SCALE-UP STRATEGIES IN STIRRED AND AERATED BIOREACTOR

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To my beloved mother and father

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ABSTRACT

The scale-up studies based on the constant oxygen transfer coefficient (k_La) from 16 liter to 150 liter of aerated and agitated bioreactor were performed. The studies included the investigation on the significance of hydrodynamic difference between Rushton and marine impeller on the k_La at 16 liter scale. By employing both static and dynamic gassing out techniques, the k_La values were calculated at different sets of impeller speeds and air flow rates performed in various viscosities and temperatures in the 16 liter and 150 liter Bioengineering^{TM} stirred bioreactor. Empirical correlation was employed to correlate and investigate the dependence of k_La on specific power input and superficial air velocity. Our experimental results discovered that the Rushton turbine was more effective in gas distribution and provide a greater oxygen transfer rate than the marine impeller. In maintaining a constant k_La upon scale-up from 16 to 150 liter, the specific power input and the superficial air velocity cannot be maintained, adjustment has to be done. Specific power input from 0.0001 to 4.2 kW/m^3 and superficial air velocity within the range of 9×10^{-4} to 7×10^{-3} m/s was tested to maintain a constant value of k_La upon scale-up in distilled water and CMC solution model. The operating variables employed at 150 liter scale successfully gave a comparable k_La values as in 16 liter scale. Hence, the calculated scaling-up factor for impeller speed and air flow rate were 0.28 and 3.1, respectively. In order to investigate the potential of employing scaling-up protocol developed in this work, the kinetic profiles of *E.coli* batch fermentation at 16 and 150 liter were compared. By employing the scaling-up factors, the proposed scale-up protocol managed to provide the similar trend of cell growth, glucose consumption and oxygen uptake rate upon scale-up based on the constant k_La. It may be concluded that the similar k_La for both scales was successfully achieved by employing the proposed scale-up protocol.

ABSTRAK

Kajian pengskalaan naik berdasarkan pekali pemindahan oksigen (k_La) yang malar daripada 16 liter ke 150 liter telah dijalankan di dalam bioreaktor teraduk berudara. Ujikaji ini melibatkan kajian ke atas perbezaan hidrodinamik yang ketara antara pengaduk Rushton dan marin terhadap k_La pada skala 16 liter. Dengan melakukan teknik penyingkiran gas secara statik dan dinamik, nilai-nilai k_La dikira pada set kelajuan putaran pengaduk dan kadar alir udara yang berbeza, kepada pelbagai kelikatan dan suhu dalam bioreaktor (Bioengineering^{TM}) 16 dan 150 liter. Korelasi empirikal telah dilaksanakan untuk mengkorelasi dan mengkaji kebergantungan k_La terhadap kuasa masukan tentu dan halaju gas luaran. Keputusan-keputusan eksperimen menunjukkan bahawa turbin Rushton adalah lebih efektif dalam penyebaran gas dan membekalkan kadar pemindahan oksigen yang lebih daripada pengaduk marin. Dalam mengekalkan k_La yang malar semasa pengskalaan naik, kuasa masukan tentu dan halaju gas luaran tidak dapat dikekalkan, penyelarasan harus dilakukan. Kuasa masukan tentu daripada 0.0001 ke 4.2 kW/m^3 dan halaju gas luaran dalam lingkungan 9 x 10^{-4} ke 7 x 10^{-3} m/s telah diuji untuk mengekalkan nilai k_La yang malar semasa pengskalaan naik dalam model air suling dan larutan CMC. Pembolehubah operasi yang dilaksanakan memberikan nilai-nilai k_La yang boleh dibandingkan dengan nilai pada 16 liter. Oleh yang demikian, faktor pengskalaan naik yang diperolehi adalah 0.28 bagi putaran pengaduk dan 3.1 bagi kadar alir udara. Bagi mengkaji keupayaan protokol pengskalaan naik yang dibentuk, profil-profil kinetik fermentasi *E.coli* pada skala 16 dan 150 liter telah dibandingkan. Dengan menggunakan faktor pengskalaan naik, protokol pengskalaan naik yang dicadangkan berupaya memberikan perilaku yang sama dalam pertumbuhan sel, penggunaan glukosa dan kadar penggunaan oksigen ketika pengskalaan naik berasaskan nilai k_La yang malar. Ia mungkin dapat disimpulkan bahawa k_La yang sama pada kedua-dua skala berjaya diperolehi dengan pelaksanaan protokol pengskalaan naik yang dicadangkan.

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LIST OF SYMBOLS

a - Specific interfacial area (m^{-1})

a' - Constants in Cooper's *et al.* (1944) equation

A - Parameter in Meztner-Otto's equation

b - Constants in Cooper's *et al.* (1944) equation

c - Constants in Cooper's *et al.* (1944) equation

C^* - Dissolved oxygen saturation concentration in liquid or solubility (mg/L)

C^o_L - Initial dissolved oxygen concentration (mg/L)

C_L - Dissolved oxygen concentration (mg/L)

$C_{O2,CRIT}$ - Critical value of dissolved oxygen concentration (mg/L)

c_p - Oxygen concentration measured by sensor (-)

C_X - Biomass concentration (g cell/L)

CMC - Carboxyl methyl cellulose (-)

D_i - Impeller diameter (m)

D_S - Sparger diameter

D_t - Tank/vessel diameter (m)

g - Acceleration due to gravity ($m.s^{-2}$)

H - Henry's law constant (kPa.L/mg)

H_L - Liquid height

H_T - Tank/vessel height (m)

k_G - Gas phase oxygen transfer coefficient (s^{-1} or hr^{-1})

k_L - Liquid phase oxygen transfer coefficient (s^{-1} or hr^{-1})

K_G - Overall gas phase oxygen transfer coefficient (s^{-1} or hr^{-1})

K_L - Overall liquid phase oxygen transfer coefficient (s^{-1} or hr^{-1})

k_La - Volumetric oxygen transfer coefficient (s^{-1} or hr^{-1})
m - Constant in Michel & Miller's equation
n - Flow behaviour index in power-law model (-)
N - Impeller speed (rpm)
N_p - Power number (-)
N_A - Aeration number (-)
N_{FR} - Froudes number (-)
N_{RE} - Reynolds number (-)
p_{AG} - Partial pressure of oxygen (kPa)
p_T - Total pressure of system (kPa)
P_o - Ungassed power consumption (W)
P_g - Gassed power consumption (W)
Q - Air flow rate (m^3/s)
Q_o - Overall oxygen uptake rate per unit volume of broth (mg O_2/L.s)
q_{O2} - Specific oxygen uptake rate per gram cells (mg O_2/g cell.s)
r_{O2} - Specific oxygen uptake rate per gram cells (mg O_2/g cell.s)
t - Time (s or hr)
T_i - Impeller thickness
V'_i - Impeller tip speed
V_L - Liquid volume (m^3)
V_T - Total volume
v_g - Superficial air velocity (m/s)
(w/v) - Mass per unit volume (-)
W_i - Impeller width
X - Dry cell weight (g cell/L)
y_A - Mole fraction of oxygen in gas phase (-)
y_{AG} - Mole fraction of oxygen in gas phase (-)

GREEK LETTERS

Δ_C - Spacing between impeller

Δ_i - Top impeller distance from top plate

E.coli - *Escherichia coli*

k - Consistency index in power-law model (Pa.s^n)

γ - Shear rate (s^{-1})

μ_L - Liquid viscosity (kg/m.s)

μ_{app} - Apparent viscosity in Oswald-de Waele model (Pa.s)

τ - Shear stress (N/m^2)

ρ_L - Liquid density (kg/m^3)

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CHAPTER 1

INTRODUCTION

1.1 Research Background

In the aerobic fermentations, sufficient supply of oxygen to the microorganisms is very crucial. Oxygen is sparingly soluble in the water (i.e. 10 ppm at 1 atm) and its transfer rate is always limited particularly through the gas-liquid interfaces (Bailey and Ollis, 1986). The limited solubility of oxygen in water is a physical constraint on bioreactor aerobic operation. This problem becomes worse especially in the larger scales since maintaining such homogeneous environment is no longer easy due to increased mixing time. The consequent anaerobic conditions result in lower fermentation performance and yields. Systematic engineering approaches to tackle this problem have been reported by a number of works (Arjunwadkar *et al.*, 1998; Badino Jr *et al.*, 2001, Cooper *et al.*, 1944). The oxygen transfer capacity in a bioreactor depends on the mechanical design and geometry of the air distributor, bioreactor aspect ratio, impeller type, and the agitation rate. All of them can be related to the oxygen transfer coefficient (k_La).

Cooper and his co-workers (1944) proposed that the k_La may be empirically linked to the gassed power consumption per unit volume of broth (P_g/V_L) and the superficial air velocity (v_g) as described by the following equation.

$$k_L a = a'\left(\frac{P_g}{V_L}\right)^b \left(v_g\right)^c \tag{1.1}$$

In this equation, the values of the constants 'b' and 'c' may vary considerably, depends on the bioreactor geometry and operating conditions. Data in Table 1.1 summarise the values of constant 'b' and 'c' from several works. Constant ‘b’ represents the level of dependence of k_La on the agitation, while, constant ‘c’ represents the level of dependence of k_La on the sparging rate applied to the system.

Table 1.1 Values of parameter 'b' and 'c' from several works that estimated from the empirical relationship proposed by Cooper *et al.* (1944)

Author	**Constant ‘b’**	**Constant ‘c’**	**Type of impeller**	**Liquid Model**	**Liquid Volume**
Cooper *et al.* (1944)	0.95	0.67	N/A	Air-water system	66 L
Shukla *et al.* (2001)	0.68	0.58	Disc turbine and pitched blade turbine	Air-water system	5.125 L
Shukla *et al.*((2001)	0.725	0.892	Disc turbine and pitched blade turbine	Yeast fermented broth	5.125 L
Badino Jr. *et al.* (2001)	0.47	0.39	Flat-blade disc style turbine	*Aspergillus’s* fermented broth	10 L
Martinov & Vlaev (2002)	0.84	0.4	Narcissus blade	(2% w/v) CMC solution	50 L
Martinov & Vlaev (2002)	0.82	0.4	Narcissus blade	(0.5% w/v) Xanthan gum solution	50 L
Arjunwadkar *et al.* (1998)	0.68	0.4	Disc turbine and pitched blade turbine	(0.7% w/v) CMC solution	5.125 L

As supplying adequate oxygen is the centre of the issue in aerobic fermentation, maintaining a similar oxygen transfer coefficient or k_La has been frequently employed as the basis of scaling up exercises. Scale-up criteria that commonly used to maintain constant k_La are i) the gassed power number per unit liquid volume (P_g/V_L), the superficial air velocity (v_g), the sparging rate (vvm) and bioreactor geometrical and operational constants such as ratio of liquid height to tank diameter (H_i/D_T), impeller diameter (D_i), impeller rotation number (N), impeller tip speed (ND_i), pump rate of impeller (Q), pump rate of impeller per unit volume (Q/V) and Reynolds number.

1.2 Motivation

The oxygen transfer coefficient, k_La plays an important role towards carrying out the design, scaling up and economic of the process. Efforts have been focused in improving the design and scaling up studies to achieve adequate supply of oxygen at higher scales (Martinov & Vlaev, 2001, Juarez & Orejas, 2001, Arjunwaadkar *et al.*, 1998). Their works employed the correlation proposed by Cooper *et al.* (1944) and demonstrated the effects of agitation and aeration at different combination of impellers in prediction of k_La values at the laboratory scales. The most commonly methods in determining the k_La are the static and the dynamic gassing-out techniques. As contrast to the static gassing-out technique, the live culture was used in the dynamic gassing-out technique. Both of these techniques have been employed by Martinov & Vlaev (2001), Juarez & Orejas (2001), Arjunwaadkar *et al.* (1998) and Shukla *et al.* (2001).

Scaling up studies performed in this work used the correlation developed by Cooper *et al.* (1944). The k_La values achieved at 16 liter scale were compared with the values at 150 liter scale. Since the scaling up factor is not proportionally increasing, the 'trial-and-error' within predicted range was performed. The effectiveness of this scaling up protocol was tested in the real *E.coli* fermentation. Identical growth profiles at both scales conclude that comparable oxygen transfer at 150 liter was successfully achieved. There has been a significant advance in the understanding of scale-up of stirred aerated bioreactors as reported by several authors. Shukla *et al.* (2001) works highlight on the performance of the impeller used upon scaling up of yeast biotransformation medium on a basis of constant k_La. Wong *et al.* (2003) employed the correlations proposed by Wang *et al.* (1979) in scaling up on a basis of constant k_La and air flow rate per unit volume, (Q/V). The work by Hensirisak (1997) concerned more on the performance of microbubble dispersion to improved oxygen transfer upon scale-up. The work by Wernesson &

Tragardh (1999) reported the influence of power input per unit mass on the hydrodynamics of the bioreactor.

In spite of these observations, the engineering focus continued to be on maintaining the volumetric oxygen transfer constant on scale-up. Humphrey *et al.* (1972) addressed that; researchers still do not have an absolute basis for scale-up. As a matter of fact, biochemical engineers still practice scale-up a black art in which they attempt to maintain constant and operating the aeration rate well below gas flooding conditions. In this study, scale-up strategy proposed by Shukla *et al.* (2001) and Garcia-Ochoa *et al.* (2000) will be further improved. The challenge and aims of this study is to manipulate the constant in the empirical correlation proposed by Cooper *et al.* (1944) and provide a scaling-up factor upon scale-up from 16 liter to 150 liter scale in a basis of constant k_La.

1.3 Research Objectives and Scope

The objectives of this research are:

1) To investigate the significance of hydrodynamic difference between Rushton turbine and marine impellers on the oxygen transfer in 16 liter bioreactor.

2) To develop a simple approach that provides a reliable protocol for scaling-up exercise based on constant oxygen transfer rate in stirred aerated bioreactor.

3) To evaluate the potential of employing the scaling-up protocol developed in this study in the actual fermentation.

In order to achieve these objectives, the following scope of work shall be covered:

1) Evaluation of oxygen transfer coefficient, k_La by using static and dynamic gassing-out techniques.

2) Study the effect of fermentation system and operational parameters by:

   i) Vary impeller speeds, volumetric air flow rate and temperature in 16 liter bioreactor.

   ii) Mimic a pseudoplastic behaviour by using carboxy methyl cellulose (CMC) to compare the effect of Newtonian and non-Newtonian fluids on k_La.

3) Investigate the dependence of oxygen transfer coefficient on superficial air velocity and volumetric gassed power input at 16 liter bioreactor using Rushton turbine and marine impeller.

4) Investigates the effect of impeller type on the dependence of oxygen transfer coefficient on superficial air velocity and volumetric gassed power input in:

   i) 16 liter and 150 liter at different viscosities namely 0.25, 0.5 and 1 %(w/v) of CMC solutions.

   ii) 16 liter and 150 liter bioreactor at different temperatures namely 30^o, 40^o and 50 ^oC.

5) Graphically determine, compare, and analyze the coefficients in the empirical correlation proposed by Cooper *et al.* (1944) at:

   i) 16 liters for Rushton turbine and marine impeller.

   ii) 16 liter and 150 liter at different viscosities namely 0.25, 0.5 and 1 %(w/v) of CMC solutions.

   iii) 16 liter and 150 liter bioreactor at different temperatures namely 30^o, 40^o and 50 ^oC.

6) Compare time-course profiles of growth, glucose consumption, specific oxygen uptake rate (OUR), and k_La at 16 liter and 150 liter bioreactor.

4. Similar kinetic profiles of *E.coli* growth, glucose consumption, oxygen uptake rate (OUR) and oxygen transfer rate (OTR) were most likely resulted from the similar oxygen transfer at both scales. This proved that the proposed scale-up strategy worked in predicting fermentation kinetic at higher scale.

5.2 Recommendations for Future Studies

1. As the scale increases, gas distribution in the bioreactor region becomes problematic. Therefore, investigation on the oxygen profile is crucial and worth pursuing in gaining further insight on measurement of k_La in the biorector. This investigation may be performed on the high pseudoplastic fluids i.e. Xanthan gum solution, a non-coalescent liquid i.e. Na_2SO_4 and on the filamentous culture broth.

2. The empirical correlation proposed by Cooper *et al.* (1944) only concerns on the effect of the sparging rate and the impeller rotational speed on the k_La. Study may be extended to other empirical equations developed by other workers such as Ryu and Humphrey (1972), Yagi and Yashida (1975) and Zlokarnik (1978).

3. The validity of the scale-up protocol proposed in this work may be further tested in scales higher than 150 liter bioreactor.

4. The scale-up protocol proposed in this work was based on the rules of thumb technique. Other scale-up approaches such as fundamentals method, semi-fundamentals method, dimensional analysis and time-regime analysis may be investigated in scaling-up stirred aerated bioreactor on the basis of constant k_La.

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