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Microencapsulation of Silicone Oils within Polyamide–Polyethylenimine Membranes as Oxygen Carriers for Bioreactor Oxygenation

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Abstract: Silicone oils were microencapsulated within polyamide (nylon) membranes cross-linked with polyethylenimine. Solubility of oxygen within the silicones, whether encapsulated or not, was approximately 6 mmol dm^{-3}, representing solubilities approximately 20-fold higher than that of oxygen in water. The response time of oxygen transfer from the microencapsulated oxygen carrier was less than 2·5 s; a more precise measurement was limited by the response time characteristics of the oxygen probe. Assuming that the bioreactor volume consists of 10–20% (v/v) microencapsulated silicone oil, this represents an enhancement of the oxygen reservoir by a factor ranging from 4 to 7 and improvement in oxygen transfer rates greater than 15-fold due to the greatly increased specific surface area in comparison to conventional bubble aeration. Circulation of oxygenated silicone oils encapsulated within inert polymeric membranes may provide an efficient alternative to bioreactor oxygenation with shear sensitive cell systems, or in fermentations with high oxygen requirements.

Key words: microencapsulation, oxygen carrier, aeration, silicone oils.

NOTATION

a	Microcapsule specific surface area (dm^{-1})
k_L	Oxygen transfer coefficient (dm s^{-1})
$k_L a$	Overall volumetric oxygen transfer coefficient (s^{-1})
K	Partition constant (dimensionless)
m	Slope
r_{O_2}	Oxygen mass flux (mmol dm^{-3} s^{-1})
S	Sulphite concentration (mmol dm^{-3})
V_c	Volume of oxygen carrier (dm^3)
V_s	Volume of sulphite solution (dm^3)
V_w	Volume of water (dm^3)
t	Time (s)
τ	Response time (s)
τ_p	Probe response time (s)

Subscripts

c	Carrier phase
f	Final state
i	Initial state
p	Probe
w	Aqueous phase

1 INTRODUCTION

The performance of industrial aerobic fermentations is often determined by the rate of oxygen supply. In conventional bubble-aerated bioreactors, low oxygen solubility combined with slow oxygen transfer rates can limit growth and productivity of the cells. Enhanced agitation and elevated pressure improve oxygen transport at increased cost. Also, high levels of mixing create shear stresses incompatible with the culture of fragile cells.

Many methods have been reported to improve oxygen supply to microbial cultures. Adlercreutz and

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Mattiasson[1] proposed replacing oxygen with another electron acceptor such as *p*-benzoquinone. The by-product formed, hydroquinone, may be re-oxidized to *p*-benzoquinone in a recirculation loop. Use of *p*-benzoquinone as an oxygen substitute resulted in a four-fold enhancement of the reaction rate. However, not all microorganisms are able to use *p*-benzoquinone and many will not tolerate the toxic effects over the long term. Adlercreutz and Mattiasson[1] observed a reduction of activity by a factor of 6, over a period of 8 days, when using *Gluconobacter oxydans* and *p*-benzoquinone for the oxidation of glycerol to dihydroxyacetone.

Another approach has been to generate oxygen *in situ* using either photosynthetic algae such as *Chlorella pyrenoidosa*[2,3] or by adding hydrogen peroxide and catalase for catalase negative microorganisms.[4,5] Algal growth requires light, limiting the scale of operation whilst hydrogen peroxide damages cells and retards growth.[6] In both cases, co-immobilization may cause inhibition or competition for the substrate.

Hemoglobin solutions[7] or emulsions of organic solvents such as perfluorocarbons,[7–10] might serve as oxygen carriers in bioreactors. Even though the oxygen content of the media was increased by a factor of 15,[7] hemoglobin was not stable and rapidly lost oxygen-binding capacity. Chandler *et al.*[11] showed that growth of *Escherichia coli* was inhibited both by the perfluorocarbon and the emulsifier used to aid dispersion. Electron microscopy showed vacuoles and other structural perturbations of the microorganisms after prolonged contact.

Oil-in-water dispersions have been used with a bubbling system.[12] By selecting a suitable oil (generally a perfluorocarbon), the oxygen transfer rate was enhanced by a factor of 3.[13] However, the presence of organic oils may also affect growth rate[14] and dispersion of the oxygen carrier may subject cells to conditions of high shear.

The methods described previously enhance reactor performance by a factor of approximately 4 over conventional bubble aeration. In most cases, instability over the long term was encountered either for the biocatalyst or the oxygen carrier. An alternative method might be to encapsulate the oxygen carrier within an ultra-thin, oxygen permeable membrane, providing an inert barrier between the carrier and the cells. Evaluation of oxygen carriers, microencapsulated within polyamide membranes (nylon) is described in this report.

2 MATERIALS AND METHODS

2.1 Microencapsulation of silicone oils

Silicone was microencapsulated within nylon membranes by dispersing a solution of 45 mmol dm^{-3} sebacoyl dichloride (Aldrich) in silicone oil (Dow Corning), into a 0·2 mol dm^{-3} sodium carbonate solution with the aid of an emulsifier (1 % v/v Dow Corning 190). After emulsification for 2 min, an interfacial polymerization reaction was initiated at the droplet interface by introducing an aqueous mixture of 1,6-hexanediamine (0·45 mol dm^{-3}) and polyethylenimine (10 g dm^{-3}). After 3 min of reaction, microcapsules were filtered and rinsed with distilled water.

Emulsification was carried out in a 150 cm^3 vessel (50 mm internal diameter) with a sheet or frame lattice mixer. The characteristics and dimensions of the reactor and impellers are described elsewhere.[15] The volume of organic, aqueous and amine phase were 10, 40 and 10 cm^3 respectively.

2.2 Microcapsule size distribution

Mean diameter and size distribution were determined with a Malvern sizer 2600Lc (Malvern Instrument). Data were fitted with a normal law and the geometric mean and standard deviation calculated based on the volume concentration.

2.3 Microcapsule physical properties

Microcapsules were evaluated microscopically to examine sphericity, fraction of broken microcapsules and membrane strength. Membrane strength was determined qualitatively by compression to rupture with micromanipulators. Microcapsules were classified as very good (+++), good (++), satisfactory (+) and unsatisfactory (−) based on the ease of rupture.

2.4 Oxygen solubility and partition

The reactor used for solubility and oxygen mass transfer measurements consisted of a closed cylindrical polycarbonate vessel (380 cm^3; Fig. 1). An oxygen probe (YSI model 5739) was fitted through a sealed port in the lid and reactants were injected through a second sealed port.

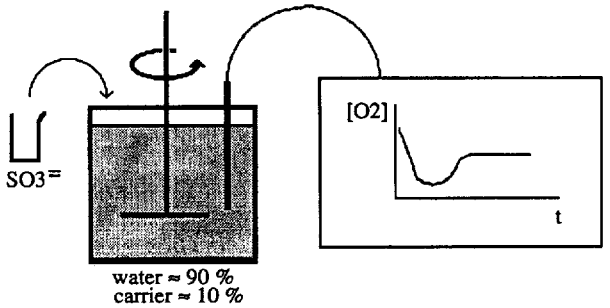

Fig. 1. Apparatus used for oxygen solubility and transfer measurement. Closed vessel was fitted with an oxygen probe and ports for the introduction of reagents.

The oxygen solubility of silicone oil was determined in a two-phase mixture of silicone/water (1/9 volume ratio). The probe was adjusted to measure oxygen tension in the aqueous phase with the two-phase mixture filling the entire volume of the reactor. After equilibration with air, sodium sulphite was added to the aqueous phase as an oxygen sink together with 0·1 mmol dm^{-3} cobalt sulphate catalyst. The concentration of oxygen in the water phase was recorded at equilibrium following incremental additions of sulphite. Solubility and partitioning of oxygen was computed as described below.

A concentrated sulphite solution was prepared just prior to use to minimize air oxidation. In all experiments, the volume of solution added was less than 0·1 % of the reactor volume and the effect on the suspension volume was negligible. The polymeric microcapsule membranes contain a number of amine groups which behave like a base. Before use, microcapsule suspensions were neutralized to ensure rapid oxidation of sulphite.[16]

A similar procedure was followed to determine the oxygen solubility of microencapsulated silicone within water suspension (1/9 volume ratio). The volume of microcapsules was determined from the mass assuming a similar density to silicone oil.

2.5 Oxygen mass transfer

Oxygen mass transfer rates were determined using the closed reactor described above and a 10% (v/v) suspension of microcapsules in distilled water (containing 0·1 mmol dm^{-3} cobalt sulphate). Sufficient sulphite ($\approx$ 0·12 mmol dm^{-3}) was added to consume half the total oxygen in the oxygen-saturated solution. Oxygen concentration in the aqueous phase was recorded with time to the new steady-state level.

3 MATHEMATICAL DESCRIPTION

3.1 Oxygen solubility and partition

Sulphite is oxidized by oxygen according to the reaction:

$$2Na_2SO_3 + O_2 \xrightarrow{Co^{2+}} 2Na_2SO_4 \qquad (1)$$

The sulphite oxidized, S, expressed in equivalent mol of oxygen reduced, is given by:

$$S = \tfrac{1}{2}[SO_3^{2-}]\,V_s \qquad (2)$$

where V_s is the volume of sulphite added. The mass balance of oxygen before and after addition of sulphite may be written as:

$$\{[O_2]_c\,V_c + [O_2]_w\,V_w\}_i = \{[O_2]_c\,V_c + [O_2]_w\,V_w\}_f + S \qquad (3)$$

Assuming a constant concentration ratio of oxygen between the water and organic phases, the partition constant, K, is defined by:

$$K = \left\{\frac{[O_2]_c}{[O_2]_w}\right\}_{\text{at equilibrium}} \qquad (4)$$

Combining eqns (3) and (4):

$$[O_2]_{wi}(KV_c + V_w) = [O_2]_{wf}(KV_c + V_w) + S \qquad (5)$$

and by grouping oxygen terms

$$(\Delta[O_2]_w = [O_2]_{wi} - [O_2]_{wf}) \qquad (6)$$

yields

$$\Delta[O_2]_w(KV_c + V_w) = S \qquad (7)$$

The oxygen partition constant may be computed using eqn (7), from the decrease in oxygen concentration, $\Delta[O_2]_w$ following the addition of sulphite, S. For greater accuracy, the oxygen concentration, $[O_2]_w$, was plotted as a function of S. The slope, m, is equal to $KV_c + V_w$ and the partition constant is calculated by:

$$K = \frac{m - V_w}{V_c} \qquad (8)$$

Expressing oxygen solubility as a partition constant is useful as this is insensitive to temperature and oxygen pressure. With knowledge of the oxygen solubility in water, the corresponding solubility in the oxygen carrier may be computed with eqn (4). Dividing the solubility by the oxygen pressure leads to Henry's constant, another method of defining oxygen solubility independent of the oxygen pressure.

3.2 Oxygen mass transfer measurements

The rates of oxygen transfer from microencapsulated oxygen carriers may be treated in an analogous manner to that of an air bubbling system. The mass transfer equation would then be:

$$r_{O_2} = k_L a(K^{-1}[O_2]_c - [O_2]_w) \qquad (9)$$

The overall volumetric oxygen transfer coefficient $k_L a$ may be replaced by the inverse of the response time, τ, resulting in:

$$r_{O_2} = \frac{(K^{-1}[O_2]_c - [O_2]_w)}{\tau} \qquad (10)$$

The time-course may be analyzed as illustrated in Fig. 2. Sulphite addition initiates a rapid consumption of oxygen in the aqueous phase, resulting in the depletion of the aqueous oxygen content.

$$\Delta[SO_3^{2-}]_w = 2\Delta[O_2]_w \text{ and } [O_2]_w \to 0 \qquad (11)$$

The oxygen is transferred from the encapsulated carrier fluid, but directly consumed in oxidizing residual

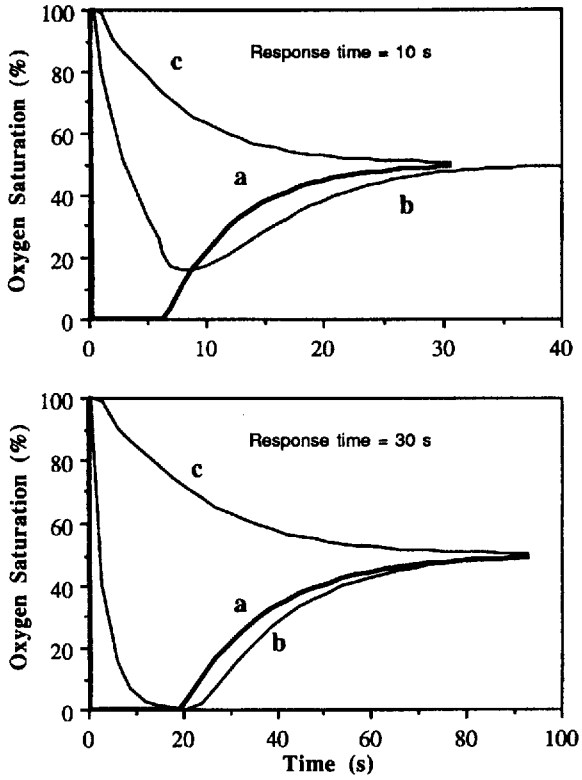

Fig. 2. Simulation of the real oxygen concentration (a) compared to the oxygen probe reading (b) for system response times of 10 s (upper) and 30 s (lower). τ_p for simulation is 5 s. The actual change in oxygen content within the microcapsules (c) is also plotted following the addition of sufficient sulphite to consume half the oxygen within the reactor.

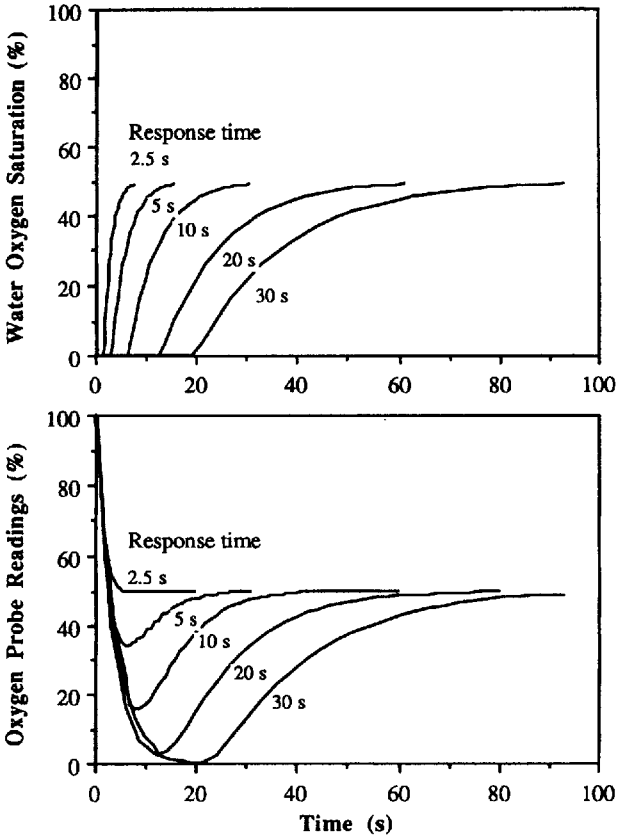

Fig. 3. Simulation of the actual oxygen concentration (upper) compared to the oxygen probe readings (lower) for system response times of 2·5 to 30 s, following the addition of sufficient sulphite to consume half the oxygen within the reactor. τ_p for simulation is 5 s.

sulphite, maintaining a low level of oxygen in the aqueous phase.

$$V_w \frac{d[SO_3^{2-}]_w}{dt} = V_c \frac{d[O_2]_c}{dt} = V_w r_{O_2} \text{ until } [SO_3^{2-}]_w = 0 \quad (12)$$

When the sulphite is totally consumed, continued oxygen transfer from the microcapsules replenishes oxygen in the aqueous phase until the new equilibrium is reached.

$$V_w \frac{d[O_2]_w}{dt} = V_c \frac{d[O_2]_c}{dt} = V_w r_{O_2} \text{ until } [O_2]_w = K^{-1}[O_2]_c \quad (13)$$

Figure 2 illustrates typical oxygen profiles in the aqueous phase as described. However, the oxygen concentration is monitored with an oxygen probe constrained by its own response time. The apparent oxygen concentration (probe reading, $[O_2]_p$) is then related to the real oxygen concentration ($[O_2]_w$) by:

$$\frac{d[O_2]_p}{dt} = \frac{([O_2]_w - [O_2]_p)}{\tau_p} \quad (14)$$

where τ_p is the probe response time. Curve b in Fig. 2 represents the probe reading corresponding to the real oxygen concentration (curve a). Figure 3 illustrates simulated results for increasing values of the oxygen transfer response time, τ, based on oxygen probe readings where τ_p is 5 s. Oxygen transfer response times τ of 10 and 30 s correspond to the illustrated examples in Fig. 2. With more rapid oxygen transfer response times, the deviation of the probe response from the system response becomes increasingly evident.

4 RESULTS

4.1 Preparation of microcapsules

Table 1 summarizes the formulation conditions tested for nylon membrane microencapsulation via interfacial polymerization. Microencapsulation of the silicone oils yielded spheres with smooth and transparent membranes (Fig. 4). The strength of the nylon membrane was enhanced by cross-linking with 2% (w/v) polyethylenimine added to the diamine aqueous solution. Of the four surfactants tested, only Dow 190 promoted good emulsification. The concentration of surfactant affected

TABLE 1
Conditions Tested in the Formation of Microcapsules Containing Silicone Oil[a]

Dichloride:	Terephthaloyl	0·04–0·1 mol dm^{-3}	–	No capsule
	Trimesoyl	0·04–0·1 mol dm^{-3}	–	No capsule
	Sebacoyl	< 0·05 mol dm^{-3}	+ + +	
		> 0·05 mol dm^{-3}	+ +	Water entrainment
Silicone viscosity:		0·01 $m^2 s^{-1}$	+ + +	Volatile, expensive
		0·05, 0·1 and 0·5 $m^2 s^{-1}$	+ +	
Hexanediamine:		0·1–0·2 mol dm^{-3}	+ +	
		0·4–0·6 mol dm^{-3}	+ +	Not very strong
+ Poly(ethylenimine)		2% w/v	+ + +	Strong
Aqueous solution pH:		8	+	
		9	+ +	
		10	+ + +	
		11	+ +	
Phase volume ratio:		1:4	+ + +	
		1:3	+ +	
		1:2·5	+ +	
Turbine:	Sheet		+ +	
	Frame		+ + +	
Turbine speed:		250 rev min^{-1}	+	Poor emulsion
		300–400 rev min^{-1}	+ +	
		450 rev min^{-1}	+ + +	
		> 450 rev min^{-1}	+ +	
Surfactant:	Tween 20	1 to 5% v/v	–	Poor emulsion
	Tween 80	1 to 5% v/v	–	Poor emulsion
	Pluronic	1 to 5% v/v	–	Poor emulsion
	Dow 190	1 to 8% v/v	+ + +	Good emulsion
Emulsion time:		1 min	+	
		1·5 min	+ +	
		2 min	+ + +	
		2·5 min	+ +	
Reaction time:		2 min	+	
		2·5 min	+ +	
		3 min	+ + +	
		> 3 min	+ +	

[a] Microcapsule classification: + + + very good, + + good, + satisfactory, – unsatisfactory.

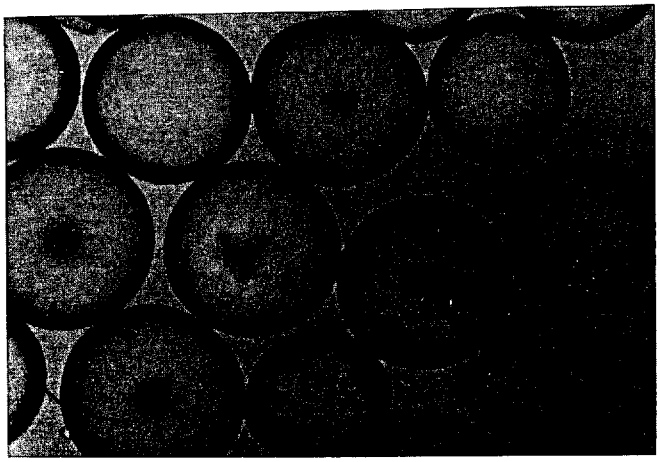

Fig. 4. Photomicrograph of silicone oils microencapsulated within nylon membranes cross-linked with polyethylenimine.

the mean size of the preparation (shown below) but did not affect the appearance. Intact microcapsules were obtained at all rotational speeds tested with a turbine impeller, but stronger microcapsules, with the lowest broken fraction, were obtained at 450 rev min^{-1} when using a frame lattice mixer.

Low viscosity (0·01 $m^2 s^{-1}$) silicone resulted in stronger microcapsules, and the acid dichloride was not completely soluble in high viscosity silicone (0·5 $m^2 s^{-1}$). Using 40–100 mmol dm^{-3} terephthaloyl or trimesoyl chloride as cross-linking agents resulted in reduced yields. Sebacoyl chloride promoted strong membranes at an optimum concentration of 45–50 mmol dm^{-3}. At higher concentrations, microcapsules were less stable or were irregularly shaped.

Different concentrations of hexanediamine, with or

without the addition of branched polyamine (polyethylenimine), were tested. Intact microcapsules with stronger membranes were obtained using 0·4 mmol dm^{-3} hexanediamine and 2% (v/v) polyethylenimine solution. The pH of the aqueous solution did not appear to strongly influence polymerization. However, the best results were obtained with pH 10.

Table 1 shows that emulsification and reaction time are optimum at 2 and 3 min respectively. The volume ratio of silicone was maintained less than 20% of the emulsion volume.

4.2 Microcapsule size distribution

Silicone microcapsule preparations resulted in broad size distributions (Fig. 5). The distribution curves were generally unimodal with a prominent peak in the 80 to 400 μm size range. The mean size varied between 100 and 300 μm. The standard deviations represented 35–47% of the mean diameter.

The mean diameter of the microcapsule preparation decreased from 160 to 110 μm as the surfactant concentration was increased from 1 to 8% (v/v) during emulsification (Fig. 6). Control of the mean diameter was also possible by varying the mixer rotational speed, as illustrated in Fig. 7. The mean diameter ranged from 130 to 330 μm when the speed was varied between 200 and 700 rev min^{-1}.

Other parameters tested (Table 1) had minimal impact on mean diameter. No real dependence was observed between the standard deviation and the operating conditions.

4.3 Oxygen solubility in silicone oils and other solvents

Oxygen solubility and partition coefficient were determined using sulphite as an oxygen sink. Table 2 presents data for silicones of varying viscosity, in comparison to that of microencapsulated 0·1 $m^2 s^{-1}$ silicone oil. The solubility of oxygen in silicone oil relative to that of water was enhanced by approximately 20-fold, including that of the microencapsulated form. Solubilities ranged from 5·3 to 6·0 mmol dm^{-3}, with no correlation evident to the silicone viscosity. As a comparison, reported values of oxygen solubility in a variety of other solvents are listed in Table 3. Partition

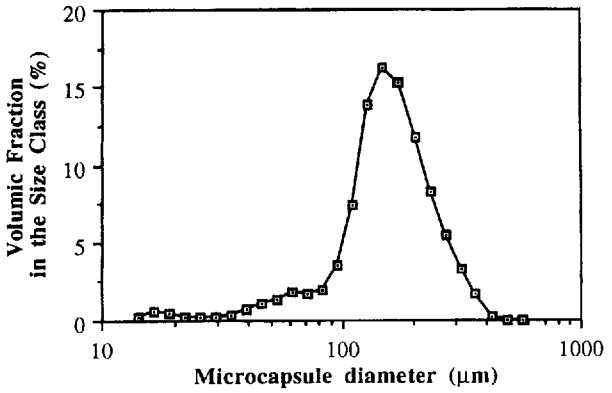

Fig. 5. Volumetric size distribution of polyamide microcapsules containing silicone oil.

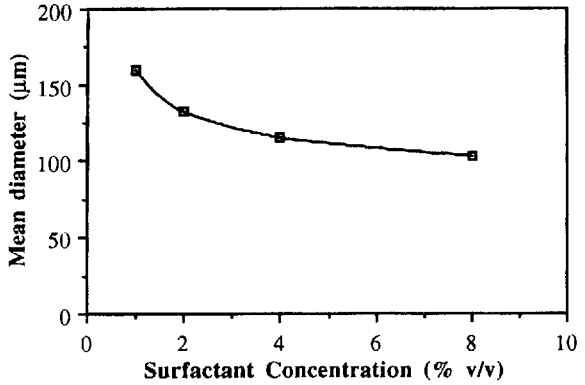

Fig. 6. Variation of microcapsule mean diameter with increasing concentration of surfactant.

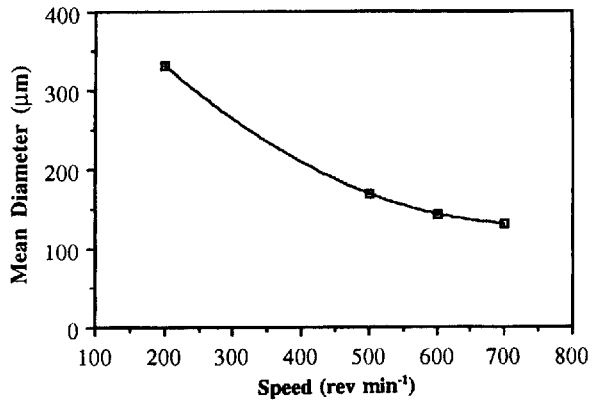

Fig. 7. Variation of microcapsule mean diameter with increasing rotational speed of the mixer.

TABLE 2
Oxygen Solubility and Partition Coefficient in Silicone Oils of Varying Viscosity and Within Microencapsulated Silicone Oil at 22°C

	Water	*Silicone oil viscosity* ($m^2 s^{-1}$)				*Microcapsules*
		0·01	*0·05*	*0·1*	*0·5*	
$[O_2]$ (mmol dm^{-3})	0·28	6·0	5·4	5·8	5·3	6·0
K	1	21	19	20	18	24

TABLE 3
Oxygen Partition Coefficient Relative to that of Water for Different Oxygen Carriers. [Accurate comparisons are unable to be made as measurements were taken at different temperatures]

Compound		*K*	*T* (°C)	*Reference*
Hexane		6·9	30	12
Hexadecane		4·0	18	10
Paraffin oil		3·9	30	17
		3·3	30	12
Perfluorodecalin		16·9	37	14
		9·2	37	18
		12·6	25	19
Fluorocarbon	FC-40	11·6	30	12
	FC-45	11·3	30	12
	FC-75	15·9	30	12
Polydimethylsiloxane		14·0	30	18
Hemoglobin (350 g dm^{-3})		66·4	20	20

constants ranged widely from 3·3 for paraffin oil to 16·9 for perfluorodecalin. Hemoglobin was estimated to bind oxygen to levels of 66·4 times that of oxygen solubility in water. Oxygen solubility in the silicone oils (Table 2) was almost double that of the fluorocarbons FC-40 and FC-45 and 1·3 times higher than that of FC-75 (Table 3).

Oxygen solubility is plotted as a function of pressure in Fig. 8 for several solvents including that of microencapsulated hemoglobin and red blood cells. A linear correlation was observed for silicone oil and fluorocarbon FX-80, while a sigmoidal-type relationship represented oxygen binding to red blood cells and microencapsulated hemoglobin.

4.4 Mass transfer of oxygen from microencapsulated oxygen carriers

Before determining a system response time, it is important to ensure that other processes do not interfere with the measurement. Oxidation of sulphite by oxygen in the presence of divalent cobalt is a rapid zero order reaction provided neutral pH is maintained. Sulphite mixing was evaluated by the addition of a similar volume of dye to the reactor under identical mixing conditions. Mixing time was evaluated as equal to or less than 2 s. The response time of the oxygen electrode was determined by addition of an excess of sulphite to an oxygen-saturated solution and recording the probe response to the step change in oxygen concentration as a function of time. Using the equation

$$\frac{\ln [O_2]_w}{[O_2]_{wo}} = -\frac{t}{\tau_p} \qquad (15)$$

to fit the data leads to the probe response time, τ_p, of 5 s. Oxygenation due to surface aeration was investigated by

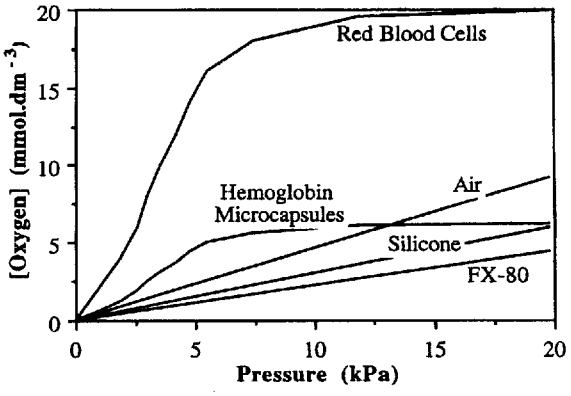

Fig. 8. Oxygen concentration or binding capacity as a function of pressure for silicone and perfluorocarbon oils, red blood cells and hemoglobin microcapsules.[20]

TABLE 4
Response Time Determined for Various Components of the Overall Oxygen Transfer Process

Process	*Response time*
Sulphite oxidation	Very fast[a]
Sulphite mixing	< 2 s
Probe response	5 s
Surface aeration	44 min
Oxygen transfer from microcapsules	< 2·5 s

[a] Zero order reaction.

continuing to follow the probe response after the sulphite was consumed and fitting oxygen concentration data during reoxygenation to an equation similar to that of eqn (15). The response time of oxygenation due to surface aeration was evaluated as 44 min, as summarized in Table 4.

Consumption of oxygen in the aqueous phase, due to oxidation of sulphite, would instantaneously reduce the oxygen concentration to zero, based on the amount of sulphite added. Reaeration from the microcapsules would be evident once the sulphite had been consumed, reaching the final equilibrium level (expected at 50% of saturation). From the experimentally-measured probe response in Fig. 9, the oxygen transfer process is evidently proceeding at rates much higher than that of the probe response. Comparing the experimental curve (Fig. 9) with the simulation curves (Fig. 3) indicates that the response time representing oxygen transfer from the microcapsules is equal to or less than 2·5 s.

5 DISCUSSION

5.1 Selection of an oxygen carrier

Oxygen carriers may be divided into two classes as a

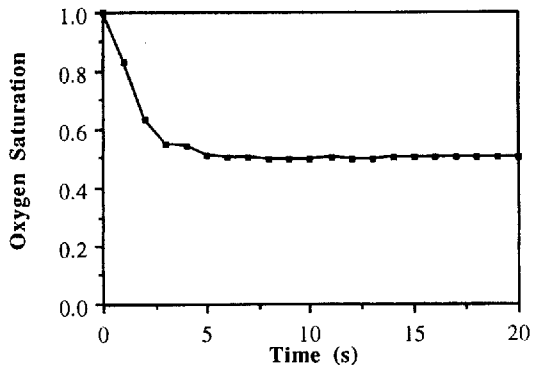

Fig. 9. Experimentally-measured probe response to a sulphite-induced step change in oxygen concentration, in the presence of microencapsulated silicone oil.

function of the mechanism of oxygen fixation (Fig. 8). Oxygen concentration is directly proportional to the oxygen pressure for solvents which absorb oxygen. In contrast, chelation of oxygen by hemoglobin leads to a more complex relationship between oxygen concentration and pressure. The binding or dissociation of four oxygen atoms on hemoglobin results in a strongly positive cooperative behavior leading to a sigmoidal oxygen saturation curve.

The concentration of oxygen in oxygenated red blood cells is approximately double that of air and triple that of silicone at atmospheric pressure (Fig. 8). Moreover, oxygen release from hemoglobin solution is accomplished within a small range of oxygen pressure. This oxygen binding/release behavior of hemoglobin may be of interest in the oxygenation of bioreactors due to its high oxygen capacity, and ability to maintain a constant oxygen pressure. However hemoglobin is an unstable molecule, susceptible to oxidation *in vitro*. Oxidized hemoglobin (met) is unable to function as the oxygen carrier. The hemoglobin concentration is very high in red blood cells (around 300 g dm^{-3}). Encapsulation of hemoglobin has been successful only at lower concentrations (less than 100 g dm^{-3}).[21] Moreover, the nylon encapsulating membranes are permeable to hemoglobin (unpublished data). Encapsulation itself denatures hemoglobin mainly by cross-linking the hemoglobin to the membrane during formation. Finally, the pressure of oxygen during release is less than 4 kPa,[22] too low for practical applications.

Air may be considered as a good oxygen carrier (9·2 mmol dm^{-3} at one atmosphere or 20 kPa of oxygen, Fig. 8) but due to the low oxygen transfer capabilities, only a small part of this oxygen is really available to the cells in a typical bioreactor. Typical response times for conventional bubble aeration range from 30 s to 15 min.[19,23]

Oxygen is soluble at high levels (4–6 mmol dm^{-3}) in many organic solvents and solvent–water oxygen exchange has proven to be faster than in air–water systems.[13] Silicones and perfluorocarbons represent the highest affinity solvents for oxygen, yet only one third that of red blood cells in contact with air. However, when the oxygen pressure exceeds 15 kPa, the oxygen concentration in red blood cells and in hemoglobin microcapsules remains constant. In contrast, silicone and other oxygen absorbents (Fig. 8) show proportional increases. If economically feasible, use of pure oxygen would lead to a concentration of 30 mmol dm^{-3} in silicone, higher than might be expected from concentrated hemoglobin solutions.

Besides the high capacity for oxygen, desirable characteristics that were sought when selecting suitable oxygen carriers included a low toxicity to biological cells, stability for extended periods and low volatility. The carrier must either be water insoluble or have a high molecular weight, to maintain the compound within semipermeable encapsulating membranes, facilitating recovery and recycle. For many engineering applications, such as oxygenation of potable water or wastewaters in purification operations, low cost of the material is a priority.

Based on these criteria, silicone 0·1 $m^2 s^{-1}$, was selected in the present study.

5.2 Microencapsulation of oxygen carriers

The low reactivity of silicones enables the use of interfacial polymerization with diamine and dichloride to form polyamide (nylon) membranes. Nylon membranes are thin (200 nm) in comparison to other types of membranes such as collodion (3 μm), enhancing oxygen transfer. The addition of a preformed branched polymer may result in stronger membranes.[24] In the present study, addition of 2% (w/v) polyethylenimine to the diamine solution yielded stronger membranes.

The use of 0·01 $m^2 s^{-1}$ silicone resulted in stronger microcapsules but, due to volatility, slight water solubility and cost (ten times the cost of 0·1 $m^2 s^{-1}$), 0·1 $m^2 s^{-1}$ silicone was preferred. To achieve a narrow size distribution, the discontinuous and continuous phases during emulsification should have similar viscosities and densities. The different characteristics of microcapsules with silicones of increasing viscosity may be related to decreasing solubility of the acid dichloride.

The microcapsules containing silicones resulted in a large dispersion of size. Control of the oxygen transfer, mechanical resistance[25] and hydrodynamic properties[26] of microcapsules may be difficult under such conditions.

5.3 Oxygen transfer from microcapsules

A comparison of Figs 3 and 9 suggests that the response time of oxygen release from microcapsules is less than 2·5 s. The method used in the present study limits more precise determination of the oxygen release response time. Methods similar to the determination of relaxation

time may solve this problem. However, even if faster measurement were possible, the oxygen release response time would be similar to that of mixing time and probably reaction time.

Response times less than 2·5 s are short when compared to that observed in conventional bubbling systems. A value greater than 30 s was reported for a system with intense mixing and greater than 15 min reported under poor mixing conditions.[27] The rapid response time for the release of oxygen from microcapsules may be attributed to the small diameter (250 μm) compared to that of bubbles (up to several millimeters in a large-scale reactor). Also, McMillan and Wang showed that exchange through the gas–liquid interface was slower than through liquid–liquid interfaces.[13] Taking into account both the increase of specific surface area (inversely proportional to bubble diameter) and the increase of the transfer coefficient (k_L),[13] expected oxygen transfer rates from microcapsules were 15 times faster than efficient bubbling systems, with a response time of approximately 2 s anticipated.

The nylon membrane did not appear to reduce the diffusion rate of oxygen from the microcapsules. Nylon membranes contain up to 85% water and pore sizes are large, permitting diffusion of molecules as large as hemoglobin. Concerning molecular oxygen, the nylon membrane appears more like a grid with large openings than a real barrier to mass transfer.

5.4 Oxygenation capacity by microencapsulated silicone compared with non-bubble systems

Oxygen solubility in silicone microcapsules is approximately 6 mmol dm^{-3} compared to 0·3 mmol dm^{-3} for water. Assuming that the microcapsules represent 10–20% of the reactor volume, the oxygen reservoir is multiplied by a factor of 4–7 (Fig. 10). If the microcapsules were re-generated with pure oxygen, the oxygen reservoir will increase by a factor of up to 30 (Fig. 10).

Response time corresponds to the time at 63% of the overall response. Thus oxygen concentration within the microcapsules will drop to 37% of the initial concentration after each 2 s assuming that the cells rapidly consume oxygen, maintaining the concentration in the aqueous phase near zero. Since this is generally not the case, the release rate of oxygen from microcapsules will be reduced. Clearly, the microencapsulated oxygen carrier must be recirculated and regenerated to provide sufficient oxygen to the bioreactor.

A bioreactor for shear sensitive cell culture (mammalian and plant cells) may supply oxygen in the influent or the recirculation loop. The maximum oxygen concentration in the fluid is then limited to 0·3 mmol dm^{-3} (oxygen water saturation). Using a 10% (or 20% v/v) microcapsule suspension as influent yields 0·9 mmol dm^{-3} or 1·5 mmol dm^{-3} in oxygen, respectively. Moreover, the flow of liquid and microcapsules may be independent. By adjusting the density of the capsules, their residence time in the reactor may be shorter than the residence time of the liquid. The microcapsules may then be recirculated at higher concentration (up to 50%) while maintaining their concentration at a low level in the reactor (around 20%). The oxygen concentration in the highly loaded influent may then reach 3·3 mmol dm^{-3} to be compared with 0·3 mmol dm^{-3} for an oxygen-saturated water recirculation. If pure oxygen is used for the microcapsule reaeration, the concentration of oxygen may be increased by a factor approaching 50. Taking this into account the recirculation flow may be reduced while increasing the oxygen supply to the reactor, improving the efficiency of reaeration. The use of pure oxygen may then become economically and technically feasible.

In this specific case, the performance reached with encapsulated oxygen carriers may be compared with that obtained with non-encapsulated oxygen carriers.[28] Using air for reaeration, bioreactor performance was increased by a factor of 4–5.[18] A similar performance may be expected when using microcapsules and the encapsulation of the oxygen carrier facilitates the control of the permanent dispersion without the need for a surfactant.[15] The membrane may also reduce potentially toxic effects observed with perfluorocarbons on cells.[13]

5.5 Oxygenation capacity by microencapsulated silicone compared to bubble systems

The residence time of a bubble in a bioreactor is short and difficult to control, often insufficient to release all the oxygen. Increasing gas flow through a reactor generally results in an increase of the bubble diameter, decrease in the bubble surface area per volume and in the residence time. Oxygen transfer capabilities are then not directly related to the gas flow rate. On the other hand, the microcapsule residence time may be optimized to increase oxygen transfer efficiency maintaining a high concentration of oxygen.

5.6 Oxygenation capacity by microencapsulated silicone in viscous media

When the culture media is viscous or at elevated cell concentrations, bubbles or microcapsules pass with difficulty through the reactor. Oxygen may be supplied by diffusion though a membrane or a grid. The residence time of the bubble in a column is short (4 s m^{-1} height).[29] Comparing this to the response time of oxygen release from bubbles, only a fraction of the oxygen is transferred to the medium (less than 10% m^{-1} height). Use of microcapsules allows optimization of the residence time of the oxygen carrier in the column and will then allow maximization of the oxygen concentration in the column whilst reducing the cost.

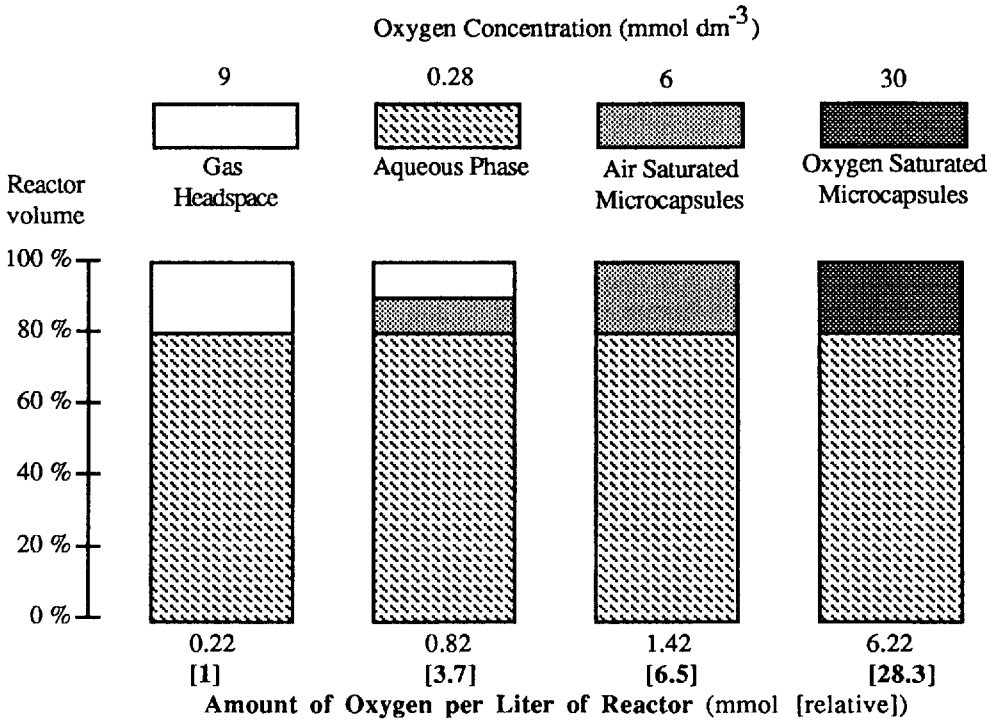

Fig. 10. Relative amounts of oxygen available to organisms in a bioreactor containing various proportions of oxygen carrier relative to the reactor liquid volume. The oxygen in the headspace was assumed to be unavailable to the cells.

6 CONCLUSIONS

Silicone oils as oxygen carriers were successfully microencapsulated within nylon membranes cross-linked with polyethylenimine. The microencapsulated oxygen carriers greatly increase the oxygen reservoir and transfer rates in comparison to conventional bubble aeration. Important enhancements in the oxygen supply to bioreactors, resulting in improvements in bioreactor performance, may be expected in systems with shear sensitive cell lines or in fermentations with high oxygen requirements. The oxygen permeable polymeric membrane coating the oxygen carrier should reduce toxic or inhibitory effects previously observed during use with live organisms.

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