MATERIAL AND ENERGY BALANCES AND TRANSFER RATES IN AEROBIC FERMENTATIONS

by

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THIS IS AS RECEIVED FROM CUSTOMER.

CHAPTER I

INTRODUCTION

Aerobic fermentations depend on a continuous supply of oxygen to the liquid phase for microorganisms to grow and function properly. Aeration also provides mixing which frequently prevents the settling of solids.

Oxygen and heat removal requirements are affected by the choice of carbon substrate. Yeast produced on a normal alkane will require roughly three times as much oxygen as yeast grown on carbohydrate. Hydrocarbon fermentations produce more than twice as much heat per gram of cells produced compared to carbohydrate fermentations. From an engineering point of view, there is a need to design fermenters with improved oxygen and heat transfer systems so that higher production rates can be achieved.

One purpose of this report is to review the available literature on oxygen requirements and heat evolution in aerobic fermentations. The methods for determining oxygen transfer rate and oxygen transfer coefficients are also reviewed. Relationships between oxygen transfer requirements and oxygen transfer rates are presented and used to estimate the mass transfer coefficient.

CHAPTER II

LITERATURE SURVEY ON OXYGEN MATERIAL BALANCES AND REQUIREMENTS IN AEROBIC FERMENTATIONS

A major factor in aerobic fermentations is oxygen transfer from the gas phase to the aqueous medium. In microbial propagation oxygen transfer frequently limits the production rate. Therefore, the real capacity of the fermenter is often determined by the quantity of oxygen the fermenter is able to transfer to the microorganisms. Material balances are needed to relate the oxygen transfer rate to the rate of cell production. Literature is reviewed on the stoichiometric relationships between oxygen consumption and cell production in aerobic fermentations.

Darlington (1) assessed for the first time the merits of biosynthesis from hydrocarbon and carbohydrate starting materials by using a material balance. He made the following assumptions in his calculations:

a) The composition of the product from the carbohydrate and hydrocarbon fermentations is identical.

b) A typical microbial composition is 47% carbon, C; 6.5% hydrogen, H; 7.5% nitrogen, N; 8% ash; and 31% oxygen, O.

c) The dry weight yield of yeast from carbohydrate is 50%.

d) The dry weight yield of yeast from hydrocarbon is 100%.

With these assumptions the empirical C, H, O formula for 100 g. yeast of this composition would be:

$$C_{3.92}H_{6.5}O_{1.94} \qquad (1)$$

and the amount of carbohydrate (CH_2O) required, based on a 50% yield, would be 200 g. or 6.67 moles CH_2O. Writing the equation:

$$6.67\ CH_2O \rightarrow C_{3.92}H_{6.5}O_{1.94} \qquad (2)$$

and balancing with the appropriate factors for O_2 consumption, CO_2 and H_2O production gives:

$$6.67\ CH_2O + 2.1\ O_2 \rightarrow C_{3.92}\ H_{6.5}\ O_{1.94} + 2.75\ CO_2 + 3.42\ H_2O \qquad (3)$$

i.e., the production of 100 g. dry yeast from carbohydrate requires 67.2 g. oxygen.

Considering the hydrocarbon fermentation in the same way the production of 100 g. dry yeast requires 100 g. hydrocarbon (CH_2) if the yield is 1 g. dry yeast per g. hydrocarbon. The balanced equation for carbon, hydrogen and oxygen is:

$$7.14CH_2 + 6.135\ O_2 \rightarrow C_{3.92}\ H_{6.5}\ O_{1.94} + 3.22CO_2 + 3.89H_2O \qquad (4)$$

The production of 100 g. dry yeast from hydrocarbon requires 196.32 oxygen.

The oxygen requirement of a hydrocarbon yeast fermentation is almost triple that of a yeast carbohydrate fermentation producing an equal amount of product. If oxygen transfer is the limiting factor in yeast propagation, then hydrocarbon fermentation must either have a significantly larger oxygen transfer rate or a much lower production rate than that of the corresponding carbohydrate.

Mateles (2) derived an equation based upon material balance to determine the amount of oxygen required per unit weight of cells produced. The assumptions for this equation are:

1) The only products of metabolism are the cells themselves, carbon dioxide and water.
2) The nitrogen source is ammonia.

The equation is:

$$\frac{\text{g oxygen}}{\text{g cell}} = 16\left[\frac{2C + H/2 - O}{Y_{\frac{x}{s}} \cdot M} + \frac{O'}{1600} - \frac{C'}{600} + \frac{N'}{933} - \frac{H'}{200}\right] \quad (5)$$

or

$$\frac{\text{g oxygen}}{\text{g cell}} = \frac{32\ C + 8\ H - 16\ O}{Y_{\frac{x}{s}} \cdot M} + 0.0174\ N' - 0.08\ H' \quad (6)$$

where,

C, H and O, represent the number of atoms of carbon, hydrogen and oxygen, respectively, in each molecule of carbon substrate; C', H', O' and N' represent the percentage of carbon, hydrogen, oxygen, and nitrogen, respectively, in the cells. $Y_{\frac{x}{s}}$ represents the yield of cells based on carbon source, gram cells per gram carbon source consumed; M represents the molecular weight of the carbon source.

Takahashi et al., (3) carried out a stoichiometric analysis on the consumption of substrate and oxygen during bacterial growth on n-pentane. As a result, the following equation was developed for 100 g. of cells:

$$1.64\ C_5H_{12} + 6.99\ O_2 + 0.62\ NO_2 \rightarrow C_{4.17}\ H_{7.50}\ O_{1.55}\ N_{0.62} + 4.10\ CO_2 + 6.16\ H_2O \quad (7)$$

i.e., the production of 100 gm. of cells from n-pentane requires 223.69 g. O_2. The microbial composition was found to be 50.09% carbon, C; 7.50% hydrogen, H; 8.74% nitrogen, N; 24.79% oxygen, O; and 8.88% ash.

Minkevich and Eroshin (4) developed the following balance equation of microbial growth on a carbon substrate of generalized compositon:

$$CH_mO_\ell + a\ NH_3 + bO_2 \rightarrow y\ CH_pO_nN_q + z\ CH_rO_sN_t + (1 - y - z)\ CO_2 + c\ H_2O \quad (8)$$

substrate — biomass — organic products

where,

y = biomass carbon yield

z = product carbon yield

m and ℓ = numbers of hydrogen and oxygen atoms per carbon atom in the substrate molecule

p, n and q are average numbers of hydrogen, oxygen and nitrogen atoms per carbon atom in the biomass.

Balancing both sides of Eqn. (8) produces a formula for yield per unit of oxygen uptake (grams of dried biomass per gram of oxygen metabolized)

$$Y_{\frac{x}{o}} = \frac{3}{2\sigma_B \gamma_b} \cdot \frac{y}{\left(\frac{\gamma_s}{\gamma_b}\right) - y} \tag{9}$$

where, σ_B is the weight fraction of carbon in the dried biomass; γ_s and γ_b are dependent on the elemental composition of the substrate and biomass; that is,

$$\gamma_s = 4 + m - 2\ell$$

and

$$\gamma_b = 4 + p - 2n - 3q$$

Quantities γ_s and γ_B characterize the degree of oxidation of the substrate and biomass, respectively. This can be seen from the balance equations for substrate and biomass combustion:

$$CH_m O_\ell + \frac{\gamma_s}{4} O_2 = CO_2 + \frac{m}{2} H_2O \tag{10}$$

$$CH_p O_n N_q + \frac{\gamma_b}{4} O_2 = CO_2 + \frac{1}{2}(p - 3q)\ H_2O + q\ NH_3 \tag{11}$$

where,

γ_s = number of gram - equivalents of oxygen necessary for complete combustion of one gram-atom of substrate carbon. In other words, γ_s is the number of available electrons in the substrate molecule per one carbon atom. The interpretation of γ_b is similar.

The value of γ_s is maximal for methane (=8), and minimal for CO_2(=0). Values of γ_s for twenty different substrates were presented by Minkevich and Eroshin.

Values of γ_b and σ_B for twenty species of microorganisms grown on various substrates were also presented by Minkevich and Eroshin. The data indicates that γ_b and σ_B are nearly constant since the variances of γ_b and σ_B are 2-4%. Minkevich and Eroshin (4) considered γ_b and σ_B as constants, and used the values of 4.2 and 0.46, respectively.

Recently, interest in the use of computers to monitor and control fermentation processes has been increasing (5,6,7). Material balances can be used to check on the consistency of process measurements and to predict unmeasured variables. The recent work of Cooney et al. (5,7) and Zabriskie (6) provide examples of how material balances can be employed.

Cooney et al., (5,7) proposed the application of computer-aided diagnostic analysis to the production of SCP from methanol and glucose. The concentration of biomass and the conversion yield of substrate to biomass were assessed by the use of material and energy balances. Then, on-line computer was used to detect deviations from optimal behavior, because this is a prerequisite for the application of automatic process control. Diagnostic strategies were developed to differentiate between the many possible reasons for a decline in productivity and/or cell yield. These strategies

included both steady state and transient analysis of the fermentation. Some of the more difficult problems which remain are prediction of uncoupling of respiration, contamination, nutrient starvation (other than oxygen and carbon), and sensor failure.

Zabriskie (6), by means of the application of computer to on-line monitoring and control of fermentation process, determined the production of biomass by using two different approaches. The first method consists of using a material balance of gas phase components and the second one involves biomass estimation by using culture fluorescence data. It was shown that both approaches possess excellent potentials for real-time biomass concentration estimates based on fermentation sensor. A more accurate oxygen analyzer could improve the results of biomass estimation.

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NOMENCLATURE

C = number of atoms of carbon in the substrate

C' = percentage of carbon in biomass

H = number of atoms of hydrogen in the substrate

H' = percentage of hydrogen in biomass

ℓ = number of oxygen atoms/carbon atom in substrate

m = number of hydrogen atoms/carbon atom in substrate

M = molecular weight of the substrate

n = number of oxygen atoms/carbon atom in biomass

N' = percentage of nitrogen in biomass

O = number of atoms of oxygen in the substrate

O' = percentage of oxygen in biomass

p = number of hydrogen atoms/carbon atom in biomass

q = number of nitrogen atoms/carbon atom in biomass

y = biomass carbon yield, $\frac{\text{g biomass carbon}}{\text{g substrate carbon}}$

$Y_{\frac{x}{o}}$ = yield per unit of oxygen uptake, $\frac{\text{g biomass}}{\text{g } O_2}$

γ_b = degree of oxidation of biomass, $4 + p - 2n - 3q$

γ_s = degree of oxidation of substrate, $4 + m - 2\ell$

σ_B = weight fraction of carbon in biomass

CHAPTER III

HEAT GENERATION AND OXYGEN CONSUMPTION RELATIONSHIPS IN AEROBIC FERMENTATIONS

SCP production requires the removal of large amounts of heat which are produced during growth. If expensive refrigeration systems are required for this purpose, the cost is important. Knowledge of the thermodynamic and energetic properties of the cells is needed to design the cooling system.

A search of the literature produces some correlations which can be used for predicting the heat evolution and provide a basis for design and scale-up of heat removal systems for fermentation vessels. Guenther (1) estimated a value of 3.97 K cal./g oxygen consumed in his evaluation of heat production during hydrocarbon fermentations. He made several assumptions for the calculation of heat evolution:

a) The yield of the yeast from hydrocarbon is 100%.

b) The heat of combustion of 100 g. of dried yeast is 363 K cal. This is derived by using a value of 109 K cal. as the heat of combustion of 100 g. of pressed yeast and assuming a solids content of 30%.

c) The heat of combustion of 100 g. of hydrocarbon (CH_2) is 1143 K cal. This is derived from the heat of combustion of hexadecane, 2559 K cal./mole.

His stoichiometric equation was:

$$7.14\ CH_2 + 6.135\ O_2 \rightarrow C_{3.92}\ H_{6.5}\ O_{1.94} + 3.22\ CO_2 + 3.89\ H_2O \qquad (12)$$

or 100 g. of hydrocarbon for which the heat of combustion is 1143 K cal. produce 100 g. of yeast with a heat of combustion of 363 K cal., resulting in a

net energy change of 780 K cal., more than twice that produced from carbohydrate fermentation (383 K cal.).

Cooney et al., (2) estimated a value of 3.44 K cal./g oxygen consumed in their experimental determination of heat evolution. The method is based on measuring the rate of temperature rise of the fermentation broth resulting from metabolism, when the temperature controller was turned off. The heat measured in this manner was later corrected for heat losses and gains. Experiments were performed using bacteria (E. Coli and B. subtilis), a yeast (C. Intermedia), and a mold (A. niger). The substrates investigated included glucose, molasses, and soybean meal. The correlation thus obtained is independent of the growth rate, slightly dependent on the substrate, and possibly dependent on the type of organisms grown.

Minkevich and Eroshin (3) estimated a value of 3.38 K cal./g of oxygen consumed by cells grown on any organic substrate. They studied the dependence of productivity and heat generation on biomass carbon yield (y) and degree of oxidation of substrate (γ_s). Productivity of the fermenter (W) is determined by rate of oxygen transfer (P) and yield per unit of oxygen uptake ($Y_{\frac{x}{o}}$). Mathematically,

$$W = P\, Y_{\frac{x}{o}} \tag{13}$$

In Eqn. (13), the value of P is determined by fermenter construction whereas $Y_{\frac{x}{o}}$ is determined by cell metabolism.

The value of $Y_{\frac{x}{o}}$ was calculated before (Equation (9)):

$$Y_{\frac{x}{o}} = \frac{3}{2\sigma_B \gamma_b} \cdot \frac{y}{\left(\frac{\gamma_s}{\gamma_b}\right) - y} \tag{9}$$

Eqn. (9) gives a basis for comparative evaluation of various substrates concerning yield per unit of oxygen uptake ($Y_{\frac{x}{o}}$). To make such evaluation γ_s and y are required. It is advisable to compare the substrates when the carbon yield is maximal. In fact, the more the substrate is reduced the larger the chemical energy store of substrate molecules. This indicates that higher values of y are possible on more reduced substrates. The interrelation between γ_s and substrate chemical energy store is of great interest. This energy in turn is dissipated as heat during complete combustion of substrate to CO_2 and H_2O.

The balance equation for combustion is:

$$CH_m O\ell + (4 + m - 2\ell) \frac{O_2}{4} \rightarrow CO_2 + \frac{m}{2} H_2O \tag{14}$$

In Eqn. (14), $\gamma_s = 4 + m - 2\ell$.

The balance equation of dried microbial biomass combustion is:

$$CH_p O_n N_q \ldots\ldots + (4 + p - 2n - 3q) \frac{1}{4} O_2 \rightarrow$$

$$CO_2 + \frac{1}{2}(p - 3q)\ H_2O + q\ NH_3 \tag{15}$$

In Eqn. (15), $\gamma_b = 4 + p - 2n - 3q$.

Defining η, as the ratio of biomass heat of combustion to the carbon substrate heat of combustion, we have,

$$\eta = \frac{\gamma_b\ Q_{ob}}{\gamma_s\ Q_{os}}\ y \tag{16}$$

where,

Q_{ob} = heat of combustion of biomass, K Cal./g equiv. oxygen

Q_{os} = heat of combustion of substrate, K cal./g equiv. oxygen

γ_b = degree of oxidation of biomass

γ_s = degree of oxidation of substrate

Minkevich and Eroshin (3) presented values of Q_{ob} for 12 cultures. The mean value of Q_{ob} is equal to 26.94 K cal., with a variance of 4%. Also, values of Q_{os} for 25 organic substrates are presented. The mean value of Q_{os} is equal to 26.92 K cal., with a variance of 4%. For the purpose of calculations Q_{ob} and Q_{os} can be considered to be the same and equal to 27 K cal.

Replacing Eqn. (16) in Eqn. (9) produces:

$$Y_{\frac{x}{o}} = \frac{3}{2\sigma_B \gamma_b} \frac{\eta}{\frac{Q_{ob}}{Q_{os}} - \eta} \tag{17}$$

Substitution of σ_B, γ_b, Q_{ob}, Q_{os} by the mean values, result in

$$Y_{\frac{x}{o}} = 0.777 \frac{\eta}{1 - \eta} \tag{18}$$

Eqn. (18) indicates that the energetic yield of growth (η) is the principal factor on which $Y_{\frac{x}{o}}$ is dependent during cultivation of various microbial cultures on various substrates under oxygen limitation. If the rate of oxygen transfer P is fixed for a fermenter, the productivity of the apparatus is determined by η.

The heat evaluation is equal to the difference between the heat of combustion of substrate and the heat of combustion of biomass formed. Mathematically,

$$\frac{\gamma_s Q_{os}}{y} - \gamma_b Q_{ob} \tag{19}$$

The metabolic heat generation per 1 gram of dried cells, Q, is

$$Q = \frac{\sigma_B}{12}\left(\frac{\gamma_s Q_{os}}{y} - \gamma_b Q_{ob}\right) = \frac{\sigma_B \gamma_b Q_{ob}}{12}\left(\frac{1}{\eta} - 1\right) = Q_c\left(\frac{1}{\eta} - 1\right) \qquad (20)$$

where, Q_c = heat of combustion per 1 g of biomass.

Using Eqn. (17), Eqn. (20) can be transformed to

$$Q = \frac{Q_{os}}{8} \cdot \frac{1}{Y_{\frac{x}{o}}} + \frac{\sigma_B \gamma_b}{12}\left(Q_{os} - Q_{ob}\right) \qquad (21)$$

Since the average Q_{os} and Q_{ob} are equal,

$$Q = \frac{Q_{ob}}{8} \cdot \frac{1}{Y_{\frac{x}{o}}} = 3.38 \frac{1}{Y_{\frac{x}{o}}} \qquad (22)$$

The product $QY_{\frac{x}{o}}$ is the heat generation per gram of oxygen consumed by the cells. According to (22) this quantity equals $\frac{Q_{ob}}{8} = 3.38$ kcal during aerobic growth of any cells on any organic substrate.

RELATIONSHIP BETWEEN THE EQUATIONS DEVELOPED BY MINKEVICH AND MATELES

Mateles (2) developed the following equation for determining the unit mass of oxygen consumed per unit mass of cells produced:

$$\frac{gO_2}{g\ cell} = 16\left[\frac{2C + H/2 - O}{Y_{\frac{x}{s}} \cdot M} + \frac{O'}{1600} - \frac{C'}{600} + \frac{N'}{933} - \frac{H'}{200}\right] \tag{5}$$

Eqn. (5) can also be written as:

$$\frac{gO_2}{g\ cell} = \frac{16}{2}\left[\frac{4C + H - 2O}{Y_{\frac{x}{s}} M} + \frac{2O'}{160} - \frac{4C'}{1200} + \frac{3N'}{1400} - \frac{H'}{100}\right] \tag{23}$$

Using Minkevich notation for Eqn. (23), C = 1, m = H and ℓ = 0, we have,

$$\frac{gO_2}{g\ cell} = \frac{16}{2}\left[\frac{4 + m - 2\ell}{y \cdot M} + \frac{2O'}{160} - \frac{4C'}{1200} + \frac{3N'}{1400} - \frac{H'}{100}\right] \tag{24}$$

Also, $$\frac{\sigma_B \gamma_b}{12} = \frac{4C'}{1200} + \frac{H'}{100} - \frac{2O'}{1600} - \frac{3N'}{1400} \tag{25}$$

where,

σ_B = fraction of carbon in biomass

$\gamma_b = 4 + p - 2n - 3q$

The molecular weight of the substrate can be represented by:

$$M = 12 + m + 16\ell \tag{26}$$

and the yield of cells based on carbon source (y) would be:

$$Y_{\frac{x}{s}} = \left(\frac{12 + p + 16n + 14q}{12 + m + 16\ell}\right) y \tag{27}$$

The fraction of carbon in biomass (σ_B) is equal to:

$$\sigma_B = \frac{12}{12 + p + 16n + 14q} \tag{28}$$

Multiplying Eqns. (26) and (27), we get

$$M \cdot Y_{\frac{x}{s}} = (12 + m + 16\ell)\left(\frac{12 + p + 16n + 14q}{12 + m + 16\ell}\right)y = \frac{12\ y}{\sigma_B} \tag{29}$$

or,
$$y = \frac{Y_{\frac{x}{s}}\ M\ \sigma_B}{12} \tag{30}$$

Substituting Eqns. (25) and (29) into Eqn. (24) and since $\gamma_s = 4 + m - 2\ell$, we get

$$\frac{gO_2}{g\ cells} = \frac{16}{2}\left[\frac{\gamma_s}{12\frac{y}{\sigma_B}} - \frac{\sigma_B\gamma_b}{12}\right] \tag{31}$$

or,
$$\frac{gO_2}{g\ cells} = \frac{2}{3}\ \sigma_B\ \left[\frac{\gamma_s - y\gamma_b}{y}\right] = \frac{1}{Y_{\frac{x}{o}}} \tag{32}$$

We can also write Mateles' equation in a more simplified form:

$$\frac{gO_2}{g\ cells} = 8\left[\frac{4\ C + H - 2\ O}{Y_{\frac{x}{s}}\ .\ M} - \frac{\sigma_B\gamma_b}{12}\right] \tag{33}$$

Considering γ_b and σ_B as constants, and using the values of 4.2 and 0.46, respectively, Eqn. (33) becomes

$$\frac{gO_2}{g\ cells} = 8\left[\frac{4C + H - 20}{Y_{\frac{x}{s}}\ .\ M} - \frac{(0.46)(4.2)}{12}\right] = Y_{\frac{o}{x}}$$

$$\frac{gO_2}{g\ cells} = \left[8\left(\frac{4C + H - 20}{Y_{\frac{x}{s}}\ .\ M} - 1.288\right] = Y_{\frac{o}{x}}\right. \tag{34}$$

In Eqn. (18) $Y_{\frac{x}{o}} = 0.777 \frac{\eta}{1 - \eta}$

$$\frac{1}{\eta} - 1 = \frac{0.777}{Y_{\frac{x}{o}}} = 0.777\ Y_{\frac{o}{x}} \tag{35}$$

$$\frac{1}{\eta} - 1 = 0.777(8)\left[\frac{4\ C + H - 2\ O}{Y_{\frac{x}{s}} \cdot M} - 1.288\right] \tag{36}$$

$$\eta = \frac{0.16\ Y_{\frac{x}{s}} \cdot M}{4\ C + H - 2\ O} \tag{37}$$

and

$$Y_{\frac{x}{s}} = \frac{6.22\eta(4\ C + H - 2\ O)}{M} \tag{38}$$

ILLUSTRATION PROBLEMS ON HEAT GENERATION AND OXYGEN CONSUMPTION RELATIONSHIPS IN AEROBIC FERMENTATIONS

Problem 1

It is desired to produce single cell protein using n-hexadecane as the only carbon source and a yeast Candida lipolytica, in an airlift fermenter at 30°C and pH 5.5. The microbial composition is 47% carbon, C; 6.5% hydrogen, H; 7.5% nitrogen, N; 8% ash; and 31% oxygen, O.

Assume:

a) the only products of metabolism are the cells themselves, carbon dioxide and water.

b) the nitrogen source is ammonia

c) the dry weight yield of yeast from hydrocarbon is 100%.

Determine:

a) the amount of oxygen required per g of cell produced by using the Mateles and Minkevich expressions, Eqns. (5) and (9).

b) the experimental value of Cooney et al. (3.44 kcal per gram of oxygen) and the value of Minkevich (3.38 kcal per gram of oxygen).

c) the energetic yield, η

d) the amount of ammonia utilized

Solution

a) The mass balance equation for biomass production using n-hexadecane as the sole carbon source and ammonia as the sole nitrogen source is:

$$C_{16}H_{34} + NH_3 + O_2 \rightarrow \text{Biomass} + CO_2 + H_2O \qquad (38)$$

Mateles equation is:

$$\frac{\text{g oxygen}}{\text{g cell}} = 16\left[\frac{2C + \frac{H}{2} - O}{Y_{\frac{x}{s}}\, M} + \frac{O'}{1600} - \frac{C'}{600} + \frac{N'}{933} - \frac{H'}{200}\right] \qquad (5)$$

$$= \frac{32C + 8H - 160}{Y_{\frac{x}{s}} M} + 0.010' - 0.0267\ C' + 0.01714\ N' - 0.08H' \qquad (6)$$

$$= \frac{(32)(16) + 8(34) - 16(0)}{(1)(226.45)} + (0.01)(31) - 0.0267(47) + 0.01714(7.5) - 0.08(6.5)$$

$$\frac{\text{g oxygen}}{\text{g cell}} = 2.12$$

The heat evolved per g cells produced is:

$$3.44 \frac{\text{kcal}}{\text{g oxygen consumed}} \times \frac{2.12 \text{ g oxygen consumed}}{\text{g cell produced}} = 7.29 \frac{\text{kcal}}{\text{g cell}}$$

Minkevich equation is:

$$Y_{\frac{x}{o}} = \frac{3}{2\sigma_B \gamma_b} \frac{y}{\frac{\gamma_s}{\gamma_b} - y} \qquad (9)$$

y is calculated from:

$$C_{16} H_{34} + NH_3 + O_2 \rightarrow \text{Biomass} + CO_2 + H_2O \qquad (39)$$

$$\frac{1}{226.45} \times 16 \times 12 = 0.847 \qquad 0.47$$

$$y = \frac{0.47}{0.847} = 0.554$$

$\sigma_B = 0.47$

$\gamma_b = 4 + p - 2n - 3q = 4.26$

$\frac{\gamma_s}{\gamma_b} = 1.43$

$$Y_{\frac{x}{o}} = \frac{3}{(2)(0.47)((4.26)} \frac{0.566}{(1.43 - 0.554)} \frac{\text{g cell}}{gO_2}$$

$$Y_{\frac{x}{o}} = 0.471 \frac{\text{g cell}}{gO_2}$$

$p = \frac{(6.5)(12)}{47} = 1.659$

$n = \frac{(31)(12)}{(47)(16)} = 0.494$

$q = \frac{(7.5)(12)}{(47)(14)} = 0.136$

$\eta = \frac{34}{16} = 2.125$

$\gamma_s = 4 + 2.125 + 0 = 6.125$

$$\frac{gO_2}{g\ cells} = 2.12$$

b) The heat evolved per g cells produced is:

$$3.38\ \frac{kcal}{g\ oxygen\ consumed} \times \frac{2.12\ gO_2\ consumed}{g\ cells\ produced} = 7.17\ \frac{kcal}{g\ cells}$$

c)

$$\eta = \frac{\frac{Y_{\frac{x}{o}}}{}}{\frac{Y_{\frac{x}{o}}}{} + 0.777}$$

$$\frac{Y_x}{o} = 0.471\ \frac{g\ cells}{O_2}\ \text{(from Minkevich equation)}$$

$$\frac{Y_x}{o} = 0.471\ \frac{g\ cells}{O_2}\ \text{(from Mateles equation)}$$

$$\eta = \frac{0.471}{0.471 + 0.777} = 0.38$$

$$\eta = \frac{0.471}{0.471 + 0.777} = 0.38$$

d) A stoichiometric equation for the aerobic production of biomass from n-hexadecane as the sole carbon source and ammonia as the sole nitrogen source is:

$$C_{16}H_{34} + \frac{b}{32}O_2 + \frac{a}{17.03}NH_3 \rightarrow \frac{yCH_p}{100}O_n N_q + zCH_r O_s Nt + (1-y-z)\ CO_2 + c\ H_2O \qquad (40)$$

where

b and a = grams of oxygen and ammonia consumed, respectively, y, z, (1-y-z) and c = grams of cells, product, CO_2 and H_2O produced, respectively.

p,n,q = g. atoms of hydrogen, oxygen and nitrogen respectively, contained in 100 g of dried cells.

r,s,t = g. atoms of hydrogen, oxygen and nitrogen contained in formed product.

Neglecting amount of organic product being formed, and taking mass balance for nitrogen in Eqn. (40), we obtain

$$N = \frac{a}{17.03} = \frac{qy}{100}$$

$$a = \frac{(17.03)(qy)}{100} = (17.03)(0.535) = 9.11 \text{ g } NH_3 \text{ /100 g cells}$$

Problem 2

Rework the above problem using glucose as substrate but assuming that the dry weight yield of yeast from carbohydrate is 50%.

Solution

a) The mass balance equation for biomass production using glucose as the sole carbon source and ammonia as the sole nitrogen source is:

$$C_6 H_{12} O_6 + NH_3 + O_2 \rightarrow \text{Biomass} + CO_2 + H_2O$$

Mateles equation is:

$$\frac{\text{g oxygen}}{\text{g cell}} = 16\left[\frac{2C + \frac{H}{2} - O}{Y_{\frac{x}{s}} \cdot M} + \frac{O'}{1600} - \frac{C'}{600} + \frac{N'}{933} - \frac{H'}{200}\right]$$

$$= \frac{32C + 8H - 16\,O}{Y_{\frac{x}{s}} \cdot M} + 0.010' - 0.0267\,C' + 0.01714\,N' - 0.08\,H'$$

$$= \frac{(32)(6) + 8(12) - 16(6)}{(0.5)(180)} + (0.01)(31) - 0.0267(47) + 0.01714(7.5) - 0.08(6.5)$$

$$= 0.796$$

The heat evolved per g cells produced is:

$$3.44 \frac{\text{kcal}}{\text{g oxygen utilized}} \times 0.796 \frac{\text{g oxygen consumed}}{\text{g cell produced}} = 2.74 \frac{\text{kcal}}{\text{g cell}}$$

Minkevich equation is:

$$Y_{\frac{x}{o}} = \frac{3}{2\sigma_B \gamma_b} \frac{y}{\frac{\gamma_s}{\gamma_b} - y}$$

y is calculated from:

$$C_6 H_{12} O_6 + NH_3 + O_2 \rightarrow \text{Biomass} + CO_2 + H_2O$$

$$\frac{1}{180.16} \times 6 \times 12 = 0.399 \qquad (0.47)(0.5) = 0.235$$

$$y = \frac{0.235}{0.399} = 0.588$$

$$\sigma_B = 0.47$$

$$\gamma_b = 4 + p - 2n - 3q$$

$$p = \frac{(6.5)(12)}{47} = 1.659$$

$$\eta = \frac{(31)(12)}{(47)(16)} = 0.454$$

$$q = \frac{(7.5)(12)}{(47)(14)} = 0.136$$

$$\gamma_b = 4.26$$

$$\gamma_s = 4 + m - 2$$

$$m = \frac{12}{6} = 2$$

$$\ell = \frac{6}{6} = 1$$

$$\gamma_s = 4$$

$$\frac{\gamma_s}{\gamma_b} = 0.938$$

$$Y_{\frac{x}{o}} = \frac{3}{(2)(0.47)(4.26)} \quad \frac{0.588}{(0.938 - 0.588)} = 1.25 \frac{\text{g cell}}{\text{g}0_2}$$

$$Y_{\frac{o}{x}} = 0.79 \frac{\text{g}0_2}{\text{g cell}}$$

b) The heat evolved per g cells produced is:

$$\frac{3.38 \text{ kcal}}{\text{g oxygen consumed}} \times 0.75 \frac{\text{g}0_2 \text{ consumed}}{\text{g cells produced}} = 2.67 \frac{\text{kcal}}{\text{g cells}}$$

c) $$= \frac{\frac{Y_x}{o}}{\frac{Y_x}{o} + 0.777} + \frac{0.79}{0.79 + 0.777} = 0.50$$

d) A stoichiometric equation for the aerobic production of biomass from glucose as the sole carbon source is:

$$C_6H_{12}O_6 + \frac{6}{32}\,O_2 + \frac{a}{17.03}\,NH_3 \rightarrow \frac{yCH_pO_nN_q}{100} + qCH_rO_sN_t + (1-y-z)CO_2 + CH_2O$$

where,

b and a = grams of oxygen and ammonia consumed, respectively,

y, z, (1-y-z) and c = grams of cells, product, CO_2 and H_2O produced, respectively.

p,n,q = g. atoms of hydrogen, oxygen and nitrogen, respectively, contained in 100 g of dried cells.

r,s,t = g atoms of hydrogen, oxygen and nitrogen contained in formed product.

Neglecting amount of organic product being formed, and taking mass balance for nitrogen in the above eqn., we obtain:

$$N = \frac{a}{(17.03)} = \frac{9y}{100}$$

$$a = \frac{(17.03)(9y)}{100} = \frac{(17.03)(0.535)}{2} = 4.55 \text{ g } \frac{NH_3}{100 \text{ g cells}}$$

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NOMENCLATURE

a	=	amount of ammonia consumed, g
b	=	amount of oxygen consumed, g
c	=	amount of water produced, g
C	=	number of atoms of carbon in each molecule of carbon source
C'	=	percentage of carbon in the cells
H	=	number of atoms of hydrogen in each molecule of carbon source
H'	=	percentage of hydrogen in the cells
M	=	molecular weight of the carbon source
N'	=	percentage of nitrogen in the cells
O	=	number of atoms of oxygen in each molecule of carbon source
O'	=	percentage of oxygen in the cells
p	=	g. atoms of hydrogen contained in 100 g dried cells
P	=	oxygen transfer rate
q	=	g atoms of nitrogen contained in 100 g. dried cells
Q	=	metabolic heat generation/g. cells
Q_c	=	heat of combustion/g. cells
Q_{ob}	=	heat of combustion of biomass, Kcal/g. equiv. oxygen
Q_{os}	=	heat of combustion of substrate, kcal/g equiv. oxygen
r	=	g. atoms of hydrogen in organic product
s	=	g. atoms of oxygen in inorganic product
t	=	g. atoms of nitrogen in organic product
w	=	productivity of the fermenter
y	=	biomass carbon yield
$Y_{\frac{x}{o}}$	=	yield per unit of oxygen uptake

$Y_{\frac{x}{s}}$ = yield of cells based on carbon source, g cells/g substrate

$1-y-z$ = amount of carbon dioxide produced, g

z = amount of organic product formed, g

γ_s = degree of oxidation of substrate, $4 + m - 2$

η = energetic yield

σ_B = weight part of carbon in biomass

CHAPTER IV

LITERATURE SURVEY OF THE PRINCIPAL METHODS FOR $K_L a$ DETERMINATION

The literature on oxygen transfer coefficient determination is so large and varied that its full presentation is beyond the reach of this survey. However, an attempt will be made to survey the important methods which are used to determine the oxygen transfer coefficient. Literature based on each technique is grouped together. The surveyed methods are:

a) Sodium Sulfite

b) Dynamic Techniques

c) Measuring respiration rate of microorganisms

d) Separate measurements of K_L and a

e) Mass Balance

a) SODIUM SULFITE

Many researchers have used sulfite oxidation instead of fermentation broth to characterize mass transfer of fermentation equipment. Cooper, Fernstrom and Miller (1) pioneered the use of sulfite medium for characterization of fermentation systems. Cooper et al. (1) initiated the measurement of oxygen transfer coefficient in an aerated vessel with the sulfite oxidation method. Vaned disk and flat paddle impellers ranging from 6 to 96 inches and different size vessels were used. The procedure of making a run was as follows: Tap water at about room temperature was placed in the tank and the agitator started. Sufficient sodium sulfite crystals to make the solution approximately 1 normal in sulfite ion and cupric sulfate to produce a Cu^{++} concentration of at least 10^{-3} molar were added and allowed to dissolve. The air was then turned on, and a timer

was started when the first bubbles of air emerged from the sparger. The oxidation was allowed to continue for 4-20 minutes, after which the air stream, agitator and timer were stopped at the same time. During the aeration interval, the agitator power was measured several times. Normally the power requirement was quite constant for a given run. Rate of oxygen absorption was measured by determination of the unoxidized sulfite-ion content of the solution before and after each run. Samples of 5-100 ml., depending on the size of the tank, were pipetted from the solution before and after each run. Each sample was run immediately into an excess of fully pipetted standard iodine reagent, the tip of the sulfite pipet being held not more than 0.5 inch from the iodine solution surface. Analysis was completed by an iodometric procedure of titration with standard thiosulfate solution to a starch indicator end point. The only precaution necessary to prevent further oxidation of the solution during transfer was to flush the sampling pipet with nitrogen for a few minutes previous to sampling. Determinations were made in duplicate and usually agreed to within 2%. This analytical method was considered sufficiently reliable to obviate the necessity of an oxygen balance on the system.

Power requirements of the agitators were observed for all except the largest equipment by measuring the torque of the agitator. Absorption coefficients, lb moles oxygen absorbed/(ft^3 sulfite solution) (atm. oxygen partial pressure)(hr) and oxygen efficiencies were calculated for each run.

Cooper et al. (1) correlated their data on the absorption coefficient K_La with the power input per unit volume P_v and superficial gas velocity V_s. It was reported that K_{La} was proportional to $(P_v)^{0.95}$ at constant V_s and to $(V_s)^{0.67}$ at constant P_v.

Cooper et al. (1) assumed that the oxygen transport was liquid film controlled and that the sulfite reaction was of zero order with respect to both sulfite and sulfate concentration. They were able to correlate data for vaned-disk impellers and flat paddles covering, respectively, three and tenfold variations of scale size. Power magnitudes ranging from 10 to 3000 $\frac{\text{Ft-lb}}{(\text{min})(\text{cu. ft})}$ and gas rates ranging from 20 to 360 ft/hour are reported. Cooper et al. (1) concluded that the oxidation by air of aqueous sodium sulfite solutions containing Cu^{++} ion is a useful reaction in the study of the design variables of agitated gas-liquid contactors.

Hixon and Gaden (2) later demonstrated the practicability of the sulfite system and since then most researchers have preferred it to using microbial fermentations. Several problems are encountered when using the sulfite medium. For fermentation systems of low shear (as in the airlift) coalescence becomes an important factor. Also, Pirt (3) found the $K_L a$ determined by the sulphite method to be 1.4 to 2.0 times as high as in the fermentation system. It is therefore quite difficult to simulate accurately oxygen transfer in the fermentation system using sulfite oxidation. The importance of $K_L a$ in describing the aeration efficiency of fermenters was perhaps fully recognized by Olson and Johnson (4). Using the sulphite oxidation method, they showed that yield of microbial mass production critically depended on the aeration rate of the fermenter. Maxon and Johnson (5) reported a similar observation on the effect of aeration rate on yeast fermentation. Smith and Johnson (6) showed that the yield of the highly aerobic bacteria Serratia marcesceus was directly proportional to the aeration efficiency of the culture vessel as measured by sulfite oxidation method.

Friedman and Lightfoot (7) reported their data on the effect of aeration and agitation on mass transfer with flat bladed impellers. They found that the air rate had little effect on power input and mass transfer in a fully baffled tank except at very low impeller speed or for very small impellers. The mass transfer coefficient was found to be proportional to N^3D^5 where N and D were respectively the speed and diameter of the impeller.

The effects of impeller geometry on oxygen transfer and power consumed were also investigated by Hamer and Blakebrough (8). For turbine impellers they experimentally correlated the power consumed with a factor referred to as power factor or impeller factor. The power or impeller factor was expressed in terms of an impeller dimension. The advantage of Co^{+2} over Cu^{+2} as a catalyst in sulfite oxidation reaction, was experimentally verified by Pirt et al. (9). They compared the rates of oxygen absorption by sodium sulfite solution using the two different catalysts. The results showed that the oxygen absorption rates were 2 to 5 times greater when Co^{+2} was used instead of Cu^{+2}.

The effects of temperature, oxygen partial pressure, salt concentration, catalyst concentration, and pH value on the oxidation rate of sulfite solutions were investigated by Fan and Wang (10). Optimal values of these parameters were determined experimentally. The information so obtained will be useful for designing fermenters with high oxygen transfer rates.

b) DYNAMIC TECHNIQUES

Bandyopadhyay and Humphrey (11) developed a rapid technique to measure the volumetric oxygen transfer coefficient, K_La in fermentation systems.

The method consists of following the dissolved oxygen concentration of the fermenting broth during a brief interruption of aeration. Only a fast response, sterilizable dissolved oxygen probe is necessary to obtain the data needed. The oxygen concentration trace thus obtained can be analyzed to determine the values of $K_L a$.

There are some difficulties with this method such as a slow release of bubbles in highly viscous fermentation broths and effects of surface aeration at high speeds. One limitation of this technique is the fact that oxygen starvation produces a temporary damage in the enzyme system of the microorganisms. The recovery by yeast from starvation effects is exponential with time.

Hsu, Erickson and Fan (12) employed a modified dynamic method to determine $K_L a$ in tower systems, because termination of gas flow to the system reduces the mixing and creates regions in the tower with different cell concentrations, oxygen uptake rates and dissolved oxygen concentration. The modification of the dynamic method consisted of increasing (step up) or decreasing (step down) the air flow rate after which the transient dissolved oxygen concentration was measured. The experiments were carried out at several different air flow rates using a mixed culture in cocurrent tower fermenter with Koch motionless mixers.

Dunn and Einsele (13) developed some mathematical models useful in the dynamic method for $K_L a$ measurements. The dynamics of the gas phase and the oxygen probe response time were studied. Computer simulation was used to show the effect of neglecting the gas phase dynamics. Correction factors are presented for a range of dimensionless parameters. In general, they showed that the errors resulting from neglecting the gas phase dynamics

and the oxygen probe response time can be large at high oxygen transfer rates to the gas dispersed in the liquid. An exact description of the gas phase is important for proper evaluation of $K_L a$ using the dynamic method, when $K_L a$ is large.

The dynamic method is accurate for small values of $K_L a$. Probes with rapid response times should be used. When the values of $K_L a$ become large, the method becomes unreliable unless the probe response and the gas phase dynamics are considered.

c) MEASURING RESPIRATION RATE OF MICROORGANISMS

Tsao (14) developed a method of oxygen transfer measurement using glucose oxidase. Pseudomonas ovalis was utilized in the experiment. This microorganism stoichiometrically converts glucose and oxygen to gluconic acid and the rate of gluconic acid formation was used as a measure of the rate of oxygen transfer. During aeration, the glucose concentration was measured by titration. From the rate of change of glucose concentration and from a plot of dissolved oxygen concentration in the aqueous solution recorded simultaneously, the rate of oxygen transfer from air into aqueous systems can be determined.

d) SEPARATE MEASUREMENT OF K_L AND a

It is apparent from a search of the literature that no method has been published which has been used to determine K_L and a separately in aerobic fermentations. Calderbank, (15) by using agitated vessels, could report mass transfer coefficients for gas-liquid systems.

Calderbank, (15) measured the specific interfacial area using a light transmission method on sieve plates for air, carbon dioxide and Freon

bubbling through water, aliphatic alcohol and glycol and combined the results with $K_L a$ values which were determined in the same system in order to evaluate K_L from the eqn. $K_L = K_L a/a$.

Calderbank and Moo-Young (16) reported a light transmission method for measuring interfacial areas in not too optically dense dispersions and also a light reflection technique for dense dispersions of optically transparent phases. They showed that the value of the liquid phase diffusion coefficient was the major factor which influenced the value of the mass transfer coefficient.

Robinson and Wilke (17) developed a new measuring technique for simultaneously evaluating K_L and a of slightly soluble gas dispersions in stirred tanks containing an aqueous solution of inorganic electrolites. The new technique involves unsteady state desorption of oxygen from an aqueous electrolyte solution containing a low concentration of hydroxyl ions accompanied by concurrent, pseudo steady state absorption with chemical reaction of CO_2 from the sparged gas. The oxygen desorption rate was measured by a dissolved oxygen probe, the response of which is used to evaluate $K_L a$. The rate of absorption with CO_2 is used to evaluate a, the corresponding value of K_L was calculated by combining the two results.

e) GAS PHASE MASS BALANCE METHOD

Siegell and Gaden (18), in presenting an approach for controlling dissolved oxygen levels, described the mass balance method of measuring oxygen transfer rates. Volumetric oxygen uptake rates may be obtained at any time by using the gas law and the difference values between inlet and

outlet gas streams reported by the analyzers. The volumetric rate of oxygen supply, r_s, in moles/(volume)(time) is:

$$r_s \cong \frac{FP}{RTV} (y_{in} - y_{out}) \tag{42}$$

where, F is the gas flow rate (Volume/Time) measured at pressure P and temperature T; R is the gas constant in appropriate units; and y is the oxygen mole fraction.

A simple material balance across the fermenter shows that oxygen supply rate equals oxygen uptake rate plus or minus the change in dissolved oxygen level. Since the dissolved oxygen level is held constant, the oxygen uptake rate must equal the measured rate of supply.

Using the familiar rate equation for absorption of oxygen into solution, absorption coefficients may be calculated directly from the information reported by the system:

$$r_s = K_L a \ (P_G - P_L) \tag{43}$$

where, $K_L a$ is the absorption coefficient; P_G is the oxygen partial pressure in inlet gas; and P_L is the oxygen tension in liquid. Knowing the supply rate and the inlet gas composition (P_G) from the gas-stream monitors and the liquid phase oxygen tension (P_L) from the electrode, $K_L a$ is readily determined.

In order to compare the oxygen balance method to the sulfite method, oxygen uptake measurements were made using the system before described simultaneously with the sulfite sampling technique. Volumetric uptake rates rather than calculated absorption coefficients were compared since the former involves few assumptions.

The oxygen balance method indicates higher uptake rates than are obtained from sulfite determinations on samples.

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NOMENCLATURE

a = gas-liquid interfacial area per unit liquid volume

D = impeller diameter

F = gas flow rate

K_L = liquid-phase mass transfer coefficient

K_La = overall volumetric mass transfer coefficient based on liquid phase

N = impeller speed

P = pressure

P_L = liquid phase oxygen tension

P_G = oxygen partial pressure

P_V = power per unit volume

R = gas constant

r_s = volumetric rate of oxygen supply

T = absolute temperature

V = fermenter volume

V_S = superficial velocity

y = oxygen mole fraction

CHAPTER 5

DETERMINATION OF OXYGEN TRANSFER COEFFICIENTS IN HYDROCARBON FERMENTATIONS BY USING A MATERIAL BALANCE METHOD AND A COMPUTER PROGRAM

Introduction

Oxygen transfer is one of the major engineering problems in hydrocarbon fermentations because of its low solubility in aqueous broths and its significantly greater stoichiometric requirement compared to carbohydrate fermentations. The rate of oxygen supply is frequently the rate limiting factor of the overall fermentation process.

The dynamic method for oxygen transfer rate determination does not give accurate results at high oxygen transfer rates (1,2). Mass balance methods are frequently used at these high transfer rates under fermentation conditions. In this work, the measured values of cell concentration, hydrocarbon substrate concentration, and ammonium hydroxide addition are used to predict the oxygen transfer rate. The oxygen transfer coefficient is estimated using the predicted transfer rate and measured values of dissolved oxygen concentration.

This work describes the oxygen transfer coefficient determination in a hydrocarbon fermentation with a yeast, Candida lipolytica, grown both in batch culture and continuously on n-hexadecane as the main source of carbon. In the following calculations these assumptions are made:

a) The microbial composition is 47% carbon, C; 6.5% hydrogen, H; 7.5% nitrogen, N; 31% oxygen, O and 8% ash.

b) Uniform oxygen concentration throughout the fermenter

c) Cell composition remains invariable

d) Substrate carbon is distributed only between bio-mass carbon and CO_2

e) The mass balance equation for bio-mass production is:

$$C_{16}H_{34} + NH_3 + O_2 \rightarrow \text{Biomass} + CO_2 + H_2O$$

Material and Methods

Equipment

The airlift fermenter utilized in this study consisted of a column and an internal draft tube with an air sparger at the base. The outside column had a 6 inch inside diameter and was made of pyrex glass. The draft tube was a plexiglass column of 4 3/8 inches inside diameter. Proper support of the draft tube was achieved by flanging the inside of the draft tube to a plate at the bottom of the tower. The column dimensions are shown in Fig. 1.

Temperature was controlled at 30°C by running cold and hot water through a rubber hose that surrounded the outside column. The temperature sensor was placed in the annular region next to the draft tube outside wall. Temperature readings were continuously recorded. The pH was maintained automatically at 5.5 by addition of 5% NH_4OH using the New Brunswick model pH-122 control system. The pH electrode was maintained inside the draft tube at the top of the fermenter. The pH was constantly recorded.

Dissolved oxygen was continuously measured and recorded using the New Brunswick Scientific Model DO-81. The oxygen probe was maintained in the annular region at the top.

Microorganisms

Candida lipolytica ATCC 8662 was cultured using n-hexadecane as the sole carbon source.

Medium Composition.-

The main culture medium was the simplified medium of Aiba et al. (3) except that Biocert was used instead of yeast extract. The medium composition was:

Constituents	Concentration, g/ℓ
$(NH_4)_2SO_4$	5
Na_2HPO_4	1.5
KH_2PO_4	3.5
$MgSO_4$	0.25
Biocert	0.1
Hexadecane	100 mℓ/ℓ

The medium used for the preculture and stock had the following composition: 40 g. of glucose, 1 g of Biocert and 100 mℓ of distilled water. For the stock culture, 15 g /ℓ agar was added.

Analytical Methods

Biomass concentration was estimated using optical density measurements. A sample of about 40 mℓ was taken from the column and homogenized manually. From this, a 5 mℓ sample was removed and combined with 15 mℓ of mixed solvent containing butanol, ethanol and chloroform (10:10:1 v/v). After mechanical shaking, the mixture was centrifiged at 3500 rpm for 15 minutes. The cells were washed with distilled water, centrifuged and resuspended in distilled water. The optical density was determined at 610 mμ. Cell dry weight measurements were also made periodically to develop the calibration curve and as a check on the accuracy of the calibration curve.

Hexadecane concentration was determined using gas chromatography after extraction with a mixed solvent. Samples of 10 to 15 g were

extracted in a separatory funnel with a 30 mℓ. hexane-chloroform mixture (1:1 v/v) containing an appropriate known concentration of tetradecane. The operating condition of the gas chromatograph was as follows: the column was 5 feet x 1/8 inch stainless steel column packed with 10% Carbonwax 20 M on Chromosorb W; nitrogen was used as the carrier gas at the flow rate of 25 mℓ/min.; the temperature was 140°C (isothermal operation); the detector was FID.

The respiration rate of the culture was calculated using the material balance method. The composition of the cells and the yield were considered to be constant. Step changes in air flow rate were made and measurements in steady state dissolved oxygen concentration were used to determine the effect of air flow rate on the oxygen transfer coefficient.

Inoculation

Yeast cells from a slant culture which were incubated at 30°C for 24 hr. were inoculated into 250 mℓ shaking flasks containing 50 mℓ of medium. After 24 hrs. cultivation, these flasks were used to inoculate 2000 mℓ shaking flasks containing 100 mℓ of medium. Two of these flasks were used to inoculate the tower fermentor.

Material Balance

The elemental balance equation of microbial growth on n-hexadecane is as follows:

$$C_{16}H_{34} + NH_3 + O_2 \rightarrow \text{Biomass} + CO_2 + H_2O \qquad (42)$$

Assuming that the microbial composition is:

C - 47%

H - 6.5%

N - 7.5%

O - 31%

Ash - 8%

The following variables are to be used:

Z = mℓ of NH_4OH - 5% dilution - consumed per ℓ -hr.

X = amount of cells produced per ℓ -hour

Y = yield, g cell produced/g oil utilized

CO_2 Balance: The carbon dioxide produced is found using a carbon balance. The substrate consumed is given by $\frac{X}{Y}$. For the carbon balance,

$$\frac{g\ C}{\ell\ -hr.} = \frac{X}{Y} \cdot \frac{1929.\ C}{226\ g\ C_{16}H_{34}} = 0.849\ \frac{X}{Y}$$

The carbon converted to biomass is given by

$$\frac{g\ C}{\ell\ -hr.} = 0.47\ X$$

The CO_2 produced is

$$\frac{g\ CO_2}{\ell\ -hr.} = A =]0.849\ \frac{X}{Y} - 0.47\ X]\ \frac{g\ C}{hr.} \cdot \frac{44\ g\ CO_2}{12\ g\ C}$$

$$A = 3.115\ \frac{X}{Y} - 1.72\ X \qquad (43)$$

H_2O Balance: The water production rate is found using a hydrogen balance. The hydrogen in the NH_3 entering as NH_4OH is

$$\frac{g\ H}{\ell\ -hr.} = Z \times 0.05 \times \frac{3}{35} = 0.0043\ Z$$

The hydrogen balance is

$$\frac{g\ H}{\ell\ -hr.} = (\frac{X}{Y})(\frac{34}{236}) + 0.0043\ Z - 0.065\ X]\frac{18}{2}$$

$$B = 1.35(\frac{X}{Y}) + 0.038\ Z - 0.585\ X \qquad (44)$$

Oxygen Balance: The oxygen balance gives

$$\frac{g\ O_2}{\ell\ -hr.} = Q_{O_2} = 0.31\ X + \frac{32}{44}\ A + \frac{16}{18}\ B$$

$$Q_{O_2} = 0.727\ A + 0.88\ B + 0.31\ X \qquad (45)$$

The oxygen transfer coefficient is

$$K_L a = \frac{Q_{O_2}}{C^* - C} \tag{46}$$

however, if C* and C are in mg/ℓ and Q_{O_2} is in g /ℓ-hr., then

$$K_L a = \frac{1{,}000\ Q_{O_2}}{C^* - C} \tag{47}$$

Using the above equations, values of $K_L a$ were determined for each of the air flow rates for each experiment. These results are shown in Tables 1, 2, 3, and 4.

Results were also obtained assuming the nitrogen input to be given by the nitrogen content of the cells rather than by the measured flow of NH_4OH. The following equations were used:

$$\frac{gO_2}{g\ cells} = \left[8\left(\frac{4C + H - 2O}{Y_{\frac{x}{s}} \cdot M}\right) - 1.288\right] = Y_{\frac{o}{x}} \tag{34}$$

$$Q_{O_2} = Y_{\frac{o}{x}} \cdot X$$

$$K_L a = \frac{1{,}000\ Q_{O_2}}{C^* - C} \tag{47}$$

These results are presented in Tables 5, 6, 7, and 8.

TABLE 1

Estimated oxygen transfer coefficients for batch cultivation of Candida lipolytica in an airlift fermenter from measured values of cell production, hexadecane utilization, and ammonium hydroxide solution.

Air Flow Rate $ft.^3/hr.$	Dissolved oxygen concentration, %	C ppm	Q_{O_2} $\frac{g}{(\ell)(hr.)}$	$K_L a$ $hr.^{-1}$
165	28	2.128	4.01	734
197.5	35	2.66	4.01	813
212.5	38	2.88	4.01	852
225	40	3.04	4.01	890
257.5	44	3.344	4.01	943
287.5	51	3.876	4.01	1078
320	55	4.18	4.01	1174

$X = 1.33$ g /ℓ hr.

$Y = 0.83$ g cell/g oil utilized

$Z = 12$ mℓ NH_4 OH/ℓ. hr.

Dilution rate = $0.15\ hr.^{-1}$

TABLE 2

Estimated oxygen transfer coefficients for continuous cultivation of Candida lipolytica in an airlift fermenter from measured values of cell production, hexadecane utilization, and ammonium hydroxide addition.

Air Flow Rate $ft.^3/hr.$	Dissolved oxygen concentration, %	C ppm	Q_{O_2} $\frac{g}{(\ell)(hr.)}$	K_La $hr.^{-1}$
150	65	4.94	3.02	1137
180	66	5.01	3.02	1168
212.5	68	5.168	3.02	1240
240	72	5.472	3.02	1420
275	75	5.70	3.02	1592
300	78	5.928	3.02	1801

Dilution rate = 0.25 $hr.^{-1}$

Cell concentration = 2.96 g /ℓ

Oil concentration = 9.18%

Y = 0.672 g cell/g oil utilized

X = 0.74 g /ℓ hr.

Z = 8.6 m . NH_4 OH/ℓ hr.

TABLE 3

Estimated oxygen transfer coefficients for continuous cultivation of Candida lipolytica in an airlift fermenter from measured values of cell production, hexadecane utilization and ammonium hydroxide addition.

Air Flow Rate $ft.^3/hr.$	Dissolved oxygen concentration, % sat.	C ppm	Q_{O_2} $\frac{g}{(\ell)(hr.)}$	K_La $hr.^{-1}$
150	57	4.33	5.01	1534
180	57	4.33	5.01	1534
212.5	60	4.56	5.01	1650
240	63	4.78	5.01	1779
275	65	4.94	5.01	1886
300	70	5.32	5.01	2200

Dilution rate = 0.20 $hr.^{-1}$

Cell concentration = 6.88 g./ℓ

Oil concentration = 8.67%

Y = 0.724 g cell/ g oil utilized

Z = 13.6 mℓ NH_4 OH/ℓ. hr.

X = 1.38 g /ℓ hr.

TABLE 4

Estimated oxygen transfer coefficients for continuous cultivation of Candida lipolytica in an airlift fermenter from measured values of cell production, hexadecane utilization, and ammonium hydroxide addition.

Air Flow Rate $ft.^3/hr.$	Dissolved oxygen concentration, %	C ppm	Q_{O_2} $\frac{g}{(\ell)(hr.)}$	$K_L a$ $hr.^{-1}$
150	50	3.8	4.43	1166
180	56	4.25	4.43	1323
212.5	60	4.56	4.43	1458
240	64	4.86	4.43	1618
275	68	5.16	4.43	1816
300	72	5.47	4.43	2081

Dilution rate, $hr.^{-1}$ = 0.15

Cell concentration = 0.12 g /ℓ

Oil concentration = 8.32%

Yield = 0.81 g cell/g oil utilized

X = 1.38 g /ℓ. hr.

Z = 13.9 mℓ NH_4 OH/ℓ hr.

TABLE 5

Estimated oxygen transfer coefficients for batch cultivation of Candida lipolytica in an airlift fermenter from measured values of cell production and hexadecane utilization.

Air Flow Rate $ft.^3/hr.$	Dissolved oxygen concentration, %	C ppm	Q_{O_2} $\frac{g}{(\ell)(hr.)}$	K_La $hr.^{-1}$
165	28	2.128	3.73	680
197.5	35	2.66	3.73	755
212.5	38	2.88	3.73	790
225	40	3.04	3.73	817
257.5	44	3.344	3.73	876
287.5	51	3.876	3.73	1000
320	55	4.18	3.73	1090

X = 1.33 g /ℓ hr.

Y = 0.83 g cell/g oil utilized

Z = 12 mℓ NH_4 OH/ℓ hr.

TABLE 6

Estimated oxygen transfer coefficients for continuous cultivation of Candida lipolytica in an airlift fermenter from measured values of cell production and hexadecane utilization.

Air Flow Rate $ft.^3/hr.$	Dissolved oxygen concentration, %	C ppm	Q_{O_2} $\frac{g}{(\ell)(hr.)}$	K_La $hr.^{-1}$
150	65	4.94	2.84	1067
180	66	5.01	2.84	1096
212.5	68	5.168	2.84	1167
240	72	5.472	2.84	1334
275	75	5.70	2.84	1494
300	78	5.928	2.84	1698

X = 0.74 g /ℓ hr.

Y = 0.672 g cell/g oil utilized

Z = 8.6 mℓ NH_4 OH/ℓ hr.

Dilution rate = 0.25 $hr.^{-1}$

Oil concentration = 9.18%

Cell concentration = 2.96 g /ℓ

TABLE 7

Estimated oxygen transfer coefficients for continuous cultivation of Candida lipolytica in an airlift fermenter from measured values of cell production and hexadecane utilization.

Air Flow Rate, $ft^3/hr.$	Dissolved oxygen concentration, % sat.	C ppm	Q_{O_2} $\frac{g}{(\ell)\ hr.)}$	$K_L a$ $hr.^{-1}$
150	57	4.33	4.83	1477
180	57	4.33	4.83	1477
212.5	60	4.56	4.83	1588
240	63	4.78	4.83	1712
275	65	4.94	4.83	1815
300	70	5.32	4.83	2118

X = 1.38 g /ℓ hr.

Y = 0.724 g cell/g oil utilized

Z = 13.6 mℓ NH_4 OH/ℓ. hr.

Dilution rate = 0.20 $hr.^{-1}$

Oil concentration = 8.67%

Cell concentration = 6.88 g./ℓ.

TABLE 8

Estimated oxygen transfer coefficients for continuous cultivation of Candida lipolytica in an airlift fermenter from measured values of cell production and hexadecane utilization.

Air Flow Rate, $ft.^3/hr.$	Dissolved oxygen concentration, % sat.	C ppm	Q_{O_2} $\frac{g}{(\ell)(hr.)}$	K_La $hr.^{-1}$
150	50	3.8	4.13	1086
180	56	4.25	4.13	1232
212.5	60	4.56	4.13	1358
240	64	4.86	4.13	1507
275	68	5.16	4.13	1692
300	72	5.47	4.13	1938

$X = 1.38$ g /ℓ hr.

$Y = 0.81$ g. cell/g oil utilized

$Z = 13.9$ mℓ NH_4OH/ℓ hr.

Dilution rate = $0.15\ hr.^{-1}$

Oil concentration = 8.32%

Cell concentration = 9.12 g /ℓ

RESULTS

The overall volumetric mass transfer coefficient $K_L a$ has been determined by using the mass balance method for batch and continuous cultivation. Biomass concentration and oil concentration were measured and from these variables the productivity and the yield were calculated. Dilution rates of 0.25, 0.20 and 0.15 $(hrs)^{-1}$ were used in the continuous culture studies. Figure 2 shows the effect of volumetric gas flow rate on the mass transfer coefficient. The values shown are from Tables 5, 6, 7 and 8. The $K_L a$ values determined by this method are about twice as high as those found by Orazem (6) using the sulfite method in airlift fermenters. Larger values of $K_L a$ under fermentation conditions have also been reported by Hirose et al. (7) and Phillips and Johnson (8).

The results in Tables 1, 2, 3, and 4 can be compared with those in Tables 5, 6, 7 and 8. The predicted values of $K_L a$ in Tables 5, 6, 7 and 8 are smaller than those predicted in Tables 1, 2, 3 and 4 because the value used for nitrogen input was smaller. The differences in the estimated values of $K_L a$ are all less than 10%.

Errors in this method may occur because of inaccurate measurements in dissolved oxygen concentration, cell productivity and carbon substrate concentration. Sensitivity analysis may be used to investigate the effect of measurement errors on the results. Using the results in Table 8 for an air flow rate of 240 $ft^3/hr.$, the effect of 5% erros in carbon substrate yield, Y, dissolved oxygen concentration, C, and productivity, X were investigated. Increasing the yield by 5% to 0.85 g. cells/g oil reduced $K_L a$ by 7% to 1403 hr^{-1}. Increasing the dissolved oxygen concentration by 5% to 5.10 ppm increased the oxygen transfer coefficient to

1659 hr^{-1} which is a 10% increase. Increasing the cell productivity by 5% to 1.45 g./ℓ-hr. resulted in an increase in K_La of 5.1% to 1585 hr^{-1}. These results show that errors in carbon substrate yield, dissolved oxygen concentration, and cell productivity frequently result in percentage errors in K_La which are as large or larger than those of the input variables.

The effect of air flow rate on the oxygen transfer coefficient is shown in Figure 2. These results are based on the assumption that the oxygen transfer rate remains constant as air flow rate changes. A linear relationship between volumetric gas flow rate and K_La is shown in Figure 2.

The differences in results from run to run at the same gas flow rate may be partly due to actual differences in oxygen transfer coefficient values due to changes in surface tension, spreading coefficient, and cell concentration; however, these differences may also be partly due to measurement errors. In the experimental work liquid-liquid interfacial tensions were measured (9); however, surface tensions were not measured.

CONCLUSIONS

A mass balance method for estimating oxygen transfer rates from measured values of dissolved oxygen concentration, cell productivity, and carbon substrate yield has been introduced. The method predicts values for the oxygen transfer coefficient which are somewhat larger than those found for sulfite oxidation in similar airlift systems. The results show that large oxygen transfer rates can be achieved in airlift systems. Since Hatch (5) has shown that the oxygen transfer efficiency in airlift fermenter can be as much as 2000% higher than that in agitated vessels, further attention should be given to the use of airlift fermenters in hydrocarbon fermentations. A linear relationship between volumetric gas flow rate and $K_L a$ was found.

Errors in this method may occur because of inaccurate measurements in dissolved oxygen concentration, cell productivity and carbon substrate concentration.

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NOMENCLATURE

A = CO_2 produced

B = water obtained

C = concentration of oxygen at any particular point in the fermenter

C* = equilibrium oxygen concentration in the liquid

$K_L a$ = volumetric oxygen transfer coefficient

X = g cells/ℓ -hr.

Y = yield, $\frac{\text{g cell}}{\text{g. substrate utilized}}$

Z = ml. of NH_4OH consumed/ℓ.hr.

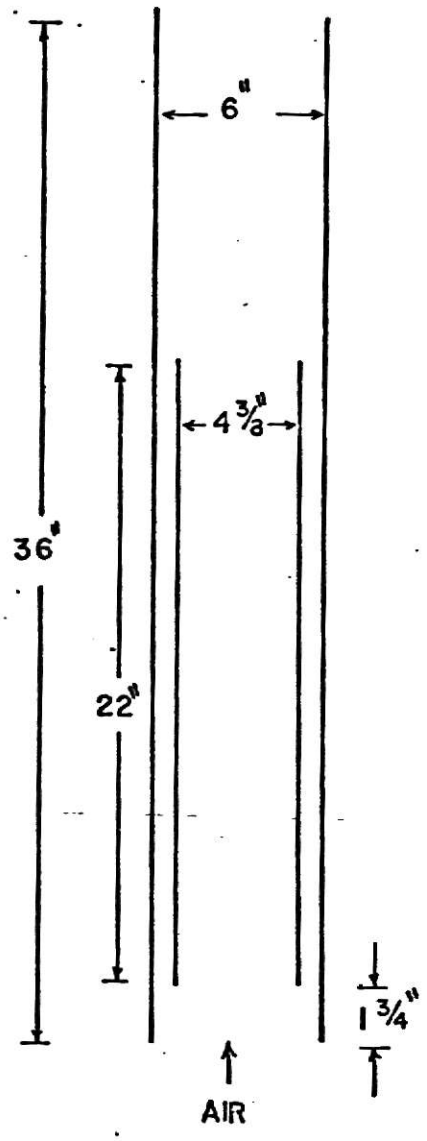

Fig. 1. Dimensions of the airlift system.

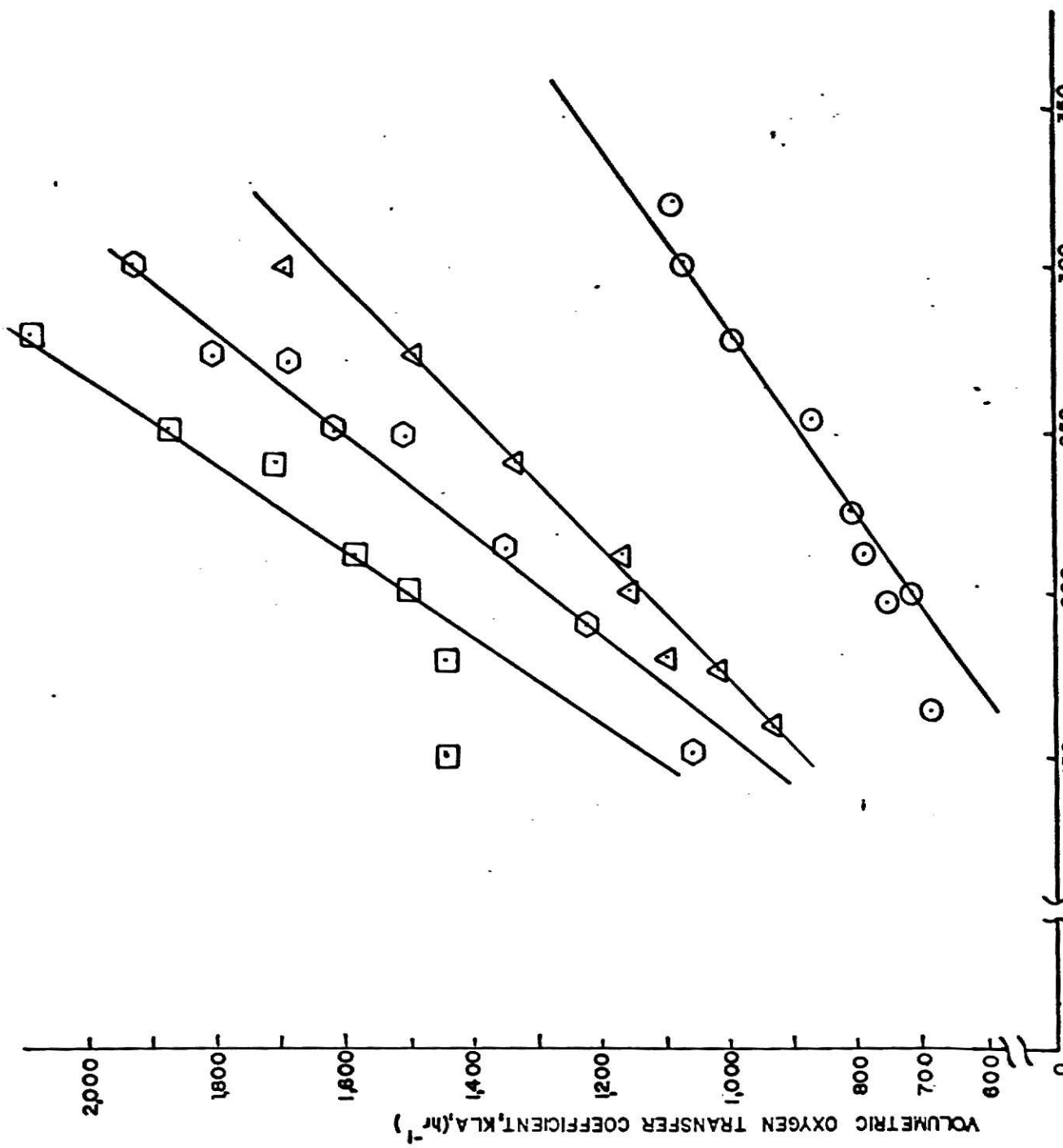

Figure 2. Effect of Air Flow Rate on K_{LA} for Batch and Continuous Cultivation of Candida lipolytica. Data for curves ⊙, △, □, and ⬡ from Tables 5, 6, 7, and 8, respectively.

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MATERIAL AND ENERGY BALANCES AND TRANSFER RATES IN AEROBIC FERMENTATIONS

by

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AN ABSTRACT OF A MASTER'S REPORT

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A literature survey on oxygen material balances and requirements and on heat generation and oxygen consumption relationships in aerobic fermentations was carried out. Methods to determine the oxygen transfer coefficient were also reviewed.

A mass balance method has been developed to measure the volumetric oxygen transfer coefficient, $K_L a$, in fermentation systems. The method consists of establishing a mass balance equation relating biomass production, carbon substrate consumption, and oxygen requirement. An airlift lift fermenter was employed to obtain both batch and continuous operation data to estimate the values of $K_L a$ based on the method.