J. Chem. Tech. Biotechnol. 1997, 68, 37–46

Enhancement of Oxygen Transfer Rate Using Microencapsulated Silicone Oils as Oxygen Carriers

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(Received 4 March 1996; accepted 18 July 1996)

Abstract: Silicone oils were microencapsulated within polyamide (nylon) membranes cross-linked with polyethylenimine for use as an oxygen carrier in aerobic fermentation. The use of 20% microcapsule dispersions enabled a four- to five-fold increase in volumetric oxygen transfer coefficient ($k_L a$), with or without the presence of cells. The improvement in oxygen transfer rates was due to the greatly increased specific surface area of the carrier in comparison to conventional bubble aeration. The production rate of dihydroxyacetone (DHA) from glycerol with *Gluconobacter oxydans* was increased from 1·5 to 9 mmol dm^{-3} DHA h^{-1} with the introduction of 20% of microcapsules in a batch fermentation and from 6 to 8 mmol dm^{-3} DHA h^{-1} in a fluidized bed fermentation. It is expected that the oxygen-permeable polymeric membrane coating the silicone oils should reduce the toxic or inhibitory effects previously observed with other oil-based oxygen carriers, and will eliminate the need for toxic chemical dispersants in the medium. Also, microencapsulated oxygen carriers avoid the need for vigorous agitation to maintain a dispersion of the oxygen carrier.

Key words: microencapsulation, oxygen carriers, oxygenation, *Gluconobacter oxydans*, silicone oils

NOTATION

- a Interfacial area per unit volume of dispersion (cm^2 cm^{-3})
- C^* Saturation concentration of dissolved oxygen at bubble surface (mol dm^{-3})
- C_L Bulk concentration of dissolved oxygen (mol dm^{-3})
- C_{L0} Dissolved oxygen concentration at $t = 0$ (mol dm^{-3})
- k_L Liquid phase oxygen transfer coefficient (cm s^{-1})
- $k_L a$ Overall volumetric oxygen transfer coefficient (h^{-1})
- K_p Probe constant (s^{-1})
- p_0 Oxygen partial pressure of the exhaust gas (atm)
- Q_{O2} Specific rate of oxygen consumption (mol g^{-1} h^{-1})
- T_p Probe time constant (s)
- X Biomass concentration (g dm^{-3})
- Y_p Normalized probe response
- Y_L Normalized oxygen concentration in liquid phase
- α_e The area above the curve of the transient aeration experiment
- α_s The area above the curve of the probe step response experiment

1 INTRODUCTION

In industrial fermentation technology, the rate of oxygen supply to submerged cultures has often been identified as a limiting factor. This occurs when the oxygen transfer rate from sparged air is less than the

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J. Chem. Tech. Biotechnol. 0268-2575/97/$09.00 © 1997 SCI. Printed in Great Britain

cellular oxygen consumption rate, resulting in dissolved oxygen levels below the critical concentration needed to maintain metabolic activity. In conventionally aerated bioreactors, low oxygen solubility (0·28 mmol dm^{-3} at 20°C) combined with slow oxygen transfer rates often results in reduced growth and culture productivity.

Various techniques used in conventional biorectors to improve the rate of oxygen transfer from the gas phase include increasing the agitation or aeration rates, raising the partial pressure of oxygen in the gas phase or modifying the vessel design. Often turbulence and shear associated with high rate mixing and aeration are incompatible with fragile cells.

Non-conventional methods reported to enhance oxygen supply include oxygen generation *in situ* with hydrogen peroxide/catalase,[1,2] or by introducing hydrogen peroxide to the medium of catalase-positive organisms.[3] However, hydrogen peroxide is toxic, resulting in growth rate reductions.[2] An alternative is to replace oxygen with a more soluble electron acceptor such as *p*-benzoquinone[4,5] which resulted in the enhancement of glycerol oxidation to dihydroxyacetone (DHA) with *Gluconobacter oxydans* by four-fold, in comparison to regular bubble aeration. The drawback of this techniqe is that not all organisms are able to use *p*-benzoquinone as an oxygen substitute and toxic effects were observed over the long term. It was observed that productivity decreased from 60 to 10 mmol h^{-1} g^{-1} in an 8-day period when using *Gluconobacter oxydans* and *p*-benzoquinone for the oxidation of glycerol to dihydroxyacetone.[4]

A biological approach to bioreactor aeration has also been reported, involving *Gluconobacter oxydans* co-immobilized with the photosynthesizing algae *Chlorella pyrenoidosa.*[6] Dihydroxyacetone productivity was enhanced 5·4-fold when compared with pure culture. However, the oxygen production is limited and co-immobilization may cause inhibition or competition for the substrate.

Yet another novel method reported is cloning and expression of haemoglobin into the host system for enhancing the utilization of oxygen. However, this technique can only be applied to a limited number of biological systems.

Oxygen supply may be enhanced by the use of oxygen carriers which are compounds with high oxygen solubility. Examples include haemoglobin,[7] perfluorochemicals[8,9] and low viscosity silicone oils.[10,11] Haemoglobin has limited application since it is not stable in fermentation medium. It is oxidized to met-haemoglobin, concomitant with a loss in oxygen absorption capacity.[7] Moreover, there are numerous reports of cell growth reduction in the presence of perfluorocarbons and/or the emulsifiers used as dispersants. The growth rate of *Escherichia coli* is inhibited both by perfluorocarbons and the emulsifiers used as dispersants. For example, Lowe *et al.*[12] showed that some perfluorocarbons led to inhibition of growth and have other negative effects on mammalian cells.

Silicone oils have also been used to supply oxygen to liquid medium. Chibata *et al.*[10] observed that silicone oil has at least twice the oxygen solubility of perfluorocarbon liquids. Leonhardt *et al.*[11] reported that the L-amino acid oxidase activity of immobilized cells was increased by a factor of four in the presence of silicone emulsion. However, after prolonged contact, the culture system can become unstable due to loss of activity of the microbial cells,[13] toxicity of the oxygen carriers[12] or the increased cell adsorption at the water–oil interface.[14]

It has been proposed to encapsulate the oxygen carriers within ultra-thin oxygen-permeable membranes such as polyamide films, eliminating direct contact between the cells or liquid medium.[15] Toxicity problems related to direct contact between the cell and the oxygen carrier can be avoided, and there is no longer a need for potentially toxic dispersants. Silicone oils microencapsulated within nylon membranes enhanced the oxygen reservoir in water containing 10–20% microcapsules by a factor ranging from four to seven.[15]

The aim of this study was to examine the enhancement of oxygen transfer due to microencapsulated silicone oils during aerobic fermentation. Determination of oxygen solubility and transfer rate from microcapsules, and evaluation of fermentation productivity using microencapsulated oxygen carriers in different types of fermentation systems are described.

2 MATERIALS AND METHODS

2.1 Microencapsulation of silicone oil

Nylon membranes were formed by interfacial polymerization around an emulsified organic phase containing silicone oils (Dow Corning 200 Fluid: 0·1 m^2 s^{-1}) as described previously.[15]

2.2 Microcapsule size distribution analysis

Mean diameter and size distribution of the silicone oil microcapsules were determined with a Malvern Sizer 2600Lc (Malvern Instruments, UK). The particle analyser determined the volume distribution of the microcapsules and calculated an average size based on a log normal distribution. The arithmetic standard deviation was calculated from diameters at 16 and 84% of the cumulative distribution curves $[\sigma_a = (d_{84\%} - d_{16\%})]$.

2.3 Oxygen electrode dynamics

The response of the oxygen electrode is subject to a delay, mainly caused by the oxygen diffusion through

the electrode membrane and electrolyte layer separating the inside surface of the membrane from the cathode. The delay in probe response may cause an error in dynamic k_La measurements if the rate of oxygen transfer to the liquid is comparable to the rate of oxygen diffusion through the electrode membrane to the cathode. Fujita and Hashimoto[16] described a graphical approach to correct for problem response involving the integrated form of eqn (1).

$$\text{OTR} = k_La(C^* - C_L) \quad (1)$$

where OTR is the oxygen transfer rate, k_L is the liquid phase oxygen transfer coefficient (cm s^{-1}), a is the interfacial area per unit volume of dispersion (cm^{-1}), C^* is the saturation concentration of dissolved oxygen at the bubble surface (mol dm^{-3}) and C_L is the solution dissolved oxygen concentration (mol dm^{-3}). The term k_La (h^{-1}) is referred to as the overall volumetric oxygen transfer coefficient and is used to characterize the performance of aeration devices. A normalized variable-normalized probe response, Y_p, is introduced into eqn (1):

$$Y_p = (C^* - C_L)/(C^* - C_{L0}) \quad (2)$$

where C_{L0} is the initial dissolved oxygen concentration, and upon integrating the following relationship is obtained:

$$Y_p = \exp(-k_La \cdot t) \quad (3)$$

Taking into account the probe dynamics, the probe response is expressed by the first order lag function:

$$dY_p/dt = K_p(Y_L - Y_p) = (Y_L - Y_p)/T_p \quad (4)$$

where Y_L is the normalized oxygen concentration in the liquid phase, K_p is the probe constant (s^{-1}) and T_p is the probe time constant (s). Setting Y_p and Y_L equal to 1 at $t = 0$, yields the following relationship:

$$Y_p = [K_p \exp(-k_Lat) - k_La \times \exp(-K_pt)]/(K_p - k_La) \quad (5)$$

Determination of k_La could be achieved either by non-linear regression estimation techniques or, as done in the present study, by the method of moments.[17]

All k_La measurements were performed in a 2 dm^3 glass bench-top fermenter. The working volume was 1–1·5 dm^3 at ambient pressure. The reactor had an inner diameter of 15 cm, a height of 30 cm and was equipped with a vertical agitator shaft turning three (or two) sets of impeller blades mounted along the shaft. Gases were introduced with a sparger placed beneath the impellers. Four vertically mounted baffles along the sides of the reactor enhanced the mixing. The agitator was powered by a variable speed motor (0–850 rev min^{-1}). The impeller speed was measured by a tachometer (Cole Parmer). The aeration rates were measured with a rotameter at the inlet. The air flow could be varied from 0·1 to 2 dm^3 min^{-1}.

Dissolved oxygen was measured with an Ingold permeable membrane probe and a Cole Parmer 0197100 digital dissolved oxygen meter giving readings as mg dm^{-3}. The probe was installed near the side wall in the bottom half of the vessel. The dissolved oxygen probe was calibrated and tested before insertion into the vessel.

2.4 Culture

Gluconobacter oxydans subsp. *suboxydans* was obtained from the American Type Culture Collection designated as ATCC 621. Lyophilized cultures were stored at −18°C. Rehydrated cells (1 g dm^{-3} dry weight) were cultured in liquid medium for 24 h at 28–30°C. The medium was sterilized for 30 min at 121°C prior to use.

The stock culture was maintained under refrigeration on agar slants containing (in g dm^{-3}): glycerol (50), yeast extract (5), peptone (3), NH_4Cl (0·8), $Na_2HPO_4.2H_2O$ (0·6), KH_2PO_4 (0·4), $MgSO_4.7H_2O$ (0·2) and agar (20). Liquid growth medium had the same composition, except agar was not added. In experiments involving alginate-immobilized cells, 10 mmol dm^{-3} $CaCl_2$ was added to the medium in order to stabilize the beads.

2.5 Estimation of biomass concentration

Optical Density (OD) was measured at 600 nm with a Varian Cary UV-visible spectrophotometer using distilled water as reference. One (1) OD unit corresponds to 706 mg dm^{-3} dry weight cells. A standard curve for cell density yields: Cell Density (g dm^{-3}) = (Absorbance − 0·026)/1·38.

Biomass concentration was also measured by the dry weight of a centrifuged cell pellet (6000 rev min^{-1} for 25 min).

2.6 Quantification of dihydroxyacetone

DHA was determined by the DNS-method of Miller *et al.*,[18] modified by Adlercreutz and Mattiasson.[7]

2.7 Batch fermentation

Cells were cultured in 500 cm^3 Erlenmeyer flasks containing 150 cm^3 culture medium, on a rotary shaker (220 rev min^{-1}) at 28°C. After 24 h, 50 cm^3 of the medium was transferred to 150 cm^3 fresh medium and

shaken for 24 h. This culture was used as the inoculum for a 2 dm^3 batch fermenter. The medium in the 2 dm^3 fermenter was inoculated with 10% (v/v) inoculum.

Batch fermentation was carried out at 28°C, 1 atm and pH 6·0. Fermentation was performed in a 2 dm^3 fermenter of 1·3 dm^3 working volume. The agitation and aeration rates were 450 rev min^{-1} and 0·3 vol vol^{-1} min^{-1} respectively.

2.8 Fluidized bed fermentation

A cell suspension was mixed with an equal volume of sterile 3·6% (w/w) sodium alginate. The mixture was dropped through a sterile needle (0·9 mm i.d.) into sterile 0·1 mol dm^{-3} $CaCl_2$, forming beads with a mean diameter of 0·2 cm. After 1 h, the beads were then washed and stored in the pre-sterilized peptone water containing 10 mmol dm^{-3} $CaCl_2$ at 4°C. The cell density in the beads was 35 mg cell (dry weight) per g wet gel.

Beads were introduced into a fluidized bed reactor with full and continuous recycle as illustrated in Fig. 1. The reactor was constructed of transparent acrylic glass with an inner diameter of 3·8 cm and a height of 16 cm. Both ends of the reactor were sealed with stainless steel mesh screen of pore size 0·12 × 0·12 cm^2. The purpose of the mesh screen was to retain the Ca-alginate gel beads in the reactor while allowing the microencapsulated silicone oil (mean diameter = 100 μm) free passage.

After passage through the fluidized bed, oxygen-spent medium was pumped to a reaerator which ensured efficient oxygenation by spraying the medium though a shower head into air-bubbled medium. The aeration section had an inner diameter of 3·8 cm and a height of 20 cm. The liquid level was 11 cm and the head space was 11 cm. Sterile air was introduced at 0·35 dm^3 min^{-1} and purged from the top. The reaerated culture medium was then circulated into the reactor. The total liquid volume in the fluidized bed fermenter was 400 cm^3. The liquid circulation rate was 100 cm^3 min^{-1}. The dissolved oxygen concentration was measured by oxygen probes which were placed in both the inlet and outlet of the reactor.

3 RESULTS

3.1 Microencapsulated silicone oil

Microencapsulation of the silicone oil yielded spheres with smooth and transparent nylon membranes. The mean diameter ranged from 100 to 300 μm, depending on the formulation conditions.[15] Microcapsules were sufficiently resilient to withstand autoclaving temperatures and pressures.

3.2 k_La determination

The k_La was determined using an Ingold dissolved oxygen electrode. The time constant of the electrode was 10·5 s following a step change in dissolved oxygen concentration (fully depleted to fully saturated oxygen), as seen in Fig. 2. The air flow rate was

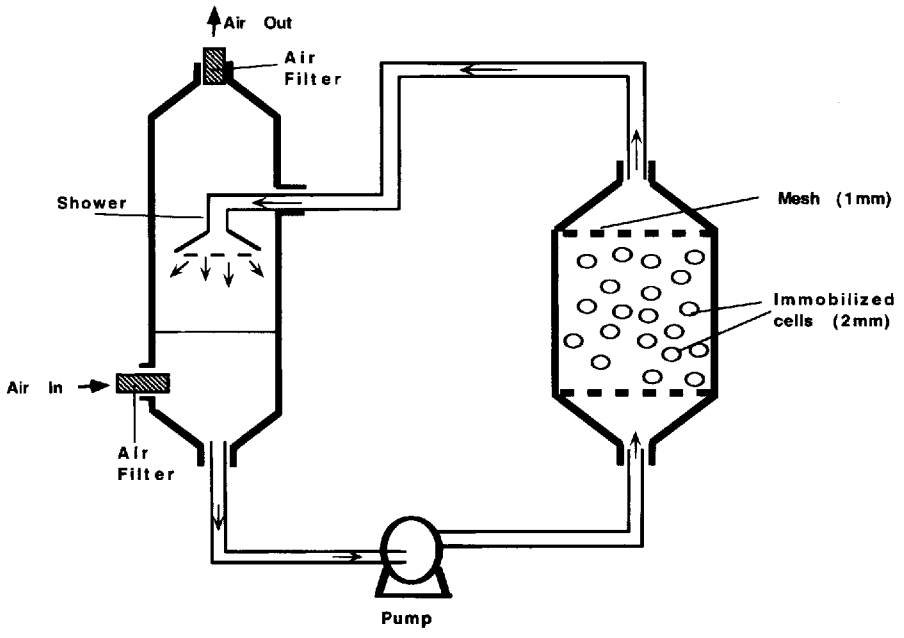

Fig. 1. Schematic of fluidized bed reactor containing alginate-immobilized cells, with recycle of medium and oxygen carriers to reaeration section.

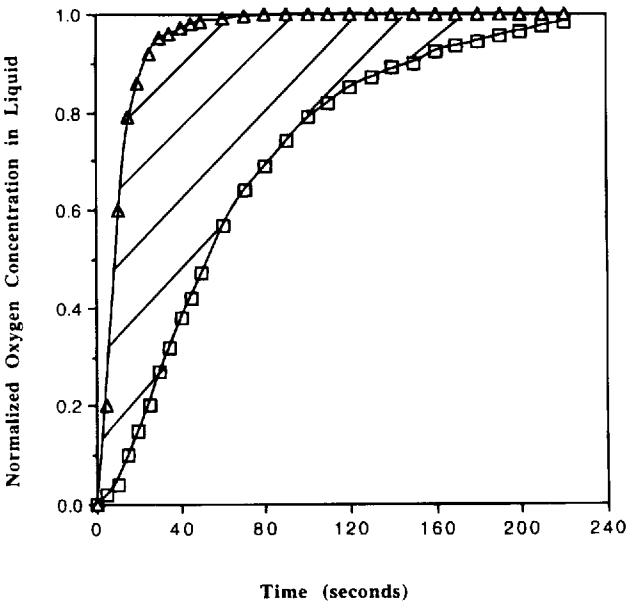

Fig. 2. Determination of k_La by the method of moments. Experiment was conducted in a 1·5 dm^3 vessel at 0·15 vol vol^{-1} min^{-1} aeration and 550 rev min^{-1} agitation. Plot illustrates the aeration response with no cells present (□), compared with the probe response (△). The k_La measured by the dynamic method was 64 h^{-1} and by the method of moments was 77 h^{-1}.

0·15 vol vol^{-1} min^{-1} and the agitation rate was 550 rev min^{-1}. The method of moments was used to evaluate k_La by comparing the probe response curve to the measured response determined in the bioreactor. In the following equation:

$$1/k_La = \alpha_e - \alpha_s = \int (1 - Y_p)dt - \int (1 - Y'_p)dt \quad (6)$$

Y'_p represents the electrode response to a step change in oxygen concentration, while Y_p was the normalized trace of the aeration experiment. The area between the two curves ($\alpha_e - \alpha_s$), is the reciprocal of k_La. The k_La measured by the dynamic method in a 1·5 dm^3 vessel was 64 h^{-1} compared with 77 h^{-1} corrected for the probe response time by the method of moments (see Fig. 2).

The k_La for oxygenation solely via microencapsulated oxygen carrier (in the absence of bubble aeration) using different microcapsule volume fractions was measured by the dynamic method. As the volume fraction of the microcapsules was increased from 10 to 30%, the k_La value increased from 125 to 188 h^{-1}, as seen in Fig. 3. The point on the ordinate represents oxygen transfer through surface aeration.

The k_La was also determined at various aeration rates ranging from 0·05 to 0·48 vol vol^{-1} min^{-1} (500 rev min^{-1}), with and without microcapsules present. Figure 4 shows that for conventional bubble aeration, the k_La values increased with the air flow rate to a maximum of approximately 180 h^{-1} at

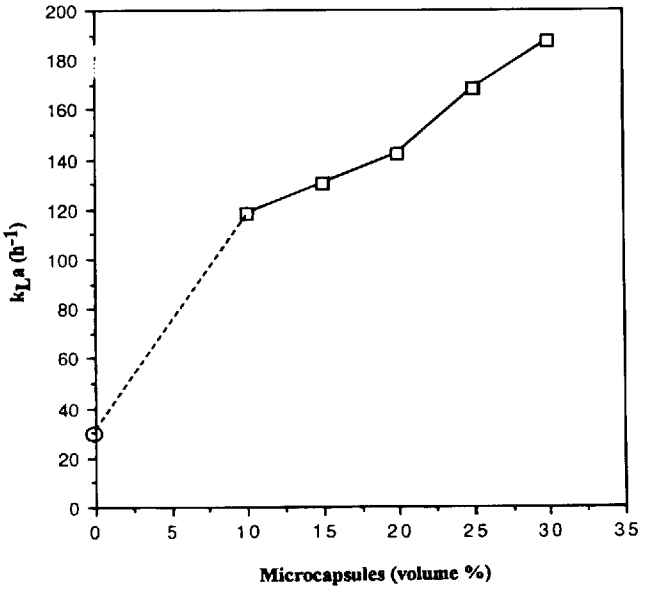

Fig. 3. k_La values with increasing microencapsulated oxygen carrier volume fraction. The point at the origin represents the k_La for surface aeration alone.

0·48 vol vol^{-1} min^{-1}. In the presence of 20% microencapsulated silicone oils, the k_La values followed a similar trend, but at enhanced levels.

A high oxygen transfer coefficient (k_La = 180 h^{-1}) can be achieved in the conventional bubbling system at an air flow rate of 0·48 vol vol^{-1} min^{-1}. However, high levels of mixing and aeration may be problematic for some shear-sensitive cells. With a 20% (v/v) microcapsule suspension, the same oxygen transfer rate can be reached at 0·15 vol vol^{-1} min^{-1} of air or less than a third of that required with air alone. At very low aeration rates (0·05 vol vol^{-1} min^{-1}), the k_La was

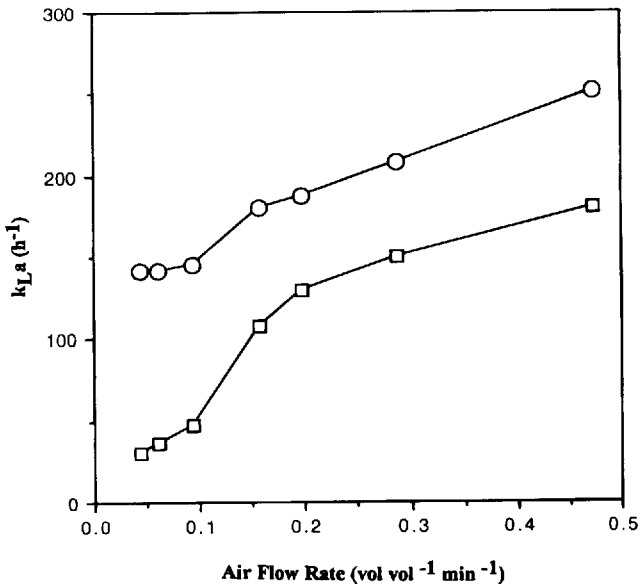

Fig. 4. Variation of k_La with aeration rate in the presence of 20% silicone microcapsules (○) or in the absence of microcapsules (□).

increased by a factor of five, in the presence of the oxygen carriers. The k_La value achieved (145 h^{-1}) is similar to that of the non-aerated system using a 20% microcapsule suspension (Fig. 3).

The oxygen transfer characteristics were strongly influenced by the agitation speed, as seen in Fig. 5. The oxygen transfer coefficient was approximately proportional to the agitation rate, over the range of 200 to 800 rev min^{-1}. Again, enhanced k_La values were observed in the presence of the oxygen carriers in comparison to bubble aeration alone.

3.3 DHA production and growth with oxygen carriers

The oxygen uptake rate ($Q_{O2}X$) and the specific oxygen uptake rate (Q_{O2}) of *Gluconobacter oxydans* during exponential growth was 4·6 mmol O_2 dm^{-3} h^{-1} and 19·3 mmol O_2 g^{-1} dry cell h^{-1} respectively, as determined from the rate of oxygen depletion profile in Fig. 6. During subsequent reaeration of the bioreactor, the rate of change in oxygen concentration is given by:

$$dC_L/dt = k_La(C^* - C_L) - Q_{O2}X \quad (7)$$

The k_La determined from a plot of C_L versus $dC_L/dt + Q_{O2}$ was 34 h^{-1} in the absence of microcapsules and 135 h^{-1} in the presence of 20% microencapsulated silicone oil. The oxygen transfer capacities appear to be increased by a factor of four in the presence of the microencapsulated oils, similar to that obtained in the absence of cells (Fig. 4).

Gluconobacter oxydans was cultivated in standard medium containing 15% (v/v) silicone oil emulsion or 15% (v/v) microencapsulated silicone oil. An inoculated

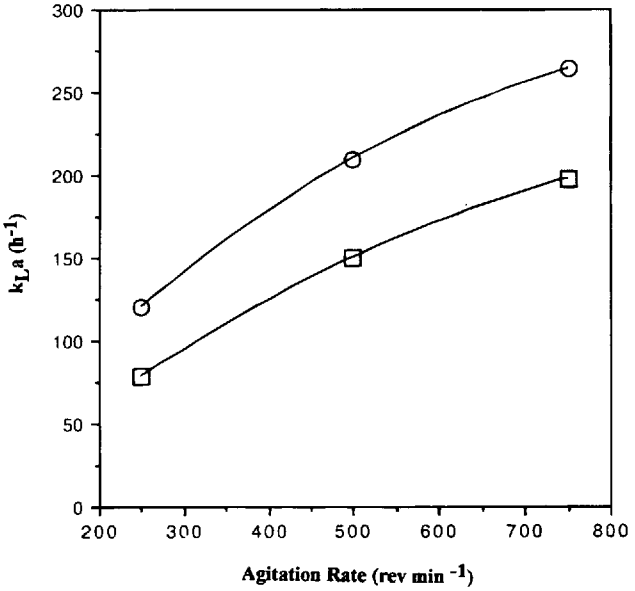

Fig. 5. Variation of k_La with increasing agitation rate in the presence of 20% microencapsulated oxygen carrier (○) or with bubble aeration alone (□).

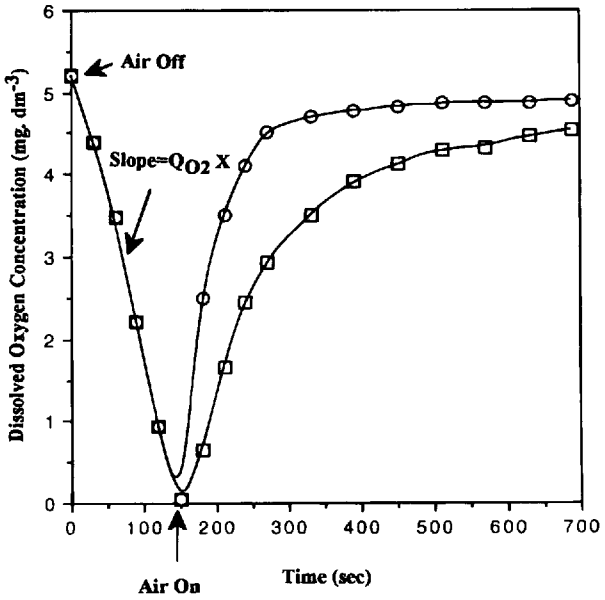

Fig. 6. Dissolved oxygen concentration profile during dynamic k_La measurement in 1·5 dm^3 bioreactor with *Gluconobacter oxydans*, with 20% microencapsulated oxygen carriers (○) or in the absence of the oxygen carriers (□). Aeration rate in both cases was 0·3 vol vol^{-1} min^{-1}.

control contained medium only. The DHA production rate of the shake flask cultures was monitored. Figure 7 indicates that both the non-encapsulated and encapsulated silicone oils enhanced the production rate by about 33% over that of the control.

Dissolved oxygen profiles and rates of DHA production during the course of duplicate 1·5 dm^3 aerated batch fermentations with *Gluconobacter oxydans* are illustrated in Fig. 8. Aeration commenced at 1 h, at which point cells began producing DHA at a stable rate of 1 mmol dm^{-3} h^{-1}. After 8·5 h, 20% silicone micro-

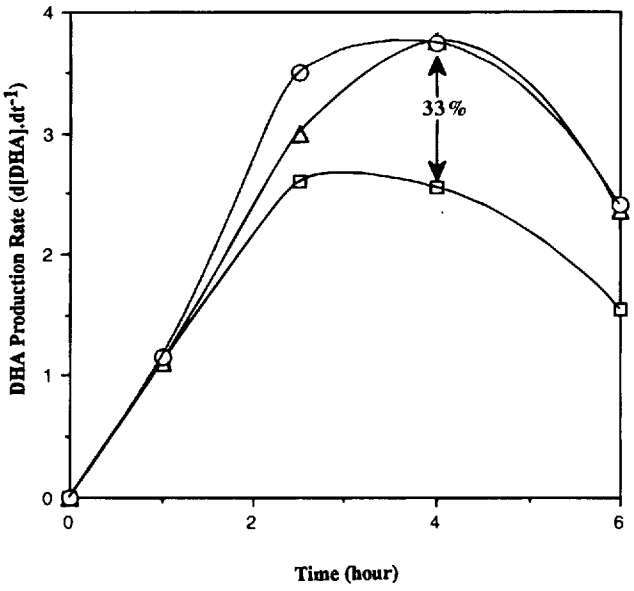

Fig. 7. Shake flask experiment with 15% silicone oil (△) or 15% microencapsulated silicone oil (○) added. The control experiment (□) was conducted in the absence of oil.

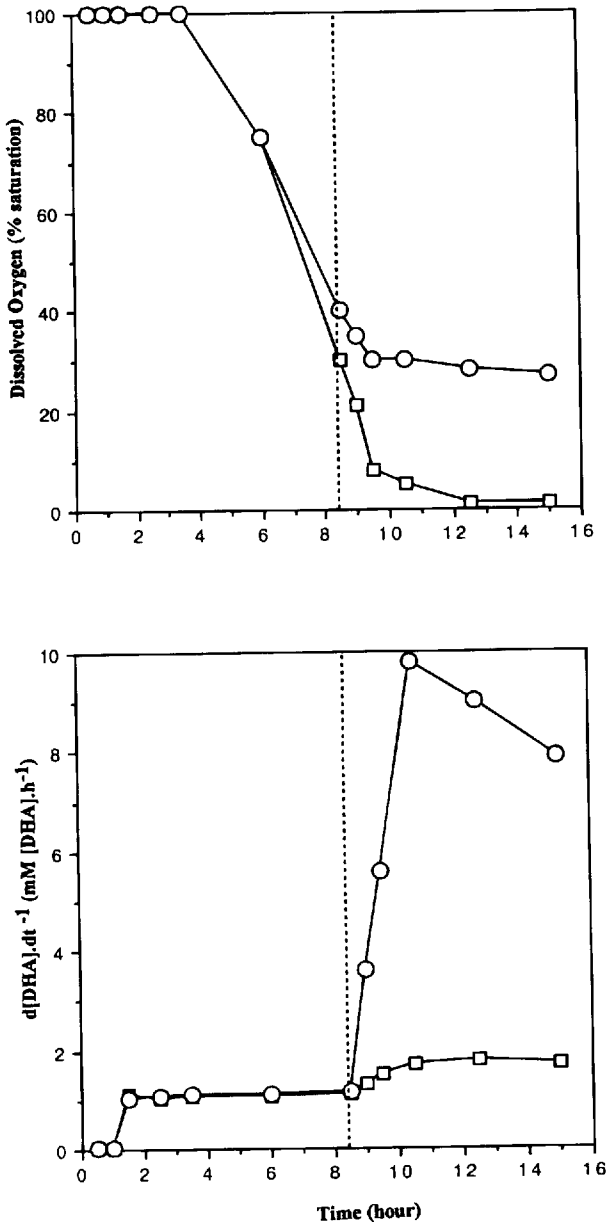

Fig. 8. Batch fermentation of *Gluconobacter oxydans* with (○) and without (□) microencapsulated silicone oils. Rate of DHA production and dissolved oxygen profiles are plotted. Microcapsules were added at 8·5 h. Fermentations (1·5 dm^3) were run at 0·3 vol vol^{-1} min^{-1} air and 450 rev min^{-1}.

capsules were added to one of the bioreactors, which triggered a dramatic increase in the rate of DHA production to 10 mmol dm^{-3} h^{-1}. In contrast, the control fermentation maintained under conventional aeration remained stable or increased slightly to 1·8 mmol dm^{-3} h^{-1} as cells entered the exponential growth phase. The dissolved oxygen was nearly depleted during the control fermentation, while in the presence of the microcapsules, oxygen levels stabilized at approximately 25–30% of saturation. In the absence of supplemental oxygen supply (k_La: 34 h^{-1}), the culture becomes oxygen limited as it enters the exponential phase, in which case oxygen demand exceeds

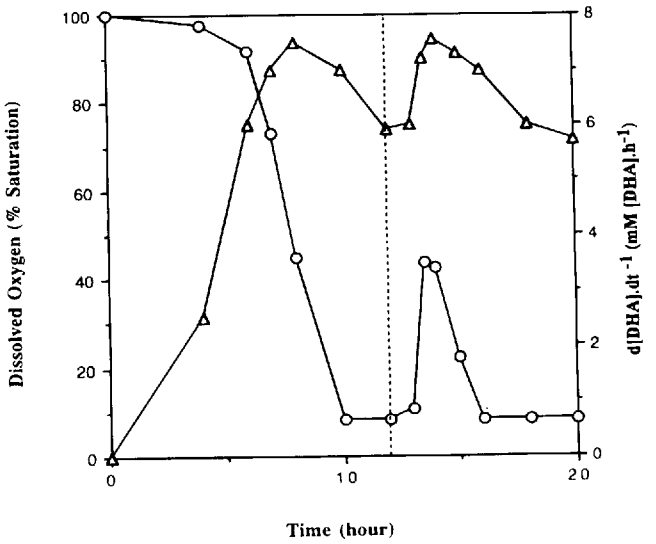

Fig. 9. Dissolved oxygen levels (○) and DHA production rates (△) with time in fluidized bed reactor operating with alginate-immobilized *Gluconobacter oxydans*. Microencapsulated silicone oils were added at 12 h.

oxygen supply. In the presence of the silicone microcapsules (k_La: 134 h^{-1}), oxygen limitation was avoided, resulting in a dramatic enhancement in productivity.

Microencapsulated oxygen carriers were also evaluated in a fluidized bed reactor, containing alginate-immobilized cells. The bioreactor was loaded with 22 g (wet weight) of alginate beads containing 0·8 g (dry weight) cells. Figure 9 illustrates the dissolved oxygen profile, measured in the reactor effluent stream. Oxygen levels decreased to below 10% of saturation, resulting in a decrease in the rate of DHA production. Dissolved oxygen levels measured at the inlet to the bioreactor consistently remained at full saturation.

At 12 h, 20% of microencapsulated silicone oils were introduced. Microcapsules were free to circulate through the reactor, and out through the reaeration loop due to the small diameter (50–200 μm), while the alginate-immobilized cells were retained within the bioreactor. Addition of the oxygen carrier resulted in an increase in dissolved oxygen to about 40%, corresponding to an enhancement in the rate of DHA production from 6 to 8 mmol dm^{-3} DHA h^{-1}.

4 DISCUSSION

4.1 Selection of an oxygen carrier

Desirable characteristics for oxygen carriers to be used in bioreactors include a high capacity for oxygen (and potentially carbon dioxide), biocompatibility, stability for extended periods and low volatility. The carrier must be water insoluble, or be of sufficiently high molecular weight to be contained within an encapsulating membrane to facilitate recovery and recycle. Silicone

and perfluorocarbon oils appear to be the most suitable, and in the present study silicone oil was selected due to its lower cost and higher oxygen capacity.[15] The buoyancy of the oils also facilitated recovery and recycle.

The low reactivity of silicone oils enables the use of interfacial polymerization with diamine and dichloride to form polyamide (nylon) membranes. The addition of 2% (v/v) polyethylenimine to the diamine solution yielded membranes resistant to autoclaving temperatures and pressures.

4.2 Enhancement of k_La with oxygen carriers

The oxygen transfer coefficient, k_La, for microcapsulated silicone oil was 187 h^{-1} using 30% microcapsules in the non-bubbling system. This was equivalent to a k_La value obtained using a 0·5 vol vol^{-1} min^{-1} air flow rate for conventional air bubbling at the same rate of agitation. The oxygen carriers not only increase the oxygen reservoir but also the transfer rates in non-bubbling systems. Furthermore, the k_La values increased linearly from 125 to 187 h^{-1} when the microcapsule volume fraction in the bioreactor was increased from 10 to 30%. Goma *et al.*[19] demonstrated a similar relationship between the volume fraction of oxygen carrier and the k_La.

Microcapsules in combination with conventional bubble aeration enhanced the k_La from 1·5 to 5, depending on the conditions. Similar enhancements were observed in the presence of cells. The k_La obtained using 20% (v/v) microcapsules ranged from 120 to 258 h^{-1}, which are higher than values observed in conventional bubbling systems. A value less than 120 h^{-1} was reported with intense mixing, and less than 4 h^{-1} under poor mixing conditions.[20]

The high oxygen transfer coefficient may be attributed to the small diameter (50–150 μm) compared with that of air bubbles (few millimetres in large scale reactors[20] or 1·5 mm in perfluorocarbon chemical dispersions[21]). Furthermore, Wang and McMillan[21] and Goma *et al.*[19] showed that exchange through the gas–liquid interface is slower than through liquid–liquid interfaces. This implies that the resistance to oxygen transfer of the oxygen carrier/water system is located in the water boundary layer. Taking into account both the increase of specific surface area (inversely proportional to particle diameter) and the increase of the transfer coefficient (k_L)[22] oxygen transfer rates from microcapsules would be faster than efficient bubbling systems under high mixing.

Previous studies on oxygen carriers in bioreactor aeration focused on perfluorocarbons[7,13,21,23] (e.g. FC-43 and FC-72) and hydrocarbons[19,24] (e.g. *n*-dodecane). Silicone oils have received limited attention.[10,11] However, the solubility of oxygen in silicone oils is higher than many other oxygen carriers.[15] Even though the enhancements of oxygen transfer capacity were 3·5-fold using 10–30% *n*-dodecane,[24] and four-fold using 40% perfluorocarbon,[25] the emulsions are stabilized with surfactants such as Pluronic F-68,[7] by mechanical agitation or ultrasonic treatment. The surfactant was found to be toxic to certain types of microorganisms[13] and cells.[12] Also, ultrasonic treatment and intense mixing may cause shear damage to the cells.

Microencapsulated oils do not require the addition of surfactant or other energy-intensive modes of dispersion. In addition, the k_La was observed to be higher than those reported with other oxygen carrier dispersions. The presence of an encapsulating membrane will also protect the cells from the toxic effects of the oxygen carrier itself.

4.3 DHA production with oxygen carriers

Gluconobacter oxydans was chosen for use in the present study due to its high oxygen demand.[26,27] In shake flask culture, available oxygen was assumed to be limiting since the only source was surface aeration.

The addition of 20% silicone oil, whether encapsulated or not will increase the oxygen availability by a factor of 6·5, in comparison to the reference culture (without silicone oils—see Fig. 10 in Ref. 15). Furthermore, the oxygen transfer capacities increased dramatically when cultivation was carried out in microcapsule-in-water dispersions. Therefore, the productivity was expected to be improved. As shown in Fig. 7, both encapsulated and non-encapsulated silicone oils enhanced the production rate of DHA by 33%. This also indicates that the encapsulating membrane is not a barrier to oxygen transfer.

In the 1·5 dm^3 batch fermentation, the productivity enhancements were more noticeable. The measured k_La during the exponential phase was 34 h^{-1}. Therefore, growth would be limited by the low oxygen transfer even though under agitation and aeration. As shown in Fig. 8, the dissolved oxygen levels remained low during the exponential growth phase. However, after the microencapsulated silicone oils were introduced in the medium, the dissolved oxygen level increased and remained stable at 30% saturation compared with a state of oxygen depletion in the reference culture. The oxygen-limited state was avoided by the addition of microcapsules, suggesting that the carriers act as an oxygen reservoir.

The k_La was measured to be about four to five times higher after the addition of microcapsules with or without the presence of cells (Figs 4 and 6). In batch fermentation, improved oxygen transfer capacity and the removal of oxygen limitation translated into greater productivity. The production rate increased by a factor of five (Fig. 6). Toxic effects or inhibition were not observed in the course of fermentation. Therefore, the

microencapsulation technique avoids the toxic effects as well as maintaining the high oxygen transfer capacity. Overall, observed increases in productivity indicate that a small fraction of microencapsulated silicone oils suspended in fermentation broth may potentially increase revenues per unit reactor volume per unit time.

4.4 Fluidized bed reactor

In conventional fluidized bed fermentations, the principal problem is often maintaining sufficient oxygen supply. Sparging gas into a liquid fluidized bed reduces the effective liquid density and the fluidized particles settle more rapidly.[28] Pre-dissolved air in the liquid feed provides sufficient oxygen for only a short period of fermentation due to the low oxygen solubility in water.[28] In this study, there was no direct bubbling to the reactor, the oxygen availability was determined solely by the feed. As seen in Fig. 9, the oxygen-limited growth period began after 8 h, concomitant with a decrease in the production rate of DHA. The introduction of 20% (v/v) microcapsules at 12 h enhanced the oxygen saturation from 0 to 40%, due to the high oxygen solubility in the microcapsules (6 mmol dm^{-3}) compared with water (0·3 mmol dm^{-3}).

The specific oxygen uptake rate of the bacteria was 19·3 mmol dm^{-3} O_2 g^{-1} dry cell h^{-1} and thus the oxygen uptake rate during the exponential phase was at least 15·4 mmol dm^{-3} h^{-1}. Without microencapsulated silicone oils, the oxygen-saturated feed stream provided only 0·03 mmol dm^{-3} min^{-1} (1·8 mmol dm^{-3} h^{-1}) to the reactor at a liquid flow rate of 100 cm^3 min^{-1}. This was insufficient to satisfy the high oxygen demand from the cells and therefore the productivity decreased as oxygen-limited growth began. Using 20% microencapsulated silicone oils as influent yielded 1·5 mmol dm^{-3} of oxygen concentration which was five times higher than liquid medium alone. This increase in oxygen capacity enhanced fermentation productivity.

5 CONCLUSIONS

Silicone oils as oxygen carriers were successfully microencapsulated within nylon membranes cross-linked with polyethylenimine. The microencapsulated oxygen carriers (20% v/v) increase the oxygen reservoir by a factor of seven as well as the oxygen transfer rates by a factor of five in comparison to conventional bubble aeration. Enhancement in the oxygen supply to bioreactors may result in an improvement of bioreactor productivity in systems with shear-sensitive cell lines or with high oxygen demand. The oxygen-permeable polymeric membrane coating the oxygen carrier, and lack of a chemical dispersant should reduce toxic or inhibitory effects previously observed during use with live cells.

ACKNOWLEDGEMENTS

The authors acknowledge financial support from the Natural Sciences and Engineering Research Council of Canada and the Brace Research Institute of McGill University.

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