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New Correlation of Volumetric Oxygen Mass Transfer Coefficient for Scale-up in Aerobic Fermentation

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An appropriately available correlation through a series of formulas derivation and reasonable simplifications was established using some hypotheses. Then a wide range of culture conditions such as gas flow rate , stirrer speed and volume of the liquid in the vessel were correlated with volumetric oxygen mass transfer coefficient and the correlation is determined as: $k_La=KQ^xN^yV_L^{-y/3.15}$. The correlation factors *K*, *x*, *y* for the 30 l bioreactors calculated by sodium sulfite oxidation method were 0.004, 0.525 and 1.685 respectively. The correlation proposed here could guide the scale-up of fermentation to obtain an optimized initial volume of fermentation broth from lab-scale to a new higher scale and could inspect intuitively to the relationship between k_La and the other three parameters.

Key words: Correlation; Volumetric oxygen mass transfer coefficient; Scale-up; Fermentation.

It's confirmed that the only non-contentious fact about scale-up is that it is one of the most complicated processes and challenging endeavors in the field of biochemical engineering (Reuss 1993). Over the past five decades, extensive work has been done in the area of the scale-up of fermentation systems. And it is usually based on various criteria such as: geometrical similarity; power input; volumetric oxygen transfer coefficient; mixing time; and bioreactor fluid dynamics (Dunn et al 1992; Miura, 1976). As things stand, there is a general consensus that the most widely used scale-up method is based on the utilization of the oxygen mass transfer coefficient (k_La) in aerobic fermentation process (Flores et al, 1997). The problem with this process has been the various methods by which the transfer coefficient has been obtained. Different methods, such as sulfite oxidation (Farrell ea al, 2012), absorption of CO_2 (Danckwerts & Gillham, 1966), chemical gassing-out (John et al, 2003), dynamic method (Linek & Sinkule, 1991) and other methods (Ortiz-Ochoa et al, 2005; Carbajal & Tecante, 2004) have been used to take the value of k_La by different investigators.

There are both dimensional and dimensionless Equations for the k_La as a function of different variables that have been proposed (Costa et al, 1982; Garcia-Ochoa & Gomez, 1998). Among these Equations, however, little has shown that the relationship between k_La and some intuitive parameters that are the important manipulated variables for scaling up in aerobic fermentation process. Seldom investigators take notice of the initial fermentation capacity in scale-

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up process. Actually, the initial fermentation capacity does play a non-neglectable role. Based on this, the paper proposes a new model solving these problems and some empirical correlations were used, then the correlation, obtained by reasonable assumptions and derivation of Equations, can guide to the scale-up of fermentation intuitively and is essential to successfully scale up the fermentation process to a larger scale through calculating the optimization initial culture volumes.

MATERIALS AND METHODS

Materials

Sodium sulfite, sodium thiosulfate and sodium iodide were purchased from Solarbio Company (China). Potassium dichromate potassium permanganate, sodium carbonate and copper sulfate were supplied by Zhengzhou YF-CHEM Ltd. Company (China). All solvents and reagents were analytical grade.

Mathematical model

Extensive literature indicates that the correlations between k_La and P_g/V_L have been widely applied in the past dozens of years. To conclude, the classical mathematical model of these correlations can be expressed as:

$$k_La=C(P_g/V_L)^aV_s^b\mu^c \quad ...(1)$$

The k_La values were correlated with the combination of stirrer speed, N, superficial gas velocity, V_s, volume of the liquid in the vessel, V_L, and liquid effective viscosity, μ. The constant C depends on the geometrical parameters of the vessel and the stirrer employed and the exponent values (a, b and c) have a wide variation range in the different correlations proposed by different authors.

For the parameter P_g presented above in Equation (1), it can be represented by the expression:

$$P_g=m(P_0^2ND_i^3Q^{-0.56})^{0.45} \quad ...(2)$$

where P_g is gassed power, P_0 is power input in un-aerated systems, N is the impeller speed, Q is the volumetric gas flow rate, D_i is the impeller diameter and m is a parameter that depends on the style and geometric sizes.

And P_0 is that of Pauline M. Doran (1995) which is shown below in *Eq*uation (3):

$$P_0=N_p\rho_LN^3D_i^5 \quad ...(3)$$

where P_0 is power, N_p is power number, $\acute{A}_L$ is fluid density, N is stirrer speed and D_i is impeller diameter. Among these, N_p is a function of Reynolds number, but it is independent of Reynolds number in turbulent flow. Therefore, for a given flow regime in a bioreactor, the term $N_p\cdot\acute{A}_L$ can be considered to be a constant.

Let $k=N_p\cdot\rho_L$, the Equation leads to the following:

$$P_0=kN^3D_i^5 \quad ...(4)$$

Substituting the Equation (4) into Equation (2) gives:

$$P_g=mk^{0.9}N^{3.15}D_i^{5.85}Q^{-0.252} \quad ...(5)$$

For a given bioreactor, these three terms (m, k and D_i) can be combined together. Let $m_1=mk^{0.9}D_i^{5.85}$, then:

$$P_g=m_1N^{3.15}Q^{0.252} \quad ...(6)$$

$$\text{As, } V_L=Q/A \quad ....(7)$$

where A is cross sectional area of vessel (m^2).

Then substituting the Equation (6) and (7) into Equation (1) gives:

$$k_La=C\mu^cA^{-a}Q^{a-0.252b}m_l^bN^{3.15b}/V_L^b \quad ...(8)$$

The term A^{-a} and μ^c in Equation (8) are assumed constant in a particular system. Let:

$$K=C\mu^cA^{-a}m_l^b \quad ...(9)$$

$$x=a-0.252b \quad ...(10)$$

$$y=3.15b \quad ..(11)$$

Finally, substituting the Equation (9), (10) and (11) into Equation (8), the desired Equation is represented by the expression:

$$k_La=KQ^xN^yV_L^{-y/3.15} \quad ...(12)$$

In Equation (12), the parameter K is a parameter that depends on the style and geometric sizes of fermenter and the value of k_La is the function of such parameters as stirrer speed, volume of the liquid in the vessel and the volumetric gas flow rate.

Measurement of k_La

The experiment is based on sodium sulfite oxidation method (Cooper, 1944). The procedure is as follow: fill the bioreactor with a 1 M sodium sulfite solution containing 10^{-3} M of Cu^{2+} ion, then turn on the air and starting the time when the air emerges from the sparger. After the oxidation reaction keeps 10 minutes, stop the air flow, agitating and take samples of 2ml at every 30 minutes. And each sample was mixed with an excess of standard iodine reagent in a 250 ml iodine flask that can prevent iodine form evaporating. Finally, the intermixture was titrated with standard sodium

tiosulfate solution ($Na_2S_2O_3$ 0.3M) to a starch indicator end point.

The mass balance for the dissolved oxygen in the well-mixed liquid phase can be established as:

$$\frac{dC}{dt} = OTR - OUR \quad ...(13)$$

where *dC/dt* is the accumulation oxygen rate in the liquid phase, *OTR* represents the oxygen transfer rate from the gas to the liquid, and *OUR* is the oxygen uptake rate by the microorganisms. The term *OTR* can be expressed as:

$$OTR=k_La(C^*-C) \quad ...(14)$$

where C^* is the oxygen saturation concentration in the bulk liquid in Equilibrium to the bulk gas phase, and *C* is the dissolved oxygen concentration in the bulk liquid.

In the absence of biomass or with non-respiring cells, when biochemical reactions do not take place, OUR=0. In this case, *Eq*. (13) can be simplified to:

$$\frac{dC}{dt} = OTR = k_La(C^* - C) \quad ...(15)$$

As mentioned above, the reaction is fast, so C is assumed to be zero. Therefore,

$$\frac{dC}{dt} = k_LaC^* \quad ...(16)$$

Once the sulfite concentration is measured versus time, the rate of sulfite consumption is determined and k_La may be calculated from:

$$-\frac{dC_{Na_2SO_3}}{dt} = 2k_LaC^* \quad ...(17)$$

then $$k_La = -\frac{dC_{Na_2SO_3}}{2dt} \cdot \frac{1}{C^*} \quad ...(18)$$

where C* can be obtained from the aid of Henry's law, which is shown below:

$$H = \frac{y_{O_2}P}{\frac{C^*}{\sum C_i}} \quad ...(19)$$

Namely, $$C^* = \frac{y_{O_2}P}{\sum C_iH} \quad ...(20)$$

where *H* is Henry's coefficient (in bar), which is 1.116 MPa/mol m^3 using the method of Perry's chemical engineers' handbook (Perry, 2008) in the condition that the concentration of the Na_2SO_3 solution at 32°C is 1M, y_{O2} is the volume fraction of oxygen in the gas phase, *P* is the system pressure, and $\sum C_i$ is the approximately Equal to molar concentration of water(55.55mol/L).

Therefore, k_La is calculated by the following Equation:

$$k_La = -\frac{dC_{Na_2SO_3}}{2dt} \bullet \frac{\sum C_iH}{y_{O_2}P} \quad ...(21)$$

RESULTS

Effects of stirrer speed and aeration rate on k_La values

In a wide range of volumetric gas flow rate and agitation speed, the k_La values were estimated as shown in Table 1 and 2. The gas flow rate was maintained constant at 10 L/min while the agitation speed was changed and the agitation speed was fixed at 900 rpm while the gas flow rate was varied.

The k_La was estimated at various volumetric gas flow rate and agitation speed and the data shows that the volumetric gas transfer

Table 1. Effects of stirrer speed on k_La values as the aeration rate keeps constant of 10

Stirrer speed *N*(*r/min*)	300	500	700	900	1100	1300
$k_La(h^{-1})$	76.61	190.34	352.71	559.12	698.82	813.26

Table 2. Effects of aeration rate on k_La values as the stirrer speed keeps constant of 900 rpm

Aeration rate *Q*(*L/min*)	4	6	8	10	12
$k_La(h^{-1})$	417.15	509.92	585.42	654.84	727.45

coefficient increases as the volumetric gas flow rate and agitation speed increases respectively in the fermenter.

Oxygen Transfer Correlation

Numerous experimental studies have been performed to relate k_La to other fermentation variables. As previously deduced in the mathematical model part, those fermentation variables found to correlate with k_La are the volumetric gas flow rate, volume of the liquid in the vessel and agitation speed, providing the correlation: $k_La = KQ^xN^yV_L^{-y/3.15}$, where K, x, and y are the desired correlation factors.

Therefore, for the 30 l fermenter, the correlation factors, x and y, can be obtained first through the linear regression of the logarithmic plot shown in figure 1 and 2, ln(k_La) *vs* ln$Q$ and ln (k_La) *vs* ln$N$. The estimated values of x and y were 0.525 and 1.685 respectively (R^2 is 0.9930, 0.9879 in each plot). And the value of R^2 was calculated to indicate the degree to which the induced Equation fit the observed data. This linear fitting equation is in good agreement with the concept by the model and the logarithmic plots in figure 1 show the dependence of k_La on the gas flow rate and stirrer speed.

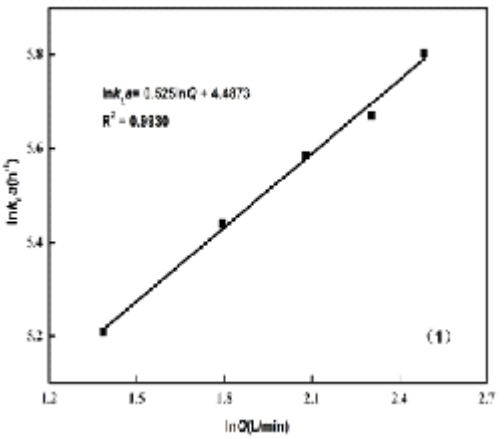

Fig. 1. Dependence of ln(k_La) on the ln$Q$ at the steer speed as a constant of 900 *rpm*

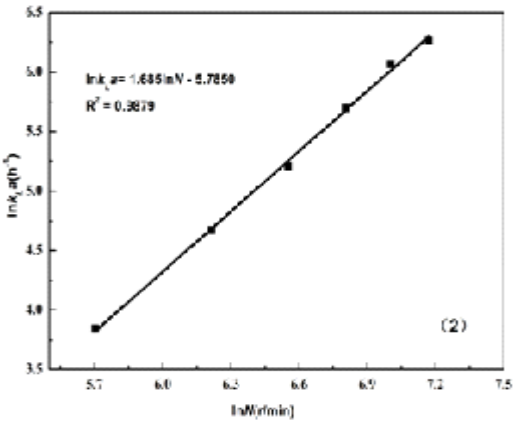

Fig. 2. Dependence of ln(k_La) on the ln$N$ at the gas flow rate as a constant of 10 *L/min*

Dependence of ln(k_La) on the ln$N$ at the gas flow rate as a constant of 10 *L/min*

Then, the constants K, as estimated through submitting the values x, y, K_Q and K_N in the Equation (19) and (22), was 0.004 for the 30 l one's. Consequently, the oxygen transfer correlation in this experiment has been determined as $k_La=0.004Q^{0.525}N^{1.685}V_L^{-0.535}$.

DISCUSSION

The correlation which provides a simple but rational mathematical model for translating an optimized lab-scale fermentation performance to a larger ones' relates k_La to the key intuitive fermentation parameters (agitation speed, gas flow rate and initial culture volume) and is essential to successfully scale up the fermentation process to the larger scale. The most important point is the potential of the mathematical model when utilizing the dynamic method in fermentation presented by (Bandyopadhyay and Humphrey, 2004) in measuring k_La. Because of its intuitiveness and liable to be related, it is suggested that other investigators should consider this model in their quest for obtaining data for scale-up of aerobic fermentation processes. Further, re-correlating the model with those parameters (such as feed rate of carbon and nitrogen nutrient) which are crucial for the feeding stage will probably bring out a new approach that can be manipulated automatically in the process of fermentation.

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