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Research Article

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New correlation of volumetric oxygen mass transfer coefficient for scale-up in aerobic fermentation of recombination *E. coli*

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ABSTRACT

This paper presents an appropriately available correlation through a series of formula derivations and reasonable simplifications using some hypotheses. Then wide ranges of culture conditions such as gas flow rate , stirrer speed and initial culture volume 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 12.8 L and 30 L bioreactors calculated by sodium sulfite oxidation method were 0.01, 0.4613, 1.5131 and 0.004, 0.525, 1.685, respectively. The correlation proposed here could guide the scale-up of fermentation to obtain an optimized initial volume of fermentation broth (15.4 L) for 30 L bioreactor form 12.8 L via the criteria of maintaining p^*k_La as a constant. The final DCW of 59.7 g L^{-1} and HLC concentration of 6.25 g L^{-1} were obtained on the pilot-scale upon maintenance of DO at the same level, resembled to the lab-scale of 80.13 g L^{-1} and 13.1 g L^{-1}.

Keywords: Correlation, Volumetric oxygen mass transfer coefficient, Scale-up, Fermentation, *E. Coli*

INTRODUCTION

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 [1]. 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 [2, 3].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 [4-8]. The problem with this process has been the various methods by which the transfer coefficient has been obtained. Different methods, such as sulfite oxidation [6], absorption of CO_2[9], chemical gassing-out[10],dynamic method[11] and other methods [12-16] have been used to take the value of k_La by different investigators. As well known, oxygen is sparingly soluble in water and culture medium at conditions normally encountered in biotechnology applications and therefore, growth and productivity of aerobic microorganisms is often controlled by oxygen availability. The oxygen availability in the fermentation broth could be rate-limiting especially in high cell density culture of the fast-growing aerobes such as recombinant *E. coli*. Therefore, the scale-up success of such aerobic fermentation process depends on satisfying the oxygen demand all through the culture volume of interest in the fermenter.

There are both dimensional and dimensionless equations for the k_La as a function of different variables that have been proposed [17-24]. Among these equations, however, little has shown that the relationship between k_La and some intuitive parameters which are the important manipulated variables for scaling up in aerobic fermentation process. Seldom investigators take notice of the initial fermentation capacity in scale-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, which can guide to the scale-up of fermentation intuitively, was obtained by reasonable assumptions and derivation of equations and does a good job in scaling 12.8 L up to 30 L through calculating the optimization initial culture volumes. The product yields and consistency of product quality have been achieved by maintaining the DO at a similar level.

EXPERIMENTAL SECTION

Microorganism and medium

Recombinant *E. coli* BL21 pNWCP31 expressing human like collagen was used in this study [25]. The plasmid, which contained a kanamycin-resistance gene and allowed temperature induction, was constructed in our laboratory. The compositions of culture media and feeding media were identical to those reported previously [26].

Inoculum preparation

Colonies from LB agar plates were inoculated into 300 mL flasks containing 50 mL LB media incubated on a shaker at 32°C and 200 rpm. 10 hours later, the first inoculums were inoculated into 300 ml flasks with 50mL LB media respectively for further cultivation at the same conditions as the first inoculum cultivation. After 10 h, the second inoculum was used for inoculation (10%, v/v) of the lab-scale stirred-tank reactor (Model L1523, Bioengineering Co. Switzerland) or for the pilot-scale stirred-tank reactor (Model NF 30, Bioengineering Co. Switzerland).

Culture conditions

The cultivation temperature was maintained at 32°C unless described otherwise. The air flow-rate was maintained at 18 L min^{-1} for lab-scale fermentor and 12 L min^{-1} for pilot-scale fermentor. The initial culture volumes were 6 L for lab-scale and 15.4 L for pilot-scale, respectively. The pH value was maintained at 6.8 by automatically adding NH_4OH (25%, w/w). The dissolved O_2 (DO) was maintained above 20% air saturation by manually increasing the agitation rate at 0.1Mpa pressure for the lab-scale fermentor (initially 300 rpm) and at 0.15 MPa over pressure for the pilot-scale fermentor (initially 300 rpm).

As soon as the initial carbon source was consumed, which indicated by the rapid increase of DO, the fed-batch culture was initiated. The probing feeding method proposed by Åkesson *et al*. was used to control the nutrient feeding manually [27]. To track the acetate threshold more quickly, the initial feeding rate was calculated to achieve the specific cell growth rate about 0.15 h^{-1} And the feeding rate was controlled to maintain the constant specific cell growth rate between the pulses with the pseudo-exponential feeding method proposed by Duan*et al*. [28]. For induction, the cultivation temperature was increased to 42°C and maintained for 3 h and was then reset to 39°C for several hours, to achieve a high-level expression of the target protein. All experiments were carried out three times under the same culture conditions.

Analysis methods

Cell density was measured turbidimetrically at 600 nm with spectrophotometer (UNICO Model 2082PCS, USA). The cell concentration was determined by the DCW: the broth sample was centrifuged at 9000r·min^{-1} for 10min, washed three times with distilled water and dried to a constant mass in a 105°C oven. Acetic acid concentration was acquired by BioProfile analyzer 300(NOVA biomedical, USA). Human-like collagen was determined with hydroproline colorimetry[29]. Temperature, pH, DO, rotation speed, airflow rate, zymotic fluid volume and the pressure of fermentor were controlled by the digital unit of the bioreactor.

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:

$$P_g=m(P_0^2ND_iQ^{-0.56})^{0.45} \quad (2)$$

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 Equation (3)[30]:

$$P_0=N_p\rho_L N^3 D_i^5 \quad (3)$$

where P_0 is power, N_p is power number, ρ_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 $N_p\rho_L$ can be considered to be a constant.

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

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

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:

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

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:

$$k_La=c\mu^c A^{-a}Q^{a-0.252b}m_1^{\ b}N^{3.15b}/V_L^{\ b} \quad (8)$$

The term A^{-a} and μ^c in Equation (8) are assumed constant in a particular system. Let:
$$K=C\mu^c A^{-a}m_1^{\ b} \quad (9)$$
$$x=a-0.252b \quad (10)$$
$$y=3.15b \quad (11)$$

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 parameters such 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 such a reaction that a reducing agent(sodium sulfite) react with the dissolved oxygen to produce sulfate in the presence of a catalyst (usually a divalent cation of Cu^{2+} or Co^{2+}) [31]. The reaction can be expressed as:

$$2Na_2SO_3+O_2\rightarrow 2Na_2SO_4$$

Since this reaction is so fast, the oxygen concentration in the bulk liquid can be assumed to be zero. As a result, the rate of oxidation is controlled by the rate of mass transfer because the reaction rate is much faster than the oxygen transfer rate. The mass transport rate can be determined as long as the overall rate is measured. Notably, the experimental results depend upon the purity of sulphite solutions more or less. De Waal (1996) stated that the results are influenced by the different batches of sodium sulphite[32]. A complex function is presented between the reaction rate and the catalyst concentration and the operational conditions that must be controlled in order to obtain reproducible measurements [33].

The experimental procedure is as follow: fill the bioreactor with a 1 mol L^{-1}sodium sulfite solution containing 10^{-3} mol L^{-1} 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, agitation 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 ($Na_2S_2O_3$ 0.3 mol L^{-1}) to a starch indicator end point. The reactions are expressed as:

$Na_2SO_3+I_2+H_2O \rightarrow Na_2SO_4+2HI$
$I_2+2NaS_2O_3 \rightarrow 2NaI+Na_2S_4O_6$

The mass balance for the dissolved oxygen in the well-mixed liquid phase can be established as:
$dC/dt=OTR-OUR$ (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:

$OTR=k_La(C^*-C)$ (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:
$dC/dt=OTR=k_La(C^*-C)$ (15)

As mentioned above, the reaction is fast, so C is assumed to be zero. Therefore,
$dC/dt=k_LaC^*$ (16)

Once the sulfite concentration is measured versus time, the rate of sulfite consumption is determined and k_La may be calculated from:
$-dC_{Na2SO3}/dt=2k_LaC^*$ (17)

then:
$k_La=-dC_{Na2SO3}/dt/2C^*$ (18)

where C^* can be obtained from the aid of Henry's law, which is shown below:
$H=y_{O2}P\sum C_i/C^*$(19)
Namely,
$C^*=y_{O2}P/H/\sum C_i$ (20)

where H is Henry's coefficient (in bar), which is 1.116 MPa mol^{-1} m^{-3} using the method of Perry's chemical engineers' handbook[34] in the condition that the concentration of the Na_2SO_3 solution at 32□ is 1 mol L^{-1}, $y_{O2}$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^{-1}).

Therefore, k_La is calculated by the following Equation:

$$k_L a = -\frac{dC_{Na_2SO_3}}{2dt} \bullet \frac{\sum C_i H}{y_{O_2} P} \quad (21)$$

RESULTS AND DISCUSSION

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.

According to this correlation, let Q and V_L keep constant:

$k_La=K_NN^y$ (22)
where,

$K_N=KQ^xV_L^{-y/3.15}$ (23)
Take the logarithm on both sides of the Equation (22), this gives:

$\ln(k_La)=\ln K_N+y\ln N$ (24)
Meanwhile, $N$and V_L were kept constant:

$k_La=K_QQ^x$ (25)
where,

$K_Q=KN^yV_L^{-y/3.15}$ (26)
Then take the logarithm on the Equation (25), this gives:

$\ln(k_La)=\ln K_Q+x\ln Q$ (27)

Therefore, for the 12.8 L fermenter, the correlation factors, x and y, can be obtained first through the linear regression of the logarithmic plot shown in Figure 1(a) and 1(b), $\ln(k_La)$ *vs.* $\ln Q$ and $\ln(k_La)$*vs.*$\ln Q$. The estimated values of x and y were 0.4613 and 1.5131 respectively (R^2 is 0.9971, 0.9969 in each plot). For the 30 L fermenter, the correlation factors, x and y, shown in the figure 1(c) and 1(d), were 0.525 and 1.685, respectively (R^2 is 0.9930, 0.9979 for each). 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.

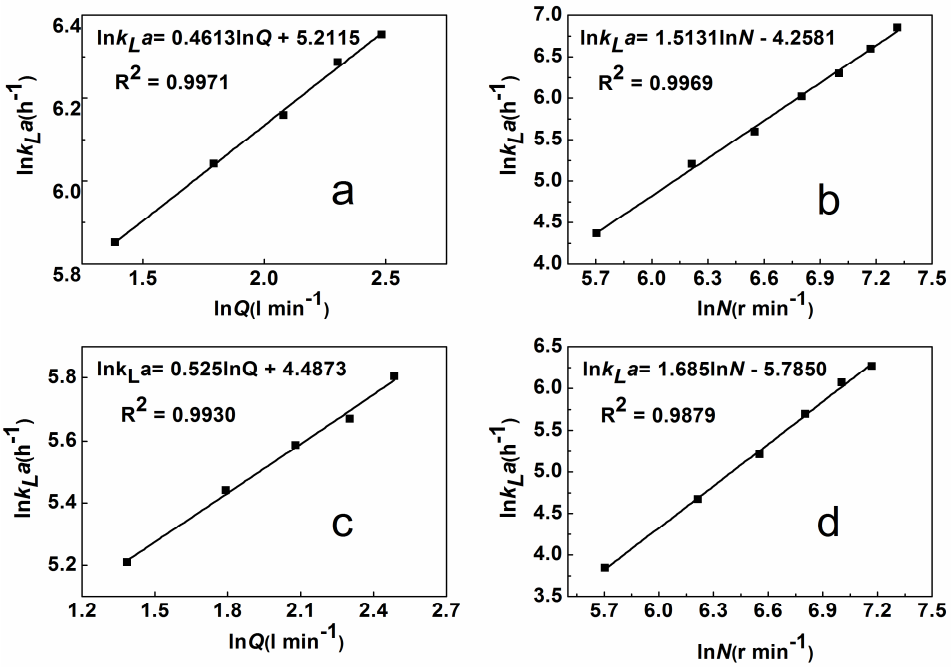

Fig. 1 Dependence of ln(k_La) on the value of ln$Q$ and ln$N$ respectively

The logarithmic plots in Figure 1 show the dependence of k_La on the gas flow rate and stirrer speed. It was found that the gas flow rate and steer speed in the 30 L vessel have higher influence compared to the 12.8 L one's. However, the slopes of the trend achieved are similar in both scales of operation.

Then, the constants K, as estimated through submitting the values x, y, K_Q and$K_N$in the Equation (23) and (26), were 0.01 and 0.004 for 12.8 L and 30 L each.

The determination of the initial volume
In order to achieve the peak of fertility of *E. coli* in fed-batch mode for the lab-scale and pilot-scale, the maximum capacity of the Equipment would be necessary to be employed. Therefore, for the 12.8 L bioreactor, the impeller speed is 1500 r min^{-1}, the gas flow rate is 18 L min^{-1} and the optimized initial culture volumes are 6 L, which can calculate the value of k_La is 982.5 h^{-1}. And for the 30 L one, the maximum impeller speed and the gas flow rate are

1400 r min^{-1} and 12 L min^{-1}, respectively. Then the initial culture volumes for the 30 L bioreactor could be obtained as 15.4 L on the basis of the product $p_* k_L a$ as a constant which is because the fermentation is highly oxygen-dependent process through scaling up.

The effects of scaling up from 12.8 L to 30 L

The cultivation profile for the 12.8 L scale with the probing feeding strategy is presented in Fig. 2, including the batch growth stage prior to feeding (0-5 h). Biomass accumulated to 80.13 g L^{-1} DCW with no obvious acetate accumulation being observed. During the initial batch stage, acetate accumulated to above 0.7 g L^{-1} but was re-consumed, and a biomass level of approximately 19.8 g L^{-1} DCW was reached prior to feeding. The specific cell growth rate was maintained at about 0.15-0.2 h^{-1} at the initial phase of fed-batch culture until DO reaches a minimum acceptable value (measured as the dissolved oxygen tension). After 10 hours later, the cells were implemented inductionat the middle logarithmic growth phase and the specific cell growth rate was maintained about 0.05 h^{-1} upon this phase. The final HLC was increased to 13.1 g L^{-1} during the induced cultivation.

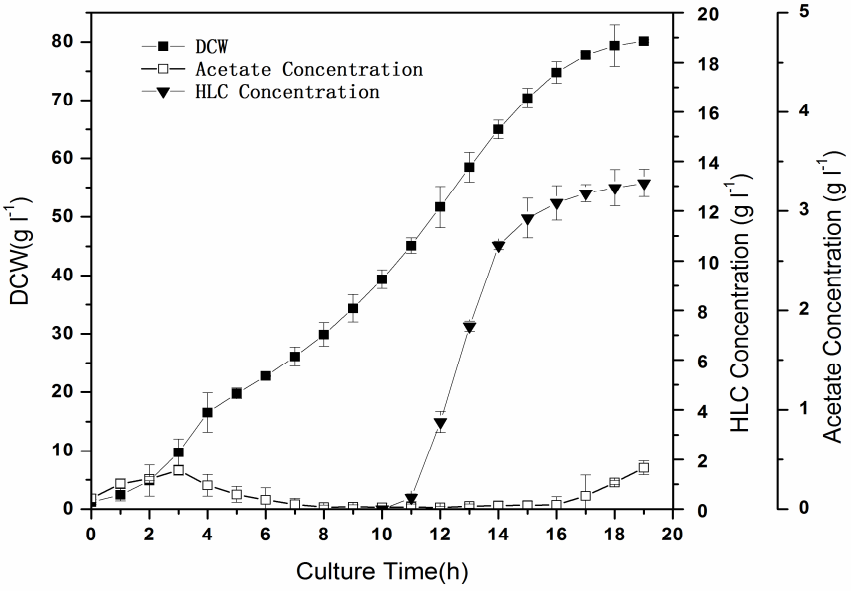

Fig. 2 Characteristics of fermentation of recombinant *E. coli* containing HLC cDNA with the probing feeding strategy on lab-scale

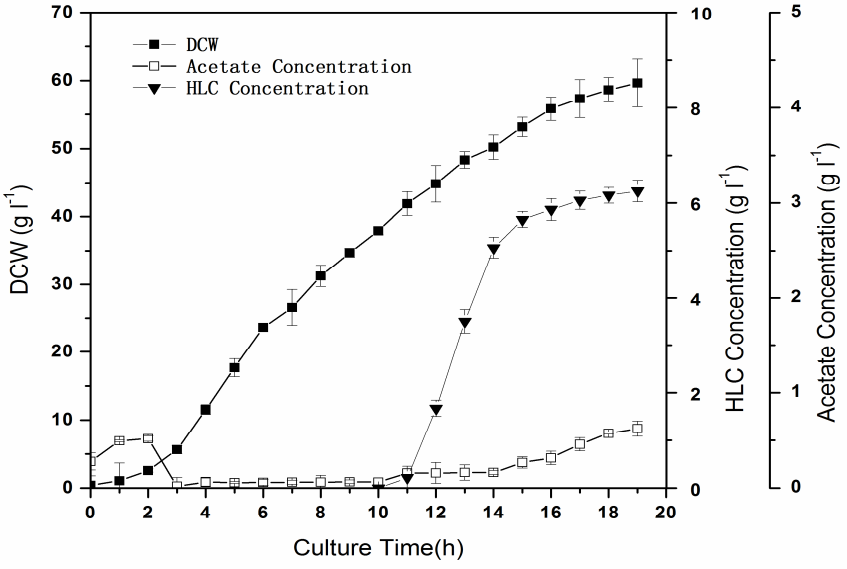

Fig. 3Characteristics of fermentation of recombinant *Escherichia coli* containing HLC cDNA with the probing feeding strategy on pilot-scale

Similarly, the profile for the 30 L scale fermentation when using the new model and maintaining p_*k_La as a constant is presented in fig 3. As shown below, the time courses of dry-cell mass differed slightly compared to the data measured in the lab-scale fed-batch process: on the 30 L scale the acetic acid concentration was higher. The difference may be caused by the step of preparation for inoculum. The final DCW of 59.7 g L^{-1} and the production of HLC of 6.25 g L^{-1} were obtained, which was lower than that obtained on lab-scale. Meanwhile, the acetic acid was higher in the induction phase. The reason led to these results would probably ascribe to the lower oxygen transfer capacity on 30 L scale. This can be explained by the fact that cells at the top of fermenter are exposed to excess glucose concentrations and simultaneously suffer from oxygen limitations, whereas those at the bottom are exposed to glucose starvation. An amount of glucose concentration that is more than necessary and a simultaneous oxygen limitation result in acetate overproduction [35-37].

CONCLUSION

The aim of this work was to provide a simple but rational mathematicalmodel for translating an optimized lab-scale fermentation performance to a pilot-scale successfully. The correlation, which could be used to design a new higher scale bioreactor, relates k_La to the key operational 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 in measuring k_La[11]. Being intuitionistic and liable to interalated, 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 etc) which are crucial for the feeding stage will probably bring out a novel approach to create a intelligent controller that can be manipulated automatically in the process of fermentation.

As clearly evident from the results of this study, in maintaining a constant value of p^*k_La upon scale-up from 12.8 L to 30 L at same policy, similar trend of growth and productivity were successfully achieved. A simple protocol for scaling-up exercise in the stirred aerated bioreactors was developed.

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REFERENCES

[1] Garcia-Ochoa F., and Gomez E., "Bioreactor scale-up and oxygen transfer rate in microbial processes: an overview", *Biotechnol. Adv., 27*, 153(**2009**).
[2] Dunn I. J., E. Heinzle, J. Ingham, and PrenosilJ. E.,"Biological reaction engineering. 2nd ed.", pp 117-140 (**1992**).
[3] BerovičM. "Scale-up of citric acid fermentation by redox potential control", *Biotechnol. Bioeng. , 64*, 552-557(**1999**).
[4] Flores E. R., F. Pérez, and de la Torre M., "Scale-up of*Bacillus thuringiensis* fermentation based on oxygen transfer", *J. Ferment. Bioeng., 83*, 561-564 (**1997**).
[5] Gill N. K., M. Appleton, F. Baganz, and Lye G. J., "Quantification of power consumption and oxygen transfer characteristics of a stirred miniature bioreactor for predictive fermentation scale-up", *Biotechnol. Bioeng., 100*, 1144-1155 (**2008**).
[6] Garcia-Ochoa F., and Gomez E., "Oxygen Transfer Rate Determination: Chemical, Physical and Biological Methods",*Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology* (**2010**).
[7] Farrell P., J. Sun, M. Gao, H. Sun, B. Pattara, A. Zeiser, D'Amore T.,"Development of a scaled-down aerobic fermentation model for scale-up in recombinant protein vaccine manufacturing", *Vaccine*.30(38), 5695-5698(**2012**).
[8] Shin W. S., D. Lee, S. Kim, Y.S. Jeong, and Chun G.T.,"Application of Scale-up Criterion of Constant Oxygen Mass Transfer Coefficient (k_La) for Production of Itaconic Acid in a 50L Pilot-scale Fermentor by Fungal Cells of Aspergillus terreus" *J. Microbiol. Biotechnol.,* (**2013**).
[9] Danckwerts P. V., and Gillham A. J., "The design of gas absorbers, I-methods for predicting rates of absorption with chemical reaction in packed columns, and tests with 11/2 in. Raschig rings", *Trans. Inst. Chem. Eng., 44*,42-54 (**1966**).

[10] John G.T., I. Klimant, C. Wittmann, and Heinzle E., "Integrated optical sensing of dissolved oxygen in microtiter plates: a novel tool for microbial cultivation", *Biotechnol. Bioeng.*, 81, 829-836 (**2003**).
[11] Bandyopadhyay B., A. E. Humphrey, H. Taguchi, and Rao G., "Dynamic measurement of the volumetric oxygen transfer coefficient in fermentation systems",*Biotechnol. Bioeng., 104*, 841-853 (**2009**).
[12] Ju L. K.,and Chase G. G., "Improved scale-up strategies of bioreactors", *Bioprocess. Eng. 8*: 49-53 (**1992**).
[13] Duetz W. A., L. Rüedi, R. Hermann, K. O'Connor, J. Büchs, and Witholt B., "Methods for intense aeration, growth, storage, and replication of bacterial strains in microtiter plates", *Appl. Environ. Microbiol., 66*, 2641-2646 (**2000**).
[14] Carbajal R.I., and Tecante A., "On the applicability of the dynamic pressure step method for $k_La$determination in stirred Newtonian and non-Newtonian fluids, culture media and fermentation broths", *Biochem. Eng J., 18*, 185-192 (**2004**).
[15] Hill G. A., "Measurement of overall volumetric mass transfer coefficients for carbon dioxide in a well-mixed reactor using a pH probe", *Ind. Eng. Chem. Res., 45*, 5796-5800 (**2006**).
[16] Scargiali F., A. Busciglio, F. Grisafi, and Brucato A., "Simplified dynamic pressure method for k_La measurement in aerated bioreactors",*Biochem. Eng. J.,49*, 165-172 (**2010**).
[17] Costa E., A. Lucas, J. Aguado, and AvilaJ. A., "Transferencia de materia en tanques agitados: burbujeo de gases en líquidos newtonianos y no newtonianos. I. Turbinas de 6 paletas y difusor plano", *An. Quim., 78*, 387-92 (**1982**).
[18] Schlüter V., and Deckwer W. D., "Gas/liquid mass transfer in stirred vessels", *Chem. Eng. Sci.,47*, 2357-2362 (**1992**).
[19] Garcia-Ochoa F., and Gomez E., "Mass transfer coefficient in stirred tank reactors for xanthan gum solutions", *Biochem. Eng. J.*, 1, 1-10 (**1998**).
[20] Vilaça P. R., J. A. C. Badino, M. C. R. Facciotti, and Schmidell W., "Determination of power consumption and volumetric oxygen transfer coefficient in bioreactors",*Bioprocess. Eng. 22*, 261-265 (**2000**).
[21] Doig S. D., S. C. Pickering, G. J. Lye, and BaganzF., "Modelling surface aeration rates in shaken microtitre plates using dimensionless groups",*Chem. Eng. Sci.,60*, 2741-2750 (**2005**).
[22] Mohamed M. S., R. Mohamad, R. N. Ramanan, M. A. Manan, and Ariff A. B., "Modeling of oxygen transfer correlations for stirred tank bioreactor agitated with atypical helical ribbon impeller", *Am. J. Appl. Sci.,6*, 848 (**2009**).
[23] Ju L. K.,"Water-in-OilCultivation Technology for Viscous Xanthan Gum Fermentation",*Bioprocessing for Value-Added Products from Renewable Resources:New Technologies and Applications*, 397(**2011**).
[24] Yu H., and Tan Z.,"New Correlations of Volumetric Liquid-Phase Mass Transfer Coefficients in Gas-Inducing Agitated Tank Reactors",*Int. J. Chem. Reactor. Eng., 10* (**2012**).
[25] Luo Y. E., D. D. Fan, and Ma X. X., "Process control for production of human-like collagen in fed-batch culture of *Escherichia coli* BL21",*Chin. J. Chem. Eng., 13*, 276-279 (**2005**).
[26] Fan D. D., Y. E. Luo, Y. Mi, X. X. Ma, and Shang L. A., "Characteristics of fed-batch cultures of recombinant *Escherichia coli* containing human-like collagen cDNA at different specific growth rates", *Biotechnol. Lett. 27*, 865-870 (**2005**).
[27] Åkesson M., P. Hagander, and AxelssonJ. P., "Avoiding acetate accumulation in *Escherichia coli* cultures using feedback control of glucose feeding", *Biotechnol. Bioeng.73*, 223-230 (**2001**).
[28] Duan M. R., Y. Mi, and Fan D. D., "Kinetics of recombinant *E. coli* expressing human-like collagen fermentation", *Chem. React. Eng.Technol., 18*, 238-243 (**2002**).
[29] Yin M. W., Y. M. Nan, and Wang X. M., "Improvement of the spectrophotometric method for the determination of hydroxyproline",*Henan. Med. Univ., 1* (**1994**).
[30] Doran P. M., "Bioprocess engineering principles", *Academic Press* (**1995**).
[31] Cooper C. M., G. A. Fernstrom,and MillerS. A., "Performance of agitated gas-liquid contactors", *Ind. Eng. Chem., 36*, 504-509 (**1944**).
[32] De Waal K. J. A., and OkesonJ. C., "The oxidation of aqueous sodium sulphite solutions", *Chem. Eng. Sci., 21*, 559-572 (**1966**).
[33] Linek V., and Vacek V., "Chemical engineering use of catalyzed sulfite oxidation kinetics for the determination of mass transfer characteristics of gas-liquid contactors", *Chem. Eng. Sci., 36*, 1747-68 (**1981**).
[34] Perry R. H., and D. W. Green, and Maloney J. O., "*Perry's chemical engineers' handbook"*(Vol. 7), New York: McGraw-Hill (**2008**).
[35] Xu B., M. Jahic, G. Blomsten, and O.Enfors S.,"Glucose overflow metabolism and mixed-acid fermentation in aerobic large-scale fed-batch processes with *Escherichia coli", Appl. Microbiol. Biotechnol.,51*, 564-571 (**1999**).
[36] Bylund F., A. Castan, R. Mikkola, A. Veide, and Larsson G., "Influence of scale-up on the quality of recombinant human growth hormone", *Biotechnol. Bioeng., 69*,119-128 (**2000**).
[37] Castan A., and Enfors S. O., "Formate accumulation due to DNA release in aerobic cultivations of Escherichia coli", *Biotechnol. Bioeng., 77*, 324-328 (**2002**)