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Increased Oxygen Transfer in a Yeast Fermentation Using a Microbubble Dispersion

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ABSTRACT

A microbubble dispersion (MBD) was used to supply oxygen for aerobic fermentations in a standard 2 L stirred tank fermenter. The microbubble dispersion was formed using only surfactants produced naturally. Growth rates of *Saccharomyces cerevisiae* cultures were found to be equal or greater with MBD sparging than with gas sparging. The oxygen transfer coefficent with MBD sparging was found to be 190/h and independent of impeller speed from 100–580 rpm. The oxygen transfer coefficient with air sparging rose from 55 to 132/h over the same range of impeller speeds. Power requirements for the fermenter systems were estimated.

Index Entries: Microbubble dispersion; oxygen transfer; *Saccharomyces cerevisiae.*

INTRODUCTION

Mass transfer processes have a major impact on the growth of microorganisms in industrial fermentations. Nutrients must be continuously replenished in the liquid layers closest to the microorganisms since the microorganisms are constantly consuming them. Nutrients that dissolve easily in water, such as glucose or ammonia, present little problem since they can be present in the fermentation media in concentrations on the order of 1 mol/L. For organisms requiring oxygen, however mass transfer can play a major limiting role. Under normal fermentation conditions,

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oxygen is only sparingly soluble. At 35°C, the solubility of oxygen in pure water is 0.217 mmol/L, and the presence of salts and other nutrients required for the growth of any organism decreases this value. Oxygen is normally supplied to the fermentation in the form of air, which must be filtered and compressed in order to pump it into the fermenter. Air processing is a major cost factor in the operation of the fermenter.

The efficiency of oxygen transport in fermenters is roughly proportional to the ratio of the bubble surface area to the bubble volume. Therefore, oxygen transport is roughly proportional to the inverse of the radius of the gas bubbles, and in general, the smaller the bubbles, the greater the oxygen transfer rate in the fermenter. Normally, however, small air bubbles coalesce quickly, and the energy spent to decrease their size is wasted. In industrial fermenters, contactors and stirrers cause a decrease in bubble size in their immediate vicinity. However, bubbles in the rest of the fermenter are approximately 3–5 mm in diameter, and they aren't affected by the amount of stirring in the fermenter (*1*). If gas bubbles are stabilized with a surfactant film, they will tend to maintain their small size with or without stirring. The use of surfactant-stabilized gas bubbles may present a method of taking advantage of the mass transport effects of small bubbles.

A microbubble dispersion (MBD) consists of very small surfactant-stabilized bubbles, typically formed as a 50–65% dispersion of a gas in liquid. The microbubbles have diameters of 20–1000 μm, compared to diameters of 3–5 mm for normal bubbles in a fermenter. Because of their small size, these microbubbles rise slowly in normal fluids. Such microbubbles are distinctive in that they are very small and sturdy, and can be pumped, already formed, into a fermenter. Surfactant-stabilized microbubbles tend to resist coalescence because the surfactant tends to orient at the air–liquid interface, forming a charged bubble surface that repels other bubbles. Since growing microorganisms produce large quantities of surfactant, and surfactant-stabilized microbubbles have characteristics that enhance oxygen transfer, surfactant-stabilized microbubbles could be an efficient means of transferring oxygen to fermentation systems.

The fermentation industry primarily uses stirred tank fermenters. Since laboratory scale stirred tank reactors are generally overstirred, a stirred tank fermenter will probably demonstrate the least amount of improvement when a surfactant-stabilized microbubble dispersion is used. The power input per volume of broth in a laboratory fermenter can be as much as 1000 times greater than the power input per volume of broth in an industrial fermenter. Any improvements seen in a laboratory scale fermenter will be significantly greater in an industrial fermenter.

Oxygen transport is very important in aerobic fermentation systems because the solubility of oxygen in liquid media is rather low, less than 10 mg/L. Thus, the oxygen in the liquid is rapidly depleted by growing organisms and must be replenished constantly. In fermenters, the transport of oxygen from a gas to a liquid is controlled by the liquid side mass transfer coefficient, k_L, which is usually reported in the form $k_L \times a$. Wallis et al. (*2*)

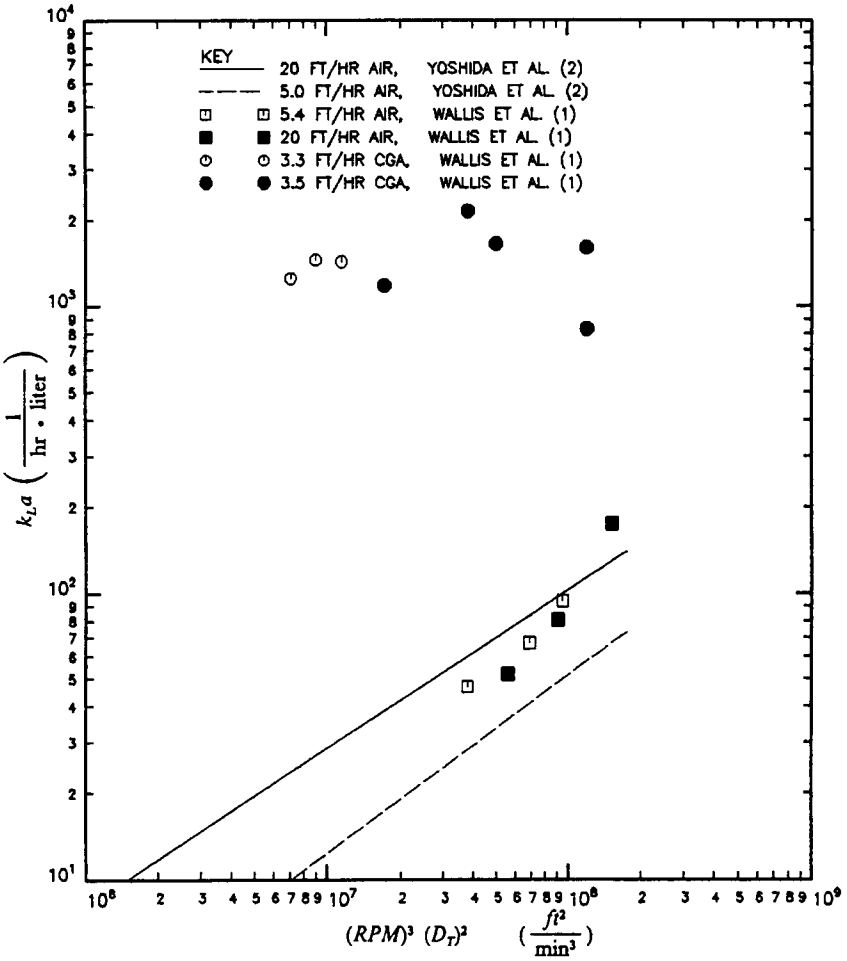

Fig. 1. Oxygen transfer coefficient in sparged air and sparged MBD systems (*1,2*).

duplicated Yoshida et al.'s oxygen transport experiments (*1*) using a MBD to supply oxygen for the chemical oxidation of sulfite to sulfate in the presence of a copper catalyst. The k_La's obtained with MBD sparging were an order of magnitude higher than for pure air sparging (Fig. 1).

The use of surfactant-stabilized microbubble dispersion to supply oxygen to yeast cultures is an offshoot of early microbubble research by Sebba (*3*). These microbubbles were referred to as colloidal gas aphrons (CGA) to underscore the colloidal properties of these very small bubbles. A CGA consists of an inner pocket of air surrounded by an aqueous double layer film that is surrounded by the continuous phase (Fig. 2). Both the gas interface and the film/continuous phase interface of the film have higher surfactant concentrations than the center of the film. This double layer phenomenon stabilizes the CGAs by preventing them from coalescing. First, an electric potential gradient is set up by the orientation of the sur-

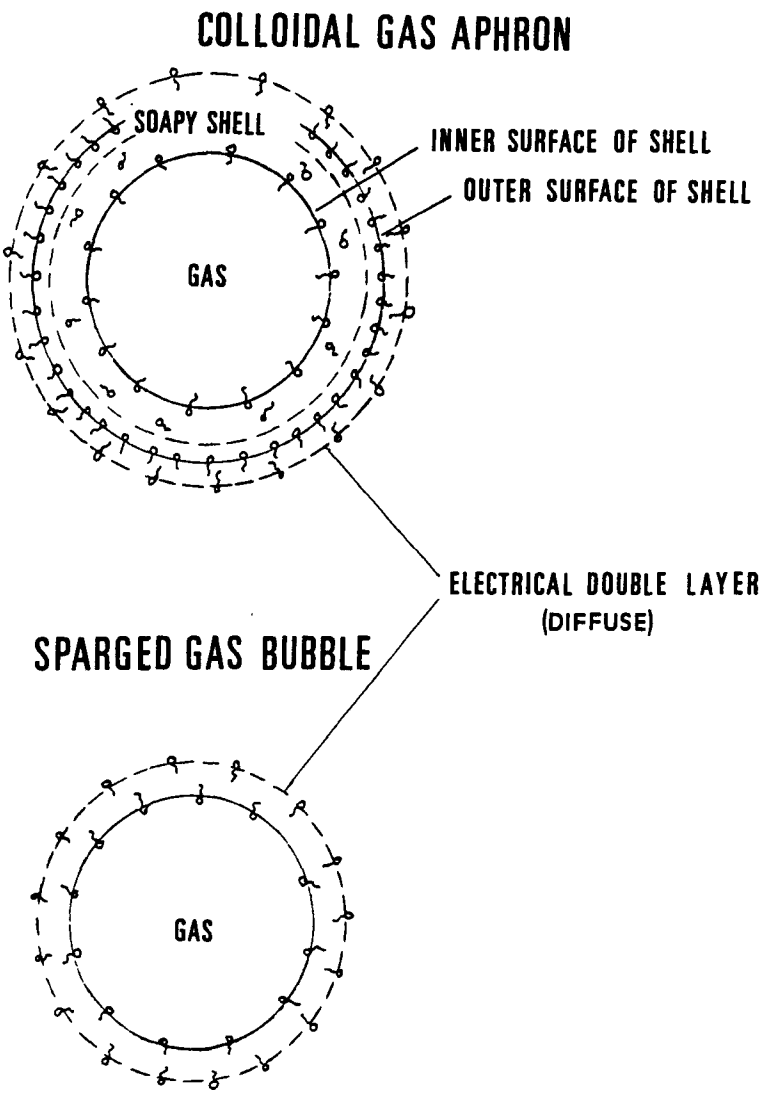

Fig. 2. Colloidal gas aphron and sparged gas bubble structures.

factant molecules at the interfaces. CGAs created with the same surfactant will have similar surface charges and will repel each other, preventing contact. Also, the film acts as a slightly springy wall when CGAs come close to each other. The combination of these two effects results in a foam that is stable enough to be pumped, has a very large surface to volume ratio, and a slow rise velocity.

In initial studies, CGAs were produced by a venturi device that required large recirculation velocities in order to form a uniform bubble size distribution. An improved CGA generator using a spinning disk and capable of producing 1-2 L CGAs/min was introduced by Sebba in 1985 (4). This device consists of a disk approximately 5 cm in diameter bracketed by

a pair of baffles with no more than 3 cm clearance. The disk needs to rotate at approximately 4000 rpm to achieve an average bubble size of 20–100 μm. Since then, a continuous spinning device has been standardized to produce 20–30 L/min of microbubbles, and a 100 L/min unit is in the early stages of testing (*5,6*). The possible applications of these microbubbles to biological systems were recognized by Barnett (*7,8*) and Wallis (*1*) as a means of transporting oxygen. The microbubble dispersion used in Wallis's and the present studies consisted of a mixture of CGA-sized bubbles (20–100 μm) and bubbles as large as 3–5 mm. The MBD generator used in this study is based on the CGA generator designed by Sebba (*4*).

The growth rate, oxygen uptake rate, and the overall oxygen transfer coefficient of a culture grown in a stirred tank fermenter were measured using either sparged air or surfactant-stabilized microbubbles as the oxygen source. A culture of *Saccharomyces cerevisiae* (baker's yeast) was used in these experiments since this organism has been widely used in similar studies, is easy to grow, and is resistant to contamination. In this study, k_La values were determined by the direct, dynamic, and yield coefficient methods. The results from these three methods were compared to determine the best value for the oxygen transfer coefficient in the fermenter. This project was designed to prove that microbubbles are more efficient oxygen transport vehicles than ordinary sparged air bubbles.

METHODS

All fermentations were run in a 2 L Multigen bench top fermenter, New Brunswick Scientific (NBS, Edison, NJ). The vessel was temperature and pH controlled. The temperature was maintained at 35°C. The pH was measured and recorded by a NBS pH controller and an Ingold pH electrode. The pH was kept at 5.0 or above by the automatic addition of 1.0*N* ammonium hydroxide. Dissolved oxygen levels in the tank were measured and recorded by a galvanic probe and a NBS DO controller. The oxygen concentration in the off gas was measured by a paramagnetic oxygen analyzer (Taylor Servomex, Sussex, England), and recorded by a Fisher Recordall 5000 chart recorder. Air and MBD flow rates were measured using the calibrated scale on a Masterflex pump. The glucose feed was metered through a Varioplex II pump (Pharmacia, LBK Biotechnology, Piscataway, NJ).

For the MBD runs, the same fermentation vessel was used with the addition of a MBD generator on a recycle loop (Fig. 3). In this case, samples were taken off the MBD delivery line from a tube that was clamped at all other times. The recycle pump was set at approximately 130 mL/min, and the level of liquid in the MBD generator was found to be self controlling. The MBD or air was delivered through the hollow impeller shaft and exited through holes at the bottom of the shaft. A filtered vent was provided to to prevent pressure buildups in the MBD generator.

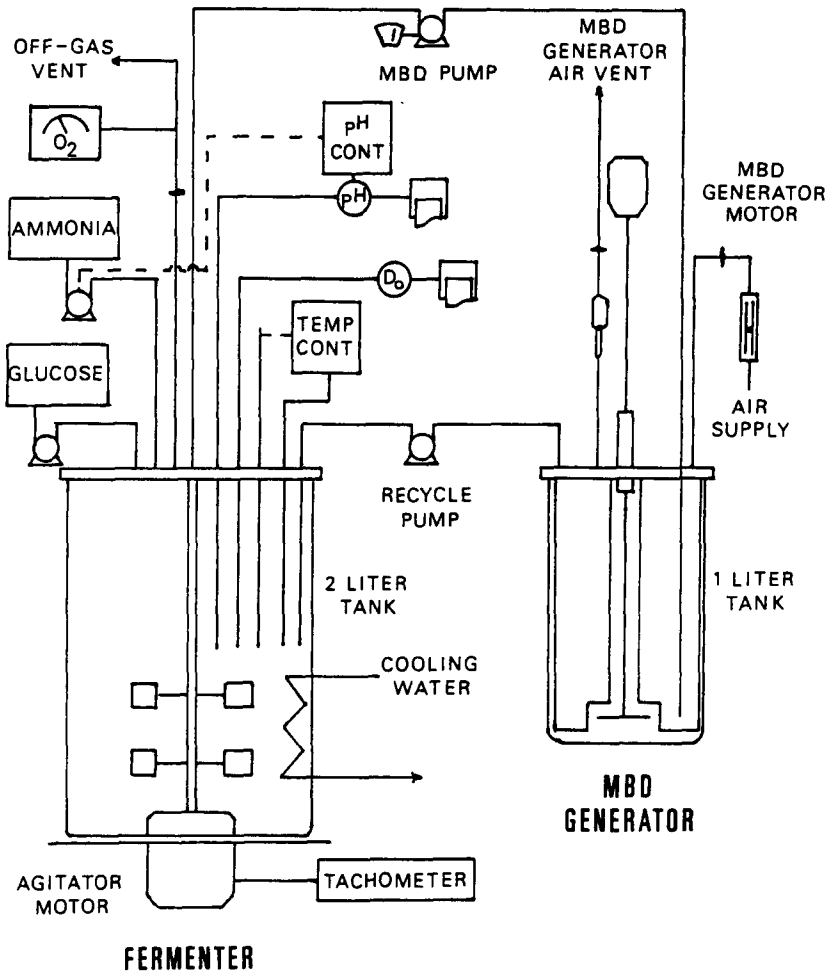

Fig. 3. The fermenter and MBD generator.

Difco YM broth was used exclusively throughout these experiments (21 g/L). One liter of YM broth was used to begin the fermentation, and the glucose feed consisted of 200.0 g glucose and 28.0 g YM broth in 300.0 mL distilled water. The glucose feed was supplied to the fermenter at a rate of 1 mL/h from hours 20–30, 3 mL/h from hours 30–50, and 4.5 mL/h up to the end of the run. *Saccharomyces cerevisiae* ATCC 4111 was the organism used in this study. Starter cultures were kept in 250 mL shake flasks (160 mL liquid volume) at 25–30°C. All fermentations were inoculated with 100 mL of starter culture for an initial yeast concentration of 0.1 g/L. Glucose concentrations were determined by the method of Park and Johnson (*9*). Cell mass concentrations in the fermenter were determined by the method of Hug and Fiechter (*10*). The dissolved oxygen probe was calibrated at the beginning of each run. The probe was zeroed by sparging purified nitrogen through the broth while it was gently stirred for 30 min.

The full range of the probe (broth oxygen concentration in equilibrium with a 21% oxygen atmosphere) was set by sparging air through the broth stirred at 580 rpm for 30 min. Impeller speeds in the fermenter were controlled by an inline variac calibrated with a phototachometer. Air or MBD was sparged into the fermenter at 400 mL/min and the agitator speed was set to either 100 or 580 rpm. Samples (10 mL) were taken approximately every 4 h for cell mass determinations.

RESULTS AND DISCUSSION

The object of this research was to determine the usefulness of a microbubble dispersion (MBD) as a means of supplying oxygen to systems containing growing organisms. To determine the effects of a MBD on growth and oxygen transfer, the growth patterns of *S. cerevisiae* were found for fed-batch fermentations with air sparging and with MBD sparging. These experiments were run using two agitation rates in the fermenter: a normal agitation rate for a laboratory fermenter (580 rpm) and a minimal agitation rate (100 rpm).

An initial set of batch and fed-batch runs served to debug the fermenter system and provide a baseline for the later experimental runs (Fig. 4). A best possible growth curve was obtained from a compilation of the points from these fermentations that showed the most growth. These points were least squares fitted by the equation

$$X = 0.1e^{(0.2642t)} / (1.0 - 0.01153(1.0 - e^{(0.2642t)})) \quad (1)$$

where: X = cell mass concentration (g/L) and t = time from the start of the fermentation (h). The air flow rates for these runs were approximately 1.35 vol air/volume liquid/min (VVM), compared to 0.4 VVM for the experimental control runs and 0.26 VVM for the MBD runs. The air flow rates for the experimental runs was reduced to create oxygen limiting conditions. Figures 5–8 show the dissolved oxygen and cell mass profiles for the air and MBD sparged fermentations. The "hypothetical best possible growth curve," included in each of these figures, was derived from Eq. (1).

The cultures in the MBD sparged and the well-stirred air sparged fermentations looked essentially identical. All three appeared to be homogeneous mixtures of cells with small- to medium-sized bubbles rolling through the fluid. The lightly-stirred air sparged fermentation was different, in that the culture was much thinner, but still homogenous. The large bubbles (2 mm and larger) did not roll with the fluid, but rather rose immediately to the surface. In all the runs, the yeast was a whitish tan in appearance. Some of this color could be owing to pigments from the YM broth, which is straw colored. Also, the cultures tended to build up a head of foam toward the end of the ethanol oxidation phase. The yeast that was

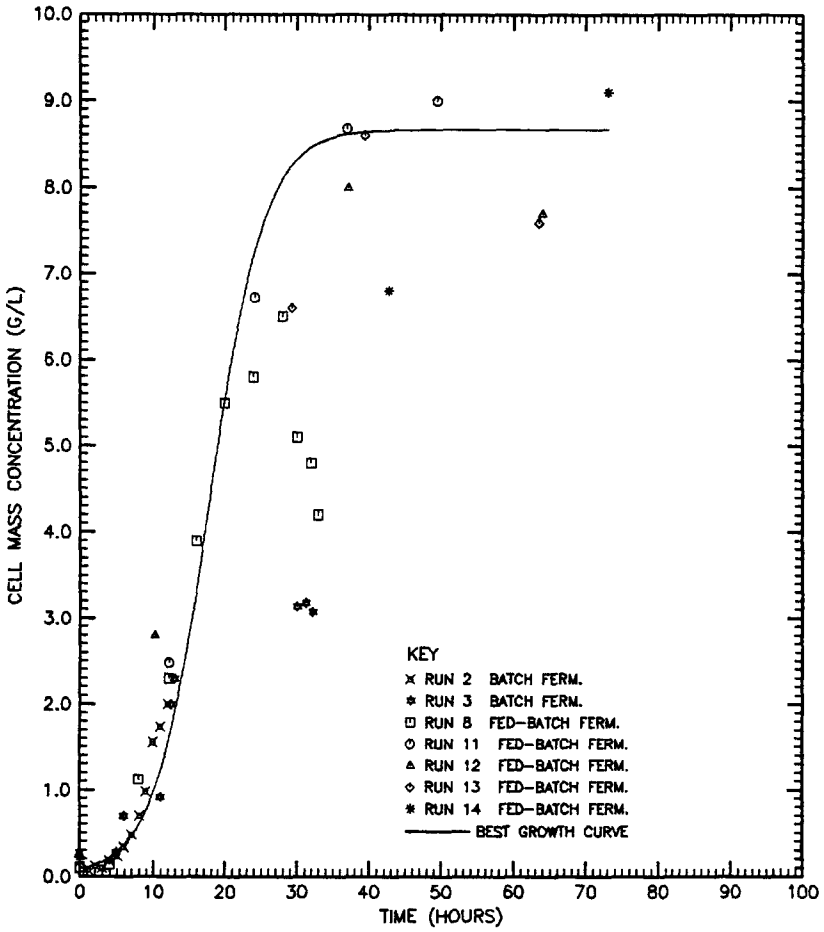

Fig. 4. Yeast growth and dissolved oxgyen concentration: batch and fed-batch runs, air sparged.

carried up the walls of the fermenter with this foam appeared to thrive, although it was a much darker tan in color. The maximum cell mass concentration in this series of runs was 9.7 g/L, slightly more than the maximum in the initial fed-batch runs.

All the fermentations were started out in batch mode and should have nearly identical growth profiles through the first 10–12 h. During this time, most of the initial glucose in the media was metabolized to ethanol. From h 10–20, the yeast was oxidatively metabolizing the ethanol to carbon dioxide and water. After 20 h, the glucose feed was started. Then, the yeast should have metabolized glucose directly to carbon dioxide and water. If the yeast growth was oxygen limited, a divergence from the normal growth pattern should be seen after 15–20 h of growth.

At 580 rpm, there is little, if any, difference between air and MBD sparging. The MBD run may have a slightly faster growth rate, but more fermentations would be needed to prove whether there is a significant difference.

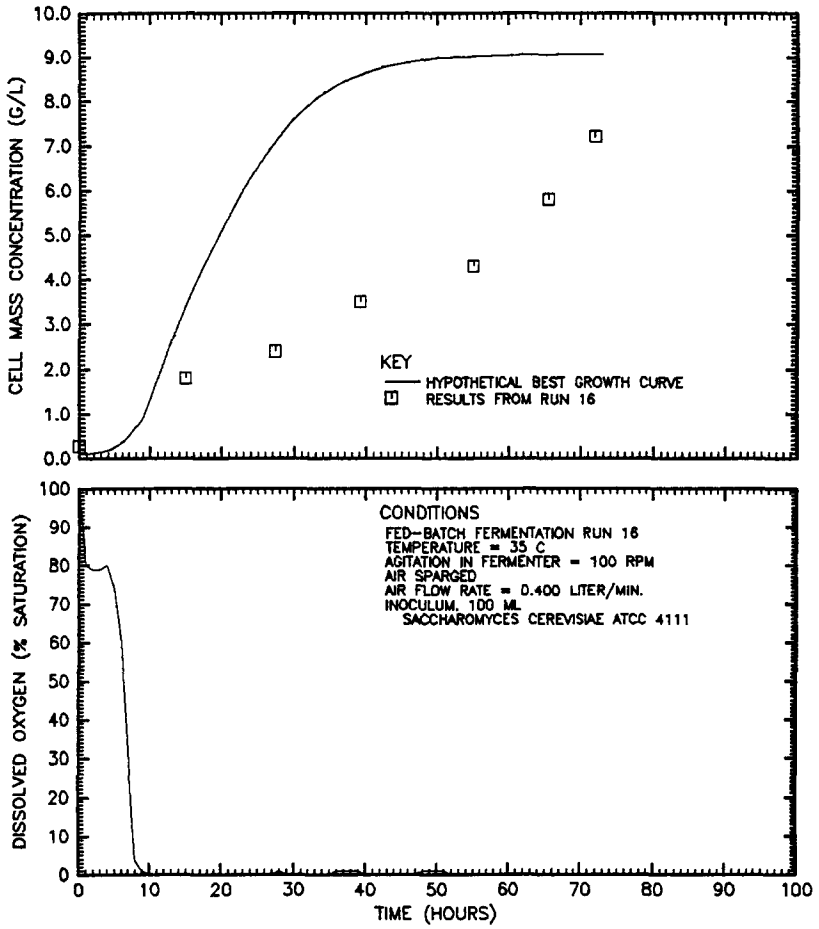

Fig. 5. Yeast growth and dissolved oxygen concentration: reduced agitation (100 rpm) and air sparging, run 16.

With minimal stirring (100 rpm), the results were significantly different. The MBD sparged fermentation (run 17) had a growth curve nearly identical to the well-stirred case. The oxygen transfer characteristics of MBD were independent of the fermenter agitator speed over the range of speeds studied. The air sparged fermentation (run 16) showed a much slower growth rate. This slower growth rate is most probably owing to oxygen limitation since the concentration of oxygen in the fermenter was nearly zero throughout the run. The oxygen limitation in run 16 is interesting because the conditions in this run come closest to approximating the conditions in an industrial fermenter. Runs 17 and 19, the MBD sparged runs, had essentially constant dissolved oxygen profiles. The lightly agitated fermentation (run 17, Fig. 6) showed a higher average dissolved oxygen concentration than the normally agitated fermentation (run 19, Fig. 8). The fact that the MBD fermenter showed no change in growth characteristics between the high and low agitation runs could point to a possible use of MBDs as a viable oxygen source for traditional tank fermenters.

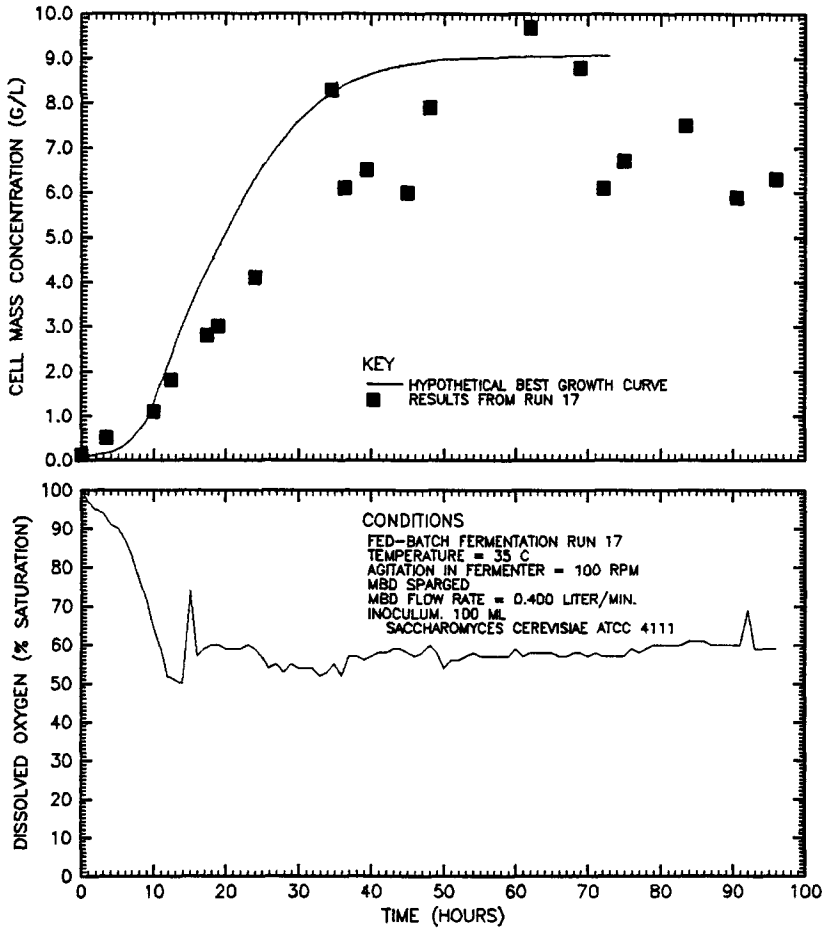

Fig. 6. Yeast growth and dissolved oxygen concentration: reduced agitation (100 rpm) and MBD sparging, run 17.

Values for the oxygen transfer rate, k_La, in runs 16–19 (Table 1) were calculated by the yield coefficient method, using the amount of yeast produced per gram of glucose consumed to calculate the amount of oxygen consumed. These k_La values fall in the range of the k_La values calculated in earlier runs by the dynamic and direct methods. Figure 9 shows the k_La values for fermentations 16–19 compared to Cooper's correlation (*11*). The k_La values for the 580 rpm agitation runs follow the trend of Cooper's data. For the reduced agitation runs (100 rpm), the k_La's fall well above Cooper's correlation. This could be a result of the small size of the fermenter. It is significant, however, that the oxygen transfer rates in the MBD sparged runs were independent of the power input in the fermenter.

The k_La values obtained from these experiments were approximately one-fifth of the k_La values that Wallis et al. (*1*) found for CGAs with the sulfite oxidation method. This was not unexpected. Approximately 300 ppm surfactant was added to the liquid used for microbubble generation

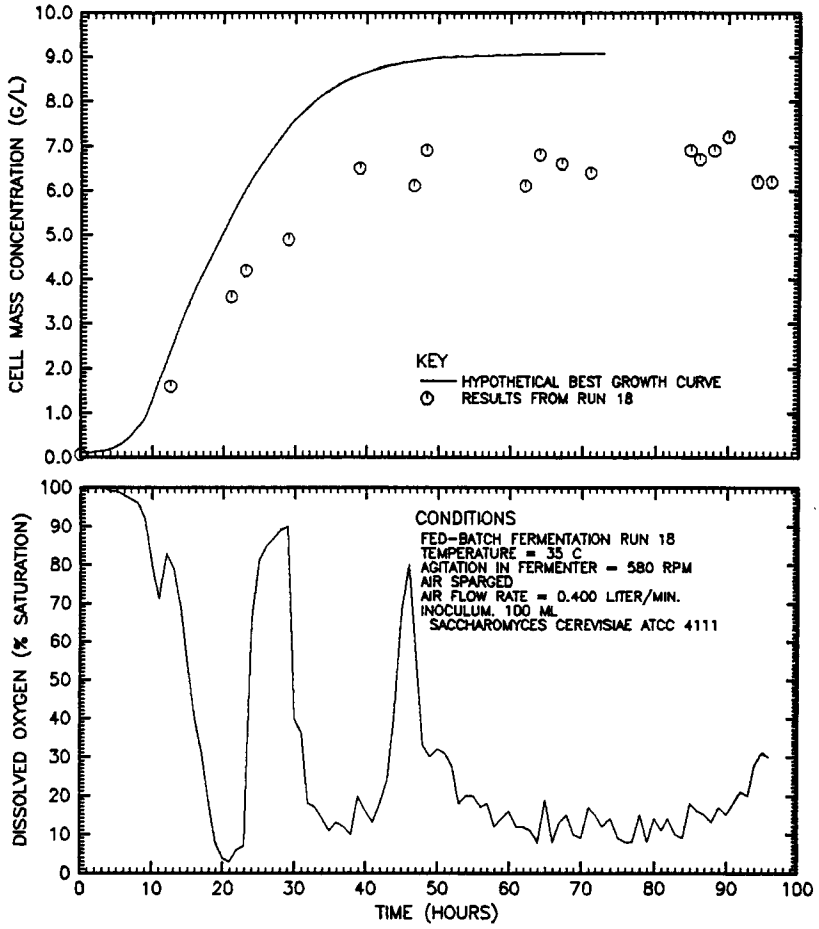

Fig. 7. Yeast growth and dissolved oxygen concentration: high agitation (580 rpm) and air sparging, run 18.

during sulfite oxidation. This addition assured smaller and more stable microbubbles when injected into water containing sodium sulfite. Also, k_La's measured using the sulfite oxidation method are normally higher than the k_La's in actual fermentations. Fermentation broths contain many surface active agents that can have a detrimental effect on oxygen transfer. The microbubble dispersion used in our experiments tended to degrade somewhat before entering the fermenter, reducing the k_La further. Although the k_La values in runs 17 and 19 were lower than the k_La values obtained by chemical oxidation, they show that MBD has excellent mass transfer characteristics for biological systems.

The apparent increase in k_La in the MBD fed runs (17 and 19) was owing to oxygen transport enhancement by the microbubble mixture containing 65% air. An oxygen mass balance around the fermenter reveals that only about 10% of the oxygen required for yeast growth could have been supplied by the liquid. For example, in run 17 at 20 h from off gas

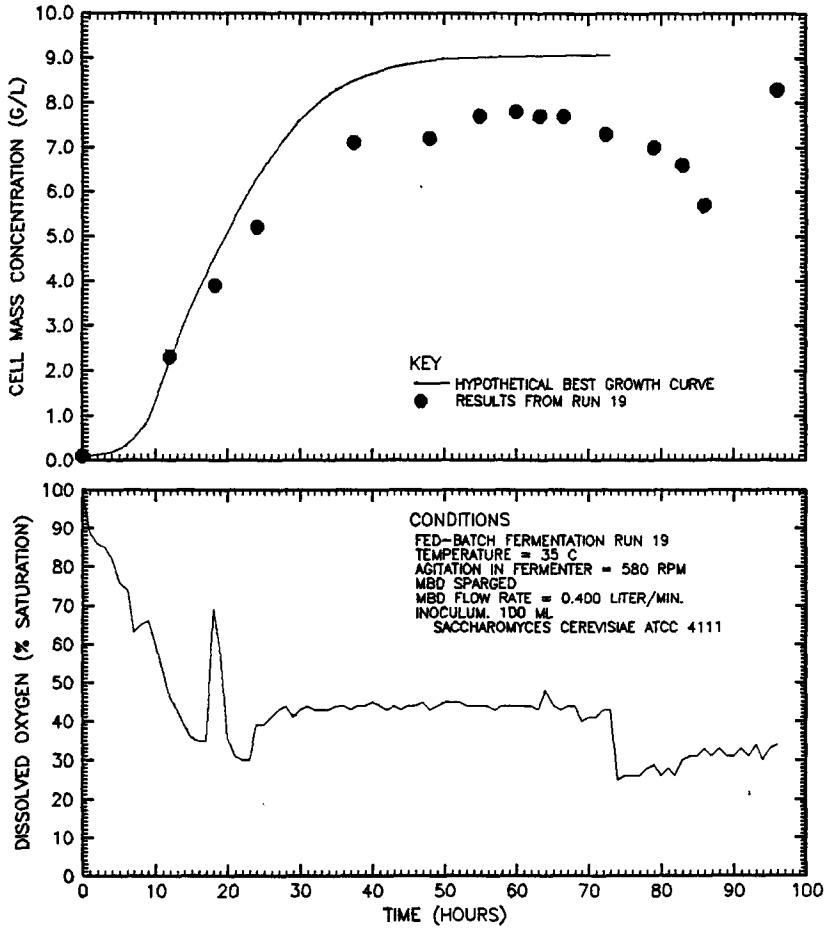

Fig. 8. Yeast growth and dissolved oxygen concentration: high agitation (580 rpm) and MBD sparging, run 19.

Table 1
Maximum Growth Rates and Oxygen Transfer Coefficients

Run	Oxygen supply	μ_{40}	$k_La_{40,3}$	k_La_{max} (manual fit)	k_La Calculation
19	MBD	0.119	180	129	Yield coefficient (*12*)
18	Air	0.124	132	90	Yield coefficient
17	MBD	0.108	202	228	Yield coefficient
16	Air	0.068	55	60	Yield coefficient
14	MBD	-	195	-	Dynamic
13	Air	-	206	-	Dynamic
12	Air	-	126	-	Direct

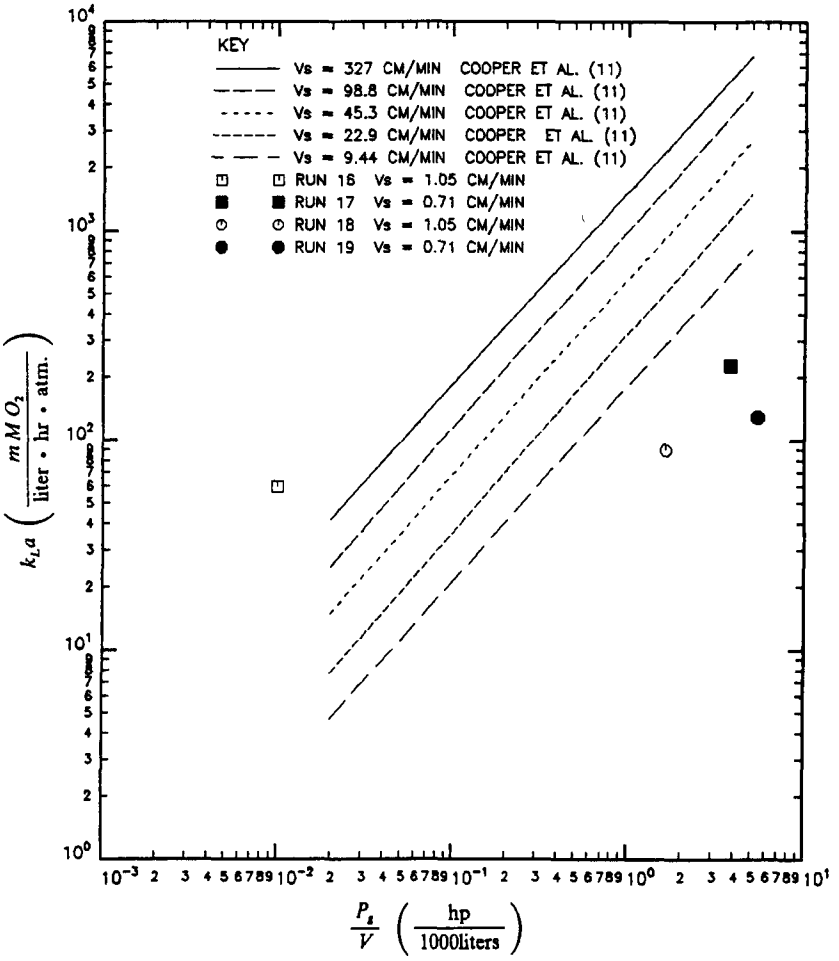

Fig. 9. Experimental oxygen transfer rates.

analysis, 15 mmol/h of oxygen was being consumed. In that same time, 8.4 L of liquid, that could hold a total of 1.7 mmol of oxygen, was pumped from the AC MBD generator Thus, the increased oxygen transfer was owing to the microbubbles.

From these calculations, it can also be seen that only 15 mmol oxygen/h were being consumed. In this same amount of time, 133 mmol of oxygen were supplied to the fermenter. This means that a maximum of 15% of the supplied oxygen was being used. Since the oxygen usage was low and the dissolved oxygen concentration was relatively high in the fermenter in runs 17 and 19, the MBD generator was oversized in relation to the 1 L fermenter. A 5 L fermentation would require about the same recycle flow rate as used in these experiments. The use of MBD as a means of oxygen transport shows a great deal of potential in this application.

Table 2
Estimated Power Requirements

Run	16	17	18	19
Oxygen supply	Air	MBD	Air	MBD
Agitation	100 RPM	100 RPM	580 RPM	580 RPM
Power requirement	(hp / L × 10^6) (*13*)			
Fermenter agitator	6.94	6.94	1640	1640
Compression	13.76	9.18	14	9
MBD generation	-	6800	-	6800
Total	20.7	6816	1654	8449

The MBD generator to fermenter volume ratio was about 1:10 for these experiments, and the residence time of the broth in the fermenter was about 7.5 min. The pumping rate this residence time entails is acceptable for a 1 L fermenter, but in scaling this to industrial size, the pumping rate would probably become excessive. As can be seen in the oxygen balances around the fermenter, less than 15% of the oxygen fed into the system was actually used. Also, the oxygen concentration in the broth was 50% of saturation or more in the lightly agitated MBD fermentation (run 17). Therefore, it should be possible to use a larger percentage of the oxygen by increasing the fermenter to generator volume ratio to about 50:1. This would increase the residence time of the broth in the fermenter to about 38 min and reduce the recirculation rate to about 3% of the reactor volume per minute, which could lead to more manageable pumping requirements.

The estimated power requirements for the four experimental fermentations in Table 2 point out one part of the original design that requires improvement. The 400 mL/min of microbubble dispersion fed to the 1 L working volume fermenter led to an estimated power consumption of 6800×10^{-6}hp/L (5.0 W/L) (*6*). Since the dissolved oxygen concentration remained above 50% at all times during run 17, a MBD feed of 75 mL/min to this fermenter might be sufficient, requiring only 1280×10^{-6}hp/L (1.0 W/L) for a reduced agitation, MBD sparged fermentation. A low volume MBD generator within the fermenter would probably produce a smaller, more stable MBD, leading to higher mass transfer rates and even lower power requirements.

The impellers in the fermenter were set to provide turbulence throughout the fermenter while spinning above about 200 rpm. For the MBD runs, especially at lower rpms, they were of little use. The MBD provides a good deal of turbulence by itself, but only after it has dispersed. The most useful location for impellers in a MBD aerated fermenter would be at the bottom of the tank. Stirring is needed at the bottom to keep the yeast from settling. The bottom is also where the MBD should be dispersed to cover the entire cross-section of the tank.

Initially, it was thought that there might be difficulties in controlling the level of liquid in the MBD generator and in the fermenter. The liquid levels were found to be self controlling. With a set volumetric flow rate to the fermenter, the percentage of the MBD that was air and the volume of liquid in the generator were controlled by the flow rate of liquid from the fermenter. With increased liquid flow rate, the MBD quality declined, allowing more liquid to be pumped back to the fermenter. Thus, the system was self controlling and self-damping.

In conclusion, it appears that a MBD does provide enhanced oxygen transfer, especially under conditions of reduced fermenter agitation. This enhancement is significant in that the agitation in most industrial fermenters corresponds to the agitation found in the reduced agitation runs. The use of a MBD as an oxygen transport media shows even more promise than would at first be assumed from these results. The system used was expected to show little difference. Experiments with a more concentrated yeast culture (up to 100 g/L) and a better fermenter to MBD generator volume ratio (about 10:1, rather than 2:1) should show even greater improvements in oxygen transfer with a MBD over air sparging.

The use of surfactant-stabilized microbubbles as a method for oxygen transport to microorganisms can be advantageous, especially in cases where microorganism sensitivity and power costs preclude the use of intensive agitation in the fermenter. MBDs have the same oxygen transport characteristics as intensely agitated (580 rpm) sparged air. The oxygen transport characteristics of MBDs are not affected by the amount of agitation in the vessel over the range of agitation studied (580 to 100 rpm). Over the same range, the k_La for ordinary sparged air dropped from 132/h at 580 rpm to 55/h at 100 rpm. The difference in oxygen transport seen between the MBD and sparged air runs at 100 rpm were not owing to oxygen transported in the liquid. Liquid oxygen transport can only account for a maximum of 15% of the oxygen transported. The MBD generator used in these experiments could easily supply a 5 L working volume fermenter. This work has shown that a MBD can increase oxygen transport to systems with minimal agitation, but it did not prove the economic feasibility of this process. A larger vessel would allow the quantification of the economics of the process.

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bubbles in biological applications before his untimely death in February 1986 at age 32.

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