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Enhancement of the Oxygen Transfer in a Circulating Three-Phase Fluidized Bed Bioreactor

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ABSTRACT

The addition of α-alumina to the aqueous solution of sodium alginate for immobilization of viable cells allows the production of denser particles than the ones obtained with sodium alginate alone. This biocatalyst was used in the continuous oxidation of ethanol to acetic acid, by a strain of *Acetobacter* isolated from an ethanol plant. The continuous fermentation was carried out in a circulating three-phase fluidized bed bioreactor.

Denser particles allow a greater oxygen transfer, resulting in increased volumetric production rate of acetic acid, because oxygen is usually the limiting factor of this process. The performance of the biocatalyst was analyzed in terms of respiration rate, oxyen diffusivity, volumetric mass-transfer coefficient, and global productivity.

Index Entries: Immobilized cells; mass transfer; *Acetobacter*.

NOMENCLATURE

C^*	Saturated dissolved oxygen concentration in liquid medium (kmol m^{-3})
C_{do}	Dissolved oxygen concentration in liquid medium, (kmol m^{-3})

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C_{HAc1}, C_{HAc2}	Initial and final acetic acid concentrations (kmol/mL)
D	Dilution rate, h^{-1}
Deff	Oxygen effective intraparticle diffusion coefficient, (m^2/s)
g	Gravitional constant (m^2/s)
K_L	Mass-transfer coefficient (m/s)
K_La	Volumetric mass-transfer coefficient (s^{-1})
K_m	Michaelis-Menten constant ($kmol/m^{-3}$)
Pr	Total acetic acid production rate (g/L·h)
Q_{o2}	Specific respiration rate (kmol/kg[dry wt]h)
$Q_{o2\ actual}$	Actual specific respiration rate of immobilized cells for 1.7 mm diameter particles (kmol/kg[dry wt]h)
$Q_{o2\ free\ of\ diffusion\ limitations}$	Specific respiration rat3, in the absence of diffusion resistance, of immobilized cells for 0.1 mm diameter particles (kmol/kg[dry wt]h)
R	Particle radius (m)
X	Total cell concentration (g/L)
β	Saturation parameter
ϵL	Liquid holdup
ϕ	Thiele modulus
η	Effectiveness factor

INTRODUCTION

The immobilization of cells presents several advantages in the fermentation process. However, a primary limitation in these systems is the oxygen mass transfer, caused by diffusional resistances, especially when cells with high respiration activity are involved.

Acetobacter sp. is very sensitive to oxygen deficiency, and cells can undergo complete inhibition when aeration is not sufficient. Therefore, under these conditions, one may not expect a higher acetic acid productivity in submerged vinegar fermentation by using an immobilized cell process, instead of the free cell process (*1*). In order to conduct an aerobic high-cell-density culture successfully, it is necessary to supply a large amount of dissolved oxygen to the culture (*2*). Therefore, efforts must be made to improve the oxygen transfer in the system.

The entrapping supports for whole cells are usually polymeric gels (e.g., calcium alginate) that are very light, presenting a density near to that of water. Sun et al. (*3*) found that the mass-transfer coefficient, K_La, in a three-phase fluidized bed reactor of Ca-alginate beads was lower than that in bubble columns for all particle diameters tested. The main reason was the low density of the beads.

Ostergaard (*4*) reported that in a bed containing 6-mm glass beads, the volumetric mass-transfer coefficient was greater than that in bubble columns. However, 1-mm particle beds gave lower coefficients when

compared to the bubble columns. Fan and Newcomer (5) suggested that larger and denser particles should break the bubbles more easily than the smaller and lighter ones, resulting in a greater interfacial area. Osuga et al. (6) used a fluidized bed reactor for the study of acetic acid production, using immobilized whole cells, and Black et al. (7) proposed a system that allowed a circulating mixing strain of low-density particles, in order to improve mass transfer. This study aims to show the feasibility of the process and to examine the effect of biocatalysts of higher density than those used by Sun et al. (1) in a circulating fluidized bed bioreactor.

MATERIALS AND METHODS

Microorganisms

The microorganism used in this study was an acetic acid bacteria isolated from a sugar and alcohol production mill, at the cane juice extraction point. The cells were obtained from the Tropical Culture Collection (Brazil) as *Acetobacter* sp. FTPT 2026.

Materials

The alginate was purchased from Wako Pure Chemicals Co. (Japan). Other reagents were of analytical grade.

Culture Media

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

Propagation medium (B): 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 (C): 10 g yeast extract, 10 g peptone, 10 g glucose, 50 mL ethanol and 5 mL acetic acid, 1 L distilled water.

Ethanol and acetic acid were added after media autoclaving.

Preculture

A lyophilized ampule was rehydrated with 0.1 mL of sterile distilled water for 15 min. This suspension was transferred to 5 mL of maintanence broth A and was incubated on a reciprocal shaker at 30°C, 180 strokes/min for 24 h. Then, 0.5 mL of this culture was inoculated onto the surface of ten Petri dishes containing propagation agar medium B. The dishes were incubated at 30°C for 36 h. The Petri dishes were carefully scraped, and the grown cells were suspended in 100 mL of the fermentation medium C. This cellular suspension was incubated in a 500-mL conical flask, under reciprocal shaking (180 strokes/min) at 30°C for 24 h. The dense

cellular broth obtained was used as seed culture for the following experiments. This cultivation methodology was based on the work of Moraes (*8*), and it ensures an *Acetobacter* cell concentration of about 10^7 CFU/mL, enough to maintain high cell viability after immobilization.

Immobilization of the Bacteria

The seed culture collected after 24 h of aerated cultivation was mixed, under room temperature, with either sterile 4% Na-alginate or 2% α-alumina + 2% Na-alginate, in a ratio of 1:1. The mixture was dropped into a solution of $CaCl_2$ from a height of 5 cm, using a 30-mL syringe supplied with a needle. Calcium alginate gel beads obtained by this method had about 1.7 mm diameter and entrapped approx 10^7 CFU/g gel of living cells.

Analyses

Dissolved oxygen concentration was measured with a polarographic oxygen sensor, YSI model 5300, which was connected to an oxygen monitor and a chart recorder. Acetic acid was estimated by titration with a 0.1*N* NaOH solution using phenolphthalein as the indicator. The medium optical density was measured at 600 nm and converted into cell mass concentration using a calibration curve.

Experimental Setup

Figure 1 shows the sketch of the experimental setup for the respiration rate measurement and the continuous operation of the bioreactor. The reactor was made of glass with 60 mm inner diameter and 265 mL of effective reaction volume. A porous flat plate was fixed at the end of the aeration tube, placed close to the bottom of the reactor. This setup promoted a circulating particle movement that is also indicated in Fig. 1. The liquid medium was continuously supplied to the reactor by a peristaltic pump from a 14-L storage tank. The oxygen sensor was installed 5 cm above the bottom, forming a 45° angle upward with the horizontal line as shown in Fig. 1, number 12. Aeration was carried out by an odontological compressor. The air was passed through two humidifiers, a cotton filter, a millipore membrane filter, and a capillary flowmeter. The reactor was placed in a 30°C water bath controlled by a thermostat. This water bath was also connected to the oxygen bath assembly (YSI 5301). By protecting the electrode membrane from direct contact with the air bubbles in the reactor, it was possible to make the readings with minimum oscillation.

Measurement of Oxygen Uptake Rates

Samples of either immobilized or free cells were added to an aerated fermentation medium in the electrode vessel (of 10 mL) to give a final vol

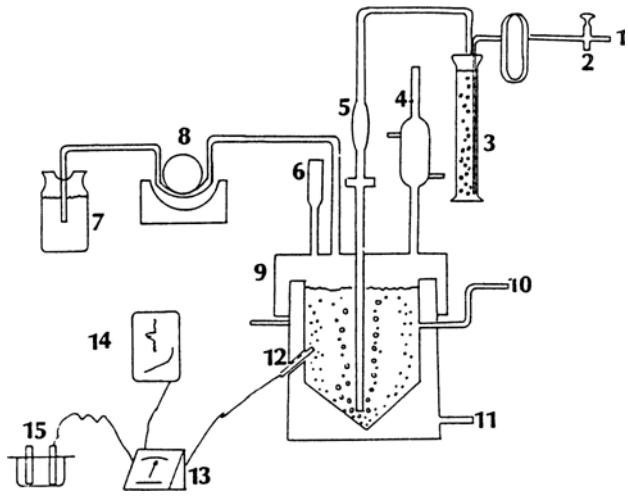

Fig. 1. Experimental setup: 1. air from compressor; 2. oxygen from cylinder; 3. humidifier; 4. condenser; 5. air filters; 6. antifoam; 7. medium tank; 8. peristaltic pump; 9. reactor; 10. product exit; 11. thermostatted water; 12. oxygen electrode; 13. monitor; 14. chart recorder; 15. bath assemble.

of 6 mL. Oxygen uptake rates were measured in the closed vessel as indicated in Fig. 1. Measurements were made with immobilized cells immediately after the gel beads had been prepared, to avoid any physiological changes in the biocatalyst.

Diffusivity Calculations

The oxygen diffusivity in gel beads was estimated using the effectiveness factor.

$$\eta = Q_{o2\,\text{actual}} / Q_{02\,\text{free of diffusion limitation}} \quad (1)$$

$$\phi = 1/\eta\,[(1 + \beta) / \beta]\,\sqrt{2}\,[\beta - \ln(1 + \beta)]^{1/2} \quad (2)$$

$$\beta = C^* / K_m \quad (3)$$

$$\text{Deff} = R^2\,Q_{o2\,\text{free of diff. limitation}} / (9\,K_m\,\phi^2) \quad (4)$$

Production Rate Calculation

$$Pr = D\,(C_{HAc2} - C_{HAc1}) \quad (5)$$

Volumetric Mass-Transfer Coefficient Calculation

$$K_L a = Q_{o2}\,X / (C^* - C_{do}) = Pr / [60\,\epsilon L\,(C^* - C_{do})] \quad (6)$$

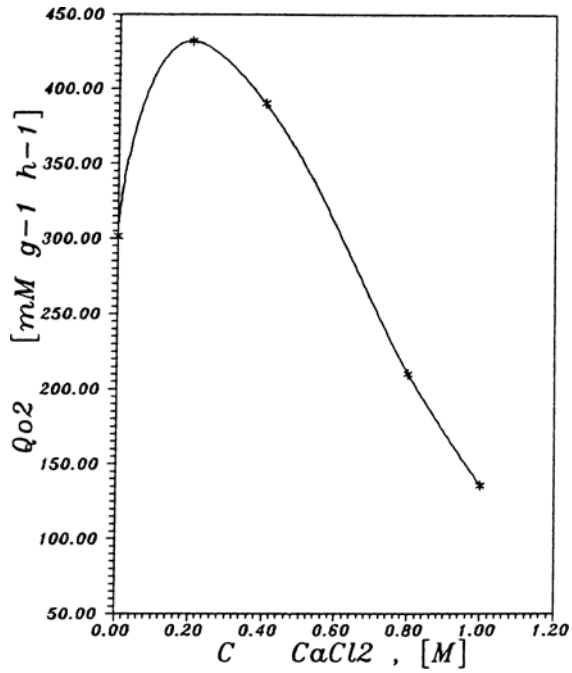

Fig. 2. Oxygen uptake rates for free cells as a function of calcium chloride concentration.

RESULTS AND DISCUSSION

Effect of Gel Preparation Variables on the Activity of the Biocatalyst

The effect of $CaCl_2$ on the oxygen uptake rate by free cells was studied. Figure 2 shows that there is an increase in the respiration rate for concentrations of $CaCl_2$ between 0.0 and 0.2*M*. After this, the respiration rate begins to decrease for higher $CaCl_2$ concentrations. The inhibition of respiration by the electrolyte is drastic at concentrations higher than 0.4*M*. Its physiological meaning cannot be explained by these data. However, it is important to observe that it might be preferable to use a lower concentration (ca. 0.2*M*) of $CaCl_2$ in the gelification step because of the obtention of a more active biocatalyst, although there may be a decrease in the mechanical strength of the bead with lower cencentrations of the electrolyte in the gelification solution.

Table 1 shows that as the time of gelling increases, the specific respiration rate decreases. The maximum reduction in the oxygen uptake rate for the alginate beads exposed to 0.2*M* solution, by 20 and 1200 min, was about 30%. A similar pattern was observed for the α-alumina + alginate, where the maximum decrease in the oxyten uptake rate was 24%. The

Table 1
Specific Oxygen Uptake Rates as a Function of the Time of Gelification and the $CaCl_2$ Concentration for Immobilization of Cells in Alginate and α-Alumina + Alginate[a]

Gelification time, min	$CaCl_2$ concentration, *M*	Q_{o2}, m*M*/g·h	
		Alginate	α-Alumina + Alginate
20	0.2	365.33	306.80
40	0.2	337.50	273.35
1200	0.2	281.25	233.44
1200	0.5	247.50	202.95
1200	1.0	247.50	202.95

[a]Cell concentration was X = 0.075 g/g gel.

Table 2
Specific Oxygen Uptake Rates as a Function of the Time of Gelification and the $CaCl_2$ Concentration for Immobilization of Cells in Alginate and α-Alumina + Alginate[a]

Gelification time, min	$CaCl_2$ concentration, *M*	Q_{o2}, m*M*/g·h	
		Alginate	α-Alumina + Alginate
20	0.2	9.04	7.55
40	0.2	8.72	7.15
1200	0.2	0.87	0.72
1200	0.5	0.83	0.69
1200	1.0	0.75	0.62

[a]Cell concentration was X = 3.7 g/g gel.

depletion of oxygen is slower in this biocatalyst, and even after 1200 min of exposure, there is more oxygen available than in the previous case. The oxygen uptake rate by the immobilized cells also diminished as the concentration of $CaCl_2$ used in the gelling increases.

As can be seen in Fig. 2, the specific respiration rate of free cells is 301.6 m*M*/g h, thus being lower than the values found for the immobilized cells (0.2*M* $CaCl_2$ solution for 20 and 40 min). The cell mass concentrations are the same for both experiments. In spite of the diffusion limitations, the specific respiration rates under immobilized cell conditions were higher (for $CaCl_2$ concentrations < 0.2*M*) than those observed for free cells. This might be because of physiological changes in the cell.

The increase in the amount of the immobilized cells is followed by a sharp decrease in the specific oxygen uptake rate, as can be observed by comparing Table 1 (X = 0.075 g/g gel) and Table 2 (X = 3.7 g/g gel). The

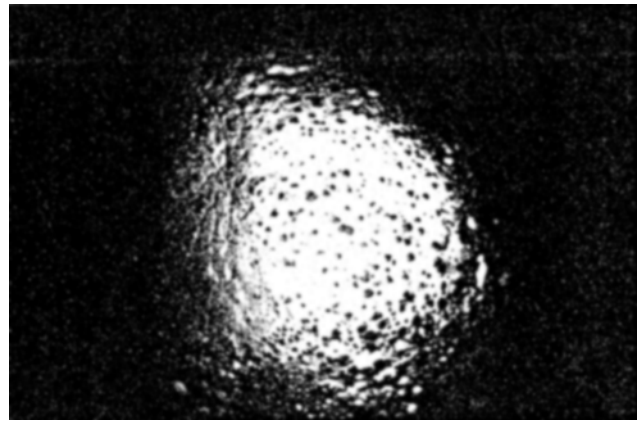

Fig. 3. Photomicrograph of an α-alumina + alginate bead, stained with saphranine, with a light microscope.

oxygen uptake rate decreases from ca. 300 m*M*/g·h to <10 m*M*/g·h. As can be observed from Tables 1 and 2, the oxygen permeation rate was always ca. 18% lower in the presence of α-alumina.

The photomicrograph (magnification 500x) of Fig. 3 was taken with a light microscope after properly staining the bead with saphranine. It shows a great number of *Acetobacter* microcolonies on the surface of an α-alumina + alginate gel bead.

Oxygen Diffusivity

From the slopes of the curves that relate the depleted oxygen concentration with time, the effectiveness factor for the 1.7 mm diameter particle can be calculated. The kinetic parameters used in the equations were calculated previously by Dabdoub (*9*). The diffusivity of oxygen in the alginate particles was found to be $4.566*10^{-7}$ cm²/s, and for the α-alumina + alginate particles, the oxygen diffusivity was $3.592*10^{-7}$ cm²/s. Thus, the presence of α-alumina does not affect the oxygen diffusivity. These values are approx 98% lower than the oxyten diffusivity in pure water at 30°C.

Continuous Acetic Acid Production in the Bioreactor

The continuous cultivations were carried out using free and entrapped cells in alginate with the presence or absence of α-alumina. Figure 4 shows production rate vs dilution rate for the three systems. Free cells present the typical behavior, where production rate increases with dilution rate up to the washout point. Under immobilization conditions, both free and immobilized cells contributed to the overall production either in the presence or absence of α-alumina. The level reached after the washout of free

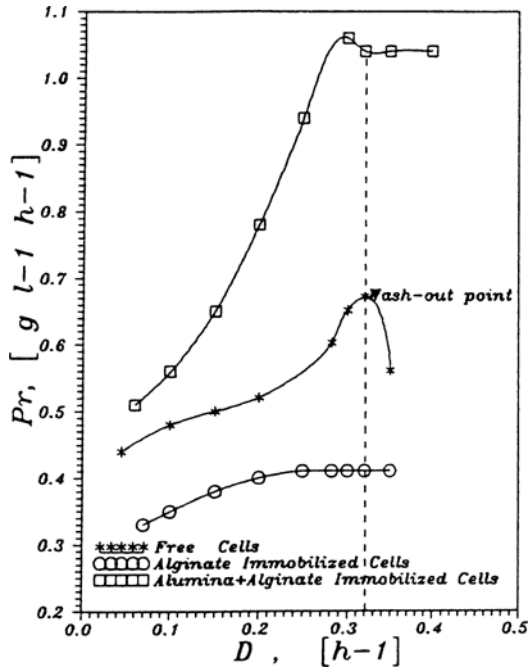

Fig. 4. Acetic acid production rate as a function of the dilution rate. Aeration rate: 4.5 vvm. Solid fraction: 0.11. Particle diameter: 1.7 mm. Alginate particles density: 1038 kg/m^3. α-Alumina + alginate particles density: 1270 kg/m^3.

cells indicates the maximum contribution of immobilized cells. The sharp decrease observed under low dilution rates (<0.3 h^{-1}) for entrapment with α-alumina + alginate reflects directly on the respiratory inhibition caused by the high acetic acid concentration.

Figures 5 and 6 show the behavior of dissolved oxygen and cell mass concentration for the three systems tested. Immobilized systems showed a small variation in the dissolved oxygen concentration regardless of the dilution rates, indicating the effective contribution of immobilized cells, whereas the free cellular system showed a sharp increase in C_{do} as the cellular level dropped, when the dilution rate reached the washout point. Figure 6 indicates a gradual decrease in cell mass concentration with increasing dilution rates, reaching a minimum for the immobilized systems, which indicates the actual contribution of immobilized cells to the suspended mass cell concentration in the system.

As can be observed from Figs. 4, 5, and 6, the contribution of immobilized cells to the production rate is greater in the α-alumina + alginate system, for dilution rates >0.1 h^{-1}. For free cell systems, an increase in dilution rates is followed by an increase in the volumetric oxygen mass-transfer coefficients (*1,6*). According to the surface renewal theory, K_L depends on Deff$^{1/2}$, and the diffusivity increases as cell concentration

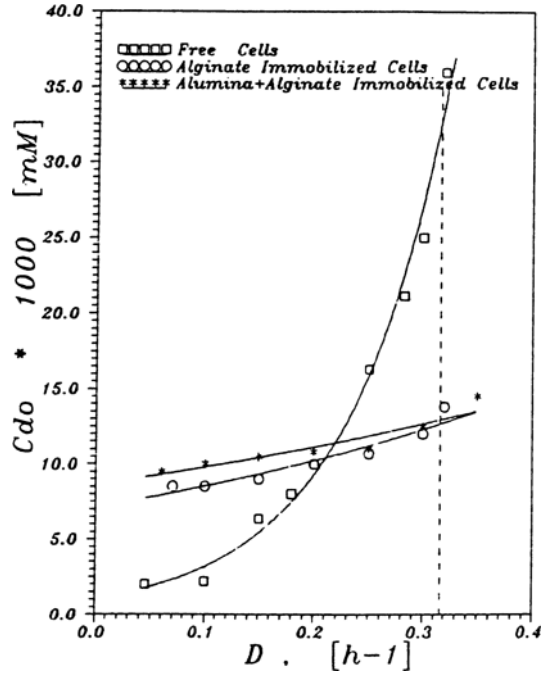

Fig. 5. Dissolved oxygen concentration as a function of the dilution rate. Experimental conditions: the same as in Fig. 4.

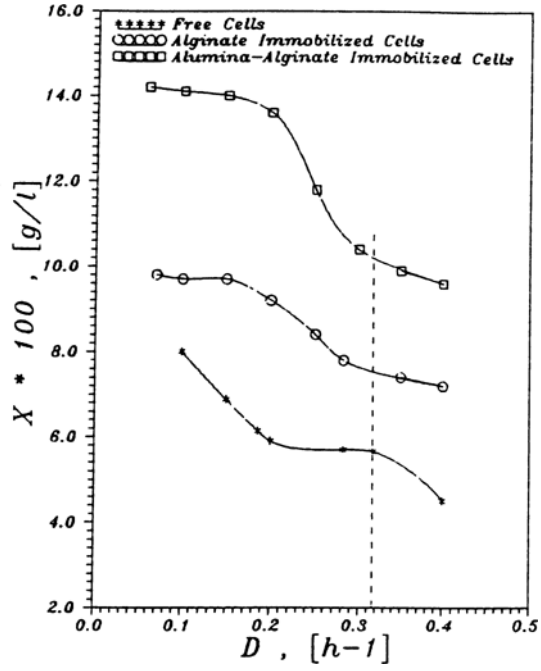

Fig. 6. Biomass concentration as a function of the dilution rate. Experimental conditions: the same as in Fig. 4.

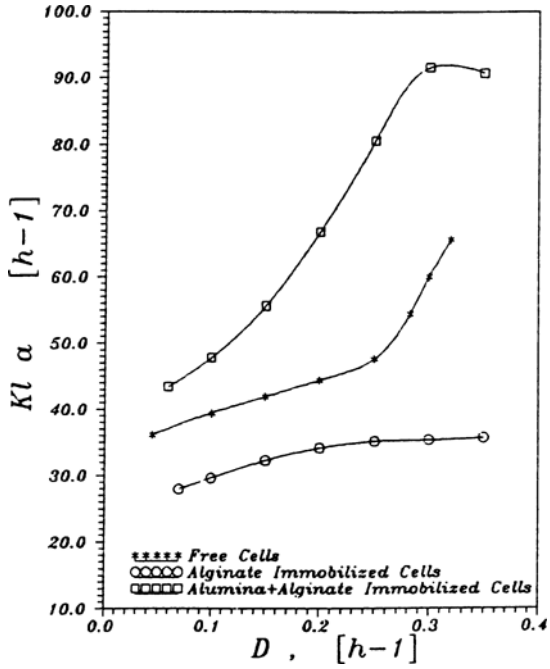

Fig. 7. Volumetric oxygen-transfer coefficient as a function of the dilution rate. Experimental conditions: the same as in Fig. 4.

decreases (*10–12*). This behavior is confirmed by the results in Fig. 7. Denser particles seem to promote greater interfacial areas and mass-transfer coefficients, so that the volumetric mass-transfer coefficient increase with higher solid density. This fact is reflected by the higher production rates observed for the α-alumina + alginate particles.

CONCLUSIONS

The activity of alginate gels biocatalysts depends on the preparation variables, as the gelification time and the concentration of electrolyte and viable cells. The gelification time must be as short as possible. The $CaCl_2$ concentration must be kept lower than 0.2*M*, and the immobilized biomass should be enough to ensure activity and stability for long times.

Particles with α-alumina present similar oxygen uptake rates and diffusivities as systems containing alginate alone. The productivity of α-alumina systems was greater because of better gas–liquid mass-transfer characteristics. Once mass transfer is improved inside the reactor, the denser particles become more active than the lighter ones, mainly because of the greater availability of oxygen.

The addition of α-alumina allowed a better performance of the system for the acetic acid production. It would be interesting to use this kind of immobilization in other reactors and with other microorganisms.

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