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Application of Scale-Up Criterion of Constant Oxygen Mass Transfer Coefficient (k_La) for Production of Itaconic Acid in a 50 L Pilot-Scale Fermentor by Fungal Cells of *Aspergillus terreus*

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The scale-up criterion of constant oxygen mass transfer coefficient (k_La) was applied for the production of itaconic acid (IA) in a 50 L pilot-scale fermentor by the fungal cells of *Aspergillus terreus*. Various operating conditions were examined to collect as many k_La data as possible by adjusting the stirring speed and aeration rate in both 5 L and 50 L fermentor systems. In the fermentations performed with the 5 L fermentor, the highest IA production was obtained under the operating conditions of 200 rpm and 1.5 vvm. Accordingly, we intended to find out parallel agitation and aeration rates in the 50 L fermentor system, under which the k_La value measured was almost identical to that (0.02 sec^{-1}) of the 5 L system. The conditions of 180 rpm and 0.5 vvm in the 50 L system turned out to be optimal for providing almost the same volumetric amount of dissolved oxygen (DO) into the fermentor, without causing shear damage to the producing cells due to excessive agitation. Practically identical fermentation physiologies were observed in both fermentations performed under those respective operating conditions, as demonstrated by nearly the same values of volumetric (Q_p) and specific (q_p) IA production rates, IA production yield ($Y_{p/s}$), and specific growth rate (μ). Specifically, the negligible difference of the specific growth rate (μ) between the two cultures (*i.e.*, 0.029 h^{-1} *vs.* 0.031 h^{-1}) was notable, considering the fact that μ normally has a significant influence on q_p in the biosynthesis of secondary metabolites such as itaconic acid.

Keywords: *Aspergillus terreus*, itaconic acid, fungal fermentation, oxygen mass transfer rate coefficient (k_La), scale-up

Introduction

Itaconic acid (IA) (methylene butanedioic acid; common synonyms: methylene succinic acid, 3-carboxy-3-butanoic acid, propylenedicarboxylic acid) is an unsaturated dicarboxylic acid produced as a secondary metabolite by the fungal cells of *Aspergillus terreus* [29]. It has been reported that IA is biosynthesized *via* decarboxylation of *cis*-aconitate produced in the normal tricarboxylic acid cycle reactions [10]. IA has been used as a copolymer in acrylic resins and efficient commercial polymeric materials of and its derivatives in medicine and cosmetic preparations

[31]. Recently, polyitaconic acid, a water-soluble polymer, has been proven to be an attractive replacement to the well-established petroleum-based polyacrylic acid, with a wide range of applications, including super absorbents, anti-scaling agents in water treatments, co-builders in detergents, and dispersants for minerals in coatings [20]. Notably, in 2004, IA was identified by the Department of Energy of USA as one of the top 12 value-added chemicals to be produced from biomass, based on the growing concerns regarding sustainability, environmental conservation, renewable resources, and rising energy costs [30].

Secondary metabolites such as IA are usually formed

under obligate aerobic conditions. It is well known that the solubility of oxygen in aqueous solutions under 1 atm of air is extremely low, which is in the order of 10 parts per million near the ambient temperature, resulting in only a small reservoir in solution available for the producing cells [8]. In particular, the gas-liquid oxygen mass transfer rate (OTR) has been shown to become significantly diminished during mycelial growth in suspended cultures, due to the three-dimensional structure of the filamentous organisms, which imparts very high non-Newtonian viscosities to the fermentation broth [13, 27]. As a consequence, the availability of oxygen for microbial use depends not only on the solubility, but also on the mass transfer rates of this gas in the fermentation broth. Since the synthesis of secondary metabolites is generally considered to depend heavily on the energy generated through primary metabolism [22, 28], it is quite natural to find that many published reports have emphasized the importance of control of oxygen supply rate to a bioreactor system in secondary metabolite fermentations [1, 8, 19, 24].

Effective scale-up is essential for the successful production of IA in industrial-scale fermentors. Once a particular bioprocess is accomplished successfully in lab-scale experiments, the bioprocess is then usually carried out in a number of bioreactors of increasing scale (normally the scale-up ratio of 1:10), the final process optimization being performed at pilot plant scale (50 L to 300 L fermentor volumes) where the operational conditions and the hydrodynamic and mixing are very similar to those used industrially on the production scale. Scale-up of the fermentation process can be done successfully by applying the rules of thumb method. In this method, the following scale-up criterion and percentage of each criteria are normally used in the fermentation industry: constant specific power input (P/V) (30% of use); constant volumetric mass transfer coefficient (k_La) (30%); constant impeller tip speed of the agitator or shear (20%); and constant dissolved oxygen (DO) concentration (20%) [18]. The different scale-up criteria could result in different process conditions on a production scale. It is well known that the influence of oxygen gas-liquid mass transport is the most significant factor for the scale-up of aerobic fermentations. Therefore, scale-up in aerobic fermentation is frequently carried out on the basis of keeping the volumetric oxygen mass transfer coefficient (k_La) constant in different sized fermentors [7, 11, 15, 26].

Despite the detailed knowledge of the enzymes involved in the biosynthetic pathway of IA [20] and basic fermentation studies [2], there is still no published information regarding the engineering aspects of fermentation scale-up

for IA production. Owing to the altered catalytic properties often caused by different fermentation environments, filamentous fungal cells usually exhibit a different physiology and morphology during the scale-up process [9]. In this paper, we propose to apply the scale-up criterion of constant oxygen mass transfer coefficient (k_La) for the successful production of IA in a 50 L pilot-scale fermentor system by the filamentous fungal cells of *Aspergillus terreus*. As an initial step, several bioreactor cultures were carried out in a 5 L stirred-tank fermentor with various agitation speeds, in order to investigate the effect of DO level on the IA production capability of the producing cells. The resulting key fermentation parameters such as specific IA production rate (q_p), IA production yield coefficients ($Y_{p/s}$), and specific growth rate (μ) were quantified and then systematically analyzed. For scale-up of the IA fermentation process, the strategy was adopted to provide almost equivalent oxygen mass transfer coefficient (k_La) to the different-sized fermentor systems (5 L lab-scale and 50 L pilot-scale fermentors), thus ensuring to supply almost the same amount of DO into each fermentation broth. For this purpose, various operating conditions were examined in order to collect as many k_La data as possible by independently adjusting the agitation speed and aeration rate in each fermentor system. In the end, these results were utilized for effective scale-up of the fermentation process, with the purpose of successful production of IA in the 50 L pilot-scale fermentor.

Materials and Methods

Culture Media

Solid agar medium, growth medium (GM), and production media (PM) were used in this study. The GM, which was able to support high cell growth, was a complex medium composed of glucose (55 g/l), corn steep liquor (3 g/l), NH_4NO_3 (5 g/l), $MgSO_4$ (4 g/l), and KH_2PO_4 (0.5 g/l). For the production of IA, a sucrose-based production medium, optimized statistically using response surface methodology (RSM) in our laboratory, was utilized. The sucrose-based PM contained sucrose (100 g/l), yeast extract (2 g/l), $(NH_4)_2SO_4$ (3 g/l), $MgSO_4$ (0.5 g/l), KH_2PO_4 (0.5 g/l), PEG (5 ml/l), and 1.0 (ml/l) of a trace metal solution. The trace metal solution contained 8.8 mg/l $ZnSO_4 \bullet 7H_2O$, 0.1 mg/l $MnCl_2 \bullet 4H_2O$, 0.005 mg/l Na_2MoO_4, 0.4 mg/l $CuSO_4 \bullet 5H_2O$, 0.2 mg/l $FeSO_4 \bullet 7H_2O$ and 1 L of water. The pHs of the production media were initially adjusted to 2.1 using 2 N HCl. PDA medium containing 20 g/l of agar was utilized as a solid agar medium for a large production of spores used as inoculums into a flask growth-culture.

Strain and Cultivations

IA high-yielding mutants of *Aspergillus terreus* (R104 strains) were used in this study, which was improved through rational

screening strategies in our laboratory, and have the features of resistance to various anti-metabolites such as nystatin and/or arsenate. To minimize variation of the activity of the high-yielding producer, the following preservation method was adopted: 5 ml of 1.5 day old liquid culture, which was cultivated in GM, was transferred to a 30 ml vial containing a sterile mixture of 2.5 ml of glycerol and 2.5 ml of distilled water. After thoroughly mixing the solution, it was stored in a –121°C freezer.

For the preparation of enough amount of inoculums, 5 ml of spore suspension (1×10^9 spores/ml) scraped from the PDA solid agar slant using aliquots of glycerol solution was transferred into 40 ml of the sterile GM (5% (v/v)) in a 250 ml Erlenmeyer flask. The pH of GM was initially adjusted to 5.0 with KOH (1 N) before sterilization. The flask was then incubated on a rotary shaker at 37°C and 230 rpm for 2 days. At this stage, the cell mass was approximately 13 g DCW/l. Then the flask seed was transferred into a 5 L production fermentor for a final production-culture (5% (v/v) inoculums), and/or into a 5 L growth fermentor for another growth-culture (5% (v/v) inoculums). This 5 L growth-culture cultivated for 2 days was then used for inoculation (5% (v/v)) into a final 50 L production fermentor. Both the lab-scale (5 L) and the pilot-scale (50 L) production-cultures were performed at 34°C for 5~6 days at various aeration rates and agitation speeds, as specified separately in the section of Results and Discussion. The foam was controlled by automatic addition of antifoam SAG 471 (Schering Plough, USA) *via* an antifoam controller equipped in the fermentor system.

Specifications of 5 L Lab-Scale and 50 L Pilot-Scale Fermentors

Lab-scale fermentations for IA production were carried out in a 5 L lab-scale fementor (Best Korea, South Korea), and the scale-up fermentations were run in a 50 L pilot-scale fermentor (KBT, South Korea). The specification of each fermentor system is as follows:

- For the 5 L fermentor: top-driven; type of impeller, turbine of 1 mm thickness; number of impellers, 2; number of blades, 6; liquid height, 155 mm; tank diameter, 165 mm; impeller diameter, 85 mm; type of sparger, ring type with 12 holes of 78 mm diameter; number of baffles, 3 of 14 mm width; working volume, 3.5 L.
- For the 50 L fermentor: bottom-driven; type of impeller, turbine of 2 mm thickness; number of impellers, 2; number of blades, 6; liquid height, 485 mm; tank diameter, 300 mm; impeller diameter, 133 mm; type of sparger, ring type with 12 holes of 120 mm diameter; number of baffles, 3 of 30 mm width; working volume, 35 L.

Both systems were equipped with the controllers for agitation speed, temperature, pH, and DO. On-line computer monitoring and control of the fermentation process were carried out using a fermentation software program (Autolab-LK930A from Lokas, South Korea) installed in each system. The DO level of the fermentation broth was adjusted to 100% before inoculation, using air as the inlet gas at the fermentation temperature and

pressure. An electric zero was used for the calibration at 0% saturation. The electric zero calibration was confirmed by exchanging air with pure nitrogen gas. A polarographic type DO probe (Mettler-Toledo, Switzerland) with replaceable membrane, which is connected to a DO analyzer of the computer-controlled fermentor system, was used. The pH value of the culture solution was measured by means of a sterilizable combination electrode (Mettler-Toledo, Switzerland). For the 50 L fermentor system, the supplied air, exhaust pipes, and other parts were sterilized *in situ*. The culture vessel and broth were sterilized by a steam-heated jacket. The temperature of the 50 L tank (operating temperature and sterilization temperature) was controlled by means of a pressurized closed-loop hot water system.

Measurement of Cell Growth and Analysis of Itaconic Acid

Approximately 30 ml of broth sample was obtained from the bioreactors at every sampling time. Two 10 ml each of sample(s) were taken for dry cell mass (DCW) determination, and the other 10 ml sample was used for IA analysis by HPLC. For measurement of DCW, the culture broth was filtered through filter paper (Whatman No. 1, USA). Wet mycelia on the filter paper were washed twice with 0.85% saline water and then with distilled water to completely remove the residual nutrients. Then, the washed pellet was dried at 90°C for 12 h to obtain a constant DCW. For measurement of IA secreted into the culture broth, the 10 ml sample was centrifuged and then 1 ml of the supernatant was filtered through a 0.2 mm filter paper (Millipore, USA) and transferred to a microtube for HPLC injection. Authentic IA standard was purchased from Sigma-Aldrich Chemical, Inc. (USA), and the following conditions were used for analysis of IA by HPLC:

Column: C18 reverse phase-column (4.6 × 250 mm) (Kanto, Japan) at 35°C
Mobile phase: 2% acetonitrile
Detector: M 720 UV detector (Young-Lin Instrument, South Korea), 254 nm
Pump and flow rate: M930 (Young-Lin Instrument, South Korea), 1.2 ml/min

Determination of Volumetric Oxygen Mass Transfer Coefficient in 5 L and 50 L Fermentor Systems

For determining the volumetric oxygen mass transfer coefficient (k_La) in the 5 L and 50 L fermentor systems, a static gassing-out method was used [8]. Oxygen was stripped from the liquid medium by purging with inert nitrogen gas. Time-course saturation of DO was then monitored as the air supply and stirring conditions were resumed (Fig. 1). The liquid balance of DO during this short time period can be expressed as follows:

$$dC_L/dt = k_La\,(C_L^* - C_L)$$

where k_L is the liquid film oxygen transfer coefficient (cm/h), a is the gas-liquid interfacial area per unit volume of liquid (cm²/cm³), and C_L^* and C_L are the saturation and local dissolved oxygen
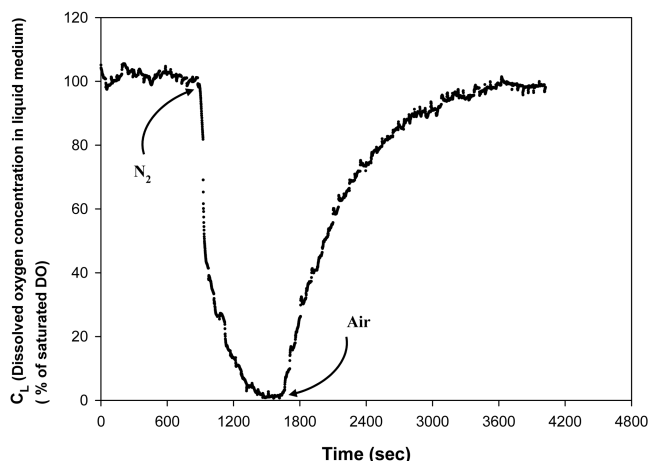
Fig. 1. Static gassing-out method for determining the volumetric oxygen mass transfer coefficient ($k_L a$) in the fermentor system. Stripping of dissolved oxygen from the liquid medium was done by purging with inert nitrogen gas and then resuming the air supply. Time-course profiles of dissolved oxygen were then monitored as the air supply and stirring conditions were resumed.

Fig. 2. Determination of the $k_L a$ value from the slope of the linear plot of the equation, $\ln((C_L^*-C_{L1})/((C_L^*-C_{L2})) = k_L a\,(t_2-t_1)$.
This graph shows the experimental data for estimating $k_L a$ values according to various aeration rates (vvm) at a fixed agitation speed of 180 rpm in a 50 L pilot-scale fermentor system.

concentrations in the liquid medium (mmol/l), respectively. Assuming $k_L a$ and C_L^* are constant during the process, integration of the above equation gives the following equation:
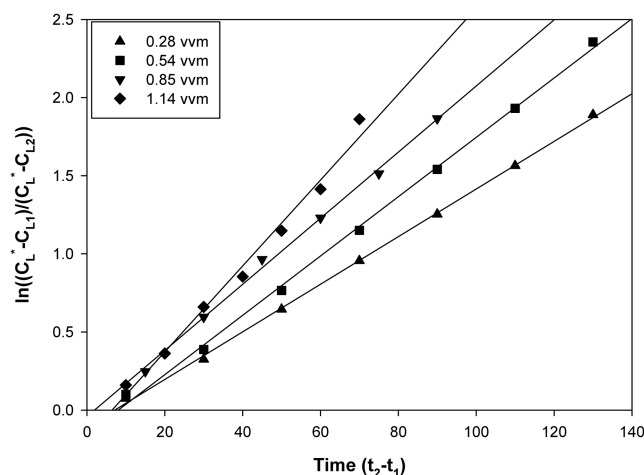
$$\ln((C_L^*-C_{L1})/((C_L^*-C_{L2})) = k_L a\,(t_2-t_1)$$

where C_{L1} and C_{L2} are the local dissolved oxygen concentrations in the liquid medium (mmol/l) at t_1 and t_2, respectively. Consequently, the slope of this equation can be regarded as the volumetric oxygen mass transfer coefficient ($k_L a$). Fig. 2 reveals the accuracy of the static gassing-out method used in this study for the determination of $k_L a$ according to various agitation speeds (rpm), as demonstrated by the linear relationship and the y-axis value of zero for all the stirring conditions.

Results and Discussion

Influence of Agitation Speed on Itaconic Acid Production

It is well known that the microbial physiology of filamentous fungal cells is significantly influenced by the DO level in suspended cultures [5, 16, 17, 32]. It was suggested that the critical DO level for fungal cells in culture should be greater than around 20% of the saturated DO value. In this study, in order to investigate the effect of DO on IA production by the mutant cells of *A. terreus* (R104 strain), 5 L bioreactor cultures were performed for 108 h under various agitation speeds (100, 150, 200, and 250 rpm), but with a constant aeration rate of 1 vvm. In the case of DO-limited cultures, where agitation speeds were kept at 100 and 150 rpm respectively, DO levels were

rapidly exhausted at around 36 h in both fermentations. On the contrary, DO levels were maintained above 20% in the cultures of 200 and 250 rpm agitation throughout the whole incubation period owing to facilitated oxygen mass transfer (data not shown). Summarized in Table 1 are the major fermentation parameters obtained under the various agitation speeds as specified above. Profound differences in the fermentation physiology can be seen, when the specific IA production rate (q_p) of each fermetation is compared. The value of q_p for the 200 rpm culture was approximately 1.95-fold and 1.37-fold higher, respectively, than those for the parallel cultures of DO-limitation (*i.e.*, 100 and 150 rpm cultures). A strong positive effect of the optimal supply of oxygen on IA production could be also inferred by referring to $Y_{p/x}$ (IA production yield on DCW) and Q_p (volumetric IA production rate). In summary, a greater portion of the available carbon source(s) appeared to be utilized for IA biosynthesis rather than for cell growth and cell maintenance in the optimally DO-controlled fermentations. In general, it is well known that secondary metabolites such as IA are produced through complex anabolic pathways, requiring a sufficient amount of ATP (and thus O_2) for their biosynthesis [6, 28]. Our group also observed that IA productivity by the recombinant strain of *A. terreus* transformed with a bacterial (*Vitreoscilla*) hemoglobin (*vhb*) gene was 37% higher compared with the parallel non-transformed strain, revealing the importance of DO availability for the enhanced production of IA [25].

Table 1. Comparison of fermentation parameters obtained in the 5 L lab-scale fermentations performed with the mutant cells of *A. terreus* R104 strain.

| Fermentations	P_f	X_f	$Y_{p/x}$	Q_p	q_p
A (100 rpm)	25.2	12.5	2.02	0.263	0.021
B (150 rpm)	35.5	12.4	2.86	0.370	0.030
C (200 rpm)	48.3	12.3	3.93	0.503	0.041
D (250 rpm)	30.1	12.1	2.49	0.314	0.026

Four sets of comparative fermentations were carried out for 96 h under the agitation speeds of 100, 150, 200, and 250 rpm, respectively. An aeration rate of 1 vvm was used for all the cultures.

A, 100 rpm and 1 vvm culture; B, 150 rpm and 1 vvm culture; C, 200 rpm and 1 vvm culture; D: 250 rpm and 1 vvm culture; P_f, Maximum IA production (g IA/l); X_f, Maximum dry cell weight (g DCW/l); $Y_{p/x}$, Specific IA production (g IA/g DCW); Q_p, Average volumetric IA production rate (g IA/l/h); q_p, Average specific IA production rate (g IA/g DCW/h).

At this moment, it should be emphasized that the fermentation parameters $Y_{p/x}$, Q_p, and q_p obtained at the excessive agitation speed (250 rpm) were 36.6%, 37.6%, and 36.5% lower, respectively, compared with those of the parallel 200 rpm culture, suggesting rather negative influences of the potential shear stress on IA biosynthesis. It has often been reported that as agitation speed increases excessively in order to supply sufficient oxygen into the fermentation broth, shear stress concurrently becomes large, thus influencing the physiology as well as morphology of the mycelial microorganisms such as filamentous fungi and *Streptomycetes* [4, 5, 16, 21]. Based on these results, we intended to find the optimal value(s) of the volumetric oxygen mass transfer rate coefficient ($k_L a$) in the stirred-tank bioreactor systems. In the end, these results were effectively utilized for successful scale-up of the IA fermentation process, as will be discussed in the later sections.

Comparison of Volumetric Oxygen Mass Transfer Coefficient (k_La) as a Function of Agitation Speed and Aeration Rate in 5 L and 50 L Fermentor Systems

The influences of physical and biological factors on the volumetric oxygen mass transfer coefficient ($k_L a$) were examined by investigating experimentally the effects of agitation speed and aeration rate in the 5 L and 50 L fermentor systems, respectively. These studies were undertaken for effective scale-up of the fermentation process, with the purpose of successful production of IA in a 50 L pilot-scale fermentor. As already mentioned, scale-up of aerobic fermentations is usually carried out on the basis of a constant oxygen mass transfer coefficient ($k_L a$) to ensure
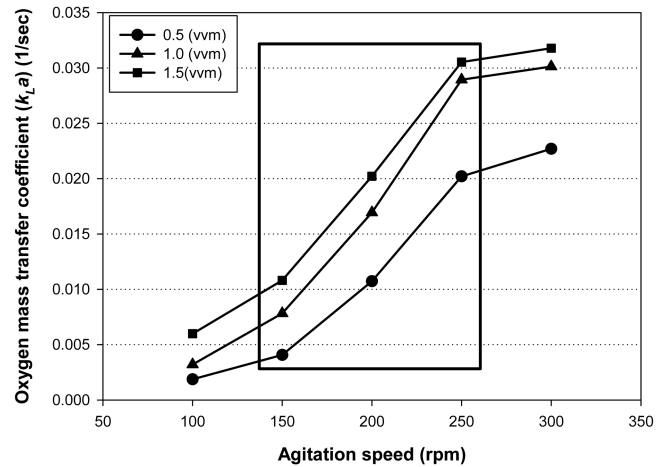
Fig. 3. Comparison of the oxygen mass transfer coefficient ($k_L a$) as a function of agitation speed (rpm) and aeration rate (vvm) in a 5 L lab-scale stirred-tank bioreactor system.

optimal supply of dissolved oxygen into a large fermentor system.

As shown in Fig. 3 and Fig. 4, $k_L a$ values were obtained as a function of the variation of agitation speed at various aeration rates (*i.e.*, 0.5, 1.0, and 1.5 vvm for the 5 L system (Fig. 3), and 0.28, 0.58, 0.85, and 1.14 vvm for the 50 L system (Fig. 4), respectively). The range of agitation speed examined in this experiment was between 100 and 300 rpm, and between 100 and 200 rpm for each system, respectively. The reason that the lower ranges of agitation and aeration rate were investigated in the 50 L system was due to the
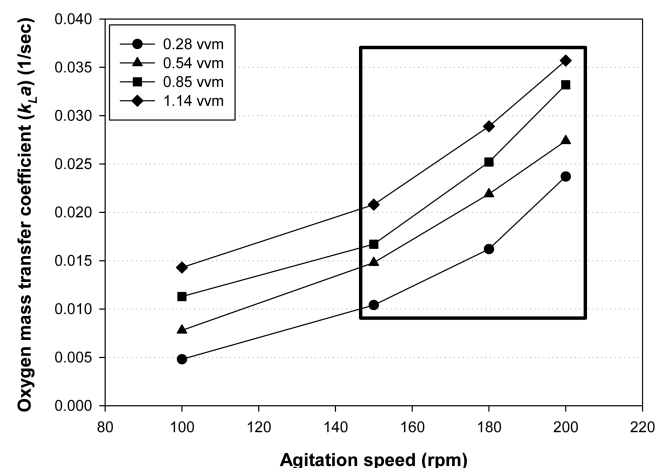
Fig. 4. Comparison of the oxygen mass transfer coefficient ($k_L a$) as a function of agitation speed (rpm) and aeration rate (vvm) in a 50 L pilot-scale stirred-tank bioreactor system.

substantial negative effects of high shear stress, which were observed when the 50 L pilot-scale fermentor was operated at above 200 rpm and 1.14 vvm (data shown below). In practice, it was assumed that the shear damages imposed on the producing mycelial cells at vigorous agitations could lead to morphological and physiological changes, leading to a decrease in IA productivity [17, 20].

The k_La measurement was performed by employing the static gassing-out method with no cells inside the bioreactors, as explained in detail in the Methods section. As revealed in Fig. 3, k_La values in the 5 L fermentor were relatively insensitive to the increase in aeration rate at above 1.0 vvm, and more insensitive to the increment of agitation speed at above 250 rpm. The lower sensitivity of k_La values at the higher aeration rates appeared to be partly attributable to the formation of large single bubbles and pronounced gas channeling, which might generate a low interfacial area and mean residence time. In this respect, Schügerl [23] explained that it was impossible to increase the aeration rate indefinitely owing to the flooding of the impellers with air, which decreased their dispersion efficiency. In their experiment with a bubble column reactor, Gbewonyo and Wang [12] observed large single bubbles surging through the center of the column at a high aeration rate (2 cm/sec) after a certain threshold cell concentration, thus promoting bubble coalescence in the column. These phenomena, however, were not observed in our 50 L system (Fig. 4), because all the experiments were carried out at relatively mild operating conditions (*i.e.*, below 1.14 vvm and 200 rpm), thus resulting in a steady increase in k_La values according to the increased agitation and aeration rates.

In Fig. 3 and Fig. 4, the higher sensitivity of k_La values on the agitation speed rather than on the aeration rate (boxed area) should be noted, as revealed by the steeper slope in each system. These results suggest that it was not possible to obtain sufficient increment in k_La through increase in aeration rate alone. Notably, much higher k_La values ranging from 0.0048 (1/sec) to 0.0357 (1/sec) were obtained in the 50 L system, when the agitation speed was varied from 100 to 200 rpm with various aeration rates (approximately 2.5-fold and 1.5-fold increases, respectively, as compared with the parallel k_La values of the 5 L system). The remarkable improvement of oxygen mass transfer capacity in the 50 L fermentor appeared to result from the different configurations of the impeller and air sparger systems as described in the Methods section. In summary, these results illustrate the importance of the role of power

input (rpm) for the enhancement in oxygen gas-liquid mass transfer rate.

5 L Lab-Scale and 50 L Pilot-Scale Fermentation Studies: Scale-Up Based on Oxygen Mass Transfer Coefficient (k_La)

In order to predict the results of a pilot-scale fermentation by using the data collected from a lab-scale fermentor, a careful analysis of the influences of operational conditions on the biological behavior in each scale system should be carried out. In this study, for Scale-up of the IA fermentation process from 5 to 50 L, the strategy was adopted to provide almost equivalent k_La values to each fermentor system, thus ensuring the same volumetric OTR into the respective fermentor. Fig. 5 shows the comparison of the k_La values of each system, as represented as functions of agitation speed and aeration rate. In the 5 L fermentations, the operational conditions of 200 rpm and 1.5 vvm were observed to show the highest IA productivity, as will be revealed below. Accordingly, we intended to find out the parallel agitation and aeration rates in the 50 L fermentor system, under which the k_La value measured was almost identical to that (0.02 sec^{-1}) of the 5 L system. As specified by the arrow in Fig. 5, the appropriate operating conditions for the 50 L system turned out to be around 180 rpm and 0.5 vvm. The reason for choosing the relatively mild operational conditions (*i.e.*, 180 rpm and 0.5 vvm) in
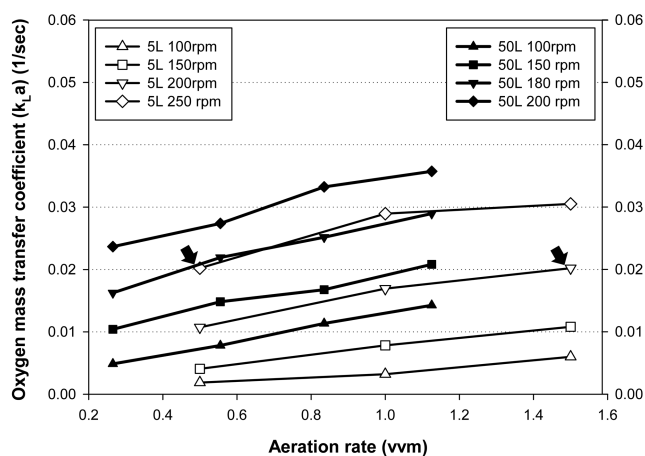
Fig. 5. Comparison of the oxygen mass transfer coefficient (k_La) between the 5 L lab-scale and the 50 L pilot-scale stirred-tank bioreactor systems.

The k_La values were estimated as a function of agitation speed (rpm) and aeration rate (vvm).
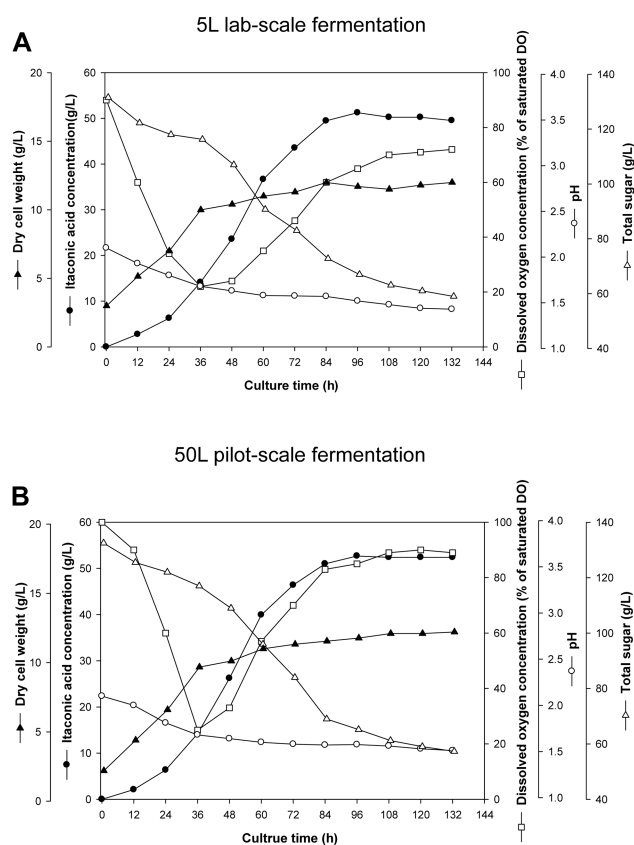
Fig. 6. Time-course profiles of cell growth, sugar consumption, DO concentration, pH, and itaconic acid production by mutant cells of *A. terreus* R104 (**A**) in the 5 L lab-scale fermentor and (**B**) in the 50 L pilot-scale fermentor.
The 5 L fermentation was performed under the operating conditions of 200 rpm and 1.5 vvm, and the 50 L fermentation under 180 rpm and 0.5 vvm.

the 50 L system was due to the shear damages imposed on the mycelial producers when the 50 L fermentations were carried out at above 200 rpm. Under such high shear environments, the shear stress was found to negatively affect the cellular secondary metabolism, resulting in a remarkable reduction in the IA biosynthetic capability of the producing microorganisms (data not shown).

Under these respective conditions (*i.e.*, 200 rpm and 1.5 vvm for the 5 L system; 180 rpm and 0.5 vvm for the 50 L system), it was possible to obtain almost equivalent time-course profiles of cell growth, IA production, sugar consumption, DO, and pH (Fig. 6). Exponential increases in cell mass were observed until 36 h of incubation in both cultures. From around 84 h to the end, the specific growth rate decreased down to zero, despite large amounts of residual sugars still available, implying that other nutrients

and/or pH should be limiting factor(s). The final cell mass reached 12 g/l and 12.2 g/l, respectively. Similar patterns of sugar consumption were also observed in both systems. The final sugar concentrations dropped down to 59.0 g/l in the 5 L system and 57.3 g/l in the 50 L system, respectively (the initial sucrose concentration was about 132 g/l in both fermentations, thus about 55.3% and 56.6% being consumed respectively during the entire fermentation time). The sugar consumptions during the later stage of exponential phase (*i.e.*, from 24 to 84 h) appeared to be more closely related to the IA production rather than to the cellular growth in both cultures. Namely, from after 24 h of incubation, active biosynthesis of IA was observed, corresponding to the significant reduction in the residual sugar concentrations from 120 g/l at 24 h to 70.8 g/l at 84 h. After 84 h, however, the IA production rate was remarkably slowed down. From these results, it can be assumed that IA is biosynthesized in a partially growth-associated mode, which has been frequently observed in the production of many secondary metabolites such as antibiotics and organic acids [20, 28]. Notably, the time-course profiles of IA production were almost identical in the two fermentations, producing maximum amounts of 51.2 and 52.7 g/l, respectively (only 2.8% increase in the 50 L culture).

It can be seen in Fig. 6 that DO levels of each system remained at above 20% of the saturated DO concentration during the entire incubation period. Accordingly, DO did not appear to act as a growth-limiting nutrient in both systems. For the IA fermentation process, the critical DO level was observed at around 15% of the saturated DO (data not shown). These DO environments were necessary, because it is well known that the oxygen uptake rate is significantly influenced by the DO concentration of the fermentation broth, especially when it is below a critical DO level, thus leading to reduced production of secondary metabolites [3, 8, 14]. The very low levels of pH should be noted during the mid- to the late-exponential growth stages in both fermentations, casually coinciding with the period of the most active IA biosynthesis (*i.e.*, from 24 to 72 h) (Fig. 6). The pHs remained of around 1.5 at the end of both incubations, possibly due to various organic acids (including IA) accumulated inside the fermentation broth. The data for cell growth *vs.* pH at around 36 h indicate that the producing fungal cells might experience significant cellular-growth limitations at the very low pH of 1.6. As for IA production, however, the low pHs ranging from 1.8 (at 24 h) to 1.57 (at 84 h) appeared to contribute to promoting IA production to a great extent. In fact, several reports have

Table 2. Comparison of fermentation parameters obtained from the 5 L lab-scale and the 50 L pilot-scale fermentations performed with the mutant cells of *A. terreus* R104.

	P_f	X_f	S_f	Q_p	q_p	$Y_{p/x}$	$Y_{p/s}$	μ
A (5L)	51.2	12.0	59.0	0.533	0.045	4.376	0.711	0.029
B (50L)	52.7	12.2	57.3	0.548	0.046	4.477	0.715	0.031

The 5 L fermentation was performed under the operating conditions of 200 rpm and 1.5 vvm, and the 50 L fermentation under 180 rpm and 0.5 vvm.
A, 5 L lab-scale fermentation; B, 50 L pilot-scale fermentation; P_f, Maximum IA production (g IA/l); X_f, Maximum dry cell weight (g DCW/l); S_f, Final residual glucose concentration (g glucose/l); Q_p, Average volumetric IA production rate (g IA/l/h); q_p, Average specific IA production rate (g IA/g DCW/h); $Y_{p/x}$, Specific IA production (g IA/g DCW); $Y_{p/s}$, IA production yield based on glucose (g IA/g glucose); μ, Average specific growth rate (1/h).

been published that very low pH values of around 2.0 were beneficial for enhancing IA productivity, despite retarded cellular growth under these pH ranges [19]. In summary, these results imply that, for maximal utilization of the producers' fermentation physiology, the pH should be optimized for the IA production phase, separately from the cellular growth phase.

Compared in Table 2 are the fermentation parameters obtained from the 5 L and 50 L cultures performed under the environments of almost equivalent k_La value. Similar trends of fermentation physiology were observed, as expressed in terms of IA production (P_f), maximum cell growth (X_f) and residual glucose concentration (S_f). The average volumetric (Q_p) and specific (q_p) production rates of IA, a measure of fermentation efficiency and cell activity, respectively, also reflect the successful scale-up of the IA fermentation process: Q_p and q_p of the 50 L culture were only 2.7% and 2.1% higher, respectively, relative to the parallel parameters of the 5 L culture. Efficient utilization of the carbon source(s) and highly active biomass in the 50 L system can be inferred by comparing the IA production yield ($Y_{p/s}$) and specific IA production ($Y_{p/x}$) to those of the 5 L system (about 0.5% and 2.2% higher, respectively, over the 5 L system). The IA production yield ($Y_{p/s}$) was calculated based on sugar concentration, since its consumption kinetics revealed that this substrate had not been limited until the end in each fermentation process (Fig. 6). In general, owing to the complexities of secondary metabolic pathways and the altered catalytic properties often caused by different fermentation environments, filamentous fungal cells usually exhibit a different physiology and morphology during a scale-up process. In this study, however, difference of the specific growth rate (μ) between the 5 L and 50 L systems was observed to be negligible (0.029 1/h *vs.* 0.031 1/h) (Table 2). This result is notable, considering the well-known fact that specific growth rate (μ) normally has a great influence on specific production rate (q_p), especially in the cultures for secondary metabolites production [3, 8].

In summary, all of these comparative results imply that the application of the criterion of constant oxygen mass transfer coefficient (k_La) was successful for the scale-up of the IA fermentation process (the scale-up ratio of 1:10).

At this moment, it should be mentioned that the 5 L and 50 L fermentation processes have not been optimized yet. Besides strain improvement and statistical medium optimization, the bioprocess operation could be further improved in order to maximize the IA biosynthetic capability of the producing cells. For example, it would be possible to develop a two-stage culture system on the basis of the different pH effects on the cell growth and IA production, as already discussed in this paper. To this end, a fed-batch fermentation process is currently being developed in our laboratory, demonstrating a promising potential for the industrial production of IA. The results from the fed-batch bioreactor operation will be presented in a subsequent article, placing a special emphasis on enhanced IA productivity in the pilot-scale fermentor system.

Acknowledgments

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