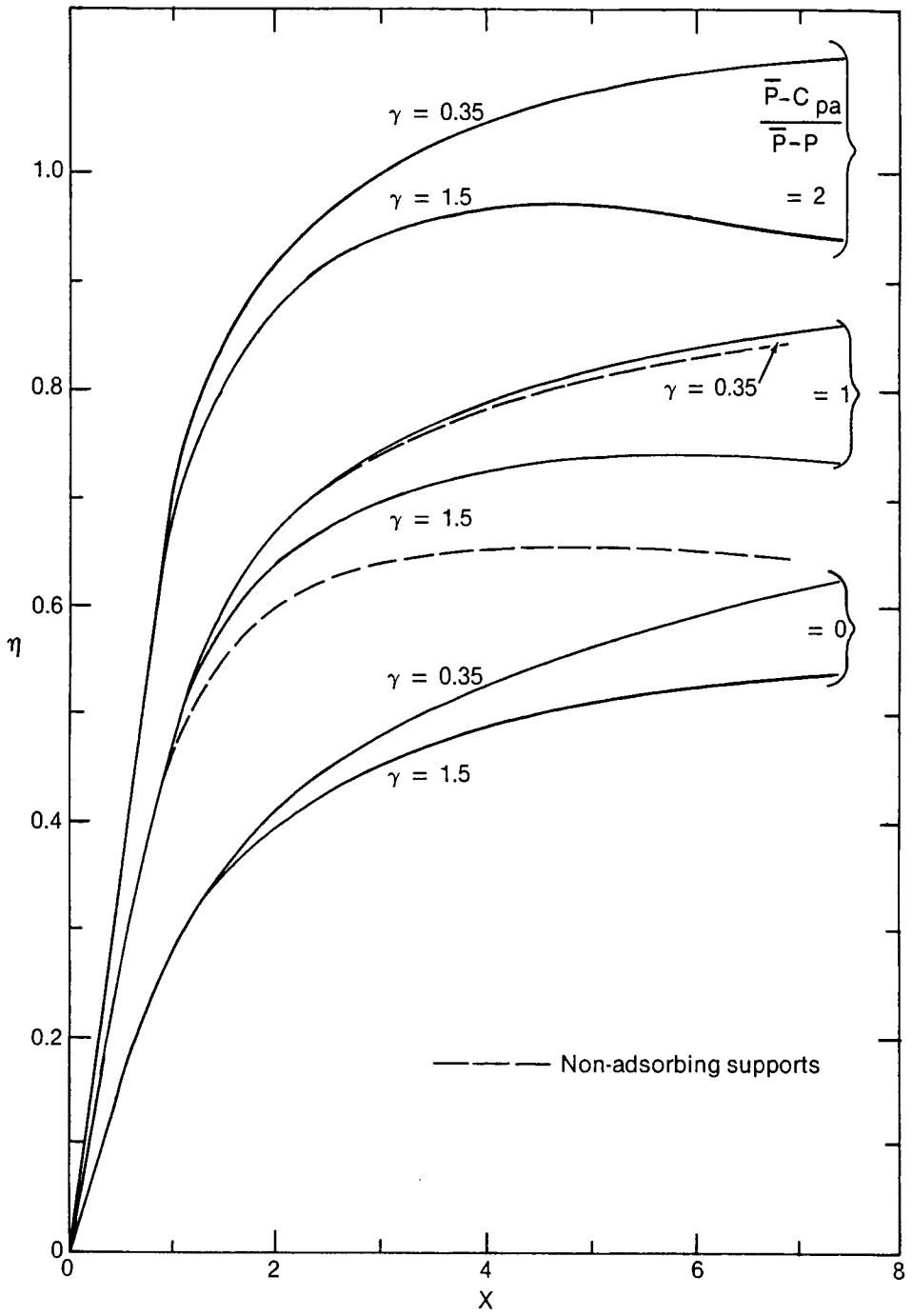

Fig. 3. Total effectiveness factor.

$$\rho\,[T(x_o - x_i) + uX_o] = Y_s\,[T(q_{si} - q_{so}) + u(S_i - S_o)] = Y_p\,[T(q_{po} - q_{pi}) + u_{po}] \quad (5)$$

The carbon entering the bed should contain neither substrate nor product ($q_{si}=q_{pi}=0$) and the substrate conversion in the reactor should be almost complete ($Tq_{so}+uS_o<<uS_i$). If, in addition, we assume that the carbon leaving the bed is saturated with product, ($q_{po}=Q_p$) and that the fraction of the cells that are washed off the particles into the liquid phase is small [$T(x_o-x_i)>>uX_o$] then

$$\frac{u}{T} = \frac{\text{carbon residence time}}{\text{liquid residence time}} = \frac{\Delta x}{Y_s S_i}$$

$$\frac{TQ_p}{TQ_p + uP} = \frac{\text{product leaving bed adsorbed}}{\text{total product formed}} = \frac{Y_p Q_p}{\rho \Delta x} \quad (6)$$

The final equation illustrates the difficulty of designing these reactors. Y_pQ_p/ρ is approximately 0.3, so if Δx is taken as 7, only 4% of the product formed will emerge from the reactor adsorbed on the carbon, the other 96% being in the liquid. This 4% would not justify the cost of the equipment required for desorption and carbon recycling. Increasing the fraction that emerges adsorbed requires either a better carbon (higher Q_p) or a lower Δx. However, a lower Δx means a lower gradient of particle settling velocities through the bed, and thus less efficient bed stratification and particle transport up the bed.

Bed Height

A reasonable compromise between the conflicting requirements of reactor productivity, stratification, and the efficient use of carbon would be $x_i=2$, $x_o=6$. Andrews and Tien (*12*) have shown that as a first approximation the fractional increase in bed height as a biofilm develops is equal to the average value of x in the bed. Therefore, we would require that the bed expand to five times the height of the bed of activated carbon with no biofilm.

EXPERIMENTS

Adsorption Isotherms

It has been found in previous experiments on the production of ethanol with *Zymomonas mobilis* (*5*) that attachment of microorganisms to Purasiv activated carbon was accelerated if the carbon was pretreated by soaking in 3.1 *N* nitric acid for 2 d, and then rinsing repeatedly with distilled water. Single solute isotherms for adsorption on this treated carbon were measured in the usual way by mixing various amounts of carbon with different strength solutions of glucose and lactic acid in 250 mL Ehrlenmeyer flasks. The flasks were sealed and placed in a rotating shaker at

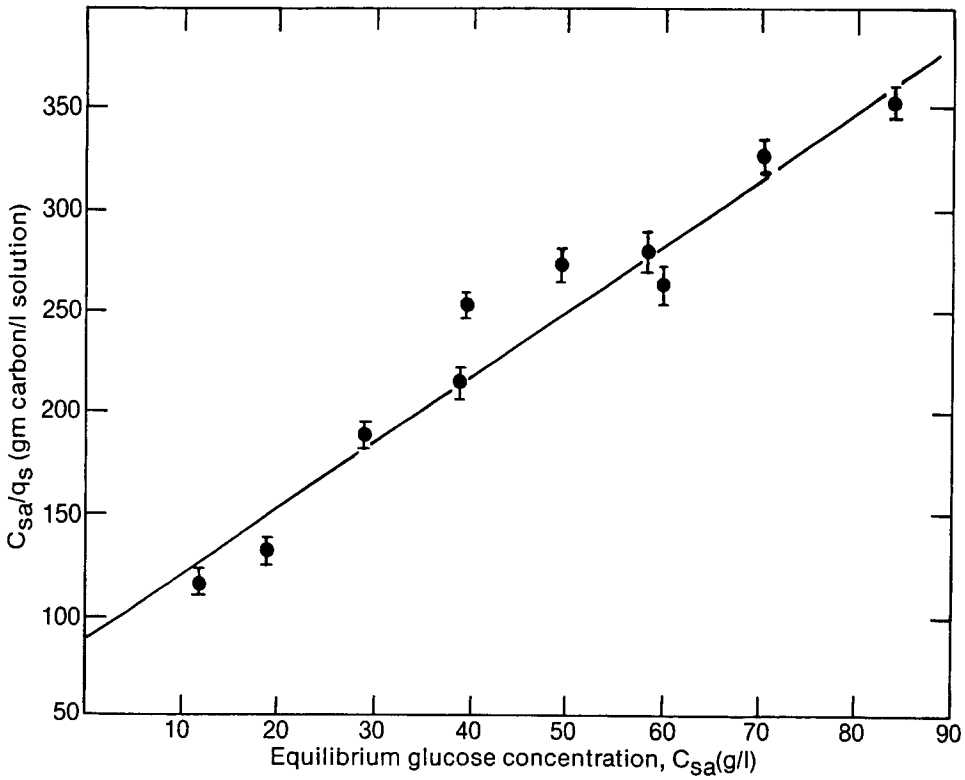

Fig. 4. Single solute glucose adsorption isotherm.

room temperature for 2 d in order to reach adsorptive equilibrium. The adsorption of organic acids is known to be pH-dependent, so the lactic acid isotherms were measured at three different pH values. In order to keep the pH at the required values during the equilibration procedure, dilute acid or base was added as required every 12 h.

Figures 4 and 5 show the fit of the data for glucose and lactic acid to the Langmuir adsorption isotherm.

$$q = \frac{QbC_a}{1 + b\,C_a} \tag{7}$$

The resulting values of the saturation capacity Q and the affinity parameter b are shown in Table 1. The decrease in lactic acid adsorption with increasing pH is important because it means that the acid can be desorbed from activated carbon removed from the bed by soaking in a basic solution.

Strain Isolation

Since no bacterium was readily available that would both carry out the homolactic fermentation and form a cohesive biofilm, one was isolated from yogurt (*9*). Test tubes containing a sucrose/yeast extract media were incubated without shaking at 31°C for 1–3 d, and organisms were then

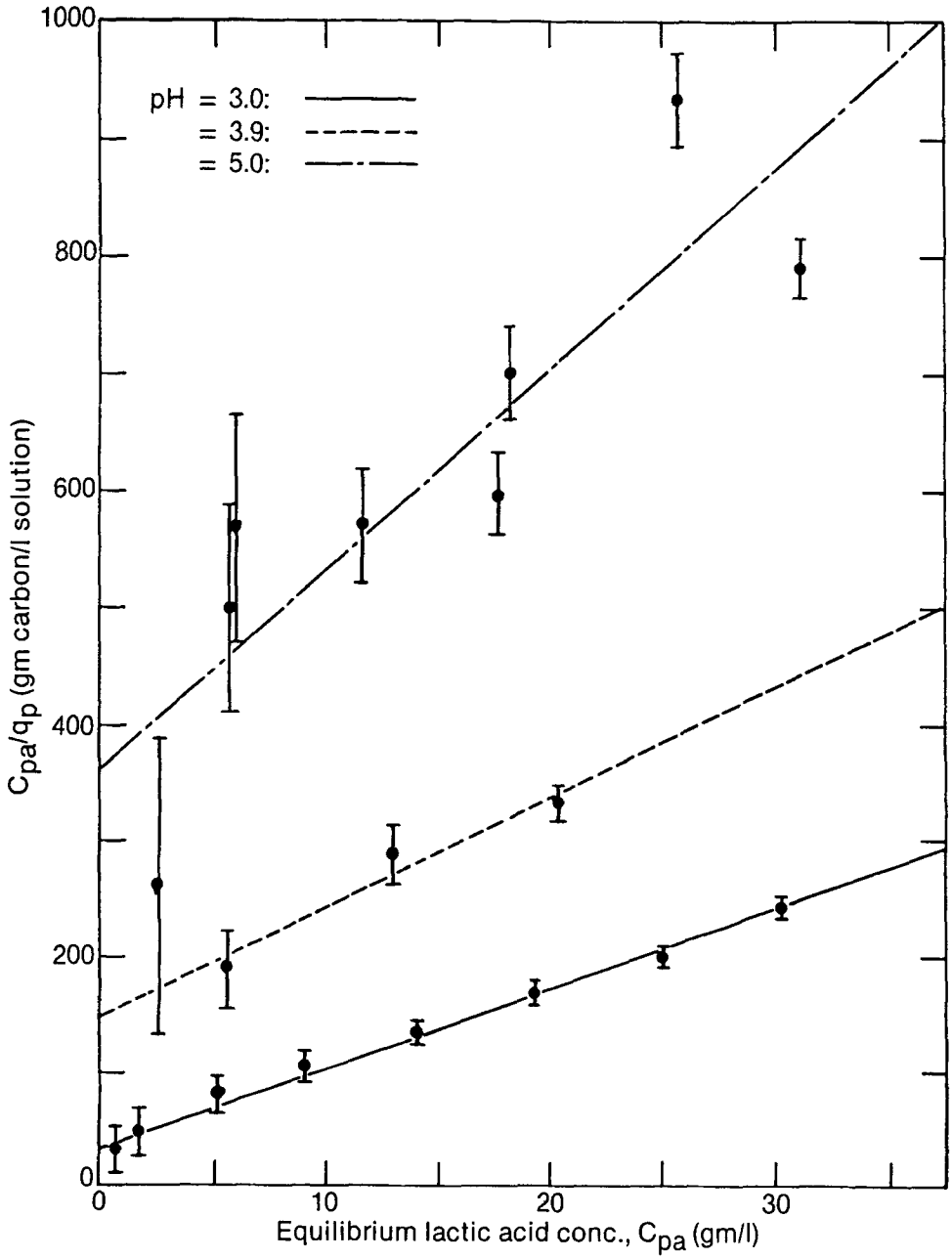

Fig. 5. Single solute lactic acid adsorption isotherm.

removed for transfer to fresh media by scraping an inoculating loop along the tube wall. After ten such transfers, one tube developed a voluminous slimy biofilm that adhered strongly to the tube wall. Examination of the organism showed it to be nonmotile, gram positive bacterium that grows as long chains of cocci enclosed in capsular polysaccharides. From these characteristics, its ability to grow on sucrose, and the appearance of its

Table 1
Adsorption Parameter Values

Solute	pH	Saturation capacity Q g/cm^3	Affinity parameter b L/g
Glucose	7.0	0.320	0.036
Lactic acid	5.0	0.146	0.040
	3.9	0.260	0.065
	3.0	0.368	0.194

colonies, the organism was identified as a mutant of *Streptococcus thermophilus* (*9*).

Column Experiment

Experiments were conducted in a plexiglas laboratory reactor 5.8 m high (Fig. 6). The working section was 2.54 cm diameter but the upper 1.05 m was 3.81 cm diameter and the bottom 0.115 m consisted of a conical entrance section. The top of the reactor was plugged with glass wool. Liquid samples could be taken through septa at points Pl (1.05 m) and P2 (2.06 m) and four outlet ports (01–04, in Fig. 6) were available for solids removal. The solids could be reinjected into the base of the bed using a funnel (IF), a syringe (IS) and a system of tube clamps. The reactor was fed with tap water passed through a particle filter (F1), an activated carbon filter to remove chlorine (F2), and a sterilizing filter to remove bacteria (F3). It was mixed at the base of the column with a concentrated glucose solution from a 50 L carboy and a yeast extract solution from a 8 L carboy. These three liquid streams were passed through separate pump heads on a single peristaltic pump in order to ensure a constant media composition. The composition was, per gram of glucose, 0.21 g of yeast extract, 0.21 g sodium acetate, 0.105 g dibasic ammonium citrate, 0.105 mg K_2HPO_4, 0.084 g $MgSO_4$, 0.021 g $MnSO_4$. The pH of this mixture was 6.0.

The carboys, tubing, and a slurry of pretreated activated carbon in distilled water were all steam sterilized. Then, after the reactor was assembled and the carbon was loaded into it, a 0.75 *N* solution of HCl was recirculated through the reactor for 5 h to complete the sterilization. After thorough rinsing with sterile water, some media was pumped into the reactor, an inoculum of cells was injected through port P1, and this mixture was recirculated slowly through the bed so that the bacteria could colonize the surface of the activated carbon. After 80 h, during which more nutrients and glucose were injected into the reactor once, the recycle line (R in Fig. 6) was clamped off switching the reactor to once-through operation. The total liquid flowrate was increased to 140 mL/min, which fluidized the bed to a porosity of 59%. The temperature at the reactor inlet was kept at 30°C by means of heating tape on the water inlet line. Insulation on the reactor kept the temperature drop along its length to less than 2°C.

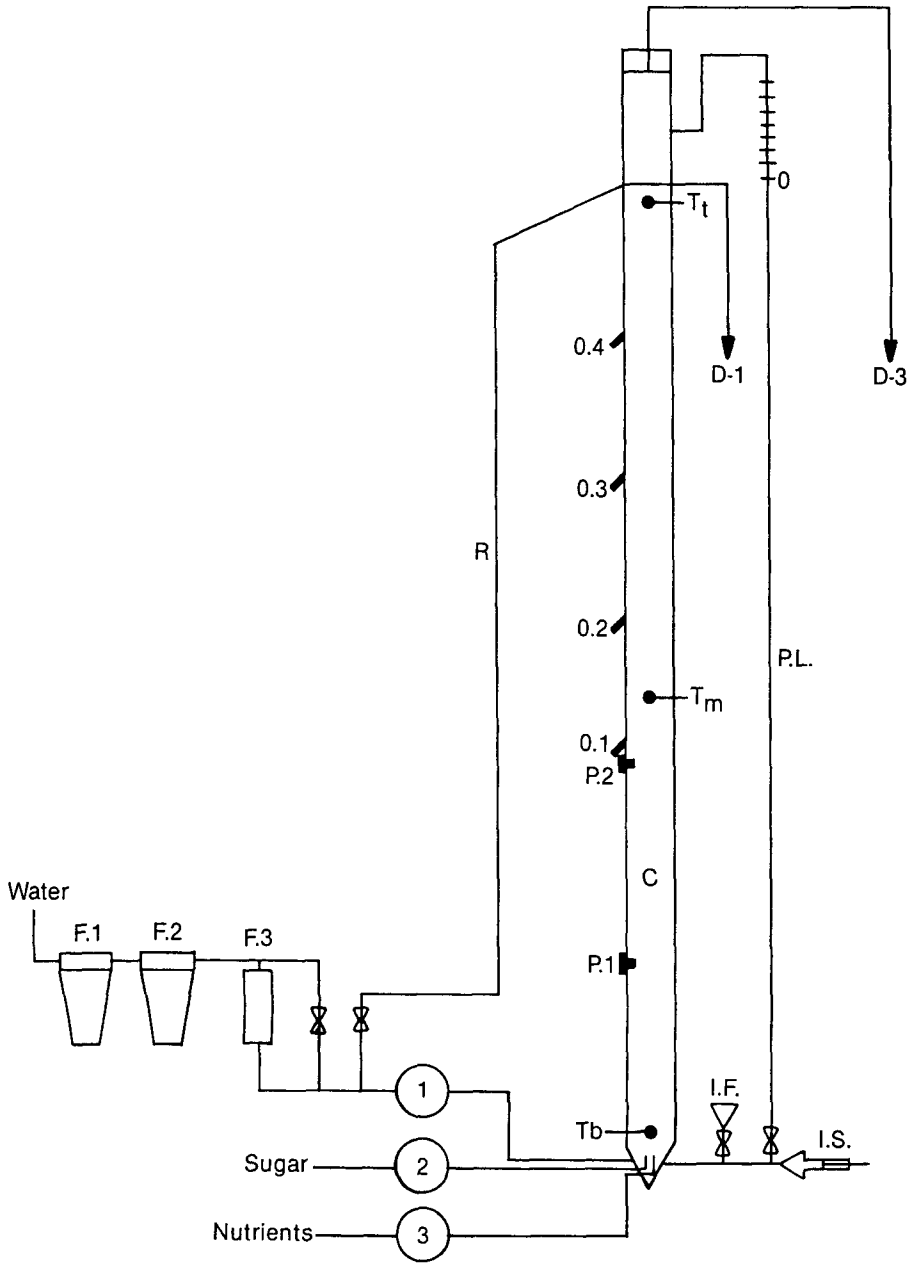

Fig. 6. The experimental reactor

Liquid samples were taken daily from ports P1 and P2 and the outlet stream. Glucose and lactic acid concentrations were measured by liquid chromatography.

A quasi-steady state condition was established by removing, twice daily, any particles that were above a selected outlet port. The particles were collected in a beaker containing a pH 11–12 solution of NaOH in order to desorb the lactic acid. After rinsing and measurement of the volume of the carbon removed, it was reinjected into the base of the bed. The results

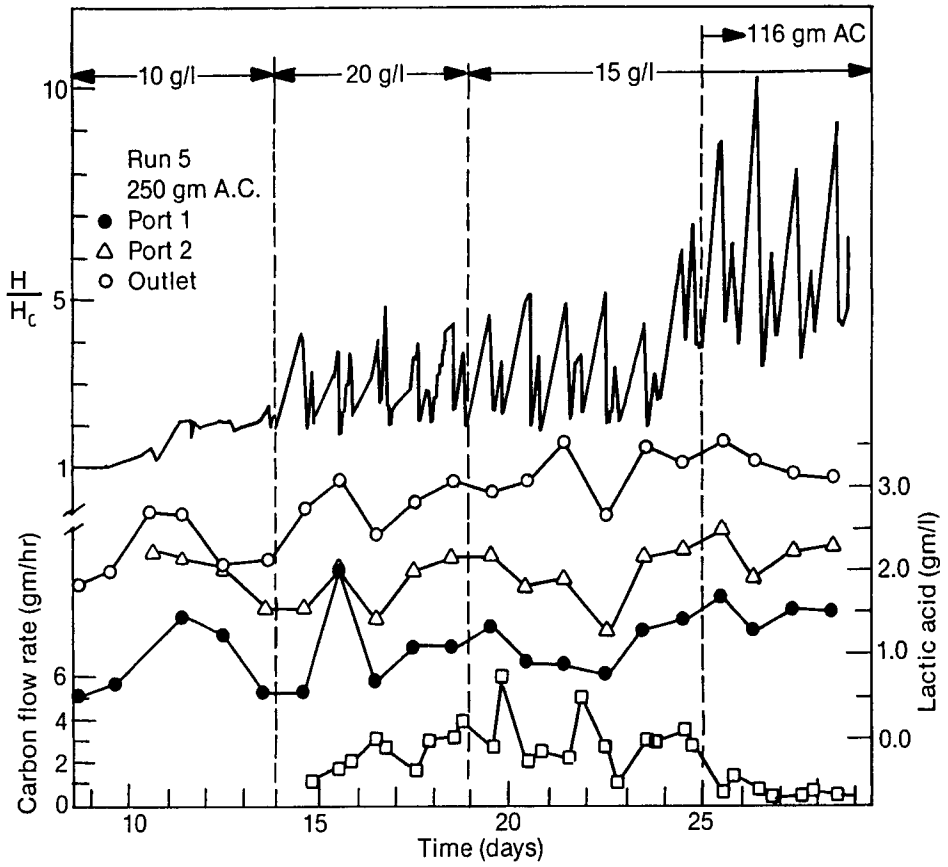

Fig. 7. Continuous lactic acid production.

(Fig. 7) show that with the initial mass of carbon put into the bed (250 g dry wt, which, given the dry particle density of 1.01 g/cm^3, corresponds to a biofilm-free bed height $H_c = 1.20$ m) three such quasi-steady-states were achieved at inlet glucose concentrations of 10, 15, and 20 g/L. After d 24, some carbon was removed from the bed and not replaced, and a new quasi-steady state achieved with bed containing 116 g carbon ($H_c = 0.56$ m).

After 20 d of operation the sterilizing filter F3 was clogged and had to be bypassed allowing nonsterile water into the reactor. Initially, this seemed to have no effect on the reactor performance, as was the case with experiments on ethanol production (*5*). However, growth of a foreign organism, believed to be the sheathed iron bacterium *Crenothrox polyspora*, became visible after a few more days. It eventually plugged the reactor bringing the experiment to an end.

DISCUSSION OF RESULTS

A practical limitation on the laboratory-scale investigation of fluidized-bed fermentors is that the liquid velocity in them is fixed by the need to fluidize the particles. If this velocity is large, as in this case, the bed diam-

eter must be kept small or the amount of substrate needed to feed the bed becomes unmanageable. Some wall effects must be tolerated. More seriously, since the velocity is fixed, the only way to increase the hydraulic residence time is to increase the column height, which is constrained by the ceiling height. These considerations explain the relatively low inlet sugar concentrations used in those experiments. Preliminary calculations using the model of Andrews and Fonta (*6*) indicated that almost complete conversion of a 20 g/L glucose solution should be possible in the available reactor height. In fact, the results showed a substrate conversion of approximately 18%.

The main reason for this is that the calculations were based on a biofilm thickness on the carbon returned to the bed equivalent to $x_i = 1$ and this was not possible in the experiments. The biofilm produced by this organism lacked cohesion and was almost stripped off when the particles were removed from the bed or reinjected into it. It is probable that only a monolayer of cells remained attached to the particles ($x_i = 0.02$), which produced a region at the base of the bed where the cell concentration was very low. This had several undesirable consequences for the operation of the reactor. The low cell concentration gave a very low local reactor productivity. Also, since cell growth is a self-catalyzed process, thin biofilms grow slowly and it is film growth (measured as dx/dt) that moves the particles up the bed. So although the bed expanded rapidly and could double its height overnight (Fig. 7) the solids taken from the top of the bed were mainly biomass (x_o estimated from photographs was approximately 15) and the actual carbon flowrate was quite low. No more than 1% of the lactic acid produced emerged adsorbed on the carbon. A comparison with the theory shows why this is so. The average value of x in the experiments (approximately equal to $(H/H_c) - 1$)(*12*) was in the range 3–6 that the theory (Figs. 2 and 3) shows to be desirable. However the spread of the x values was $\Delta x = 15$, much higher than the desirable values of 4–5. Equation (6) shows that this has a serious effect on the efficiency with which the carbon is used.

Despite the low conversions some important results were obtained. The liquid phase did approximate plug flow, as is shown by the steady increase in lactic acid concentrations up the bed (Fig. 7). The solid phase did stratify based on biofilm thickness as expected. This could be seen directly, because particles with a thin biofilm were black and accumulated at the bottom of the bed, whereas particles with a thick biofilm were light brown and could be seen at the top. Solids mixing is known to be less intense in small diameter fluidized beds as a result of wall effects, so there is no guarantee that this stratification would occur in full-size beds. However evidence for stratification of full-size bioreactors, even in the presence of gas bubbles, is available from tower fermentors used for beer production (*13*). Large yeast flocs are invariably found at the base of the bed, and small ones at the top.

No active pH control was applied to the reactor, so the pH dropped rapidly from 6.0 at the inlet to 4.3 at P1, 3.8 at P2, and 3.4 at the outlet. The resulting inhibition is the other reason for the low substrate conversion. At pH 3.4 the lactic acid concentration that completely inhibits product formation is $\bar{p} = 1.1$ g/L. Even allowing for the approximate nature of this value it was therefore surprising to observe active metabolism near the reactor outlet. This was shown by the rapid film growth mentioned above and by the increase in product concentration from 2.0 to 3.3 g/L between P2 and the outlet giving an overall volumetric productivity of over 10 g/L h. This metabolic activity in a highly inhibitory environment may result from microbial adaption or it may be due to a beneficial interaction between carbon adsorption and product inhibition that is not reflected in the theory. As the pH drops, inhibition increases because more of the lactic acid appears in the undissociated form, and it is this form that inhibits the bacteria. However, the undissociated form is also selectively adsorbed on activated carbon, which is why lactic acid adsorption increases with decreasing pH (Fig. 5). Near the top of the reactor the biofilm is thick and diffusion of substrates and products through it is slow. The combination of this mass transfer resistance with the adsorption of the undissociated acid may make conditions at the base of the film more favorable than in the liquid. Research is continuing on this point.

CONCLUSIONS

The proposed reactor is uniquely suited to the production of organic acids such as lactic and acetic. These fermentations do not produce any gas bubbles that can strip the biofilm from the particles. Also, it is the undissociated form of the acids that is primarily responsible for microbial inhibition and it is the same form that adsorbs on activated carbon. Adsorption, therefore, decreases the concentration of the inhibitor both directly and indirectly by keeping the pH higher than it would otherwise be and thus increasing the dissociation of the acid.

The laboratory reactor produced lactic acid with a productivity comparable to other innovative reactor designs despite: (a) using a newly isolated microbial strain not selected for its productivity; (b) having highly inhibitory conditions in the upper parts of the reactor; (c) having a low cell concentration in the lower part of the reactor. Problems (a) and (b) can easily be solved by a strain improvement program and pH control, respectively. Problem (c) is caused by the fragile biofilm produced by this organism, which results in the film being almost completely stripped from the particle during the handling and regeneration of the activated carbon. It could perhaps be solved by finding a microbial strain that produces a more cohesive biofilm or by surrounding the activated carbon with a macroporous layer in which the cells could grow. Having a biofilm thick-

ness corresponding to $x_i = 1$–2 on the carbon returned to the bed would greatly increase reactor productivity, the flowrate of activated carbon through the bed, and the efficiency with which the carbon was utilized.

ACKNOWLEDGMENT

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REFERENCES

1. Vickroy, T. B. (1985), *Comprehensive Biotechnology,* Moo Young, M., ed., **3,** chap. 38.
2. Vickroy, T. B., Blanch, H. W., and Wilke, C. R. (1983), *Biotechnol. Lett.* **5,** 665.
3. Steiber, R. W. and Gerhardt, P. (1981), *Biotechnol. Bioeng.* **23,** 535.
4. Linko, P. (1984), *Ann. NY Acad. Sci.* **434,** 406.
5. Andrews, G. F. and Fonta, J. P. (1988), *Bioreactor Immobilized Enzymes and Cells,* Moo Young, M., ed., Elsevier, 111–125.
6. Andrews, G. F. and Fonta, J. P. (1986), *Biotechnol. Bioeng. Symp. Ser.* **17,** 317.
7. Andrews, G. F. and Przezdziecki, J. (1986), *Biotechnol. Bioeng.* **28,** 802.
8. Luedeking, R. and Piret, E. L. (1959), *J. Biochem. Micro. Tech. Eng.* **1,** 393.
9. Fonta, J. P. (1987), PhD Thesis, Dept. of Chemical Engrg., State Univ. of NY, Buffalo, NY.
10. Rogers, L. A. and Whittier, E. O. (1928), *J. Bacter.* **16,** 211.
11. Longsworth, L. G. and McInnes, D. A. (1938), *J. Bacter.* **32,** 567.
12. Andrews, G. F. and Tien, C. (1981), *AIChE J.* **27,** 396.
13. Royston, M. G. (1966), *Proc. Biochem.* **1,** 215–221.