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.

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

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.