Increased Oxygen Transfer in a Yeast Fermentation

Using A Microbubble Dispersion

by

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(ABSTRACT)

The use of a microbubble dispersion (MBD) for oxygen transport in a yeast fermentation was studied. No surfactants were added to the fermentations; the microbubbles were formed using only the surfactants naturally produced by the yeast. The growth rate of a *Saccharomyces cerevisiae* culture was found to be almost twice as great for a microbubble dispersion sparged fermentation, as for a gas sparged fermentation when equivalent volumes of air or microbubble dispersion were used. The growth rate of the MBD sparged fermentation was 0.108/hr, as opposed to 0.068/hr for the air sparged fermentation. Oxygen transfer rates were measured by the yield coefficient, dynamic, and direct methods. The oxygen transfer coefficient was found to be approximately 190/hr and independent of the fermenter impeller speed, over the range of 100 - 580 RPM, for the microbubble dispersion fermentation. Over the same range, the oxygen transfer coefficient for the air sparged fermentation rose from 55 to 132/hr. The power requirements for the fermenter systems were estimated. It was determined that a five fold reduction in the fermenter to MBD generator volume ratio could be made.

Acknowledgements

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1.0 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 as 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 one mole per liter. For organisms requiring oxygen however, mass transfer can play a major limiting role. Under normal fermentation conditions, oxygen is only sparingly soluble. At 35°*C*, the solubility of oxygen in pure water is 0.217 mmoles/liter and the presence of salts and other nutrients required for the growth of any organism only decreases this value.

Because of its sparing solubility, the oxygen in the fermentation broth can be quickly depleted by growing organisms. A culture of ***Saccharomyces cerevisiae*** growing in respiratory mode at a rate of 1.0 $\frac{\text{grams new cell mass}}{\text{liter} \cdot \text{hour}}$ on glucose will consume 62.5 mmol of O_2 This consumption rate is 4.8 times the maximum oxygen content of the broth. Oxygen, therefore, needs to be constantly replenished. Oxygen is normally supplied to the fermentation in the form of air, which must be filtered and compressed compressed in order to pump it into the fermenter. Therefore, air processing is a major cost factor in the design of the fermenter.

Three major design types of fermenters are common today, each of which uses a different means of maximizing the efficiency of oxygen transfer. Stirred tank fermenters use turbines, paddles,

or propellers to create turbulence. The turbulence breaks up gas bubbles and increases the residence time of the bubbles and the area available for mass transfer. Airlift fermenters use the rising of bubbles in one part of the fermenter to create ordered circulation. This circulation is facilitated by a physical barrier that divides the fermenter into two sections: the riser and the downcomer. In the riser, sparged air bubbles pull fluid with them as they rise to the surface. In the downcomer, the descending fluid carries small bubbles back down to the bottom of the fermenter. The long residence time of the small bubbles allows for more complete use of the oxygen they carry. Bubble column fermenters are simple columns that use the rolling of fluid caused by rising bubbles to mix the broth. A simple bubble column has oxygen transfer enhancing features. Oxygen transfer in this type of fermenter is simply guaranteed by the large volumes of air pumped through the fermenter. In all of these systems, less than 40% of the oxygen that is supplied to the fermenter is used. The sterilization and transfer of oxygen can still account for up to 25% of the final product costs in aerobic fermentations [1].

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 though, small air bubbles tend to 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, the 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. If gas bubbles are stabilized with a surfactant film, however, 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 to 65% dispersion of a gas in liquid. The microbubbles have diameters of 20 to 1000 microns as compared to diameters of 3 to 5 millimeters for normal bubbles in a fermenter. Because of their small size, these microbubbles rise relatively 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. The orientation of surfactant molecules forms a charged bubble surface that repels other bubbles, which leads to reduced coalescence. 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 purpose of this project was to explore the use of surfactant-stabilized microbubbles in fermentation systems as a means of increasing oxygen transport. This was accomplished by first determining which fermenter/organism combination would be most suitable for determining oxygen transport in a live system and for comparison to other researchers results. Then, the fermenter and the microbubble dispersion (MBD) generator had to be modified to insure a sterile environment for the organism. Finally, a system of variables had to be determined and measured that would quantitatively show any differences between oxygen transport with normally sparged air and oxygen transport with surfactant-stabilized microbubbles. Specifically, the growth rate, oxygen uptake rate, and the overall oxygen transfer coefficient of a culture grown in a stirred tank fermenter were determined using either sparged air or surfactant-stabilized microbubbles 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 and is easy to grow and resistant to contamination.

2.0 LITERATURE REVIEW

2.1 Yeast

In selecting the best organism for this study, three criteria had to be met. First, the organism had to consume oxygen during growth. It wasn't necessary for the organism to require oxygen in order to grow, but it had to consume oxygen if any was supplied. Second, the organism had to have been well-studied; preferably, some of the studies of the organism should have included reactor design. Finally, the organism had to be easy to grow and resistant to contamination. *Saccharomyces cerevisiae*, or baker's yeast, fit all three of these requirements.

The production of baker's yeast has been an industrial process for more than one hundred years. Initially, in the early to middle 19th century, baker's yeast was produced in a manner similar to that used to produce beer. Mash and yeast were added to a tank batchwise, and alcohol and yeast were produced simultaneously. Around 1860, the effect of oxygen on the growth of the yeast was recognized and producers began adding oxygen to their tanks. By about 1920, the fed-batch or Zulauf process was recognized as the best method of producing yeast. In a fed-batch yeast fermentation, a starter culture of yeast is added to a small amount of growth media. Then, additional media is added as the yeast consumes the nutrients in the tank. This process is still used today [2].

The baker's yeast organism, ***Saccharomyces cerevisiae***, has been extensively studied in the laboratory as well as in production. The first such studies by Pasteur pointed out the need for increased air flow to the fermenter. Since that time, much research has been done on the effects of oxygen [3] and carbon sources [2, 4] on the growth of yeast. Recently, improving strains of ***S. cerevisiae*** with gene transfer techniques has become very popular with microbiologists [5]. Of main interest in this study are the effects of oxygen and glucose on yeast growth.

S. cerevisiae is a facultative anaerobe. If no oxygen is present in the immediate environment, ***S. cerevisiae*** will convert glucose to carbon dioxide and ethanol. The presence of oxygen tends to slow down the growth rate of the yeast, but it increases the amount of yeast that is produced per gram of glucose. Under strictly aerobic conditions, 0.54 grams of cell mass per gram of glucose can be achieved compared to 0.075 grams of cell mass per gram of glucose for anaerobic growth [4]. Furukawa et al [3] found that a dissolved oxygen concentration of at least 1.0 mg/l was necessary for efficient yeast production. Yet, the use of hyperbaric oxygen feeds tend to decrease the efficiency and growth rate of the yeast. Oura [6] found that yeast grows most efficiently with an oxygen supply containing between 21% and 30% oxygen.

Glucose concentrations also have interesting effects upon the growth rate and efficiency of ***S. cerevisiae***. In batch growth on glucose, ***S. cerevisiae*** initially produces both carbon dioxide and ethanol, and then converts the remaining ethanol to carbon dioxide when the glucose is completely gone. This effect is seen even in aerobic fermentations. When this phenomenon was first noted, neither the carbon dioxide produced, nor the oxygen consumed could be measured. It was thought that the presence of glucose repressed the production of respiratory enzymes [7, 8]. This mechanism was questioned by Barford and Hall in 1978 [9]. They and others [10-15] have shown that this is not the case. Actually, the utilization of glucose is regulated by the fact that ***S. cerevisiae*** can only produce a limited amount of respiratory enzymes. If there is more glucose present than can be metabolized oxidatively, then some will be metabolized anaerobically. Furukawa et al found that ***S. cerevisiae*** grows most efficiently when the glucose concentration in the fermentation broth remains below 100mg/l [3].

The theoretical maximum yield of cell mass on glucose Y_G is 0.54 $\frac{\text{grams of cell mass}}{\text{grams of glucose}}$. This value is very difficult to achieve in a batch fermentation. This difficulty is primarily due to the fact that most of the glucose is first broken down to ethanol. Then, the ethanol is consumed in a second growth phase. This two phase process is rather inefficient from a production standpoint as can be seen from von Meyenburg's work [18]. In his classic study of yeast growth in a batch fermentation, he was able to achieve a maximum cell mass yield of only 0.43 $\frac{\text{grams of cell mass}}{\text{grams of glucose}}$, see Table 1 on page 7.

In continuous mode, one is able to approach the theoretical maximum cell mass yield on glucose of 0.54 $\frac{\text{grams of cell mass}}{\text{grams of glucose}}$. This theoretical maximum is approachable because, irregardless of the glucose concentration of the feed, the glucose concentration in the fermenter will remain near zero. The low glucose concentration in the fermenter causes the yeast to completely oxidize all of the sugar it consumes. In continuous fermentations, known as chemostats, once a steady state is reached, the dilution rate (the rate of feed/liquid volume in fermenter) is exactly equal to the growth rate. Thus, the maximum possible growth rate for an organism is easily found from chemostat culture studies. The maximum growth rate, μ_{max}, for *S. cerevisiae* is approximately 0.29/hr. Several researchers have studied *S. cerevisiae* growth at or near this maximum growth rate (see Table 1 on page 7). The greatest glucose yields were obtained using feeds with glucose concentrations of 10 gm/l or less. This is a result of the non-ideality of a real chemostat. In an ideal chemostat, the concentration of glucose is always essentially zero because an ideal chemostat is perfectly mixed. A true chemostat, however, can be well-mixed, but not perfectly mixed. Therefore, the more concentrated the feed is, the better the probability that a yeast cell will find an abundance of glucose. In the presence of excess glucose, the yeast converts some of it to ethanol, thereby reducing the cell mass yield on glucose. Thus, in order to get a high conversion of glucose to cell mass, the glucose concentration in the fermenter must remain near zero and new glucose must be added continually.

In order to obtain highly concentrated yeast growth, a fed-batch mode must be used. A fed-batch fermentation takes advantage of the fact that *S. cerevisiae* grows most efficiently when glucose is present in small amounts. Fed-batch fermentations are normally fed solutions containing

Table 1. Cell Mass Concentrations of Saccharomyces cerevisiae in Batch and Continuous Culture

Reference	Subject	Cell Mass (g/l) Start	Cell Mass (g/l) Max.	Initial or Feed Glucose Conc. (g/l)	Ferm. Mode	Time or Dilution Rate
Beck and von Meyenburg [17]	Metabolism	0.16	3.4	9.2	Batch	22. hrs
von Meyenburg [18]	Metabolism	0.06	3.9	9.0	Batch	18. hrs
von Meyenburg [18]	Metabolism	-	14	30.0	Continuous	0.25/hr
Beck and von Meyenburg [17]	Metabolism	-	4.3	9.2	Continuous	0.14/hr
Barford and Hall [9]	Metabolism	-	5.2	10.0	Continuous	0.12/hr
Borzani, Gregori and Vario [19]	Metabolism Oscillations	-	3.75	29.4	Continuous	0.131/hr
		-	5.3	32.5	Continuous	0.168/hr
		-	5.7	34.9	Continuous	0.135/hr
		-	6	30.0	Continuous	0.192/hr
Furukawa, Heinzle and Dunn [3]	Metabolism	-	14	30.0	Continuous	0.25/hr
	O_2 Effects	-	13	30.0	Continuous	0.27/hr
de Kok and Roels [20]	Energy Balances	-	3.8	10.0	Continuous	0.008/hr
		-	3.17	10.0	Continuous	0.047/hr
		-	4.41	10.0	Continuous	0.072/hr
		-	3.8	10.0	Continuous	0.092/hr
		-	4.5	10.0	Continuous	0.118/hr
Meyer and Beyeler [21]	Control	-	14	30.0	Continuous	0.29/hr
Parulekar et al [22]	Metabolism	-	5.25	10.0	Continuous	0.2/hr
Rieger, Käppeli and Fiechter [13]	Metabolism	-	15	30.0	Continuous	0.23/hr
		-	2.7	5.0	Continuous	0.29/hr
Rieger, Käppeli and Fiechter [14]	Metabolism	-	14.2	30.0	Continuous	0.29/hr
		-	4.7	10.0	Continuous	0.29/hr
		-	2.4	5.0	Continuous	0.29/hr
Rogers and Stewart [23]	Metabolism	-	5.2	10.0	Continuous	0.07/hr
Sonnleitner and Käppeli [15]	Metabolism	-	4.8	10.0	Continuous	0.29/hr
Fiechter [11]	Metabolism	-	12	20.0	Semi-contin.	0.1/hr

Table 2. Cell Mass Concentrations of Saccharomyces cerevisiae in Fed-Batch Culture

Reference	Subject	Cell Mass (g/l) Start	Max.	Feed Glucose Conc. (g/l)	Ferm. Mode	Time or Dilution Rate
Aiba, Nagai and Nishizawa [24]	Computer Control	13 15	28 31	300 300	Fed-batch Fed-batch	4 hrs 4 hrs
Woehrer and Roehr [10]	Metabolism	3.5	10.1	250	Fed-batch	8 hrs
Williams, Yousefpour and Wellington [25]	Control	10	31.5	270	Fed-batch	7 hrs
Wang, Cooney and Wang [26]	Control	2.6	42	395	Fed-batch	26 hrs
Dairaku et al [27]	Control	9	24	-	Fed-batch	11 hrs
Kishimoto et al [28]	Control	1	95	600	Fed-batch	28 hrs
Fukuda et al [29]	Models	10 10 15 10 15 30	21 83 75 100 128 137	47.5 700 285 - 300 700 285 - 300 700	Fed-batch Fed-batch Fed-batch * Fed-batch * Fed-batch * Fed-batch	14 hrs 18 hrs 18 hrs 18 hrs 17 hrs 18 hrs
Fukuda et al [30]	Metabolism	15 50 70 15 15	75 100 110 147 130	333 333 333 100? 350	Fed-batch Fed-batch Fed-batch * Fed-batch * Fed-batch	28 hrs 16 hrs 16 hrs 14 hrs 19 hrs
Netto and Goma [31]	Biomass Kinetics	4 4 4 4	66 52 64 45	130.5 130.5 130.5 130.5	** Cont. Fed. ** Cont. Fed. ** Cont. Fed. ** Cont. Fed.	100 hrs, 0.305/hr 110 hrs, 0.199/hr 210 hrs, 0.100/hr 600 hrs, 0.049/hr

* Fed-batch - Most of the broth drained off at set intervals and replaced with new broth.
** Cont. Fed. - Grown in a tower fermenter, actually continuous but with little or no yeast loss with the expended broth.

approximately 300 $\frac{\text{grams glucose}}{\text{liter}}$. A highly concentrated feed is used to reduce dilution effects when the glucose is added. The control of a fed-batch fermentation can be difficult. The glucose needs to be fed to the yeast at a rate exactly equal to the rate at which the yeast can consume it. Since the amount of yeast is constantly changing, the glucose feed rate must also change. The importance of this can be seen in the large body of fed-batch fermentation control research (see Table 2 on page 8). A normal fed-batch fermentation can result in final cell mass concentrations of up to 40 gm/l. Kishimoto et al [28] were able to get up to 100 to 120 gm/l but they were using a concentrating procedure to obtain these results. A high concentration of yeast cell mass increases the viscosity of the fermentation broth, and consumes large amounts of oxygen. A highly concentrated yeast fermentation would therefore be advantageous for oxygen transfer studies.

From the information gathered above, optimal growth conditions for *S. cerevisiae* can be determined. The fermenter should be fed-batch, with a high glucose concentration in the feed to limit dilution effects. Oxygen levels in the media should be as close to the saturation value under an air atmosphere as possible. Sugar levels in the fermenter should not exceed 100 mg/l and the initial concentration of yeast needs to be constant from one experiment to the next.

2.2 Fermenters

Three main types of fermenters have been used in industry to grow microorganisms. Airlift fermenters are tower fermenters with riser and recycle sections that use air sparged into the riser to effect both oxygen transfer and mixing of the fluid. Bubble column fermenters are simple tower fermenters that use the turbulence caused by rising bubbles to mix the broth and they rely on the bubble path length to allow time for oxygen transfer. Stirred tank fermenters use turbines or impellers to stir the broth and to break up sparged air bubbles creating more surface area for mass transfer. ***Saccharomyces cerevisiae*** has been grown in all three types of fermenters.

Industrially, *S. cerevisiae* is produced in bubble columns. The baker's yeast producers, however, are a rather small group. The fermentation industry in general mainly uses stirred tank fermenters. A stirred tank fermenter will probably demonstrate the least improvement in the laboratory with the use of a surfactant-stabilized microbubble dispersion for oxygenation, since laboratory stirred tank reactors, as a general rule, are over-stirred. 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. Although a laboratory stirred tank reactor will probably show the least improvement with a microbubble dispersion, if any improvements are seen, the results can be immediately applied to existing industrial fermenters.

2.3 Oxygen Transfer

2.3.1 Oxygen Transfer in Fermenters

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. For scale-up purposes, this figure is usually reported in conjunction with the surface area available for mass transfer, a, in the form $k_L \times a$.

Cooper et al. [32] were the first to correlate k_La as a function of power input per volume and superficial gas velocities. They measured oxygen transport in stirred tanks using sulfite oxidation to absorb the oxygen in the liquid. Their data, as shown in Figure 1 on page 12, was fit by

$$k_La = K\left(\frac{P_g}{V_L}\right)^{0.95} V_s^{0.67} \qquad (1)$$

where: K = constant

P_g = gassed power requirement (hp)

V_L = liquid volume (1000 liters)

V_s = superficial gas velocity (cm/min.)

In 1961, Richards [33] used the data of Cooper et al. plus some of his own sulfite oxidation data to derive the following correlation:

$$k_L a = c \left(\frac{P_g}{V_L} \right)^{0.4} V_s^{0.5} N^{0.5} \tag{2}$$

where: c = constant

N = impeller speed (RPM)

A graph of Richardson's data is shown in Figure 2 on page 13.

Yoshida et al [34] also collected a large amount of sulfite oxidation oxygen transfer data. They found $k_L a$ to be a function of the following:

$$k_L a = c \, V_3^m \, (N^3 L^2)^n \tag{3}$$

where: V_s = superficial gas velocity (ft/hr)

c = function of V_s

m = function of $N^3 L^2$

n = function of V_s

N = agitator speed (RPM)

L = tank diameter (ft)

Wallis et al [1] duplicated Yoshida's experiments with colloidal gas aphrons (CGA's) supplying the oxygen. The results of Yoshida et al and Wallis et al are shown in Figure 3 on page 15. The $k_L a$'s obtained with CGA sparging were significantly higher than for pure air sparging. Before fur-

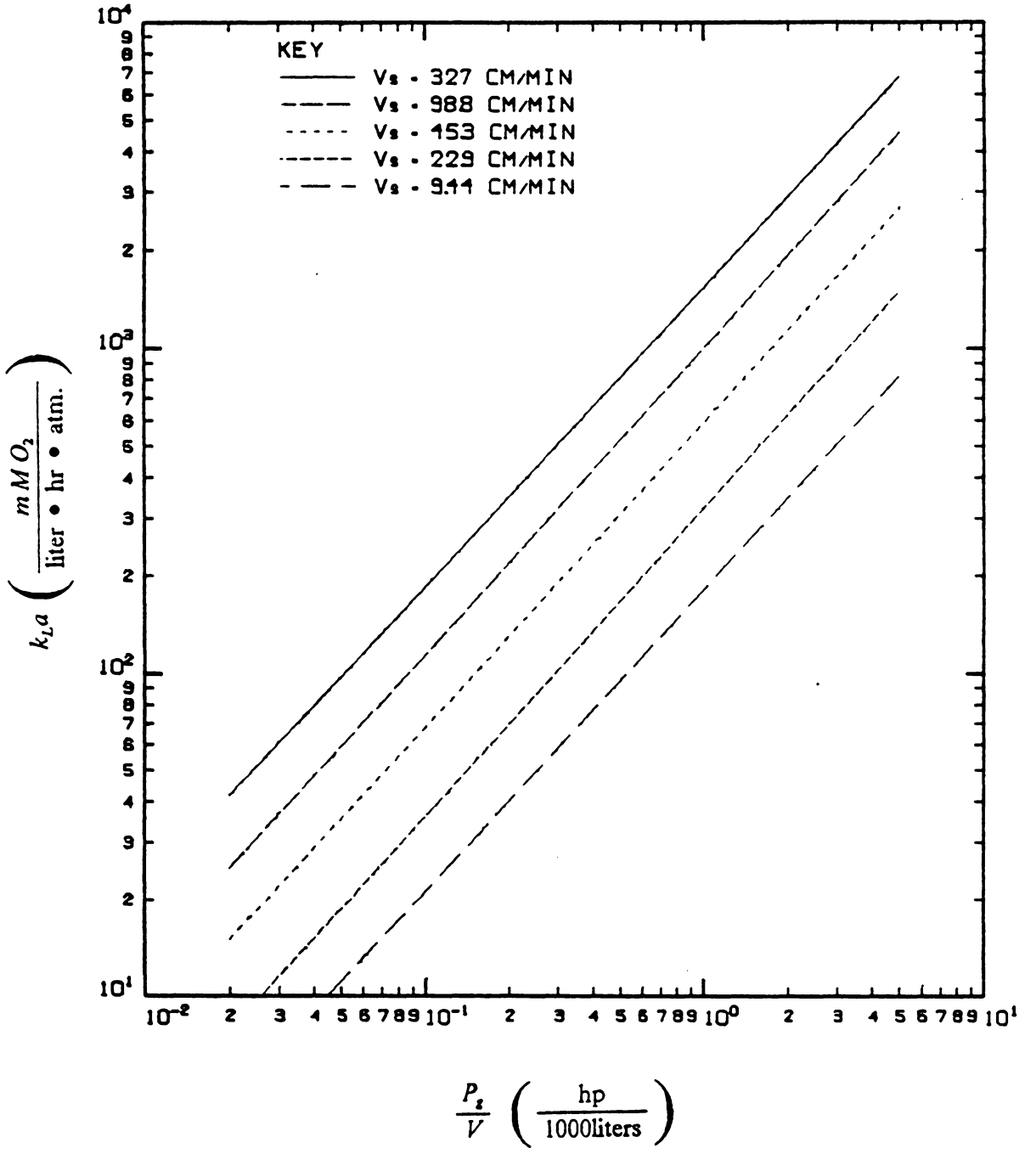


Figure 1. Oxygen transfer coefficient as a function of power input to the fermenter (Cooper et al [32]).

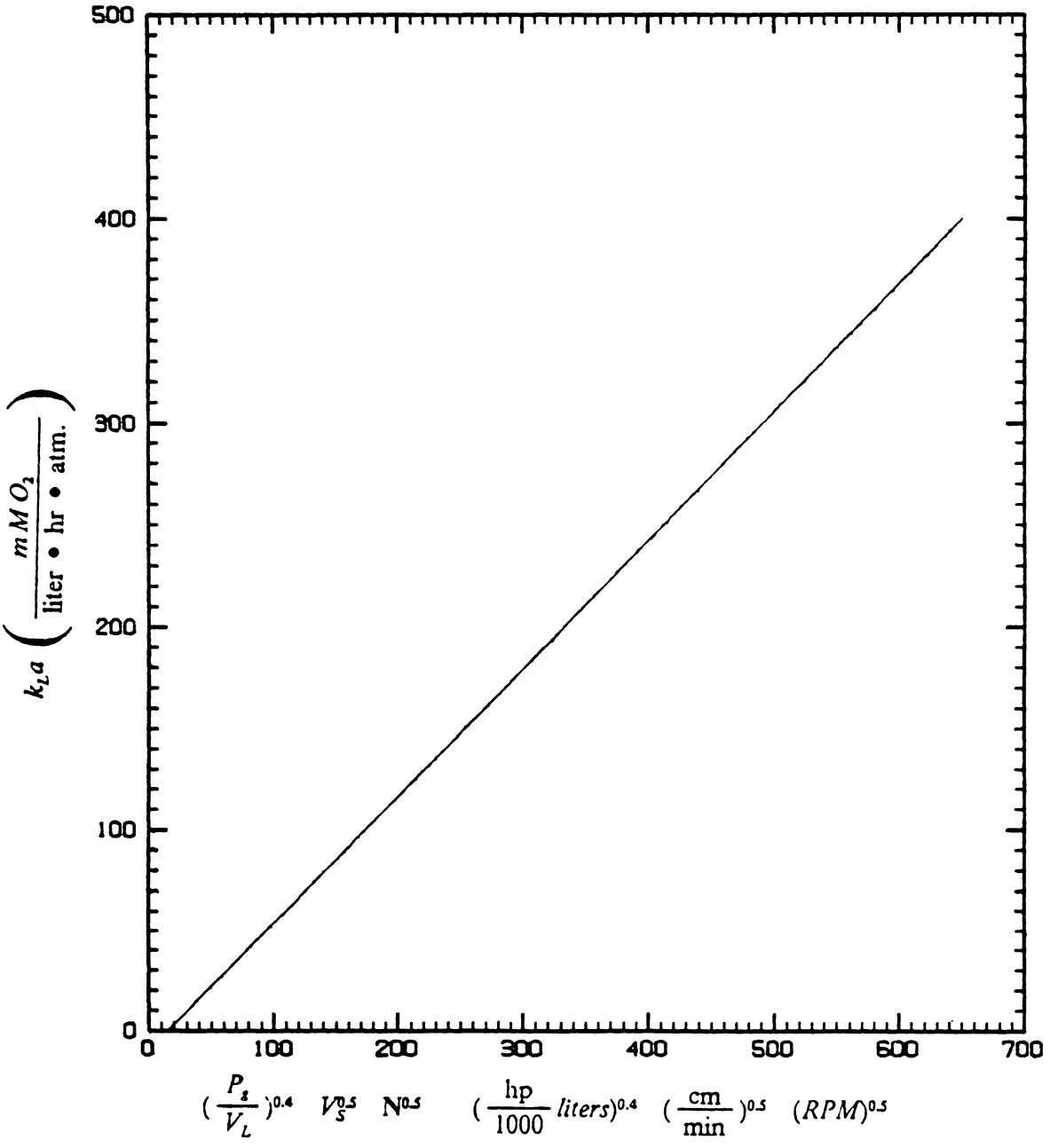


Figure 2. Oxygen transfer coefficient as a function of ower input to the fermenter and impeller speed (Richardson [33]).

ther comparisons can be made, the methods of calculating k_La 's and the limitations of these methods should be examined.

2.3.2 Oxygen Transfer Measurement Methods

In aerobic fermentations, the rate of oxygen transfer needs to match the rate of oxygen use by the microorganisms. The rate of oxygen transfer to the media can be described by

$$N_A = k_La\,(C_L^* - C_L) \tag{4}$$

where: N_A = rate of oxygen transfer to liquid (mol/l • hr)

k_L = liquid side mass transfer coefficient (1/hr • m^2)

a = gas/liquid surface area (m^2)

C_L^* = equilibrium dissolved oxygen concentration (mol/l)

C_L = actual dissolved oxygen concentration (mol/l)

Since the liquid film resistance is limiting [35], k_La is a critical parameter in the design of fermenters (see Figure 4 on page 16). To determine k_La, the rate of oxygen transfer, N_A , needs to be found first. Several methods of determining N_A are widely used in the literature. Described below are the four most important methods: direct measurement, sodium sulfite oxidation, dynamic measurement, and the yield coefficient method.

2.3.2.1 Direct Measurement

Direct measurement [36] of the oxygen transfer rate is the most accurate method, but, it also requires the most instrumentation. The temperatures and pressures of all gas inlet and outlet streams are required, as well as all flow rates and the oxygen concentration in all streams. The oxygen transfer rate is then described by

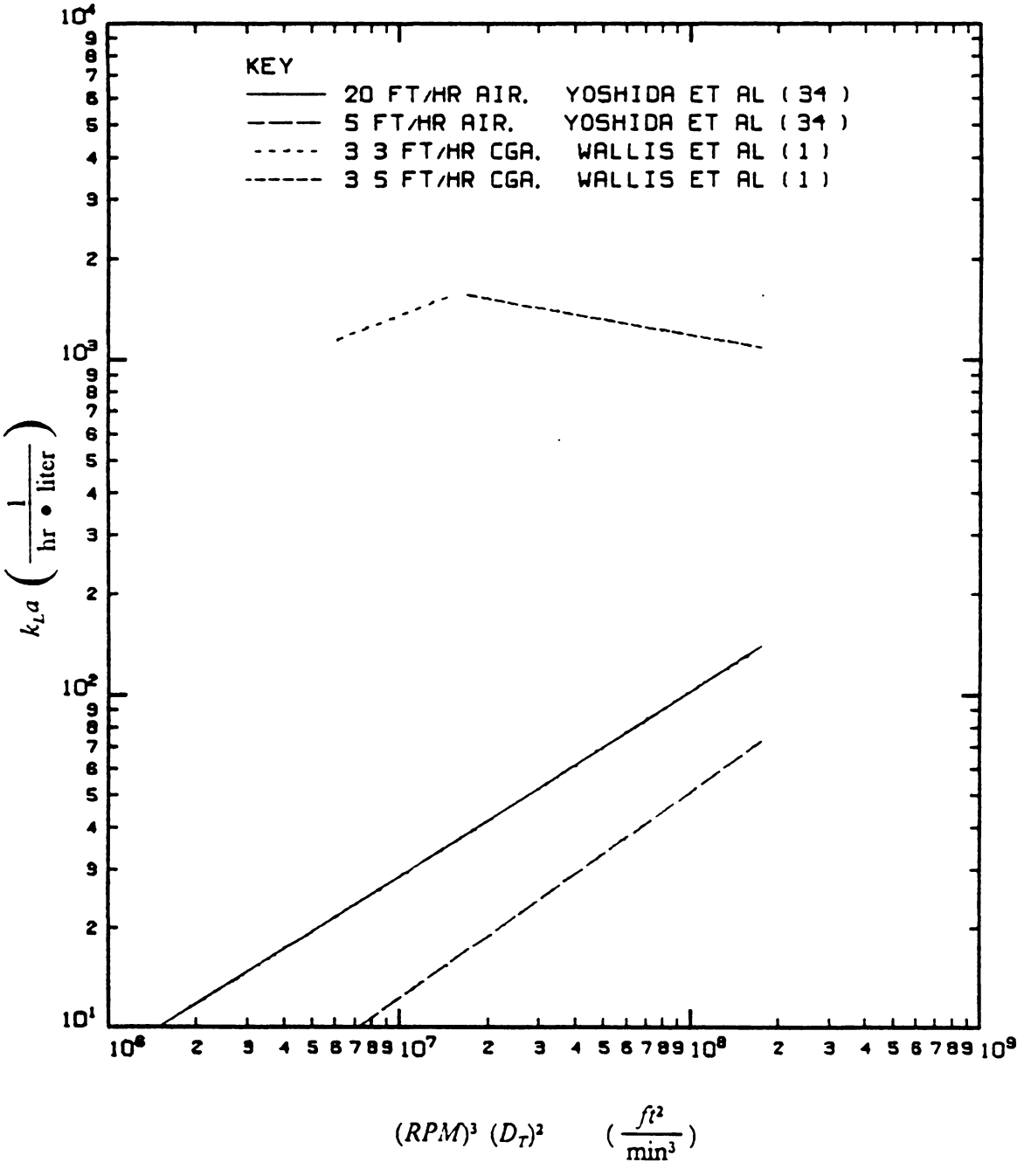


Figure 3. Oxygen transfer coefficient in sparged air and sparged CGA systems [34, 1]

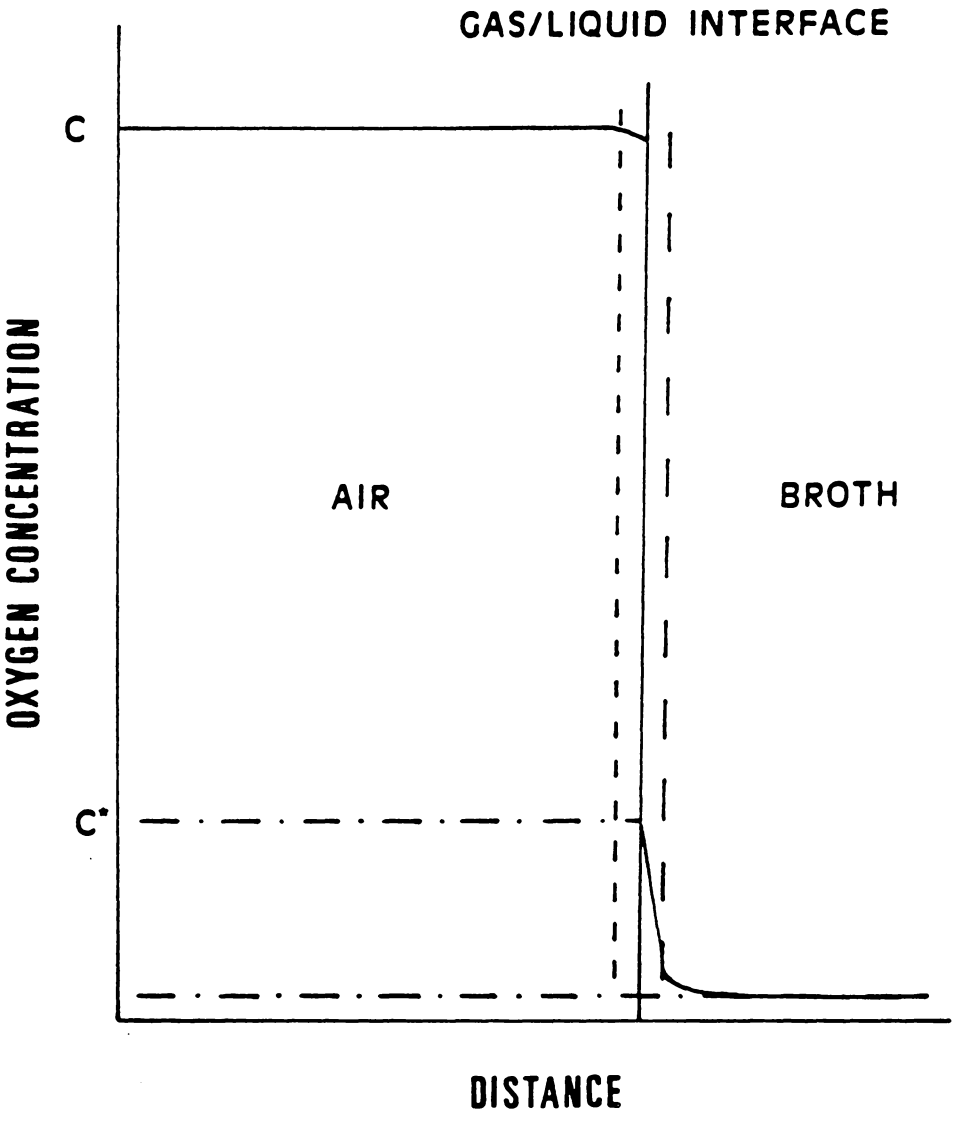


Figure 4. Oxygen transfer from a gas into a liquid.

$$N_A = \frac{7.32 \times 10^5}{V_L} \left(\frac{Q_I P_I y_I}{T_I} - \frac{Q_O P_O y_O}{T_O} \right) \quad (5)$$

where: I = inlet value

O = outlet value

Q = volumetric air flow rate (min^{-1})

P = pressure (atm.)

y = mole fraction of oxygen

T = temperature (°K)

V_L = volume of broth (liters)

In this equation, the constant, 7.32×10^5 , is a combination of several conversion factors, (60min/hr)(mole/22.4liters)(273°K/1.0atm.) .

In order to determine k_La, only the concentration of oxygen in the broth and the equilibrium oxygen concentration need to be found. For a well mixed reactor, which includes most laboratory units, the concentration in the broth can be considered to be constant and the equilibrium concentration can be taken to be the concentration in equilibrium with the exit gas.

$$k_La = \frac{N_A}{(C_O^* - C_L)} \quad (6)$$

where: C_O^* = dissolved oxygen concentration in equilibrium with the outlet gas

In a fermenter that isn't well mixed, the oxygen concentration will vary with position in the vessel. The equilibrium of dissolved oxygen concentration will also change, due to variations in broth composition. A log mean concentration is required to model this case:

$$k_La = \frac{N_A}{(C^* - C_L)_{\log mean}} \quad (7)$$

The direct method is most likely to be used on an industrial scale fermenter where errors, especially in the flow rates, are likely to be smaller percentages of the total values. Most of the early data on mass transfer from small scale fermenters was collected using the sulfite oxidation method.

2.3.2.2 Sodium Sulfite Oxidation

This method uses the oxidation of sodium sulfite to sodium sulfate as a means of measuring the maximum oxygen transfer rate [19, 36]. The oxidation reaction requires the presence of a catalyst (Co^{++}or Cu^{++}) and follows the equation

$$Na_2SO_3 + \frac{1}{2}O_2 \xrightarrow{Cu^{++}\text{or } Co^{++}} Na_2SO_4 \tag{8}$$

This reaction is used because its rate is essentially independent of both sulfite concentration and oxygen concentration. A 1.0 M sodium sulfite solution with a copper ion concentration of 10^{-3} M is allowed to react with the air sparged into the fermenter. The unreacted sulfite is then placed in an excess iodine solution and back titrated with a standard sodium thiosulfate solution. Because of the fast kinetics of this reaction, the concentration of oxygen in the liquid is essentially zero at all times. The oxygen transfer coefficient is given by

$$k_L a = \frac{N_A}{C_L^*} \tag{9}$$

The sulfite oxidation method is relatively quick and easy. However, since about 1970, its relevance to real systems has come into question [36, 37]. Researchers are turning to methods that can be applied to living systems.

2.3.2.3 Dynamic Measurement

The dynamic oxygen electrode method of measuring k_La was introduced by Bandyopadhyay et al in 1967 [38]. Several improvements have been published since then [39-48], but the basic method gives a reasonably accurate measurement of k_La. It is based on the material balance of the dissolved oxygen in a growing culture

$$\frac{dC_L}{dt} = k_La(C_L^* - C_L) - rX \tag{10}$$

where: r = the specific oxygen uptake rate (OUR) $\left(\frac{\text{mmol } O_2}{\text{gm cells} \cdot \text{hr}}\right)$

X = dry weight of cells per liter (gm cells/l)

and how the dissolved oxygen concentration reacts to a step function change in the oxygen supply.

First, the oxygen supply is cut to zero, usually by changing from air to nitrogen sparging. This removes the oxygen transfer term leaving

$$\frac{dC_L}{dt} = -rX \tag{11}$$

so that the oxygen consumption term (rX) is simply the negative of the dissolved oxygen concentration vs. time curve. Then the oxygen feed is restarted as a step increase. This procedure will result in a dissolved oxygen profile similar to Figure 5 on page 20.

Equation (10) can be rearranged to give

$$C_L = C_L^* - \frac{rX + \frac{dC_L}{dt}}{k_La} \tag{12}$$

A plot of C_L vs. $(rX + \frac{dC_L}{dt})$ will have a slope of $-\frac{1}{k_La}$. To avoid errors with this method, the assumptions it entails need to be recognized. First, a step decrease in the oxygen supply is assumed. Any oxygen left in the head space will throw off the value for rX by supplying oxygen via

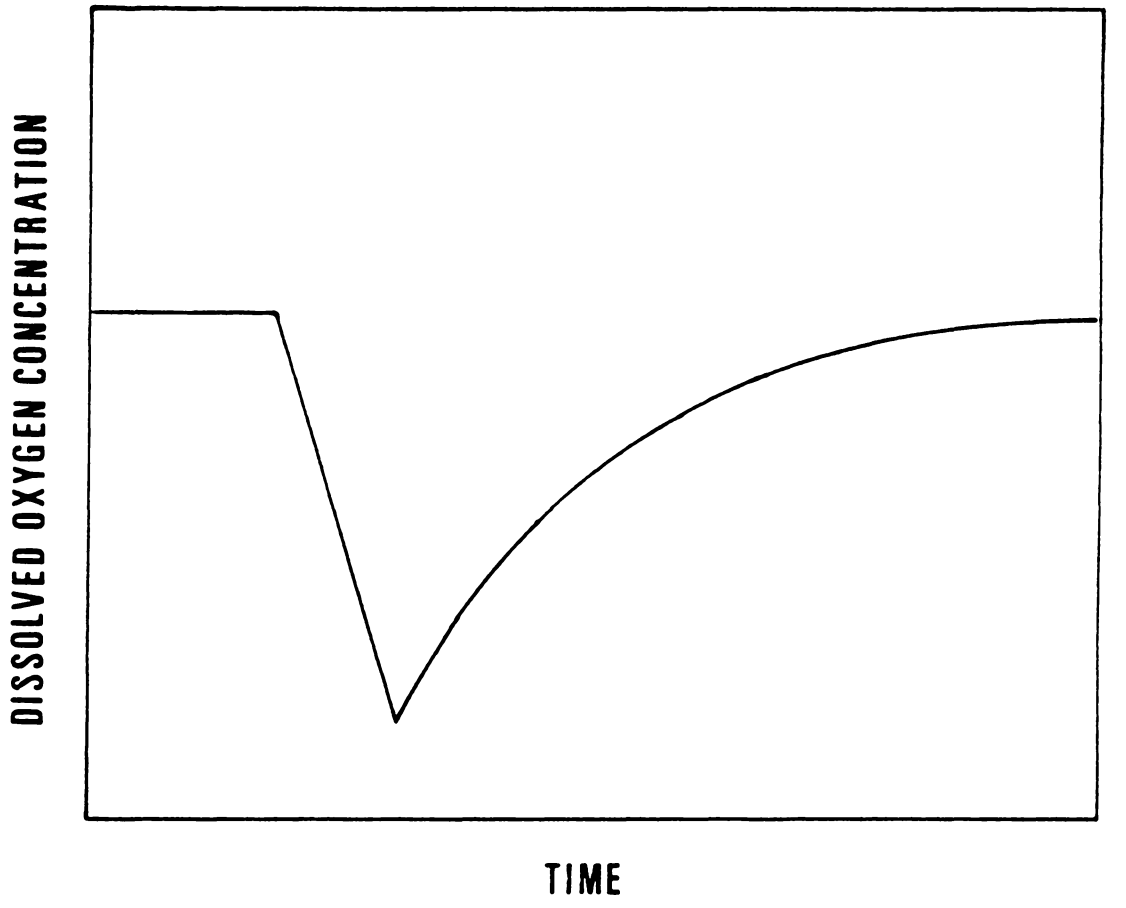


Figure 5. Dissolved oxygen response during a dynamic oxygen transfer coefficient measurement.

entrainment. Second, the value of r is assumed to be constant, so the minimum oxygen concentration should be above oxygen limiting levels. Finally, the response time of the oxygen electrode needs to be an order of magnitude or more greater than $\frac{dC_L}{dt}$ so that the response time of the probe doesn't significantly affect the oxygen reading.

2.3.2.4 Yield Coefficient Method

For this method, the oxygen uptake rate of the organism is examined, rather than the rate of depletion of oxygen in the gas or liquid phases. A material balance on oxygen at the cell gives [36]

$$N_A = \mu X\left(\frac{K'}{Y_{O_2}}\right) \tag{13}$$

where: μ = specific growth rate of the organism (hr^{-1})

Y_{O_2} = yield coefficient on oxygen (grams cells/grams O_2).

K' = conversion constant = $31.25 \frac{\text{mmol } O_2}{\text{grams } O_2}$

Then, if Y_{O_2} is known, only the growth rate is necessary in order to calculate N_A. Figure 6 on page 23 is a graph of the relationship of the oxygen yield to the substrate yield for yeast on several substrates as found by Mateles [37] This is a very quick and easy method of determining the oxygen transfer rate, but equation (13) is valid only when the substrate is completely converted into carbon dioxide, water and cell mass.

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.

2.4 Oxygen Solubility

k_La is important to fermenter design because oxygen is only sparingly soluble in water. When air is sparged into a fermenter under normal conditions, the maximum concentration in the liquid phase will be of the order of 10 mg/l or 0.3 mmol/l. Finding the actual equilibrium concentration of oxygen in the fermenter is further complicated because any salts or other additives tend to reduce the solubility of oxygen. Popovic et al [49] and Quicker et al [50] have compiled data on salt and sugar effects. They found they were able to calculate the oxygen solubility to within about $\pm 2\%$ assuming that individual influences were log additive. Their results for C_L^* were used in the calculation of k_La 's to determine the effect of MBD on oxygen transport.

2.5 Colloidal Gas Aphrons

In this study, a surfactant-stabilized microbubble dispersion was used to supply oxygen to growing yeast cultures. This is an offshoot of research with aphrons conducted Dr. Felix Sebba at VPI. The possible applications of Dr. Sebba's colloidal gas aphrons (CGA's) to biological systems was seen early on, and both Wallis at VPI and Barnett at Rhode Island tried to show the benefits of CGA's as a means of transporting oxygen. The work done in this study used a microbubble dispersion that consisted of a mixture of CGA-sized bubbles (20-70 μ) and bubbles as large as 3-5 mm. The generator design, however is based on the CGA generator designed by Sebba, and the fermenter system used is an improved version of the Wallis fermenter. Therefore, the history of CGA's as used in biological systems is necessary for a full understanding of how and why this study was implemented.

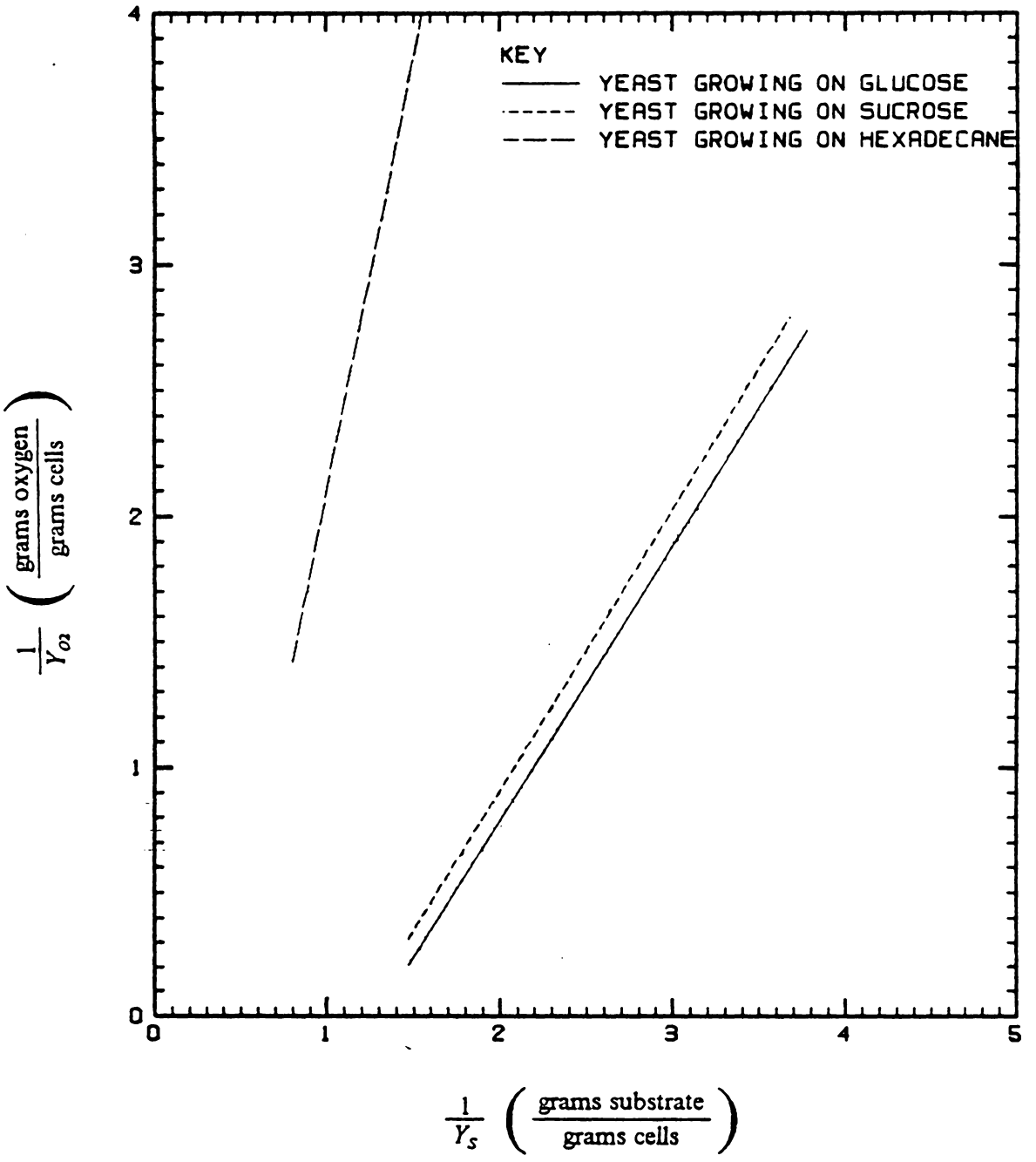


Figure 6. Oxygen yield as a function of substrate yield for yeast (Matcles [37]).

2.5.1 History and Formation

The term aphron was chosen by Dr. Felix Sebba in the early 70's to denote a 50 to 65% dispersion of bubbles with an average diameter of 25 - 100 μ bubbles. Sebba found that such a foam has several interesting characteristics, due both to the small size of the bubbles and to the type of surface which surrounds each aphron. He observed that a foam created with a venturi device consisted of bubbles of an average diameter of 50 - 100 μ The bubbles rose very slowly because of their small size and they tended to resist coalescence [51]. He also found that the surface of the CGA bubbles was more akin to a soap bubble than to an air bubble in water Figure 7 on page 25 depicts the structure of a colloidal gas aphron as compared to the structure of a normal sparged gas bubble.

A CGA consists of an inner pocket of air surrounded by a double layer film. The complete CGA is surrounded by the continuous phase. 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 CGA's by preventing them from coalescing. First, an electric potential gradient is set up by the orientation of the surfactant molecules at the interfaces. CGA's 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 CGA's 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.

The venturi device required large recirculation velocities in order to form a uniform bubble size distribution. This created problems for scale-up. In 1985, Sebba introduced an improved CGA generator design [52], a spinning disk generator capable of producing about 4 liters of CGA's per minute. This devise, shown in Figure 8 on page 27, 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 50 to 100 μ

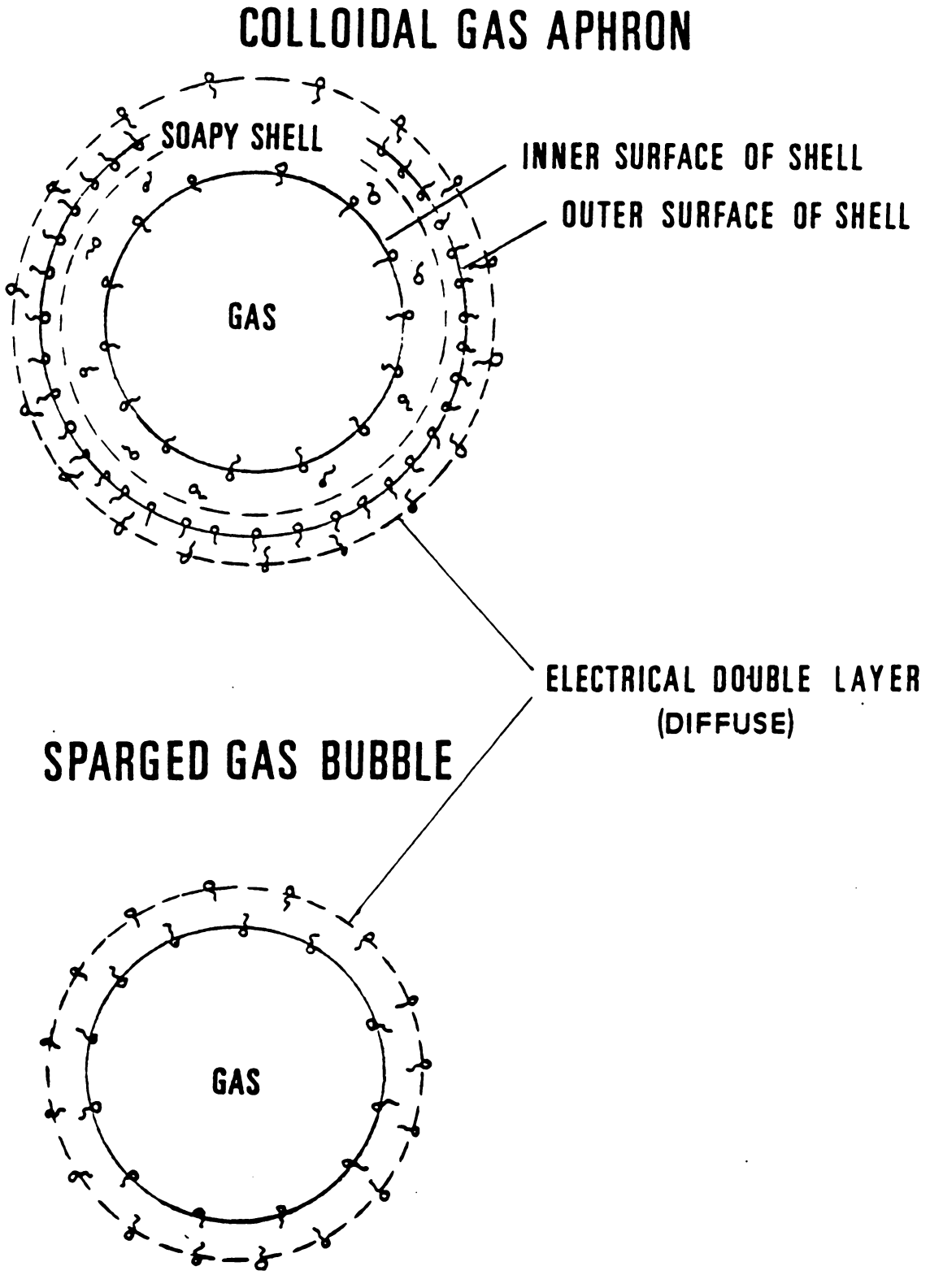


Figure 7. Colloidal gas aphron and sparged gas bubble structures.

2.5.2 Colloidal Gas Aphrons in Fermentations

CGA's have been used in two fermentation systems to date. Barnett and co-workers [53, 54] have used a two column bubble/airlift reactor to try to show the energy requirements of a CGA fermenter. Wallis and co-workers [1] have looked at the use of a bubble column with CGA recycle to grow ***Penicillium chrysogenum***. From the work of these two groups, it is evident that the use of CGA's in fermentation systems shows promise, but as yet has not been quantitated.

Wallis used a system consisting of a 1500 ml column connected to a 1 liter CGA generator (see Figure 9 on page 28). The CGA generator was a spinning disk type per Sebba's design [52], with additions to insure aseptic conditions within the generator. The system held 1300 ml of broth and the CGA recycle rate was 250 ml/min of a 65% air solution. This reactor was rather primitive, with no monitoring of pH or dissolved oxygen in the fermentation column. Essentially all they were trying to prove was that one can grow organisms in a reactor using CGA's as the source of oxygen. They were able to grow the ***P. chrysogenum*** quite well and so proved their point.

Barnett's fermenter, as shown in Figure 10 on page 29, consists of two columns connected at the top with an overflow tube. The CGA recycle exits the bottom of one column and enters the bottom of the other. The CGA generator used in this fermenter is a venturi type originally presented by Sebba in 1971 [51]. This fermenter apparently had no in-line pH control, nor dissolved oxygen measuring equipment. The volume of the entire apparatus is approximately 8.5 liters, with a working volume of approximately 6 liters [54, 55]. Two papers have been presented or published on the use of this fermenter for growing different organisms.

Saccharomyces cerevisiae was the first organism grown in Barnett's fermenter, as described in a paper presented at the 1981 meeting of the American Chemical Society by Bradley and Barnett [54]. In this paper, they report specific growth rates of 0.31-0.44 /hr, which they compared to a standard of 0.35-0.45 /hr. Substrate yields (gm cells/gm glucose) of 0.28 were reported and compared to a standard value of 0.37. The air flow rate was given as 0.017 volumes of air/volumes

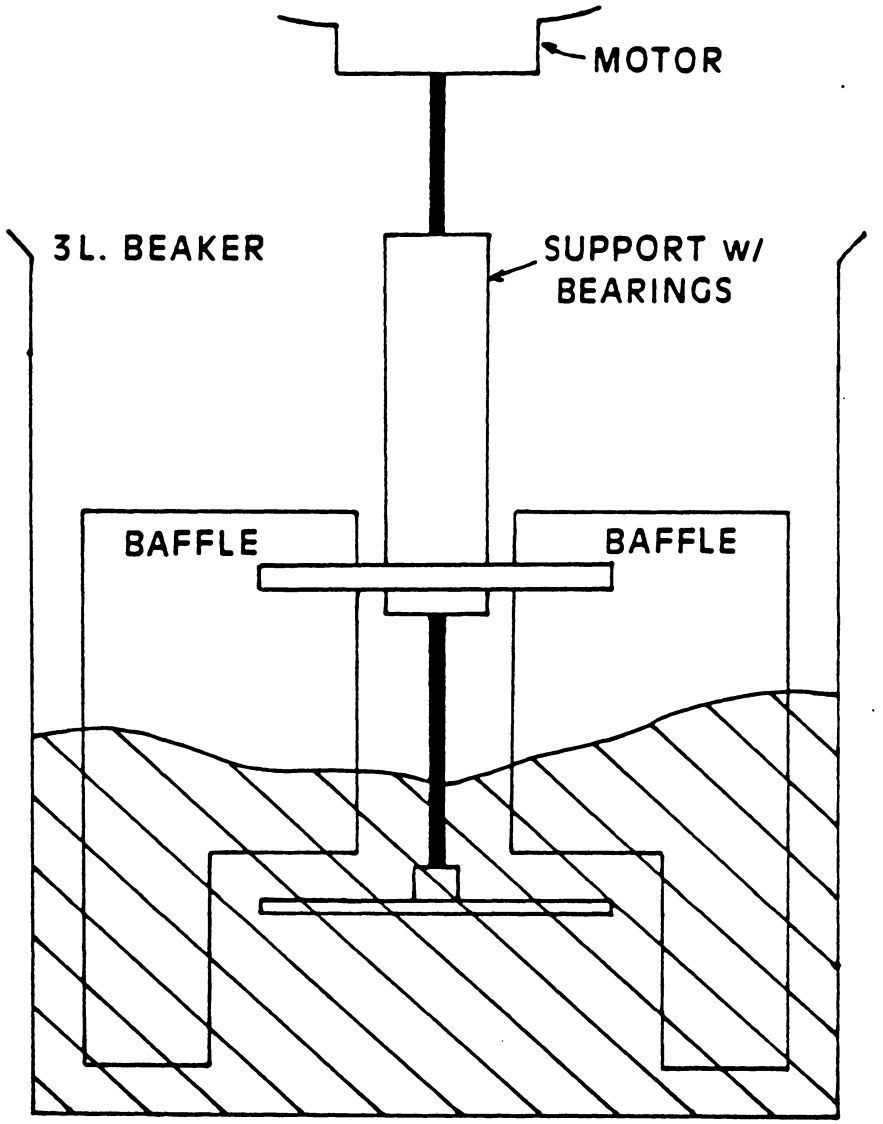


Figure 8. The spinning disk CGA generator (Sebba [52]).

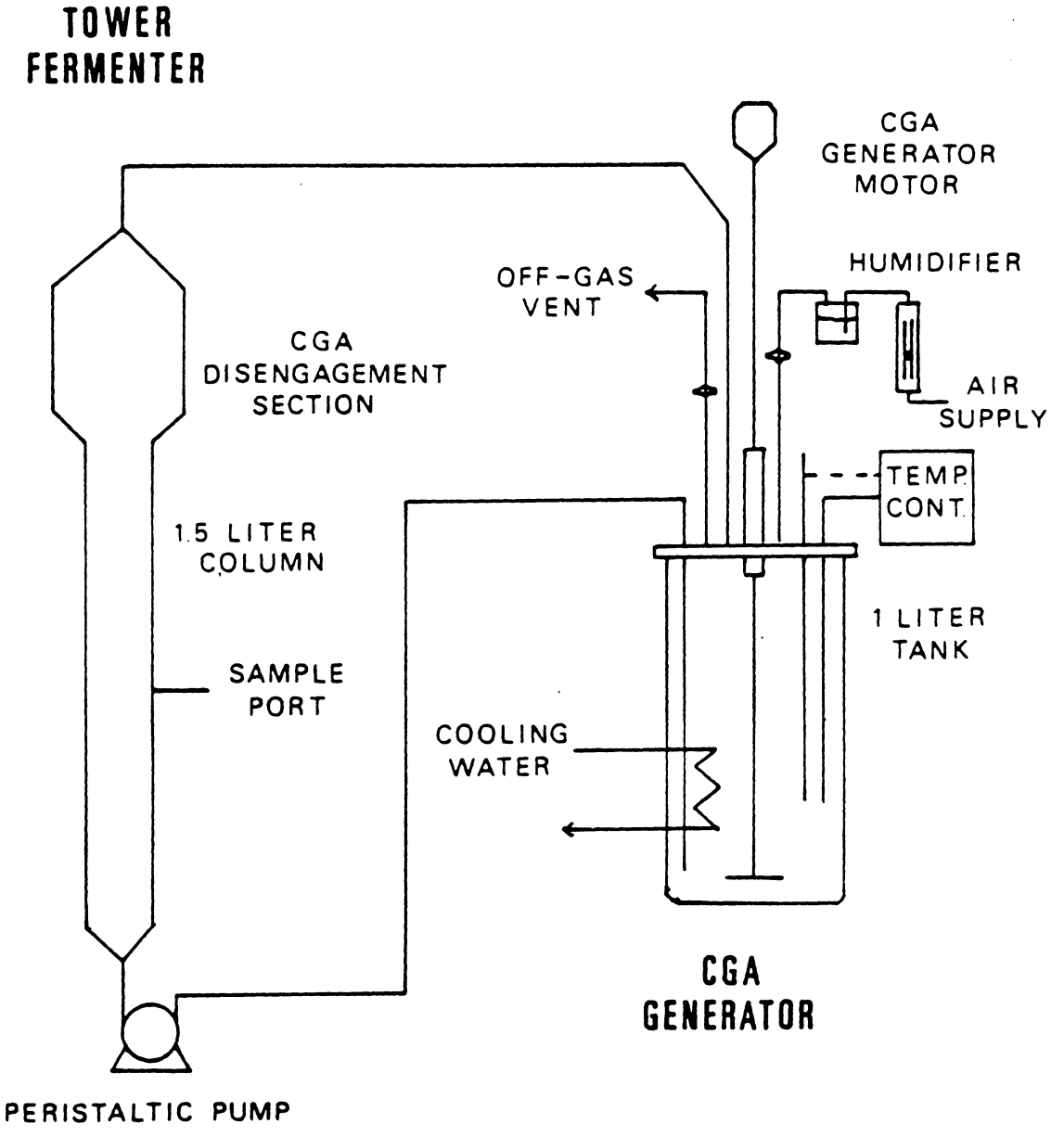


Figure 9. Bubble column CGA fermenter (Wallis et al).

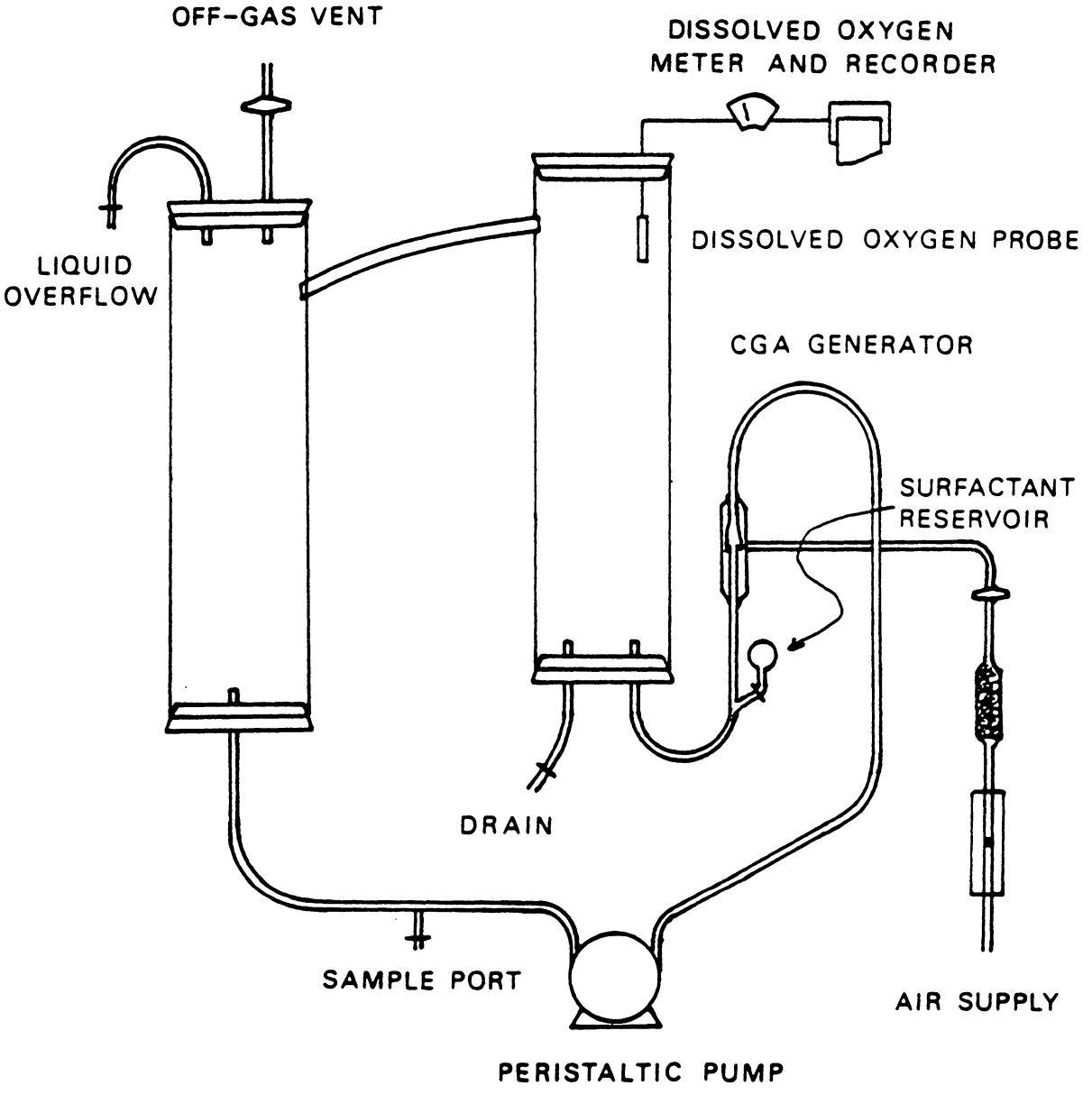


Figure 10. Foam fermenter (Barnett et al).

liquid min. (VVM), and the power input per volume was given as 5.9/6 liters. They compared these values to standard values of 55.2/6 liters for a stirred tank and 20.0/6 liters for an airlift fermenter.

According to their figures, they only used 0.017 volumes of air per volume of broth per minute. If this was the case, their fermenter could only supply a maximum of 2% of the oxygen needed for aerobic yeast growth. They say that power requirements for the CGA fermenter are nearly an order of magnitude smaller than in a stirred tank reactor.

The second set of experiments using Barnett's fermenter was presented by Misra and Barnett at the World Congress III of Chemical Engineering in Tokyo, Japan in 1986 [53]. These experiments used ***Xanthomonas campestris***, a xantham gum producing organism, to test the effectiveness of CGA's in supplying oxygen to high viscosity media. For these experiments, an oxygen probe was added to the top of the first column to measure the dissolved oxygen in the fermenter. They reported a maximum specific growth rate of 0.22 /hr and a maximum k_La of 0.58/min.. The liquid volume of the fermenter was not reported, nor was the CGA recycle rate. They conclude that the dead spaces have been removed from their fermenter, although their figures do not support this. From their design (Figure 10 on page 29), it appears that there should be dead spaces at the bottoms of both columns.

In conclusion, Wallis and Barnett have shown that several organisms can be grown in fermenters using CGA's as the oxygen source. They have not shown, however, that CGA's are any more efficient at oxygen transfer than normal sparged air in a fermentation environment.

This project was designed to prove that CGA's are more efficient oxygen transport vehicles than ordinary sparged air bubbles. During the experiments, however, it was found that the CGA generator was producing a mixture of CGA's and some larger bubbles. Therefore, the more general term microbubble dispersion (MBD) was applied. It is probable that Wallis' "CGA" fermenter was actually a MBD fermenter.

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Fermenter

All fermentations were run in a two liter Multigen bench top fermenter, New Brunswick Scientific (NBS). The vessel was temperature and pH controlled. pH was measured and recorded by a NBS pH controller and an Ingold pH electrode. The pH was kept at a constant value 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), and recorded by a Fisher Recordall 5000 chart recorder. Air flow into the fermenter in the initial runs was measured by a variable area flow meter (Cole Parmer) but was determined to be of limited reliability. Air and MBD flow rates in the later runs were measured using the calibrated scale on the Masterflex pump. The glucose feed was metered through a Varioplex II pump (LBK). All other measurements were done on a discrete basis and will be described later.

3.1.1.1 Control - Stirred Tank Fermenter

The control runs were done in a standard stirred tank configured as shown in Figure 11 on page 33. Samples were removed from a special sampling port at the top of the fermenter. The temperature in the fermenter was held constant at 30°*C* for most of the initial runs and 35°*C* for the actual control runs. For those runs held at 30°*C*, cooling was necessary and cooling water was run at a constant rate through a "cold finger" inserted into the top of the fermenter. 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.

3.1.1.2 Microbubble Dispersion Fermenter

For the MBD runs, the same fermentation vessel was used with the addition of a MBD generator on a recycle loop (Figure 12 on page 34). 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 was delivered through the same holes in the bottom of the impeller shaft as the air was in the control runs. For the MBD runs and the final control runs, a filtered vent from the MBD generator was provided to prevent pressure buildups in the MBD generator.

The MBD generator was built along the lines of Sebba's CGA generator [52]. Sterility was a special problem with the MBD generator because the driving shaft of the rotating disk had to come in from the top and it had to spin at approximately 4000 RPM. A coupling designed to reduce the chances of dirt and bacteria entering along this shaft was added to the generator. Figure 13 on page 36 is a schematic of the MBD generator with its coupling.

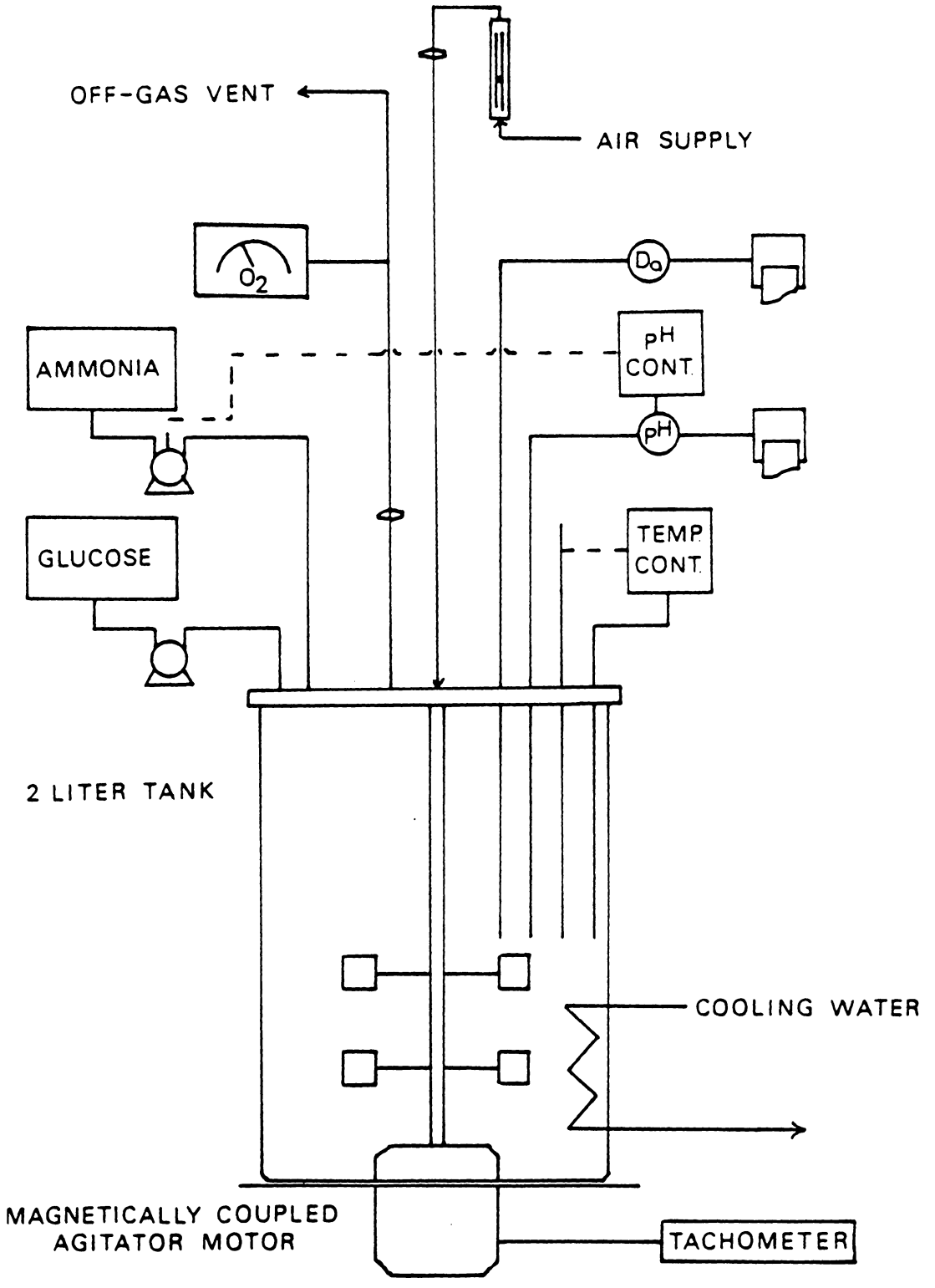


Figure 11. Fermentation system: air sparged runs.

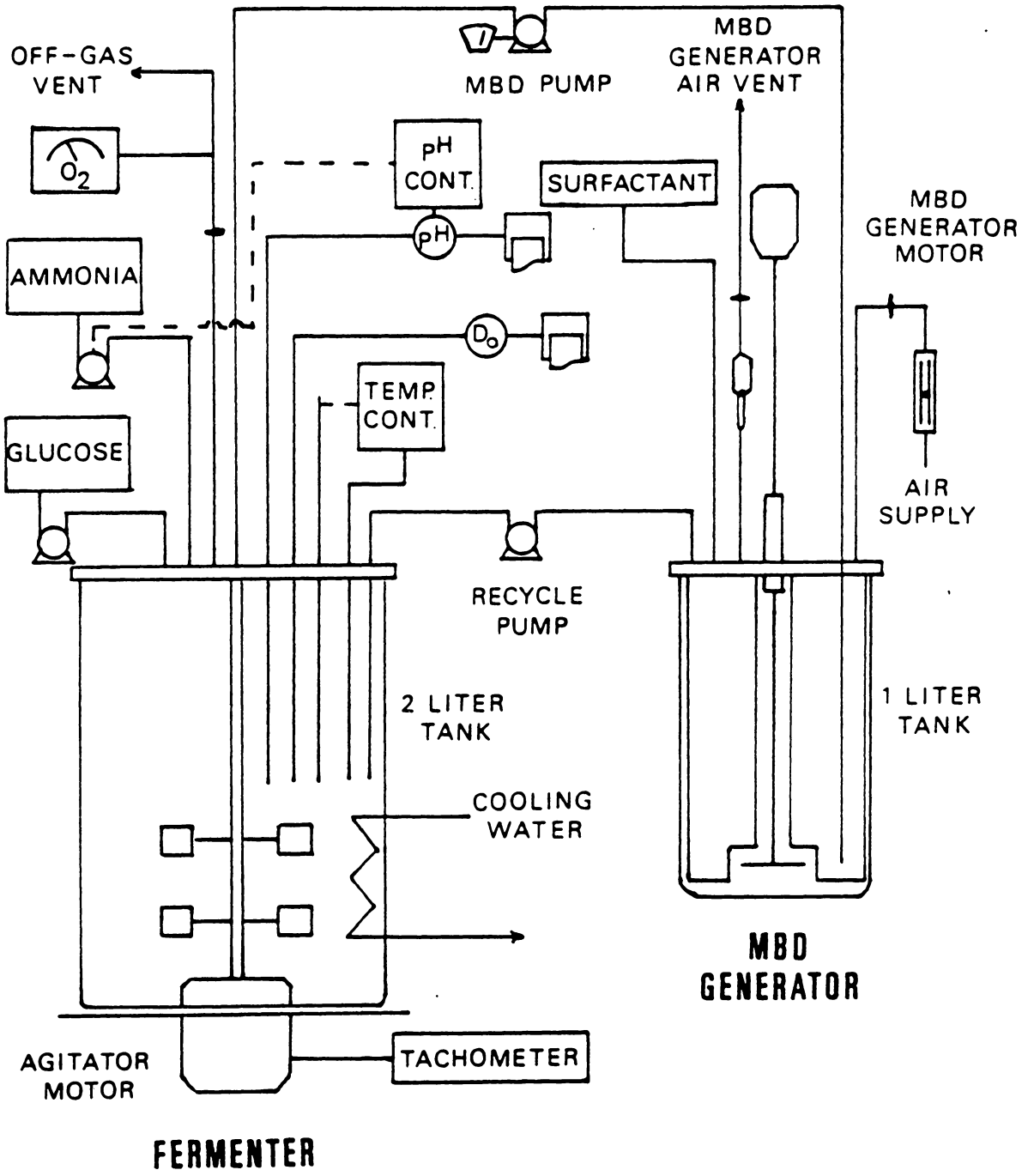


Figure 12. The fermenter and MBD generator: MBD sparged runs.

3.1.2 Media

Difco YM broth was used exclusively throughout these experiments. 150 ml of standard YM broth (21 grams per liter of distilled water) was used for all starter cultures. For the actual fermentations, unless noted otherwise, 1 liter of standard YM broth was used to begin the fermentation and the glucose feed consisted of 200.0 grams of glucose and 28.0 grams of YM broth in 300.0 ml of distilled water. The composition of the standard YM broth is given in Table 3 on page 37.

3.1.3 Organism

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 (approximately four days old) unless otherwise noted. New cultures were taken at the same time as inoculation by transferring 10 ml of the original culture to a fresh sterile shake flask.

3.2 Methods

3.2.1 Assays

Glucose concentrations and cell mass concentrations were determined from the samples taken throughout each fermentation. Glucose was determined by the method of Park and Johnson [56], which is based on the reduction of ferricyanide ions in alkaline solution by a reducing sugar (i.e.

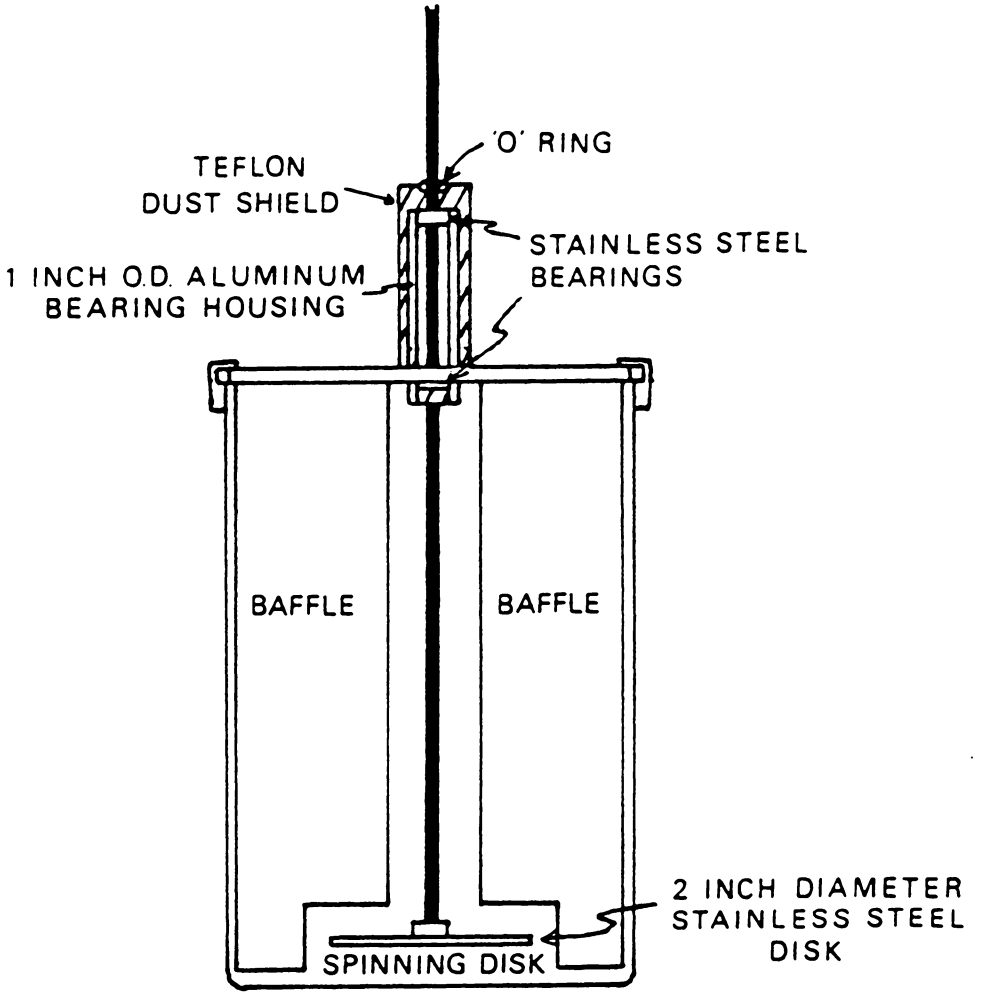


Figure 13. Aseptic microbubble dispersion generator design.

Table 3. Standard YM Broth Composition

Distilled water		1 liter
YM Broth		
Yeast Extract	3 grams	
Malt Extract	3 grams	
Peptone	5 grams	
Dextrose	10 grams	
Total Solids		21 grams

glucose). The ferrocyanide then reacts with another ferricyanide to produce ferric-ferrocyanide (Prussian blue) complex. The Prussian blue concentration was measured photometrically (see Appendix A.1).

Cell mass concentrations in the fermenter were determined by the method of Hug and Fiechter [57], using a solvent wash, followed by filtration, then drying to constancy (see Appendix A.2).

Dissolved oxygen in the fermenter was measured using a galvanic probe that 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 thirty minutes. 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 thirty minutes.

pH was measured with an Ingold pH electrode that was calibrated with 4.00 and 7.00 buffered solutions before broth sterilization. The calibration of the probe was rechecked at the end of each run and any adjustments needed were assumed from the beginning of run.

Gas or MBD flow rates during runs 15-19 were measured using a calibrated peristaltic pump. The pump was calibrated by measuring the amount of water displaced by air from a sealed side arm flask during one minute intervals.

Impeller speeds in the fermenter were controlled by a calibrated inline variac. The variac's calibration was checked using a phototachometer.

3.2.2 Fermentations

3.2.2.1 Batch Runs

The batch fermentations were started with the addition of 75 ml of yeast starter culture to 1500 ml of the standard YM broth at 30°C. The initial pH of the YM broth was 6.25 $\pm$ 0.05. The pH of the fermenter was essentially only monitored during these runs. Control was attempted in

fermentation 3, but the 0.1 N NH_3OH used was too weak to affect the pH in a significant way. Samples were taken approximately every hour and analyzed for cell mass. The optical density at 580 nm was also taken for each sample.

3.2.2.2 Fed-Batch Runs

The initial fed-batch runs were trial runs for determining optimum feed rates, feed concentrations, pH control variables, and temperature. Because of this, conditions varied from run to run and a summary of those conditions can be found in Table 4 on page 40. All fed-batch runs were started as batch runs with the standard YM broth, then after 18 to 22 hrs., glucose feeding commenced.

3.2.2.3 Fed-Batch Control and Microbubble Dispersion Runs

The control and MBD runs were started with the addition of 100 ml. of yeast starter culture to 1000 ml. of YM broth at $35°C$. The initial pH of the YM broth was 6.25 ± 0.05 and was held at 5.0 or above by the addition of 1.0 N NH_3OH. Air or MBD was sparged into the fermenter at 400 ml./min. and the agitator speed was set to 100 or 580 RPM. Samples were taken approximately every four hours for cell mass determinations.

Table 4. Conditions of the Fermentations

Run	Run Type	MBD or Air	Flow Rate (l/min)	Agitation in the Fermenter (RPM)	Temp. Set (°C)	Initial Volume Broth (ml)	Volume. of the Innoc. (ml)	Glucose feed x 100 (g/w/ym*)
2	Batch	Air	1.35	420	30	1500	75	---
3	Batch	Air	1.35	420	30	1500	75	---
4	Batch	Air	1.35	420	30	1500	100	---
8	Fed-batch	Air	1.35	530	30	1500	100	3/5
9	Fed-batch	Air	1.35	530	30	1500	100	3/5
10	Fed-batch	Air	1.35	475	30	1000	100	3/4.5/.21
11	Fed-batch	Air	1.35	475	30	1000	100	3/4.5/.42
12	Fed-batch	Air	1.35	640	30	1000	100	3/4.5/.42
13	Fed-batch	Air	1.35	640	35	1000	100**	3/4.5/.42
14	Fed-batch	MBD	0.450	585	35	1500	100	3/4.5/.42
15	Fed-batch	Air	0.400	100	30***	1000	100	2/3/.28
16	Fed-batch	Air	0.400	100	35	1000	100	2/3/.28
17	Fed-batch	MBD	0.400	100	35	1000	100	2/3/.28
18	Fed-batch	Air	0.400	585	35	1000	100	2/3/.28
19	Fed-batch	MBD	0.400	585	35	1000	100	2/3/.28

* grams glucose /grams water/ grams dry YM broth
** inoculum 100 ml of Alltech yeast
*** temperature was not constant during run.

4.0 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. Having chosen to grow ***Saccharomyces cerevisiae*** and having decided on a stirred tank fermenter, there still remained many variables to set. These included the air flow rate, the agitation rate, the temperature, and the concentration of the pH controlling species. The best values for these items were determined from data gathered in three batch fermentations. With the move to a fed-batch mode, several more variables were introduced. The two most important of these were the concentration of nutrients in the feed and the feed rate. The feed rate and composition were set from the results of six fed-batch runs with air sparging. Finally to determine the effects of a MBD on growth and oxygen transfer, the growth pattern of ***S. cerevisiae*** was 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).

4.1 Initial Runs

The initial batch runs served two purposes. The first was to define operating ranges for air flow, agitation, pH, and temperature. The second was to determine the growth characteristics of *Saccharomyces cerevisiae* ATCC 4111. The growth curves of the batch runs are shown in Figure 14 on page 43. For comparison, the "classic" results of von Meyenburg [18] are also included. The batch data agreed, at least through the first 15 hours, and showed that the fermenter and its control systems worked respectably. From these runs, it was determined that the ammonium hydroxide used for pH control had to be greater than 0.1 N. For the remainder of the runs a 1.0 N ammonium hydroxide solution was used for pH control. The yeast seemed to grow as individual cells, and the broth appeared homogenous throughout the run. There was a slight amount of foaming once the culture began oxidizing ethanol. A plot of pH versus time for runs 2 and 3 (Figure 15 on page 44), shows a characteristic minimum, followed by a slight rise, and then a gradual decline. The initial minimum in the pH coincides with von Meyenburg's shift from glucose to ethanol consumption. Therefore, the batch fermentation had proceeded as expected, and fed-batch fermentations were begun using this data.

The trial fed-batch runs were used to define the best operating conditions and the best results possible with this fermenter. The initial minimum in the pH curve found during the batch fermentations was used to determine the best time to begin adding the glucose feed. To insure complete oxidation of the fed glucose, it had to be added after all of the glucose had been consumed, but before all of the ethanol was oxidized. A feed start time of 1.8 × the time at which the initial minimum in the pH occurred was chosen as the best possible time, since this would supply more glucose just as the last of the ethanol was oxidized, and before the yeast began to die. Next, the concentration of the glucose feed and the best rate of feeding were determined. Runs 8 and 9 were fed a slightly dilute pure glucose feed (300g glucose per 500ml water). In run 8, this was fed in too quickly, as can be seen in the drop in cell mass concentration starting at approximately 20 hours (Figure 16 on page 46). For the remainder of the runs, a more concentrated glucose feed (300g

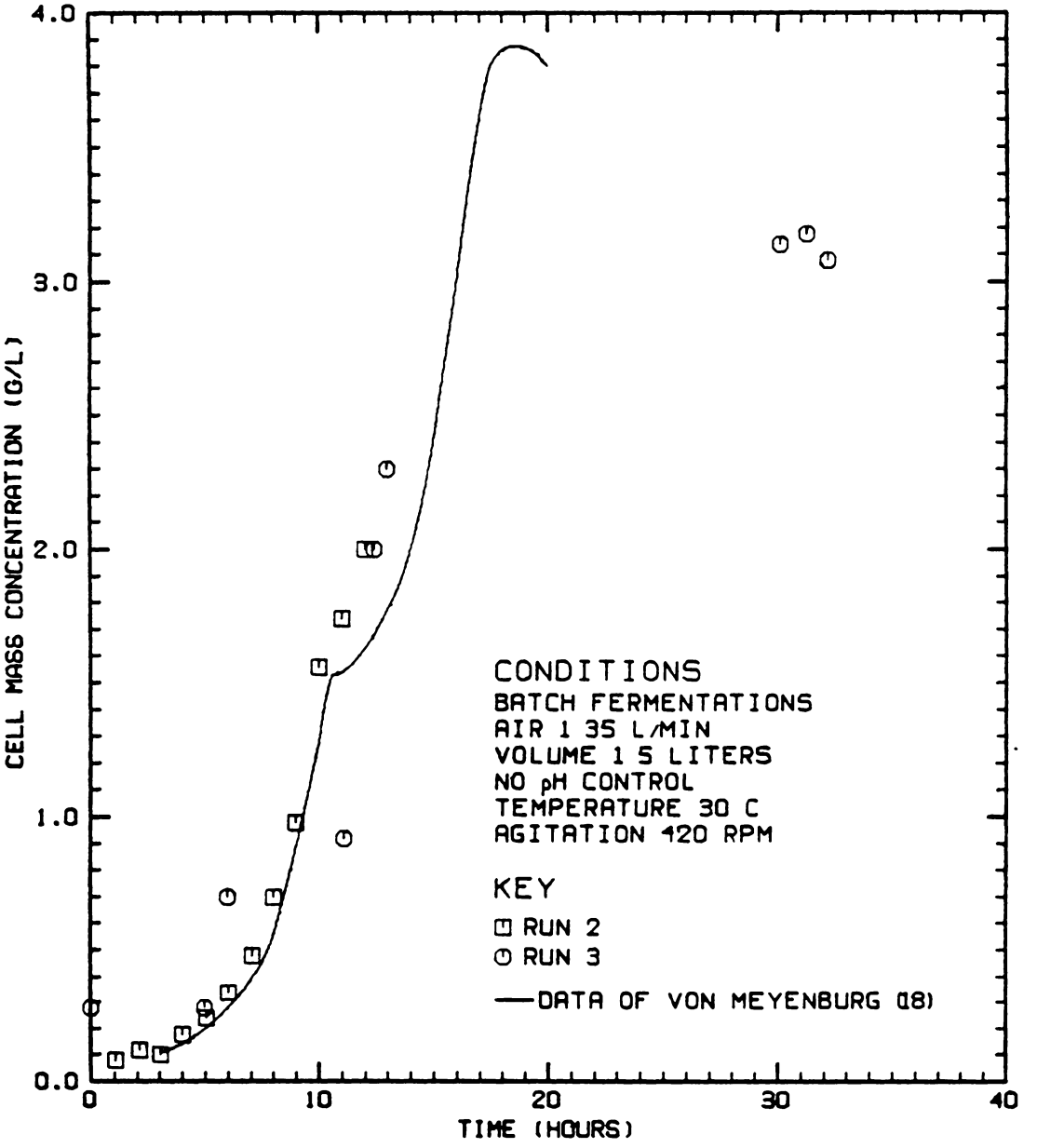


Figure 14. Yeast growth during initial batch fermentations.

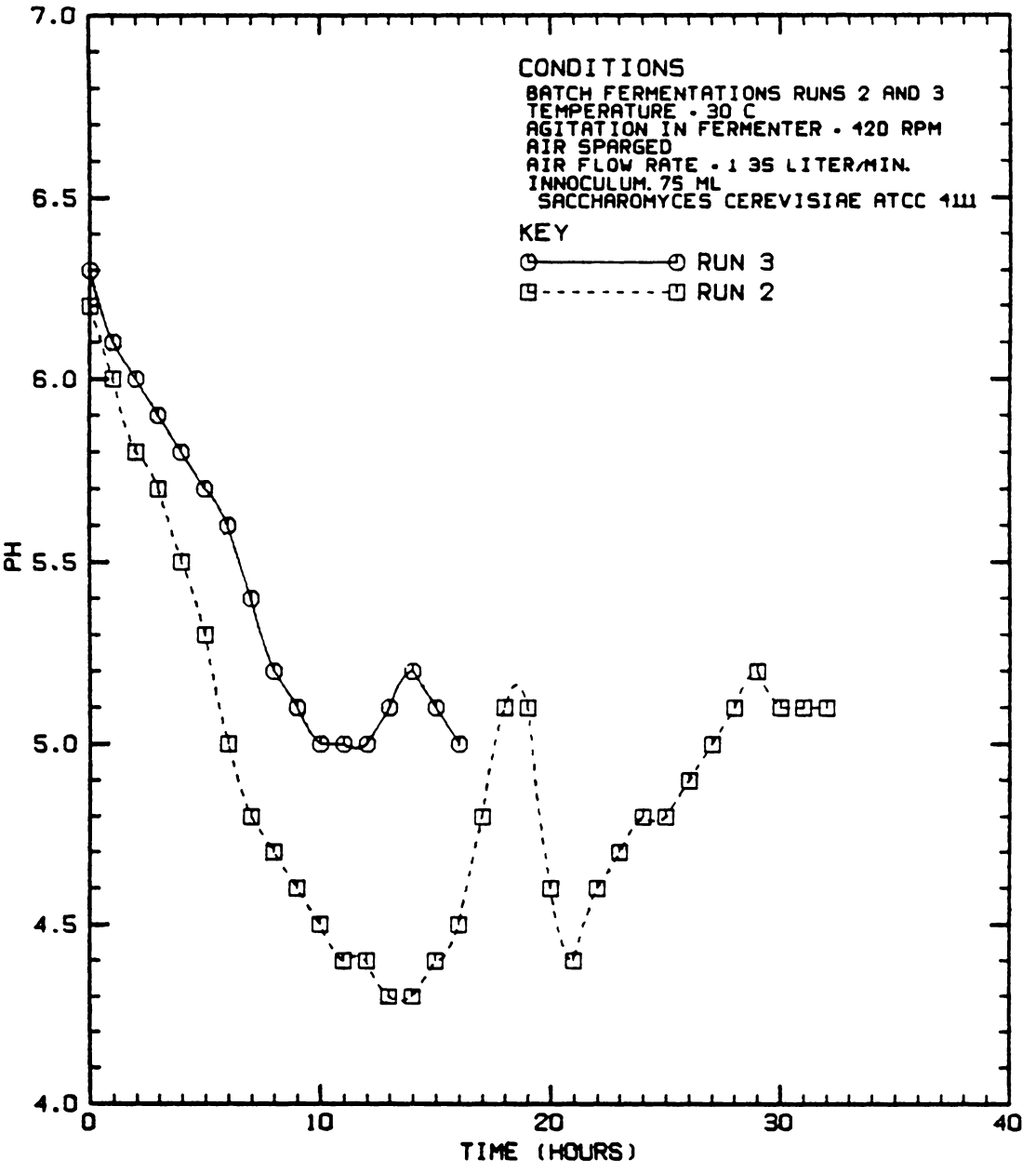


Figure 15. pH during initial batch fermentations.

glucose per 450ml water) was used. In runs 10 and 11, additional YM broth in the glucose feed resulted in increased growth. Runs 12 and 13 used an excessive agitator speed (640 RPM), which reduced the growth rate by about 10%. Run 13 also used a commercial yeast culture (Alltech Yeast), which grew at about the same rate as the ATCC 4111. Run 14 was the initial trial run for the MBD generator system. Figure 16 on page 46 shows the results of five of the initial fed-batch runs. The maximum cell mass concentration obtained through 100 hours in these runs was 9.2 gm/l dry wt. yeast. Most cell growth occurred within the first 50 hours. After this time, the cell mass in all runs remained essentially constant or fell slightly.

The initial batch and fed-batch runs, fermentations 1 through 15, served both to debug the fermenter system and to provide a base line for the later experimental runs. A compilation of the data from the initial runs is shown in Figure 17 on page 47. A best possible growth curve was obtained from a compilation of the points which showed the most growth. These points were least squares fit by the equation:

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

where: X = cell mass concentration (gm/l)

t = time from the start of the fermentation (hrs)

This curve will be included with all subsequent data plots. The air flow rates for these runs were approximately 1.35 volumes of air per volume of liquid per minute (VVM), as compared to 0.4 VVM for the experimental control runs, and 0.26 VVM for the MBD runs.

4.2 Experimental Runs

The four main fermentations (runs 16 - 19) varied agitation speeds and oxygen inputs as shown in the experimental conditions grid in Figure 18 on page 50. The cell mass concentration and

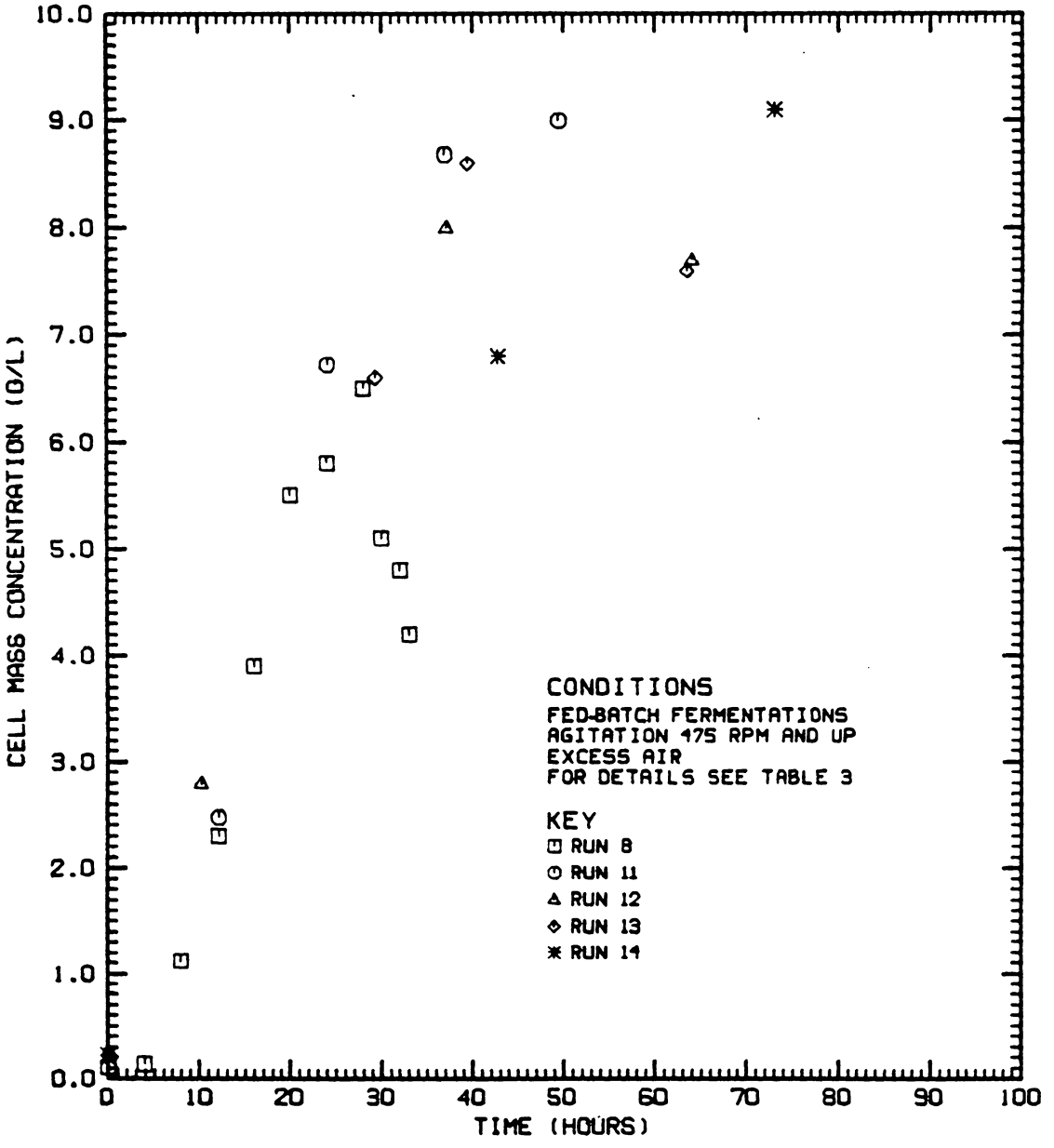


Figure 16. Yeast cell growth during fed-batch fermentations: Initial runs.

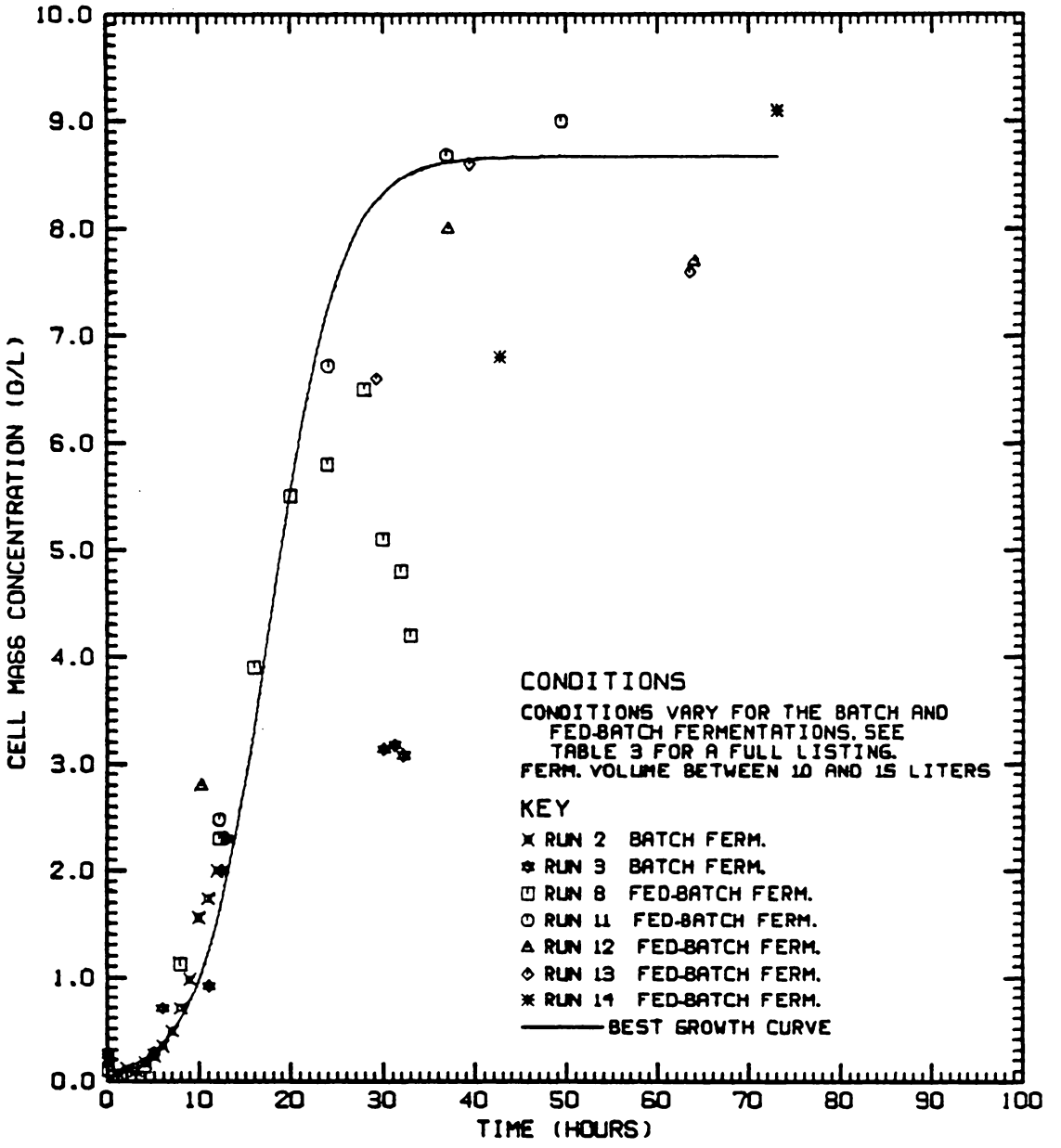


Figure 17. Yeast growth during initial runs and extrapolated best possible growth curve.

dissolved oxygen profiles of the four main fermentations are shown in Figures 19 - 22 on pages 51 - 54. Included in each of these figures is a line giving the "hypothetical best possible growth curve". This line was derived from Figure 17 on page 47 and is valid for growth that is not oxygen limited. As can be seen in these figures, the lightly agitated air sparged run (Figure 19 on page 51) was the only oxygen limited run and had the slowest yeast growth. Runs 17 and 19, the MBD sparged runs, had essentially constant dissolved oxygen profiles. The lightly agitated fermentation (run 17) showed a higher average dissolved oxygen concentration than the normally agitated fermentation (run 19). Run 18, (Figure 22 on page 54) the normally agitated air sparged run, shows some anomalies in the dissolved oxygen profile. These anomalies are probably due to the feeding regimen, but it is not clear why the MBD runs do not react similarly. The cell growth was essentially the same in all three runs.

The cultures in runs 17, 18, and 19 looked essentially identical. All three appeared to be homogenous mixtures of cells, with small to medium-sized bubbles rolling through the fluid. Run 16 was much 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 of the runs, the yeast was a whitish tan in appearance. Some of this color could be due 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 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 gm/l, slightly more than the maximum in the initial fed-batch runs.

All of the fermentations were started out in batch mode and should have nearly identical growth profiles through the first ten to twelve hours. During this time, most of the initial glucose in the media is metabolized to ethanol. From hours ten to twenty, the yeast were oxidatively metabolizing the ethanol to carbon dioxide and water. After twenty hours, the glucose feed was turned on. Then the yeast should have been metabolized glucose directly to carbon dioxide and water. If the yeast growth is oxygen limited, a divergence from the normal growth pattern should be seen after fifteen to twenty hours of growth.

The combined results of the two well mixed runs (Figure 23 on page 55) show that at 580 RPM there is little if any difference between air sparging and MBD sparging. The MBD run may have had a slightly faster growth rate, but more fermentations would be needed to prove whether or not there is a significant difference. The final cell mass concentration for the MBD run was 8.3 gm/l, more than 25% greater than the cell mass concentration in the previous sample. The final sample was taken directly from the fermenter, instead of from the sample port, as was done for all four runs. The anomaly in the cell mass concentration in run 19 is probably due to yeast settling before reaching the line that led to the sample port. This settling could have occurred because the recycle line draws liquid out of the fermenter at a slow rate through an 8 inch long vertical tube.

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 (Figure 24 on page 56). Thus, 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. Differences in the growth rates between the MBD sparged and the air sparged fermentation are readily apparent when the growth curves are superimposed (Figure 25 on page 57). The slower growth rate of the air sparged fermentation is most probably due to oxygen limitation, since the concentration of oxygen in the fermenter was nearly zero throughout the run.

Fermentation 16 was definitely oxygen limited, not only from the above arguments, but also from the evidence of the dissolved oxygen in the fermenter. The oxygen limitation in run 16 is interesting because the conditions in this run come closest to approximating the conditions in an industrial fermenter. 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 MBD's as a viable oxygen source for traditional tank fermenters.

	Fermenter Agitator Speed	
Oxygen Delivery System	100 RPM	580 RPM
Air Sparging	Fermentations 16	Fermentation 18
MBD Sparging	Fermentation 17	Fermentation 19

Figure 18. Fermentation Conditions Grid for Main Experiment

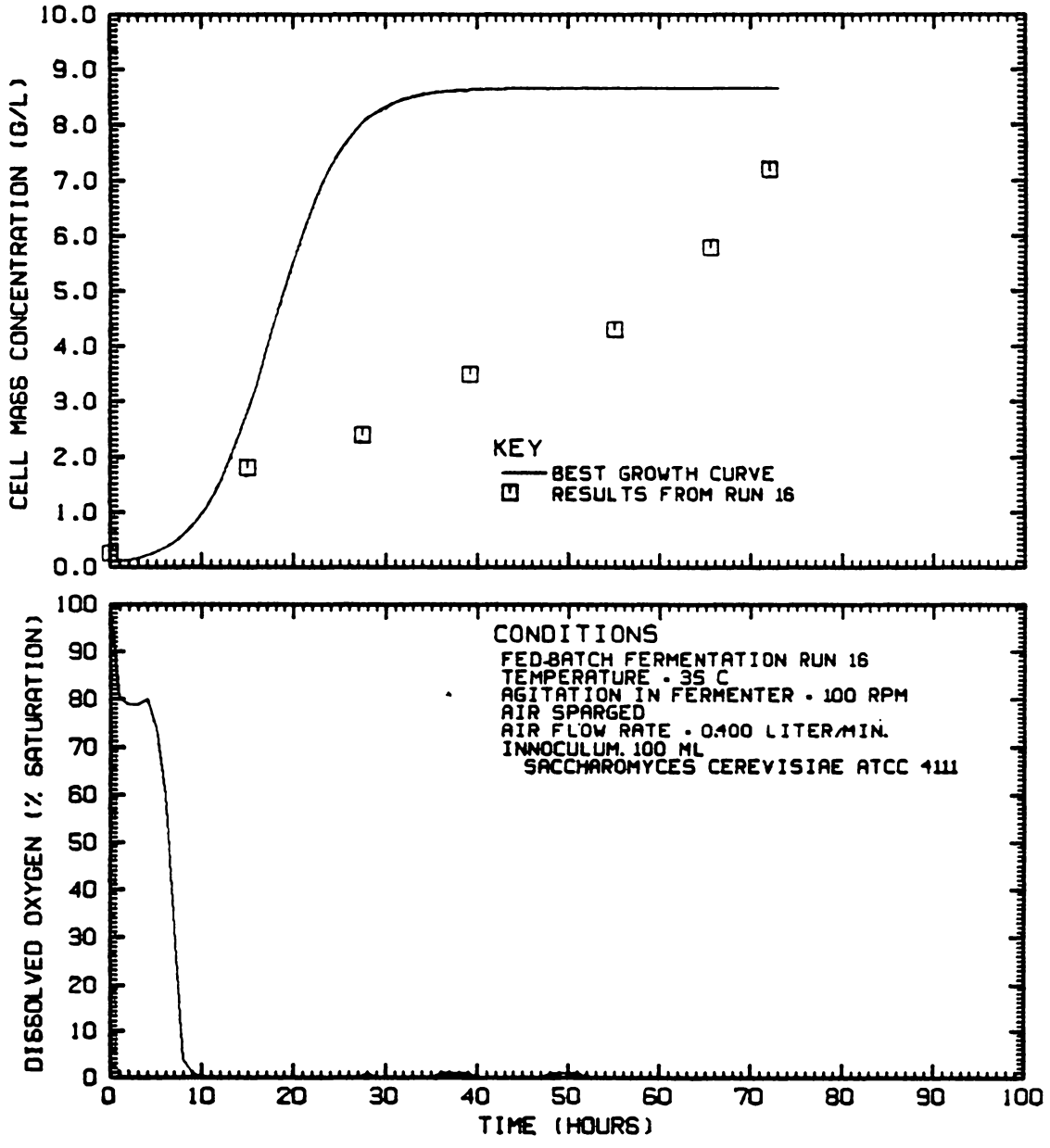


Figure 19. Yeast growth and dissolved oxygen concentration: reduced agitation (100RPM) and air sparging, run 16.

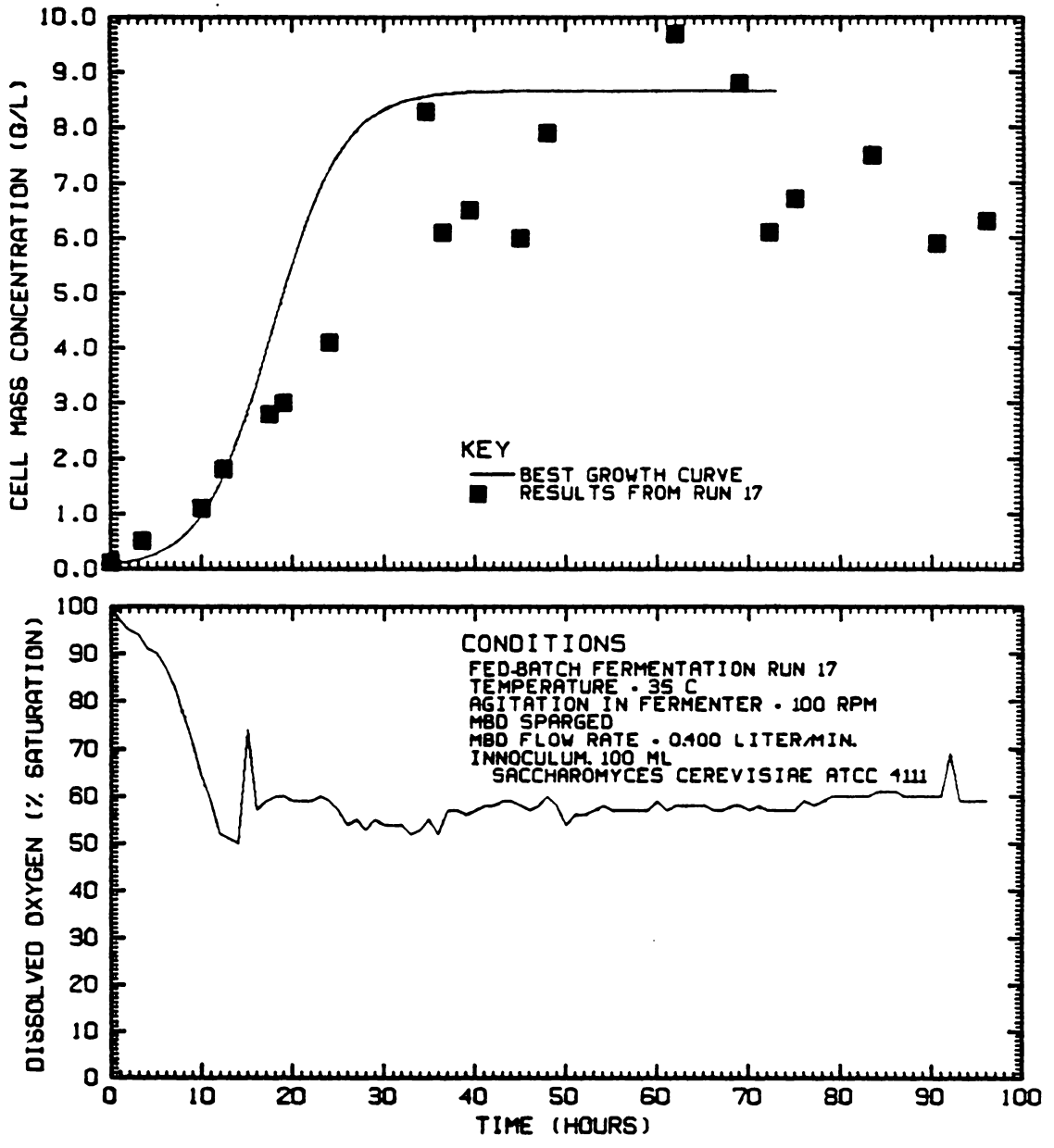


Figure 20. Yeast growth and dissolved oxygen concentration: reduced agitation (100RPM) and MBD sparging, run 17.

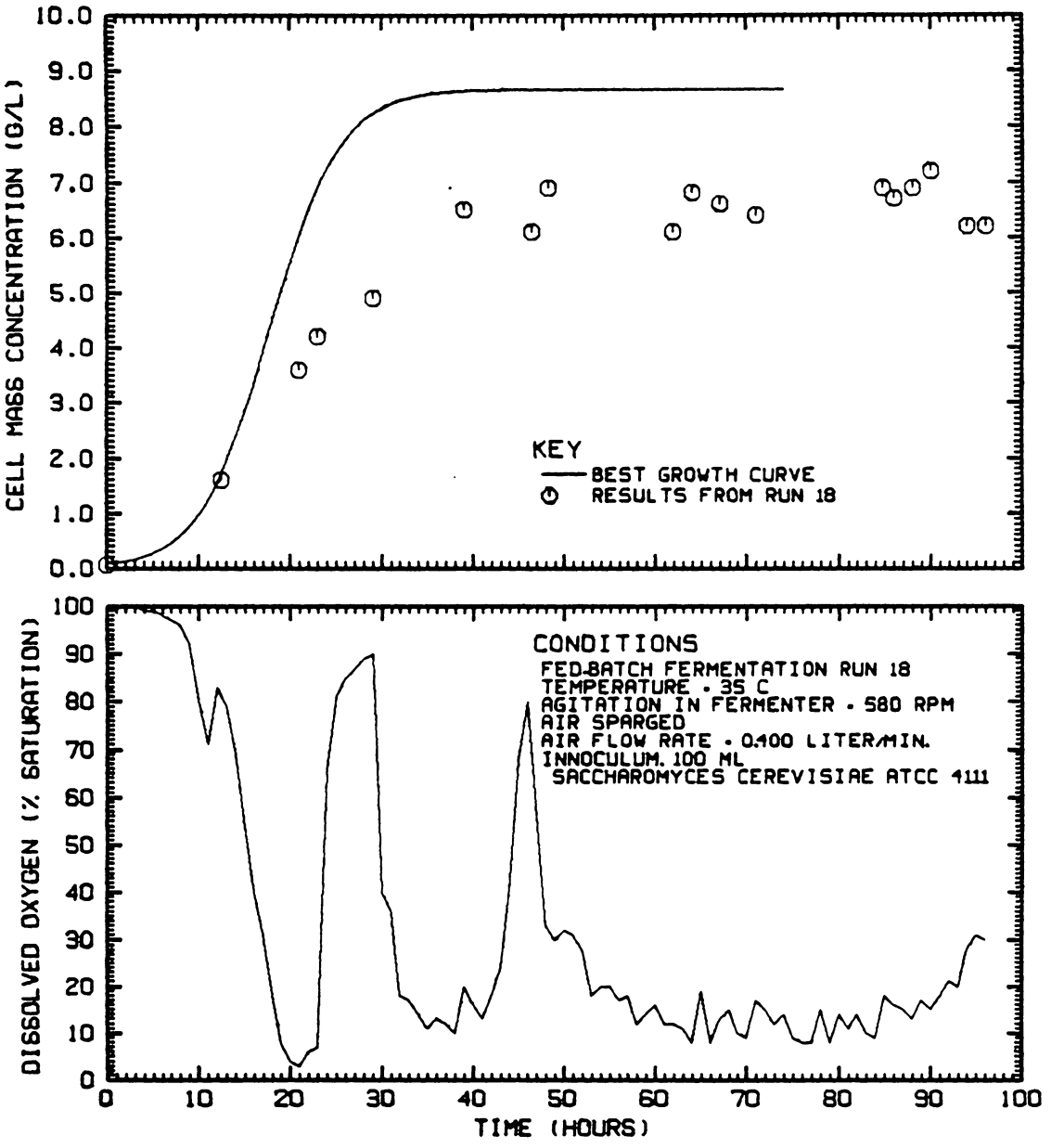


Figure 21. Yeast growth and dissolved oxygen concentration: normal agitation (580 RPM) and air sparging, run 18.

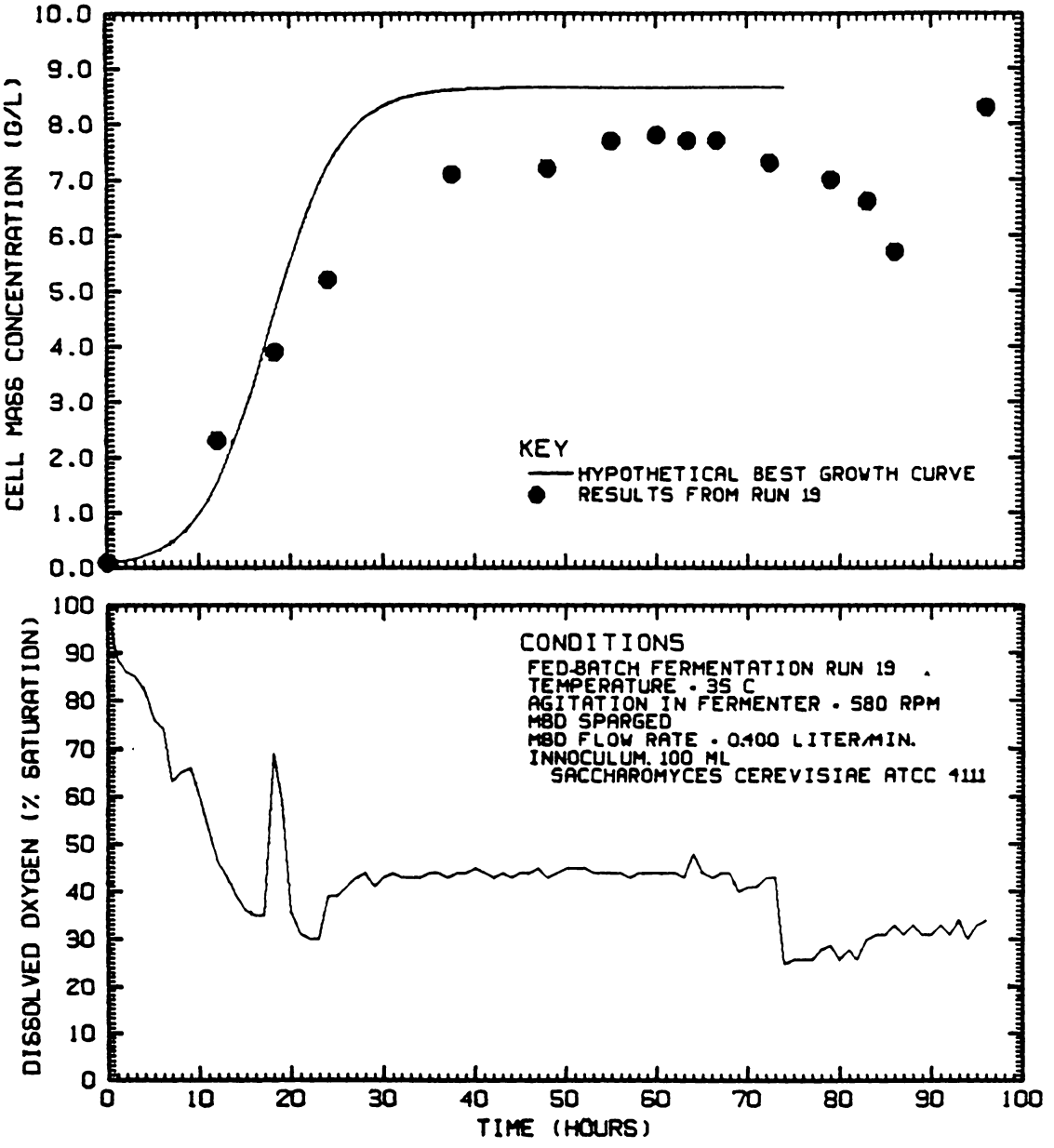


Figure 22. Yeast growth and dissolved oxygen concentration: normal agitation (580 RPM) and MBD sparging, run 19.

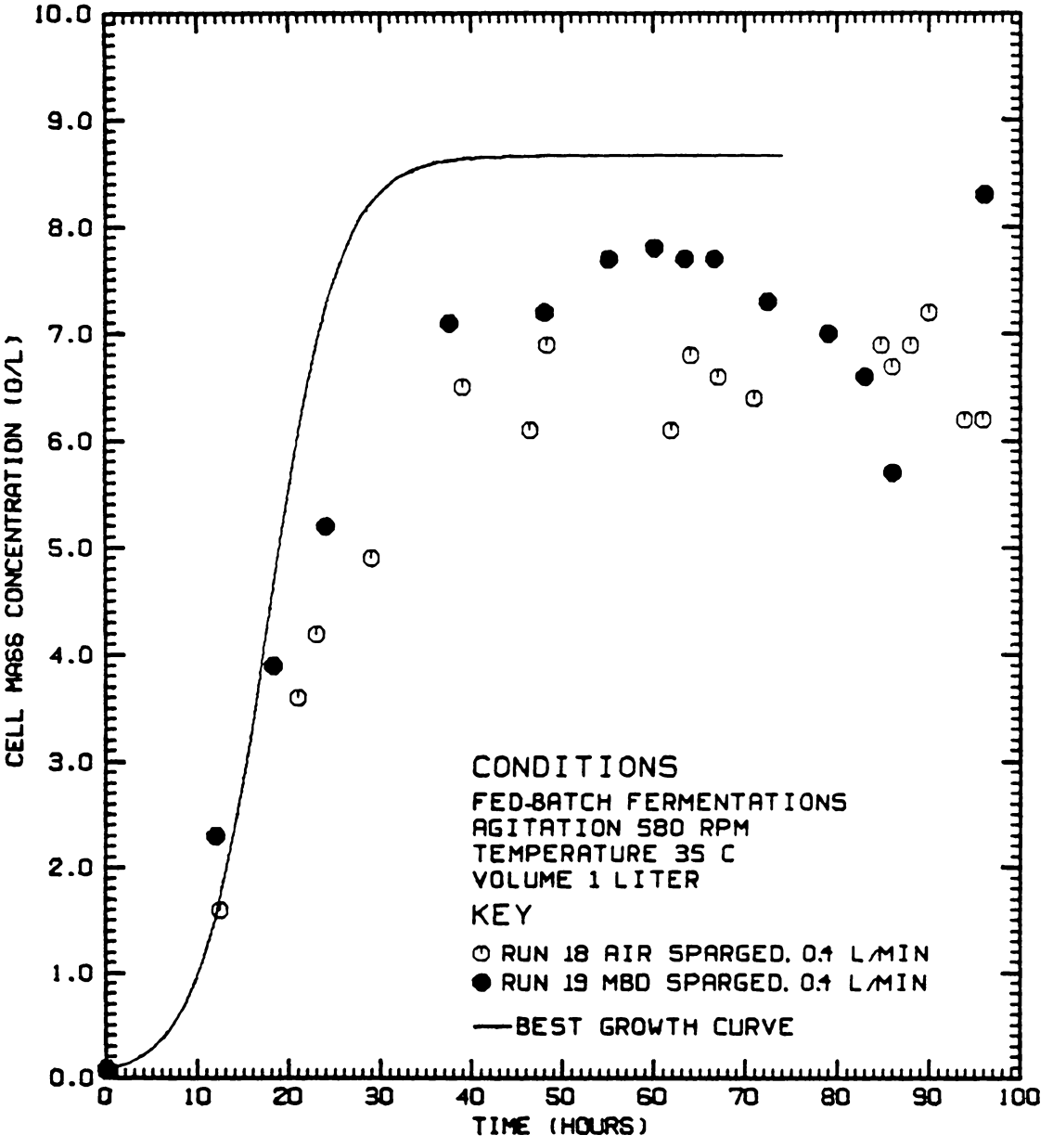


Figure 23. Yeast growth with normal agitation (580 RPM): air vs. MBD sparging.

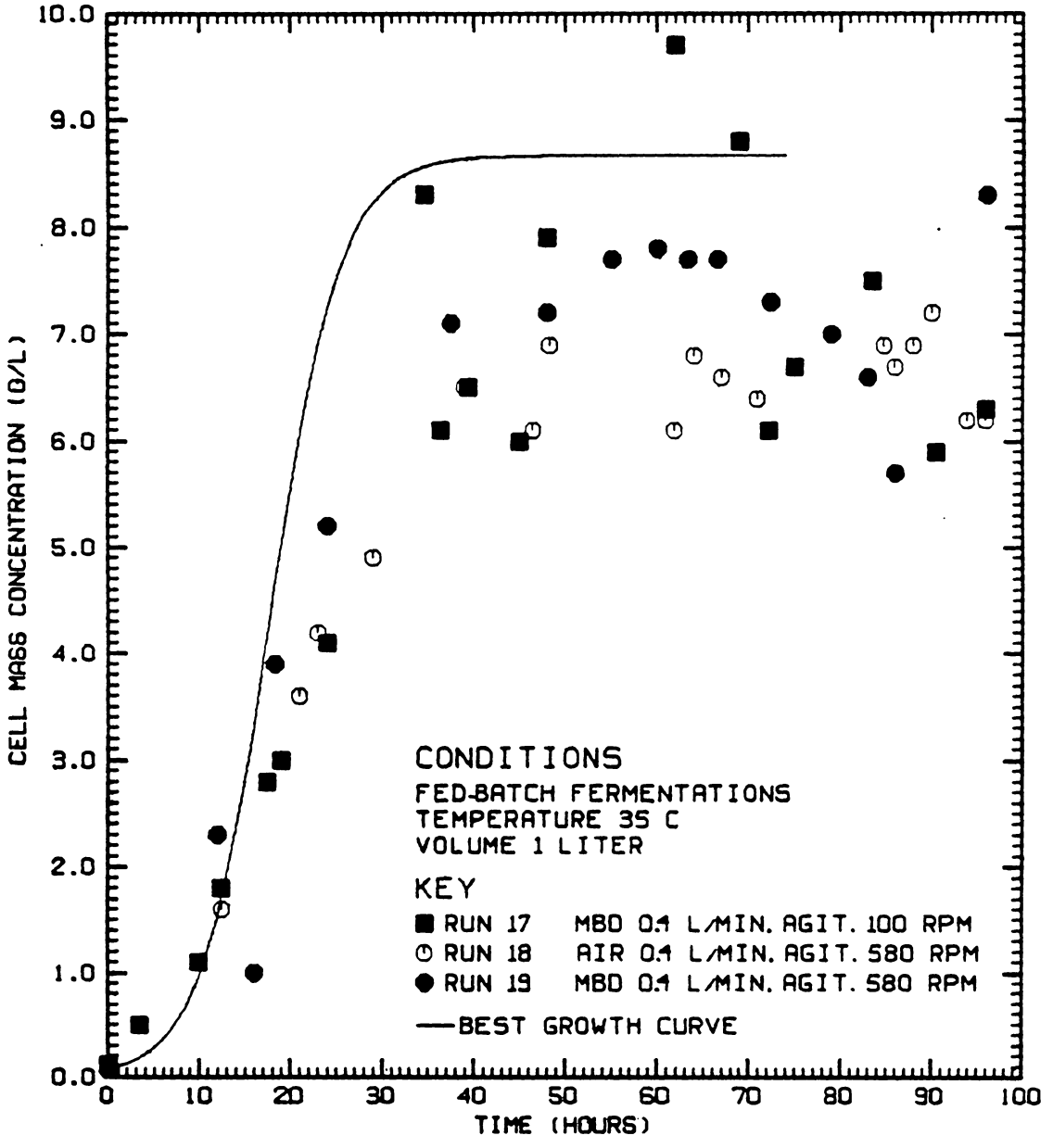
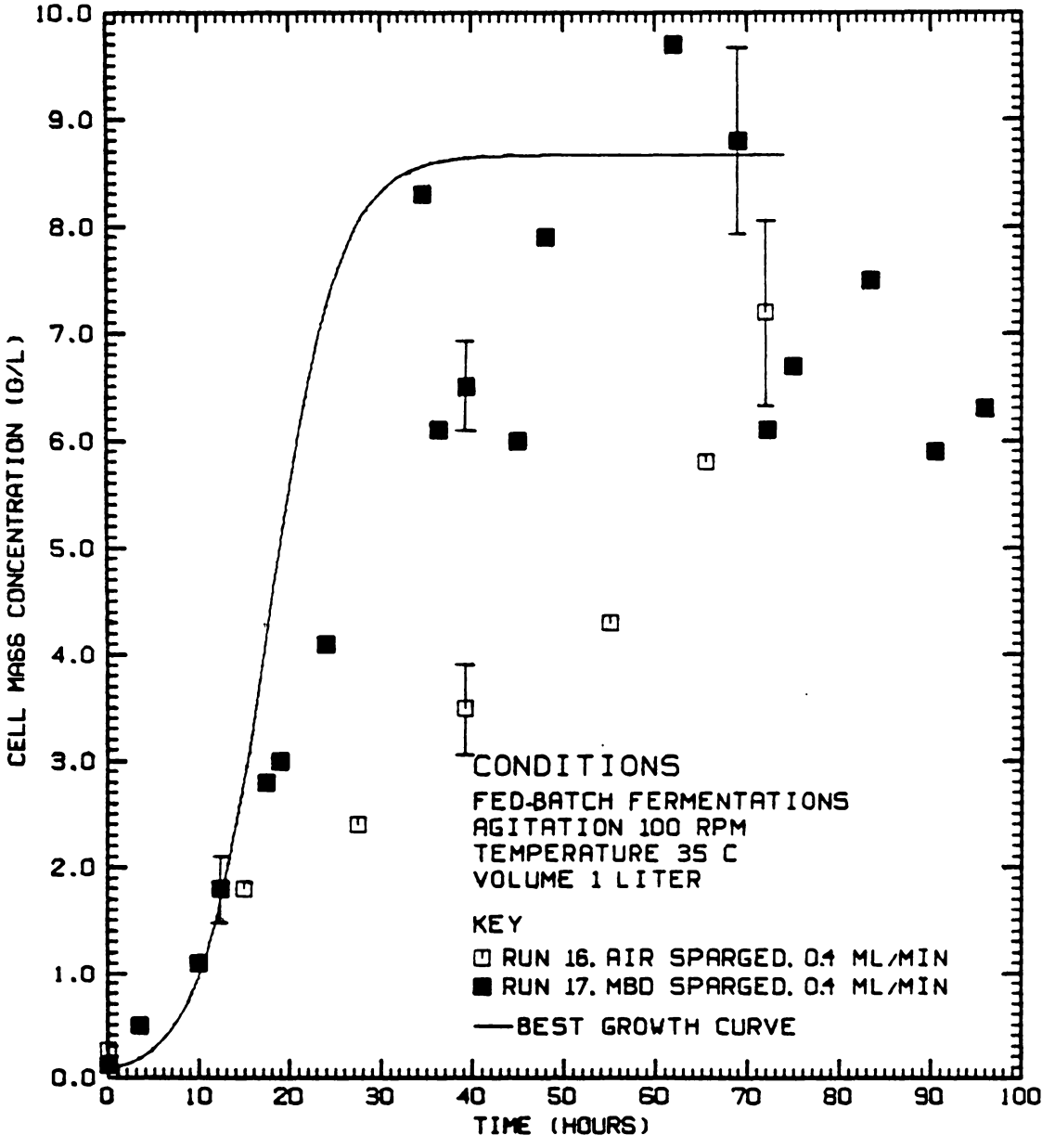


Figure 24. Yeast growth with normal agitation (580 RPM) and MBD sparging.



*The error bars represent the range of the run to run variations from the initial runs.

Figure 25. Yeast growth with reduced agitation (100 RPM): air vs. MBD sparging.

4.3 Oxygen Transfer and Yeast Growth

Values for the oxygen transfer rate, k_La , in runs 16 - 19 were calculated by the yield coefficient method and are presented in Table 5 on page 61. These k_La values fall in the range of the k_La values calculated in earlier runs by the dynamic and direct methods. Figure 26 on page 59 shows the k_La values for fermentations 16 - 19 in comparison to Cooper's correlation. The k_La values for the 580 RPM agitation runs agree with 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. However, the high k_La in the low agitation MBD sparged run is significant. 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 CGA's with the sulfite oxidation method (see Figure 3 on page 15). This was not unexpected. The CGA's used by Wallis were smaller and more uniform than the microbubble dispersion that were fed to these fermentations. Also, k_La's found by the sulfite oxidation method are normally higher than the k_La's in actual fermentations. The sulfite oxidation method uses pure water, while fermentation broths contain many surface active agents that can have a detrimental effect on oxygen transfer. Although the k_La values in runs 17 and 19 were lower than the k_La values obtained by Wallis, they show that microbubble dispersion have excellent mass transfer characteristics for biological systems.

The apparent increase in k_La in the microbubble dispersion fed runs (17 and 19) was due to oxygen transport enhancement by the microbubbles. It would seem possible at first glance that the increase in the measured k_La could have been due to oxygen transported into the fermenter by the liquid from the MBD generator. A detailed inspection of the oxygen mass balance around the fermenter reveals that only about 10% of the oxygen required for yeast growth could have been supplied in this manner, (see Appendix B).

From these calculations, it can also be seen that only 20 mmol of oxygen per hour were being consumed. In this same amount of time, 133 mmol of oxygen were supplied to the fermenter. This

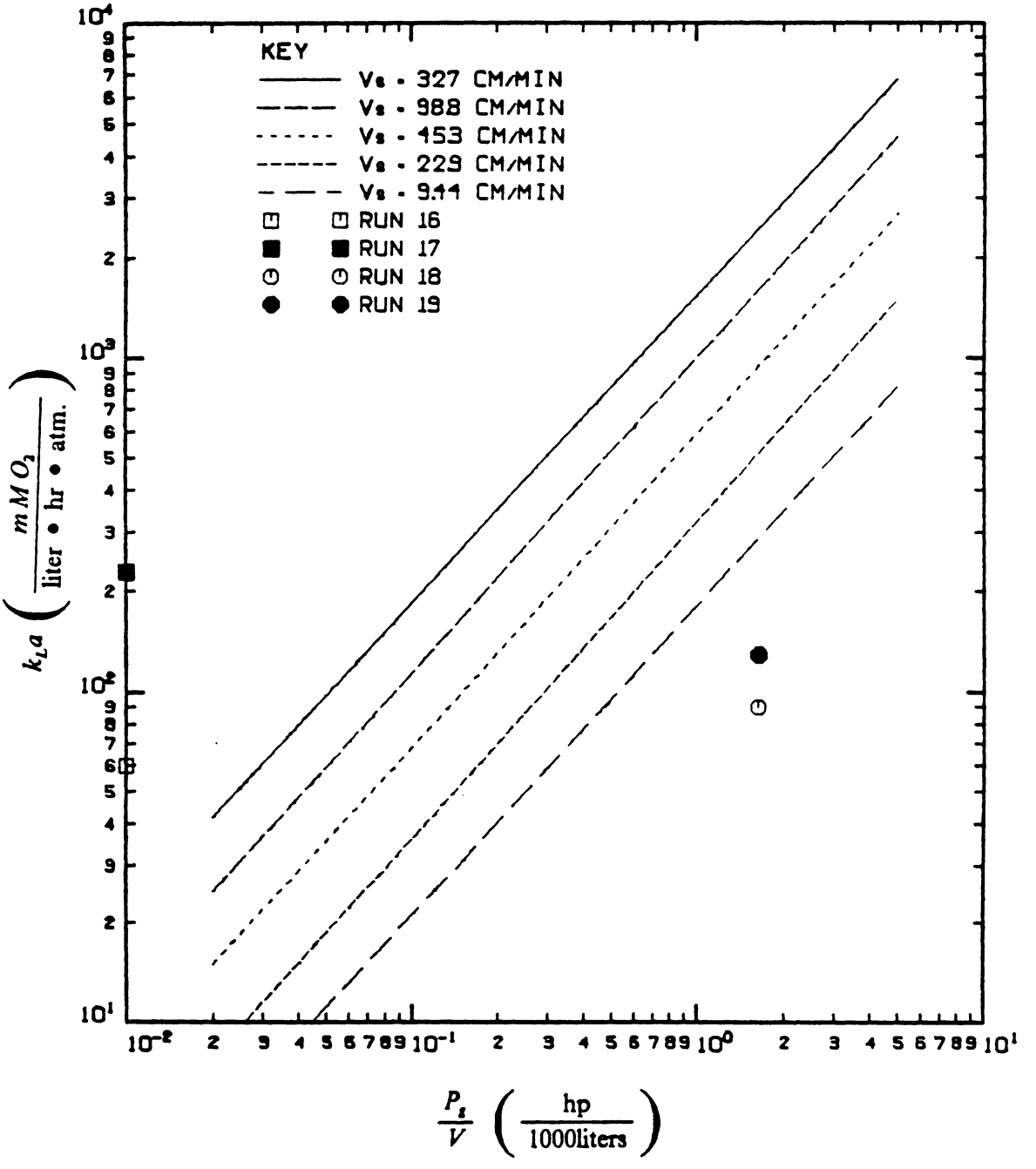


Figure 26. Experimental oxygen transfer rates.

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 one liter fermenter. A 5 liter fermentation would require about the same recycle flow rate as was used in these experiments. The use of MBD as a means of oxygen transport shows a great deal of potential in this application.

The MBD generator to fermenter volume ratio was about 1 to 10 for these experiments, and the residence time of the broth in the fermenter was about 7.5 minutes. The pumping rate this residence time entails is fine for a one liter 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 concentration of oxygen in the broth was 50% of saturation or more in the lightly agitated MBD fermentation (run 16.) Therefore, it should be possible to use a larger percentage of the oxygen by increasing the fermenter to generator volume ratio to about 50 to 1. This would increase the residence time of the broth in the fermenter to about 38 minutes 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 6 on page 63 point out one part of the original design that requires improvement. The 400 ml/min of microbubble dispersion fed to the fermenter led to a power consumption of 2900×10^{-6}hp/l Since the dissolved oxygen concentration remained above 50% at all times during run 16, a MBD feed of 200 ml/min to this fermenter should be more than sufficient. This reduction in MBD flow rate would lead to a power requirement of only 1465 hp/l for a reduced agitation, MBD sparged fermentation. It may be possible to use even smaller MBD flow rates per liter of broth, which would lead to corresponding reductions in the power requirements. Also, the MBD generating system can probably be improved. The generator used for these experiments was originally designed to produce CGA's batchwise. A MBD generator designed for inline continuous production of MBD would probably produce the MBD more efficiently. In these experiments, the MBD

Table 5. Maximum Growth Rates and Oxygen Transfer Coefficients.

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

fermentations required more power than the normally agitated control run, but the prospects for an energy efficient MBD fermenter are favorable.

Table 5 on page 61 shows the average growth rates for runs 16 - 19. These growth rates were calculated using a least squares fit of the first 40 hours of cell mass data from the four main fermentations (runs 16 - 19). These figures are slightly less than the average growth rates seen by the researches listed in Table 2 on page 8. In runs 16 - 19, glucose feed was added according to a fixed schedule. The glucose feeding was probably a major factor in the reduced growth rate. The average growth rate in the MBD fermentation system could be improved by improving the control of the glucose feed rate. Growth also leveled off after about forty-five hours in spite of new glucose additions. It appears that there is either a build-up of a growth inhibiting substance in the fermenter or a lack of a necessary nutrient in the feed. The latter was ruled out during the early fed-batch runs. When YM broth was added in the feed, some improvement in growth was seen but higher concentrations of YM broth in the feed caused little or no improvement. From the data compiled in Table 2 on page 8, the initial yeast concentration in the fermenter appears to have a great effect on the maximum obtainable yeast mass concentration. Thus, to obtain the higher yeast cell mass concentrations and higher oxygen consumption rates, the inoculum concentration and/or inoculum volume should be increased.

The dissolved oxygen probe may not have been entirely accurate throughout the entire length of each fermentation. As a general rule, the maximum obtainable dissolved oxygen reading after one hundred hours was 80%. This could be due to several things. Fouling of the probe or a reduction in the oxygen solubility seem to be the most likely. The electrolyte solution in the probe required changing every 200 hours of use due to a build-up of white crystals and a reduction in sensitivity. Measuring oxygen solubility against an outside standard would be useful in determining the accuracy of the probe, especially towards the end of a run. Oxygen is known to have reduced solubility in salt solutions and this could account for most or all of the reduction in the maximum dissolved oxygen readings. It would be interesting to see what the actual equilibrium concentration of oxygen in the broth was after a hundred hours of yeast growth, since the calculation of k_La requires C_L^* The changes in the dissolved oxygen reading should have caused little error in the k_La

Table 6. Estimated Power Requirements.

Run	16	17	18	19
Oxygen Supply	Air	MBD	Air	MBD
Agitation	100 RPM	100 RPM	580 RPM	580RPM
Power Requirements $\left(\frac{hp}{l} \times 10^6\right)$				
Fermenter Agitator	6.94	6.94	1640	1640
Compression	13.76	9.18	14	9
MBD Generation	-	2900	-	2900
Total	20.7	2916	1654	4549

results for these fermentations because the data for these calculations were obtained during the first 40 hours of the fermentation, during the log phase of yeast growth.

4.4 Fermenter Design

The fermenter used in these experiments was adequate. It provided an easily sterilizable and controllable environment for initial quantitative experiments. Several improvements could be made to enhance its effectiveness in further experiments, including the addition of foaming control, the reduction of the MBD generator to fermenter volume ratio, and the placement of impellers to improve MBD effectiveness. There were several runs that were terminated by fouling of the off gas filters. Yeast in the filters led to a pressure build-up in the fermenter and a reduction in the gas flow into the fermenter. The addition of a mechanical foam breaker in the vessel or the addition of a foam breaking chamber on the outgas line would reduce the chances of this problem recurring.

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 RPM's, 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 is 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 needs to be spread out to cover the entire cross section of the tank.

The design of the MBD generator had several good points but many improvements had to be made and several others are still needed. The Teflon dust shield and associated equipment worked well. The shield lost some of its effectiveness, though, as the hole for the shaft eroded to twice its original size. Problems in the MBD generator were observed throughout the experiments, most due to extreme amounts of vibration. The length of the shaft was also a problem because it was difficult to line up all of the bearings perfectly and binding resulted. The special baffles in the generator were not as effective as they should have been. They allowed the broth to gain an ap-

preciable horizontal rotational velocity. For the best MBD production, this velocity should be kept to a minimum. Finally, the rubber top of the generator had several plugged holes (normally there to accept probes) which tended to leak especially if there was a build-up of pressure in the generator.

Most of these problems were alleviated by reducing the shaft length. This reduced the number of bearings to be lined up and removed binding problems. It also stopped most of the vibrations found in the original design. The erosion of the Teflon shield around the shaft is probably directly attributable to vibrations. The elimination of the vibrations should eliminate this as well. For these experiments, the coupling between the motor and the shaft was a small piece of rubber tubing held on by two screw type hose clamps. This hose tended to wear out after about 1000 hours at 4000 RPM. The flexibility of this coupling probably added to the vibrational problems as well. A more rigid coupling that holds the motor shaft and generator shaft in alignment eliminated these problems. The baffles in this MBD generator were screwed into the rubber top of the vessel. This proved to be an unwise design. The baffles tended to rotate to present as little surface perpendicular to the flow as possible. Also, the holes drilled into the top to hold the bolts in the baffles tended to leak and the steel bolts rusted, both of which increased the chances for contamination. In subsequent experiments, a four baffle system with two connecting rings was added. This design seemed to interfere with MBD production. The percentage of small bubbles dropped drastically and a distinct liquid/foam interface could be seen in the delivery tubes. The removal of the lower ring (about 2.5 cm above the spinning disk) helped some. Recent problems, however, may be more a function of the level of MBD in the generator than of the baffle design. In fermentations 16 through 19, the MBD level was approximately 1/2 to 2/3 of the generator vessel height.

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 MBD quality 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. The system was then 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 to 1, rather than 2 to 1) should show even greater improvements in oxygen transfer with a MBD over air sparging. These experiments have shown MBD to be of use in a biological setting and research on the use of MBD in Bioreactors should continue.

5.0 CONCLUSIONS

1. The use of surfactant-stabilized microbubbles as a method for oxygen transport to microorganisms can be advantageous, especially in those cases where microorganism sensitivity and power costs preclude the use of intensive agitation in the fermenter.
2. MBD have the same oxygen transport characteristics as intensely agitated (580 RPM) sparged air.
3. The oxygen transport characteristics of MBD 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 $k_L a$ for ordinary sparged air dropped from 132/hr at 580 RPM to 55/hr at 100 RPM.
4. The difference in oxygen transport seen between the MBD and sparged air runs at 100 RPM were not due to oxygen transported in the liquid. Liquid oxygen transport can only account for a maximum of 15% of the oxygen transported.
5. The fermenter in this experiment was undersized for the MBD generator used. This MBD generator could easily supply a 5 liter working volume fermenter.

6.0 RECOMMENDATIONS

1. The fermenter used for these experiments was undersized for the MBD generator used. This MBD generator should probably be used with a fermenter with a working volume of about ten liters.
2. In most industrial fermenters most of the oxygen transport takes place in the first meter [58]. it would be very interesting to find out how the transport characteristics of microbubble dispersion vary with increasing path length. If MBD's have k_La's at 1.5 meters that are in the same range as those found here for the 0.1 to 0.2 meter range, they could attract a great deal of interest from industry.
3. The actual oxygen concentrations in the fermenter over the length of a run should be looked into to determine the cause of the deterioration of the maximum dissolved oxygen concentration.
4. A more defined and proven nutrient broth might increase the yeast growth. Some of the literature also suggests that final yeast concentrations depends upon the initial yeast concentration, so it would probably be a good idea to try to inoculation with a concentrated yeast culture.
5. It would also be interesting to see how well the MBD can do with no agitation. The addition of a solid cone to the oxygen delivery shaft could take care of spreading out the MBD and preventing the yeast from settling in the center of the column.

6. For the present, baker's yeast appears to be the organism of choice for continued experiments. It is easy to grow, easy to clean up after, and requires no exotic substrates.

The next step in these studies should be either to grow much higher cell mass concentrations (50 - 100%) in the existing fermenter, or to grow yeast at the present concentration in a larger vessel with the same MBD generator. 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. Concentrated yeast growth could be used to quantify the differences in oxygen transport between sparged air and microbubble dispersions.

This research has shown definite advantages in using a microbubble dispersion in fermentations and should be continued.

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Appendix A. Assays

A.1 Park and Johnson Reducing Sugar Assay [56]

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

Reagent A

0.5 g potassium ferricyanide

1000 ml distilled H_2O Store in brown bottle

Reagent B

5.3 g sodium carbonate

0.65 g potassium cyanide

1000 ml distilled H_2O Reagent C

1.5 g ferric ammonium sulfate

1.0 g sodium Lauryl sulfate

1.4 ml concentrated sulfuric acid

998.6 ml water

A.1.1 For standard curve

Nine dilutions of a 28 mg/l glucose solution. Amounts of solution 1.0 ml 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 and 0.0 ml for standard.

A.1.2 Procedure

1. Add solution to 10-20 ml test tube.
2. Top off with distilled water to a final volume of 1.0 ml.
3. Add 1.0 ml solution B.
4. Add 1.0 ml solution A.

   (DO NOT MOUTH PIPET)
5. Stir (vortex).
6. Cover test tube with a marble.
7. Place in boiling water bath for 15 minutes.
8. Remove and let cool to room temperature.
9. Add 5.0 ml solution C.

   (DO NOT MOUTH PIPET)
10. Mix thoroughly.
11. Incubate 15 minutes at room temperature.
12. Determine absorbance at 690 nm against the blank.

A.2 Biomass Determination Method of Hug and Fiechter [57]

Solution 1

Ethanol 10.0 ml

Butanol 10.0 ml

Chloroform 1.0 ml

A.2.1 Procedure

1. Weigh dried Whatman filter #934-AH.
2. Remove 5.0 ml sample from fermenter.
3. Add 5.0 ml solution 1.
4. Shake
5. Centrifuge at 10,000 RPM for 10 min.
6. Wash with 20 ml H_2O.
7. Dry to constancy at 105°C (24 hrs.)

Appendix B. Calculations

B.1 Oxygen balance around the MBD fermenter

The average cell mass yield on glucose for the exponential growth phase was approximately $\frac{0.33\text{grams cells}}{\text{grams glucose}}$. Then from Figure 6 on page 23 (Mateles [37]), the yield on oxygen was approximately 0.5 grams cells/gm oxygen. Then taking the maximum rate of cell mass increase, $1.0\frac{\text{grams cells}}{\text{hour}}$, the oxygen uptake rate (OUR) is calculated as

$$\begin{aligned} \text{OUR} &= \frac{0.25\text{grams cells}}{\text{liters} \cdot \text{hour}} \cdot \left(0.5\frac{\text{grams cells}}{\text{grams oxygen}}\right) \\ &= 0.5\frac{\text{grams oxygen}}{\text{liter} \cdot \text{hour}} \\ &= 15.6\frac{\text{mmol oxygen}}{\text{liter} \cdot \text{hour}} \end{aligned}$$

The dissolved oxygen concentration in the fermenter is a function of both the temperature and the concentration of dissolved species. From the work of Popovic et al [49] the concentration of dissolved oxygen in these fermentations was about 98% of the equilibrium value in pure water.

$$DO_{2(media,\ 35°C)} = 6.93 \times 0.98$$

$$= 6.79 \frac{\text{grams oxygen}}{\text{liter}}$$

$$= 0.21 \frac{\text{mmol oxygen}}{\text{liter}}$$

The liquid recycle rate though the MBD generator was approximately 133 ml/min which is 7.98 l/hr. Therefore, the total amount of oxygen that can be supplied by saturating the liquid with oxygen in the MBD generator is

$$\frac{7.98\text{l/hr}}{1.1\text{liter}} (0.21\text{mmol oxygen/liter}) = 1.52 \frac{\text{mmol oxygen}}{\text{liter} \cdot \text{hour}}$$

Thus, the liquid recycle can supply only about 10% of the oxygen required for yeast growth.

B.2 Oxygen transfer coefficient calculations

From the yield coefficient method:

$$\text{OUR} = 0.5 \frac{\text{g}}{\text{l hr}} = 15.6 \frac{\text{mmol } O_2}{\text{l}} \cdot \text{hr}$$

or from oxygen off-gas anaylsis OUR $\simeq 9.6 \frac{\text{mmol } O_2}{\text{l hr}}$

$DO_{2\ max} 0.19 \frac{\text{mmol}}{\text{l}}$

For the lightly sparged MBD fermentation the average dissolved oxygen concentration was approximately .6 of the initial value.

$$DO_2 = 0.6DO_{2\,max} = 0.114 \frac{\text{mmol } O_2}{\text{l}}$$

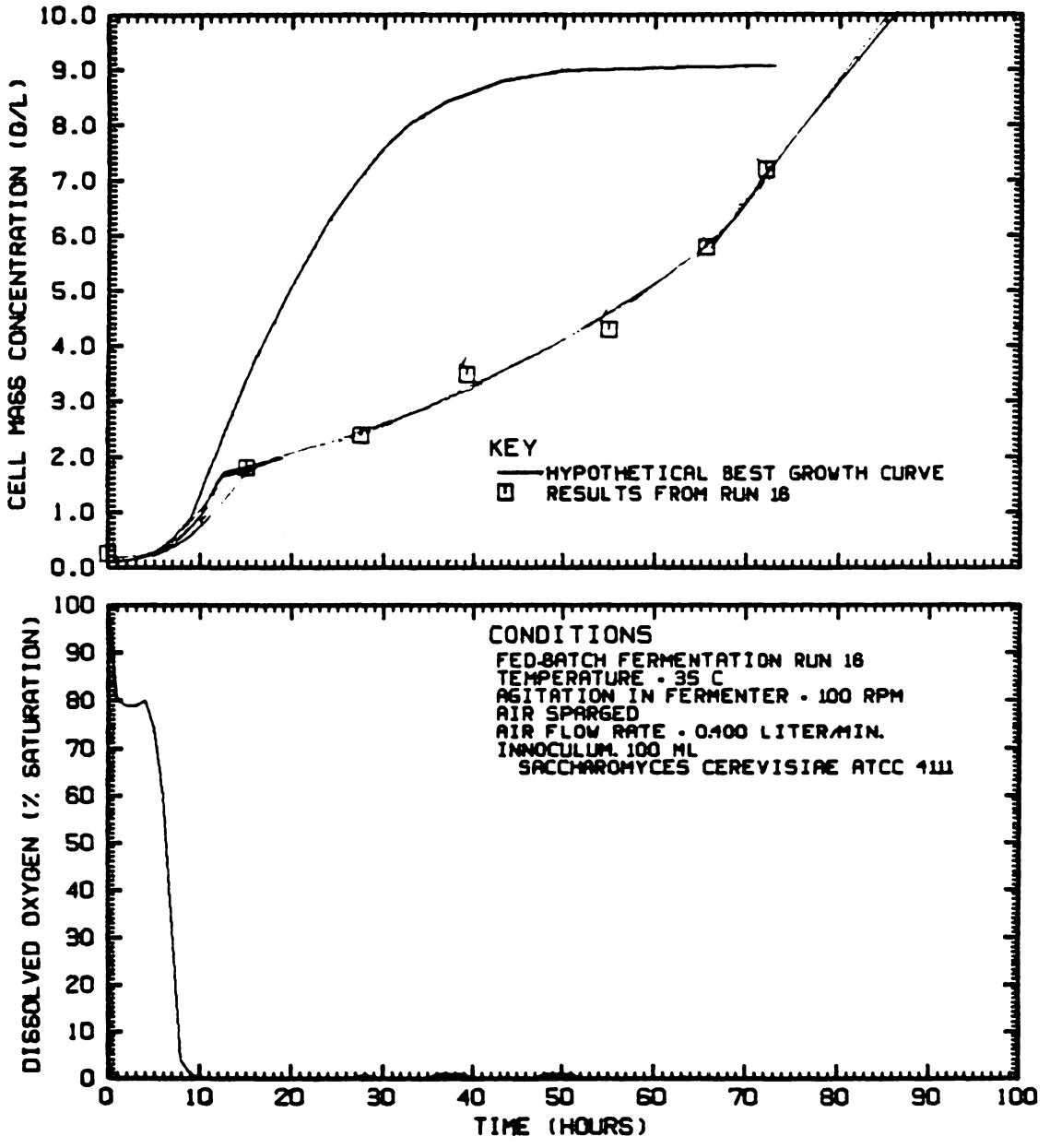
Equation (6) can be rewritten

$$K_L a = \frac{OUR}{C^* - C_L}$$

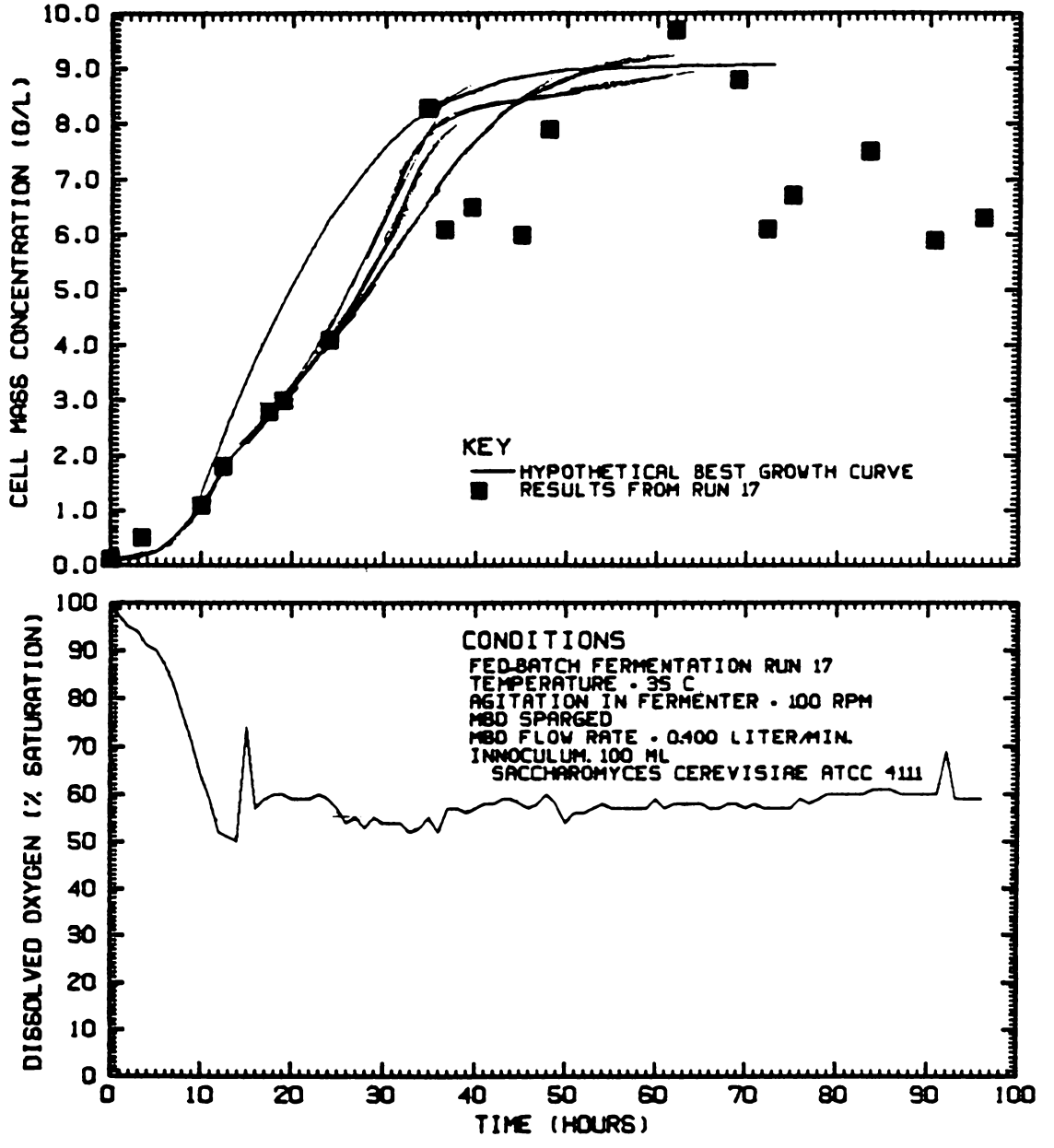
$$K_L a = \frac{15.6 \frac{\text{mmol } O_2}{\text{l} \cdot \text{hr}}}{\left(0.19 \frac{\text{mmol } O_2}{l} - 0.114 \frac{\text{mmol } O_2}{\text{l}}\right)}$$

Thus, from the Yield Coefficient Method $K_L a$ = 205/hr From off-gas analysis $K_L a$ = 126/hr

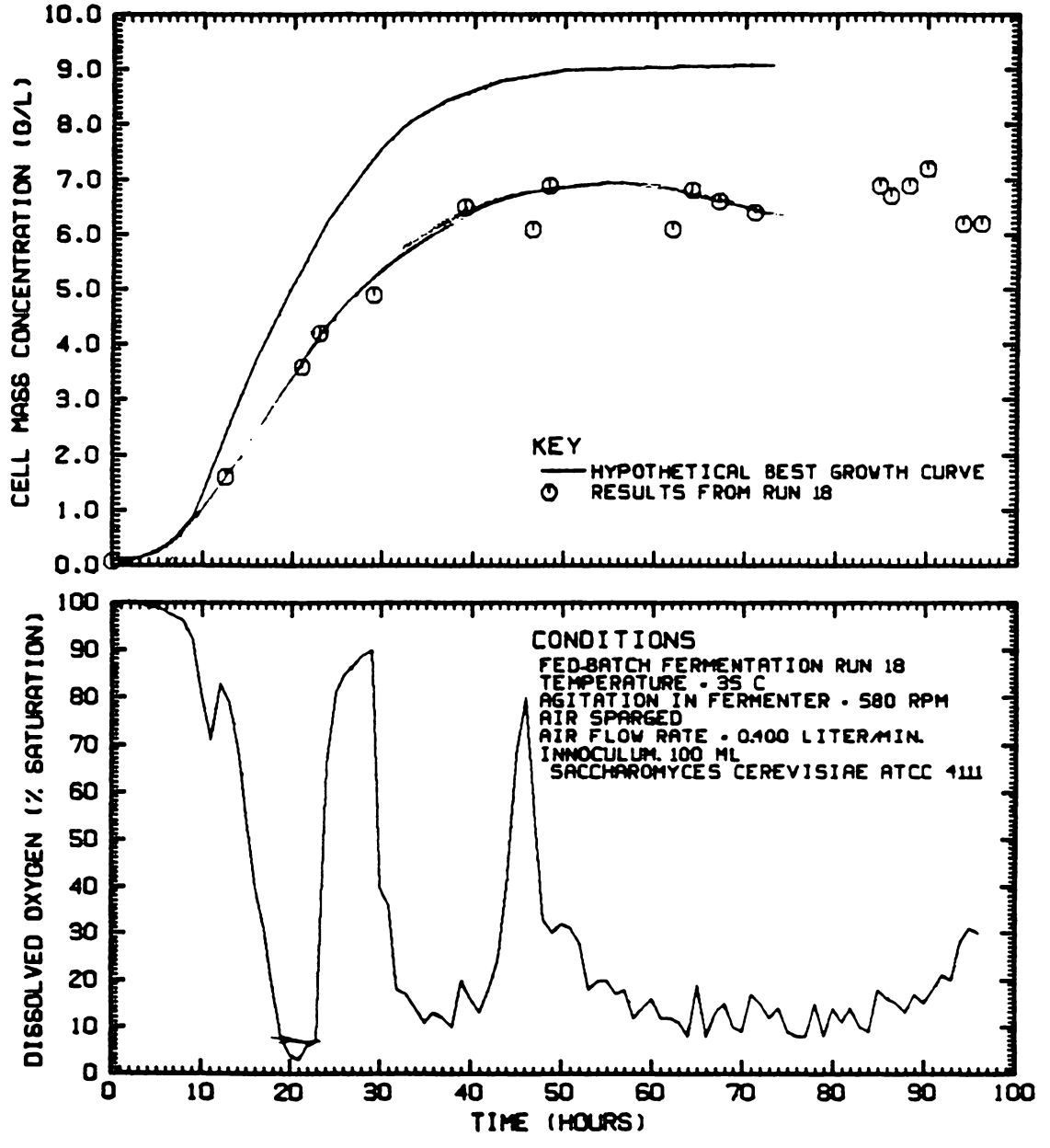
Graphs used to calculate $K_L a$ values follow.



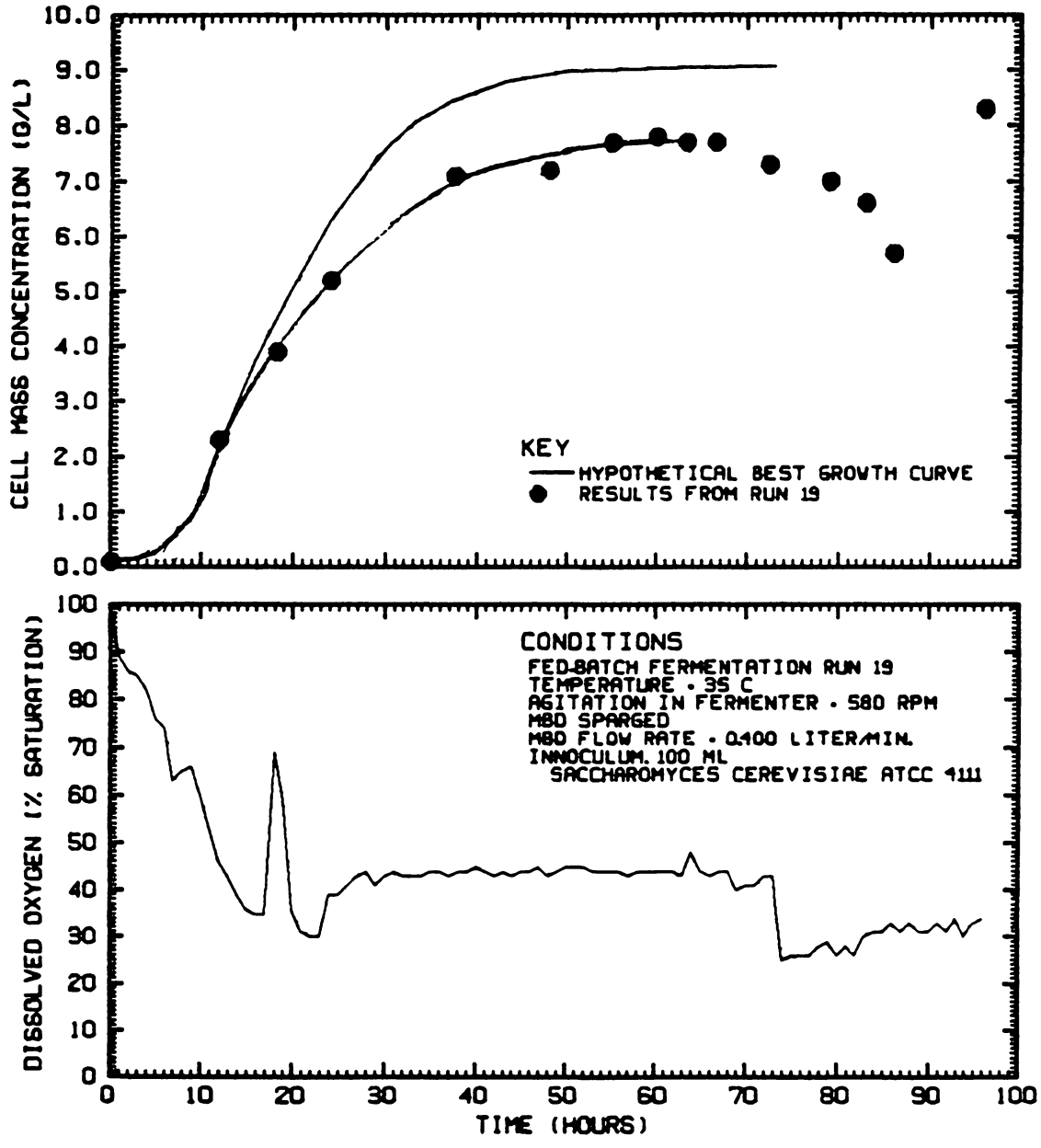
Oxygen transfer rate for run 16



Oxygen transfer rate for run 17



Oxygen transfer rate for run 18



Oxygen transfer rate for run 19

B.3 Power Estimate Calculations

Power estimates were calculated using correlations from Perry [59] pp. 19-6 to 19-8.

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

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

where: D_a = impeller diameter (ft)

N = RPM

ρ = density (lb / ft^3)

μ = viscosity (lb / ft sec)

g_c = 32.2 ft lb_m / lb_f sec^2

P = power (ft lb_f / sec)

For the fermenter used in these studies:

D_a = 0.15833

N = 100/60 or 580/60

ρ = 62.06 lb / ft^3

μ = 8.19×10^{-4} lb / ft sec

P = power (ft lb_f / sec)

The Reynold's number was calculated and the power number was taken from Figure 9-13 in Perry [59].

$N_{Re_{100}} = 3164$

$N_{P_{100}} = 4.3$

$N_{Re_{580}} = 18350$

$N_{P_{580}} = 5.2$

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

$$P_{100} = 3.8 \times 10^{-3} \frac{\text{ft lb}_f}{\text{sec}}$$

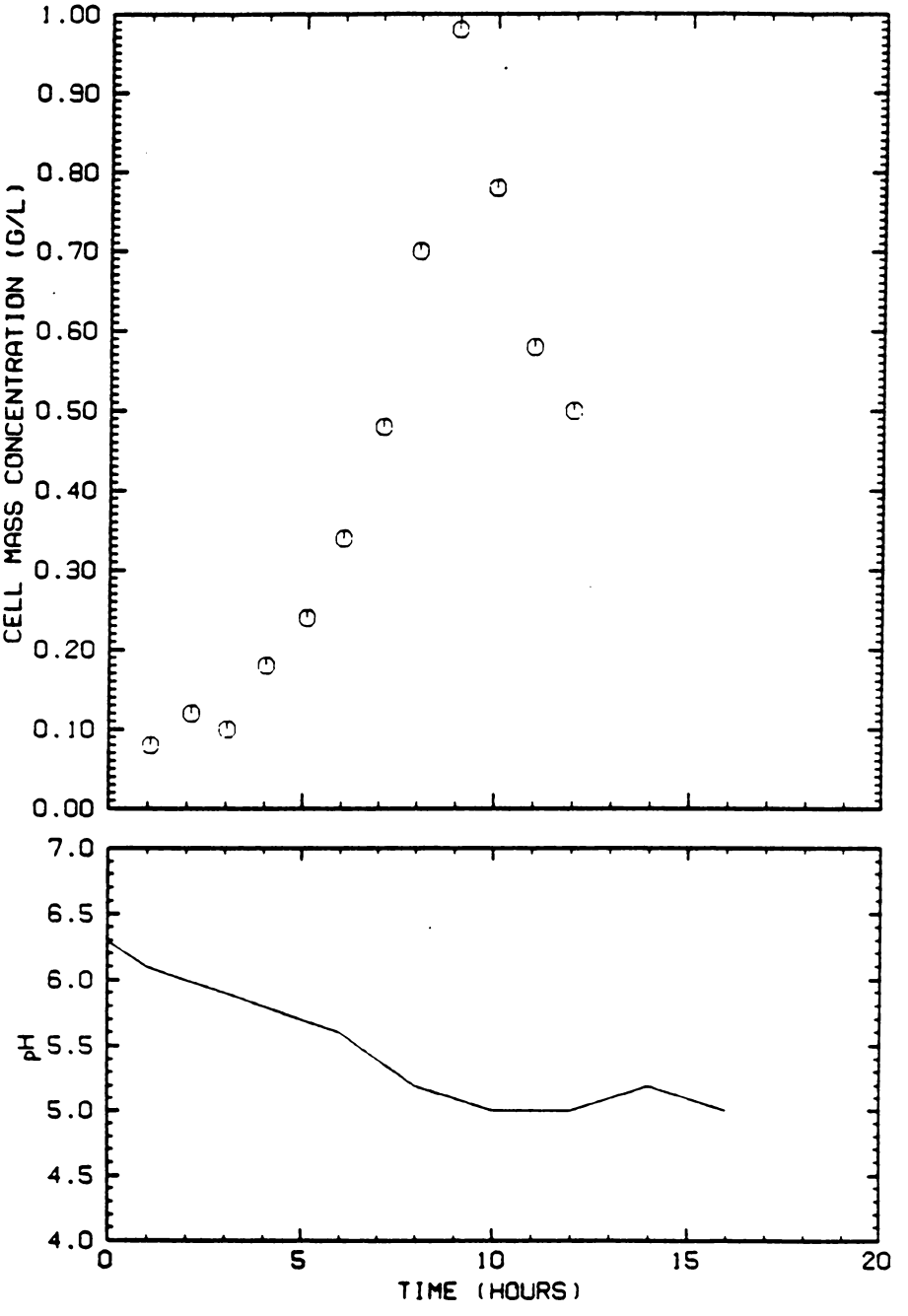
$$= 6.94 \times 10^{-6} \text{hp}$$

$$P_{580} = 0.9 \frac{\text{ft lb}_f}{\text{sec}}$$

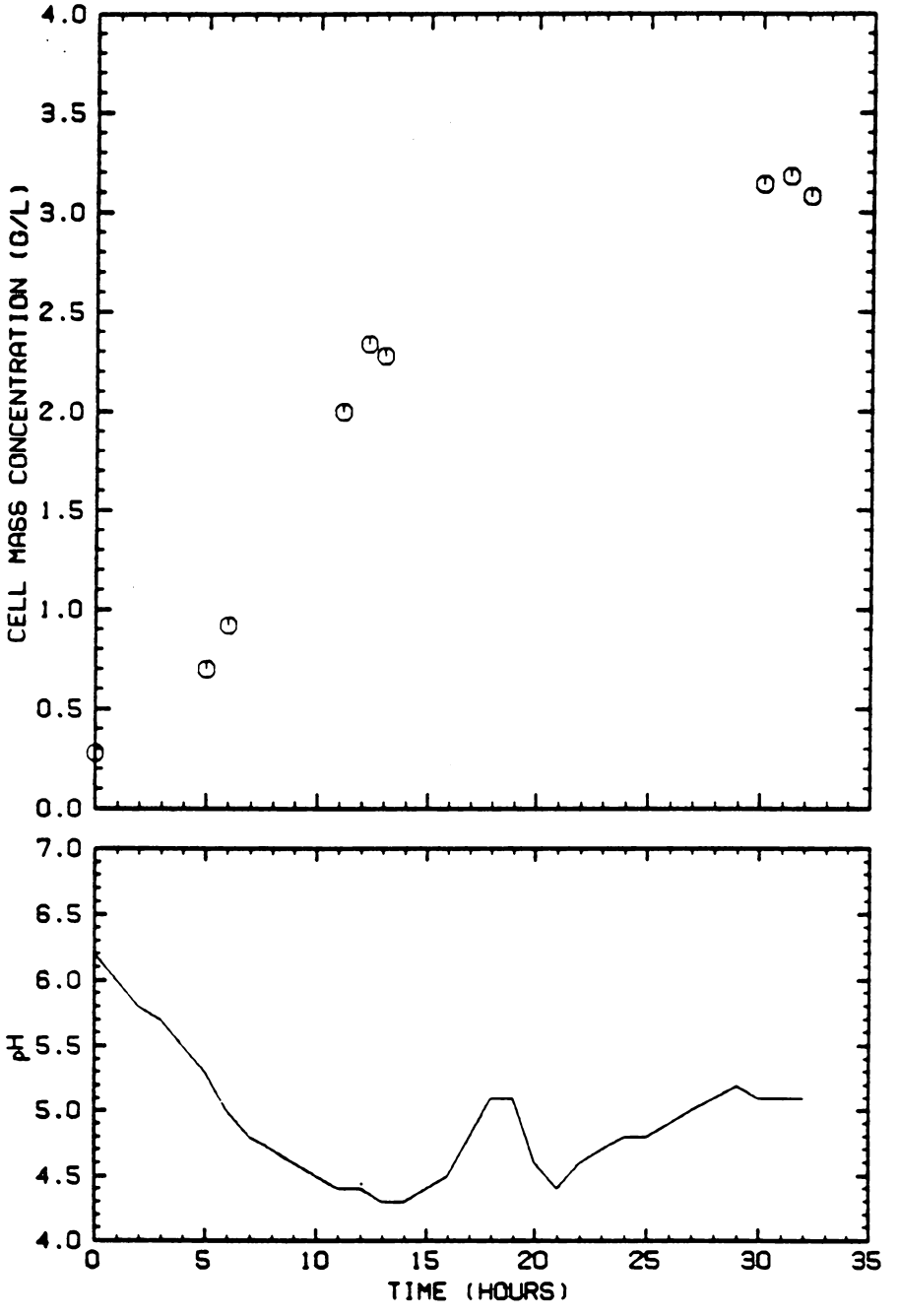
$$= 6.14 \times 10^{-3} \text{hp}$$

Appendix C. Fermentation Data

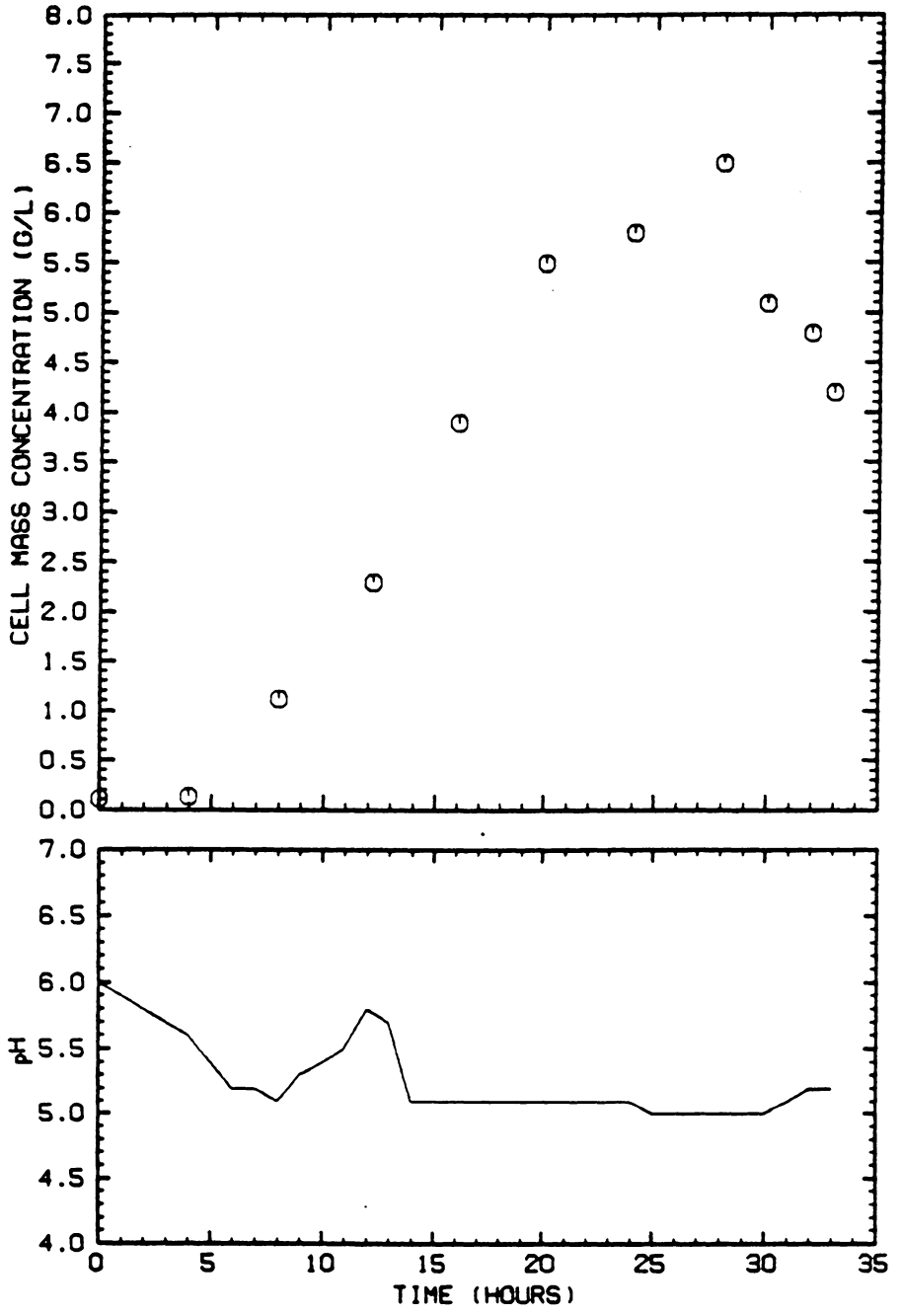
The data from the fermentations is given in graph form showing the cell mass concentration and dissolved oxygen or pH variations during the length of the runs.



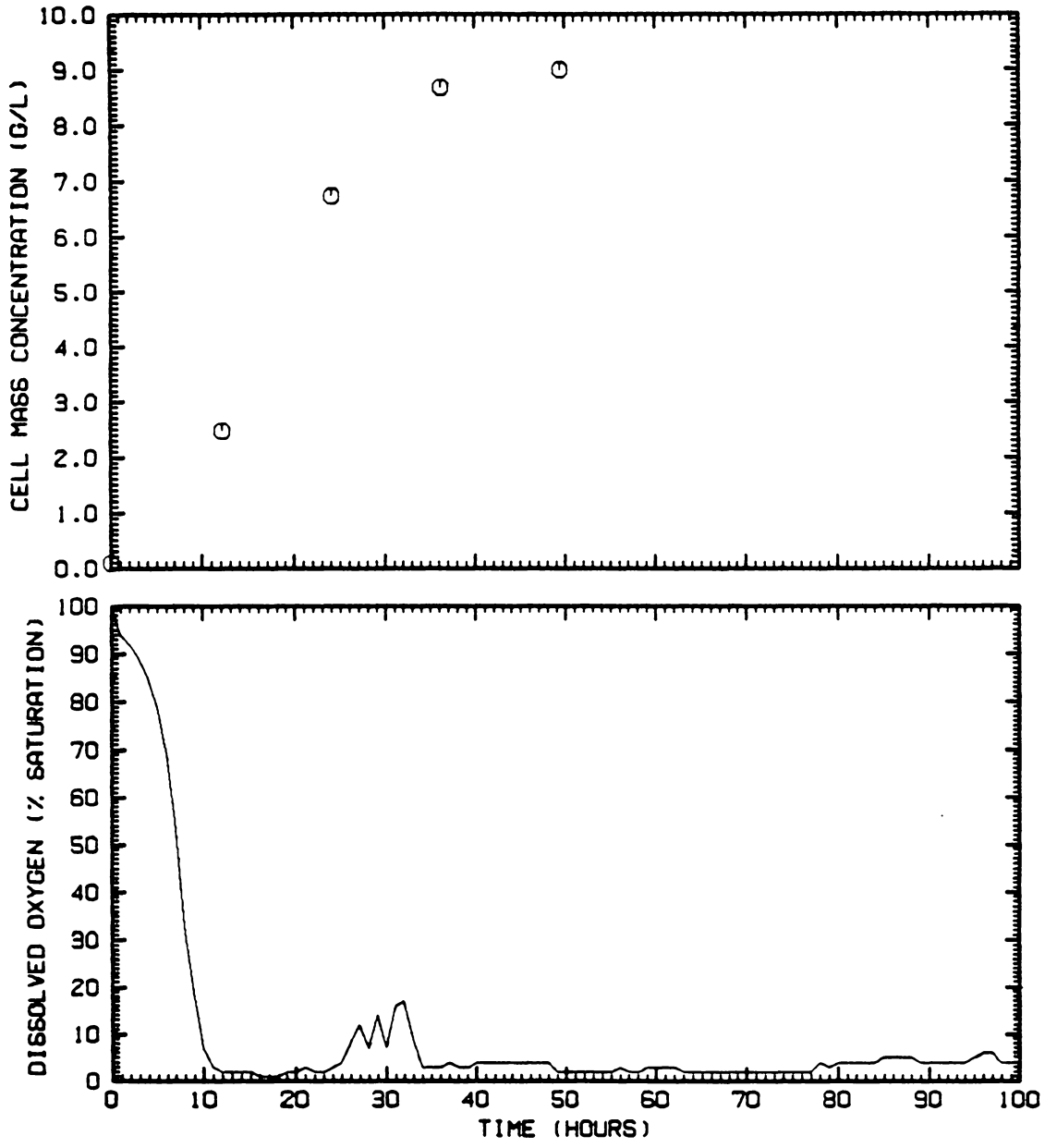
Cell mass concentrations and pH: Run 2 (Batch)



Cell mass concentrations and pH: Run 3 (Batch)



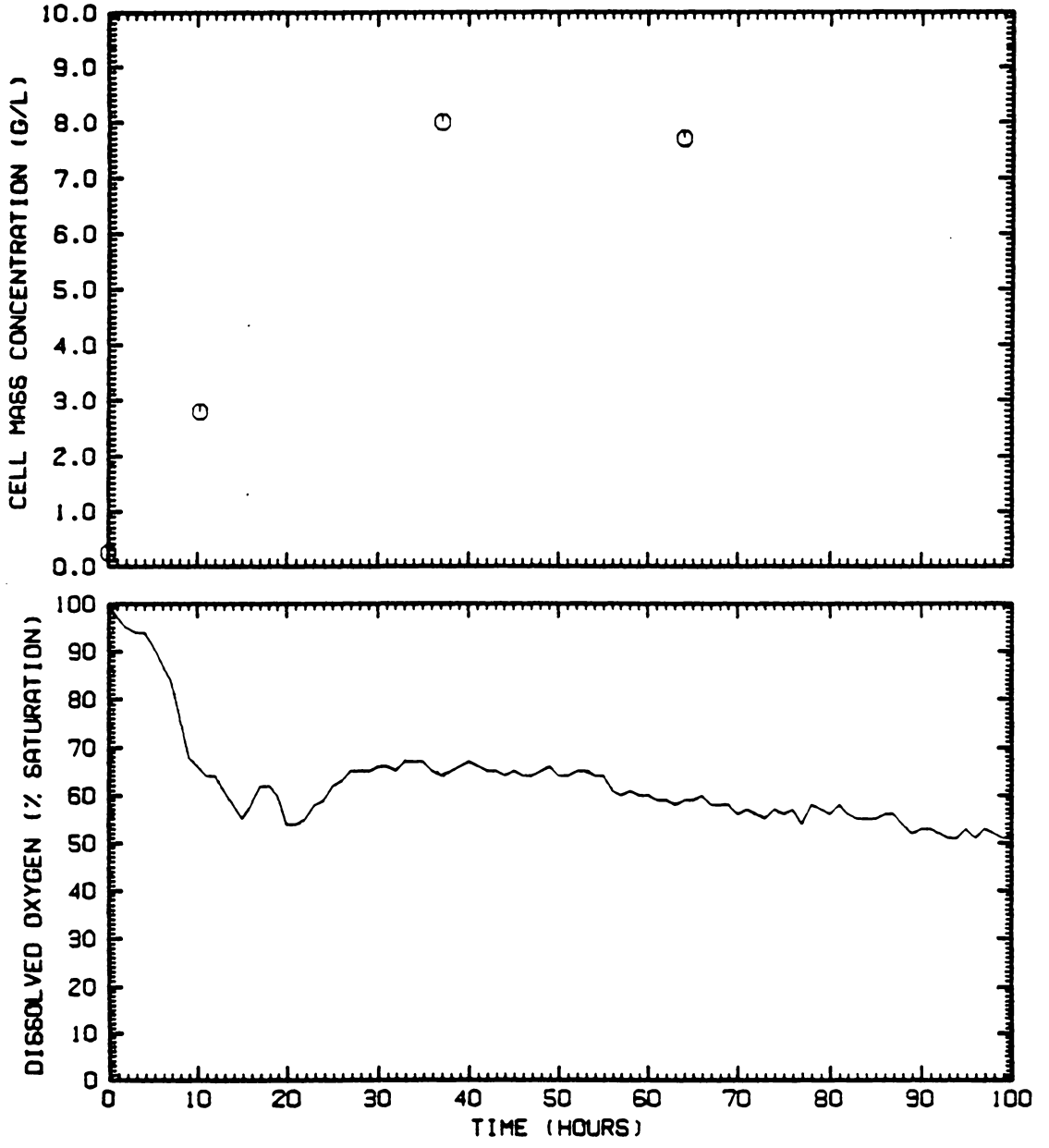
Cell mass concentrations and pH: Run 8 (Fed-batch)



CONDITIONS

Fed batch fermentation
Temperature = 30 C
Agitation 475 RPM
Air Sparged
Air flow rate = 1.35 l/min
Initial tank volume 1.0 l
Innoculum 100 ml *Saccharomyces cerivicea* ATCC 4111

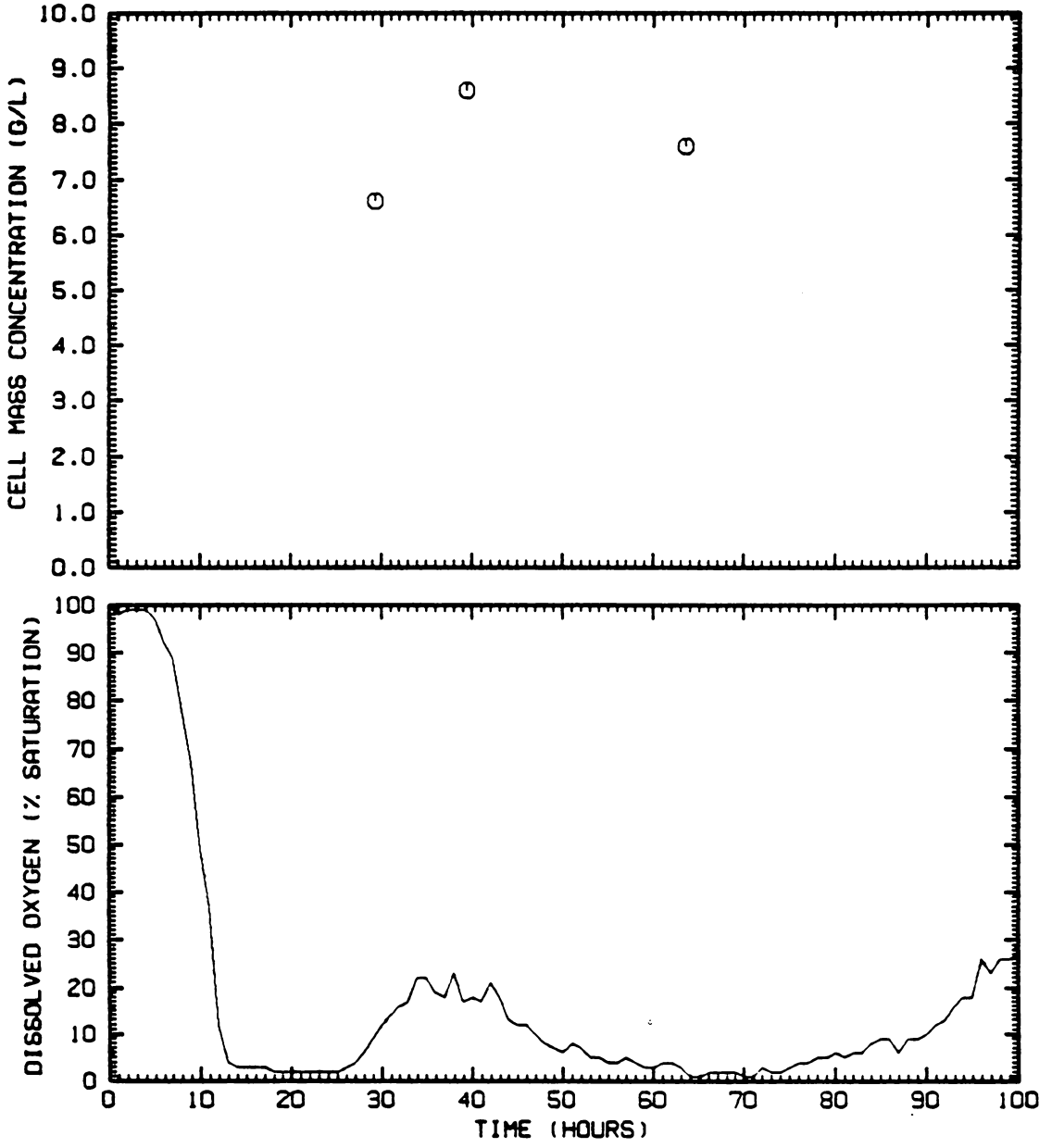
Cell mass concentrations and dissolved oxygen: Run 11 (Fed-batch)



CONDITIONS

Fed batch fermentation
Temperature = 30 C
Agitation 640 RPM
Air Sparged
Air flow rate = 1.35 l/min
Initial tank volume 1 l
Innoculum 100 ml *Saccharomyces cerivicea* ATCC4111

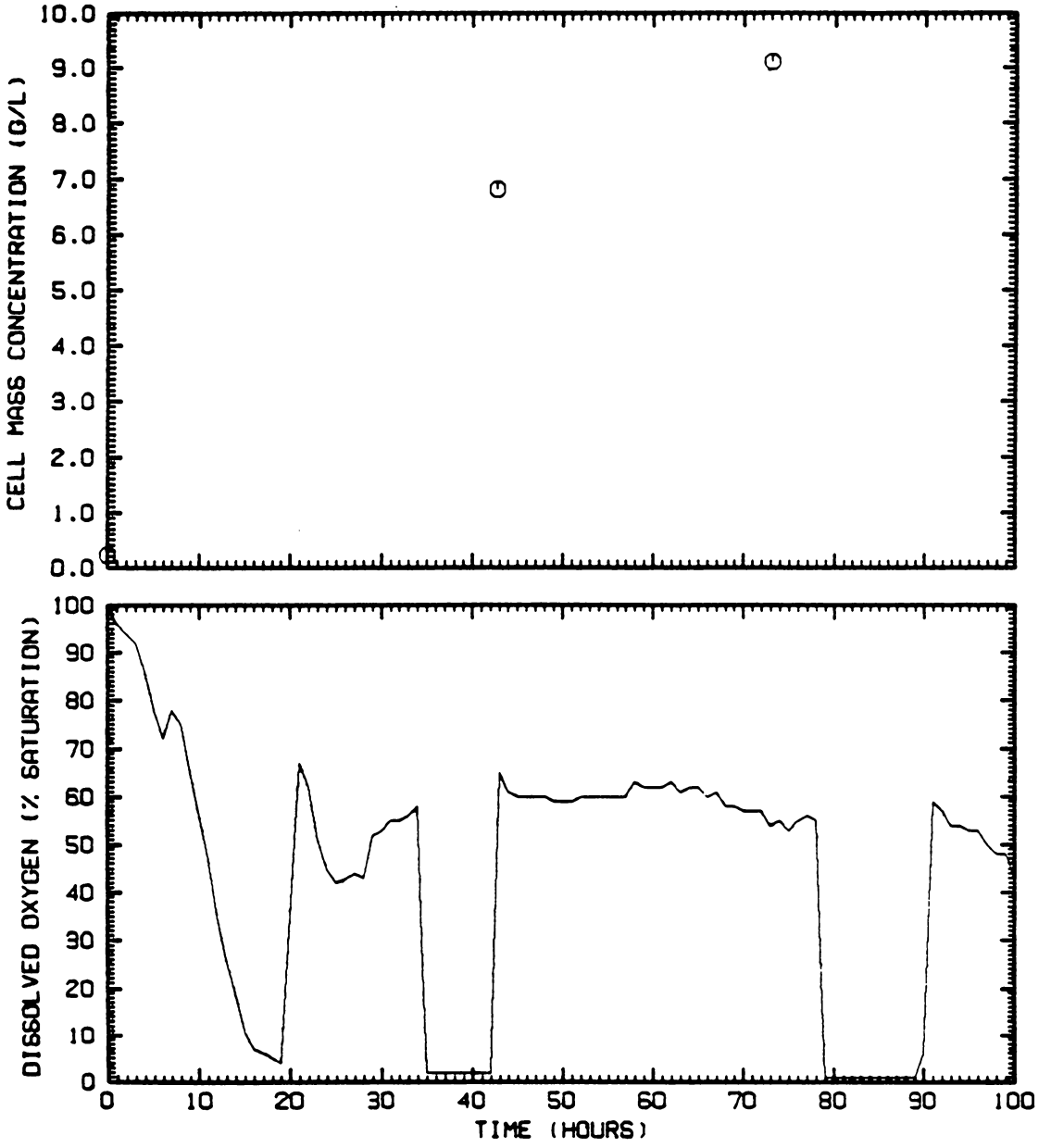
Cell mass concentrations and dissolved oxygen: Run 12 (Fed-batch)



CONDITIONS

Fed batch fermentation
Temperature = 35 C
Agitation 640 RPM
Air Sparged
Air flow rate = 1.35 l/min
Initial tank volume 1.0 l
Innoculum 100 ml Alltech yeast

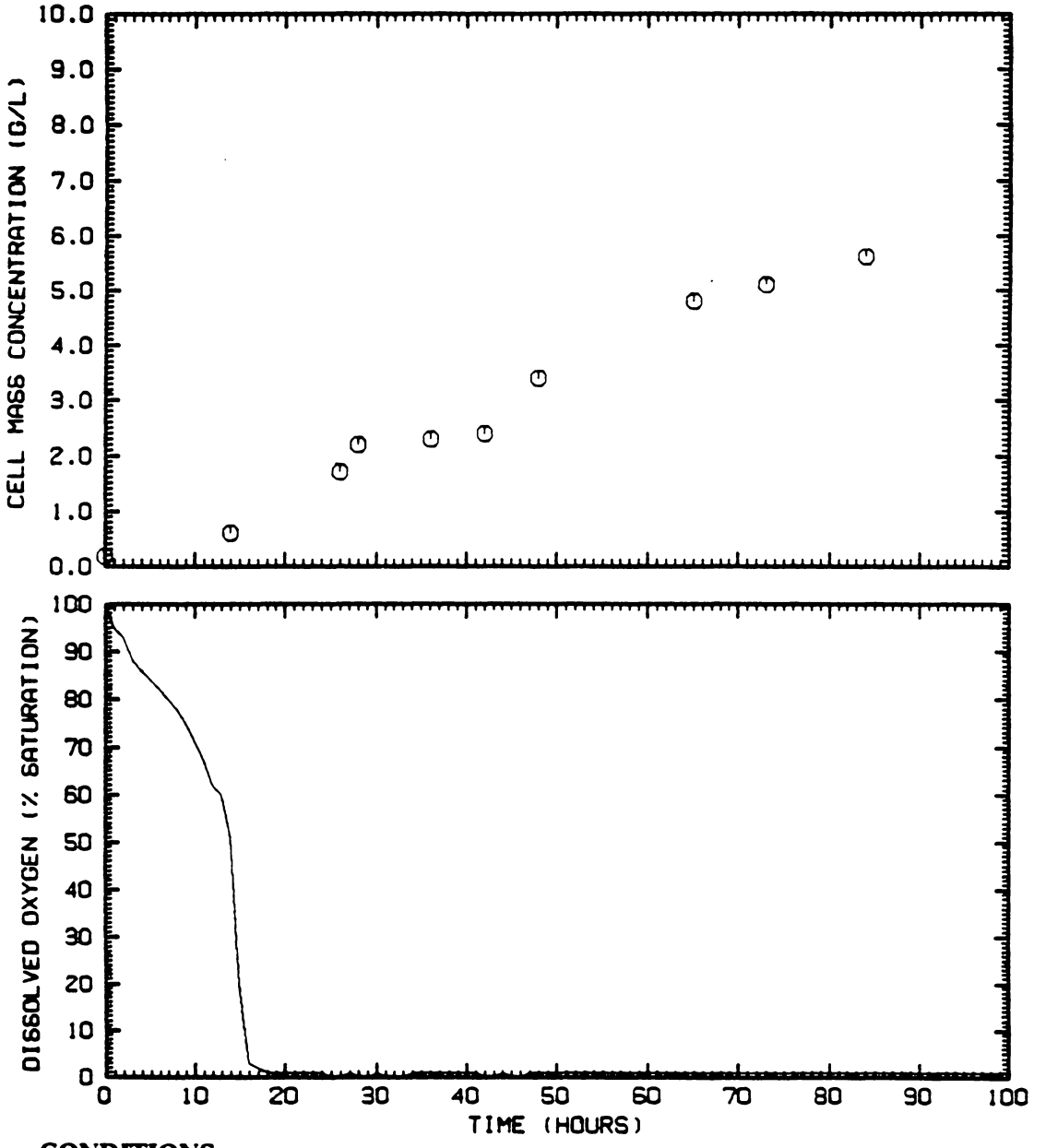
Cell mass concentrations and dissolved oxygen: Run 13 (Fed-batch)



CONDITIONS

Fed batch fermentation
Temperature = 35 C
Agitation 580 RPM
CGA Sparged
CGA flow rate = 0.400 l/min
Air flow rate = 0.30 l/min
Initial tank volume 1.0 l
Innoculum 100 ml *Saccharomyces cerivicea* ATCC 4111

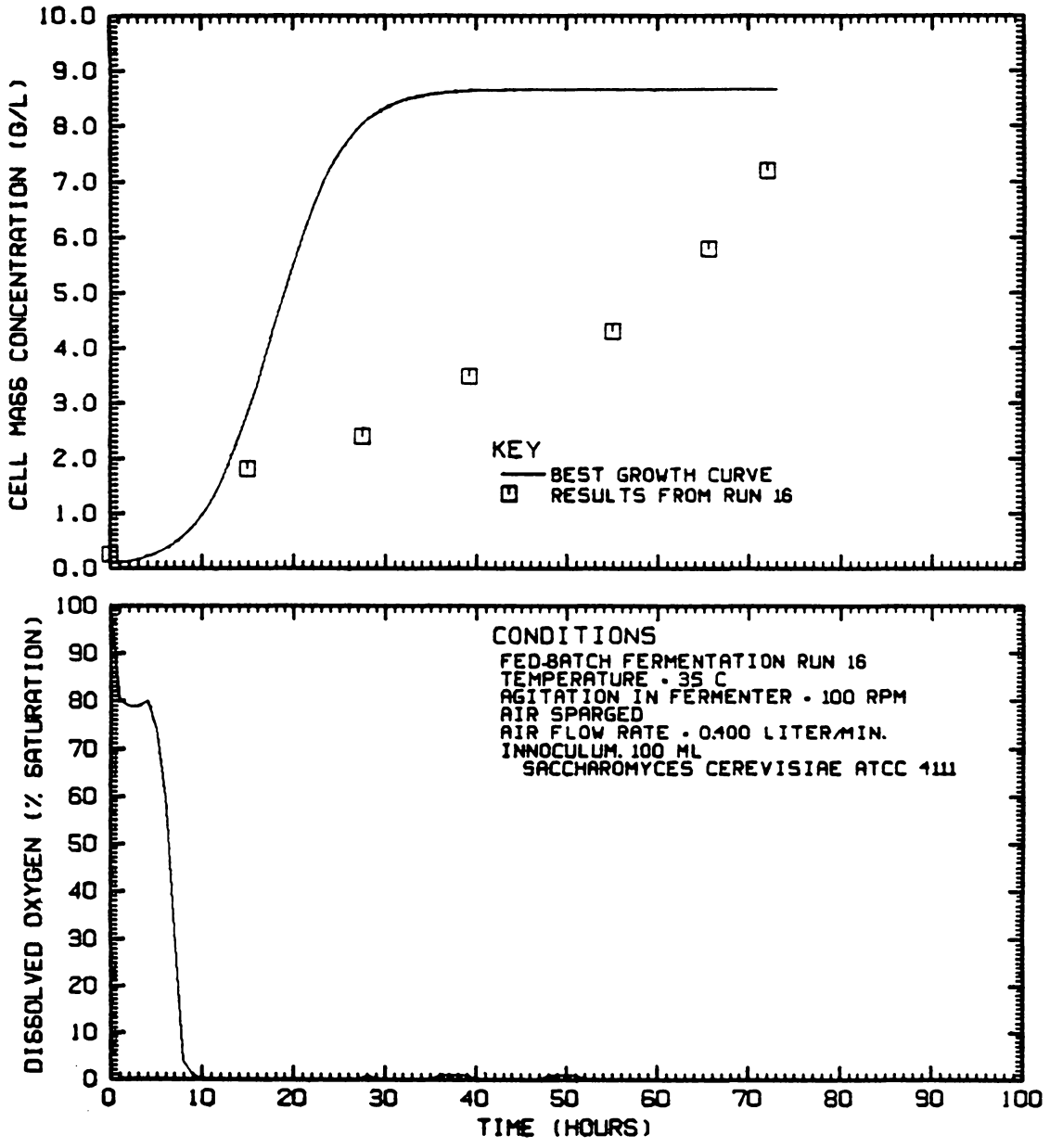
Cell mass concentrations and dissolved oxygen: Run 14 (Fed-batch)



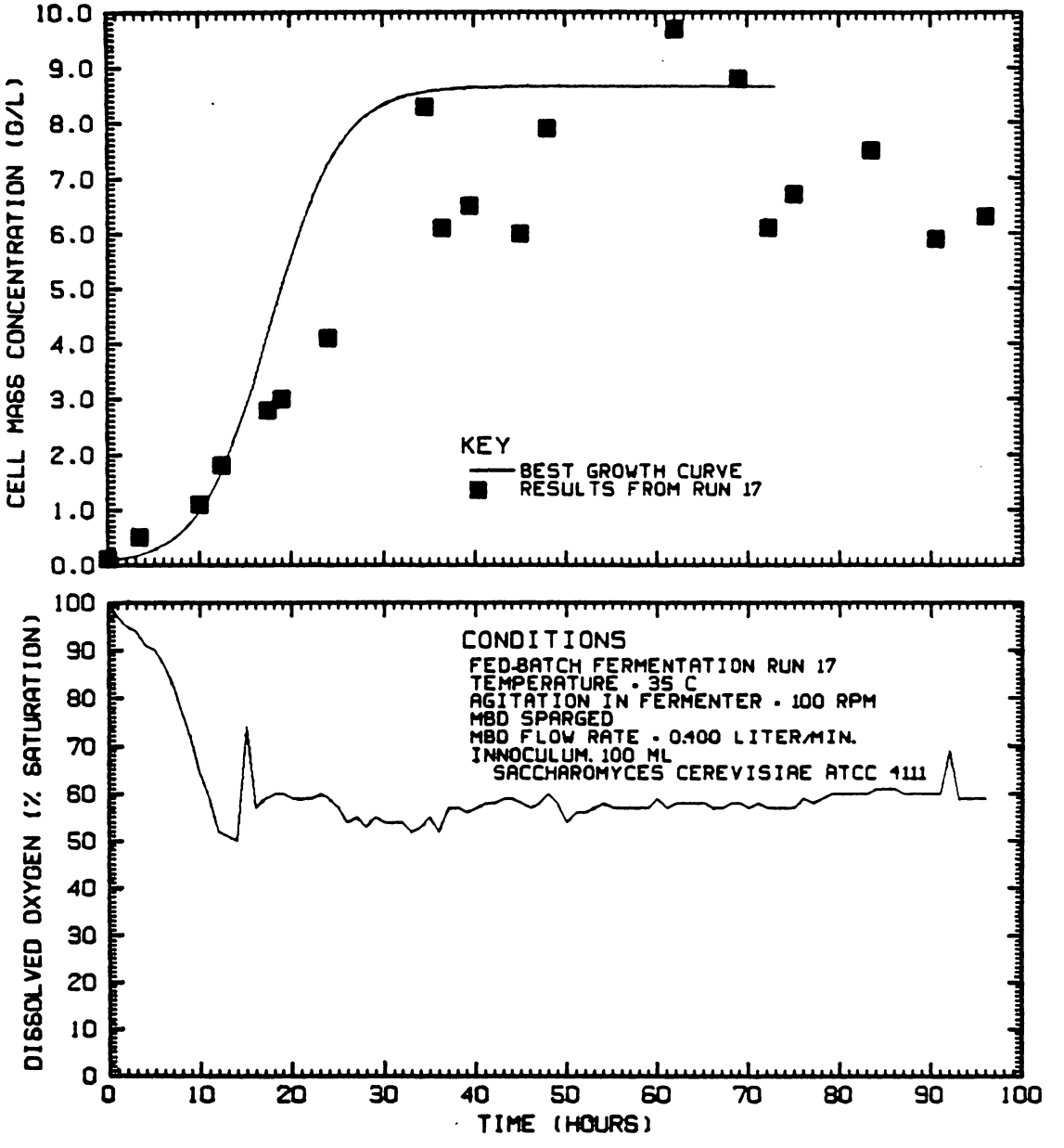
CONDITIONS

Fed batch fermentation
Temperature = 35 C
Agitation 100 RPM
Air Sparged
Air flow rate = 0.400 l/min
Initial tank volume 1.0 l
Innoculum 100 ml *Saccharomyces cerivicea* ATCC 4111

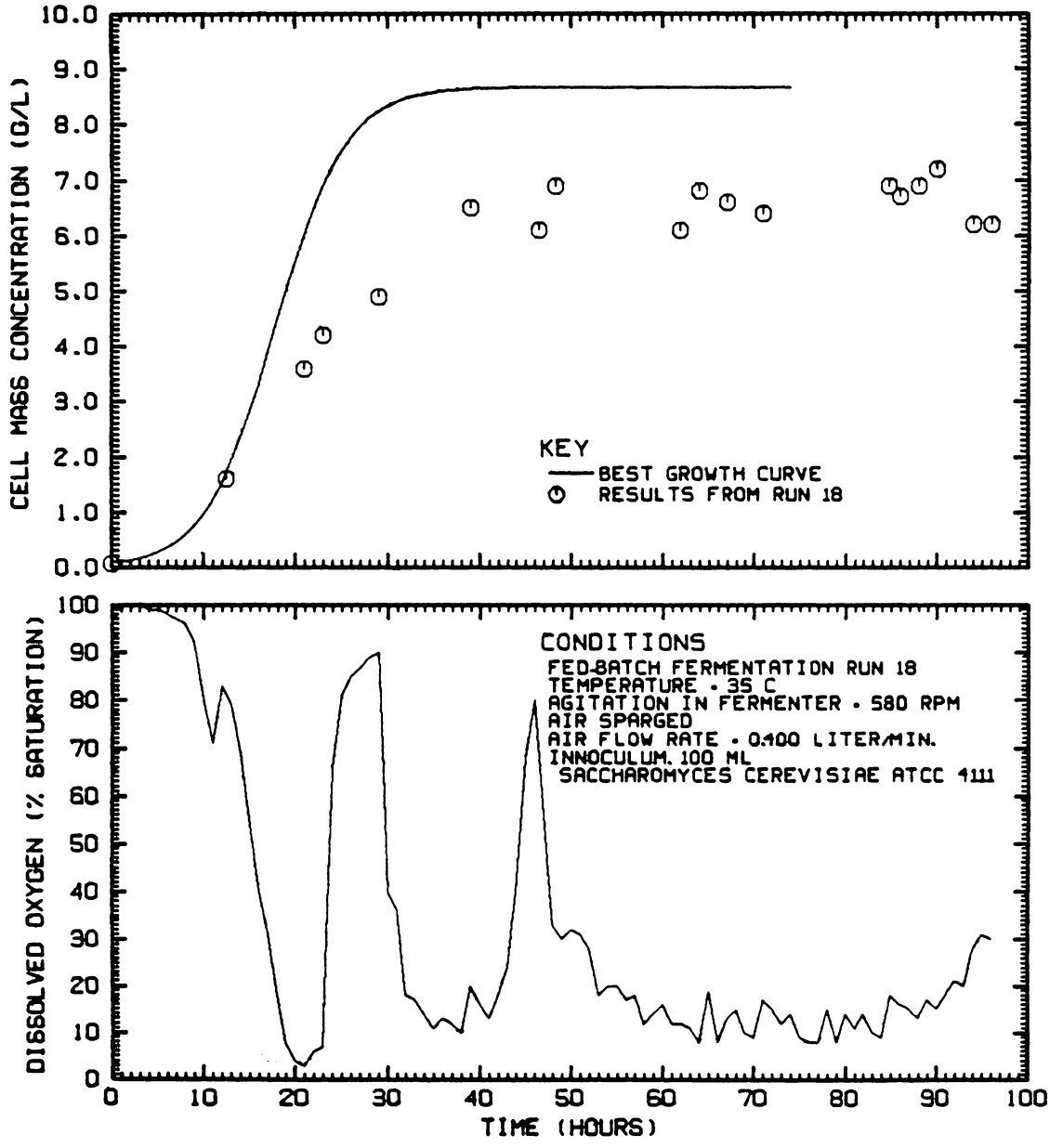
Cell mass concentrations and dissolved oxygen: Run 15 (Fed-batch)



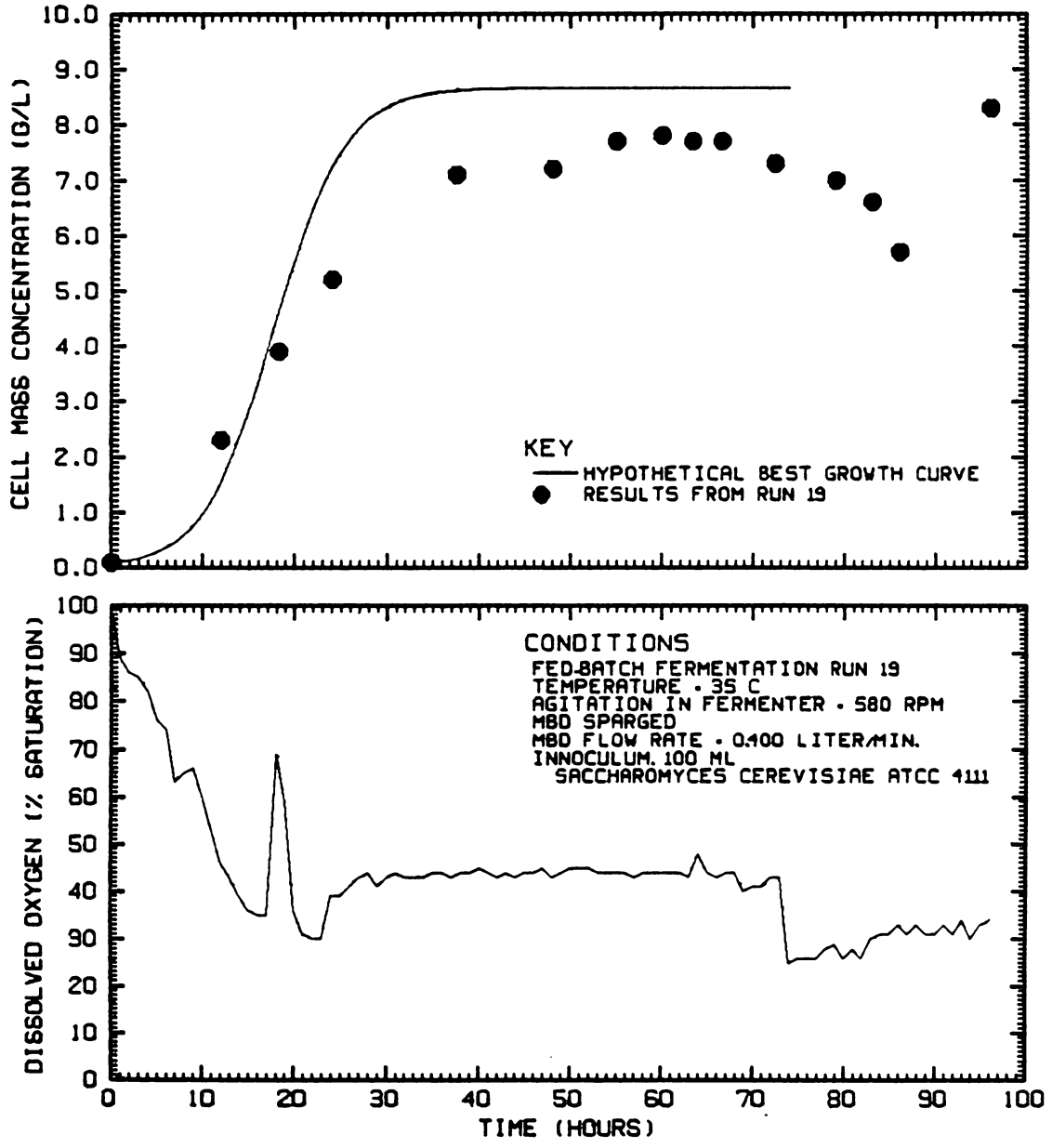
Cell mass concentrations and dissolved oxygen: Run 16 (Fed-batch)



Cell mass concentrations and dissolved oxygen: Run 17 (Fed-batch)



Cell mass concentrations and dissolved oxygen: Run 18 (Fed-batch)



Cell mass concentrations and dissolved oxygen: Run 19 (Fed-batch)

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