SCALE-UP THE USE OF A MICROBUBBLE DISPERSION TO INCREASE OXYGEN TRANSFER IN AEROBIC FERMENTATION OF BAKER'S YEAST

Patcharee Hensirisak

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APPROVED:
John S. Cundiff, Co-Chairman
William H. Velander, Co-Chairman
Kenneth C. Diehl

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by

Patcharee Hensirisak

John S. Cundiff, Co-Chairman

Biological Systems Engineering

William H. Velander, Co-Chairman

Chemical Engineering

(ABSTRACT)

A microbubble dispersion (MBD) was used to supply oxygen for an aerobic fermentation of Baker's yeast. The 1-liter microbubble dispersion generator supplied bubbles for 20-liter and 50-liter working volume fermentations in a 72-liter pilot scale fermenter. The microbubbles were stabilized by the surfactants naturally present in the culturing broth medium. The growth patterns of yeast *Saccharomyces cerevisiae*, cultured at agitation speeds of 150 rpm and 500 rpm, were compared for oxygen supplied by ordinary air sparging and by MBD sparging. Both air sparged and MBD systems were supplied air at equivalent volumetric flow rates.

The volumetric oxygen transfer coefficients (K_La) were estimated by the yield coefficient method. The K_La values increased from 142.5 to 458.3 h^{-1} and from 136.1 to 473.3 h^{-1} for 20- and 50- liter runs, respectively, as the agitation speed was increased from 150 to 500 rpm in the ordinary air sparged fermentations. The oxygen transfer coefficients in the MBD sparged fermentations were found to be independent of the fermenter agitation speed at approximately 480 h^{-1} for 20-liter runs and 340 h^{-1} for 50-liter runs. The growth rates for MBD at 150 rpm were essentially equivalent with air sparged fermentations at 500 rpm. The total power consumption per unit volume of broth for the 150 rpm, MBD fermentation was 68% lower than the 500 rpm, air sparged run for the 20-liter fermentations and was 55% lower for the 50-liter fermentations.

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CHAPTER 1

INTRODUCTION

Typical microbial culture growth patterns show a slow initial phase (lag phase) followed by a fast exponential phase (log phase), and then a stationary phase where no growth occurs due to nutrient limitations (Figure 1.1). A shortened lag and log phase is desirable for more economical large-scale cultures. Typical aerobic large-scale cultures occurring at 10,000 liter or greater are oxygen mass transfer limited. Gas-liquid interfacial surface area and bubble residence time phenomena are the limiting steps of oxygen delivery to the microorganisms.

Gas-liquid surface area can be manipulated by changing interfacial tension, but this factor is difficult to adjust in many fermentations. Thus, an increase in interfacial surface may be better achieved by increasing mechanical agitation. It is noted that an increase in interfacial area gained by a decrease in bubble size also results in a concomitant increase in bubble residence time. According to the Stokes equation, the bubble rise velocity, v, is given by

$$v = \left(\frac{g\rho}{18\mu} \right) d^2 \tag{1-1}$$

where g = acceleration due to gravity, (cm sec^{-2}),

d = sphere bubble diameter (cm),

ρ = broth density (gm cm^{-3}), and

μ = broth viscosity (gm cm^{-1} sec^{-1}).

Reducing d greatly reduces v.

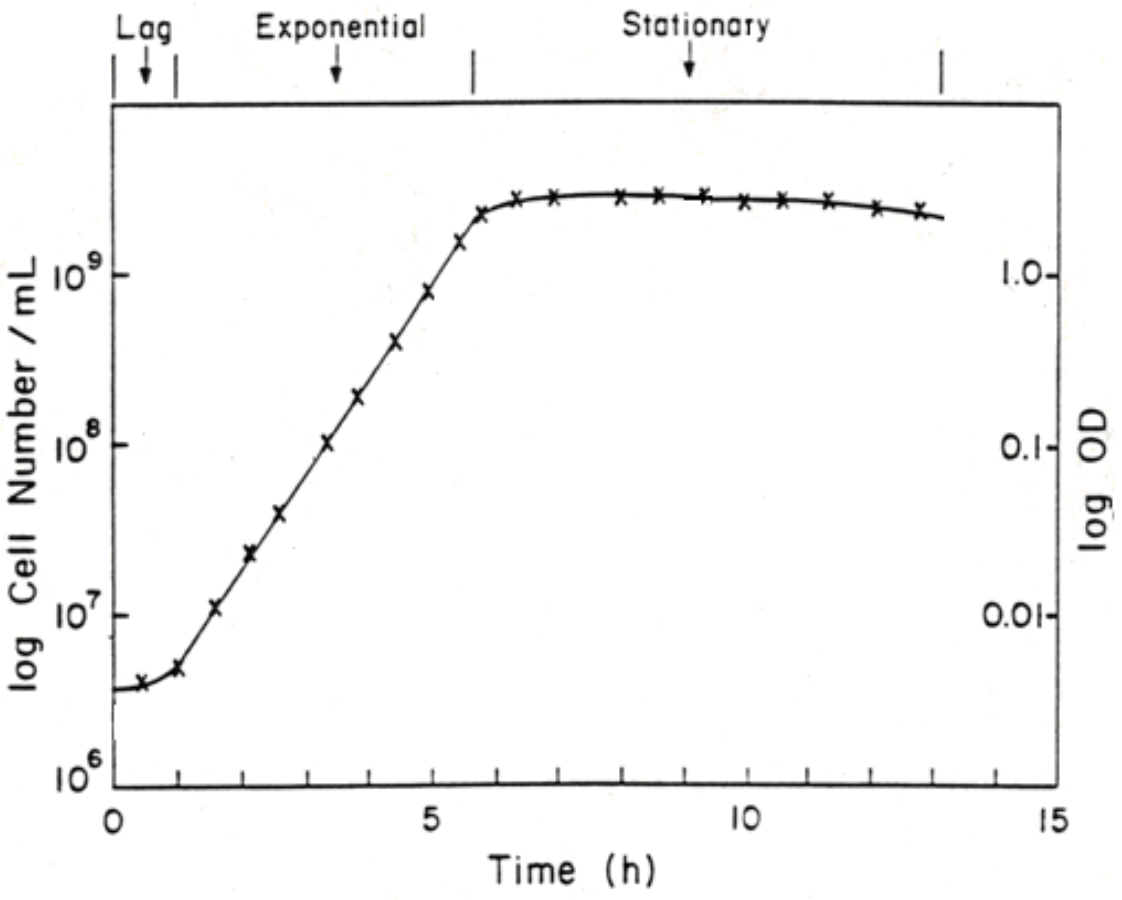

Figure 1.1 Typical microbial growth pattern.

Power consumption is a significant part of the operating cost for large-scale systems. Rushton *et al*. (1950) and Bird *et al*. (1960) have shown by dimensional analysis of the governing physics that the power delivered to an incompressible fluid by a rotating impeller in an agitated tank is proportional to the agitation rate raised to the third power and the impeller diameter raised to the fifth power. Nonlinear correlations of the following form exist for the classical agitation geometries for ungassed (unaerated) vessels :

$$N_P = \phi\,[\mathrm{Re}, \mathrm{Fr}, (\mathrm{Nt})] \tag{1-2}$$

where ϕ is a function whose form is to be determined experimentally. The three dimensionless parameters appearing in the expression are defined by

$$N_P = \text{Power number} = \frac{P}{\rho N^3 D^5}$$

$$\mathrm{Re} = \text{Reynolds number} = \frac{D^2 N \rho}{\mu}$$

$$\mathrm{Fr} = \text{Froude number} = \frac{D N^2}{g}$$

where P = power imparted by the impeller to the fluid (watts),

N = rate of rotation of the impeller (revolutions sec^{-1}),

D = impeller diameter (cm),

g = gravitational acceleration (cm sec^{-2}),

ρ = liquid density (gm cm^{-3}),

μ = liquid viscosity (gm cm^{-1} sec^{-1}), and

t = time since start of operation (sec).

In the gassed (aerated) systems, the presence of gas has an effect on power consumption. The sparged gas bubbles reduce density and therefore decrease power consumption. The concept

of aeration number, N_a, was introduced by Oyama and Endoh (1955). The ratio of gassed and ungassed power consumption was correlated to the aeration number as :

$$(P_g/P_o) = f(N_a) = f(Q/ND_i^3) \quad (1\text{-}3)$$

where P_g = gassed power (watts),

P_o = ungassed power (watts),

N_a = aeration number (dimensionless),

Q = volumetric gas flow rate (liters sec^{-1}),

D_i = impeller diameter (cm), and

N = impeller speed (revolutions sec^{-1}).

An increase in the agitation rate with a classical impeller geometry does not efficiently increase the interfacial area available for gas-to-liquid mass transfer, but greatly increases the bubble residence time. As a result, power delivered per volume of aerated broth is significantly increased. This increase produces only a modest increase in fermentation growth rate.

It has been shown that a microbubble dispersion (MBD) can be efficiently used to increase the rate of oxygen transfer in an aerobic fermentation system. The application of the MBD in aerobic stirred-tank fermentation was investigated by Kaster *et al.* (1990) in a 2-liter fermenter having a working volume of 1.5 liter. The microbubble dispersion provided efficient oxygen transfer with K_La values which were 30% greater than sparged air at equivalent agitator power to volume ratios. Microbubble dispersions consisting of very small surfactant-stabilized bubbles with diameters of 20-1000 μm were delivered to a 2-liter fermentation of *Saccharomyces cerevisiae*, a relatively shear insensitive microorganism. Compared to ordinary bubbles delivered by a conventional sparger (3-5 mm diameter), these smaller microbubbles provide higher interfacial area for oxygen transport and longer gas hold-up because of their comparatively slow rise in the fluid. If, for example, the bubble diameter is halved, the steady-state rise velocity will be 25% of the larger size bubble velocity (by the Stokes equation).

Since the micron-sized bubbles are formed in the presence of surfactants naturally present in the fermentation broth, these surfactant-stabilized bubbles are more sturdy and resistant to coalescence (Oolman and Blanch, 1983, 1986). Marrucci and Nicodemo (1967) proposed that, with the presence of the surfactants, the coalescence process between bubbles is hindered due to electrical repulsive forces which act as an energy barrier to coalescence. The surfactants tend to stabilize the bubble interface and alter the surface tension. The probability of bubble coalescence depends on the properties of the gas-liquid interface. Under the effects of the surfactant materials in fermentation broth, the equilibrium bubble size has been correlated as (Walter and Blanch, 1986)

$$d = \frac{(\sigma + E)^{0.6}}{(P/V)^{0.4}\rho^{0.2}}\left(\frac{\mu_{el}}{3\mu_g}\right)^{0.1} \tag{1-4}$$

where σ = surface tension (dyne cm^{-1}),

ρ = liquid density (gm cm^{-3}),

E = the surface elasticity induced by surfactants present in the liquid (dyne cm^{-1}),

P/V = the power input to the liquid phase per liquid phase volume (watts $liter^{-1}$),

μ_{el} = the extensional viscosity of the liquid phase (gm cm^{-1} sec^{-1}), and

μ_g = gas viscosity (gm cm^{-1} sec^{-1}).

While the microbubble dispersion showed a significant enhancement of oxygen transfer rate in laboratory-scale tests done by Kaster *et al.* (1990), typical laboratory-scale reactors are comparatively overpowered and no scale-up potential was evaluated. In these small-scale fermentations, the power input per volume of broth (P/V) can be significantly higher than the power input per volume of broth in an industrial-scale fermenter. For example, the P/V values in Streptomycin production were reported to be in the range of 0.0010 to 0.0150 hp/gal for 5-liter

laboratory fermentation while in the 15,000-gallon fermentation, the P/V were found to be from 0.0007 to 0.0041 hp/gal (Karow *et al*., 1953). Thus, the microbial growth kinetics observed in laboratory-scale experiments often do not agree with the kinetics observed in scale-up fermentations. The disagreement can be attributed to various effects such as the changes in mixing pattern, flow-motion condition, mass and heat transfer rate, etc. Microbial properties such as growth, adaptation, and shear sensitivity can also cause the complication in scale-up. Therefore, additional experiments are needed to determine if the microbubble dispersion benefit in a small laboratory-scale reactor can be used to overcome the mass transfer limitations in an industrial-scale reactor where P/V ratios are much smaller.

Yeast is a model organism that is unaffected by shear since yeast cells have a rigid cell wall which protects it from environmental shear. Some organisms are quite shear sensitive, so high agitation rates cannot be applied. Organisms employed for antibiotic production, *Aspergillus* and *Penicillium*, important industrial strains of molds (Bailey and Ollis, 1986), cannot be subjected to high agitation rates. They have a filamentous structure called a mycelium, a highly branched system of tubes containing many nuclei and cytoplasm. Trinci (1970) investigated the transport of oxygen to the center of mycelial pellets of *Aspergillus nidulans* and found that the mycelium results in a substantial mass-transfer resistance. The morphological changes arising from agitation can ultimately cause significant change in the product formation. With increasing agitation, long unbranced hyphae (the long, thin filaments of cells within the mycelium) tend to be replaced by the shorter and thicker hyphae (Metz and Kossen, 1977). A further increase leads to mechanical damage, and the antibiotic production is greatest at a level of agitation below the level where this damage occurs. The potential for microbubble dispersion sparging may be more advantageous for the culture of shear sensitive organisms.

The yeast strain *Saccharomyces cerevisiae* was chosen for use as the model aerobic organism in the experiments because : (1) it is relatively insensitive to shear, (2) it is important in the food and beverage industry, (3) its overall metabolism is simple, the only major products are yeast cells, ethanol, and carbon dioxide, and (4) it can be easily grown in culture because of it highly competitive growth characteristics which outcompete contaminating microorganisms.

In this study, scale-up of a 1-liter microbubble dispersion generator (having a working volume of 0.5 liter) was extended to 20- and 50-liter working volume fermentations to investigate the effect on oxygen transfer rate during cultivation of Baker's yeast, *S. cerevisiae* ATCC 4111. Yeast growth patterns obtained with oxygen supplied by ordinary air sparging were compared with growth patterns obtained by MBD sparging using two agitation speeds, 150 rpm and 500 rpm. The overall oxygen transfer coefficient, K_La, in the culture was determined by the yield coefficient method. Finally, the total power delivered per liter of fermentation broth for each method, air sparging and MBD sparging, was estimated.

CHAPTER 2

REVIEW OF LITERATURE

2.1 General characteristics of fermentation process

The practice of fermentation is one of man's oldest technologies. The fermentative abilities of microorganisms have been used for thousands of years. Yeasts were used to raise bread in Egypt from 4000 BC, and fermented dairy products such as cheese and yogurt were developed early in history (Scragg, 1991). The ability to cultivate large quantities of organisms are achieved by using a vessel known as a fermenter or bioreactor.

A fermenter is a vessel in which an organism is cultivated in a controlled manner to produce the organism cell mass itself, or a product produced by the cell. In some specialized cases, fermentation is used to carry out specific reactions. The use of such vessels has allowed laboratory results to be scaled up (as large as 100 000 liter) and become a commercial process.

The term "fermentation" has different meanings in practice. Its classical biochemical meaning relates to the generation of energy by the catabolism of organic compounds, whereas its meaning in industrial microbiology tends to be much broader. In industry, "fermentation" is no longer used in its original classic sense, but it has been extended to describe any chemical process catalyzed by microbial enzyme systems, which in turn are produced during the growth of microorganisms (Gaden, 1955). The production of alcohol by the action of yeast on malt or fruit extracts, and the production of organic solvents, may be described as fermentation in both senses of the word. The description of an aerobic process as a fermentation is obviously using the term in the broader sense.

Currently, there are approximately 200 commercial fermentation products. These products can be classified in three general groups (Scragg, 1991) :

(1) Those that produce microbial cells.

(2) Those where the product is produced by the cells.

(3) Those that modify a compound which is added to the fermentation, a process known as biotransformation.

The basic components of a fermentation process are given in Figure 2.1. This figure shows the flow of steps in a development program which is designed to gradually improve the overall efficiency of the fermentation. Before a fermentation process is established, a producer organism has to be isolated, and perhaps modified, such that it produces the desired product. Then, the proper environmental conditions have to be verified. Scale-up and plant design are the next step to achieve the product needed in commercial quantities. Also, the extraction process has to be established. The development program has features for the continual improvement of the process organism, the culture medium, and the extraction process.

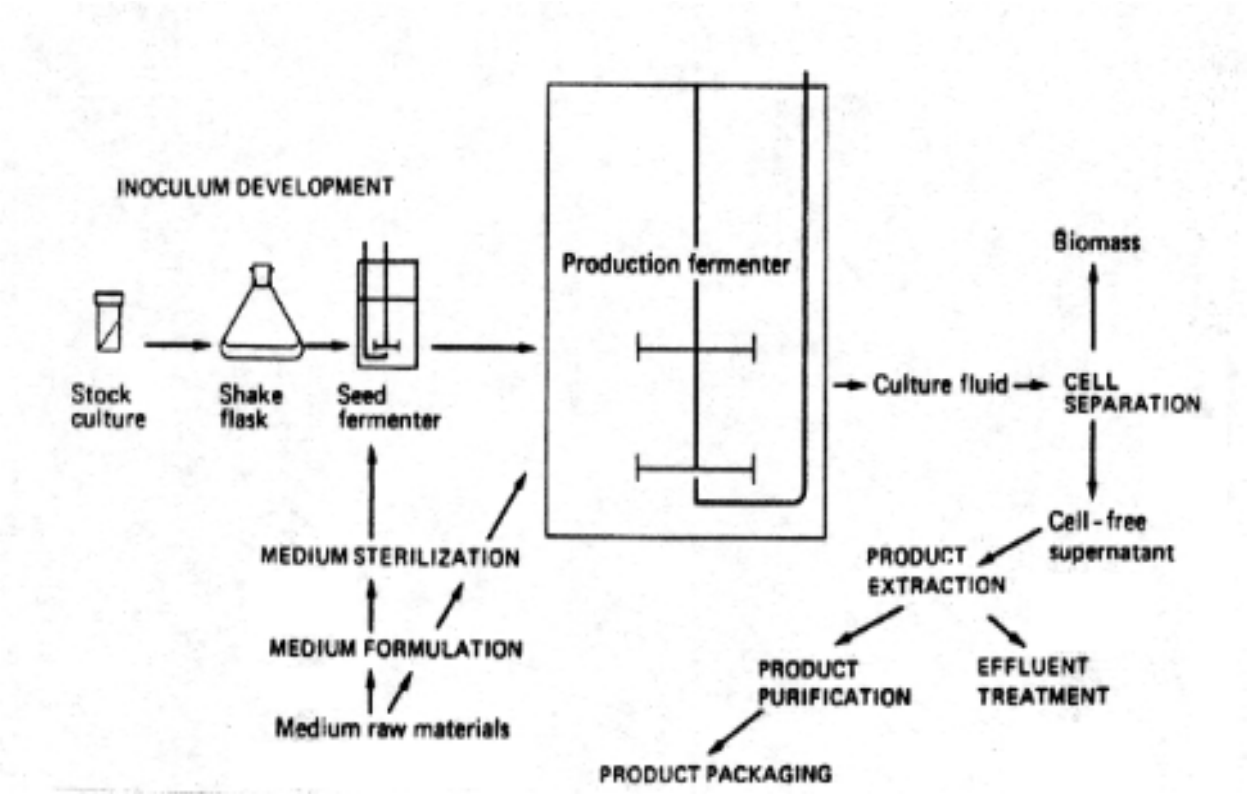

Figure 2.1 A generalized schematic representation of a typical fermentation process (Stanbury and Whitaker, 1984).

2.2 Fermentation of Baker's yeast

Microorganisms which multiply predominantly by budding are collectively called "yeasts" (Kirsop and Kurtzman, 1988). Phaff (1990) gave the definition for yeasts as unicellular eukaryotes which, at some stage in their life cycle, divide by budding. The best known of these organisms is the strain of *Saccharomyces cerevisiae* used in the brewing and baking industries. If the term "yeast" is not further specified, *S. cerevisiae* is the organism commonly referred to. This strain of yeast has been extensively studied and applied widely both in the laboratory and in industry. The characteristics and the various aspects related to the growth behavior of *S. cerevisiae* have been summarized and well documented by Heinisch and Hollenberg (1993), and the literature will not be further reviewed here.

Yeast metabolism uses nutrients to synthesize new cell material as well as to generate energy for their survival. Metabolically, yeasts are mostly facultative aerobes, capable of growing either in the absence of air (fermentative) or in its presence (oxidative). Therefore, in the absence of aeration, yeast has the ability to instantaneously change its respiratory metabolism from oxidative to fermentative (Reed and Nagodawithana, 1991). This catabolic shift is referred to as the "Pasteur effect".

Baker's yeast is a fermentation product used primarily in the preparation of bread dough. It is manufactured by large scale aerobic fermentation of selected strain, *S. cerevisiae*. Aerobic growth of *S. cerevisiae* on fermentable sugars has been studied mainly in batch culture experiments (Scragg, 1991; Orlowski and Barford, 1987; Barford, 1990; Livense and Lim, 1982).

The growth characteristics of *S. cerevisiae* are variable depending on the condition to which yeast cells are subjected. Many researchers have studied the factors affecting the growth patterns under aerobic conditions. Slonimski (1953) reported the influence of aeration on the yeast growth. Strittmatter (1957) and Bruver *et al*. (1975a, 1975b) defined the different growth patterns for different carbon sources. Ball *et al*. (1975) and Tustanoff and Bartley (1964)

investigated the adaptation of yeast to variation in sugar concentration. Subsequently, studies in applications of genetic engineering techniques become very popular due to the increasing demands of the industry to improve the strains of yeasts.

Control strategies in industrial aerobic fermentation have been developed to maximize the growth of yeast and minimize the detrimental factors affecting the yeast's growth patterns. Around 1920, the technique of substrate-feeding, referred to as fed-batch culture, was developed to maintain high biomass yields. Fed-batch operation is the process whereby nutrients necessary for cell growth are fed intermittently during the production phase (Reed and Nagodawithana, 1991). This technique has been found to be particularly effective for processes in which effects such as substrate inhibition, catabolite repression, product inhibition and glucose effects are important (Modak *et al.*, 1986). In fermentation of Baker's yeast, fed-batch processes were devised to prevent a detrimental effect to yeast growth because of a high glucose concentration . A fed batch fermentation takes advantage of the fact that yeast grows most efficiently when glucose is present in small amounts. Currently, the fed-batch technique has been recognized as the best method of commercial yeast fermentation.

2.3 Aeration and agitation in aerobic fermentation

Aeration supplies the necessary oxygen to the microorganisms, and agitation maintains uniform conditions within the fermenter. Altogether, the aeration and agitation are important in promoting effective mass transfer to liquid medium in the fermenter. The main function of a properly designed bioreactor is to provide a controlled environment in order to achieve the optimal growth and/or product formation in the particular cell system employed. In laboratory shake flasks, aeration and agitation are accomplished by the rotary or reciprocating action of the shaker apparatus. In pilot-scale and production-scale fermenters, oxygen is generally supplied by compressed air, and mechanical devices are used to agitate the liquid broth.

In aerobic fermentation processes, oxygen is a key substrate, and because of its low solubility in aqueous solutions, a number of studies to enhance the efficiency of oxygen mass transfer have been conducted. The concentration of dissolved oxygen in a suspension of respiring microorganisms generally depends on the rate of oxygen transfer from the gas phase to the liquid, on the rate at which oxygen is transported to the site of utilization, and on the rate of its consumption by the microorganism. In the conventional water soluble carbohydrate substrate processes, it has frequently been found that the rate of oxygen transfer from dispersed air bubbles can become the rate limiting factor.

Karow *et al.* (1953) found the yield of an extracellular product (penicillin) to be affected by the oxygen supply capability of the stirred-tank cultivation vessel, while Strohm *et al.* (1959) reported maximum cell yield to be limited by the rate of supply of oxygen. Hamprey (1967) has reviewed the engineering problems associated with hydrocarbon fermentations. One such problem is that of supplying sufficient oxygen to meet the relatively-high microbial respiration rate demands in these fermentations. Darlington (1964) has shown that hydrocarbon substrate growth requires about 2.7 times the rate of oxygen transfer from air as does microbial growth on glucose.

2.3.1 Oxygen transport

Oxygen is one of the most important substrates in aerobic fermentation. It is a factor which influences the direction of aerobic metabolism of microbial cells. In glucose fermentation by yeast, it is very important to increase the oxygen transfer rate to the fermentation media because oxygen can be regarded as a nutrient for aerobic microorganisms. Growing microorganisms are capable of removing (metabolizing) oxygen from the liquid faster than it can be supplied. The solubility of oxygen in liquid media is quite low, while its demand for the growth is high. If oxygen supply drops too low, the system may become anaerobic.

Using the stoichiometry of respiration, the oxidation of glucose may be represented as:

$$C_6H_{12}O_6 + 6O_2 \quad \rightarrow \quad 6H_2O + 6CO_2$$

To complete oxidation of 180 grams of glucose, 192 grams of oxygen are required. However, oxygen is approximately 6000 times less soluble in water than glucose (Stanbury and Whitaker, 1984). Thus, it is not possible to initially provide a microbial culture with all the oxygen it will need for the complete respiration of the glucose (or any other carbon source). Oxygen must be supplied during growth at a rate sufficient to satisfy the organism's demand.

During a fermentation, the transfer of oxygen from an air bubble to the cell can be represented by a number of steps as shown in Figure 2.2 (Doran, 1995). The sparingly oxygen is transferred from a rising gas bubble into a liquid phase and ultimately to the site of oxygen reaction in a cell particle. The study of oxygen transfer from air bubbles, through the liquid medium, to microbial cells is of great importance. Whether the mode of fermentation is batch, semicontinuous, or continuous, oxygen must be continuously supplied to the process, if acceptable productivities are to be achieved.

In a bioreacter, the transport of oxygen from gas phase to liquid phase is controlled by the liquid-phase mass-transfer coefficient, K_L. To determine the total oxygen transfer rate in a fermenter, the total surface area available for mass transfer, a, has to be known. Separate determination of K_L and a is difficult to evaluate and sometimes impossible. The combined term of K_La is usually reported as the mass-transfer coefficient rather than just K_L (Doran, 1995).

Rate of mass transfer is directly proportional to the driving force for transfer and the area available for the transport process to take place (Charles and Wilson, 1994). For each fluid on either side of the phase boundary, the driving force for mass transfer can be expressed in terms of a concentration difference. Therefore, rate of oxygen transfer at a phase boundary in a fermentation broth is described by the equation:

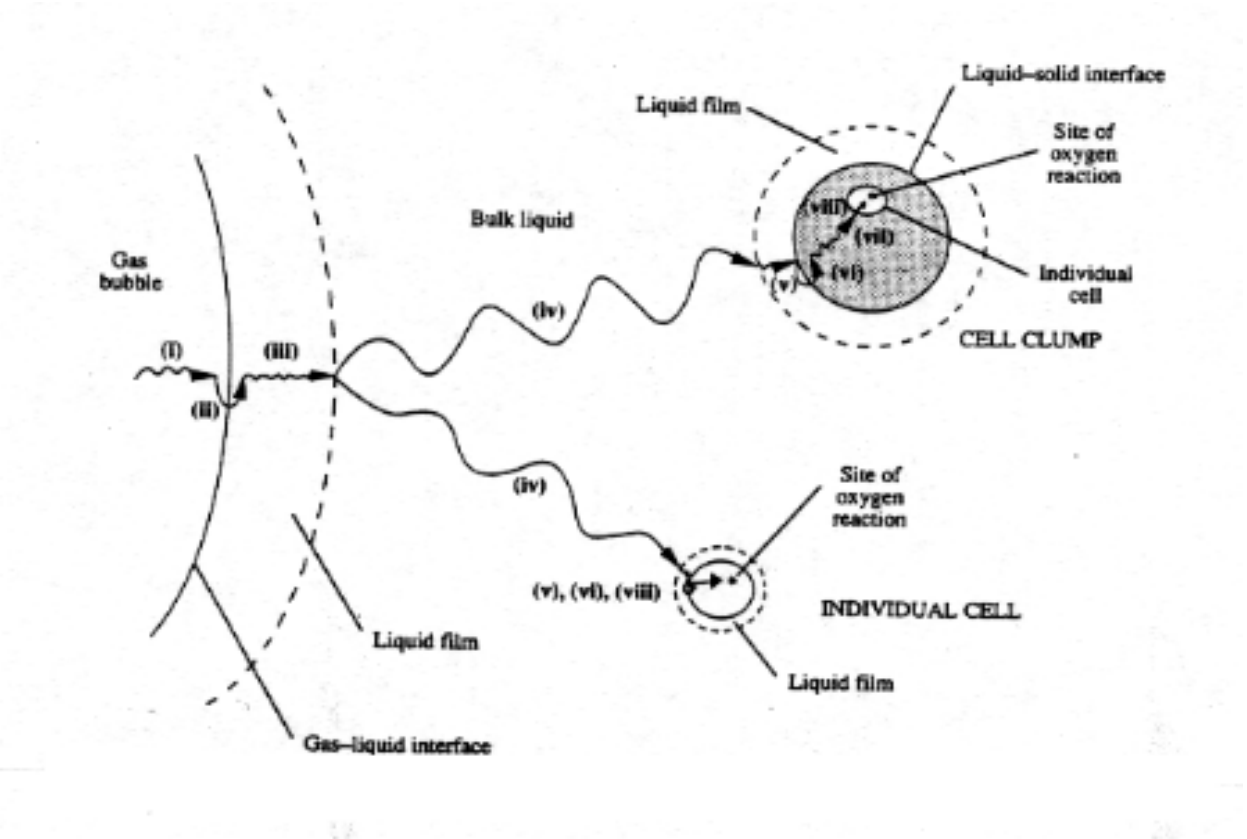

Figure 2.2 Steps for transfer of oxygen from gas bubble to cell. (i) transfer from the interior of the bubble to the gas-liquid interface; (ii) movement across the gas-liquid interface; (iii) diffusion through the relatively stagnant liquid film surrounding the bubble; (iv) transport through the bulk liquid; (v) diffusion through the relatively stagnant liquid film surrounding the cells; (vi) movement across the liquid-cell interface; (vii) if the cells are in a floc, clump or solid particle, diffusion through the solid to the individual cell; and (viii) transport through the cytoplasm to the site of reaction (Doran, 1995).

Oxygen Transfer Rate (OTR) = $K_L a\ \Delta C$

where K_L = liquid-phase mass-transfer coefficient ($h^{-1} \cdot m^{-2}$)

a = surface area available for mass transfer (m^2), and

ΔC = concentration driving force (mol l^{-1}).

ΔC is essentially the difference in oxygen concentration inside a bubble, C^*_L compared to the oxygen concentration in the surrounding liquid, C_L . $\Delta C = C^*_L - C_L$.

Therefore,

$$\text{OTR} = K_L a\ (\ C^*_L - C_L\ ) \qquad (2\text{-}1)$$

If we need to transfer as much oxygen as possible to the growth medium in a fermenter, we need to consider the physical and chemical factors that affect either the value of K_L or the value of a, or the concentration driving force, ΔC.

It has been shown that dissolved oxygen levels in culture media are affected by gas flow rate, speed of agitation, and oxygen partial pressure in the aeration gas. Attempts to control dissolved oxygen levels by varying agitator speed or gas flow rate have been done. Ahmad *et al.* (1994) found that an increase in oxygen transfer rate from 8.94 to 38.63 mmol $l^{-1} \cdot h^{-1}$ is obtained as the agitation speed is increased from 300 to 600 rpm. In addition, by increasing the air flow rate from 0.21 l min^{-1} to 1.05 l min^{-1}, the oxygen transfer rate increased from 5.7 mmol $l^{-1} \cdot h^{-1}$ to 20.5 mmol $l^{-1} \cdot h^{-1}$. Increasing the agitation rate is the traditional approach used to enhance the oxygen transfer rate. More agitation produces more gas dispersion, and more gas dispersion produces more mass transfer.

Kaster *et al.* (1990) found that by using a microbubble dispersion for oxygen supply in aerobic fermentation of yeast, the transport of oxygen can be increased, especially under conditions of reduced speed of agitation. For a given volume of gas, more interfacial area, a, is provided if the gas is dispersed into many small bubbles rather than a few large ones. Since the

efficiency of oxygen transport is approximately proportional to the ratio of the bubble surface area to the bubble volume, the smaller size of the microbubbles increased oxygen transfer rate in the fermenter. In addition, smaller bubbles have a longer dwell time in the liquid because of their slower bubble-rise velocities, allowing more time for the oxygen to dissolve.

2.3.2 Determination of K_La value

The determination of K_La in bioreactors is essential in order to establish aeration efficiency and to quantify the effects of operating variables on the provision of oxygen. A number of methods have been developed to estimate the oxygen transfer rate in a bioreactor (Sobotka *et al*., 1982). When selecting a method, several factors must be taken into account: 1) the aeration and homogenization system used, 2) the fermenter construction, 3) the physiological effects from the microorganism used, and 4) the composition of the fermentation medium (Novak and Klekner, 1988). The following are the four most common methods applied to estimate the oxygen transfer rate in the fermentation system: yield coefficient, dynamic technique, sodium sulfite oxidation, and direct method.

Yield coefficient method

This technique was found to be a reliable and quite satisfactory method for estimating the oxygen uptake rate by microorganisms during fermentation. It is based on the oxygen uptake rate of the organism rather than the rate of depletion of oxygen in the gas or liquid phase. By using a stoichiometric balance on oxygen in the cell mass together with the kinetic data from growth, the following relationship of the volumetric oxygen absorption rate during growth can be obtained (Wang *et al*., 1979)

$$\text{OUR} = \mu X \left(\frac{K'}{Y_{o_2}} \right) \tag{2-2}$$

where OUR = oxygen uptake rate ($mmolO_2\ l^{-1} \cdot h^{-1}$),

μ = specific growth rate of the organism (h^{-1}),

K' = conversion factor = 31.25 mmol O_2/gm O_2 , and

Y_{O2} = yield coefficient on oxygen (gm cell mass/gm O_2).

The oxygen yield coefficient, Y_{O2}, is dependent on substrate yield coefficient, Y_s (gm of cell mass/gm of substrate consumed). Mateles (1971) provided a generalized method to calculate the oxygen yield coefficient. The relationship of the oxygen yield to the substrate yield for bacteria and yeast on several types of substrate is show in Figure 2.3. This method for calculating the oxygen transfer rate is simple, however Eq. (2-2) is based on the assumption that substrate is completely converted into carbon dioxide, water, and cell mass. In those cases where substrate is converted to another product in addition to carbon dioxide, water, and cell mass, then Eq. (2-2) is not valid.

At steady state, the rate of oxygen transfer from the bubbles is equal to the rate of oxygen consumption by the cells. We then can set OTR in Eq. (2-1) equal to OUR in Eq. (2-2) and solve for the $K_L a$ value.

Dynamic method

Several versions of the dynamic method have been reported. The one described here was proposed by Taguchi and Humphrey (1966). It is based on the respiratory activity of organisms which are actively growing in the fermenter. While the dissolved oxygen level of the fermenter

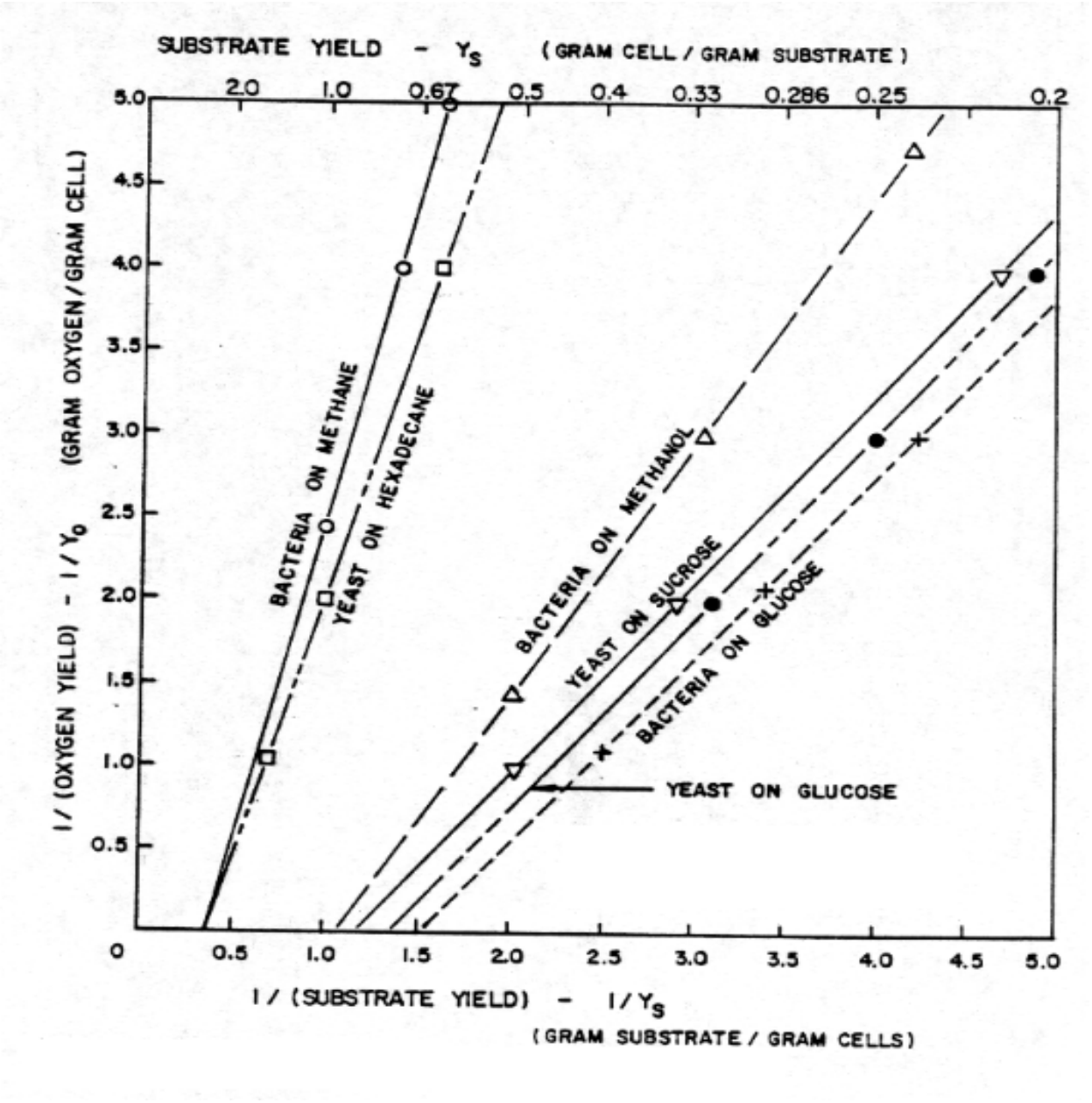

Figure 2.3 Relationship between substrate yields and oxygen yields for different microorganisms grown on different substrates (Mateles, 1971).

is steady, we suddenly stop the supply of air. Shortly after the air supply is turned off, the dissolved oxygen concentration will be decreased due to the respiration of the culture (Figure 2.4). The decrease occurs at

$$\frac{dC_L}{dt} = - r_{O2}\, C_x$$

where C_L = the oxygen concentration in the surrounding liquid (mmoles O_2 l^{-1}),

r_{O2} = the specific respiration rate (mmoles O_2 gm cells$^{-1}\cdot h^{-1}$), and

C_x = dry weight of cells per liter (gm cell mass l^{-1}).

When the aeration is turned on again, the dissolved oxygen concentration increases until it reaches the steady oxygen concentration. The oxygen material balance in an aerated batch fermenter with growing organisms is given by

$$\frac{dC_L}{dt} = K_L a\, (C^*_L - C_L) - r_{O2}\, C_x \qquad (2\text{-}3)$$

where, K_L = liquid-phase mass-transfer coefficient ($h^{-1}\cdot m^{-2}$),

a = surface area available for mass transfer (m^2), and

C^*_L = the oxygen concentration inside a bubble(mmoles O_2 l^{-1}).

This equation can be rearranged to result in a linear relationship as

$$C_L = C^*_L - \left[\left(\frac{1}{K_L a} \right) \left(\frac{dC_L}{dt} + r_{O2}\, C_x \right) \right]. \qquad (2\text{-}4)$$

The term, $r_{O2}\, C_x$, can be obtained by measuring the slope of the C_L vs. t curve in Fig. 2.4. Therefore, from Eq. (2-4), the plot of C_L versus (dC_L / dt + $r_{O2}\, C_x$) will result in a straight line which has the slope of -1/ ($K_L a$) and the y-intercept of C^*_L.

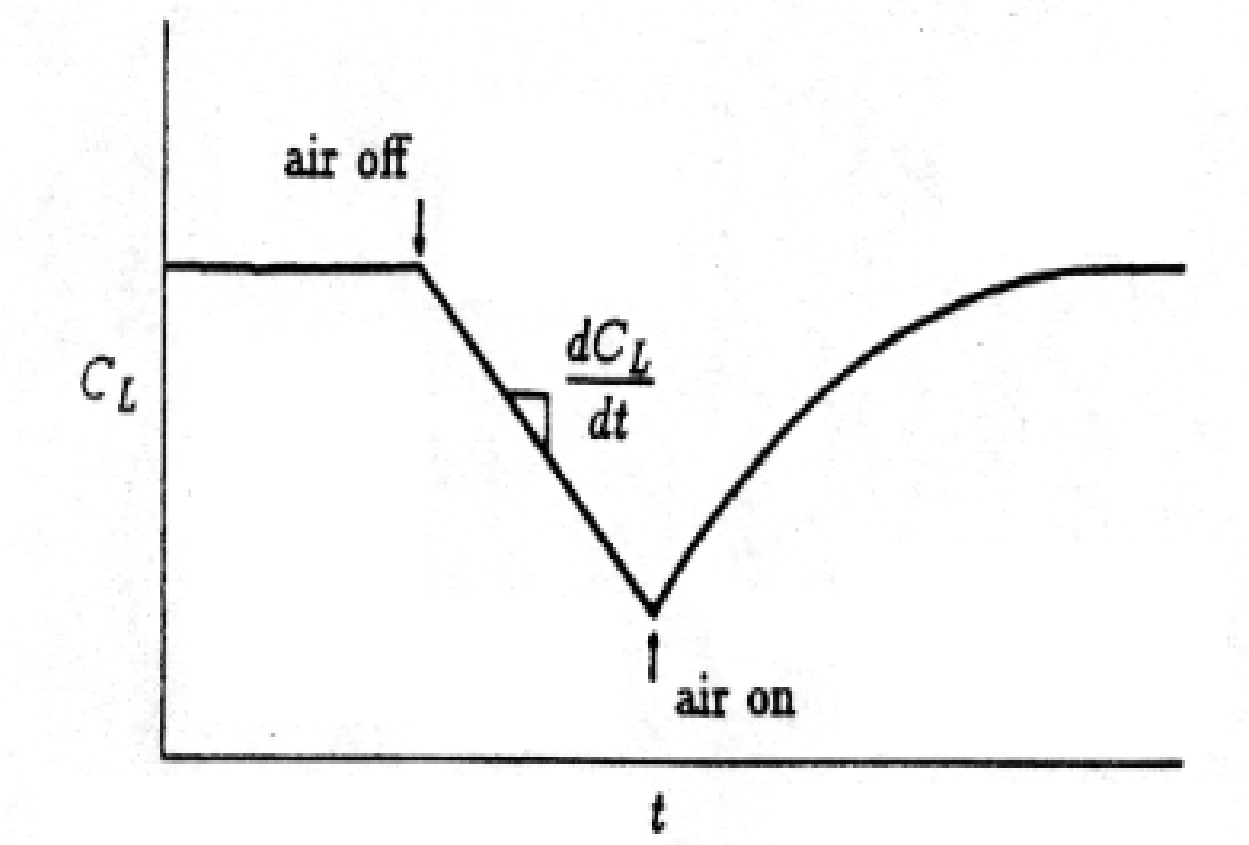

Figure 2.4 Dynamic technique for the determination of $K_L a$ (Taguchi and Humphrey, 1996).

Sodium sulfite oxidation

Cooper *et al*. (1944) first proposed this method based on the oxidation of sodium sulfite to sodium sulfate in the presence of a catalyst (Cu^{++} or Co^{++}) as

$$Na_2SO_3 + 1/2\ O_2 \rightarrow Na_2SO_4$$

This reaction has the following characteristics which can be exploited to measure the oxygen transfer rate:

1. The rate of this reaction is independent of the concentration of sodium sulfite within the range of 0.04 to 1 N.

2. The rate of reaction is much faster than the oxygen transfer rate; therefore, the rate of oxidation is controlled by the rate of mass transfer alone.

To measure the oxygen transfer rate in a fermenter, fill the fermenter with a 1 N sodium sulfite solution containing at least 0.003 M Cu^{++} ion. Turn on the air and start a timer when the first bubble of air emerges from the sparger. Allow the oxidation to continue for 4 to 20 minutes. After which, stop the air stream, agitator, and timer at the same instant, and take a sample. Mix each sample with an excess of freshly pipetted standard iodine reagent. Titrate with standard sodium thiosulfate solution ($Na_2S_2O_3$) to a starch indicator end point. Once the oxygen uptake is measured, the K_La may be calculated using Eq. (2-1) where C_L is zero and C^*_L is the oxygen concentration in the air being sparged into the fermenter.

The sodium sulfite oxidation method is relatively quick and easy. However, this method has the limitation that the solution cannot approximate the physical and chemical properties of a fermentation broth.

Direct measurement

This method uses a gaseous oxygen analyzer to measure the oxygen content of the gas stream entering and leaving the fermenter (Lee, 1992). This technique is the most reliable and accurate, but it also requires accurate instruments. An oxygen material balance can be described by

$$OUR = (Q_{in}\, C_{O2,in} - Q_{out}\, C_{O2,out})$$

where,

OUR = oxygen uptake rate ($mmolO_2\ l^{-1}\cdot h^{-1}$),

Q_{in}, Q_{out} = the volumetric air flow rate measured at fermenter inlet and outlet (h^{-1}),

$C_{O2,in}$, $C_{O2,out}$ = the oxygen content measured at fermenter inlet and outlet ($mmolO_2\ l^{-1}$).

Once the oxygen uptake is measured, the K_La can be calculated by using Eq. (2-1), where C_L is the oxygen concentration of the liquid in a fermenter and C^*_L is the equilibrium concentration of the oxygen in the air passing through the fermenter. The oxygen concentration of the liquid in a fermenter can be measured by an on-line oxygen sensor. If the size of the fermenter is rather small (less than 50 liter), the variation of the $C^*_L - C_L$ in the fermenter is fairly small. However, if the size of a fermenter is very large, the variation can be significant. In this case, the log-mean value of $C^*_L - C_L$ at the inlet and outlet of the gas stream can be used. The log-mean value is given by

$$(C^*_L - C_L)_{LM} = \frac{[(C_L{}^* - C_L)_{in} - (C_L{}^* - C_L)_{out}]}{\ln\left[\dfrac{(C_L{}^* - C_L)_{in}}{(C_L{}^* - C_L)_{out}}\right]}.$$

2.4 Development of MBD generator

The application of a microbubble dispersion was first described by Dr. Felix Sebba in the early 1970s (Chemical Engineering Department, Virginia Polytechnic Institute and State University, Blacksburg, Virginia). The microbubble dispersion was originally given the name "microfoam" due to the minute size of the bubbles. Then, with the property of a colloidal dispersion of gas with an average diameter as small as 20 μm in a liquid phase, it was later called colloidal gas aphron (CGA) (Sebba, 1971). The term “microbubble dispersion” was first used by Kaster *et al.* (1990) when the CGA generator was first applied in the aerobic fermentaion system. The more general term, MBD, was coined because it was found that the CGA generator was producing a mixture of CGA-sized bubbles (20-70 μm) and some larger bubbles (3-5 mm). The CGAs are exceptional because of the very small size of the bubbles and the type of surface which surrounds each aphron. These unusual properties made the CGAs differ markedly from ordinary bubbles. The CGA-size bubbles rise very slowly under the influence of gravity and scarcely, if ever, coalesced as long as they were completely immersed in the liquid broth.

The original CGA generator design was a simple modification of the glass aspirator known as a Bunsen suction pump. The colloidal dispersion of gas was generated by rapid flow of an aqueous solution of surfactant through a venturi throat. Gas was introduced through a fine orifice into the venturi throat (Figure 2.5). This method could produce a dispersion of micron-sized bubbles at a concentration which reached 65% by volume of gas in the water. It was found unsatisfactory on the larger scale because the production of CGA was comparatively slow. The operation required recycling of the dispersion in order to build up the concentration. It also required a powerful pump to force the water through the venturi throat fast enough to entrain the gas bubbles. If a certain minimum flow rate was not achieved, the CGA was not produced. In 1985, Sebba proposed a new method to overcome this drawback (Figure 2.6) (Sebba, 1985). This system has a thin solid metal disc of approximately 5 cm diameter, positioned horizontally

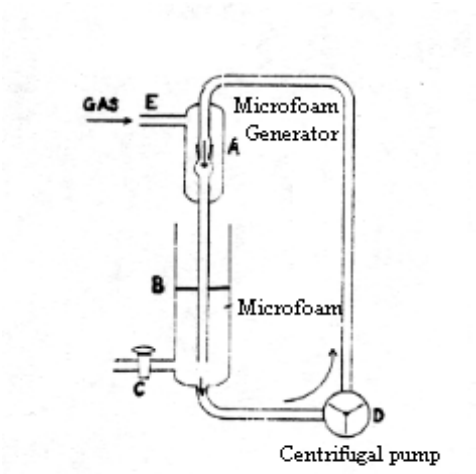

Figure 2.5 Microfoam generator (Sebba, 1971).

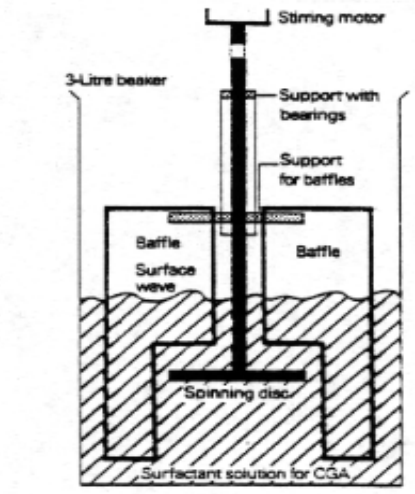

Figure 2.6 Spinning disc CGA generator (Sebba, 1985).

about 2-3 cm below the surface of the dilute surfactant solution. The disc is fixed to a vertical shaft connected to an electric motor which spins the disc at approximately 4000 rpm to create the micron-sized bubbles. The disc is also encompassed by two vertical baffles. When the disc spins fast up to the critical speed of revolution, the hydrodynamic forces adjacent to the disc produce strong waves on the surface. These waves strike the baffles and, having nowhere else to go, re-enter the liquid. As they reenter, they entrain the gas in the form of very small bubbles.

2.5 Scale-up

Ju and Chase (1992) classified the scale-up of a bioprocess into three stages: (1) *laboratory scale*, where elementary studies are carried out; (2) *pilot scale*, where the process optimizations are determined; and (3) *plant scale or production scale*, where the process is brought to economic fruition. Scale-up is a procedure for the design and construction of a large-scale system on the basis of the results of experiments with small-scale equipment.

Mavituna (1996) described three different phenomena important for process design: thermodynamic phenomena, microkinetics (intrinsic) phenomena, and transport phenomena (momentum, heat, and mass). Transport phenomena is the only one that is highly dependent on scale; thermodynamic and microkinetics are scale-independent phenomena. Moreover, it is the transport phenomena that governs the aerobic fermentation process.

Once a particular aerobic fermentation is accomplished successfully in a laboratory-scale experiment, the values of the operating variables and the physical properties are known or can be measured. This fermentation system is then examined in a number of bioreacters of increasing scale, and usually the final process optimization is carried out in a pilot plant (scale 50- 300 liter) where the process conditions are very similar to those used in the production scale. Kossen and Oosterhuis (1985) divided the approach for scale-up into four widely recognized steps: 1)

fundamental methods; 2) semifundamental methods; 3) dimensional analysis; and 4) rules of thumb.

2.5.1 Fundamental methods

These methods are based on the application of turbulence models for description of the influence of operating conditions and geometrical design of the bioreacter on the flow pattern in the reactor. Solution of the microbalance equations for momentum and mass transfer is required. These methods are very complicated, and frequently, many simplifications are required. Nevertheless, these fundamental models allow us to obtain a complete description of the process that is beneficial both for scale-up and for optimal operations at the production scale.

2.5.2 Semifundamental methods

In these methods, simplified equations are applied to obtain a practical approximation of the process. The parameters obtained will be scale dependent, and thereby the influence of scale on the process can be examined by model simulations. However, despite the extensive simplification of the problem when moving from the fundamental models to semifundamental models, the complexity of the model is still substantial.

2.5.3 Dimensional analysis

Dimensional analysis is based on keeping the values of dimensionless groups of parameters constant during a scale-up. In physical meaning, the dimensionless groups, often called

dimensionless numbers, are ratios of rates or time constants for the different mechanisms involved. Then, if all the dimensionless groups are kept constant, the relative importance of the mechanisms involved in the process will not change during scale-up.

It is often impossible to keep all the dimensionless groups constant during scale-up, hence one has to determine the most important group and deemphasize the rest. Moreover, noncritical application of the approach may lead to technically unrealistic values, e.g., for power consumption and stirrer speed in the fermenter**.**

2.5.4 Rules of thumb

The rules-of-thumb method is the most common method. The scale-up criterion most frequently used are; 1) constant specific power input (*P/V*), 2) constant K_La , 3) constant tip speed of the agitator, and 4) constant dissolved oxygen concentration. Table 2.1 shows the percentage of each criteria used in the fermentation industry. The different scale-up criteria normally result in entirely different process conditions on a production scale. It is impossible to maintain all the parameters in the same ratio to one another. The consequences of such calculations for two geometrically similar stirred tank systems with the model system volume, V_m = 10 liter and the production system volume, V_p = 10 m^3 (linear scale-up factor of 10) are shown in Table 2.2.

Application of the rules of thumb method is very simple, but it is also a very weak method. There may easily occur a complete shift in the limiting regime above a certain scale.

In practical application, all four methods are used in combination with each other and sometimes the trial-and-error method must also be included. For the scaling-up of aerobic fermentation, the effect of gas liquid mass transport is the most significant factor (Hubbard *et al*., 1994). Therefore, scale-up in aerobic fermentation is often performed on the basis of keeping the value of K_La constant. The success of scale-up processes are usually confirmed by experimental

results which show that there is no difference between small and large scale fermentation carried out under the same oxygen transfer rate.

Table 2.1 Scale-up Criteria in Fermentation Industries

Scale-up criterion used	Approximate percentage of industries using criterion
constant P/V	30
constant K_La	30
constant v_{tip}	20
constant pO_2	20

From: F. Mavituna, Strategies for Bioreactor Scale-up, In: *Computer and Information Science Application in Bioprocess Engineering* (A. R. Moreira and K. K.Wallace), Kluwer Academic Publishers, Dordrecht, Netherlands, 1996, pp.125-142

Table 2.2 Different scale-up criteria and their consequences

Scale-up criterion	Value at 10 m^3 scale (V_m=10 L)					
	P	P/V	N	ND	Re	N/D
Equal P/V	10^3	1	0.22	2.15	21.5	0.022
Equal N	10^5	10^2	1	10	10^2	0.1
Equal tip speed	10^2	0.1	0.1	1	10	10^{-2}
Equal Re number	0.1	10^{-4}	10^{-2}	0.1	1	10^{-3}
Equal shear to flow ratio	10^8	10^5	10	10^2	10^3	1

From: N. W. F. Kossen and N. M. G. Oosterhuis, Modelling and Scaling-up of Bioreactors, In: *Biotechnology 2nd edn.* (H. J. Rehm and G. Reed), VGH-Verlag, Weinheim, Germany, 1985

CHAPTER 3

EXPERIMENTAL PROCEDURE

3.1 Description of equipment

3.1.1 Laboratory-scale fermenter

Preliminary tests were run in a 2-liter bench top fermenter (Multigen F-2000, New Brunswick Scientific Co. Inc., New Jersey). The fermenter was equipped with four baffles and a six-blade turbine stirrer. pH was measured and recorded by a pH electrode (Ingold) and pH controller (Model 5997-20, Horizon Ecology Company, Chicago, Illinois). Air and MBD flow were metered with a variable speed peristaltic pump (Master Flex 7523-10, Barnant Company, Barrington, Illinois).

3.1.2 Pilot-scale fermenter

The scale-up fermentations were run in a 72-liter pilot-scale fermenter (BIOSTAT® U 50, B. Braun Company, Germany). The air supply, the exhaust pipes and other parts were sterilized *in situ*. The culture vessel and broth were sterilized by steam heated jacket. A glass storage vessel for additive solutions was sterilized separately in an autoclave.

The temperature of the vessel (operating temperature as well as sterilization temperature) was controlled by means of a pressurized closed-loop hot water system. Heating or cooling water was supplied via the integrated tubular heat exchanger with separate heating and cooling coils. The temperature inside the vessel was measured by means of a Pt-100 resistance thermometer which controlled the supply of steam and cooling water in conjunction with two pulse-triggered control values. Air was injected into the vessel bottom via a sparger having 1 mm diameter orifices. The turbulence of the broth was increased by four baffles which were attached to the inner wall of the vessel. The pH value of the culture solution was measured by means of a sterilizable combination electrode (lnFit 764-50®, Ingold Company). The dissolved oxygen was measured by means of a sterilizable pO_2 electrode (Ingold Company). This electrode works on the polarographic principle and consists of an Ag anode and a Pt cathode which are separated from the solution to be measured by a gas-permeable polymeric membrane. The stirrer drive system was designed as a bottom drive and was equipped with three 6-blade disk impellers. The actual stirrer speed was measured by a precision DC tachogenerator integrated into the motor.

3.1.3 Organism and maintenance

The microorganism used in this study was a Baker's yeast, *Saccharomyces cerevisiae*, obtained from the American Type Culture Collection designated as ATCC 4111. Lyophilized culture was rehydrated in YM liquid medium and was incubated for 18 hours at 37 C. Stock culture was maintained on a Difco YM agar medium at 4 C and was transferred onto a new medium every month to maintain viability.

3.2 Description of experiments

3.2.1 Fermentations

3.2.1.1 Preliminary experiments

Batch fermentation studies of *S. cerevisiae* in a 2-liter laboratory fermenter were undertaken to test the performance of the microbubble generator and to provide a background for the scale-up experiments. The fermentation system was controlled at 35 C and pH 6. The pH was kept at a constant value by the automatic addition of 1.0 N ammonium hydroxide.

Inoculum preparation

Starter culture was prepared by transferring two loops of the stock culture to a 500 ml Erlenmeyer flask containing 150 ml of standard YM broth (21 grams per liter of distilled water) and incubated at 30 C for 18 hours on a rotary shaker at 200 rpm.

Batch fermentation with ordinary sparged air

The 2-liter batch fermentation was started with the addition of 150 ml of yeast starter culture to 1350 ml of the standard YM broth at 35 C. The temperature was controlled by cooling water run at a constant rate through a heat exchanger tube inserted into the top of the fermenter. The agitator speeds used were set at minimum speed of 100 rpm and normal agitation speed of 600 rpm. Air was sparged into the system through the shaft of the impeller and emerged from a set of twelve 1 mm diameter holes in the impeller shaft 2 cm from the bottom of the fermenter. Samples were taken from a sampling port at the top of the fermenter approximately every hour.

Batch fermentation with natural surfactant-stabilized microbubble

For the microbubble dispersion tests, the same fermentation system was used with the addition of a microbubble dispersion generator installed in a recycle loop. Figure 3.1 illustrates schematically the experimental set-up for a MBD experiment. The peristaltic recycle pump was set at approximately 130 ml/min. The microbubble dispersion was delivered via the MBD pump at 400 ml/min through the impeller shaft. The dispersion entered the broth through the same set of twelve holes as the sparged air.

3.2.1.2 Main Experiments

Fermentations were carried out in a 72-liter pilot-scale stirred-tank fermenter with two working volumes, 20 liters and 50 liters, respectively. It is noted that the 50-liter run was done to determine if the MBD was as effective at a larger working volume. Air sparge and microbubble fermentations were run at both working volumes with two agitation speeds, 150 rpm and 500 rpm. The culture, medium, and fermentation system were maintained the same as in the laboratory-scale experiments. Samples were taken approximately every hour and the pH and pO_2 readings were recorded.

Inoculum preparation

Yeast starter culture was prepared in proportion to the scale-up volume. The ratio of the amount of starter culture volume to the volume of the sterilized medium in the fermenter was 1:20. This ratio gave an initial dry cell mass concentration of 0.1 gm per liter of broth.

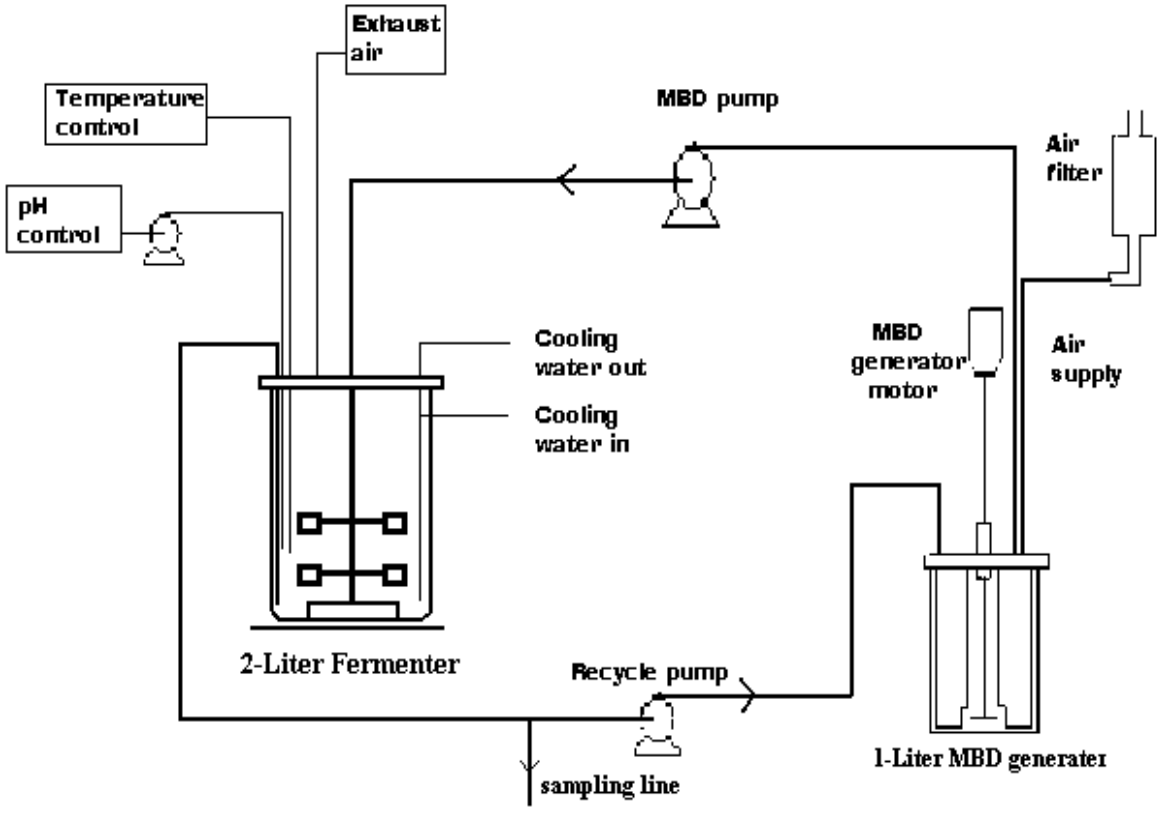

Figure 3.1 The experimental set-up of fermenter and MBD generator in laboratory-scale experiment.

Batch fermentation with natural surfactant-stabilized microbubble

As shown in Figure 3.2, the set-up of the MBD run in the pilot-scale fermentaion was generally the same as in the laboratory-scale fermentation. Volumetric flow rate was scaled-up based on the working volume (From the preliminary laboratory-scale experiment, the MBD flow rate was 270 ml min^{-1} $liter^{-1}$working volume). To deliver the MBD through the same sparger used for the ordinary air-run, the original air inlet to the fermenter was disassembled and the MBD entering the fermenter was connected to that inlet. The air flow was adapted using a tube connector and supplied to the MBD generator. To recycle the broth back to the MBD generator, the sampling port at the bottom of the fermenter was adapted using polypropylene tubing. The broth samples were taken out of the recycle loop by using a three-way splitter. In the 20-liter experiments, the volumetric flow rate of the peristaltic MBD pump used was 1700 ml min^{-1} and the recycle pump was 800 ml min^{-1}. In the 50-liter experiments, the flow rate for the MBD pump and the recycle pump was 7500 and 1350 ml min^{-1}, respectively. The MBD flow rate was 85 ml min^{-1} $liter^{-1}$working volume for the 20-liter experiments and 150 ml min^{-1} $liter^{-1}$working volume for the 50-liter experiments.

3.2.3 Assays

3.2.3.1 Glucose concentration

The method of Park and Johnson (1949) was used to determine the glucose concentration. This method is based on the reduction of ferricyanide ions in alkaline solution by a reducing sugar. The ferrocyanide produced can then react with a second mole of ferricyanide producing the ferric-ferrocyanide (Prussian blue) complex. Potassium cyanide and sodium dodecyl sulfate are included in the formulation to speed the rate of reduction and maintain the

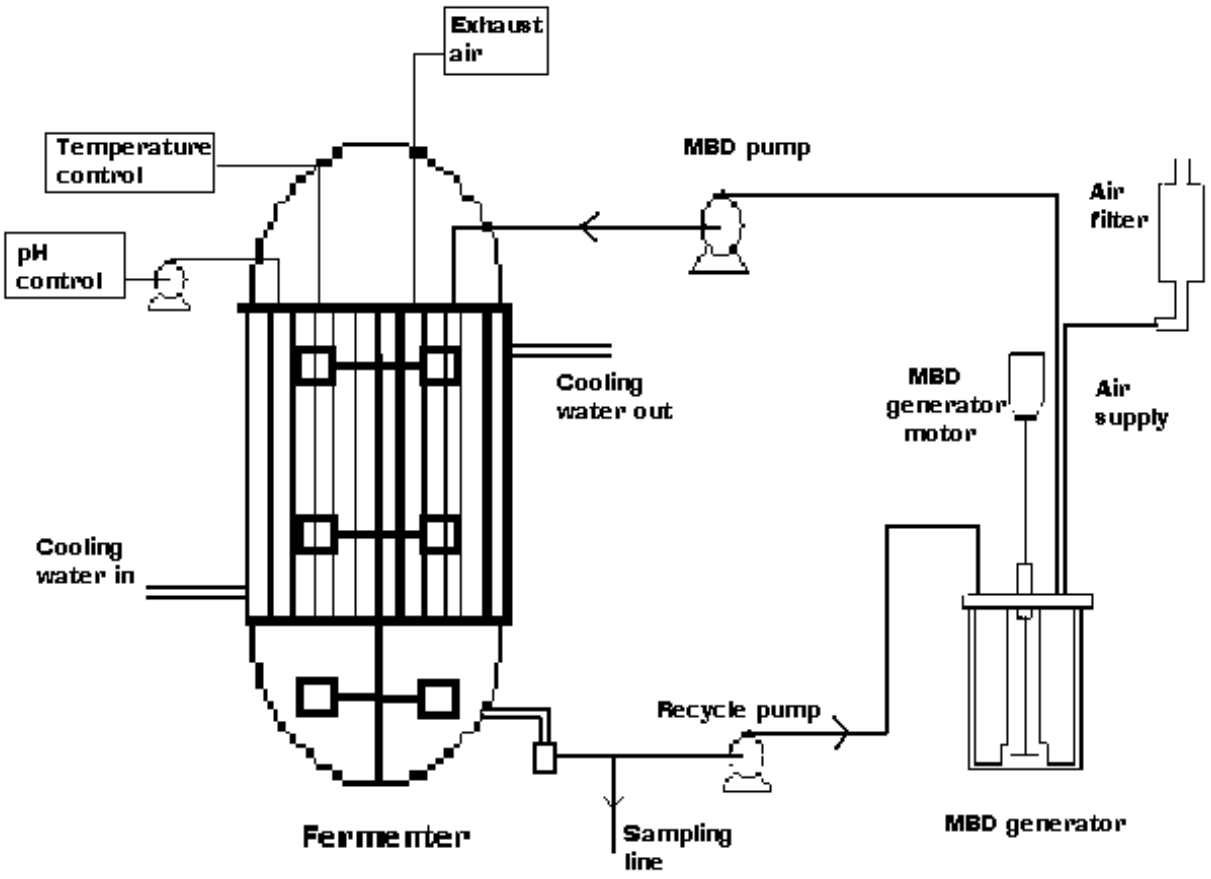

Figure 3.2 The experimental set-up of fermenter and MBD generator in pilot-scale main experiment.

Prussian blue complex in suspension. To determine the sugar concentration, the Prussian blue concentration was measured in a spectrophotometer (Spectronic 1201, Milton Roy Company).

3.2.3.2 Dry cell mass concentration

Samples of the culture were carefully pipetted into pre-weighed centrifuge tubes followed by centrifugation at 11,000 rpm (Eppendorf Centrifuge 5415C, Brinkmann Instruments Inc., New York) for 15 minutes. The broth samples were maintained at a temperature under 4 C to prevent further growth of the culture. The cell pellet was next washed with deionized water to remove any substrates, etc., which attach to the cells, and centrifuged again to draw off the water. The washed cells were then dried to constant weight. The tubes were re-weighted and the cell dry weight was calculated.

$$\text{Cell dry weight (gm l}^{-1}) = \frac{[\text{wt. of dried centrifuged tube + cells (gm) - wt. of tube (gm)}]\text{x } 10^3}{\text{sample volume (ml)}}.$$

The dry weight determination was made in duplicate in order to get a good mean value.

3.2.4 Calculations

3.2.4.1 Calculation of the major metabolic parameters

The specific growth rate was calculated from the slope of the semilog plot of cell dry mass (x) versus time (t) and the maximum specific growth rate (μ) was obtained by linear regression in the log-phase range. The cell yield on sugar, $Y_s = -(\Delta x/\Delta S)$ was defined as the amount of cells produced per gram of sugar consumed, where x = dry cell weight concentration (gm l^{-1}) and S = residual sugar concentration in fermenter (gm l^{-1}). The oxygen yield coefficient, $Y_{O2} = -(\Delta x/\Delta O_2)$, defined as the amount of cells produced per gram of oxygen consumed, was obtained from the correlation between the oxygen yield and substrate yield (Mateles, 1971).

3.2.4.2 Determination of the $K_L a$ values

Based on the metabolic oxygen uptake rate of the organism during fermentation, the overall oxygen transfer coefficients were determined by the yield coefficient method. The oxygen uptake rate was calculated using Eq. (2-2) (Sec. 2.3.2). At steady state, the oxygen absorption and consumption rates can be balanced; the OTR (oxygen transfer rate) in Eq. (2-1) must equal to the OUR (oxygen uptake rate) in Eq. (2-2). The $K_L a$ value was then obtained.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Preliminary experiments

A preliminary test in a laboratory-scale experiment was conducted to verify the efficiency of a microbubble dispersion (MBD). The dry cell mass concentrations versus time for the 2-liter fermentations are presented in Figures 4.1 and 4.2. In Figure 4.1, the growth curves for two agitation speeds (100 and 600 rpm) with ordinary air sparging are compared. The inoculum in both runs was 0.1 gm (dry cell mass) l^{-1}. In the 100 rpm run, a lag phase occurred over the first 4 hours following by 8 hours of log phase where exponential growth occurred. The final dry cell mass concentration obtained in this run was 2.2 gm l^{-1}. In the 600 rpm run, a lag phase was very short (approximately 1 h), and the log phase lasted until hour 15, yielding a stationary phase with cell mass concentration of 3.5 gm l^{-1}. Comparison of the same two fermentations with MBD sparging shows a lag phase of about 1 hour followed by a log phase of approximately 15 hours (Figure 4.2). The final dry cell mass concentrations for both runs were 3.4 and 3.5 gm l^{1}.

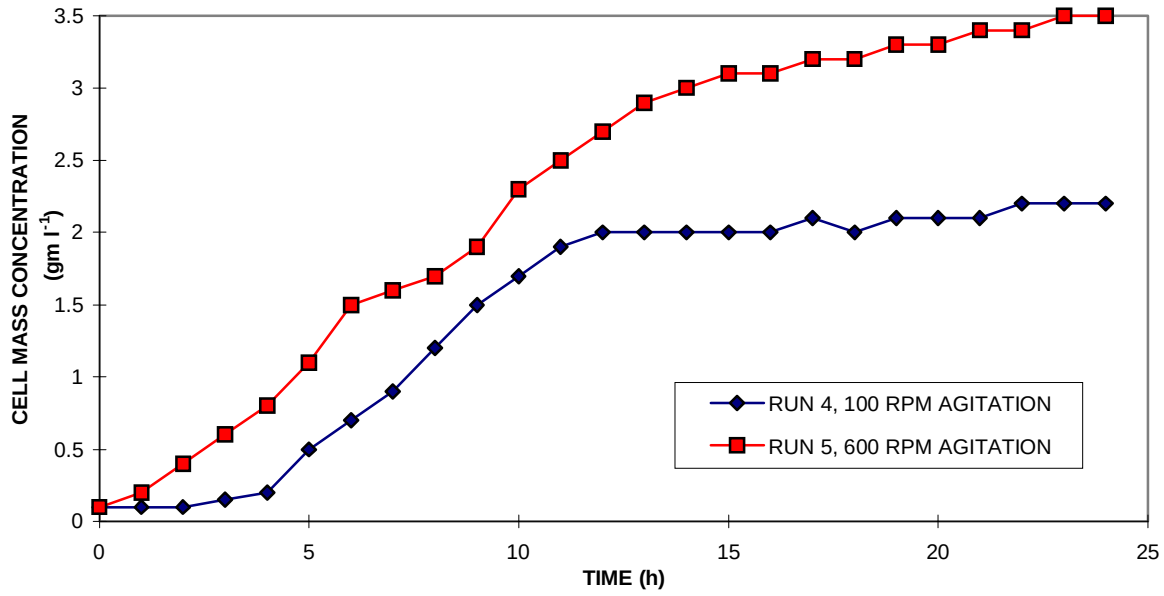

Figure 4.1 Batch cultivation of yeast *S. cerevisiae* with ordinary air sparging.

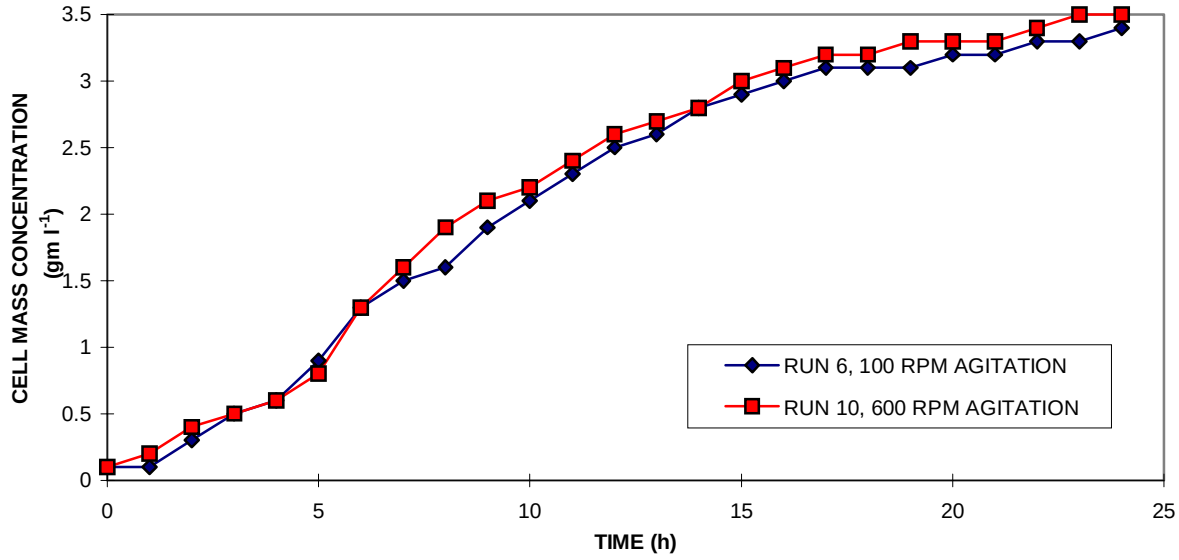

Figure 4.2 Batch cultivation of yeast *S. cerevisiae* with MBD sparging.

4.1.2 Main experiments

The scale-up application of the MBD was done using a 72-liter pilot scale fermenter for 20-liter and in 50-liter working volume fermentations with a 1-liter MBD generator. The experimental conditions are summarized in Table 4.1.

Figures 4.3 to 4.6 present the cell mass concentrations, pH, residual sugar concentrations, and dissolved oxygen curves measured during 24-hour batch cultivation in the 20-liter fermentations. The comparison of the growth pattern for all runs is shown in Figure 4.7. The 150 rpm, air sparged test had a lag phase of 1 hour following by a log phase of 6 hours. The final cell mass obtained for this run was 2.1 gm l^{-1}. In the other three runs, the growth patterns were very similar. The distinct lag phase was not seen. The yeast cells were in the log phase for approximately 16 hours until they attained steady state with a final cell mass concentrations of about 3.3 gm l^{-1}. The maximum specific growth rate in the air sparged, 150 rpm run was 0.26 h^{-1}, while in the other three runs, these values fell in the range from 0.31 to 0.33 h^{-1}.

Table 4.1 Control conditions used in main experiment.

Oxygen Supply Method	Agitation Speed (rpm)	Working Volume (liter)	Run No.
Air	150	20	17
MBD	150	20	18
Air	500	20	16
MBD	500	20	14
Air	150	50	22
MBD	150	50	21
Air	500	50	19
MBD	500	50	20

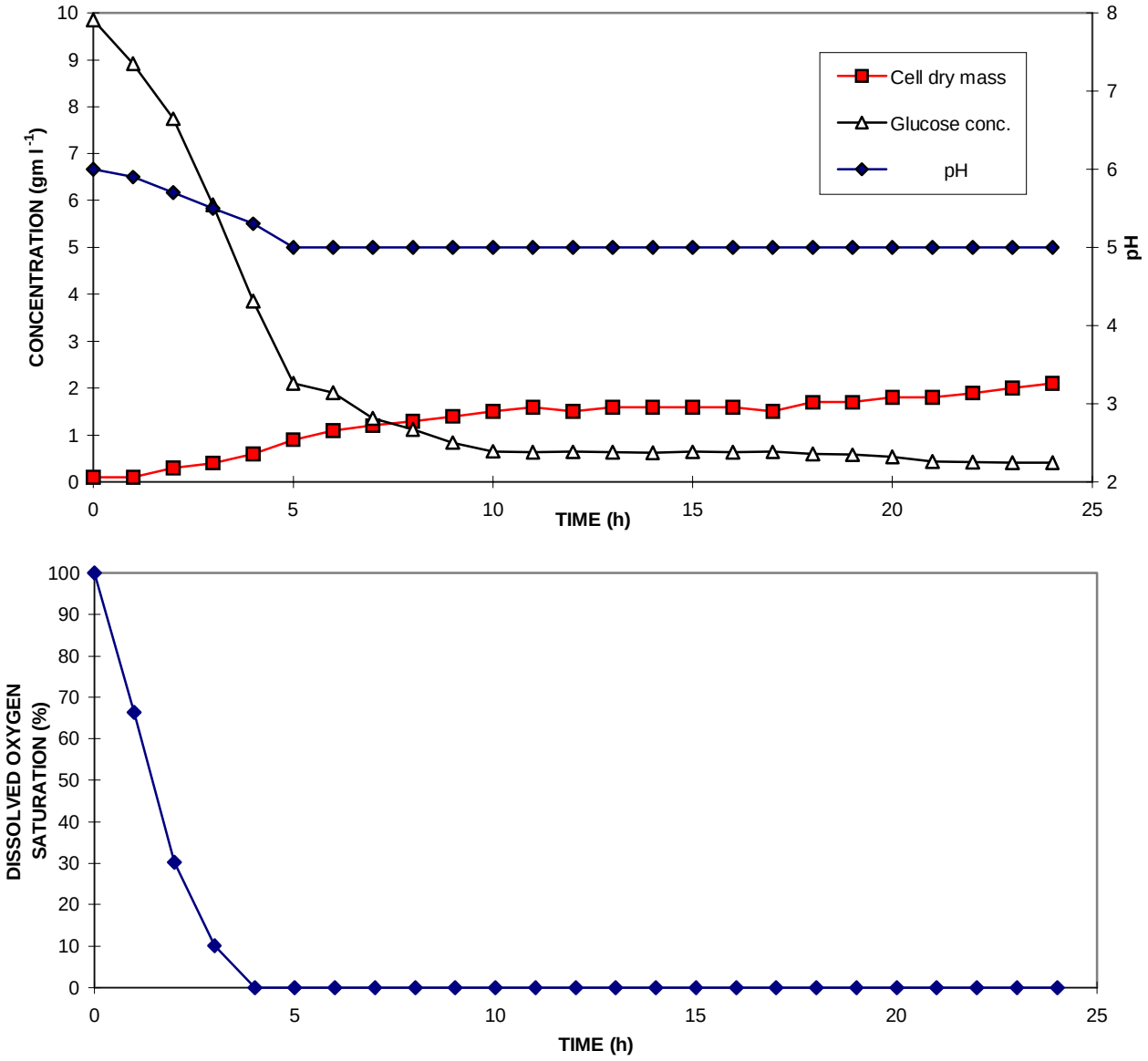

Figure 4.3 Batch cultivation of yeast *S. cerevisiae* with air sparging, 150 rpm agitation, 20 liter working volume.

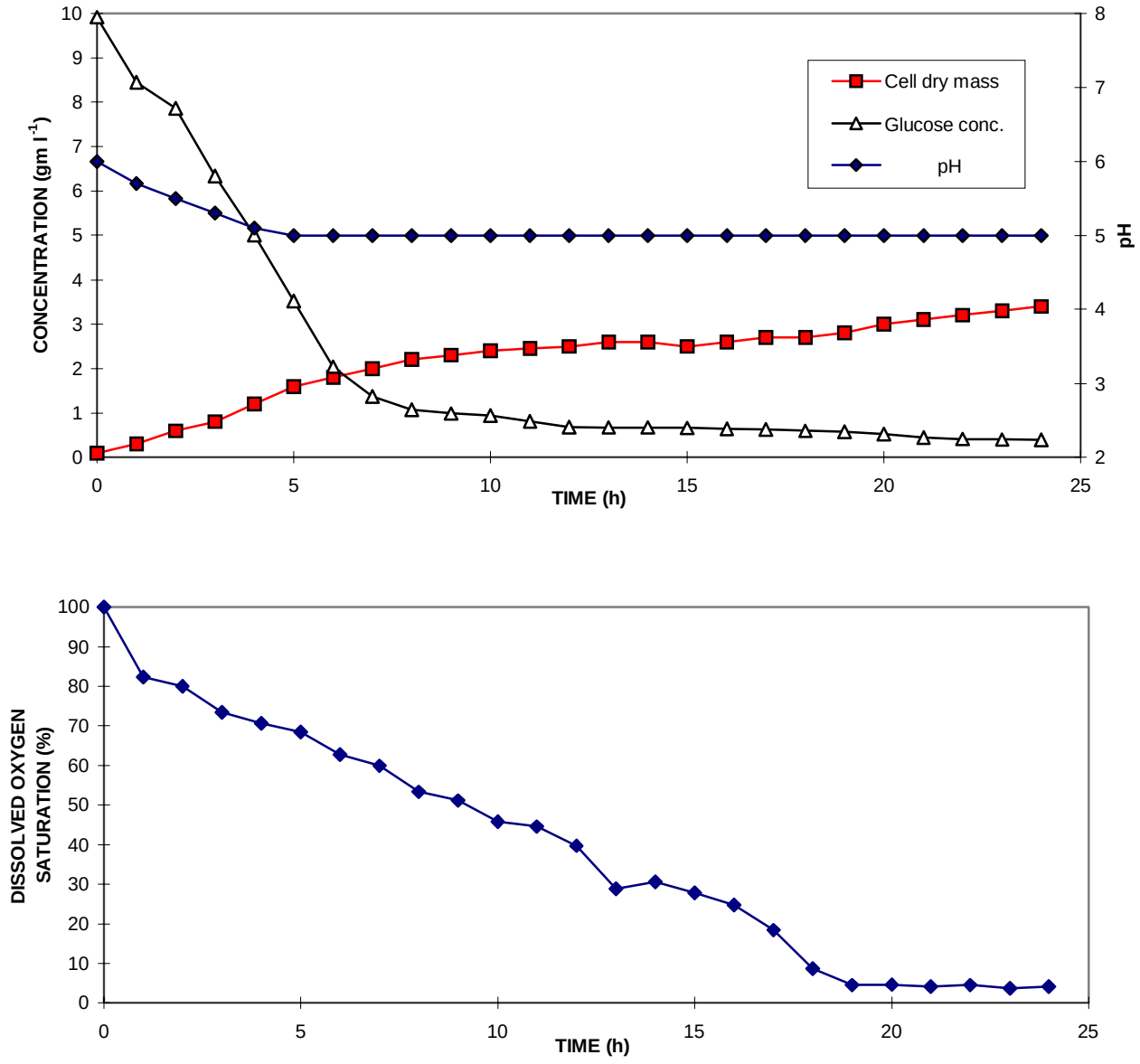

Figure 4.4 Batch cultivation of yeast *S. cerevisiae* with MBD sparging, 150 rpm agitation, 20 liter working volume.

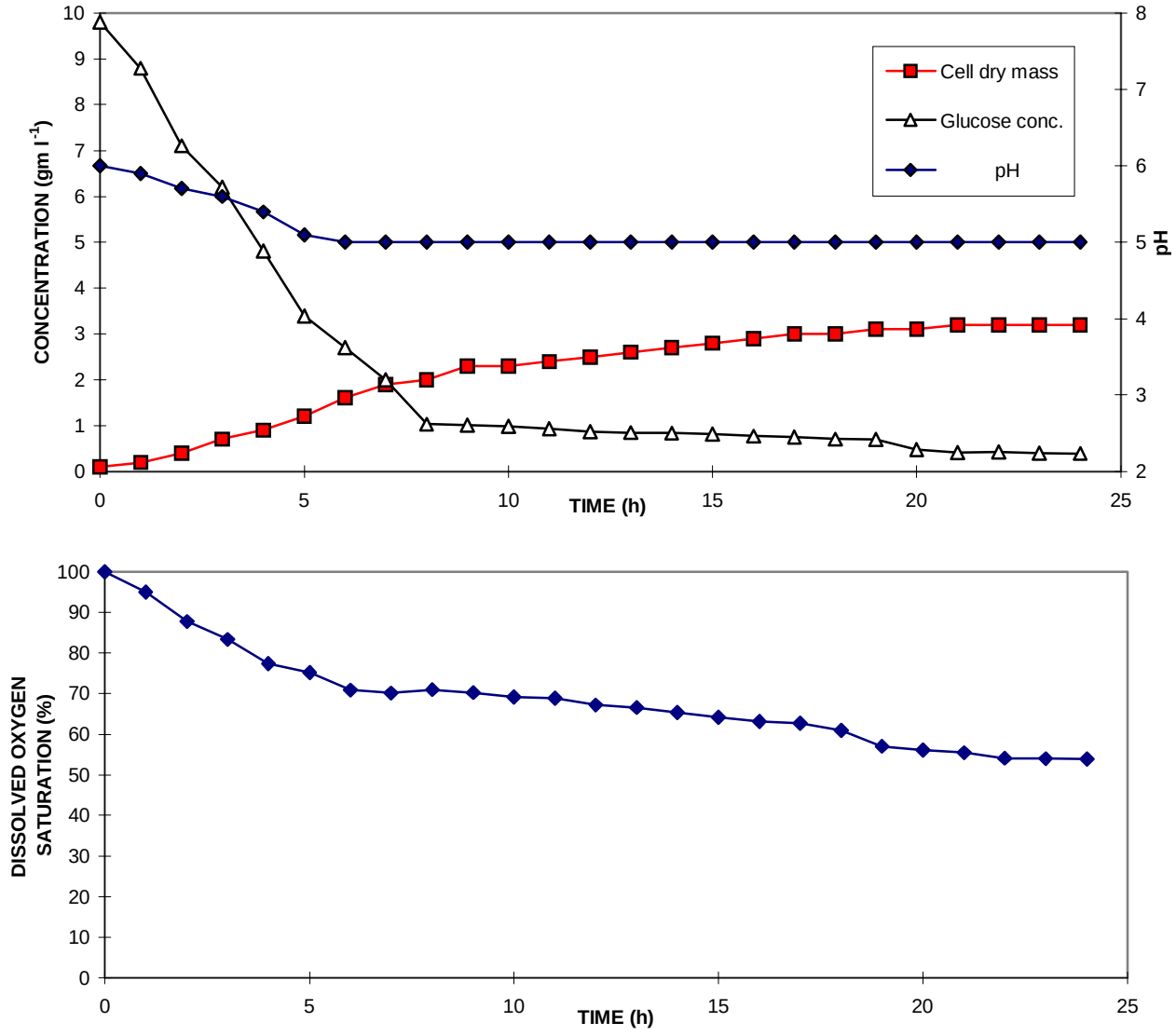

Figure 4.5 Batch cultivation of yeast *S. cerevisiae* with air sparging, 500 rpm agitation, 20 liter working volume.

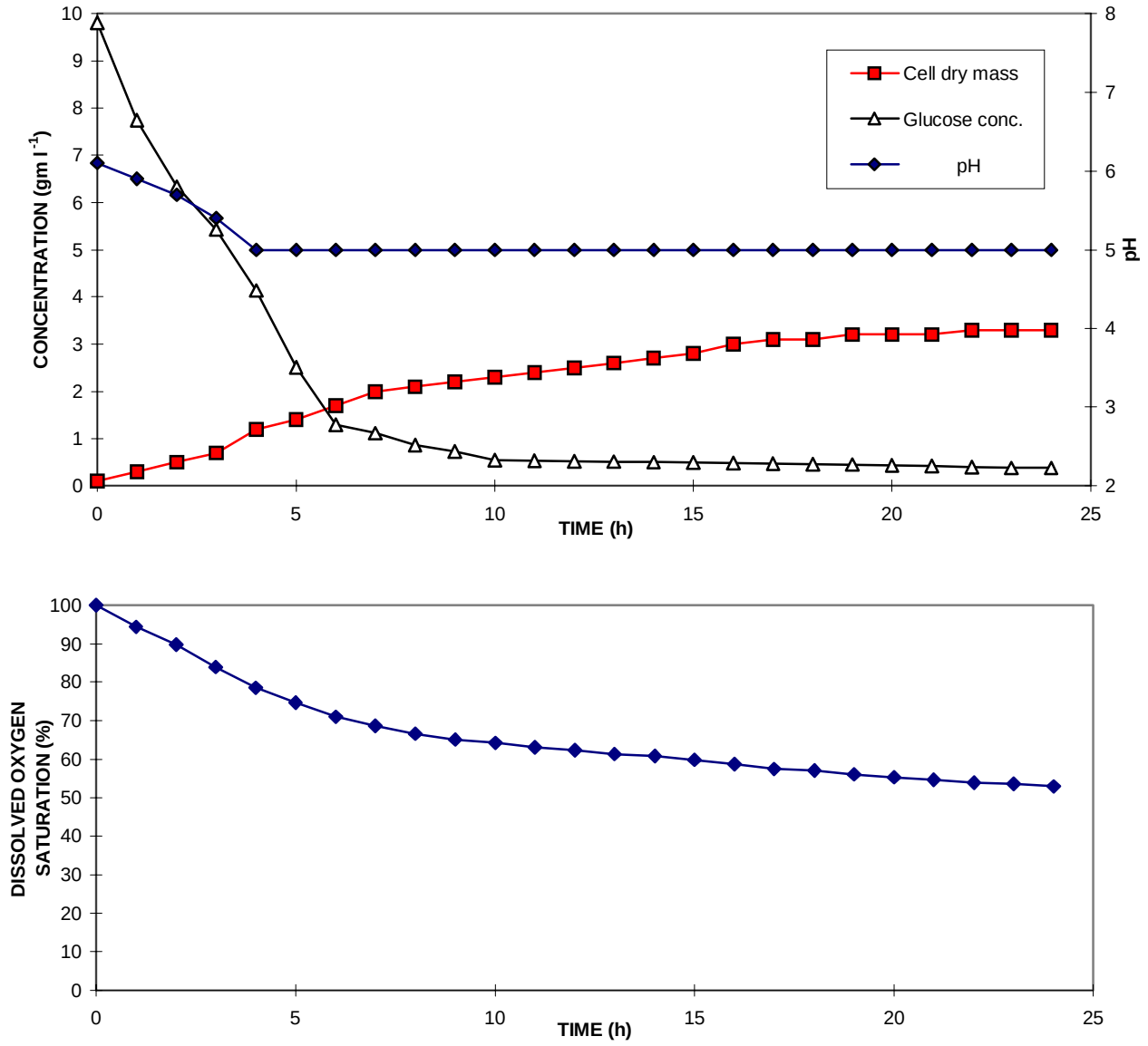

Figure 4.6 Batch cultivation of yeast *S. cerevisiae* with MBD sparging, 500 rpm agitation, 20 liter working volume.

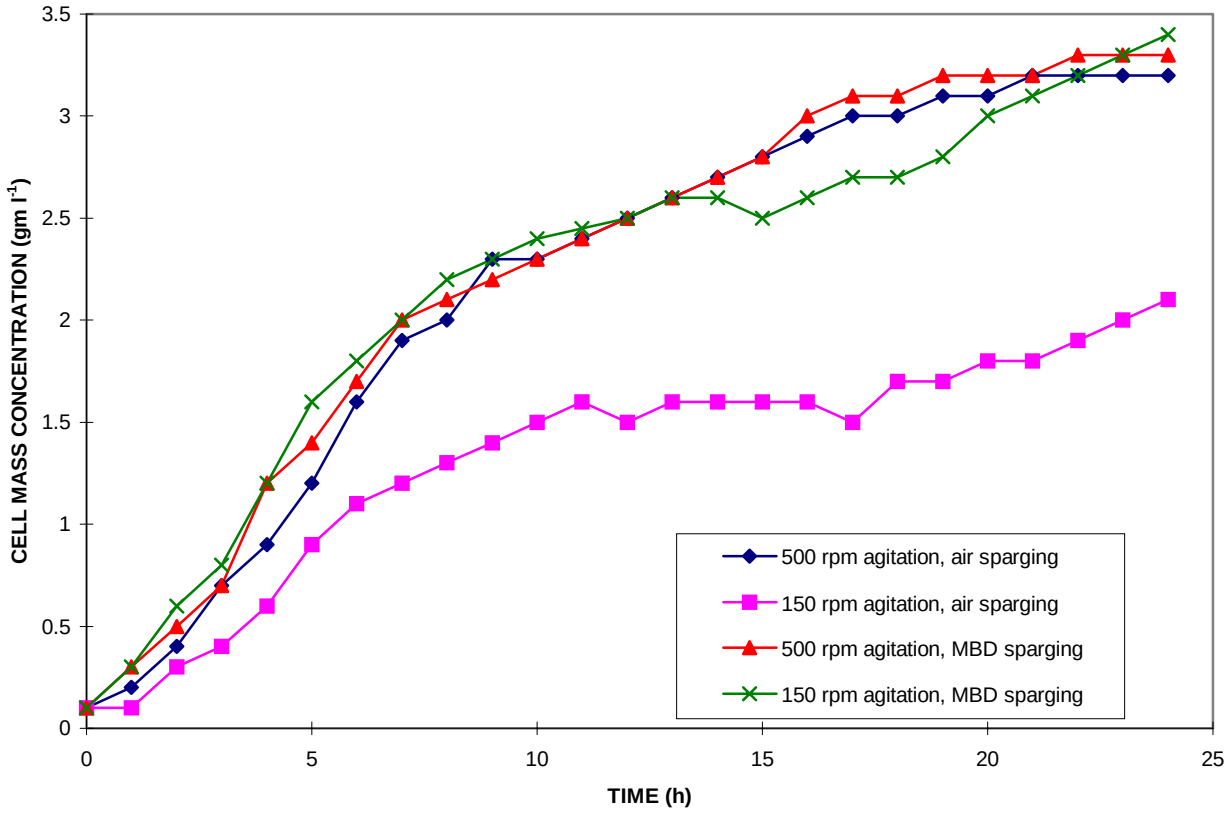

Figure 4.7 Comparison of yeast growth patterns, 20 liter working volume fermentations.

The comparison of the dissolved oxygen profiles is shown in Figure 4.8 (150 rpm agitation) and in Figure 4.9 (500 rpm agitation). In the highly agitated runs (Figure 4.9), the oxygen saturation levels of both air sparged and MBD sparged runs were held above 50% throughout the 24-hour fermentation, whereas in the low agitation runs (Figure 4.8), the oxygen level in the air sparged run fell to zero at hour 4. The oxygen level in the MBD run fell below 50% at hour 9 and continued to decline until hour 19, when it was approximately zero.

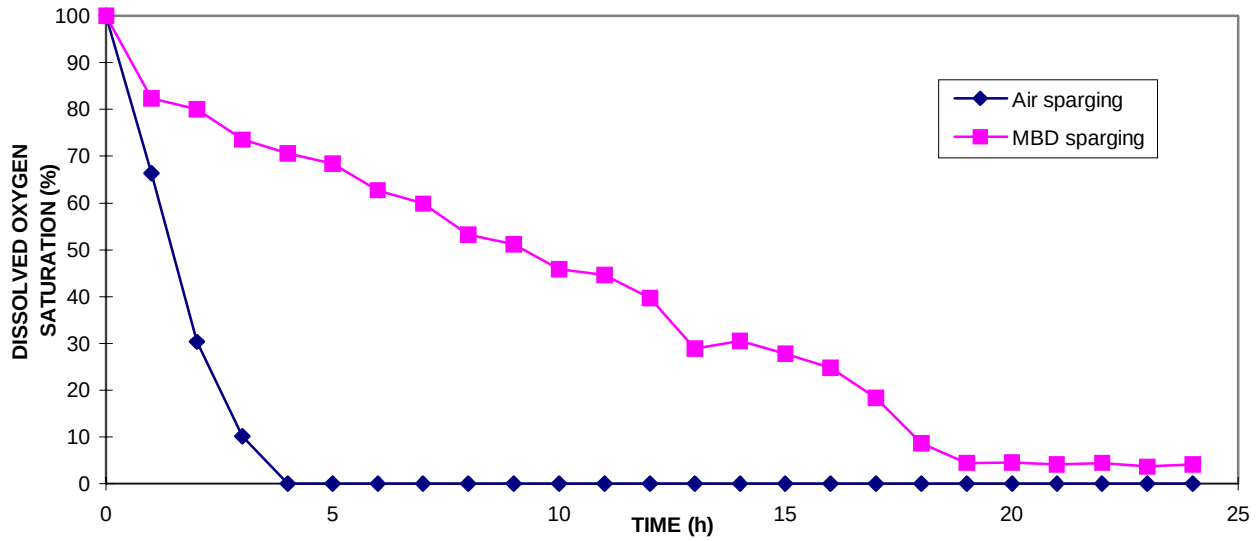

Figure 4.8 Comparison of dissolved oxygen profiles, 150 rpm agitation.

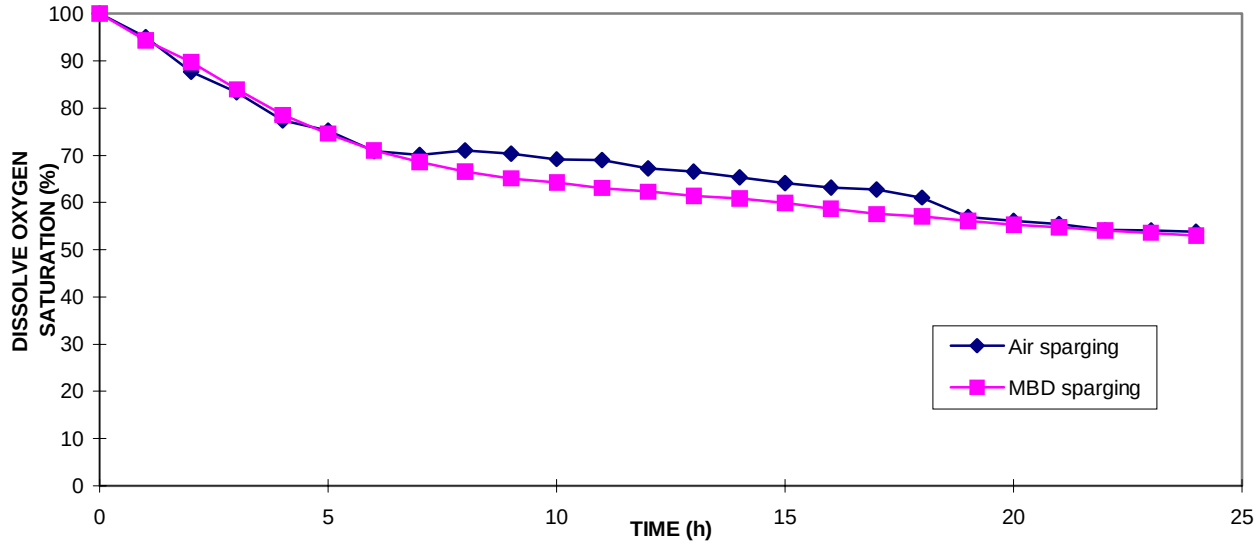

Figure 4.9 Comparison of dissolved oxygen profiles, 500 rpm agitation

Having demonstrated the capability of the 1-liter MBD generator to effectively supply the oxygen demand of the organism in a 20-liter fermentation, experiments were then done in a 50-liter fermentation to investigate the maximum efficiency of the 1-liter MBD generator. Figures 4.10 to 4.13 present the plots of cell mass concentrations, pH, glucose residue and dissolved oxygen for the four 50-liter fermentations during 24-hour batch cultivation.

The growth curves for the four 50-liter fermentations are compared in Figure 4.14. The cell growth for the air sparged, 150 rpm fermentation was the only one that definitely differed from the other runs. It had a longer lag phase of about 4 hours followed by a log phase of 8 hours and had the lowest final cell mass at 2.2 gm l^{-1}. For the other three fermentations, they all had a short lag phase of approximately 1 hour before growing exponentially and finally achieving a cell mass concentration of approximately 3.3 gm l^{-1}. However, there was an aberration during the log phase between the air and the MBD sparged runs. At hour 13, the yeast culture in both high- and low- agitated MBD sparged runs was still in the middle of the log phase. In the high-agitated air sparged run, yeast growth had started entering steady state by this hour. The yeast in both MBD sparged runs continued to grow and ultimately reached the same final cell mass at hour 22 as the 500 rpm, air sparged run.

Figures 4.15 and 4.16 compare the dissolved oxygen profiles in the 150 rpm agitation fermentations and in the 500 rpm agitation fermentations, respectively. For the two 500-rpm fermentations (Figure 4.16), the dissolved oxygen concentration were held at approximately 50% saturation throughout the experiment. In the 150-rpm runs, the MBD sparging was able to hold the dissolved oxygen above zero until hour 16, while in the air sparged run, percent oxygen saturation dropped to zero at hour 3 (Figure 4.15).

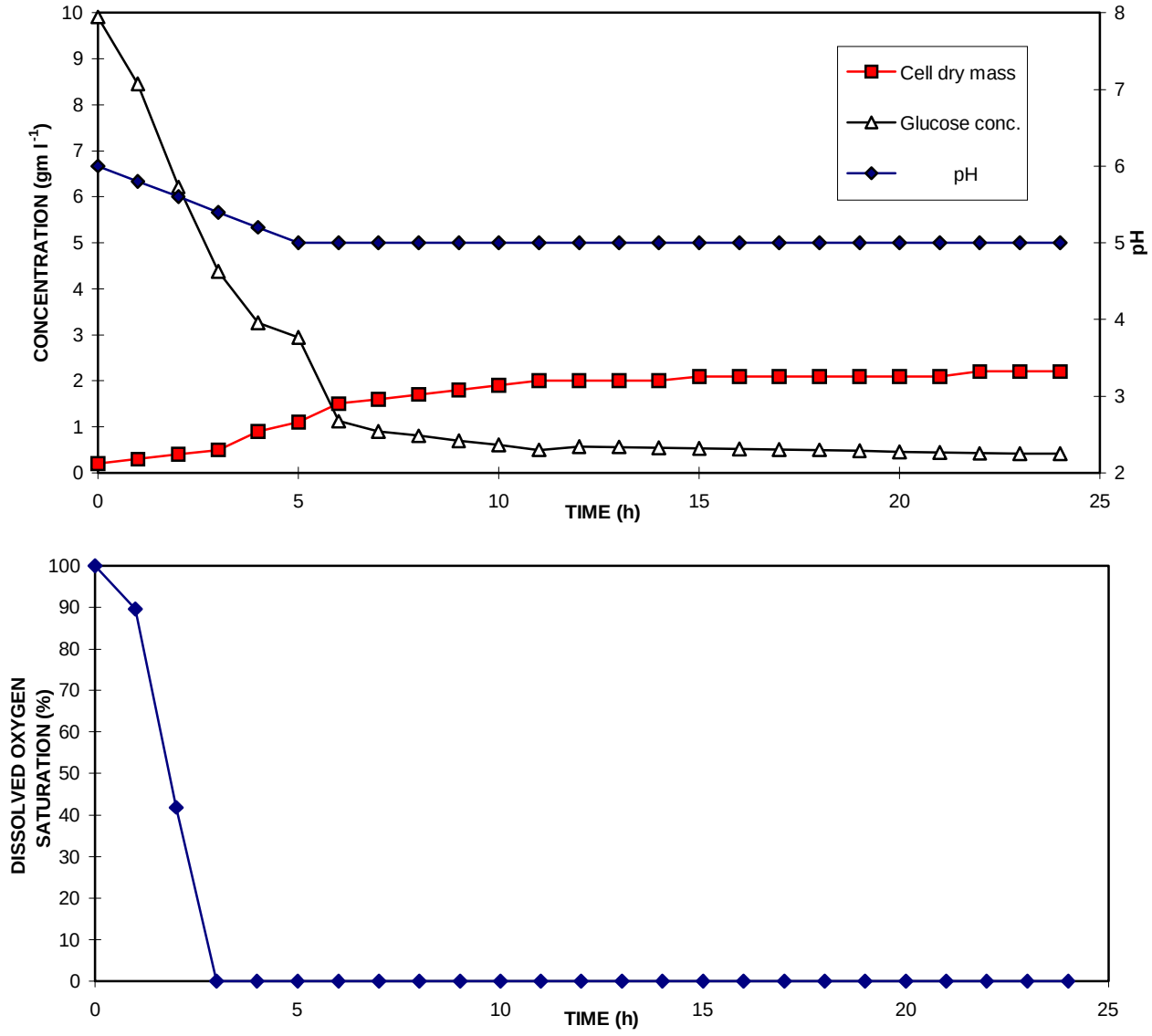

Figure 4.10 Batch cultivation of yeast *S. cerevisiae* with air sparging, 150 rpm agitation, 50 liter working volume.

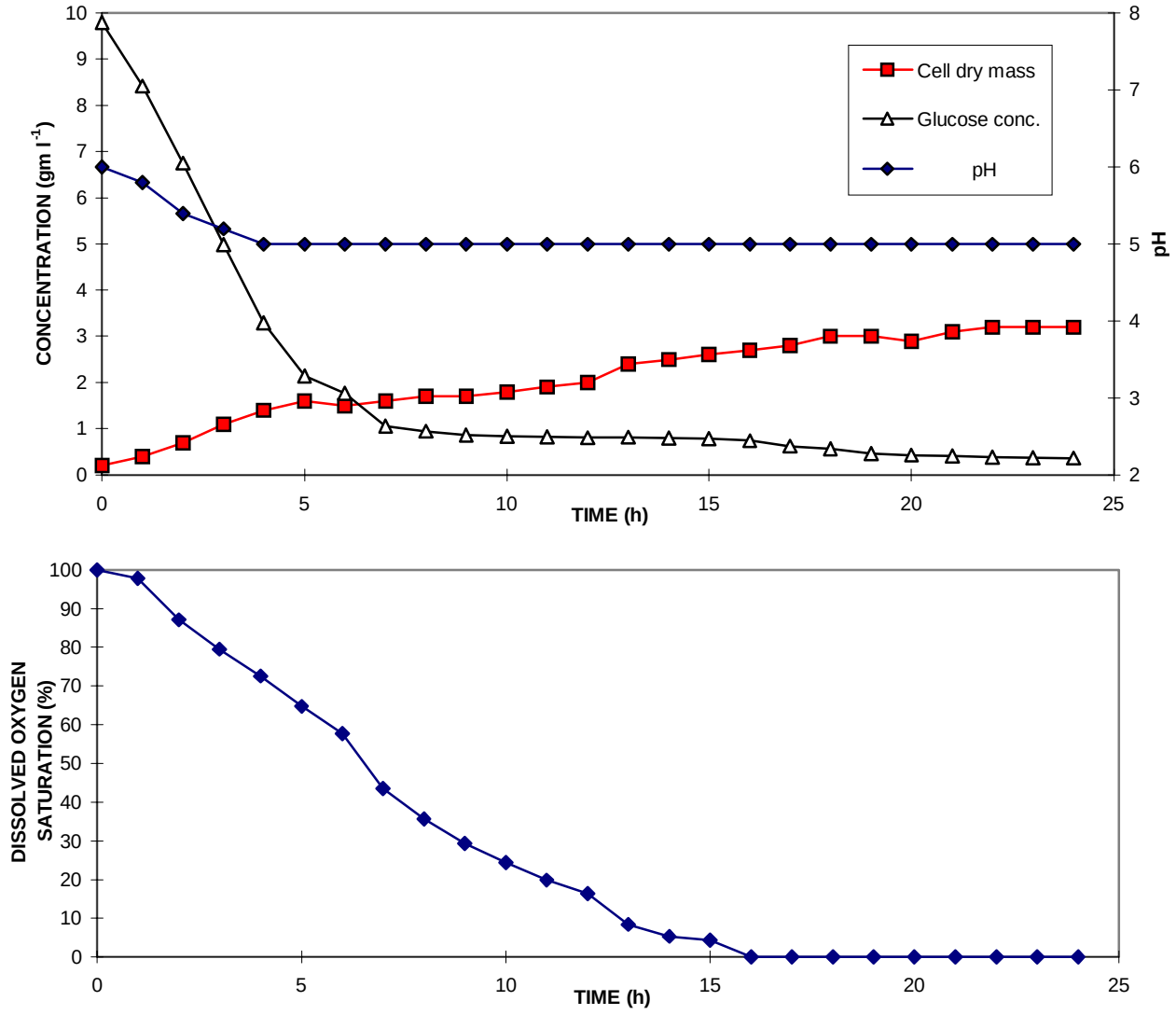

Figure 4.11 Batch cultivation of yeast *S. cerevisiae* with MBD sparging, 150 rpm agitation, 50 liter working volume.

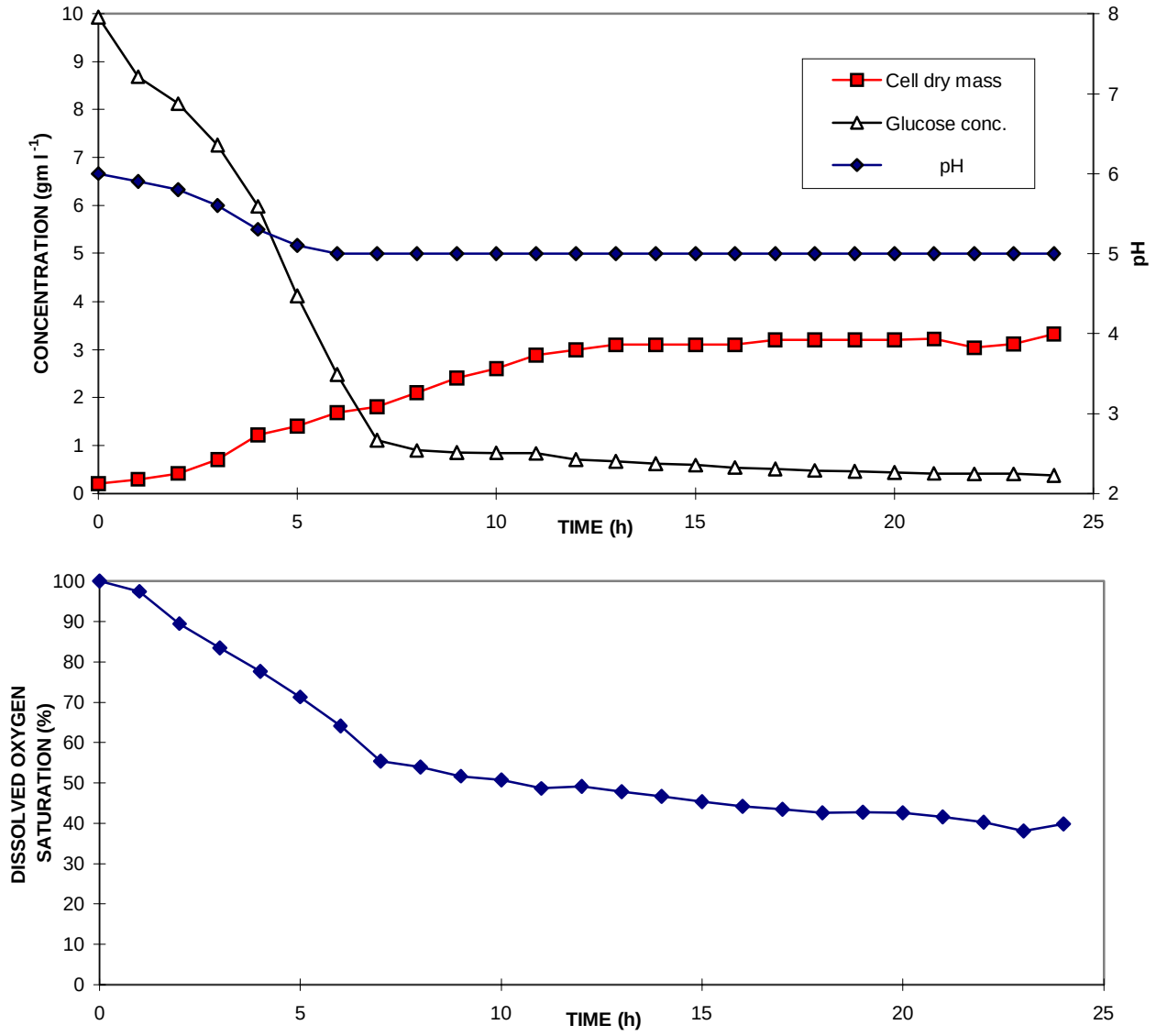

Figure 4.12 Batch cultivation of yeast *S. cerevisiae* with air sparging, 500 rpm agitation, 50 liter working volume.

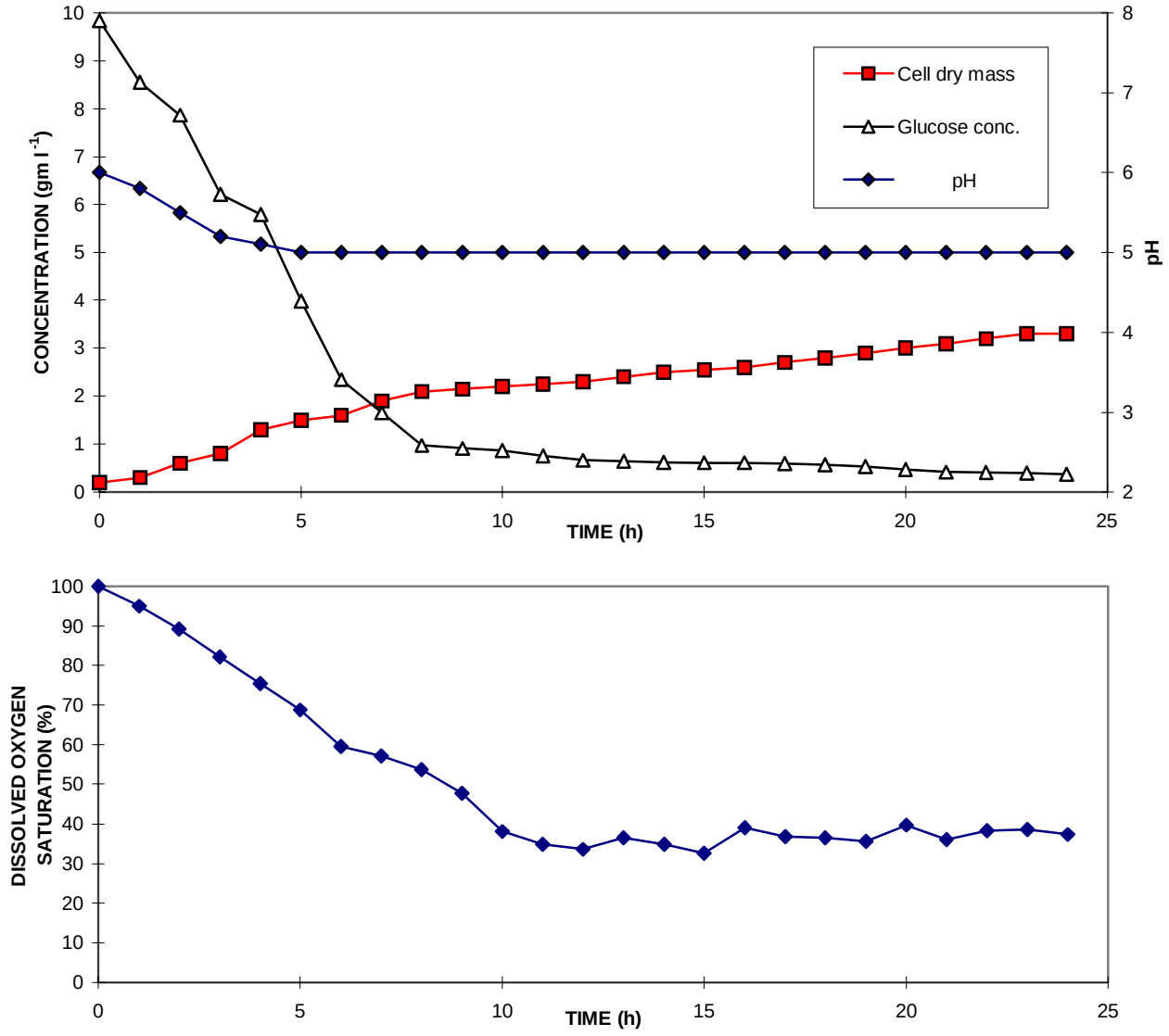

Figure 4.13 Batch cultivation of yeast *S. cerevisiae* with MBD sparging, 500 rpm agitation, 50 liter working volume.

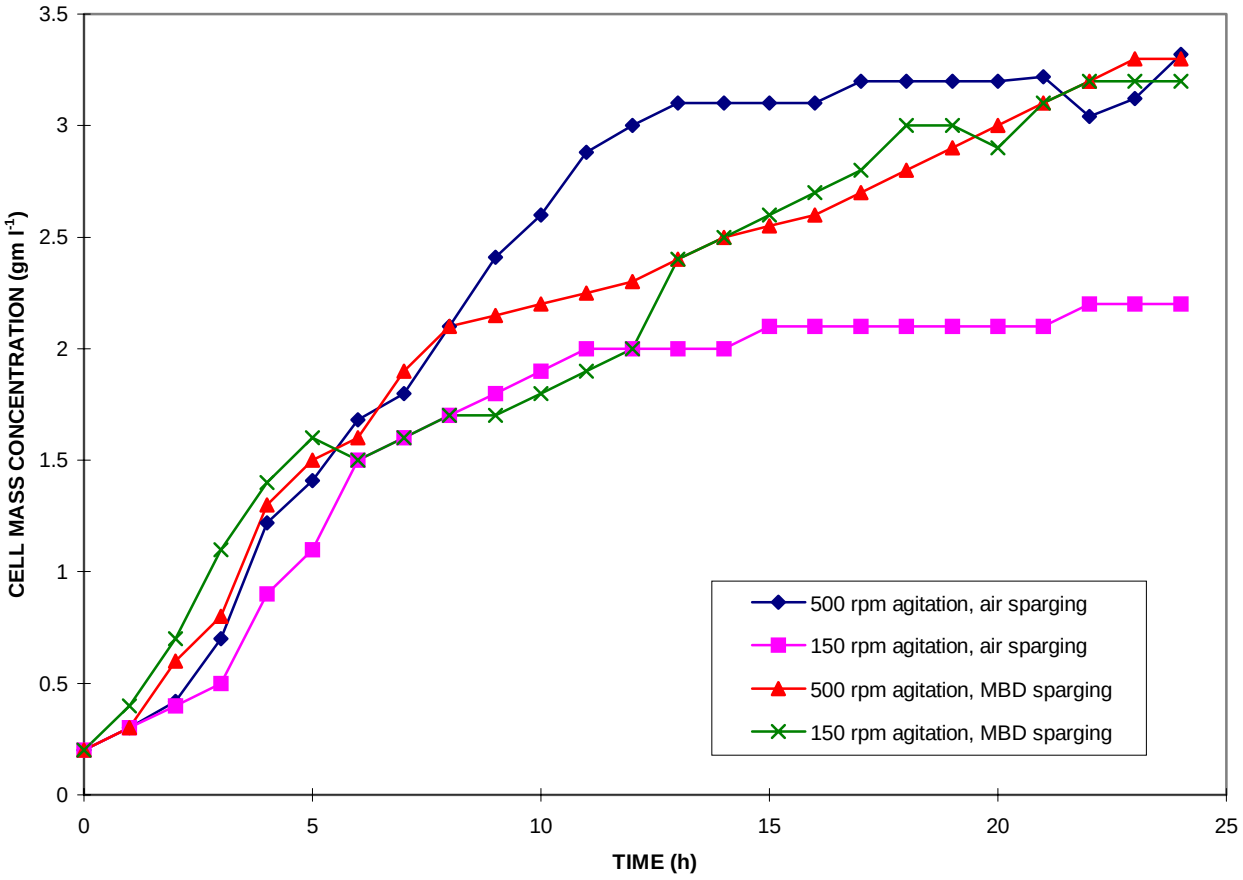

Figure 4.14 Comparison of yeast growth patterns, 50 liter working volume fermentations.

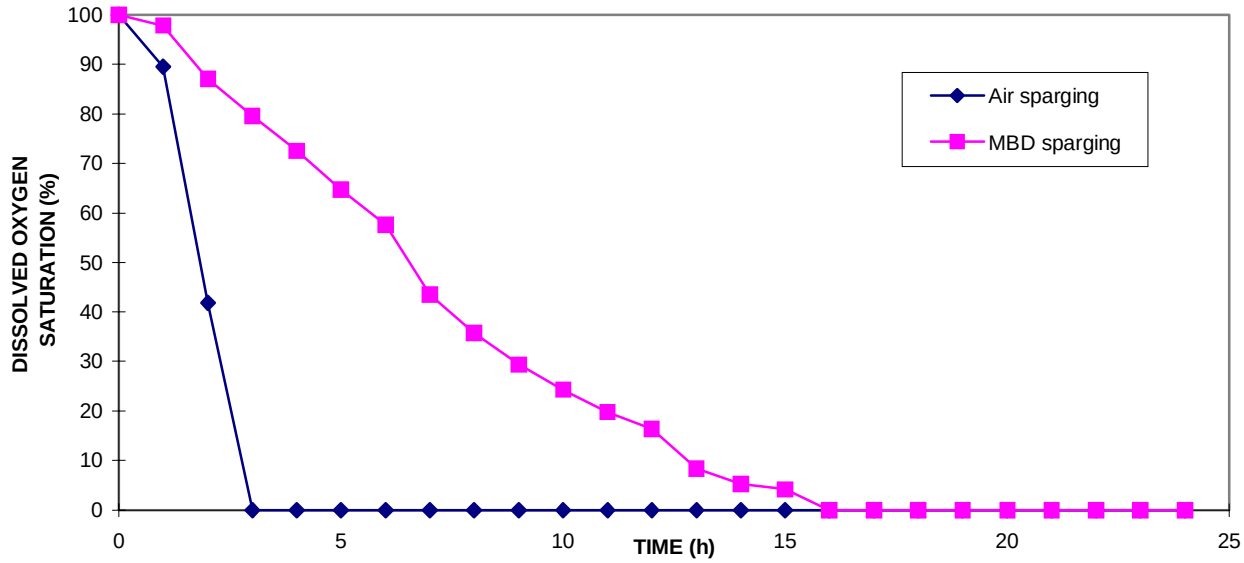

Figure 4.15 Comparison of dissolved oxygen profiles, 150 rpm agitation.

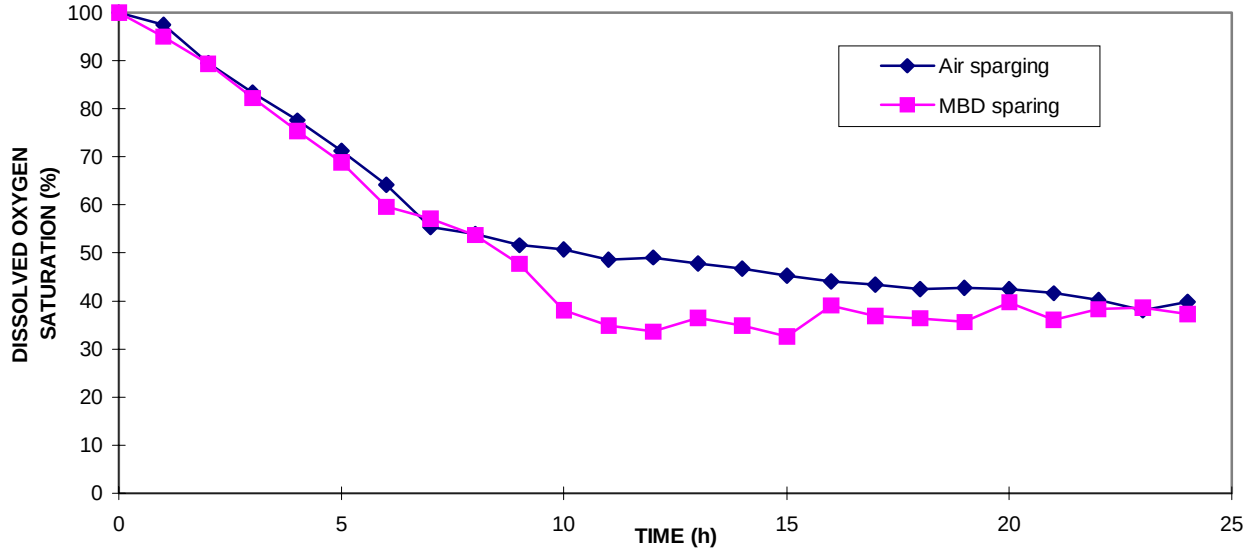

Figure 4.16 Comparison of dissolved oxygen profiles, 500 rpm agitation.

The volumetric oxygen transfer coefficients, K_La, were calculated by the yield coefficient method. The values obtained are given in Table 4.2. In the 20-liter fermentations, as the agitation speed increased from 150 to 500 rpm in the air sparged runs, the K_La values increased from 142.5 to 458.3 h^{-1}. While in the MBD sparged runs, the K_La values were 453.2 h^{-1} for the 150 rpm agitated run and 515.6 h^{-1} for the 500 rpm agitation. When the same agitation speeds were used for the 50-liter fermentations, the K_La values increased from 136.1 to 473.3 h^{-1} in the air sparged runs, and from 293.4 to 390.6 h^{-1} in the MBD runs.

The power consumption calculated for the 1.5 liter, 20 liter, and 50 liter fermentations are given in Tables 4.3, 4.4, and 4.5, respectively. The power consumption values were compared between the air and MBD sparged runs with two speed agitations. The total power consumption for each run included the power consumed by the agitator, air compressor, and MBD generator (for the MBD runs).

Table 4.3 shows the power consumption calculated for the 1.5-liter fermentations. With 150 rpm agitation in the air sparged run, the power consumed was 7.8×10^{-6} kw by the agitator and 13.4×10^{-6} kw by the air compressor. Total power was 21.2×10^{-6} kw, and the total power per unit volume of liquid broth was 14.1×10^{-6} kw l^{-1}. In the 150 rpm MBD run the power consumed was 7.8×10^{-6}, 13.4×10^{-6}, and 1081×10^{-6} kw for the agitator, air compressor, and MBD generator, respectively. The total power in this run was 1102×10^{-6} kw and the power per unit volume was 735×10^{-6} kw l^{-1}. In the 500 rpm air sparged run, the power consumed by the agitator and air compressor was 1790×10^{-6} and 13.4×10^{-6} kw, respectively. The total power per volume was 1202×10^{-6} kw l^{-1}. With the addition of 1081 kw for the MBD generator at the same agitation speed, the total power in the 500 rpm-MBD run was 2884×10^{-6} kw and the total power per unit volume of broth was 1923×10^{-6} kw l^{-1}.

Table 4.2 Calculated oxygen transfer coefficients and maximum specific growth rates.

Oxygen Supply Method	Agitation Speed (rpm)	Working Volume (liter)	Run No.	K_La (h^{-1})	μ (h^{-1})
Air	150	20	17	142.5	0.26
MBD	150	20	18	453.2	0.31
Air	500	20	16	458.3	0.33
MBD	500	20	14	515.6	0.33
Air	150	50	22	136.1	0.19
MBD	150	50	21	293.4	0.23
Air	500	50	19	473.3	0.29
MBD	500	50	20	390.6	0.28

Table 4.3 Power consumption calculated for the 1.5-liter fermentations.

Device Consuming	Agitation, Sparging Method			
Power	150 rpm, Air	150 rpm, MBD	500 rpm, Air	500 rpm, MBD
……..……kw x 10^{-6}……..				
Agitator	7.8	7.8	1790	1790
Air compressor	13.4	13.4	13.4	13.4
MBD generator	-	1081	-	1081
Total	21.2	1102	1803	2884
Total (kw x 10^{-6} l^{-1})	14.1	735	1202	1923

Table 4.4 Power consumption calculated from the 20-liter fermentations.

Device Consuming	Agitation, Sparging Method			
Power	150 rpm, Air	150 rpm, MBD	500 rpm, Air	500 rpm, MBD
……kw x 10^{-6}……				
Agitator	2088	2088	84786	84786
Air compressor	3729	3729	3729	3729
MBD generator	-	22744	-	22744
Total	5817	28561	88515	111259
Total (kw x 10^{-6} l^{-1})	291	1428	4426	5563

Table 4.5 Power consumption calculated for the 50-liter fermentations.

Device Consuming Power	Agitation, Sparging Method			
	150 rpm, Air	150 rpm, MBD	500 rpm, Air	500 rpm, MBD
..……..……kw x 10^{-6}……				
Agitator	1864	1864	81207	81207
Air compressor	7457	7457	7457	7457
MBD generator	-	30797	-	30797
Total	9321	40118	88664	119461
Total (kw x 10^{-6} l^{-1})	186	802	1773	2389

The power consumption calculated for the 20-liter fermentations is shown in Table 4.4. In the 150 rpm air sparged run, the power consumed by the agitator and air compressor was 2088×10^{-6} and 3729×10^{-6} kw, respectively. The total power requirement in this run was 5817×10^{-6} kw, and the total power per unit volume was 291×10^{-6} kw l^{-1}. In the 150 rpm MBD run, the power consumed was 2088×10^{-6}, 3729×10^{-6}, and 22744×10^{-6} kw for the agitator, air compressor, and the MBD generator, respectively. The combined power of these three components was 28561×10^{-6} kw, and the total power per unit volume was 1428×10^{-6} kw l^{-1}. In the 500 rpm air sparged run, the power consumed by the agitator and the air compressor was 84786×10^{-6} and 3729×10^{-6} kw, respectively. The total power per volume was 4426×10^{-6} kw l^{-1}. In the 500 rpm MBD run, the power consumed was 84786×10^{-6}, 3729×10^{-6}, and 22744×10^{-6} kw for the agitator, air compressor, and the MBD generator, respectively. The total power in this run was 111259×10^{-6} kw and the power per unit volume was 5563×10^{-6} kw l^{-1}.

Table 4.5 shows the power consumption calculated for the 50-liter fermentations. In the 150 rpm air sparged run, the power consumed was 1864×10^{-6} and 7457×10^{-6} kw for the agitator and the air compressor, respectively. The total power in this run was 9321×10^{-6} kw, and the power per unit volume was 186×10^{-6} kw l^{-1}. For the 150 rpm MBD fermentation, the power values were 1864×10^{-6} kw for the agitator, 7457×10^{-6} kw for the air compressor and 30797×10^{-6} kw for the MBD generator. The total power was 40118×10^{-6} kw, and the total power per unit volume of liquid broth was 802×10^{-6} kw l^{-1} In the 500 rpm air sparged run, the power consumed by the agitator and the air compressor was 81207×10^{-6} and 7457×10^{-6} kw, respectively. Total power was 88664×10^{-6} kw l^{-1}, and total power per unit volume of broth was 1773 kw l^{-1}. In the 500 rpm MBD run, the power consumed was 81207×10^{-6}, 7457×10^{-6}, and 30797×10^{-6} kw for the agitator, air compressor, and the MBD generator, respectively. The total power for this run was 119461×10^{-6} kw, and the power per unit volume was 2389×10^{-6} kw l^{-1}.

Power per unit volume of broth calculated for the 20-liter and 50-liter experiments is summarized in Table 4.6. In the 20-liter fermentations, when oxygen transfer rate was enhanced by increasing agitation speed from 150 to 500 rpm, the power per unit volume increased by 1421%. Comparing MBD sparging, 150 rpm with air sparging, 500 rpm, the total power

consumption for MBD sparging was 32% of that required for the increased agitation speed. The same oxygen transfer capability (Table 4.2) was achieved with one-third the power input. Increasing the agitation speed in the 50-liter experiments required almost a 10-fold increase in the total power per unit volume. Again comparing MBD sparging, 150 rpm with air sparging, 500 rpm, the total power consumption for MBD sparging was 45% of that required for the increased agitation speed.

Table 4.6 Power consumption per unit volume calculated for the 20-liter and 50-liter fermentations.

Oxygen Supply Method	Agitation Speed (rpm)	Power Consumption (kw x 10^{-6} l^{-1})	
		20-liter	50-liter
Air	150	291	186
MBD	150	1428	802
Air	500	4426	1773
MBD	500	5563	2389

4.2 Discussion

Favorable characteristics of oxygen transfer in an aerobic fermentation system can be indicated by the K_La value and by the pattern of product formation. In the aerobic fermentation of Baker's yeast, cell mass is the desired product; therefore, the biomass growth profile can be used as the indicator that oxygen transfer is sufficient to satisfy the demand of the organism.

4.2.1 Preliminary experiments

From the preliminary investigation in the small laboratory-scale fermentations, the results between 100 rpm and 600 rpm agitation with air sparging (Figure 4.1) clearly show the difference in the yeast growth pattern. The 100 rpm-air sparged run was oxygen limited due to the low speed agitation, and this caused the longer lag phase and the decrease in cell mass concentration. As compared to the growth profile in the two MBD runs, (Figure 4.2), there was no significant difference in growth pattern between the high and low speed agitations used. These results in the preliminary tests agreed with the earlier work conducted by Kaster *et al*. (1990). The use of MBD enhanced the oxygen transport efficiency in aerobic fermentation. The oxygen transport characteristics of the MBD were not affected by the amount of agitation in the vessel. Therefore, the low-agitated MBD sparged fermentation, which consumed approximately 40% less power than the high-agitated air sparged run, could be used in aerobic culture to overcome the oxygen limitation. The definite advantages of MBD sparging in the laboratory-scale fermenter were proven. The next step was to verify performance in the scale up to pilot scale. According to Kaster *et al*. (1990), the 2-liter fermenter was undersized for the 1-liter MBD generator. It was found that only 15% of the supplied oxygen by the MBD was being consumed. In this study, the same size MBD generator was used to supply oxygen to 20-liter and 50-liter working volumes.

4.2.2 Main experiments

In the 20-liter fermentations, it was apparent that only yeast culture in the 150-rpm, air sparged run showed significant difference in growth pattern from the other runs (Figure 4.7). Examination of the percent oxygen saturation profiles showed the inadequate quantity of oxygen in this run. The depletion of dissolved oxygen caused a decrease in the final biomass production of about 36.4% over that achieved with the other three fermentations. The K_La value decreased in the air sparged runs from 458.3 to 142.5 h^{-1} (69% decrease) when the agitation speed was reduced from 500 to 150 rpm which clearly show that the oxygen transfer characteristics were strongly influenced by the agitation speed. The same reduction in the agitation speed for the MBD runs caused a decrease in the K_La value from 515.6 to 453.2 h^{-1}, or approximately 12%. It is very significant that the low-agitated MBD run had a high K_La value. The K_La value of 453.2 h^{-1} in this run was equivalent to the K_La obtained in the 500 rpm, air sparged run (458.3 h^{-1}) and can be compared to the K_La in the 500 rpm MBD run (515.6 h^{-1}). The advantage of the low-agitated MBD fermentation as compared to the high-agitated air sparged run was similar in the 20-liter scale-up to the advantage observed in the laboratory-scale experiments. This ability of the MBD to maintain the rate of oxygen transfer from the gas phase to the liquid phase at low agitation speed was attributed to the smaller size of bubbles produced by the MBD generator. These surfactant-stabilized microbubbles provided greatly increased interfacial area and longer residence time for the oxygen to transfer. It is evident that the 1-liter MBD generator was able to overcome the mass transfer limitation and increase the oxygen transfer in the 20-liter aerobic fermentation experiment using an MBD flow rate of 85 ml min^{-1} $liter^{-1}$working volume.

The results for the 50-liter batch fermentations showed a similar trend to the results in the 20-liter experiments. For the air sparged, 150-rpm fermentation, the dissolved oxygen concentration had decreased to zero by hour 3. It is obvious that 150 rpm does not provide adequate agitation when the oxygen is supplied with normal air sparging. Using MBD sparging

did increase oxygen transfer. For the 150-rpm agitation, the dissolved oxygen concentration did not decrease to zero until hour 16 (Figure 4.15). After hour 16, the oxygen was being consumed at the rate it was being introduced. For the 500-rpm MBD run, the oxygen availability could be maintained throughout the 24-hour course (Figure 4.16). The final cell mass concentrations obtained for the MBD run with both high and low speed agitations were approximately the same as the concentration achieved with the high agitation, air sparged run. However, the benefit was not as high as observed in the laboratory-scale and 20-liter fermentations. Since the dissolved oxygen in the low-agitated MBD run decreased during the log phase, growth rate slowed. It took an additional 9 hours (from hour 13 to hour 22) for the yeast to achieve the same final cell mass concentration.

In the 50-liter fermentation experiments, the MBD runs produced an improvement in K_La values similar to the improvement found in the 20-liter experiments, but the magnitude of improvement was less. Even though the benefit of the MBD was less noticeable compared to that observed in the 20-liter fermentations, its contribution to oxygen transfer was still higher than ordinary air sparging. Therefore, the optimum performance of the 1-liter MBD generator probably falls somewhere between the 20- and 50-liter working volume fermentations.

As shown in Table 4.2, the MBD was able to maintain oxygen transfer such that the decrease in K_La was about 25% (from 390.6 to 293.4 h^{-1}) when agitation speed was reduced from 500 to 150 rpm. The same agitation speed decrease in the 20-liter fermentations produced a 12% decrease in K_La. In the air sparged runs, the same reduction in the agitation speed resulted in a 71% decrease in K_La (from 473.3 to 136.1 h^{-1}) for the 50-liter fermentations, which was approximately equal to the 69% decrease in the 20-liter runs. In the 500-rpm experiments, the air-sparged run actually had a higher K_La value (473.3 h^{-1}) than the MBD run (390.6 h^{-1}). This is significant since both runs had the same volumetric air flow rate. It is probable that the flow from the 1-liter MBD generator was insufficient to establish the oxygen supply for the organism population in a 50-liter fermentation.

It is clear that low oxygen solubility combined with slow oxygen transfer rates often results in reduced growth and culture productivity in conventionally aerated fermenters. The oxygen limited condition seriously affects the aerobic growth metabolism of yeast cells. It was reported by Gelinas and Goulet (1991) that a decrease in the availability of oxygen in the broth medium caused cell morphology damage to Baker's yeast, especially damage to the cytoplasmic membrane and mitochondria. This damage caused a malfunction in part of the aerobic metabolism. With the shift from aerobic to anaerobic environment, the yeast cells could no longer use the substrate to reproduce cell mass themselves but fermentatively converted the present glucose to CO_2 and ethanol instead.

From a power consumption point of view, the comparison in the power requirement between the low-agitated MBD and the high-agitated air sparged run points out the great economic advantage in the use of the MBD as an oxygen transfer method. The results from the 20-liter experiments showed that by using MBD with low agitation, the total power consumed per unit volume of broth could be reduced by 68% compared to conventional high agitation with air sparging. For the 50-liter experiments, the power value for the 150 rpm MBD fermentation was 55% lower than the 500 rpm air sparged run.

Increasing the oxygen transfer rate with the MBD was more effective than using intensive agitation in the fermenter and has a greater economic benefit. In commercial practice, the electrical power consumed for aeration and agitation usually comprises a major part of the production cost. The MBD can improve the economic profitability of the aerobic fermentations. Moreover, the MBD generator used in this system was not originally made for application with the stirred-tank fermenter. The proper design of an MBD generator to fit the system would improve the energy efficiency and further reduce costs in this application.

4.2.3 Discussion of the MBD flow rates

The setup of the MBD flow rates in the main experiment was initially made to produce the same ratio of the flow to working volume as in the laboratory-scale runs (270 ml min^{-1} $liter^{-1}$working volume). However, in the 20-liter experiments, the capacity of the peristaltic pump was not sufficient to achieve this ratio. The flow rate used in the experiments was the maximum flow which the available pump could provided (85 ml min^{-1} $liter^{-1}$working volume). When the 50-liter experiments were conducted, a higher capacity peristaltic pump was available. However, the desired flow rate still could not be obtained. Maximum flow was 150 ml min^{-1} $liter^{-1}$working volume. In addition, one more problem occurred in the 50-liter runs. The sparger was not originally made for connection of a MBD generator. The sparger connection was adapted by disassembling the air inlet and using a polypropylene connector to connect the MBD. The connection could not support the 150 ml min^{-1} $liter^{-1}$working volume flow of microbubbles. A bypass on the air inlet had to be used, and this possibly reduced the efficiency of the injection of MBD into the fermenter. The proportion of the 150 ml min^{-1} $liter^{-1}$ flow that entered through the bypass, rather than through the sparger, is unknown.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

The following conclusions can be drawn from this study

1. A 1-liter MBD generator can effectively increase oxygen transfer for yeast culture in 20-liter and 50-liter aerobic fermentations.

2. With MBD sparging, the oxygen transfer coefficients were found to be independent of the agitation speed, whereas, in the air sparged experiments, the oxygen transfer coefficients were strongly influenced by the amount of agitation.

3. The use of MBD to increase oxygen transfer required less power than the use of intensive agitation. The aspect of energy savings for the MBD is obviously favorable. Approximately 55% less power was required for the MBD fermentations as compared to the traditional air sparged fermentaions.

4. The improvement in oxygen transfer achieved with the MBD was highest in the 20-liter experiments. To achieve the maximum advantage of the MBD, the ratio of the fermenter volume to the microbubble generator volume must be optimized. For the 50-liter fermentations, flow of microbubbles from the 1-liter MBD generator was not sufficient to provide the needed oxygen. The optimal flow rate needs to be applied to achieve the highest benefit from the low-agitated MBD system.

Recommendations for future work :

1. MBD flow from the 1-liter MBD generator was 1.7 liter min^{-1} in the 20-liter fermentation. Flow was 7.5 liter min^{-1} in the 50-liter fermentation. The optimum MBD flow for the 20-liter fermentation is probably somewhere between 1.7 liter min^{-1} and 7.5 liter min^{-1}. For the 50-liter fermentation, the experimental results showed that a 7.5 liter min^{-1} flow of microbubbles does not provide sufficient oxygen. More experiments are needed to define the optimum flow of microbubbles.

2. The intensive use of agitation speed is limited for shear sensitive organisms. It is probable that the benefit of MBD will be greatest for the culture of these organisms. Future research should investigate the application of the MBD for shear sensitive organisms.

3. The construction of the MBD generator needs to be improved. Most of the problems were due to high speed revolution of the spinning disc, which resulted in a vibration problem. The Teflon shield around the shaft eroded and built up heat before the conclusion of this study. The placement of baffles in the generator also needs to be improved.

4. The maximum flow of microbubbles which can be produced with 1-liter generator is not known. The 1-liter generator may be able to produce more than 7500 ml min^{-1} flow.

5. Two ideas were developed to scale up the capacity of MBD generator. Two possibilities suggested here are an increase in either the spinning disc diameter (Figure 5.1) or an increase in the number of the spinning discs (Figure 5.2). Both methods increase the area of localized high shear zone between the disc surface and the liquid. It is probable that empirical relations can be developed to define the volume flow rate of microbubbles as a function of the area of the moving surface.

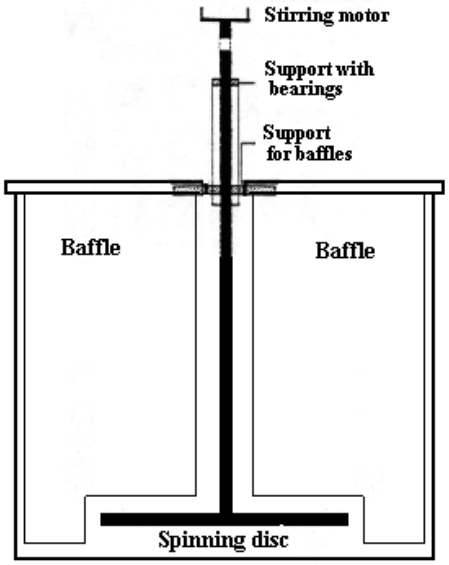

Figure 5.1 Large-disc MBD generator.

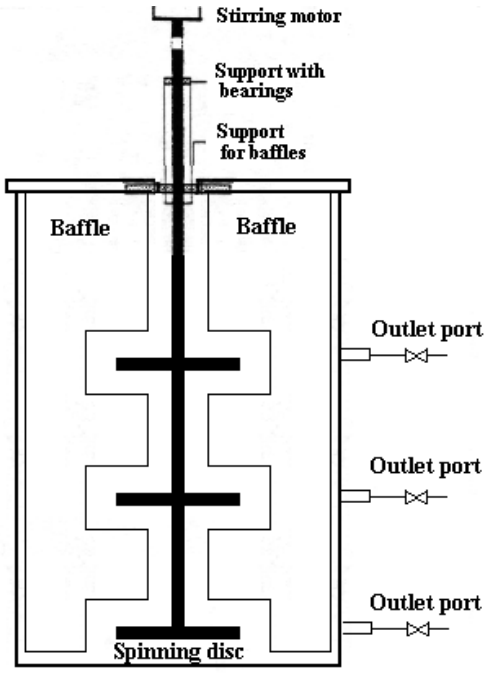

Figure 5.2 Multiple-disc MBD generator.

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APPENDIX A

ASSAYS

A.1 Glucose concentration determination

Park and John Method for a Reducing Sugar Assay

Based on the reduction of ferricyanide ions in alkaline solution by a reducing sugar. The ferricyanide produced can then react with a second mole of ferricyanide producing the ferric-ferrocyanide (Prussian blue) complex.

Reagent A

0.5 g	Potassium ferricyanide
1000 ml	Distilled water

(store in brown bottle)

Reagent B

5.3 g	Sodium carbonate
0.65 g	Potassium cyanide
1000 ml	Distilled water

Reagent C

1.5 g	Ferric ammonium sulfate
1.0 g	Sodium lauryl sulfate (Sodium dodecyl sulfate)

1000 ml 0.05 N sulfuric acid (1.4 ml concentrated sulfuric acid + 998.6 ml distilled water)

Standard curve :

1. Number ten 20 ml test tubes and place them in a test tube rack. Into each of the tubes carefully pipette one of the following volumes of a 28 mg/l glucose solution : 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml.

2. Add an appropriate amount of distilled water to each tube to give a final volume of 1.0 ml.

Procedure :

1. Add 1.0 ml sample to a 10 ml test tube.
2. Add 1.0 ml **reagent B**.
3. Add 1.0 ml **reagent A**.

   (DO NOT MOUTH PIPETTE)
4. Mix thoroughly by vortexing.
5. Cover each test tube with a marble.
6. Place in water bath (boiling water) for 15 minutes.
7. Remove and let cool to room temperature.
8. Add 5.0 ml **reagent C**.

   (DO NOT MOUTH PIPETTE)

9. Mix thoroughly by vortexing.

10. Incubate 15 minutes at room temperature.

11. Determine absorbance at 690 nm.

APPENDIX B

CALCULATIONS

B.1 Estimated power consumption

B.1.1 **Impeller power consumption** (Perry and Chilton, 1973).

$$N_{Re} = \frac{D_i^2 N \rho}{\mu}$$

$$N_P = \frac{P g_c}{N^3 D_i^5 \rho}$$

where D_i = impeller diameter (cm),

N = impeller speed, (revolutions sec^{-1}),

ρ = density (gm cm^{-3}),

μ = viscosity (gm cm^{-1} sec^{-1}),

g_c = 986 cm sec^{-2}, and

P = power transmitted by agitator shaft (ft lb_f sec^{-1}).

For the system used in this study:

D_i = 4.9 cm (2-liter fermenter), 12.5 cm (72-liter fermenter),

N = 100/60 or 600/60 sec^{-1} (preliminary experiments) and

150/60 or 500/60 sec^{-1} (main experinents),

ρ = 1.04 gm cm^{-3},

μ = 1.2×10^{-2} gm cm^{-1} sec^{-1},

The Reynold's number was calculated and the power number was taken. The impeller power (P) was finally obtained from

$$P = \frac{N^3 D_i^5 \rho N_P}{g_c}$$

The impeller power obtained was estimated from the correlation of ungassed system. For gassed system, the air sparged to a liquid broth resulted in reduction in the power absorption. Oyama and Endoh (1955) proposed the correlation of the aeration number as an independent variable to the ratio of gassed power to the ungassed power (P_g/P_o). The concept of the aeration number (N_a) was introduced by the ratio of the superficial gas velocity (Q/D_i^2) to the impeller tip velocity (N/D_i).

$$N_a = \frac{Q}{ND_i^3}.$$

where N_a = the aeration number (dimensionless),

Q = volumetric gas flow rate (cm^3 sec^{-1}),

D_i = impeller diameter (cm), and

N = impeller speed (sec^{-1}).

$$(P_g/P_o) = f(N_a) = f(Q/ND_i^3).$$

Gassed power was then taken from Oyama and Endoh ‘s correlation.

B.1.2 Air compressor power consumption (from Perry and Chilton, 1973 page 6-15 to 6-19).

$$hp = 0.0154\ Q\ p\ X$$

where Q = flow rate ($ft^3\ min^{-1}$),

p = pressure ($lb\ in.^{-2}$), and

X = factor taken from Table 6-1 (Perry and Chilton, 1973 page 6-17)

B.1.3 MBD generator power consumption

The power consumption in MBD generator came from 3 parts: (1) motor, (2) MBD delivery pump, and (3) MBD recycling pump.

$$P_{MBD} = P_{motor} + P_{MBD\ pump} + P_{recycling\ pump}.$$

(1) P_{motor}

$$Power = Current \times Voltage.$$

(2) $P_{MBD\ pump}$ and (3) $P_{recycling\ pump}$

The power requirement for MBD delivery pump and recycle pump were calculated from the *steady-state macroscopic mechanical energy balance* (Bird *et al*., 1960).

The average velocity, $\langle v \rangle$, in the pipe line is

$$\langle v \rangle = \frac{Q}{\pi R^2}$$

where Q = flow rate ($ft^3\ min^{-1}$), and

R = internal radius of smooth circular pipe (ft).

The Reynolds number is

$$\mathrm{Re} = \frac{D\langle v \rangle \rho}{\mu}$$

where D = internal diameter of smooth circular pipe (ft),

ρ = density (lb ft^{-3}), and

μ = viscosity (lb ft^{-1} sec^{-1}).

For turbulent tube flow, $2.1x10^3 < Re < 10^5$, the Friction factor, f , = $0.0791/Re^{1/4}$.

The friction loss is

$$\hat{E}_v = \sum_i \left(\frac{1}{2} \langle v \rangle^2 \frac{L}{D} 4f \right)$$

where $\langle v \rangle$ = average velocity in pipe line (ft sec^{-1}),

D = internal diameter of smooth circular pipe (ft),

L = length of pipe line (ft),

f = friction factor (dimensionless).

The contribution from to the fittings, valves, meters, etc. is

$$\sum_i \left(\frac{1}{2} \langle v \rangle^2 e_v \right)_i$$

where e_v = friction loss factor,

(obtained from Table 7.4-1, page 217 (Bird, *et al*., 1960).

Then from *the steady-state macroscopic mechanical energy balance*:

$$\Delta \frac{1}{2} \langle v \rangle^2 + g \Delta h + \int_{p_1}^{p_2} \frac{1}{\rho} dp + W + \sum_i \left(\frac{1}{2} \langle v \rangle^2 \frac{L}{D} f \right)_i + \sum_i \left(\frac{1}{2} \langle v \rangle^2 e_v \right)_i = 0$$

the work W can be calculated and finally the power delivered by the pump is obtained.

VITA

Patcharee Hensirisak was born on August 3, 1972 in Bangkok, Thailand. She graduated from Mattayom-watdusitaram High school on March 1989, and then entered Mahidol University in the same year. After graduating with a Bachelor degree of Science in Biotechnology in March 1993, she joined the graduate program in Industrial Microbiology at Chulalongkorn University. On the Fall semester 1995 she was accepted as a graduate student to pursue a Master of Science at Virginia Tech in the Biological Systems Engineering Department.