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Interfacial Adsorption of Microorganisms and Its Effect on Oxygen Absorption by Fermentation Broths

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ABSTRACT

Steady-state mass transfer experiments were done to determine whether certain strains of bacteria in homogeneous suspension will accumulate at the gas:liquid interface and thereby significantly increase the oxygen transfer rate to the suspension. In particular, with suspensions of *B. licheniformis*, the measured transfer rates are as much as three times the rate expected for a uniform suspension. In contrast, suspensions of *M. luteus* show no increase in oxygen flux above expected values. The fact that *B. licheniformis* is motile, whereas *M. luteus* is not suggests that cell motility may play an important role in the accumulation process.

Index Entries: Fermentation; oxygen transport; microbial accumulation at interfaces; *Bacillus licheniformis*.

INTRODUCTION

A continuous supply of oxygen is essential to aerobic fermentations, and often the interphase transport rate of oxygen is the limiting factor that determines the ultimate yield of cell mass in a biochemical process. Consequently, much attention has been focused on the fundamentals of oxygen transfer to microbial suspensions. Whether oxygen transfer is facilitated by surface aeration, by sparging with submerged turbines, or by some other gas-liquid contacting technique, the transfer process is complicated by complex hydrodynamic conditions, possibly by a non-

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Newtonian liquid viscosity, and by the consumption of oxygen by the cells, which is often modeled as a pseudohomogeneous chemical reaction of zero-order. Yet despite extensive research efforts, no reliable correlations have emerged that accurately predict the volumetric mass transfer coefficient k_La *a priori*, based on the physical properties of the system alone. Since the performance of gas-liquid contactors can be predicted accurately in the absence of microorganisms, it is understandable that some investigators have hypothesized that microorganisms may uniquely alter the oxygen transfer rate to the liquid phase by adsorbing or accumulating at the gas-liquid interface (*see* (*1*) for a review of early literature).

A variety of mechanisms have been proposed to estimate the potential impact that the interfacial adsorption of cells may have on oxygen transfer rate. For example, Andrews and coworkers have proposed that the presence of cells on the surface of bubbles will make the surface appear "rough" from the perspective of the broth flowing past the bubbles. The increased frictional drag will induce interfacial flows, which otherwise would be absent because of the presence of surfactants, thereby enhancing the interphase transport rate of oxygen (*2,3*). In contrast, others have proposed that interfacial accumulation of cells will retard oxygen transfer by a blockage mechanism (*4,5*). Possibly the most popular and intriguing proposal, however, is that the microorganisms actually accumulate at the interface to shorten the diffusion path for oxygen transfer to the cells (*6–12*). It has even been suggested that these adsorbed cells absorb oxygen directly from the gas phase, without any intervening mass transfer boundary layer in the liquid phase (*8*). Unfortunately, although these hypotheses have been debated for over thirty years, no experimental evidence exists that demonstrates conclusively that microbial accumulation at the gas-liquid interface can increase the oxygen transfer rate (*13–16*).

The idea that microorganisms might actively pursue the gas-liquid interface through a chemotactic response to the oxygen gradient is a fascinating possibility. However, chemotaxis is not necessarily a prerequisite for interfacial accumulation. Cells might simply become attached to the interface because of the amphiphilic nature of their cell wall, their contact with the interface being promoted by bulk convection and Brownian motion (*17,18*). Whether the accumulation process is passive or active, the extent of accumulation should be microbe-specific, each species having its own special affinity for the gas-liquid interface (*19*).

Environmental microbiologists already have done some significant work in this area to substantiate the claim that microbes can accumulate at interfaces (*20*). Some of the pioneering work was done as early as 1924 by Mudd and Mudd (*21*), who observed that high tension interfaces seem to trap bacteria, whereas low tension interfaces allow bacterial cells to stream past. They also observed that bacteria accumulate faster at interfaces of high surface tension. More recently, certain bacteria have been

observed to orient at air–water interfaces, suggesting the existence of hydrophilic and hydrophobic regions in the cell wall (22).

The objective of the present investigation is to determine whether accumulation of living aerobic cells at the air:broth interface is sufficient to increase the oxygen transfer rate to the suspension and, if so, to measure the extent of the enhancement. Because hydrodynamic effects can complicate the interpretation of results and obscure the mechanism of enhancement, the oxygen transfer rate to a quiescent pool of broth containing microorganisms is measured. An added advantage of this configuration is that the interfacial area for oxygen transfer is well defined and easily measured. Finally, the transport rate of oxygen to the microbial suspension is measured directly, by following the pressure decrease in the enclosed gas space above the quiescent broth. This technique for determining k_L is a major improvement over more common methods that depend on the measurement of dissolved oxygen concentration, because in the latter case any oxygen-consuming reaction in the liquid produces a reduction in the dissolved oxygen concentration, which may be mistakenly interpreted as a decrease in the rate of mass transfer to the liquid.

THE EXPERIMENT

Two microorganisms are used in this work: *Bacillus licheniformis* which is a motile, gram positive, facultative bacterium, and *Micrococcus luteus*, which is strictly aerobic and nonmotile. *B. licheniformis* was selected for this study because it is known to form pellicles (two dimensional spiral structures) at the interface in stagnant waters after several days, suggesting that it may have a special affinity for the air:water interface. *B. licheniformis* is also an important industrial microorganism, used as a source of antibiotics (bacitracin), proteolytic enzymes, and, at least in one case, of biosurfactants used for oil recovery. *M. luteus* was selected to determine if lack of cell motility correlates with lack of interfacial accumulation. Both cultures were maintained by monthly subculture on agar plates. The nutrient medium used was trypticase soy broth (Baltimore Biological Labs). Growth curves for these microorganisms were measured by standard techniques, cell concentration being determined by optical densitometry at 540 μm with a spectrophotometer. The specific growth rate at 25°C for *B. licheniformis* and *M. luteus* is 0.26 h^{-1} and 0.12 h^{-1}, respectively.

The experimental apparatus consists of twin stainless steel chambers connected to an ultrasensitive differential pressure transducer (MKS Baratron 310BH-10) with a full range of 0–10 mm Hg and the capability of measuring pressure differentials as small as 0.001 mm Hg (*see* Fig. 1). Absolute pressures in the room and in the absorption chamber are measured with mercury manometers. One chamber contains the fermentation broth, whereas the other is dry. The second chamber serves as the refer-

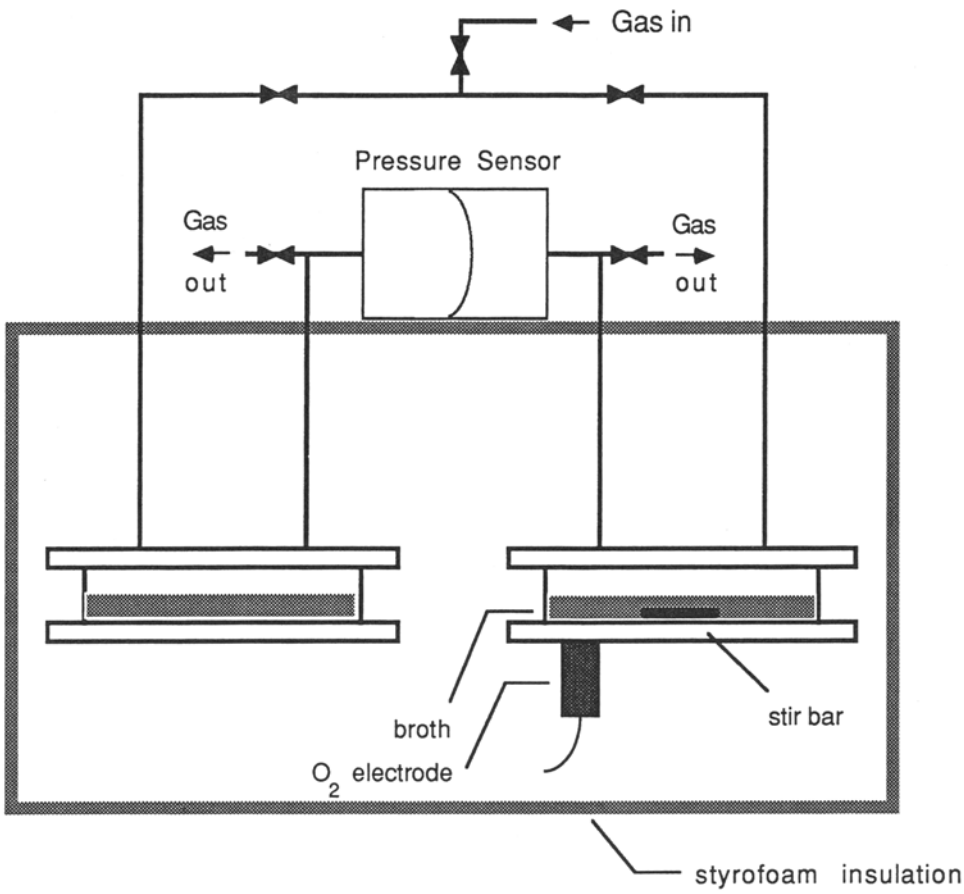

Fig. 1. Schematic diagram of the apparatus, showing the two stainless-steel chambers, the magnetic stirrer, the dissolved oxygen electrode, the differential pressure transducer, the ball valves for isolating the chambers from each other, and the styrofoam, constant-temperature box.

ence pressure for the transducer and compensates for any heat effects caused by step increases in gas pressure imposed during unsteady-state experiments. To equalize the gas phase volume in both chambers, a piece of Plexiglas of the same volume as the broth is placed in the dry chamber. Mass transfer to the broth produces a difference in pressure between the two chambers, which is sensed by the transducer and recorded continuously on a chart recorder. Because the volume of the vapor space in each chamber is 410 mL during the experiment, the high sensitivity of the transducer allows us to detect the interphase transport of as little as 0.1 μg of oxygen to the broth.

The chamber that contains the broth is provided with a polarographic oxygen electrode (YSI Instruments, Model 57) with a sensitivity limit of 0.1 mg/L and a response time of 10 s. The electrode is inserted through the bottom plate and positioned such that the electrode membrane is at the same level as the bottom of the nutrient broth. The YSI oxygen meter auto-

matically compensates for temperature and has a manual compensation adjustment for salinity, which is set to near sea water conditions for the trypticase broth. Furthermore, the temperature sensor in the meter is used to measure the temperature of the broth to within ± 0.5°C. The chamber also contains a magnetic stirrer and a small vessel containing filter paper soaked in concentrated NaOH solution to absorb any carbon dioxide liberated by the respiring organisms. The two chambers are connected to a gas mixing system by a solenoid valve. An oxygen–nitrogen mixture is fed to the chambers, the flowrate of each gas stream being controlled with a Nupro needle valve and measured with a calibrated rotameter. Oxygen levels in the gas phase are adjusted between 0.7 and 35 volume percent to vary C* from 2.8 to 14.5 mg/L. Both chambers are enclosed in a box made of inch-thick styrofoam and equipped with a fan. This arrangement provides adequate temperature control for both chambers and effectively isolates the apparatus from fluctuations in room temperature. All experiments were done at 25 ± 1°C.

Before each measurement, the absorption chamber is disinfected with an iodine solution, rinsed with distilled water, and partially filled with 100 mL of sterile nutrient broth, which creates a pool ~5 mm deep with a surface area of 204 cm^2. The system is flushed with an oxygen–nitrogen mixture of known composition and the broth is stirred until the dissolved oxygen concentration becomes constant. The gas flow is stopped, the chambers are isolated from the rest of the system by closing the valves shown in Fig. 1, and the pressure decrease inside the absorption chamber is measured by the pressure transducer. If the pressure decreases, it means that oxygen or nitrogen is still absorbing into the liquid. If the pressure is constant, the system has reached equilibrium and C* can be measured with the oxygen electrode. This measurement is important because it is used to compute the oxygen flux predicted by the mathematical model and the Henry's Law constant for the broth. For the trypticase soy broth used in our experiments, the Henry's Law constant was measured to be 3250 Pa-[mg/L]$^{-1}$.

When equilibrium is reached, at least 10 mL of a suspension of growing bacteria of known concentration is added to the stirred broth and the system is again flushed with the gas mixture. Typically, it takes less than 5 min for the dissolved oxygen concentration to become stable after inoculation, indicating that pseudosteady-state has been reached. The chambers are again isolated from each other and the oxygen transfer rate into the well stirred liquid is determined from the measured decrease of pressure with time. Because the system is at steady-state, the measured value of oxygen transfer rate equals the oxygen consumption rate by the cells:

$$\text{oxygen consumption rate} = \int \mathrm{r}X\mathrm{d}V = \mathrm{r}X_O V \qquad (1)$$

where V is the volume of broth in the chamber and $X_O V$ is the mass of cells suspended in the broth. Thus, this measurement determines the value of

specific respiration rate r at the experimental conditions and verifies that the cells indeed are in their log-growth phase.

Once the specific respiration rate has been measured, the valves are opened and gas flow to the chambers is resumed to restore the oxygen depleted by respiration. Simultaneously, the stirring is stopped and about 5 min are allowed to elapse for convection to cease and for the steady-state oxygen concentration profile to be established in the quiescent broth. In previous experiments with the same apparatus (*25*), it was found that 1–2 min is enough for the liquid to become quiescent; and mathematical modeling suggests that the pseudosteady concentration profile should be reached within a minute after the broth becomes quiescent (*24*). Thus, a 5 min time lapse is more than sufficient to achieve the desired state in the liquid phase. At this point, the valves are closed again to isolate the chambers, and the pressure difference caused by oxygen diffusion into the broth is recorded over a period of several minutes.

The pseudosteady-state nature of the process is confirmed by the constant slope of the pressure vs time trace produced by the chart recorder. This change in pressure with time, $\Delta p/\Delta t$, is then used to compute the oxygen flux to the broth N, with the aid of the ideal gas law.

$$N = -(\Delta p/\Delta t)[V_G/ART] \tag{2}$$

where V_G is the gas volume in the chamber and A is the surface area of the broth. Because the oxygen transfer rate is low compared to the total amount of oxygen available in the gas phase, the effect on flux of oxygen depletion from the gas phase can be ignored in the analysis of our data, the maximum change in C^* being less than 5%. At the end of the experiment, the suspension is sampled to obtain the cell concentration by optical densitometry. Because the cell concentration may increase by as much as 30% after innoculation, the microbial concentration measured at the end of the experiment is used to analyze the data.

MATHEMATICAL MODEL

The steady-state transfer rate of oxygen to the quiescent broth is described by the following form of the diffusion equation:

$$D\, d^2C / dz^2 = rX \tag{3}$$

where z is the distance into the bulk liquid measured from the gas:liquid interface, D is the molecular diffusivity of oxygen, C is the dissolved oxygen concentration, and rX equals the volumetric rate of oxygen consumption by the cells, whose local concentration is X. Although steady-state is assumed, the transfer process only approaches a pseudosteady-state condition because the microorganisms are always growing and dividing; thus the mass concentration of cells X is always increasing with time.

However, in our experiments the doubling time for the microorganisms is over 2.5 h, whereas the experiments take only about 10 min, before sampling to determine the mass concentration of cells. Consequently, in the mathematical model we assume that X is independent of time. Furthermore, because the cells do not settle when stirring ceases, X is also independent of position, except possibly for a thin region immediately adjacent to the interface where accumulation may take place.

The specific rate of oxygen consumption r is generally a function of the concentration of nutrients present in the broth, and, additionally, becomes a function of oxygen concentration at low levels of dissolved oxygen. Because of our desire to provide an unambiguous interpretation of our data, special care was taken to determine the nutrient level above which r is independent of nutrient concentration. Furthermore, the dependence of r on oxygen concentration was measured and modeled with Michaelis-Menten kinetics (*23*). These independent experiments reveal that the oxygen consumption rate is independent of the dissolved oxygen concentration at values above 0.4 mg/L. Although the D.O. level falls below 0.4 mg/L in a small portion of the diffusion boundary layer, it is easy to show mathematically that the assumption of zero-order kinetics for the entire layer introduces an error of less than 2%, as long as the value of K_o/C^* is less than 0.02, where K_o is the Michaelis-Menten half-saturation constant and C^* is the equilibrium solubility of oxygen (*24*).

Insight into the possible effect of microbial accumulation can be obtained by integrating Eq. (3) over the mass transfer boundary layer and requiring that the oxygen flux at $z=\delta$ be zero.

$$O_2 \text{ flux at gas:liquid interface} = -D\,[dC/dz]_{z=0} = r\int_0^{\delta} X dz \qquad (4)$$

In the above equation, δ represents the thickness of the mass transfer boundary layer, that is the distance over which the dissolved oxygen concentration drops from its value at the interface to zero. The integral of Xdz equals the total mass of microbial cells in the boundary layer per unit area of interface. Thus, as a consequence of the assumption of zero-order reaction, the oxygen flux to the broth depends on the total number of viable cells in the boundary layer, which is enriched by interfacial accumulation; but it appears to be independent of how these cells are distributed spatially. In actuality, the spatial distribution of cells does affect the interphase transport rate, but its influence is weak and is imposed through its effect on δ. This observation has two important implications to our study. On the positive side, it means that our experimental results can be interpreted adequately without concern for the details of how excess cells are distributed near the gas:liquid interface. On the negative side, it means that mass transfer experiments alone will be unable to provide detailed information about the physical form of the accumulation layer.

The solution of Eq. (3) for the oxygen flux at the interface is obtained subject to the following boundary conditions.

$$\text{at } z = 0, \qquad C = C^* = p/H \tag{5}$$

$$\text{at } z = d, \qquad C = 0 \text{ and } dC/dz = 0 \tag{6}$$

Equation (5) assumes that the resistance to mass transfer in the gas phase is negligible in comparison to that in the bulk. Thus, the oxygen concentration at the interface is the equilibrium solubility C* based on the prevailing oxygen partial pressure p in the gas phase and the Henry's Law constant H. Equation (6) acknowledges that at $z=\delta$ the dissolved oxygen concentration has fallen to zero and so no further diffusive flux can exist. Both of these conditions are needed at $z=\delta$ because the thickness of the mass transfer boundary layer δ is an unknown in the problem.

To integrate Eq. (3), a hypothesis is needed about how the cell concentration X varies with z. For discussion purposes, we assume that any accumulation of cells near the interface is confined to a thin region between $z=0$ and $z=\epsilon$. Within this region, the mass concentration of cells is presumed to be X_i, which is independent of position. Outside this region, where $\epsilon \leq z \leq \delta$, the cell concentration is uniformly the bulk concentration X_o. This means that we are ignoring the effect of any natural settling that may take place during the 10 min that the transfer rate is measured in the experiments. With this cell distribution model, the integration of Eq. (3) yields an expression for C for each of the two regions. These two expressions are then matched at $z=\epsilon$, by requiring that both the oxygen concentration and flux be continuous. After applying boundary conditions (5) and (6), we obtain the following result.

$$O_2 \text{ flux to the broth} = r\Gamma + [2\,D\,C^* r X_o - \epsilon\Gamma r^2 X_o]^{1/2} \tag{7}$$

$$\text{boundary layer thickness} = \delta = [2\,D\,C^*/rX_o - \epsilon\Gamma/X_o]^{1/2} \tag{8}$$

where Γ is the surface excess mass of cells in the interfacial region.

$$\Gamma = \int_o^\epsilon [X_i - X_O]dz = [X_i - X_O]\epsilon \tag{9}$$

If there is negligible cell accumulation, then $\Gamma \sim 0$ and Eqs. (7) and (8) reduce to the classic result of steady-state diffusion with zero-order reaction. If accumulation is significant and ϵ is less than δ, then the measured value of oxygen flux to the broth will be much greater than that expected based on bulk measurements of cell concentration.

To interpret our experimental data, we have chosen to assume that the accumulation layer is much thinner than the mass transfer boundary layer; that is, $\epsilon << \delta$. Thus

$$O_2 \text{ flux to the broth} = r\Gamma + [2\,D\,C^* r X_O]^{1/2} \tag{10}$$

RESULTS AND DISCUSSION

Selected measurements of oxygen flux vs microbial concentration are presented in Fig. 2 for *B. licheniformis* at an oxygen partial pressure such

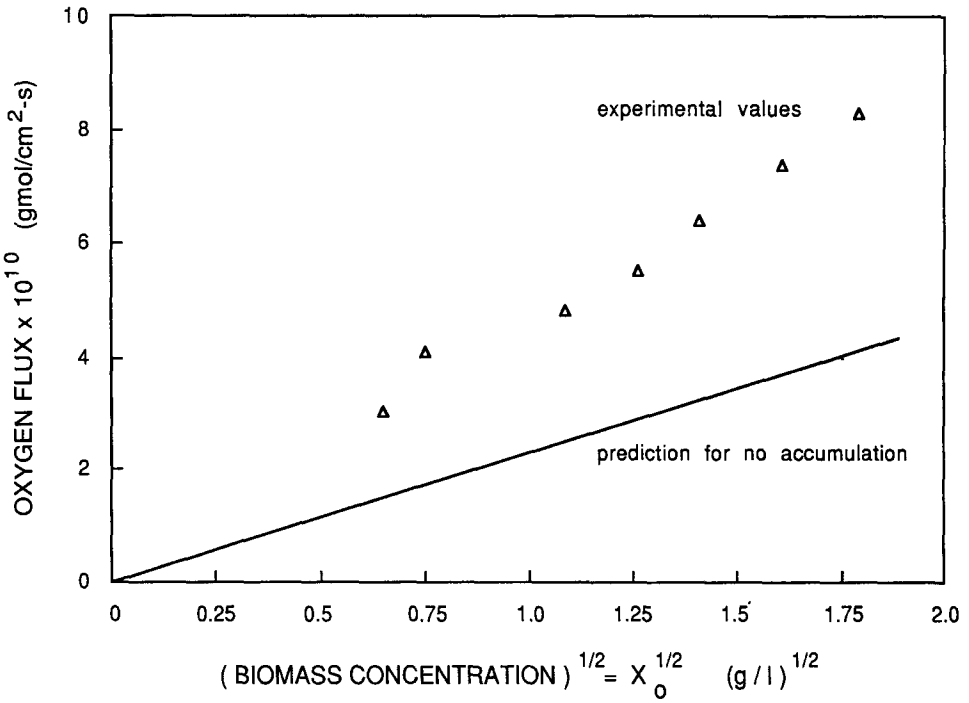

Fig. 2. Experimental measurements of oxygen flux to suspensions of *B. licheniformis*, with C* equal to 8.0 mg/L.

that C* equals 8.0 mg/L. Each data point presented in the figure is an average of at least two replicates, with a reproducibility of better than 9%. The solid line is the theoretical prediction of oxygen flux to the broth, assuming no accumulation of cells at the interface. To compute this theoretical curve, values of r and D are needed. The value of r is measured at the start of each experiment, as described earlier. The molecular diffusivity of oxygen in the broth D is measured in independent unsteady-state oxygen transfer experiments with sterile broth in the apparatus (25). At 25°C, the value of D was measured to be 2.0×10^{-5} cm^2/s in the broth and 2.2×10^{-5} cm^2/s in pure water. The value for water is within 5% of values reported in the literature (26).

It is apparent from Fig. 2 that the measured oxygen flux is substantially higher than that expected for a broth that contains a uniform distribution of viable cells. Furthermore, the difference between the predicted and measured flux increases with increasing cell concentration. Because the oxygen consumption rate by the cells was measured just prior to the measurement of oxygen flux, the only other explanations for the discrepency are that the cells are *not* uniformly distributed but rather have accumulated near the interface, or the broth is not totally quiescent and the enhancement is a result of some hydrodynamic effect. The second argument does not explain why the enhancement should depend on the biomass concentration; but in order to discard it completely, it is necessary to verify that the broth can indeed be considered quiescent. An excellent way to test

this assumption is to measure the rate of nitrogen transfer to the broth, at the same time that the oxygen transfer is occurring. If the liquid is free of convective motion, nitrogen will be transferred by physical diffusion alone, the flux being predicted by diffusion theory.

To induce nitrogen transfer, the gas composition above the broth is suddenly increased by the addition of pure nitrogen: this changes the interfacial concentration of nitrogen without altering the oxygen partial pressure. The total decay of pressure inside the chamber results from the sum of the steady-state transfer rate of oxygen and the transient mass transfer rate of nitrogen. Since the oxygen transfer rate is measured immediately before the nitrogen concentration is increased, the nitrogen transfer rate can be calculated from the difference between the two measurements. The unsteady-state diffusion rate of a gas into a semi-infinite liquid has been calculated numerous times (27). For the present case

$$\text{Nitrogen flux} = [D_N/\pi t]^{1/2}[C_N^* - C_N] \tag{11}$$

where C_N is the nitrogen concentration in the liquid before the increase in partial pressure, C_N^* is the equilibrium solubility of nitrogen after the increase, D_N is the molecular diffusivity of nitrogen and t is time.

In order to calculate the nitrogen transfer rate, one must know both the diffusivity and the solubility of nitrogen in the nutrient broth. Rather than measuring these properties separately, it is easier to measure the transfer rate of nitrogen to a quiescent sterile broth and use this value as a basis for comparison. The results of the nitrogen transfer experiments are presented in Fig. 3. For comparison, the expected result for unsteady-state nitrogen transport to pure water is also shown in Fig. 3, the solid line being computed from the above equation and literature values of the diffusivity and solubility of nitrogen in water. The fact that the experimental data in Fig. 3 fall below this line is not surprising since diffusivities and solubilities of gases in the broth tend to be lower than in water.

A comparison of these results indicates that hydrodynamic effects cannot be the cause of the anomolously high oxygen flux measurements shown in Fig. 2. Although the nitrogen flux to the microbial suspension is about 15% higher than the flux to the sterile broth, both are lower than the flux expected to a completely stagnant layer of water. Furthermore, the difference of 15% between the two sets of data are within experimental error since in each experiment the nitrogen flux contributes only 25% of the total flux measured in the experiment (the rest being oxygen transport) and reproducibility in all our oxygen transfer experiments is 5–9%. The conclusion, then, must be that the large difference between the measured and predicted oxygen fluxes in Fig. 2 is owing to bacterial accumulation at the air:broth interface.

A convenient way to illustrate the impact of the interfacial accumulation of cells on oxygen transfer rate is to compute the dimensionless oxygen flux, by scaling the measured flux by the flux expected in the absence of interfacial accumulation (*see* Eq. 10).

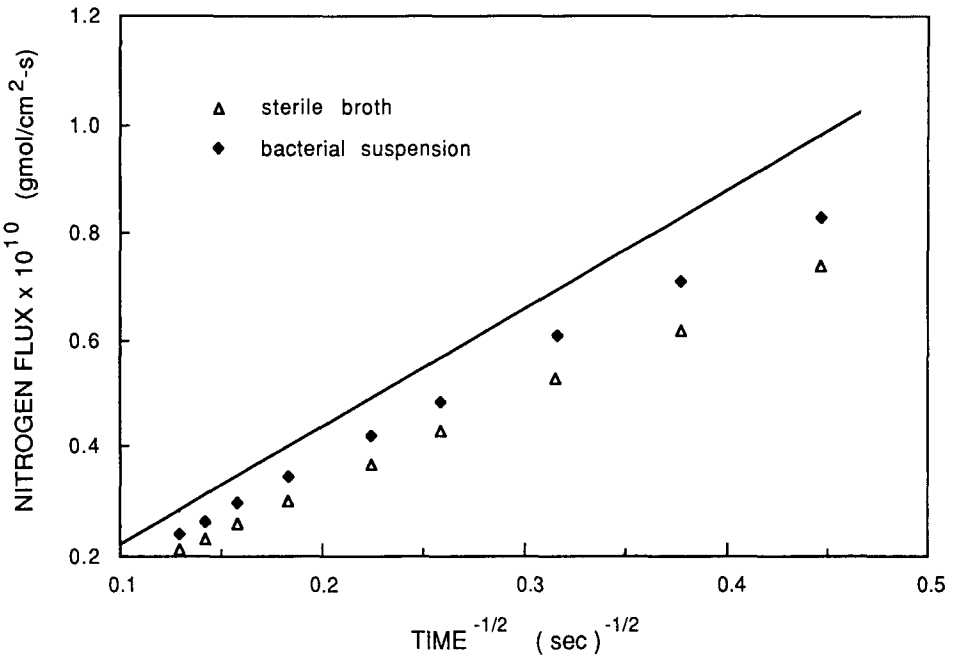

Fig. 3. Measurements of the unsteady-state flux of nitrogen to a suspension of respiring organisms. In parallel with the nitrogen flux, there is a steady-state flux of oxygen to the suspension as well. The solid line is the theoretical prediction for transient diffusion into a semi-infinite, quiescent layer of water.

$$\text{Dimensionless } O_2 \text{ flux} = \text{measured flux} / [DC^*rX_O]^{1/2} \quad (12)$$

This dimensionless flux is plotted versus microbial concentration in Fig. 4 for the case of *B. licheniformis* when C* equals 8.0 mg/L. Notice that the measured values of dimensionless flux are independent of cell concentration, the oxygen flux consistently being twice that expected, based on the bulk concentration of cells in the broth.

From Eq. (10), the dimensionless flux is expected to depend on the extent of interfacial accumulation in the following manner.

$$\text{Dimensionless } O_2 \text{ flux} = 1 + r\Gamma / [2DC^*rX_O]^{1/2} \quad (13)$$

Thus, a constant value for dimensionless oxygen flux means that the surface excess mass of cells Γ must increase as the square root of the bulk concentration of cells.

$$\Gamma = 0.0014\,(\text{g/cm})^{1/2}X_O^{1/2} \quad (14)$$

The above equation may be thought of as the *equilibrium adsorption isotherm* for *B. licheniformis* at the experimental conditions. It also suggests that the accumulation process is reversible; that is, the mechanism of accumulation involves an adsorption step (for transfer to the interfacial region) and a desorption step (for transfer away from the interface). Otherwise, given sufficient time, the surface excess mass Γ would reach a constant, saturation value that is independent of the bulk cell concentration.

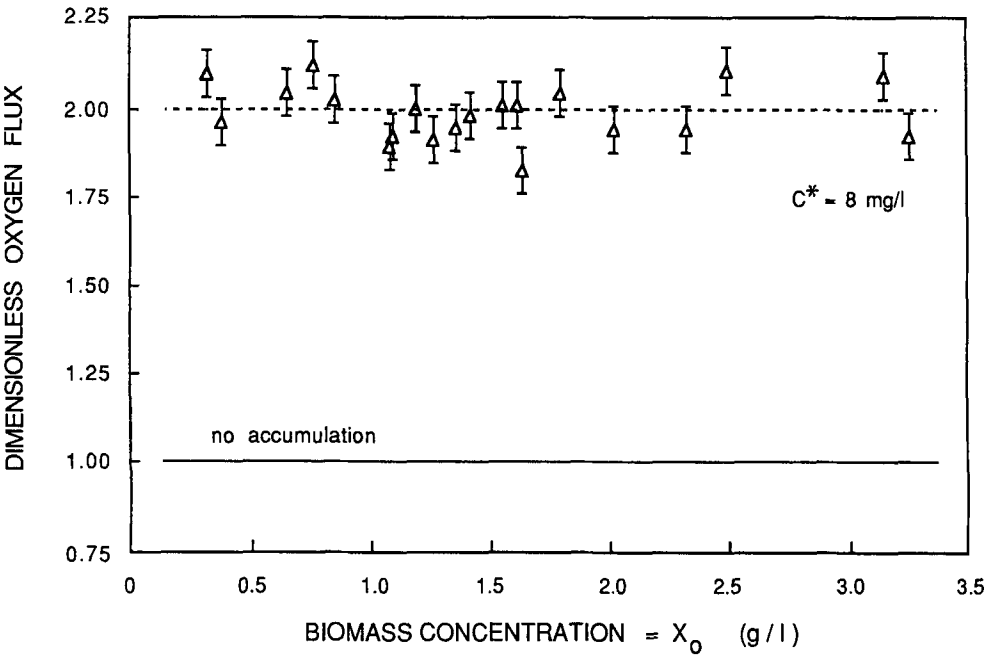

Fig. 4. Dimensionless oxygen flux vs cell concentration for *B. licheniformis*, with C* equal to 8.0 mg/L. Notice that the oxygen flux is twice that expected for a uniformly dispersed suspension of cells and that this twofold enhancement is independent of the bulk concentration of microorganisms in the broth.

Equation (13) predicts that the importance of interfacial accumulation of cells should increase as the partial pressure of oxygen in the gas phase decreases. To test this hypothesis, experiments were done with *B. licheniformis* at C* equal to 2.8 and 14.5 mg/L. The results of these experiments are summarized in Fig. 5. Notice that the dimensionless oxygen flux does indeed increase as C* becomes smaller. In fact, the oxygen flux that is measured at C* = 2.8 mg/L is over three times the flux expected, based on the assumption of no interfacial accumulation. Aside from confirming the trends predicted in Eq. (13), the data in Fig. 5 also support the concept of an equilibrium adsorption isotherm for *B. licheniformis*. The dotted lines drawn in Fig. 5 are the predictions of oxygen flux compiled from Eq. (13) based on the isotherm determined from the data at C* = 8.0 mg/L. The correspondence between these predictions and the experiments is within about 10–15%, and suggests that Eq. (14) applies quite well to all the mass transfer data obtained with *B. licheniformis*.

ACCUMULATION MECHANISM

An approximate model of the accumulation mechanism is presented in Fig. 6. The transfer of cells into and out of the interfacial region are shown as elementary steps similar to those in a reversible reaction.

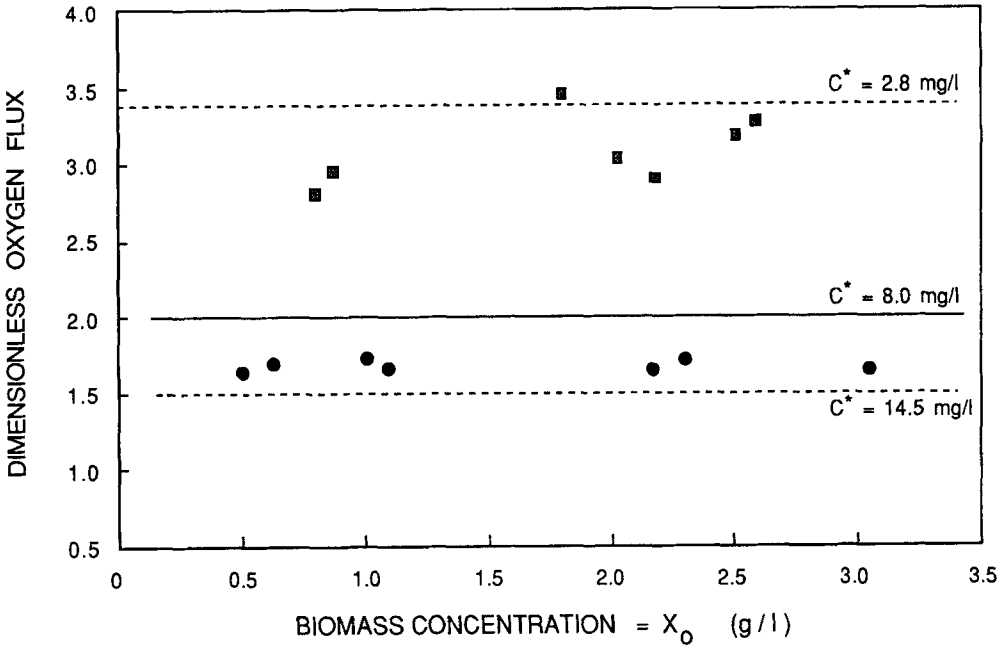

Fig. 5. The effect of oxygen partial pressure in the gas phase on the enhancement of interphase transfer by microbial accumulation of *B. licheniformis* at the interface. As the oxygen partial pressure decreases, and with it the equilibrium solubility, the enhancement in the oxygen flux increases.

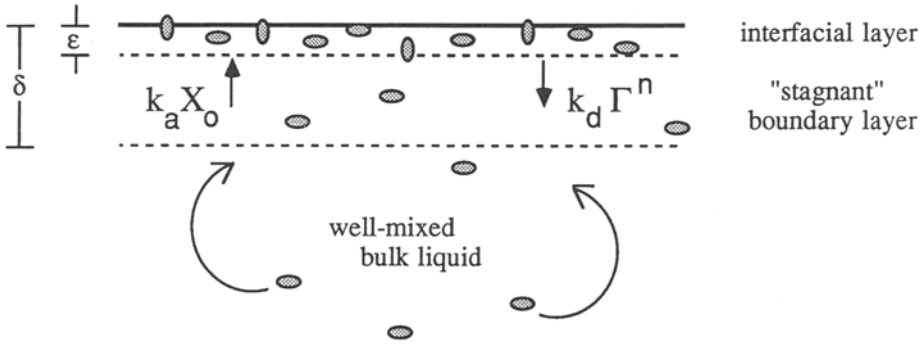

Fig. 6. A mechanistic description of cell accumulation that suggests the importance of convective transport and cell motility to the process. Because the surface excess mass increases in proportion to the number of cells in the bulk liquid, the accumulation process appears to be reversible, involving both "adsorption" and "desorption" steps that are characterized by the rate constants k_a and k_d.

$$\text{net flux of cells to interface} = k_a X_O - k_d \Gamma^n \quad (15)$$

where k_a and k_d are the rate constants for the adsorption and desorption steps. At equilibrium, the net flux of cells to the interface is zero, and the equilibrium adsorption isotherm is obtained.

$$\Gamma^n = (k_a/k_d) X_O \quad (16)$$

In order for this equation to be consistent with the experimental results summarized in Eq. (14), the value of n must be 2. This suggests that the desorption process is "bimolecular," meaning that cells in the interfacial region aggregate and, when they desorb, leave the interface in pairs.

At least two issues exist regarding the accumulation model presented in Fig. 6: (1) How thin is the interfacial region where the cells accumulate? and (2) What special properties must the cells possess in order for interfacial accumulation to take place? Regarding the first issue, an upper bound on the thinness of the accumulation layer can be deduced from calculations of the mass transfer boundary layer thickness, with the aid of Eq. (8). Since the data at all values of C* correlate well with the adsorption isotherm presented in Eq. (14), it is reasonable to expect that the thickness of the accumulation layer is small compared to the boundary layer in all these experiments. The smallest values of δ are obtained at the lowest oxygen partial pressure and the highest microbial concentration. In particular, at C* equal to 2.8 mg/L and X_O equal to 2.6 g/L the concentration profile of dissolved oxygen is 160 μm thin, and this must be significantly larger than the thickness of the accumulation layer.

Figure 6 indicates that cells must be transported to the interfacial region before they can begin to accumulate there. When the broth is stirred, this transport is by convection. Nevertheless, a stagnant zone near the interface is expected to exist since oxygen transfer to the stirred liquid is not instantaneous. In particular, $k_L a = 243\ h^{-1}$ (23), which corresponds to a "film theory" boundary layer thickness of 6 μm. This value is a lower limit on the thickness of the "stagnant" region; if any surface renewal is occurring, this thickness will be much larger. Thus, the cells may require some motility to create the flux to the interface that is required to establish a significant degree of accumulation. To test this hypothesis, a series of oxygen transfer experiments were done with the microorganism *Micrococcus luteus* because it is nonmotile but has respiration kinetics similar to *B. licheniformis*. The results of these transport experiments are summarized in Fig. 7 in the form of dimensionless oxygen flux vs bulk microbial concentration. In contrast to the data for *B. licheniformis*, the oxygen transfer measurements with *M. luteus* show no enhancement of the transfer rate whatsoever. If anything, the flux measurements are slightly below those predicted assuming no interfacial accumulation.

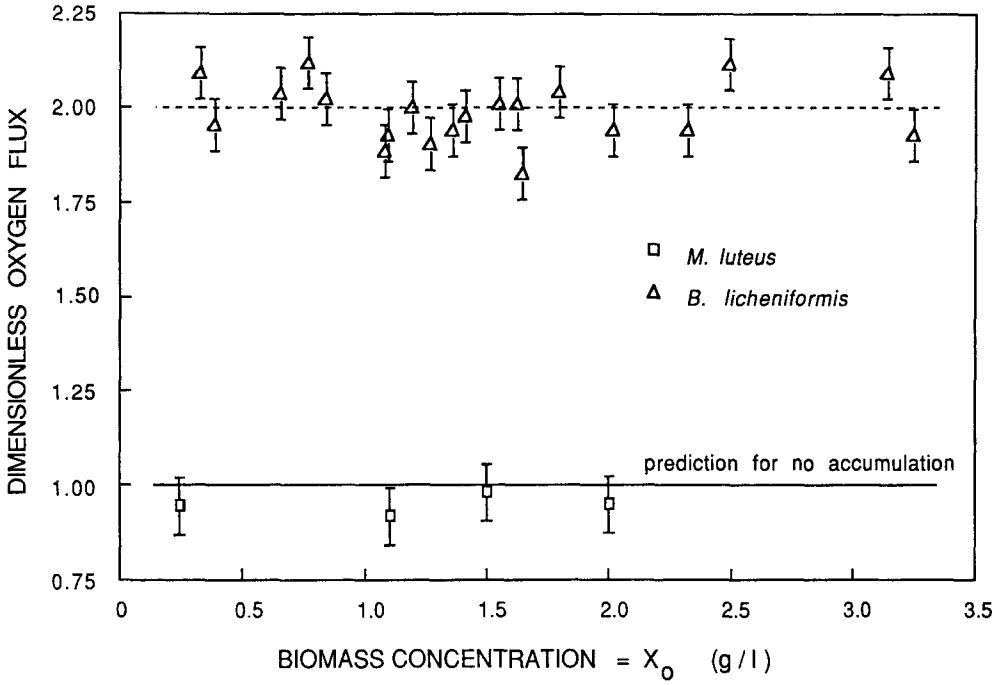

Fig. 7. The oxygen flux to suspensions of *M. luteus* as a function of microbial concentration, with C* equal to 8.0 mg/L. Experimental results are in good agreement with the theoretical model that assumes no accumulation of cells at the interface (solid line). The data for *B. licheniformis* are presented for comparison purposes.

CONCLUSIONS

Steady-state oxygen transfer rates to quiescent broths containing growing cultures of *Bacillus licheniformis* and *Micrococcus luteus* were measured at various cell concentrations ranging from 0.1 to 3.2 g dry wt/L. With suspensions of *B. licheniformis*, all the measured oxygen transfer rates were between 1.7–3.5 times the rate expected for a uniform suspension of cells. These high fluxes strongly indicate that *B. licheniformis* accumulates at the gas:liquid interface, thereby shortening the path length for oxygen transport and increasing the local oxygen consumption rate in the vicinity of the interface. In fact, all the data obtained are correlated quite well with a single equilibrium adsorption isotherm for the cells that describes the reversible accumulation of cells at the interface, as shown in Eq. (14). In contrast, suspensions of *M. Luteus* show no increase in oxygen flux above the value predicted for a uniform suspension of cells, meaning that this particular microorganism does not accumulate at the interface. Since cells of *B. licheniformis* are motile while cells of *M. luteus* are not, the

data suggest that cell motility may play an important role in the process of bacterial accumulation at the air:broth interface.

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