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Hydrodynamic and Mass Transfer Studies in an External-Loop Air-Lift Bioreactor for Immobilized Animal Cell Culture

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ABSTRACT

Air-lift bioreactors containing suspended or immobilized animal cells have been used for the production of a variety of high-value biologicals. In the bioprocessing industry, there is a need to study and quantify the relationships between bioreactor-system properties such as mixing, flow, mass transfer, and cell processes. In the present study, the performance of a 1-L external-loop air-lift bioreactor was investigated by studying gas-liquid oxygen transfer, mixing time, liquid velocity and gas hold-up at various aeration rates. These studies were performed over a range (0–25%) of loadings of small (500–800 μm) calcium alginate beads to investigate the effect of using various concentrations of cell immobilization matrices on the physical properties of the system. At an aeration rate of 0.5 vvm, the mixing time was decreased by 50%, from 75 s at 0% bead loading to 38 s at 10% bead loading. A minimum liquid velocity of 10 cm/s was required to keep the alginate beads in suspension. As bead loading increased, flow within the reactor went from turbulent conditions to the transition zone. At all bead loadings tested, the gas hold-up increased by only 2% with an increase in aeration rate from 0.1 to 1.0 vvm, regardless of whether the total reactor volume (i.e., liquid and beads) or the liquid volume was used in calculating the hold-up. A mathematical correlation was developed for expressing the dependence of the

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volumetric mass-transfer coefficient, k_la, on aeration rate (vvm) and microbead loading. With this equation it was possible to predict, within 20%, the k_la knowing the gas-flow rate and the volume percentage of microbeads present in the bioreactor. A theoretical study was also performed to calculate the oxygen transfer from the bulk liquid to the center of microcapsules containing animal cells using experimental k_la data. The results suggest that whereas there is no oxygen limitation at 10 to 15% microcapsule loading, there is a potential mass-transfer problem at 25% loading if the bioreactor is operated at an aeration rate of less than 1.06 vvm.

Index Entries: Air-lift bioreactor; animal-cell culture; hydrodynamics; mass transfer; k_la; alginate bead; microcapsule.

INTRODUCTION

Mammalian- and insect-cell cultures are the only methods that can be used for the synthesis of many complex proteins *(1,2)*. Therefore, a major focus has been on finding techniques, such as immobilized cell culture, that can improve the concentration of cell products and enhance recovery, thereby permitting cost-effective large-scale production. Some of the common methods for immobilizing cells employ gel entrapment, microencapsulation, hollow fibers, and microcarrier systems *(3)*.

Choosing the best bioreactor configuration for a given cell-culture system depends on a number of factors, including oxygen transfer, mixing requirements, and the magnitude of acceptable shear rates *(4,5)*. Since cell-culture systems are complex, it is often easier to assess a bioreactor's performance by simulating its characteristics and properties in a distilled-water system without cells present. The hydrodynamic and mass transfer properties most widely used to characterize a bioreactor are mixing time (i.e., time required to achieve homogeneity of an inert tracer), liquid velocity, bead velocity (in a three-phase system), gas hold-up, and the oxygen mass-transfer coefficient.

A comparison by Siegel et al. *(4)* demonstrates that mixing times of air-lift reactors for air-water systems can vary from 20 to 600 s depending on reactor geometry (i.e., height-to-diameter ratios and concentric vs external-loop). Also, the degree of agitation will greatly affect mixing time. Increasing gas flow in air-lift reactors or increasing stirring rates in stirred-tank reactors will decrease the mixing time. Small laboratory-scale reactors exhibit a greater degree of mixing than large-scale industrial reactors *(4)*. Also, since surfactants and components that affect bubble coalescence, such as salts, and changing fluid viscosity may influence mixing time (usually a decrease), comparison of mixing times in different bioreactors must take into account variations in fluid properties.

Liquid velocity is the characteristic parameter that distinguishes air-lift reactors from other gas-liquid contacting reactors such as bubble columns. Bubble columns generally operate at liquid velocities of less than 5 cm/s, whereas liquid velocities in air-lift reactors are approximately one order of magnitude higher *(6)*. Measurements of liquid velocity, using tracers, have been investigated in concentric air-lift bioreactors *(7)*, and in external-loop air-lift bioreactors *(8)*.

The average gas hold-up is most often determined by volume expansion *(9–11)*. Bello et al. *(12)*, and Siegel and Merchuk *(13)* used manometric measurements of hydrostatic pressure for determining the gas hold-up. The influence of superficial gas-flow rate on removal of solutes from fluids has also been studied *(14)*.

Perhaps the most important function of an animal-cell bioreactor is to provide adequate oxygen to the cells without damaging them. It is important to note that in the operation of a bioreactor, the gas-to-bulk liquid-oxygen transfer is the only resistance that can be controlled. Varying the aeration rate will affect the gas-to-liquid-oxygen transfer, which, in turn will affect the transfer of oxygen from the bulk liquid to the cell. Therefore, researchers have focused on measuring the mass transfer coefficient $k_l a$, under various operating conditions, for the purpose of developing useful correlations that may be employed as scale-up criteria for animal-cell bioreactors. A common technique used in dynamic $k_l a$ measurements involves deoxygenating the reactor contents *(15)*. Subsequently, gas of a different oxygen concentration is admitted and the oxygen profile is monitored.

For immobilized-cell systems it is not adequate to simply transfer sufficient oxygen to the bulk liquid (i.e., culture medium). Oxygen must also be transferred from the liquid to the cells. Consider for instance the transfer of oxygen from a gas bubble, through the culture medium to a microcapsule containing animal cells. The resistances to oxygen transfer from the gas phase to the inside edge of the microcapsule (i.e., gas to liquid, liquid to microcapsule, and transmembrane resistance) (Fig. 1) can be added together resulting in the following expression for the resistance to oxygen transfer, R:

$$R = \left[\frac{1}{(V_L k_l a)}\right] + \left[\frac{1}{(V_L k_s a)}\right] + \left(\frac{\left(\frac{1}{r_o}\right) - \left(\frac{1}{r_i}\right)}{(4\pi n D_{o_2 m})}\right) \qquad (1)$$

where V_L is the volume of the liquid phase, n is the number of microcapsules, r_o and r_i are the outside and inside radius of the microcapsule, respectively, $k_s a$ is the volumetric mass-transfer coefficient from a liquid to

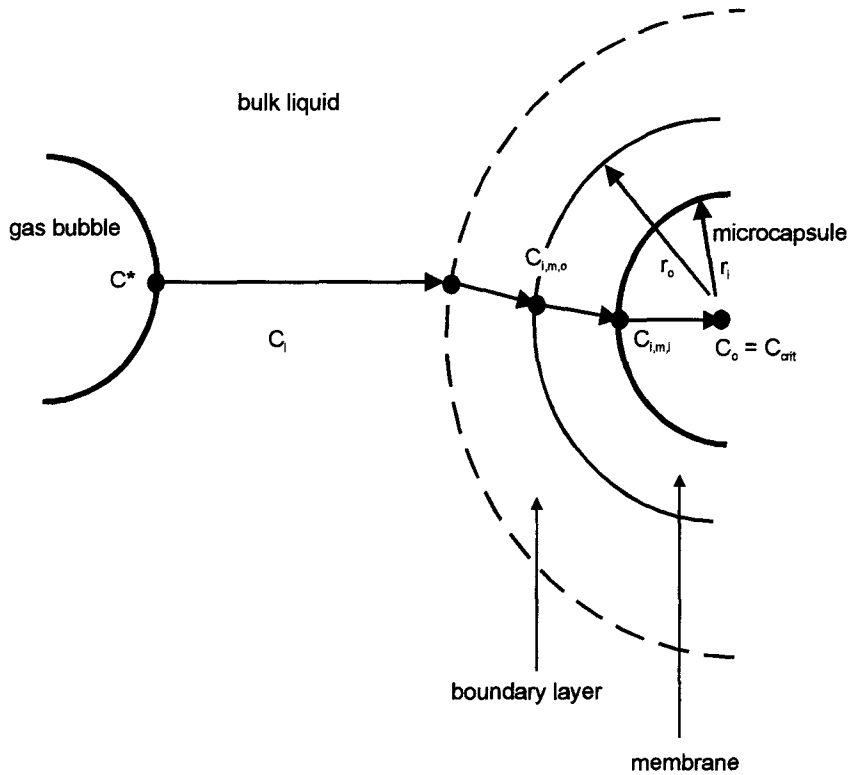

Fig. 1. Schematic diagram of oxygen profile from gas bubble to center of microcapsule.

a solid, and $D_{o_2,m}$ is the diffusivity of oxygen in the membrane. The oxygen-transfer rate (OTR) is usually expressed in the form:

$$OTR = \left[\frac{1}{(resistance)}\right](driving\ force) \qquad (2)$$

Employing Fick's law of diffusion *(16)*, it can be shown that the oxygen transfer rate from the gas phase to the inside edge of a microcapsule, OTR_G, is given by:

$$OTR_G = \left(\left[\frac{1}{(V_L k_l a)}\right] + \left[\frac{1}{(V_L k_s a)}\right] + \left[\frac{\left(\frac{1}{r_o}\right) - \left(\frac{1}{r_i}\right)}{(4\pi n D_{o_2 m})}\right]\right)^{-1} \qquad (3)$$

$$(C^* - C_{i,m,i}) \geq Q = Q_{o_2} x$$

where C^* is the oxygen concentration in equilibrium with the oxygen partial pressure in the gas phase, $C_{i,m,i}$, is the oxygen concentration on the inside of the membrane, Q is the oxygen-consumption rate of cells inside

a microcapsule (mg O_2/lh), Q_{o_2} is the oxygen-consumption rate per cell, and x is the cell density. To enable the cells in the microencapsule to survive, the oxygen-transfer rate, OTR_C, must be greater than (or at least equal to) the oxygen-consumption rate of cells inside the capsules, Q. Based on the work of Heath and Belfort *(17)*, the oxygen-concentration profile within a microcapsule can be represented by:

$$C_{i,m,i} - C_o = \left(\frac{Q}{D_{o_2a}}\right)\left(\frac{1}{6}\right)r^2 \qquad (4)$$

where r is the radius of the alginate core. If the concentration of oxygen in the center of the microcapsules, C_o, is equal to a critical oxygen concentration, C_{crit}, the concentration of oxygen at the inner surface of the membrane, $C_{i,m,i}$, can be determined from Eq. 4.

In the present study the performance of a 1-L external-loop air-lift bioreactor was investigated by studying the gas-liquid oxygen transfer, mixing time, liquid velocity, and gas hold-up at various aeration rates (0.1–1.06 vvm). The influence of suspended alginate beads on the hydrodynamics and mass transfer of the system was examined over a range of microbead loadings (0–25% by volume). The intent was to investigate the effect of using various concentrations of cell immobilization matrices on the physical properties of the system. A mathematical correlation was developed for expressing the dependence of k_la on aeration rate and microbead loading. A mathematical study of the mass-transfer resistances from the gas phase to the interior of a microcapsule was also performed, using experimentally determined k_la values, to enable the determination of the maximum bioreactor microcapsule loading.

MATERIALS AND METHODS

Formation of Calcium Alginate Beads

A 1.5% (w/v) sodium alginate solution was prepared by dissolving 1.5 g of sodium alginate powder (Keltone LV, Kelco, Chicago, IL) in 100 mL of isotonic saline (i.e., 0.85 g NaCl in 100 mL distilled water). The sodium alginate solution was extruded into a 1.5% (w/v) calcium chloride solution. Spherical droplets were formed by an air jet-syringe pump droplet generator that extruded the sodium alginate solution through a 23-gage needle located inside a sheathed tube (3 mm iD) through which air flowed at a controlled rate of 20 mL/min. As the liquid was forced out of the end of the needle by the syringe pump, the droplets were pulled off by the shearing action of the air stream. The spherical droplets had a diame-

ter of approx 500–800 microns. The calcium alginate beads were stored in a 1.5% (w/v) calcium chloride solution at 4°C prior to being used in a graduated cylinder.

The density of calcium alginate beads was determined by measuring the volume displacement of a known mass of calcium alginate beads in a graduated cylinder. At 22°C, the density of the calcium alginate beads was found to be 1.06 ± 0.02 g/mL.

Hydrodynamic Studies

Gas hold-up, mixing time, liquid velocity, and bead velocity measurements were made for alginate-bead loadings ranging from 0 to 25% (by volume). The maximum alginate-bead loading that could be maintained in suspension in the bioreactor over the range of gas flow rates investigated was 25%.

For the hydrodynamic studies the bioreactor (Fig. 2) was filled with distilled water (with or without alginate beads), and the temperature was allowed to stabilize before measurements were made. The temperature was in the range 22 ± 1°C. These measurements were carried out at aeration rates of 0.1 vvm (volume gas/volume liquid/minute) to 1.06 vvm, which corresponds to superficial gas velocities of 30 to 95 cm/min.

The external-loop air-lift bioreactor used in this study was made of borsilicate glass and had a working volume of 1060 mL. The internal diameter of the riser and downcomer sections was 3.37 cm. The overall reactor dimensions were 29.1 cm wide and 33.7 cm high (height-to-diameter ratio of 10). The reactor was fitted with threaded sample/probe ports (approx 14 cm in diameter) at the top of the riser and downcomer sections. These ports made a 45° angle with the riser and downcomer. The ports were fitted with septa for use with various probe sizes as well as being completely sealed when not needed. Aeration was accomplished by a sintered-glass sparger (fritted cylinder, Pyrex brand, Corning cat. no. 39533, Corning, NY, 12-mm disc diameter, extra coarse) fitted 4.5 cm above the bottom of the riser section. The sparger port was threaded and could be fitted with different types of spargers.

The gas hold-up was determined by measuring the increase in volume of the aerated reactor contents (liquid and alginate beads) compared to the unaerated reactor contents. The change in volume was measured by marking the liquid height in the reactor.

Mixing data were obtained by recording the liquid pH in the reactor subsequent to the addition of pulse of tracer. The tracers used in this experiment were 2.5 *M* NaOH and glacial acetic acid. These tracers were chosen because they have densities similar to that of distilled water (approx 1.08 g/mL). A 1-mL pulse of the tracer was added to the reactor

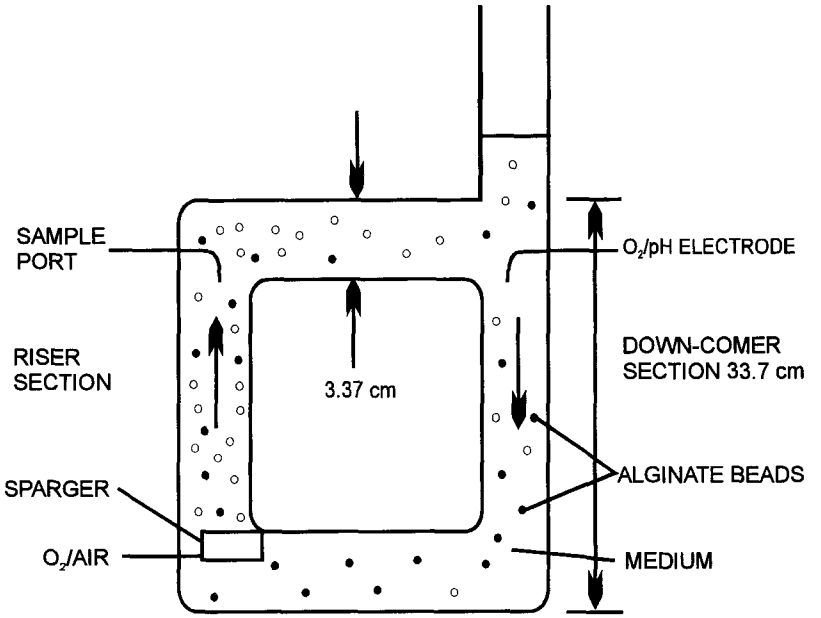

Fig. 2. Schematic diagram of external-loop air-lift bioreactor used in present study.

contents using a hypodermic syringe at the sample port. A pH electrode was placed in the other sample port and the pH was recorded. The mixing time in these experiments was defined as the time elapsed from tracer input to a degree of homogeneity of 99%. The time for one circulation (circulation time, t_c) was defined as the interval between two adjacent concentration peaks.

In order to investigate the effect of the range of pH on mixing time, the starting pH value of the reactor contents was varied between 4.0 and 9.0. The effect of direction of pH change (acidic to basic or basic to acidic) was also studied. The response time of the pH probe coupled to the recorder was measured by determining the time taken to achieve a steady-state reading after immersing the probe in pH buffer solution of 4.0 and from a pH solution of 10.0.

The average liquid velocity was calculated by dividing the length of the riser-downcomer circuit by the circulation time. The circulation time was determined from the mixing time data. The bead velocity was determined by measuring the time taken for a single alginate bead to travel a specified distance. In order to facilitate the measurements, the alginate bead was dyed by dissolving 2 drops (from a Pasteur pipet) of dextran blue in 5 mL of alginate before extrusion. The bead velocity was measured five times in each of four sections in the bioreactor (riser, downcomer, and two horizontal sections). The bead velocity was therefore an average of these 20 measurements.

For each alginate-bead loading, the only operating parameter that was varied was the air-flow rate. For two-phase systems (gas and liquid) air-flow rates were usually reported as superficial gas velocity (volumetric air flow rate/cross-sectional area of reactor) in order to facilitate comparisons with other work. However, in the presence of beads attempts were made to normalize the air-flow rate. Using the normalized aeration rate, vvm (volume gas/volume liquid/minute), allowed for comparisons on a volume of liquid basis.

Mass-Transfer Studies

The $k_l a$ values were determined experimentally by the dynamic gassing-out method as suggested by Linek et al. *(15)*. Experiments were carried out in batch liquid with a step change in the concentration of the sparged gas while the gas flow rate was kept unchanged. After saturating the liquid with nitrogen (in order to deoxygenate the liquid), the gas supply was interrupted to allow the gas bubbles to escape from the liquid. Subsequently, gas of a different oxygen concentration was admitted, in this case pure oxygen. The change in the concentration of dissolved oxygen in the liquid phase was measured with a steam-sterilizable galvanic dissolved-oxygen probe (Cole-Parmer, Chicago, IL). The dissolved-oxygen probe was connected to a New Brunswick Scientific dissolved-oxygen analyzer and the dissolved-oxygen level was recorded on a strip-chart recorder. Knowing the oxygen concentration change with time for a specific gas-flow rate, $k_l a$ was found from the slope of the graph of $Ln\ [(C^* - C(0)) / (C^* - C(t))]$ vs time.

The dissolved-oxygen probe response was obtained by immersing the oxygen probe in an Erlenmeyer flask containing distilled water saturated with nitrogen (i.e., dissolved-oxygen level of 0%). The oxygen probe was then immersed in another Erlenmeyer flask that was saturated with oxygen, and the time for the probe to reach 63.2% of the response (i.e., 63.2% of the saturated concentration) was considered to be the probe time constant.

The $k_l a$ experiments were conducted at various alginate-bead loadings and aeration rates. The aim of this work was to try and find an equation that expressed the dependence of $k_l a$, for oxygen absorption in the presence of alginate beads, on the physical properties of the liquid phase. In order to assess the effect of different types of beads, $k_l a$ measurements were also performed in the presence of ion-exchange resin beads (Rohm and Haas, [Philadelphia, PA] Amberlite synthetic ion-exchange resin, IRA-458). These ion-exchange resin particles were approximately the same size as the alginate beads. The resin beads were rigid (solid) beads with some

surface roughness, but were essentially impermeable to oxygen. The difference in porosity would be large relative to the alginate beads that contain more than 90% water.

RESULTS AND DISCUSSION

Hydrodynamic Studies

At 0.5 vvm the addition of 10% alginate beads decreased the mixing time by 50%, from 75 to 38 s, compared to the mixing time when no alginate beads were present. At the same vvm, increasing the alginate bead loading from 10 to 25% (by volume), however, increased the mixing time from 38 to 55 s. At higher bead loadings the liquid velocities were reduced (Fig. 3B) and thus longer times were required to achieve homogeneity. At greater than 0.6 vvm in the presence of alginate beads, the mixing time was approx 45 s compared to 58 s in the absence of alginate beads. This compared well with the studies of Lin et al. *(18)* in a 5.5-L external-loop air-lift reactor, which obtained a mixing time of 57 s.

In all runs the liquid velocity was greater than 5.0 cm/s, therefore, the bioreactor was being operated as an air-lift rather than a bubble column *(5,6)* (Fig. 3B). As the alginate-bead loading in the bioreactor increased, the liquid velocity decreased. This phenomenon may be attributed to an energy loss caused by friction between the liquid and the microbeads, thereby decreasing the liquid velocity. It is also interesting to note that a minimum liquid velocity of approx 10 cm/s was required to keep the alginate beads in suspension regardless of the percentage of beads in the bioreactor. However, this liquid velocity occurred at different aeration rates depending on the bead loading. For example, a liquid velocity of 10 cm/s occurred at 0.28 and 0.49 vvm for 10 and 25% bead loadings, respectively.

For the range of aeration rates studied, at 0% bead loading, the bioreactor was predominantly operating under turbulent conditions (i.e., $Re \geq 4000$). On the other hand, in the presence of 25% beads, the bioreactor operated in the transition zone between laminar and turbulent flow. We can speculate that this could affect (i.e., lower) the $k_l a$ (i.e., liquid-film volumetric mass-transfer coefficient) values because of less mixing. Turbulence may also reduce microcarrier stability. However, a recent study indicated that alginate beads tend to be more stable in air-lift reactors than in stirred tank reactors *(3)*.

Figure 3A shows the change in average bead velocity with aeration rate and alginate-bead loading. The bead velocity was the average of five replicate measurements (± SD) made in each of four sections in the reactor. The bead velocity in all four sections was found to be essentially the

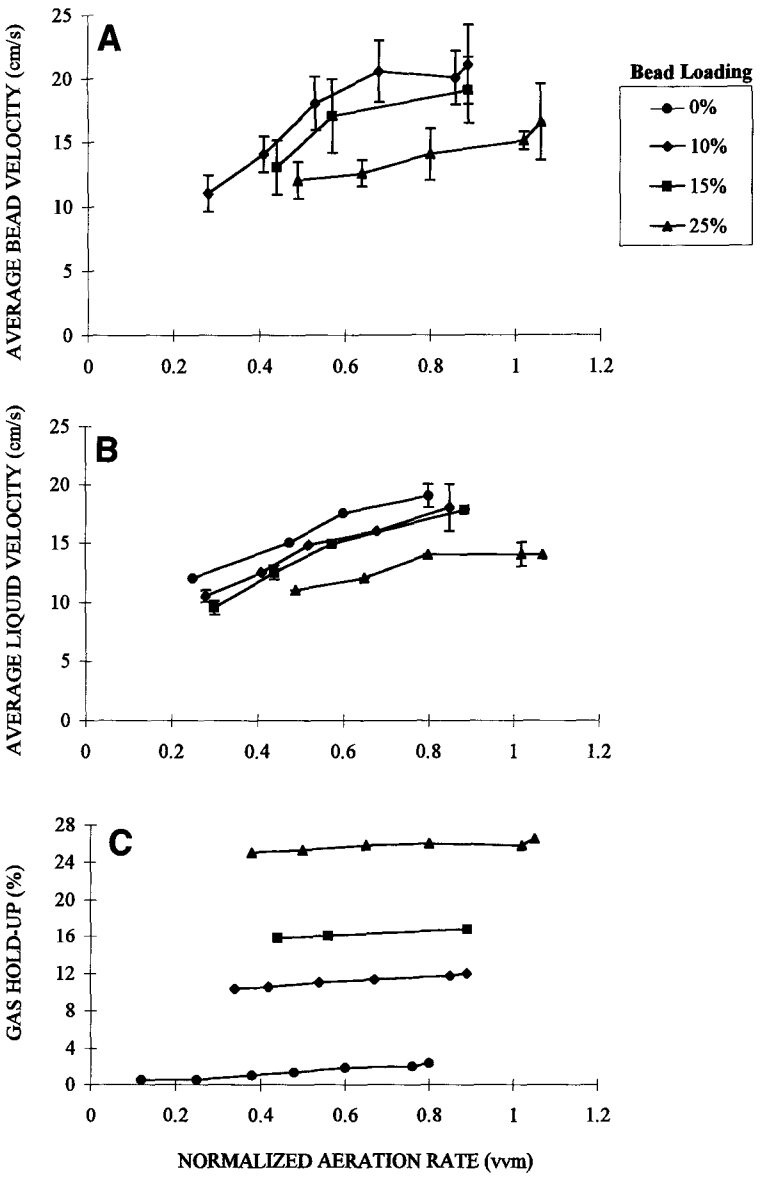

Fig. 3. Average bead velocity vs normalized aeration rate (vvm) **(A)**. Average liquid velocity vs normalized aeration rate (vvm) **(B)**. Gas hold-up vs normalized aeration rate (vvm) based on liquid volume **(C)**.

same (within the SD), therefore the results reported are the average of these measurements. For the 10% bead loading, the bead velocity increased dramatically from 11 to 20 cm/s at 0.28 to 0.70 vvm respectively, after which it leveled off. There appeared to be no significant difference in the average bead velocity between 10 and 15% alginate-bead loadings. For

25% alginate-bead loading, however, the average bead velocity decreased by approx 25%. For example, at 0.8 vvm, the bead velocities were 20 and 14 cm/s for the 10 and 25% bead loadings, respectively. The results suggest significant bead-to-bead collisions at high aeration rates and high bead loadings.

Gas hold-up was calculated based on the total reactor volume (liquid volume + bead volume) as a function of the aeration rate, vvm, for 0 to 25% bead loadings. The hold-up increased approx 2% for all bead loadings studied. For example, at 25% bead loading, the gas hold-up increased from 0.3 to 2.1% as the aeration rate went up from 0.4 to 1.06 vvm. This data also shows that increasing the volume of beads in the reactor resulted in a slight decrease in the gas hold-up for a constant vvm. At 0.5 vvm, for example, the gas hold-up at 0% bead loading was 1.1% compared to a gas hold-up of 0.5% at 25% bead loading (data not shown). When the gas hold-up was calculated based on liquid volume, the same 2% increase in hold-up was observed over the aeration rates studied (Fig. 3C). These results agree with gas hold-up studies performed by Siegel et al. *(4)* in a three-phase external-loop air-lift reactor.

Mass-Transfer Studies

According to Van't Riet *(19)*, the response lag of an oxygen probe can be neglected if the oxygen probe time constant, τ_p, is less than $[1/(5\ k_l a)]$. The oxygen-probe time constant for our studies was 27 ± 7s and the maximum $k_l a$ value found was $0.00861/s^{-1}$. Since this is approximately equal to $[1/(5\ k_l a)]$, the oxygen-probe response time was therefore not accounted for in the determination of the $k_l a$ values in our investigation.

In order to ensure that the $k_l a$ studies would be measuring the gas-to-liquid mass-transfer coefficient and not the mass transfer from the gas to the interior of the bead, the $k_l a$ in the presence of relatively porous alginate beads was compared to that in the presence of impermeable (i.e., solid) ion-exchange resin beads. The $k_l a$ for a 10% loading of alginate beads was determined to be $31.7 \pm 0.7/h^{-1}$, and for a 10% loading of ion-exchange resin beads it was $31.4 \pm 1.5/h^{-1}$, at an aeration rate of 0.67 vvm. The $k_l a$ values are essentially equal even though there was great difference in bead porosity. This suggested that the alginate beads were not acting as a "sink" for oxygen and that the desired quantity, gas-to-liquid mass-transfer coefficient, was being measured.

In two-phase (gas/liquid) systems such as bubble columns and air-lift bioreactors, the $k_l a$ is usually found to be an exponential function of the superficial gas velocity as follows *(20)*:

$$k_l a = \alpha\ v_s^{\beta} \tag{5}$$

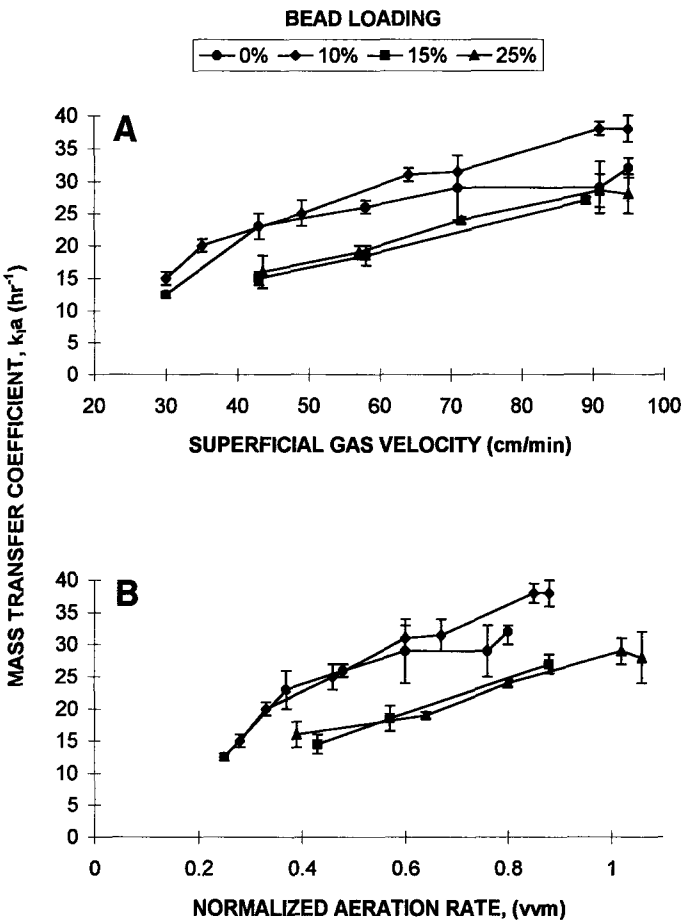

Fig. 4. Mass-transfer coefficient, k_la, vs superficial gas velocity **(A)** and normalized aeration rate **(B)**.

Figure 4A gives the values of k_la as a function of superficial gas velocity for the various alginate-bead loadings studied. All curves showed the same general trend; as the superficial gas velocity increased, the k_la increased. It was apparent, though, that a nonmonotonic relationship between bead loading and k_la existed. Relative to the value of the k_la determined in water with no beads present, the k_la increased slightly (up to 20%) when a small volume percentage (i.e., 10%) of beads was added. As more beads were added (i.e., 25% by volume) the value of the k_la decreased. Therefore, for our data other possible correlations for expressing the dependence of k_la on aeration rates and alginate-bead loading were examined.

As a first approach to correlate the k_la values with an aeration term, the k_la values were plotted vs the normalized aeration rate, vvm (Fig. 4B). In the presence of alginate beads, this resulted in a monotonic relationship,

whereby increasing the percentage of beads in the reactor decreased the k_la. However, it is important to note that this was not valid in the absence of beads (i.e., 0% loading). For the range of aeration rates studied, at 0% bead loading, the bioreactor was predominantly operated under turbulent conditions (i.e., Re ≥ 4000). On the other hand, in the presence of beads, 10 to 25%, the bioreactor was mostly being operated in the transition zone. We can speculate that this could affect the k_la values.

Using vvm instead of the superficial gas velocity accounted for the presence of "internals" (i.e., alginate beads) since vvm normalizes the gas-flow rate with respect to the liquid volume. Correlating the k_la with vvm by performing a linear regression on the data and setting the constant equal to zero using the statistical package Minitab (Version 5.1.3), gave correlation coefficients (R^2 values) ranging from 64.6% for 0% bead loading to 84% for 15% bead loading. This was not considered to be a very good fit of the data because of the low R^2 values. Also, this did not give a single correlation that would account for the presence of the beads but rather a correlation for each bead loading was required.

It was then proposed that perhaps a term was necessary in the correlation to account for the presence of alginate beads. Thus, a concentration effect, C_E, term was introduced to the correlation. To define the concentration effect, Einstein's equation for the suspension of rigid spheres was used:

$$C_E = 1 + 2.5\,\phi \tag{6}$$

where ϕ is the volume fraction occupied by the spheres (i.e., alginate beads). A multiple linear regression using Minitab was used to correlate k_la with vvm and C_E. This multiple linear regression resulted in the following expression:

$$1n\,k_la = 4.10 + 0.803\,Ln\,vvm - 1.67\,1nC_E \tag{7}$$

where k_la has units of h^{-1}.

Rearranging Eq. 7 into a more convenient form gave:

$$k_la = 60.34 vvm^{0.803} C_E^{-1.67} \tag{8}$$

This correlation had a coefficient of 0.815, which was indicative of a good fit. There were no trends in the residual plots. Also, the T ratios for the constant and coefficients in the correlation were very large, which indicated that these parameters were significant to the model. It was, therefore, concluded that this regression line was adequate to explain the data. Equation 8 was used to predict the k_la value for a given aeration rate (vvm) and alginate-bead loading and gave a good fit (Fig. 5).

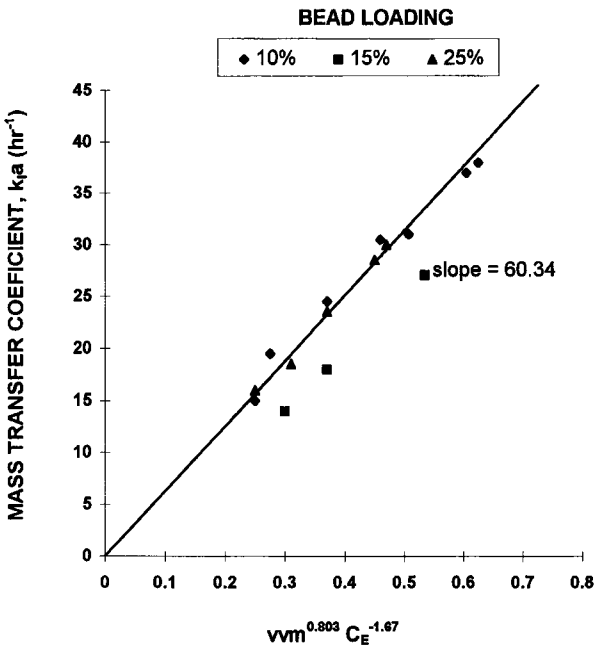

Fig. 5. Correlation between mass-transfer coefficient, normalized aeration rate, and bead loading.

A variety of other correlations were attempted to fit the data. Correlations that did not contain a term accounting for the presence of beads were rejected because for each bead loading a distinct correlation was required. When attempting to develop a correlation that can be used as a design parameter for bioreactor scale-up, a single correlation that can be applied to all systems is desirable. Correlations involving liquid velocity were not considered to be appropriate because of the dependence of the liquid velocity on the aeration rate. Although used successfully for describing two-phase systems *(13,21)* correlations involving the gassed power per unit liquid or reactor volume (P_g/V_L and P_g/V, respectively) did not account for the change in bulk fluid properties, caused by the presence of alginate beads. It is important to note that the correlation proposed (Eq. 8) does not fit the k_la data when there are no beads present because of the turbulent flow regime at 0% bead loading.

Theoretical Study of Oxygen Transfer to Microencapsulated Insect Cells

For immobilized cell systems, such as microcapsules, ensuring adequate oxygen transfer from the gas phase to the liquid medium does not necessarily ensure that adequate oxygen will reach the immobilized cells. It is possible that the k_la may not be adequate for a certain microcapsule

loading. A theoretical study of oxygen transfer to cells immobilized in microcapsules for various microcapsule loadings in the bioreactor was performed by employing Eqs. 3 and 4. A schematic diagram of the oxygen concentration profile from a gas bubble to the center of a microencapsule is shown in Fig. 1. Upon arbitrarily specifying the critical oxygen concentration in the center of the microcapsule, the rate of oxygen transfer from the gas phase to the inner surface of the microencapsule membrane (OTR_G, mg/h) for a certain microcapsule loading was compared to the oxygen demand of the cells (Q_{o_2} x) for the same microcapsule loading. The study was made for *Spodoptera frugiperda* cells cultivated in poly-L-lysine/alginate microcapsules at a maximum cell density of 8×10^7 cells/mL capsules (*22*). An oxygen demand of 1.4×10^{-10} mmole O_2/cell h was assumed. These insect cells are usually cultivated at 27 and 33°C, therefore our study was performed at both temperatures.

It was necessary to estimate several of the parameters used to evaluate the oxygen-transfer rate. The diffusivity of oxygen in sodium alginate (the immobilization agent inside the microcapsule) was estimated to be 86% of the diffusivity of oxygen in water (i.e., approximately the same as the diffusivity of oxygen in calcium alginate) (*23*). According to King et al (*22*), the membrane is 5 μm thick and is composed of approx 90% water. The diffusivity of oxygen through the membrane was therefore assumed to be equal to that of oxygen in water. A critical oxygen concentration in the center of the microcapsule was assumed to be 40% of air saturation.

Figure 6 shows the results for the oxygen transfer rate attainable for microcapsule loadings of 10, 15, and 25% at 33 and 27°C as a function of the aeration rate. The terminal settling velocity was used to calculate the Reynold's number. Comparing Figs. 6A and B, the latter uses the difference between the bead and liquid velocities (determined experimentally) to calculate the Reynold's number, indicates that there is not much difference (at most 8%) between the two methods. This suggests that the terminal velocity may be used as a good approximation of the relative velocity between the bead and the liquid if it is not feasible to determine the liquid and bead velocities experimentally.

At 33°C, for 10% bead loading, the oxygen demand of the cells was achieved at 0.29 vvm, which is the minimum vvm for suspension of the beads (Fig. 6A). On the other hand, for 25% bead loadings, a vvm of 1.06 is required to meet the oxygen demand of the cells. This is quite a high aeration rate, thus it may not be feasible to operate at 25% bead loading. Decreasing the temperature to 27°C (Figs. 6C and D) increased the oxygen transfer rate only slightly (by approx 8%). This was expected since a decrease in temperature increases the solubility of oxygen in the bulk liquid that increases the driving force for oxygen transfer. This, in turn, increases the oxygen transfer rate to the cells. The temperature did not,

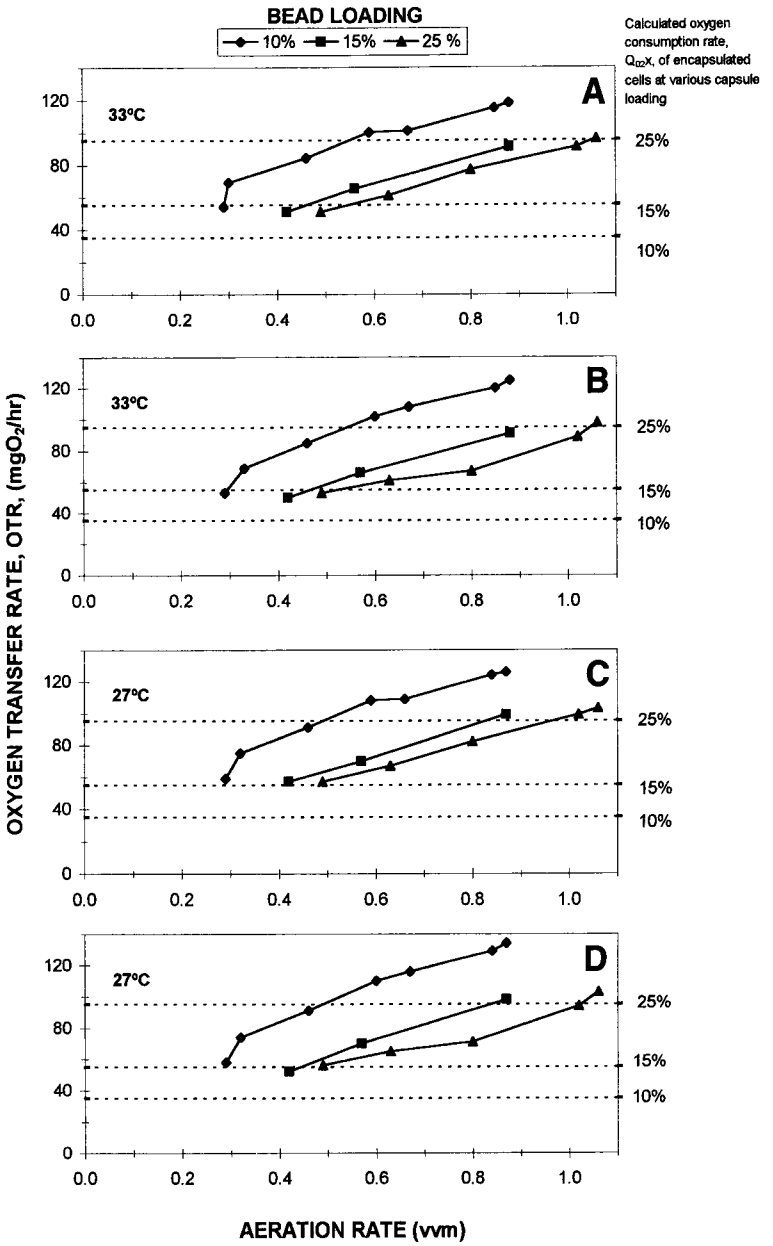

Fig. 6. Theoretical oxygen-transfer rate as a function of aeration rate for encapsulated insect cells in bioreactor at 33°C using the terminal settling velocity to calculate the Reynold's number **(A)**; OTR at 33°C using the difference between the bead velocity and the liquid velocity to calculate the Reynold's number **(B)**; OTR at 27°C using the terminal settling velocity to calculate the Reynold's number **(C)**; OTR at 27°C using the difference between the bead velocity and the liquid velocity to calculate the Reynold's number **(D)**.

however, have a very significant effect on the oxygen-transfer rate. These results suggest that for this bioreactor the cells will not be oxygen limited at microcapsule loadings at 10 and 15% (by volume), however, there is the potential for oxygen limitation at 25% microcapsule loadings if the reactor is not operated at a minimum aeration rate of 1.06 vvm.

CONCLUDING REMARKS

The addition of alginate beads to the external-loop air-lift bioreactor, at a 10% loading, decreased the mixing time by up to 50%, compared to the mixing time in the absence of alginate beads. Since the liquid velocity was found to be greater than 5 cm/s, the bioreactor was being operated as an air-lift rather than a bubble column. A minimum liquid velocity of 10 cm/s was required to keep the alginate beads in suspension, regardless of the percentage of alginate beads in the reactor. The alginate bead velocity at 10 and 15% loading was similar. However, when the alginate-bead loading was increased to 25%, the bead velocity decreased. The gas hold-up increased by only 2% with an increase in the aeration rate from 0.1 to 1.0 vvm.

A correlation for expressing the dependence of $k_l a$ on aeration rate and bead loading was developed. The correlation proposed is:

$$k_l a = 60.34 vvm^{0.803} C_E^{-1.67} \tag{8}$$

where vvm is the normalized aeration rate (volume gas/volume liquid/minute), and $C_E = 1 + 2.5\,\phi$ with ϕ being the volume fraction of solids occupied by the microbeads. Correlations that did not contain a term accounting for the presence of microbeads were rejected because for each bead loading, a distinct correlation would have been required. With the above correlation it is possible to predict, the $k_l a$ within 20% knowing the gas-flow rate and the percentage (by volume) of alginate beads present in the bioreactor.

The form of the $k_l a$ correlation, Eq. 8, may be useful to other researchers working with air lifts of different scale or geometry. They would, though, have to fit new numerical values to the parameters vvm and C_E. It is quite common with bioreactors, that the values of the exponents in $k_l a$ correlations change as the scale changes. The main contribution of the present study is that it was the first to show the impact that immobilization matrices (i.e., beads) have on the hydrodynamics and mass-transfer properties of an external-loop air-lift system.

A theoretical study to calculate the oxygen transfer from the bulk liquid to the center of a microcapsule using the experimental $k_l a$ data was

also performed. The results suggest that for a 1-L external-loop air-lift bioreactor, the cells in the center of a microcapsule will not be oxygen limited at microcapsule loadings at 10 to 15%. However, there is a potential mass-transfer limitation at 25% microcapsule loading if the bioreactor is not operated at a minimum aeration rate of 1.06 vvm.

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