
# EDGEWOOD

## CHEMICAL BIOLOGICAL CENTER

U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND

ECBC-TR-846

# OXYGEN MASS TRANSFER COEFFICIENT ($k_La'$) CONSIDERATION FOR SCALE-UP FERMENTATION SYSTEMS AT THE BIOTECHNOLOGY LABORATORY U.S. ARMY EDGEWOOD CHEMICAL BIOLOGICAL CENTER

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Volumetric oxygen mass transfer coefficients ($k_La'$) were determined at different agitation speeds, airflow rates, and overhead pressures for a Micros 30, IF 150 and IF 1500 fermentation systems at U.S. Army Edgewood Chemical Biological Center's Biotechnology laboratory. We found agitation speed as the most significant factor resulting in the highest magnitude of ($k_La'$) values. Three sets of ($k_La'$) values were further correlated in a power regression form at standard airflow rate of 1.0 volume of air per volume of fermentor per minute (vvm) for the Micros 30 and IF 150 (but 0.5 vvm for IF 1500) with a 1.0 psi overhead pressure for all. The similar ($k_La'$) value scale-up strategy was tested between the Micros 30 and IF 1500 systems using the gram-positive bacterium *Bacillus thuringiensis kurstaki* (Btk, ATCC 33679) at the ($k_La'$) value of 0.027 $sec^{-1}$ for Micros 30 (300 rpm) and 0.033 $sec^{-1}$ for IF 1500 (175 rpm). Specific accumulated oxygen uptake values were 600 and 509 mg Dissolved Oxygen/Optical Density, and the Btk spores yields were $4.7 \times 10^8$ and $4.4 \times 10^8$ Colony-Forming-Unit/mL, respectively, for the Micros 30 and IF 1500 systems. This study successfully demonstrated the scale-up fermentation strategy based on the ($k_La'$) value.

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CONTENTS

FIGURES

TABLES

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# OXYGEN MASS TRANSFER COEFFICIENT ($k_La'$) CONSIDERATION FOR SCALE-UP FERMENTATION SYSTEMS AT THE BIOTECHNOLOGY LABORATORY U.S. ARMY EDGEWOOD CHEMICAL BIOLOGICAL CENTER

## 1. INTRODUCTION

Natural and/or recombinant microbial fermentations are often aimed at achieving high cell densities, believing that higher cell densities will lead to higher product concentration (Yee and Blanch, 1992, and Lee, 1996). Environmental factors affecting the rate of cell growth under aerobic conditions can be optimized and controlled (e.g., pH, temperature, nutrient levels, etc.) during a batch fermentation run. However, dissolved oxygen (DO), necessary for sustaining high-density cell cultures is dependent on the mechanically limited aeration capacity of a particular fermentation system and often susceptible to rate-limiting conditions.

Oxygen limitation occurs when the utilization rate of DO by microbial cultures exceeds the dissolution rate of sparingly soluble oxygen gas in the aqueous phase, which is indicated by the DO level in the culture medium falling to zero. Many counter measures (e.g., higher airflow rate, mixing speed, and/or use of pure oxygen) are implemented to increase the dissolution rate of gaseous oxygen. However, as the cell density increases, a higher rate of growth-associated metabolic oxygen demand will inevitably deplete the DO that a particular aeration system is capable of delivering gaseous oxygen into the aqueous phase. At the zero level of DO, oxygen is being up-taken by respiring cells as soon as it is dissolved, resulting in a maximum oxygen transfer rate, but limited to support the intrinsic maximum specific growth of particular cell cultures. Hence, determining the aeration capacities of various sized systems in water and the metabolic oxygen demand requirements by microbial cultures can provide invaluable information for optimizing cell densities and product yields.

The rate of gas-liquid mass transfer process for sparingly soluble gases is characterized by its ($k_La'$) value, where $k_L$ and a' are liquid mass transfer coefficients and gas-liquid interfacial area per unit volume (A/V), respectively. Due to difficulties in measuring $k_L$ and a' separately, the combined term of ($k_La'$) is often measured and reported in literature (Bailey and Ollis, 1986). Incidentally, the use of $k_L$ instead of the overall mass transfer coefficient, $K_L$, implies that the resistance to overall mass transfer cross the gas-liquid interface lies on the liquid-film side.

Located within the Biotechnology Laboratory, U.S. Army Edgewood Chemical Biological Center (ECBC), the fermentation suite consists of two Micros 30 L, one IF 150 L, and one IF 1500 L scale fermentation units and their support tank systems (New Brunswick Scientific, Edison, NJ). These fermentation systems are used for research and development in bioprocess optimization (e.g., Micros 30 L system) as well as 1500 L scale-up production of various microbial cultures and/or associate products (Lukens *et al.*, 2000 and Kim *et al.*, 2003). The objective of this study is to

determine and establish oxygen mass transfer coefficients for each fermentation unit at different combinations of impeller mixing speed, airflow rate, and overhead pressure. We bring together and use these ($k_La'$) values in analyzing cell yield data obtained from fermentation run data to discuss scale-up implications.

## 2. MATERIALS AND METHOD

### 2.1 Fermentation System Description

The fermentation units' technical data are summarized below in Table 1. Each fermentation unit is fitted with four baffles and three Rushton impellers containing six blades each; impellers are approximately equally spaced below the liquid level at working volumes of 20, 100, and 1000 L, respectively, for the Micros 30, IF 1503 and IF 1500 units.

Table 1. Fermentation Units' Design/Technical Data.

| Model No. | Micros 30 | IF 150 | IF 1500 |
|---|---|---|---|
| Total Volume (L) | 30 | 150 | 1500 |
| Working Volume, max (L) | 22.5 | 115 | 1200 |
| Working Volume, min (L) | 5 | 40 | 400 |
| Working Volume for this study (L) | 20 | 100 | 1000 |
| Vessel Diameter (cm) | 27 | 45.7 | 101.6 |
| Vessel Height (cm) | 54.5 | 102 | 213.5 |
| Speed (rpm) | 50-1000 | 45-450 | 30-300 |
| Number of Rushton Impellers | 3 | 3 | 3 |
| Number of Blades | 6 | 6 | 6 |
| Number of Baffles | 4 | 4 | 4 |
| Impeller Diameter (cm) | 12.065 | 15 | 38 |
| Motor Power (hp) | 1.5 | 3 | 15 |

### 2.2 Gas-Liquid Mass Transfer Equation

The dissolution rate of oxygen gas in water, which is driven by a concentration gradient between maximum saturation DO (i.e., at a given temperature, pressure, and salinity of water), and bulk DO concentration, is defined as follows:

$$\frac{dC}{dt} = (k_La')(C_e - C) \quad (1)$$

where

dC/dt = volumetric oxygen mass transfer rate, mg/L time
($k_La'$) = volumetric $O_2$ mass transfer coefficient, time$^{-1}$
$C_e$ = saturated DO concentration at equilibrium, mg/L

C = bulk dissolved $O_2$ concentration, mg/L by substituting a dimensionless DO concentration, $C^* = (C-C_o)/(C_e-C_o)$ and integrating with the initial $O_2$ concentration, $C_o$, the equation becomes as follows:

$$\ln(1 - C^*) = -(k_L a')t \tag{2}$$

Hence, by plotting $\ln(1-C^*)$ vs. t should yield the slope being equivalent to $-(k_La')$ if time-dependent data of *C vs. t* are available.

The *C vs. t* data are obtained initially by chemically stripping off $O_2$ from each fermentation unit and then sparging back with a fixed compressed airflow rate at specified temperature, agitation speed, and overhead pressure. The increases in DO over time constitute the *C vs. t* data necessary to compute the ($k_La'$) coefficients at those particular operating conditions. By repeating the oxygen stripping process and sparging at different operating conditions, the values of ($k_La'$) as a function of agitation speed, airflow rate, and overhead pressure can be established for each fermentation unit.

## 2.3 Metabolic Oxygen Utilization Rate Equation and Accumulated Oxygen Uptake

By a mass balance over DO at any point during microbial growth, the amount of oxygen being transferred into the aqueous phase is expected to be equal to the total microbial oxygen demand (consumption) as follows (Bailey and Ollis, 1986):

$$(k_L a')(C_e - C) = \frac{X\mu}{Y_{O2}} = X\mu_{max}\left[\frac{C}{K_{O2} + C}\right]/Y_{O2} \tag{3}$$

where

X = cell density, mg/L
$\mu$ = specific growth rate of cultures, $hr^{-1}$
$\mu_{max}$ = maximum specific growth rate of cultures, $hr^{-1}$
$K_{O2}$ = half saturation constant, mg/L
$Y_{o2}$ = cell yield based on oxygen consumed, mole of cell carbon per mole of oxygen consumed

Many factors can influence the total microbial oxygen demand ($X\mu/Y_{o2}$); $\mu$ and $Y_{o2}$ are normally constant for exponentially growing cultures; but, as X increases in the batch fermentation, the normal response is a DO decrease, given a constant ($k_La'$) capacity. Hence, the idea is to maintain the DO level above a critical DO level $C_{cr,O2}$ circa $\geq 3\ K_{o2}$ to prevent DO limitation until X is maximized (i.e., depletion of nutrients). Typical $C_{cr,O2}$ values for microbial cultures are in the range of 0.005 to 0.02 mmol (0.16 to 5.12 mg/L).

Equation 1 can also be rewritten in a time incremental form (instead of continuously differential form) as follows (Gaudy Jr. and Gaudy, 1988):

$$\frac{\Delta C}{\Delta t} = (k_L a')D_t \quad (4)$$

where $D_t$ represents oxygen tension, which is a driving force for oxygen to dissolve in water. Therefore, the amount of oxygen transferred into the fermentation medium (e.g., $\Delta C$) in a small increment of time $\Delta t$ becomes:

$$\Delta C = (k_L a')D_t \Delta t \quad (5)$$

In addition, an oxygen mass balance over the aqueous fermentation medium between opposing transfers (i.e., transfer in by dissolution and transfer out by uptake (i.e., microbial utilization) results in the following equation:

$$\Delta DO_{\text{in the medium}} = \Delta DO_{\text{reaeration}} - \Delta DO_{\text{uptake}} \quad (6)$$

By substituting eq. 5, the re-aeration term, in eq. 6, the changes in DO in the medium ($\Delta DO_{\text{in the medium}}$) becomes as follows:

$$\Delta DO_{\text{in the medium}} = (k_L a')D_t \Delta t - \Delta DO_{\text{uptake}} \quad (7)$$

By solving for $\Delta DO_{uptake}$ and summing increments, the accumulated oxygen uptake can be calculated as follows:

$$\text{Accumulated } O_2 \text{ uptake} = \sum \Delta O_2 \text{ uptake} = \sum ((k_L a')D_t \Delta t - \Delta DO_{\text{in the medium}} \quad (8)$$

Hence, by knowing a predetermined *($k_L a'$)* value and *DO profile vs. time* during a fermentation run, the accumulated amount of $O_2$ uptake, by growing microbial cultures, can be calculated according to eq. 8.

## 2.4 Volumetric Oxygen Transfer Coefficient Determination Procedures

A DO probe (InPro 6800 series, 25 mm dia, 80 mm insert length, Mettler-Ingold, Bedford, MA) was inserted into each fermentation unit prior to filling with deionized water to the working volume given above in Table 1.

The desired control set points (e.g., mixing speed, airflow rate, and overhead pressure) were entered using a touch-screen PanelView 2000 PLC controller, dedicated to each respective unit. A constant temperature of 30 °C was used and maintained throughout the studies.

Compressed air was sparged into the fermentation unit with the air overlay valve closed to obtain a full DO saturation inside the unit for at least 15 min. When the DO reading stabilized, the DO probe was calibrated to the100% level using an $O_2$ transmitter 4100 e (Mettler-Toledo, Bedford, MA).

Following DO probe calibration, the compressed air flow was placed in overlay mode with the air sparge valve closed. Cobalt chloride hexahydrate (Fisher Chemicals, Pittsburgh, PA) was added, at a concentration of 0.3 mg/working liter, and served as a catalyst for the following reaction:

$$SO_3^{-2} + \frac{1}{2}O_2 \xrightarrow{Co+2} SO_4^{-2} \qquad (9)$$

Chemical stripping of DO inside the fermentation unit was achieved by adding a 2:1 stoichiometrical excess of sodium sulfite (Fisher Chemicals) to a nominal 8 mg/L DO (i.e., approximately 100% saturation level at 30 °C). A 0.134 g/working liter of $Na_2SO_3$ was dissolved in 0.5 mL of $dH_20$ then added as a concentration solution (i.e., 2.68 g sodium bisulfite in 10 mL for 20 L and 134 g sodium bisulfite in 500 mL for 1000 L).

For the 20- and 100-L fermentors, a syringe was used to add 10- and 50-mL solutions, respectively, through the top port fitted with septa; for the 1000-L fermentor, 500 mL of the concentrated sodium sulfite solution was added aseptically using a 2-L transfer bottle. The transfer bottle cap was prefitted with one unfiltered line, which extended to the bottom of the bottle and two above-the-liquid lines, both externally fitted with 0.2 µ filters. A ½-in. quick-connect was attached to the line that extended into the bottle and then connected to the inoculation port on the fermentation unit; a compressed air line (10 to 15 psi) was then connected to one of two filtered overhead lines. The air was turned on, letting the air pass through the second filtered line. Immediately after the fermentor's inoculation port was opened, the second filtered line was blocked by bending, thus forcing the sodium bisulfite solution into the fermentation unit.

As soon as the concentrated sodium sulfite solution was added to each fermentation unit, the DO decreased rapidly; when DO level reached a minimum level and slowly started to rise due to surface mixing, the compressed airflow was immediately switched to sparge mode by opening the air sparge valve and closing the air overlay valve, simultaneously.

The rise of DO level inside each fermentation unit was monitored and transmitted by the $O_2$ transmitter 4100 e to each respective PanelView 2000 PLC controller, which in turn logged the DO level by a built-in Historian Software for later analyses.

## 2.5 20- and 1000-L Scale-up Fermentation with *Bacillus thuringiensis kurstaki* (ATCC 33679)

### 2.5.1 Organism and Growth Medium

The microorganism used in this study was *B. thuringiensis kurstaki* (Btk, ATCC 33679). Freeze-dried cultures from ATCC were expanded in 5 mL of Difco nutrient broth (8 g/L, Beckon Dickinson), streaked onto nutrient agar plate (8 g/L nutrient

broth+1.5% Difco-agar, Beckon Dickinson), then incubated at 30 °C. Stock vials were prepared following standard microbial protocol. A single colony from the overnight nutrient agar plate was inoculated into an 80-mL nutrient broth in a 250-mL flask, then incubated at 30 °C and 200 rpm (Innova 4300, New Brunswick Scientific). When the optical density (OD) of the 80-mL cultures reached approximately 0.8 at 600 nm, the cultures were poured into a100-mL flask containing 20 mL of pre-sterilized glycerol (20%, v/v), completely mixed, then 2 mL aliquoted into cryotube vials (Nunc), and stored in a -80 °C freezer (Model 8600 Series, ThermoElectron) until use. Growth medium consisted of the following. For the 500-mL batch, culture nutrient broth was prepared in a 2-L flask at the same concentration described above. For the 20- and 1000-L scale up, 2X nutrient broth (16 g/L) and NZ Amine A medium, consisting of the following composition were used (g/L): 10.0 Glucose, 5.0 Casein peptone–type S, 1.0 Yeast extract, 4.0 $K_2HPO_4$, 3.0 $KH_2PO_4$, 0.134 $CaCl_2 \cdot 2H_2O$, 0.02 $FeSO_4 \cdot 7H_2O$, 0.05 $MgSO_4 \cdot 7H_2O$, 0.023 $MnSO_4 \cdot H_2O$, 0.02 $ZnSO_4 \cdot 7H_2O$. All medium ingredients were autoclaved together with the exception of glucose and metals ($CaCl_2 \cdot 2H_2O$, $FeSO_4 \cdot 7H_2O$, $MgSO_4 \cdot 7H_2O$, $MnSO_4 \cdot H_2O$, $ZnSO_4 \cdot 7H_2O$). Glucose was filtered separately (0.8/0.2 µ) then added aseptically as a concentrated sterile solution. The two metal solutions ($CaCl_2 \cdot 2H_2O$ and others) were prepared separately, filtered, and added to prevent precipitation of $CaSO_4$.

### 2.5.2 Scale-up Cultivation

The 500-mL seed cultures were prepared by inoculating with 2 vials of frozen stock and incubating at 30 °C and 200 rpm until the OD reached approximately 0.4. Then, 500-mL seed cultures were aseptically transferred into the 20-L fermentor containing either 2X nutrient broth or NZ Amine A medium. The operating conditions for the 20-L fermentor were controlled at 300 rpm, 1 vvm airflow (i.e., air volume per liquid volume per minute), 30 °C, and pH 7.0 (with 3M $H_3PO4$ and 3 M NaOH).

For the 1000-L fermentor, 500 mL of nutrient broth, 20 L of NZ Amine A medium, and 1000 L of NZ Amine A medium were used in sequence for the 1000-L scale- up fermentation. The same procedures and operating conditions for the 20-L scale-up fermentation were used at the 1000-L reaction volume with one additional step. The 20-L cultures were further transferred into the 1000-L fermentor when the OD of the 20-L fermentor reached approximately 0.4. The operating conditions for the 1000-L fermentor were controlled at 175 rpm, 0.5 vvm airflow, 30 °C, and pH 7.0 (with 3 M $H_3PO4$ and 3 M NaOH).

### 2.5.3 Analytical Methods

Cell growth was monitored spectrometrically by absorbance measurement at 600 nm (Genesys 20, ThermoSpectronic). Percentage and number of vegetative cells, sporangia, and/or spores were counted using a Petroff Hausser counting chamber (Hausser Scientific, Horsham, PA); CFUs were numerated by serially diluting samples, plating 100 µL on nutrient agar plate, and incubating at room temperature for 48 hr minimum.

## 3. RESULTS AND DISCUSSION

Figure 1 illustrates DO profiles obtained from the 20-L fermentor unit at various agitation speeds (200 - 800 rpm), 20 standard liters per minute (slpm) constant airflow, and 1 psi overhead pressure.

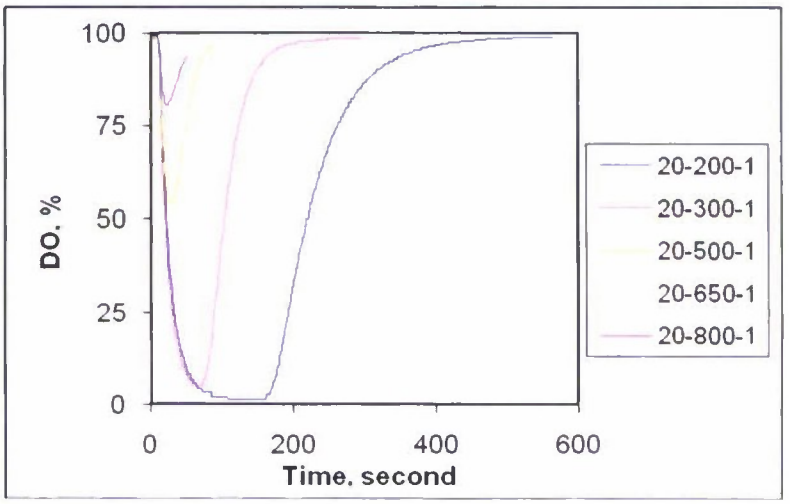


Figure 1. Time-dependent DO Profiles for the 20-L Fermentor System at Different Mixing Speeds (Airflow rate and overhead pressure were held constant at 20 slpm and 1 psi, respectively).

The effect of higher mixing speed on DO response curves was clear in that a higher residual DO (i.e., DO level immediately prior to compressed air sparging) resulted at a higher mixing speed with constant 20 slpm airflow and 1 psi overhead pressure. This difference could be attributed to the fact that higher speed (i.e., ≥500 rpm) imparts a higher degree of added turbulent mixing of air at the liquid surface. Hence, the overhead exhaust air inside of the fermentation unit could add extra demand for the 2:1 stochiometrical excess of sodium sulfite. As a result, higher residual DO is expected with a higher mixing speed. One obvious shortcoming in measuring C vs. t at higher mixing speed is that it results in a limited DO range for oxygenation, in which the data may not be suitable for subsequent ($k_La'$) determination. Nevertheless, the dimensionless DO concentration from each experiment was plotted against time for each mixing speed, using DO data during oxygenation period (Figure 2). Also, data sets of increased airflow rate and overhead pressure at a constant mixing speed of 200 rpm are shown in Figure 2 (i.e., 20-200-10 and 30-200-1). The ($k_La'$) values in Table 2 were obtained by a linear regression analysis (Microsoft Excel) on each line in

Figure 2 then plotted for those data pertaining to the constant values of 20 slpm and 1 psi (Figure 3).

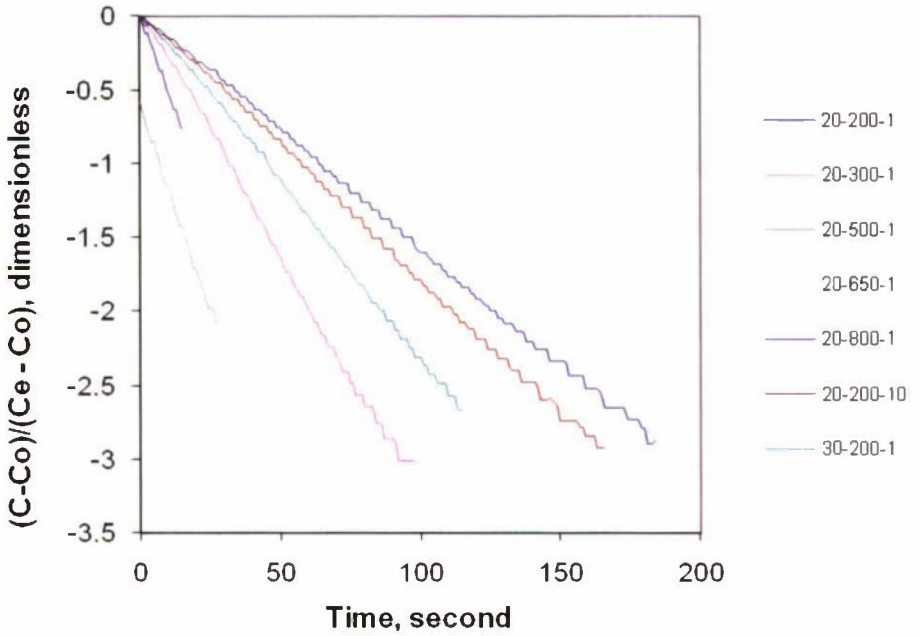


Figure 2. Dimensionless DO Concentration vs. Time for the 20-L Fermentor System.

Table 2. Summary of ($k_La'$) Values Obtained from the 20-L Fermentor System.

| Air-flow Rate (slpm) | Mixing Speed (rpm) | Overhead Pressure (psi) | Oxygen Mass Transfer Coeff. (per second) | Regression coefficient ($R^2$) |
|---|---|---|---|---|
| 20 | 200 | 1 | 0.0159 | 0.9992 |
| 20 | 200 | 10 | 0.0181 | 0.9992 |
| 30 | 200 | 1 | 0.0239 | 0.9992 |
| 20 | 300 | 1 | 0.033 | 0.9976 |
| 20 | 500 | 1 | 0.0557 | 0.9969 |
| 20 | 650 | 1 | 0.0521 | 0.9925 |
| 20 | 800 | 1 | 0.0514 | 0.9934 |

The effect of increased head pressure at 20 slpm and 200 rpm to 10 psi on the ($k_La'$) was not significant (i.e., 0.0159 vs. 0.0181 per second, 13.8%).

However, the increased airflow rate to 30 slpm at 200 rpm and 1 psi did result in a significant increase in the ($k_La'$) value (i.e., 0.0159 vs. 0.0239 per second, 50%), as can be expected from the law of mass action.

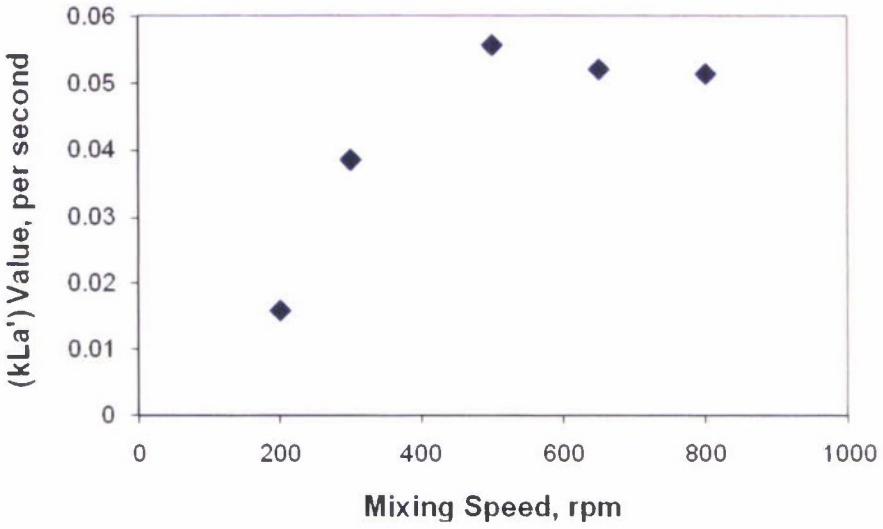


Figure 3. ($k_La'$) Values as a Function of Mixing Speed at Constant 20 slpm and 1 psi.

The ($k_La'$) values reached a plateau value of approximately 0.053 per second at 500 rpm and did not increase with higher agitation speed. To determine if the overlay airflow (used to provide a normal operating 1 psi head pressure) was interfering with the test, a second set of oxygenation tests were performed with the overlay airflow line disconnected from the Micros 30 L fermentation unit. The airflow was set at 20 slpm and vented off through the disconnected overlay line when adding sodium sulfite solution. When the DO level reached a minimum value, the airflow was switched to sparging, and DO was monitored for the ($k_La'$) determination.

These new DO profiles (Figure 4) demonstrate that isolating the airflow while adding sodium sulfite helped to minimize dissolution of oxygen because of intense surface mixing at higher mixing speeds. A broader DO range for oxygenation was observed for these measurements. For example, at 500 and 800 rpm without the overlay airflow, the lowest DO readings after the depletion of sodium sulfite were 20 and 60%, compared to the previous values of 75 and 80%, respectively.

The new set of ($k_La'$) values from the 20-L fermentor system, without the overlay airflow, is shown in Table 3. Indeed, the new ($k_La'$) values at the higher mixing speed (≥500 rpm) were higher, indicating that the surface mixing does interfere with the oxygenation test (perhaps negating the effect of sodium sulfite addition) when the air overlay was on. Nevertheless, as the mixing speed was increased, the ($k_La'$) value

approached a maximum value of 0.085 per second at approximately 650 rpm. Hence, beyond 650 rpm, there was no increase in ($k_La'$) value with an increase in mixing speed.

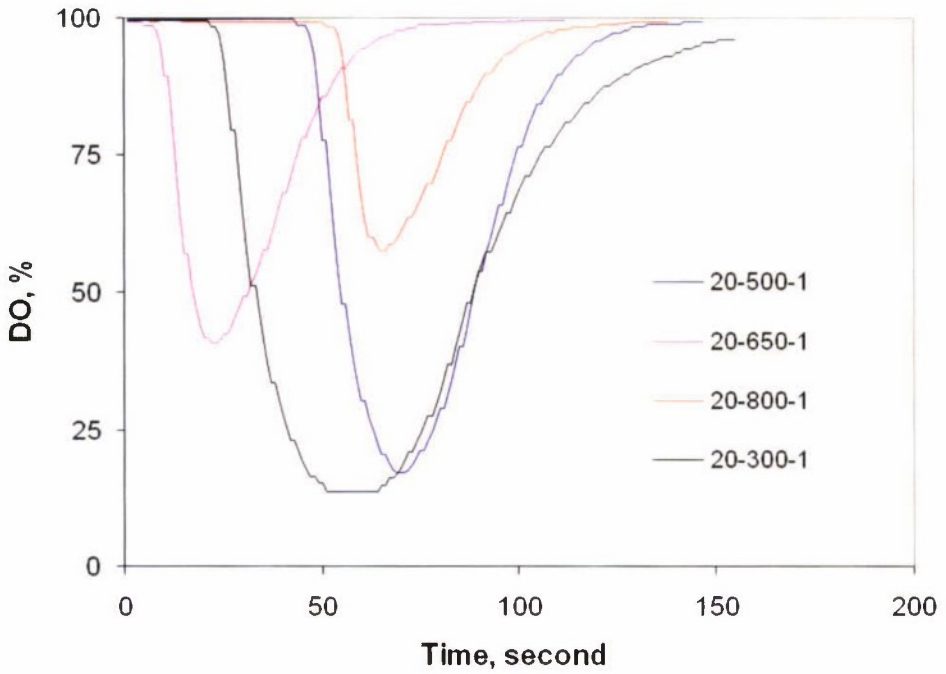


Figure 4. Time-dependent DO Profiles for the 20-L Fermentor System at Different Mixing Speeds without the Overlay Airflow.

Table 3. Summary of ($k_La'$) Values Obtained from the 20-L Fermentor System without the Overlay Airflow.

| Airflow Rate (slpm) | Mixing Speed (rpm) | Overhead Pressure (psi) | Oxygen Mass Transfer Coeff. (per second) | Regression coefficient ($R^2$) |
|---|---|---|---|---|
| 20 | 300 | 0 | 0.04 | 0.9992 |
| 20 | 500 | 0 | 0.0717 | 0.9992 |
| 20 | 650 | 0 | 0.0867 | 0.9853 |
| 20 | 800 | 0 | 0.0825 | 0.9965 |

In fact, the ($k_La'$) value at 800 rpm showed a slight decrease with a value of 0.0825 per second; this ($k_La'$) value may be physically impossible and could be attributed to a difficulty in precise DO measurement at the high mixing speed.

The corrected and combined ($k_La'$) values for the 20-L fermentor system as a function of mixing speed are shown in Figure 5. The ($k_La'$) data have been fitted as a second-degree polynomial function of mixing speed.

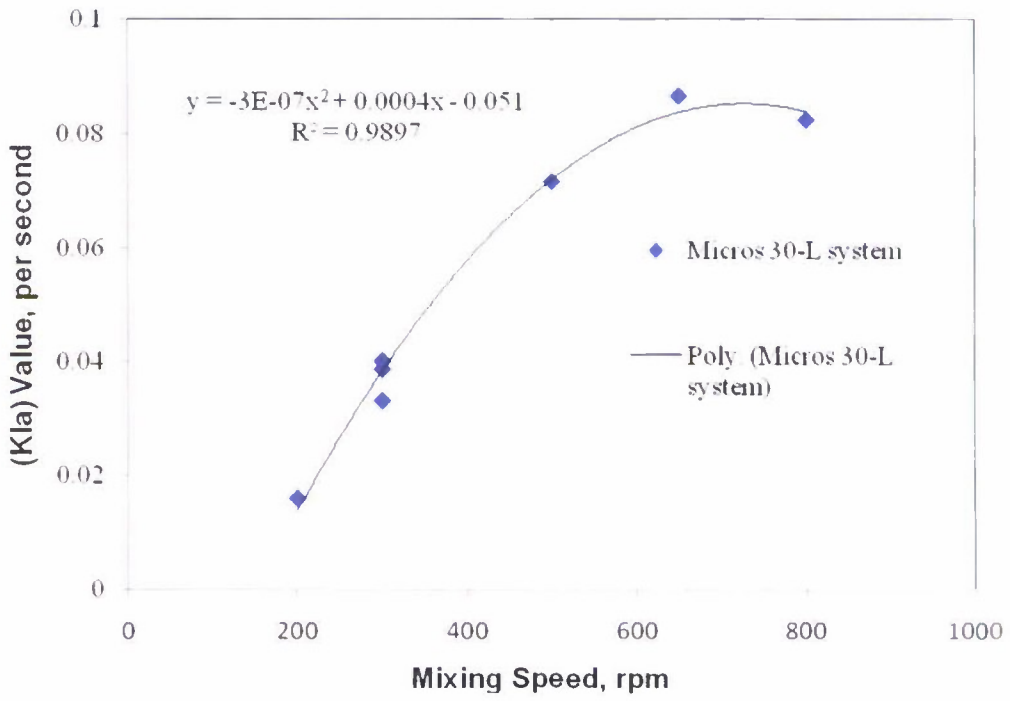


Figure 5. Combined ($k_La'$) Values as Function of Mixing Speeds at Constant 20 slpm and 1 psi.

DO profiles for the 100- and 1000-L fermentor systems are shown in Figures 6 and 7, respectively. Unlike the 20-L fermentor system, no significant interference due to surface mixing from the airflow overlay was noted.

However, a biphasic DO profile at 250 rpm was observed when the air sparge valve was inadvertently left closed. The initial increase in DO associated with 250 rpm was due to surface mixing (i.e., as the DO reached a bottom with the depletion of added sodium sulfite then began to rise due to surface mixing from the airflow overlay). The second phase of DO rise corresponds to when the air sparge valve was open.

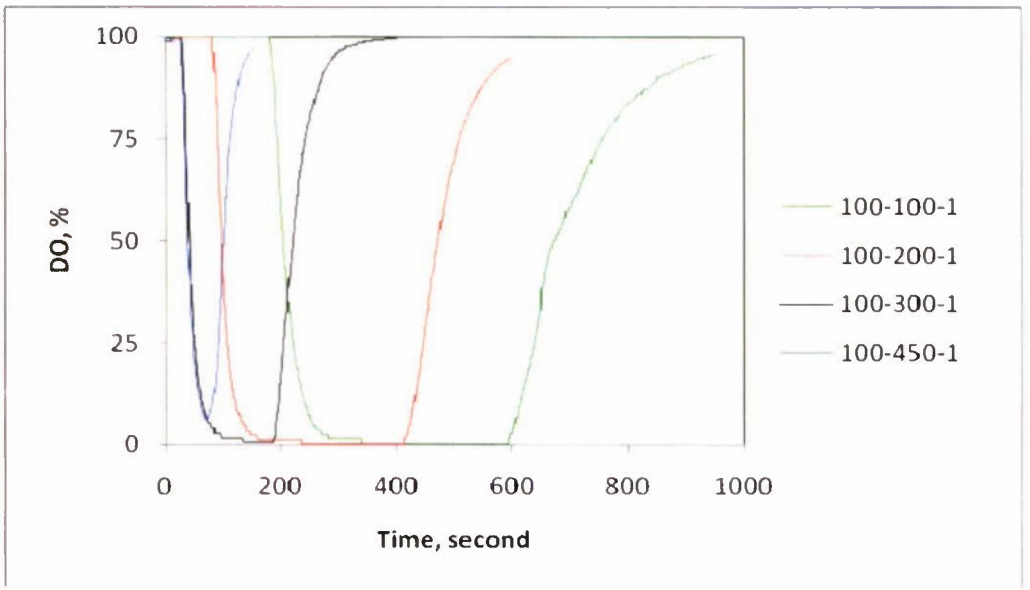


Figure 6. Time-dependent DO Profiles for the 100-L Fermentor System at Different Mixing Speeds (airflow rate and overhead pressure were held constant at 100 slpm and 1 psi, respectively).

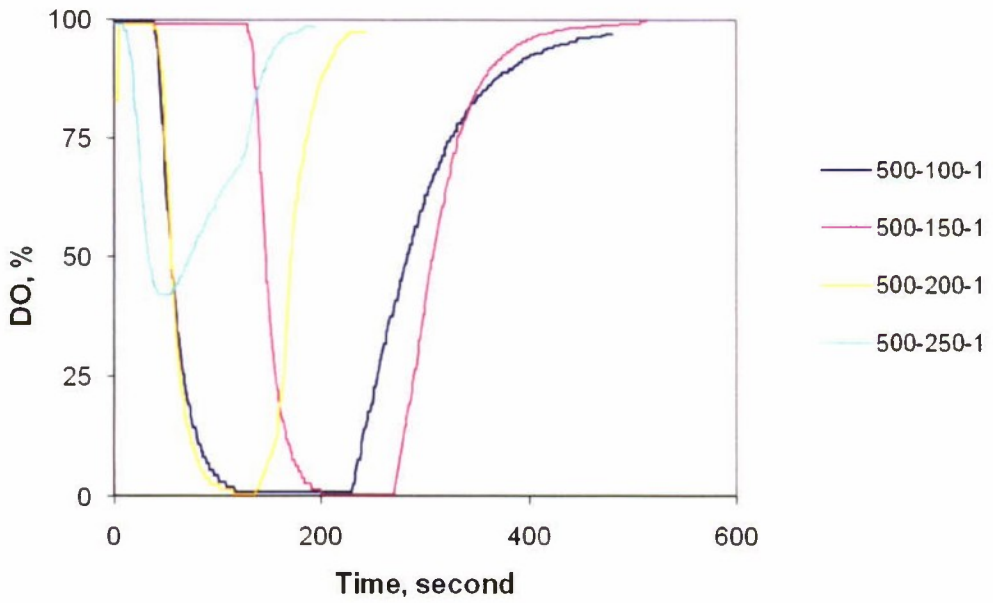


Figure 7. Time-dependent DO Profiles for the 1000-L Fermentor System at Different Mixing Speeds (airflow rate and overhead pressure were held constant at 500 slpm and 1 psi).

The calculated ($k_La'$) values from the IF 150 and 1500 L systems are shown in Tables 4 and 5, respectively.

Table 4. A Summary of ($k_La'$) Values Obtained from the 100-L Fermentor System.

| Airflow Rate (slpm) | Mixing Speed (rpm) | Overhead Pressure (psi) | Oxygen Mass Transfer Coeff. (per second) | Regression Coefficient ($R^2$) |
|---|---|---|---|---|
| 100 | 100 | 1 | 0.0091 | 0.9983 |
| 100 | 200 | 1 | 0.0184 | 0.9991 |
| 100 | 300 | 1 | 0.0312 | 0.9983 |
| 100 | 300 | 10 | 0.0314 | 0.9981 |
| 150 | 300 | 1 | 0.0433 | 0.9953 |
| 100 | 450 | 1 | 0.0555 | 0.9975 |

Table 5. Summary of ($k_La'$) Coefficient Values Obtained from the 1000-L Fermentor System.

| Airflow Rate (slpm) | Mixing Speed (rpm) | Overhead Pressure (psi) | Oxygen Mass Transfer Coeff. (per second) | Regression Coefficient ($R^2$) |
|---|---|---|---|---|
| 500 | 100 | 1 | 0.0158 | 0.9988 |
| 500 | 150 | 1 | 0.0274 | 0.9983 |
| 500 | 150 | 5 | 0.03 | 0.9862 |
| 750 | 150 | 1 | 0.0322 | 0.9988 |
| 500 | 200 | 1 | 0.0491 | 0.9964 |
| 500 | 250 | 1 | 0.055 | 0.9976 |

Similar to the 20-L fermentor system, increased overhead pressures of 10 and 5 psi at 100 slpm and 300 rpm, and 500 slpm and 150 rpm for the 100-L fermentor, respectively, resulted in minimal increases on the magnitude of ($k_La'$) Values (i.e., 0.65 and 7.3 %, respectively).

Figure 8 shows ($k_La'$) values as a function of mixing speed for the three different size fermentation units.

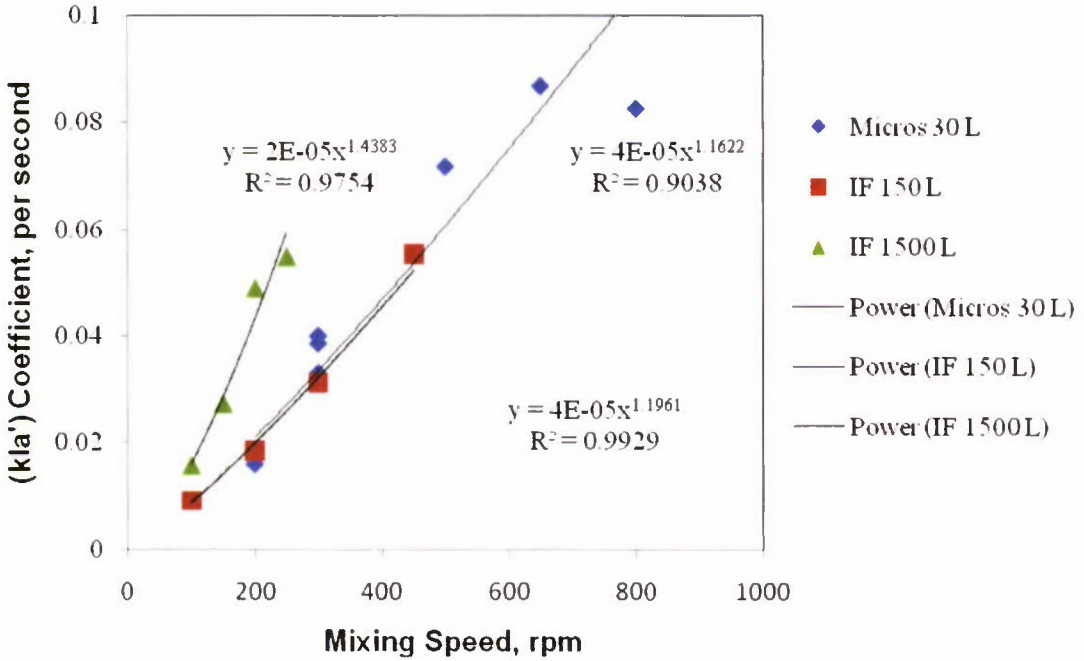


Figure 8. ($k_La'$) Value as a Function of Different Mixing Speeds with Fitted Polynomial Functions for the 20-, 10- , and 1000-L Fermentor Systems.

Each respective data line is fitted with a power regression line (shown as inserts in Figure 8 with regression coefficients) as follows:

$$y = ax^b \tag{10}$$

where

y = ($k_La'$) value, per second
x = mixing speed, rpm
a, b are empirical constants that yielded the best fit of data

Each equation can be further correlated to obtain an equivalent mixing speed required to scale-up based on the same oxygen transfer basis as follows (e.g., for 20- to 1000-L scale-up):

$$2.0 * 10^{-5} p^{1.4383} = 4.0 * z^{1.1622} \tag{11}$$

where

p = mixing speed at 1000-L scale, rpm
z = mixing speed at 20-L scale, rpm

By solving for x in term of z, eq. 6 becomes

$$p = 1.6192 z^{0.969} \tag{12}$$

Hence, to scale up a fermentation process from 20- to 1000- L based on the same oxygen transfer capacity as that for the 20-L at 300 rpm (e.g., ($k_La'$) = 0.027 $sec^{-1}$), the mixing speed at the 1000-L scale has to be equal to approximately 162 rpm. Incidentally, the closeness in terms of generating similar ($k_La'$) values of the 100 L to those of the 20 L rather than to the 1000 L is attributed to the overall geometrical similarity between the 20- and 100-L fermentor systems (Singh, 1987).

A set of 20- and 1000-L scale-up data from Btk fermentation runs for the production of Btk spores was selected to demonstrate the validity of using ($k_La'$) consideration for scale-up from 20-L to 1000-L. For 20-L scale fermentation of Btk with 300 rpm, the corresponding rpm with 1000-L scale should be 162 rpm according to eq. 7. However, 175 rpm for the 1000-L scale fermentation of Btk was used.

The DO and OD profiles vs. elapsed fermentation time (EFT) from 20- and 1000-L scales are shown in Figure 9. In both runs, no DO limitation was apparent, and the profiles appeared similar in DO and OD responses with the exception of the 1000-L final DO value. Theoretically, the final DO value should have been returned to 100% and remained at that level, as cells ceased to respire in the stationary phase as in the case of the 20-L run. However, a malfunctioning of the DO probe response may have attributed to a drifting from 90% to a final value of approximately 80% toward the end of the fermentation run, instead of an expected 100%.

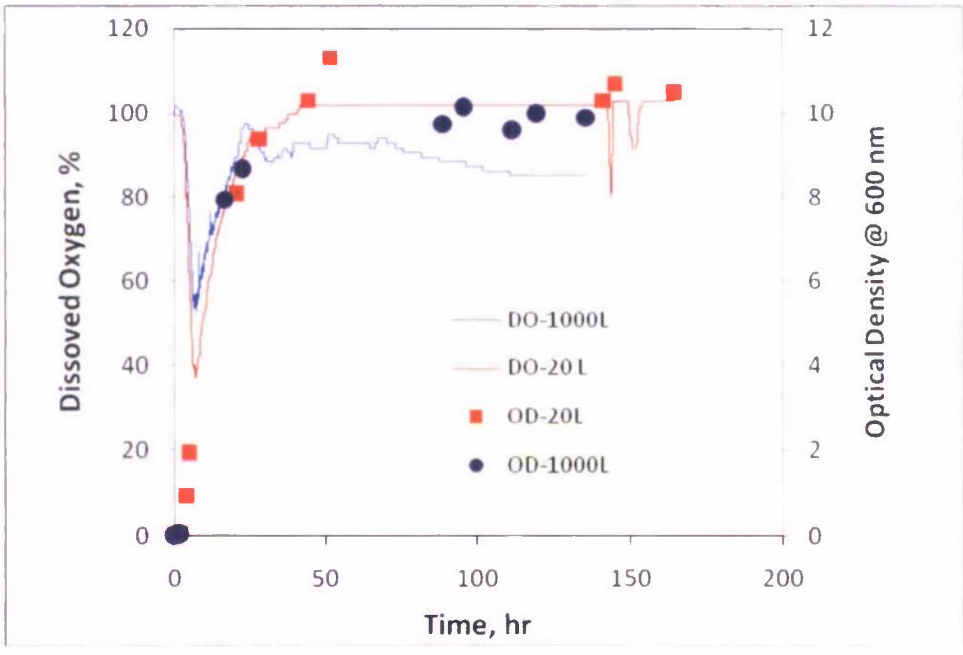


Figure 9. DO and OD Profiles for the 20- and 1000-L Fermentation Runs of Btk.

By focusing on the portion of DO responses in 20- and 1000-L fermentation runs, where there were significant metabolic oxygen demand (i.e., up to EFT = 28 hr). Eq. 8 was used to estimate the amount of accumulated $O_2$ uptake by Btk cultures for 20- and 1000-L fermentation runs up to EFT = 28 hr (Figure 10).

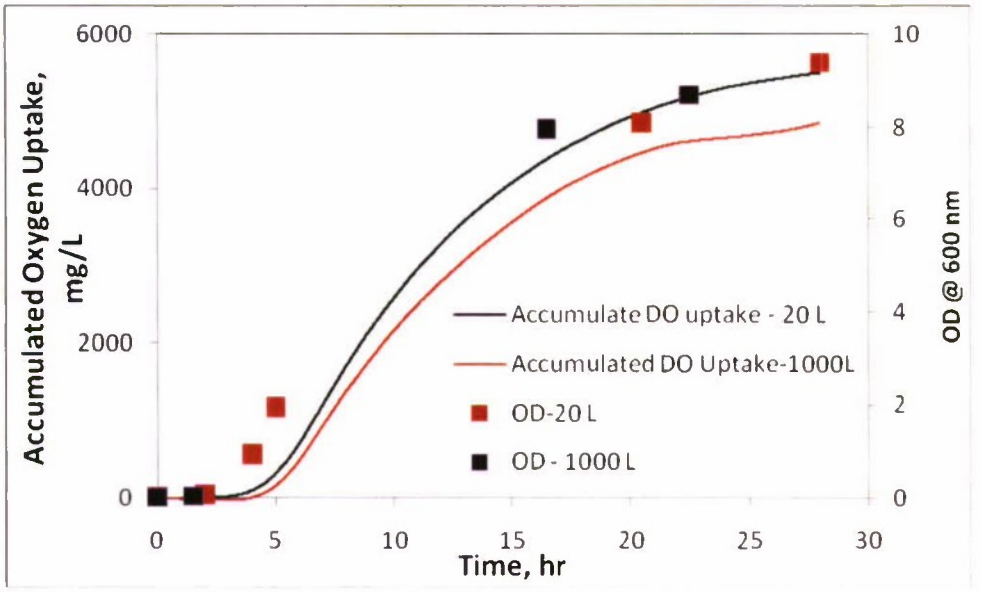


Figure 10. Accumulated DO Uptake Patterns between the 20- and 1000-L Fermentation Runs of Btk.

Theoretically, the ratio of accumulated DO uptake by any OD (i.e., specific DO uptake, $1/Y_{O2}$) at any given point in time should be valid. However, there were inherent noises in measuring low OD values in the beginning of the fermentation runs. Hence, the last two data points (i.e., EFTs = 28 and 21.5 hr, respectively, for the 20-L and 1000-L fermentation runs), where OD data are still in an exponential phase of growth, were used to calculate average specific oxygen uptake values of 600 and 509 mg $DO/OD_{600}$, respectively. These two similar average values demonstrate a validity of overall ($k_La'$) determination of each 20-L and 1000-L fermentation system using the ($k_La'$) value as the scale-up factor.

Finally, to compare product (Btk spores) yields between the 20- and 1000-L fermentation runs, Petroff Hauser and CFU counts are shown in Figure 11. CFU originating from spores (neither vegetative nor sporangia) were estimated from the same sample where Petroff Hauser count was obtained. Numbers of each vegetative cell, sporangia, and spores were counted, and the percentage of spores in each sample was applied to CFU counts for the same sample.

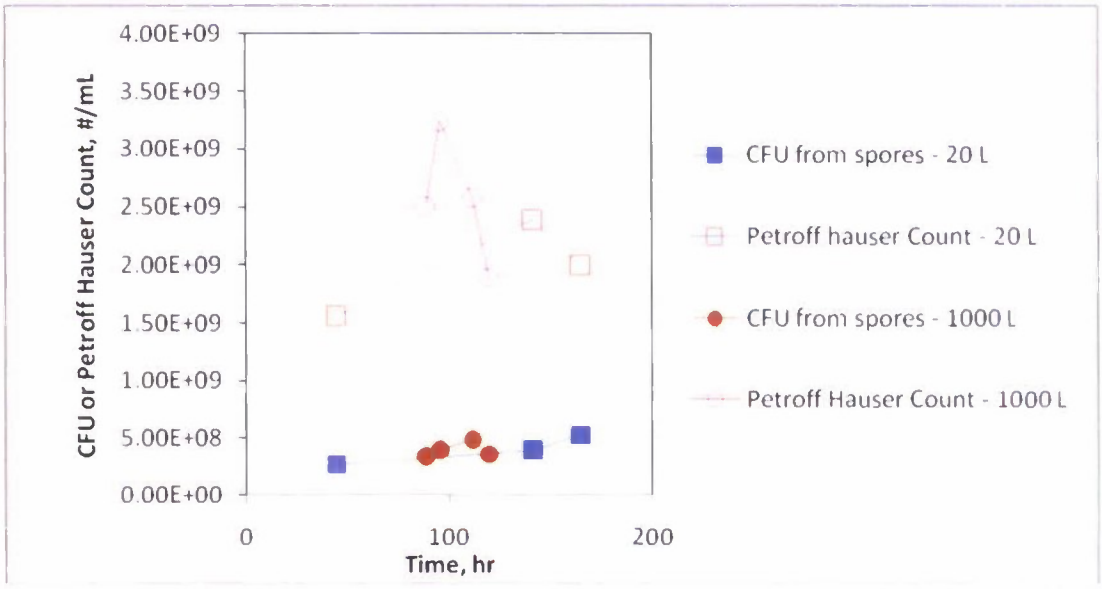


Figure 11. Product Yields in Terms of Petroff Hauser Counts and CFUs between the 20- and 1000-L Fermentation Runs of Btk.

Approximately one order of magnitude higher value in Petroff Hauser counts (e.g., average value = 2.5 x $10^9$/mL) were consistently measured in all 20- and 1000-L samples compared to their respective samples for CFU counting (i.e., 4 x $10^8$/mL). Using a Neubauer chamber resulted in a Btk spore yield of 4 to 5 x $10^9$/mL (Flores, et al., 1997) has been reported. However, a reason for apparent difference in the concentration of Btk spores by two different techniques (e.g., CFU and Petroff Hauser) is not clear. Nonetheless, consistency in the concentration of Btk spores between the 20- and 1000-L systems by either CFU or Petroff Hauser technique confirms the validity of using the ($k_La'$) value as the scale-up factor in terms of obtaining a similar product yield value at the 1000-L scale system compared to the 20-L system.

## 4. CONCLUSIONS

The ($k_La'$) values at different operating conditions of overhead pressure, mixing speed, and airflow rates were determined for the 20-, 100-, and 1000-L fermentation systems and subsequently used as the basis for 20- to 1000-L scale-up fermentation for production of Btk spores. Neither the overhead pressure nor airflow rate had much influence on the magnitude of ($k_La'$) value for all three systems. Contrastingly, the magnitude of the ($k_La'$) value was strongly dependent on the mixing speed used in each system. Standard operating conditions of airflow rate (i.e., 1 vvm for 20 and 0.5 vvm for 1000) and 1 psi overhead pressure at different mixing speeds (300 and 175 rpm, respectively, for the 20-L and 1000-L systems) were used for a scale-up Btk fermentation. The ($k_La'$) values were 0.027 and 0.033 $sec^{-1}$, respectively, for 300 rpm for 20-L systems and 175 rpm for 1000-L systems.

The use of the same ($k_La'$) values as the basis for successful scale-up strategy was demonstrated in this study. Similar values of specific accumulated oxygen uptake values (600 and 509 mg DO/OD) and practically identical values of Btk spore yields ($4.7 \times 10^8$ and $4.4 \times 10^8$ CFU/mL) were obtained for the 20- and 1000-L systems, respectively. The use of ($k_La'$) as the basis for scale-up fermentation has been recommended and frequently used among other applications (e.g., power input/volume ratio, tip velocity, etc. [Singh, 1987]). The strategy of using the same ($k_La'$) for scale-up fermentation implicitly assumes that the DO is the limiting factor and may not be applicable if other than DO becomes a limiting factor. Nevertheless, pre-determination of ($k_La'$) values for the various sized fermentation systems provides a readily available tool for a scale-up fermentation strategy to provide similar aerobic growth conditions at different sizes of fermentation systems and to achieve the desired product concentration at higher volumes.

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