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Aerobic Immobilized Cells in Alginate Gel Particles of Variable Density

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ABSTRACT

Immobilized cells of *Acetobacter* sp. were used as a model system of aerobic cells to study the influence of gel particle density in fermentation carried out in fluidized-bed bioreactor. Particles of variable density were prepared by adding different amounts of α-alumina to the gel matrix. The effect of the density of the particles was analyzed through the variation of their terminal velocity.

The behavior of continuous oxidation of ethanol to acetic acid was characterized in terms of productivity of acetic acid and gas–liquid volumetric oxygen transfer coefficient, as a function of dilution rate.

An empirical correlation among the gas–liquid volumetric oxygen transfer coefficient, volumetric particle concentration ($\varepsilon_{S/L}$), and particle Reynolds number (Re_p) was proposed in this work.

Index Entries: Acetic fermentation; fluidized bed; immobilized cells; alginate gel; gas-liquid volumetric oxygen transfer coefficient (K_La)

Nomenclature: C^*, saturated dissolved oxygen concentration in fermentation medium, (kmol/m^3); C_{do}, dissolved oxygen concentration in fermentation medium, (kmol/m^3); C_{HAc2}, C_{HAc1}, bioreactor outlet and inlet acetic acid concentration (g/L); D, dilution rate (1/h); K_La, gas–liquid volumetric oxygen transfer coefficient (1/h); M, molecular weight of acetic acid; Pr, total acetic acid production rate (g/L·h); Qo_{2t}, specific respiration rate, (kmol/kg [dry wt] h); Re_p, particle Reynolds number (–); vvm, vessel volumes per minute; X, cell concentration, (kg/m3); ε_L, liquid holdup (–); $\varepsilon_{S/L}$, volumetric particle concentration (–).

INTRODUCTION

Hydrophylic gel matrix is a useful support for viable microbial cell immobilization. Jamuna et al. *(1)* stated that sodium alginate is the most commonly used polymer matrix for the entrapment of microbial cells. Sun and Furuzaki *(2)*, and Osuga et al. *(3)* used the fluidized-bed bioreactor to study acetic acid production from ethanol with immobilized whole cells. However, these kinds of supports are very light, presenting a specific mass close to that of water. This characteristic

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is a limitation on the use of gel particles in fluidized-bed bioreactors. The use of denser gel particles permits operation of fluidized beds at higher air flow rates and promotes better motion of particles in the bed. Fan and Newcomer *(4)* suggested that larger and denser particles should break the bubbles more easily than the smaller and ligher ones, resulting in a greater interfacial area. Ghommidh et al. *(5)* affirmed that particles have to be dense enough to settle down rapidly and allow easy retention in a fluidized bed. Chase *(6)* reported that denser materials have been developed specifically for use in expanded-bed procedures. Black et al. *(7)* proposed a system that allowed a circulating mixing of low specific mass particles to improve mass transfer.

Sun et al. *(8)* proposed a dimensionless correlation for the gas–liquid volumetric oxygen transfer coefficient, $K_l a$, in a fluidized-bed bioreactor, as a function of volumetric particle concentration and particle diameter of conventional particles.

The main purpose of this article is to characterize the influence of dense gel particles in the continuous fermentation carried out in a fluidized-bed bioreactor and propose an empirical correlation for $K_l a$, including the influence of particle density.

MATERIAL AND METHODS

Microbial Strain

A high acetic-acid-producing strain of *Acetobacter* sp. CCT 2026 (Tropical Culture Collection, Campinas, São Paulo, Brazil) bacteria was used in this study.

Materials

The alginate was purchased from Wako Pure Chemical Industries Ltd. (Japan) and (α-alumina was obtained from Alcoa (Brazil). Other reagents were of analytical grade.

Culture Media

Maintenance broth: 5 g yeast extract, 3 g peptone, 25 g glucose, 1 L distilled water.

Propagation medium: 10 g yeast extract, 10 g peptone, 10 g glucose, 50 mL ethanol, 10 mL acetic acid, 20 g agar, 1 L distilled water.

Fermentation medium: 10 g yeast extract, 10 g peptone, 10 g glucose, 50 mL ethanol, 5 mL acetic acid, 1 L distilled water.

Ethanol and acetic acid were added after media autoclaving.

Preculture

A lyophilized ampule was rehydrated with sterile distilled water and transferred to 5 mL of maintenance broth for 24 h of incubation at 30°C, 180 rpm. This culture was inoculated onto 10 Petri dishes, containing propagation medium, and incubated at 30°C for 48 h. The grown cells were suspensed in 100 mL of fermentation medium and incubated at 30°C, 180 rpm for 27 h. The cellular broth obtained (about 107 CFU/mL) was used as seed culture for the immobilization of cells. This cultivation methodology was based on the work of Moraes *(9)*.

Preparation of Denser Particles of Biocatalysts

The apparatus used to prepare denser gel beads consisted of a double nozzle based on the work of De Araújo *(10)*. This apparatus was a modification of the one developed by Tanaka et al. *(11)*, producing double-layered alginate gel fibers, which prevented cell leakage from the polymeric matrix into the medium, and used by Fumi et al. *(12)* for a preliminary study on conditions affecting *Acetobacter* immobilization both in single- and double-layered alginate gel.

The particles of variable density were prepared by adding different amounts of α-alumina in the gel matrix. In order to prevent the influence of α-alumina concentration on the cells' growth and distribution in the gel matrix, particles were composed of a core-containing gel and α-alumina, coated with a thin layer of gel entrapping viable cells.

The beads were obtained by simultaneously pumping a solution of sodium alginate (1% w/v) and α-alumina at different concentrations (from 1 to 40% w/v) through an inner nozzle, and a mixture of sodium alginate (4% w/v) and seed culture collected after 27 h of aerated cultivation, in a ratio of 1:1, through an outer nozzle. Both solutions were pumped into a solution of $CaCl_2 \cdot {}_2H_2O$ 0.2*M* at 10°C from a height of 1 cm. The gel beads were allowed to solidify for 20 min in the latter solution, obtaining spheres around 3 mm in diameter.

Analyses

The amount of acetic acid was estimated by titration with 0.05*N* NaOH solution using phenolphtalein as indicator. Dissolved oxygen concentration was measured with a polarographic oxygen sensor, YSI model 5300, which was connected to an oxygen monitor and a chart recorder. The particles' terminal velocities were determined in a water column.

Experimental Setup for Continuous Fermentation

The experimental setup for respiration rate measurements and the continuous operation of the bioreactor was the same as in the work of Paz et al. *(13)*. The reactor had 150 mL of effective reaction volume. The fermentation medium was continuously supplied to the bioreactor by a peristaltic pump. The oxygen sensor was installed 5 cm above the bottom. Aeration was provided by the use of a compressor. The air passed through a humidifier, a cotton filter, a 0.45-μm Millipore membrane filter, and a capillary flowmeter. The bioreactor was placed in a 30°C water bath controlled by a thermostat. The water bath was also connected to the oxygen bath assembly (YSI 5301). A condenser was connected to the gas exit preventing ethanol and water from being carried out of the bioreactor, according to observations by Ghommidh and Navarro *(14)*.

Production Rate and Oxygen Transfer Calculations

The productivity of acetic acid in continuous fermentation, *Pr*, was calculated by the equation:

$$Pr = D\,(C_{HAc2} - C_{HAc1}) \tag{1}$$

The gas–liquid volumetric oxygen transfer coefficient, $K_l a$, was calculated from the oxygen balance in the system. At steady-state, oxygen transfer and consumption are balanced:

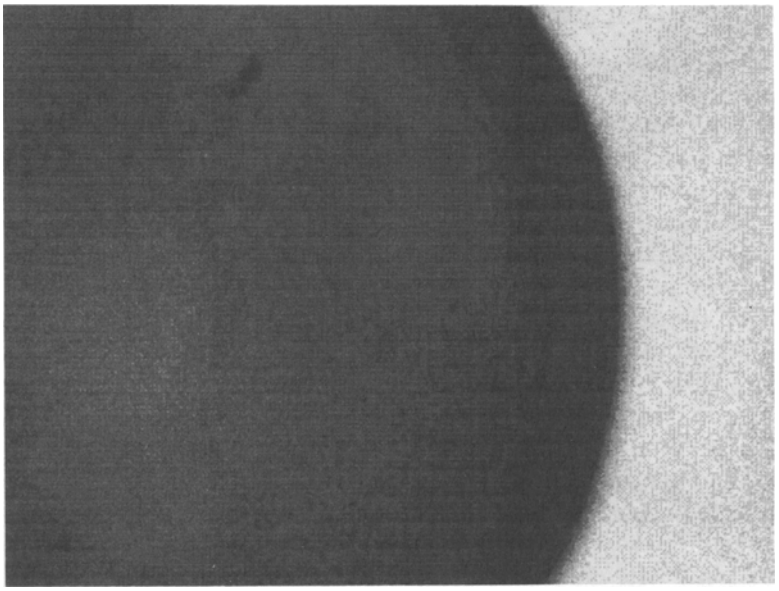

Fig. 1. Microphotograph of cross section of biocatalyst bead, after 15 d of continuous fermentation.

$$\text{Total oxygen consumption} = Qo_{2t}\, X = \text{oxygen transfer} = K_l a\, (C^* - C_{do}) \quad (2)$$

Since acetic acid bacteria oxidize ethanol in a stochiometric ratio 1:1, between oxygen consumption and acetic acid production, we can conclude that:

$$\text{Total oxygen consumption} = Qo_{2t}\, X = \text{acetic acid production} = Pr/(M\varepsilon_L) \quad (3)$$

As a consequence:

$$K_l a = Qo_{2t}\, X \,/\, (C^* - C_{do}) = Pr/(M\varepsilon_L)\, (C^* - C_{do})] \quad (4)$$

RESULTS

The microphotograph (magnification 65×) of Fig. 1 was taken with light microscope after properly staining the bead with saphranine. It shows that *Acetobacter* microcolonies grow on the thin layer of coating of gel bead.

Figures 2 and 3 show the productivity and gas–liquid volumetric oxygen transfer coefficient profiles as a function of dilution rate, for the continuous acetic fermentation in the bioreactor using free and entrapped cells in the presence or absence of α-alumina at the core of the particles.

Free cells present the typical behavior, production rate increasing with dilution rate up to the washout region ($D = 0.3$/h). Owing to *Acetobacter* growing characteristics, a film of cells adhered to the bioreactor that was responsible for the contribution of acetic acid production at high dilution rates. Under immobilization conditions, both free and immobilized cells contributed to the overall production either in the presence or in the absence of α-alumina at the core of the beads. The

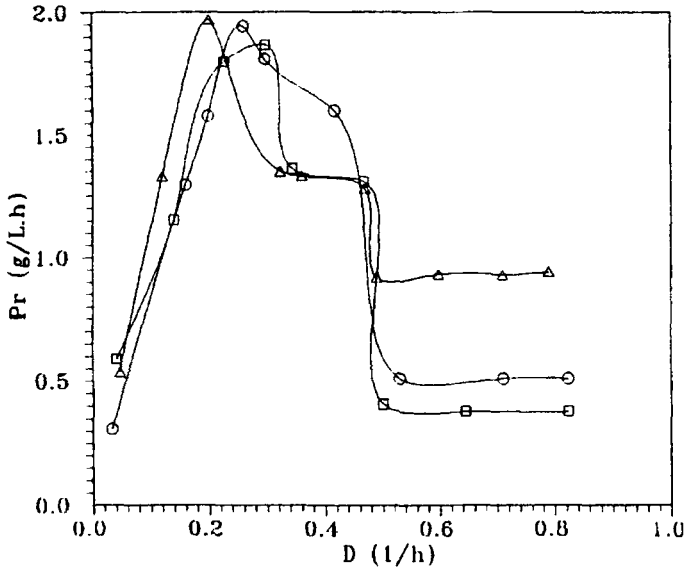

Fig. 2. Profiles of production rate vs dilution rate. Aeration rate: 8 vvm. Volumetric particle concentration: 0.066. Particle diameter: 3 mm. ○, free cells; □, alginate immobilized cells; △, alginate + alumina 5% immobilized cells.

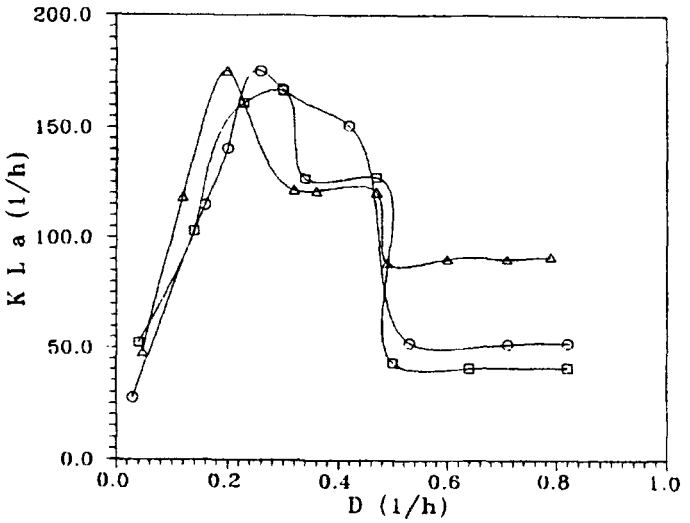

Fig. 3. Profiles of gas–liquid volumetric oxygen transfer coefficient vs dilution rate. Experimental conditions: the same as in Fig. 2. ○, free cells; □, alginate immobilized cells; △, alginate + alumina 5% immobilized cells.

levels reached after the washout region of free cells indicate the contribution of immobilized cells and cells from the film. The removal of the film is facilitated by bead movements in the bioreactor, so the contribution of only immobilized cells was observed at high dilution rates ($D > 0.5/h$).

Figure 4 shows the productivity of acetic acid as a function of the particles' terminal velocity at the low, medium, and high levels of dilution rates. Particles

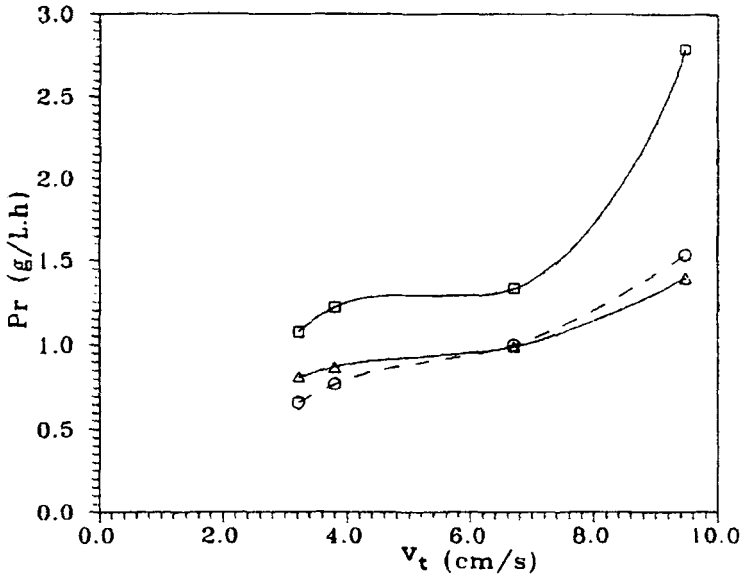

Fig. 4. Production rate vs particles' terminal velocity at representative dilution rates. Experimental conditions: the same as in Fig. 2. ○, D = 0.08/h; □, D = 0.28/h; △, D = 0.50/h.

containing 1, 5, 20, and 40% (w/v) of α-alumina at the core had a terminal velocity of 3.2, 3.8, 6.7, and 9.5 cm/s, respectively, determined in a water column at the intermediary Reynolds number region ($0.4 < Re < 500$). The influence of the particles' terminal velocity on acetic acid productivity can be seen better at the medium dilution rate (D=0.28/h), where the contribution of free cells is effective. The higher effect of the particles' terminal velocity is probably the result of the enhancement of oxygen transfer rates in the system.

Figures 5 and 6 show the productivity and gas–liquid volumetric oxygen transfer coefficient profiles as a function of dilution rate, varying volumetric particle concentrations in the bed. These particles were prepared with an inner solution of sodium alginate (1% w/v) and α-alumina (5% w/v), and an outer one of sodium alginate (4% w/v) and a seed culture obtained by the method described above, in a ratio of 1:1.

The same behavior described above for immobilized cells was observed. Both free and immobilized cells contributed to the overall production, but the levels reached after the washout region of free cells indicate the influence of volumetric particle concentrations.

An empirical correlation for the gas–liquid volumetric oxygen transfer coefficient, K_La, was obtained in terms of the particle Reynolds number, Re_p, and volumetric particle concentration, $\varepsilon_{S/L}$, at the region where the main contribution is owing to immobilized cells. The experimental data were fitted by Marquardt method using the Statistical Analysis Systems (SAS). The parameters of the proposed correlation are presented below. The standards errors were below 10.7%.

$$K_La = 96.83\ Re_p^{0.48}\ \varepsilon_{S/L}^{0.87} \tag{5}$$

$$(96 < Re_p < 285 \text{ and } 0.031 < \varepsilon_{S/L} < 0.066) \tag{6}$$

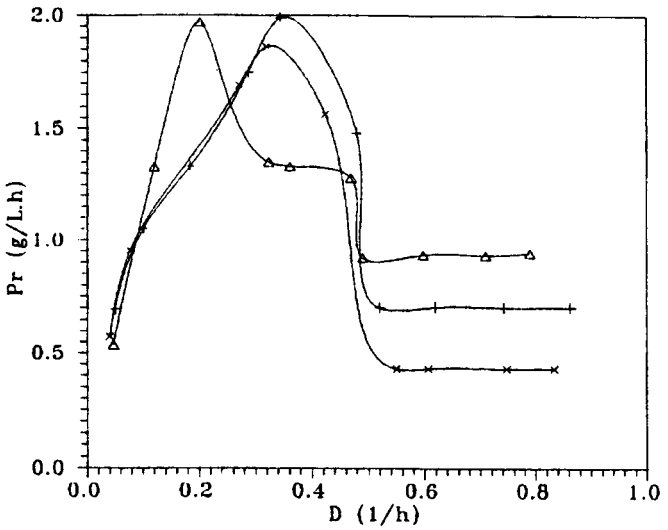

Fig. 5. Profiles of production rate vs dilution rate. Aeration rate: 8 vvm. Particles' terminal velocity: 3.8 cm/s. Particle diameter: 3 mm. △, $\varepsilon_{S/L} = 0.066$; +, $\varepsilon_{S/L} = 0.050$; x, $\varepsilon_{S/L} = 0.031$.

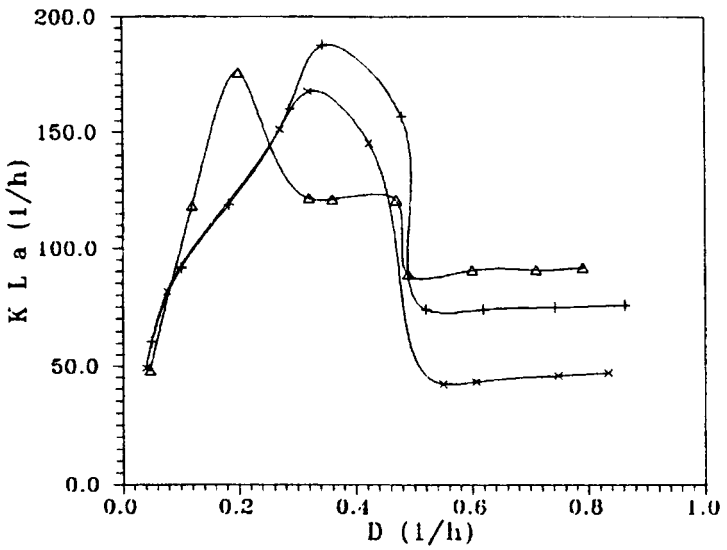

Fig. 6. Profiles of gas-liquid volumetric oxygen transfer coefficient vs. dilution rate. Experimental conditions: the same as in Fig. 5. △, $\varepsilon_{S/L} = 0.066$; +, $\varepsilon_{S/L} = 0.050$; x, $\varepsilon_{S/L} = 0.031$.

CONCLUSION

Denser particles of biocatalysts containing viable cells could be obtained using sodium alginate and α-alumina through the double-nozzle apparatus.

The fermentation carried out in fluidized-bed bioreactor with denser gel particles of biocatalysts presents a better performance than the one with conventional alginate gel particles.

Denser particles promote greater mass transfer coefficients, so that the volumetric mass transfer coefficient increases with higher particle density. The volumetric mass transfer coefficient increases also with the volumetric particle concentration. Both denser particles and higher volumetric particle concentrations seemed to promote greater interfacial areas or better mixing characteristics, giving consequently higher production rates.

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