Scale-up in bio-chemical processes

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Via Cesare da Sesto, 10
20123 Milano (Italy)
Tel. 0039 02 83241119
Fax 0039 02 8376457
www.b5srl.com

MOHIT CHOPRA

Stevens Institute of Technology
Hoboken, NJ 07030, USA
mchopra@stevens-tech.edu

ABSTRACT

This paper consists of two parts. In first part it discusses the traditional general method of scale-up and in the second part an example of scale-up of fermentation is discussed.
The scale-up of fermentor discussed consists of two parts. The first part investigates the effects of the environmental state variables on cultivation. If a wide range of the state variables has no significant effect on yield or productivity, scaling-up is not an issue in the fermentation system. If only a small range of the state variables has no substantial impact on cultivation, then pulse or periodical change of the state variables would be employed to investigate the effects of the change on cultivation. To illustrate the proposed method of scale-up, Bacillus thuringiensis was cultivated for thuringiensin production with the pH value as the environmental state variable. Different pH values at 7.0 and 8.4 had a significant effect on both cell growth and thuringiensin production. Variation of pH value for a short period of time did not have a substantial effect on either cell growth or thuringiensin production.

INTRODUCTION

Scale-up is the study of the problems associated with transferring data obtained in laboratory and pilot plant equipment to production level. Equipment for the industrial fermentors, heat exchangers, crystallizers, separators and so forth can be designed and operated properly only if scale-up technology is fully appreciated. To demonstrate the idea of scaling-up a following simple example is presented: Suppose the conditions of aeration that give maximum productivity in a specific fermentation have been established using a bench scale fermentor, and it is then proposed to transfer that to a large fermentor having same geometrical design The problem is to estimate the proper aeration rate in the large fermentor. Since physical properties of broth under consideration are same in geometrical similar fermentors. Therefore,

$$(k_L a)_1/(k_L a)_2 = [(F/V)_1/(F/V)_2]X$$
$$(H_{L1}/H_{L2})\,X\,(d_{B2}/d_{B1})^{3/2}\,X$$
$$(v_{B2}/v_{B1})^{1/2} \qquad (I)$$

where subscripts 1 and 2 relate to small and large scale equipment.
It may be assumed that $d_{B2}{\cdot}d_{B2}$ in Eq. I because the size of the bubble is considered not to differ appreciably with fermentor size. Suppose the aeration rate with large fermentor be determined by equating LHS of (I) to unity,

$$(F/V)_1/(F/V)_2 = H_{L2}/H_{L1} \qquad (II)$$

Equation II signifies for example that the aeration rate, $(F/V)_2$ with large fermentor will be $(F/V)_2 = 5^{-1} = 0.2$ vvm, provided that the value of $(F/V)_1 = 1.00$ vvm and the scale-up ratio, $H_{L2}/H_{L1} = 5$ (volume scale-up X 125).
However the effect of oxygen transfer from bubbling liquid surface and that associated with transient stage in bubbles' emergence at a sparger cannot be necessarily disregarded when the bench scale fermentor is used. If this effect is appreciated, it is suggested that the term H_{L1}/H_{L2} in the right hand side of the Equation I be replaced by $(H_{L1}/H_{L2})^{2/3}$. If this is acceptable, the aeration rate, $(F/V)_2 = 5^{-2/3} = 1.34$ vvm instead of 0.2.
It is clear that the problems of scale-up in the fermentor are associated with the behavior of the liquid in the fermentor and the metabolic reactions of the organisms.

BASIS OF SCALE-UP

Physical Concept

Physical properties of the broth in geometrically similar conditions and fully baffled fermentors are assumed to be same. Items relevant to liquid behavior in agitated fermentor vessel are:
Power requirement of the agitation, P, or power requirements of agitation in gassed systems, P_g, rotation speed of the impeller, n, and pumping rate of impeller, F.
For turbulent liquid motion,

$$P \propto n^3 D_i^5$$
$$P_g/P = f(N_a)$$

In addition,

$$V \propto D_i^3$$
$$F \propto nD_i^3$$

Physical terms useful for scale-up are:

a. Power consumed per unit volume of liquid, P/V,
$$P/V \propto n^3 Di^2 \qquad (VI)$$
b. Liquid circulation rate inside the vessel, F/V,
$$F/V \propto n \qquad (VII)$$
c. Impeller velocity, v, representing liquid shear rate
$$v \propto nDi \qquad (VIII)$$

Table I – Relationships between properties for scale-up

Property	Small scale, 80 l	Large scale, 10,000 l			
P	1.0	125	3125	25	0.2
P/V	1.0	**1.0**	25	0.2	0.0016
n	1.0	0.34	**1.0**	0.2	0.04
D_i	1.0	5.0	5.0	5.0	5.0
F	1.0	42.5	125	25	5.0
F/V	1.0	0.34	**1.0**	0.2	0.04
n/D_i	1.0	1.7	5.0	**1.0**	0.2
$nD_i r/m$	1.0	8.5	25	5.0	**1.0**

d. The modified Reynolds number, $nD_i\rho/\mu$, where ρ = liquid density and μ = liquid viscosity,

$$nD_i\rho/\mu \propto nD_i^2 \qquad (IX)$$

Table I points out that all the physical properties for large and small scale equipments cannot be equated simultaneously. Supposing that the property P/V, be equated, for instance,

From Eq. VI

$$n_2^3 D_{i2}^2 = n_1^3 D_{i1}^2$$

Then,

$$n_2/n_1 = (D_{i1}/D_{i2})^{2/3} = 5^{-2/3} = 0.34$$

From Eq. III,

$$P_2/P_1 = n_2^3 D_{i2}^5 / n_1^3 D_{i1}^5$$

Then from Eqs. III and VI,

$$P_2/P_1 = (D_{i2}/D_{i1})^3 = 5^3 = 125$$

If the scale-up is attempted on the basis of equal power per unit volume of liquid it is apparent that tip velocity of impeller increases, whereas the value of F/V or shear rate of liquid decreases.

If a fermentation can be scaled-up successfully on the basis of equal power per unit volume of liquid, the fermentation concerned is presumably one which is less sensitive to inevitable increase in tip velocity of the impeller and in the mixing time.

The proper choice of property for scale-up changes from case to case. If the heat transfer coefficient, h, of a liquid film with a coil in the agitated reactor is of interest, plausible correlation can be obtained using the values of h, P/V, and F/V. In order to achieve the same value of k_L, the following relation must hold (assuming the physical properties of the liquid are constant):

$$n_1/n_2 = (D_{i2}/D_{i1})^{(2\alpha-1)/\alpha} \qquad (X)$$

$$(P_2/V_2)/(P_1/V_1) = (n_2/n_2)^3 (D_{i2}/D_{i1})^2 \qquad (XI)$$

From Eqs. X and XI

$$(P_2/V_2)/(P_1/V_1) = (D_{i2}/D_{i1})^{2-3(2\alpha-1)/\alpha} \qquad (XII)$$

In Figure 1, the left-handed side of Equation XII is plotted against α parameters being the scale-up ratio D_{i2}/D_{i1}. It is apparent from that the "equal power per unit volume" concept can be applied effectively to the scale-up, irrespective of D_{i2}/D_{i1}, when the value of α is equal to 0.75.

If the value of $\alpha = 0.5$ can be accepted for a particular fermentation broth in terms of oxygen transfer, Figure 1 suggests that the same value of k_L as that of small scale equipment cannot be expected for large scale fermentors, when the value of P_2/V_2 equal to P_1/V_1. The value of k_{L2} is about 58% of k_{L1}, provided $D_{i2}/D_{i1} = 5$, for instance.

In microbial fermentations, power consumed per unit volume, P/V, volumetric oxygen-transfer coefficient, k_La, and the mean liquid velocity at a particular point in the vessel are three important criteria commonly used for scale-up. The last criterion is incorporated into aeration number, N_a, in gassed systems.

Biological Concept

Figure 2 shows schematically the relative concentration of final product in fermentations as affected either by power input per unit volume of broth, P/V or by volumetric oxygen transfer coefficient, k_La. This kind of hyperbolic patttern is generally observed in fermentations regardless of microbial species – bacteria, yeast or fungi.

For the solid curve in Figure 2, it is better to select an operation variable where the concentration of product levels off, whereas for broken curve, selection at maximum concentration of product is advisable. Other factors such as energy requirements, convenience of operation etc, must be taken into account before the optimum value for P/V or scale-up is determined from the plateau curve.

Even assuming that microbial metabolism is a factor in scale-up, very little information about the effect of scale on microbial metabolism is available. In order to illustrate this, a simple example of ethanol fermentation is used.

The kinetic equation established using a chemostat and anaerobic culture are:

$$\frac{dX}{dt} = \frac{\mu_0}{1 + p/k_p} \cdot \frac{S}{K_s + S} \cdot X \qquad (XIII)$$

$$\frac{dp}{dt} = \frac{\nu_0}{1 + p/k_p} \cdot \frac{S}{K_s + S} \cdot X \qquad (XIV)$$

$$\frac{-dS}{dt} = \frac{1}{Y_{X/S}} \cdot \frac{dX}{dt} = \frac{1}{Y_{p/S}} \cdot \frac{dp}{dt} \qquad (XV)$$

where

X = cell mass concentration
p = product (ethanol concentration)
S = substrate (glucose concentration)
K_S, $K_{S'}$ = saturation constants
K_p, $K_{p'}$ = empirical constants
$Y_{X/S}$ = yield ($= \Delta X/-\Delta S$)
$Y_{p/S}$ = yield ($=\Delta p/-\Delta S$)
μ_o = specific growth rate at p=0
ν_o = specific growth rate of ethanol production at p=0
t = time

A fact to be emphasized is that the metabolic patterns as represented by Eqs. XIII to XV are unchanged irrespective of the cultural procedure. The only difference is in the values of empirical constants, K_p, $K_{p'}$, K_S etc., depending on the equipment. The examples of K_p and $K_{p'}$ obtained in different environment are reported in Table II. Table II suggests that effect of ethanol on the specific growth rate and the specific rate of ethanol production, in other words, on the microbial activity of the yeast cell depends on the equipment.

This example of biological factors influencing scale-up indicates clearly the necessity of studying the metabolic patterns. There is a need for further experimentation and discussion on the values of empirical constants used in kinetic equation for a particular

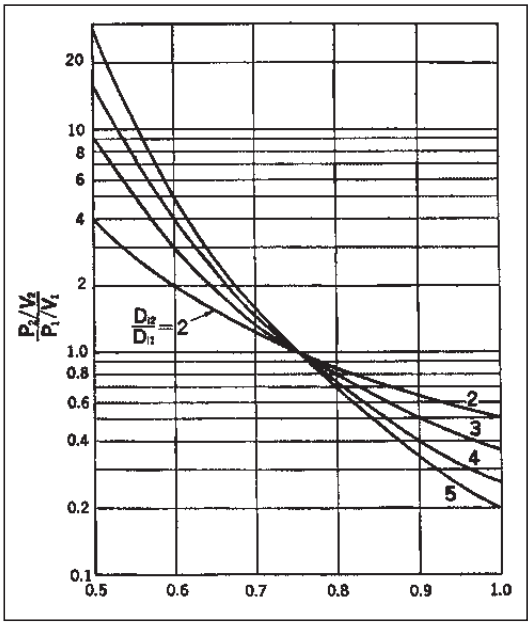

Figure 1 – P/V vs α in scale-up

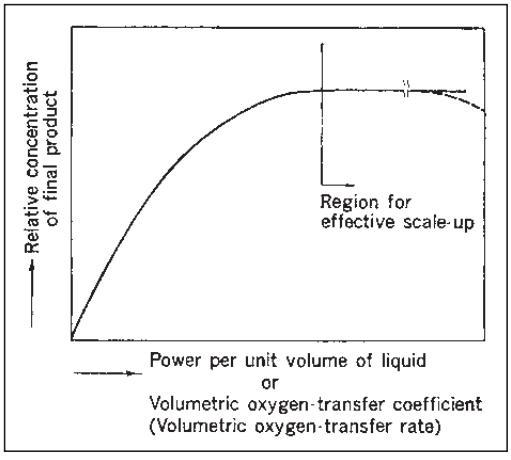

Figure 2 – Microbial performance as affected by operation variables

Table II

Culture	K_p (g/l)	$K_{p'}$ (g/l)
Batch (shaken flask for K_p, Warburg manometer for $K_{p'}$)	16.0	71.5
Continous (bench scale fermentor)	55.0	12.5

fermentation in order that the equation can be properly related to variables like P/V and/or k_La in scaling-up.

EXAMPLES OF SCALE-UP

Power per Unit Volume

Figures 3 and 4 are examples of fermentations where product concentration has been correlated with power input for different sized fermentors. The penicillin titre at the 108th hour of fermentation is plotted against power input, HP/m^3, in Figure 3; the volumetric ratio of scale-up was 1:10, while the values of v_S were roughly doubled in each fermentor tested. Within the range of these data, it appears that scale-up was successful provided that the power input exceeded 1.5 HP/m^3. It is also noted from Figure 3 that the yield of penicillin fell sharply with a power input of less than 1.0 HP/m^3. In principle it is known that the performance of impellers can be assessed accurately, irrespective of the geometrical similarity or dissimilarity of the system, if the power imposed by the impeller on unit volume of agitated liquid is selected as the basis of comparison. Therefore, the geometrical properties of the system from which the data points of Figure 3 were derived are not of prime importance.

However, in Figure 4, where novobiocin production was related to power input, the above fact may not necessarily apply. Standard flat blade turbines whose sizes ranged from 2.03 to 3.42 in terms of D_t/D_i were used in this case; it can be seen that the three curves drawn through the data points in the Figure 4 are approximately parallel to each other. It is seen from the figure that for equal values of HP/m^3 the large impeller gave lower yields of novobiocin compared with the size of impeller. These experimental facts suggests that the type of mycelium in fermentation broth may be another factor to be considered in scale-up, besides the factors associated with hydronamical behavior of the broth. It is interesting to note also that the values of HP/m^3 > 1.5 gave the best yields of antibiotics. From the reports of the values of power input in general use in chemical processes, the degree of liquid agitation with impellers may be classified as follows:

	P/V (HP/m^3)
Mild agitation	0.3 - 1.0
Strong agitation	1.0 - 4.0

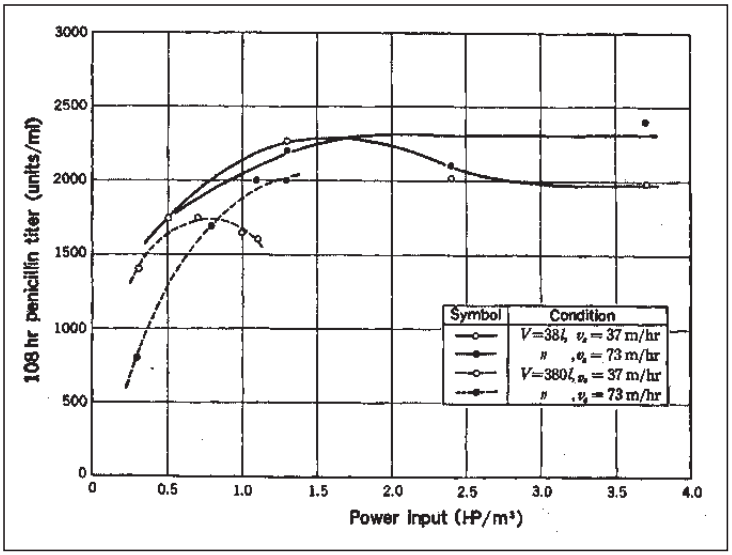

Figure 3 – Effect of different values of power input on the yield of penicillin with different fermentation conditions (6)

SCALE-UP OF FERMENTORS FOR PRODUCTION OF *B. THURINGIENSIS*

INTRODUCTION

The flow behavior and the bio reactions in the vessel are problems associated with the scale-up of a fermentor. Although there have been many studies on the flow behavior in different types of fermentors, knowledge about the behavior of the microorganisms in a fermentor under incomplete mixing is quite limited. The traditional method for scaling-up a fermentation system, as discussed is usually based on empirical criteria such as constant power input per unit volume, a constant mass transfer coefficient, constant mixing time and constant impeller tip velocity. However, these criteria are related to transport processes and consideration of cell kinetics is limited. The shortcomings of using these empirical criteria are inevitable, especially when there is a change in the controlling regime during the scale-up. The other approach, scale-down, is regarded as a suitable method for improving scale-up. Scaling-down bioreactors have been employed widely to find the key process parameters and study the effects of the various production scale conditions on a laboratory scale. A combination of transport phenomena and cell kinetics is considered in the scale-down approaches. However, direct scale-up of a fermentation system cannot be achieved using the scale-down approach. As a consequence, although many things are known about scale-up, it is still considered an art, not a science.

It is well known that microorganisms in a large-scale fermentor are exposed to varied environmental states. Hence, much work has been done on studying the transient behavior of these bioprocesses. Since a steady state of a continuous culture provides a well-controlled environment, studies of transient behavior of bioprocesses are often carried out under these steady state conditions with a step, pulse or periodic change of the substrate concentration or environmental variables. However, industrial fermentation processes are usually conducted as a batch or fed-batch system. These cultivation systems change with respect to time. Studies of the transient behavior in batch cultures are helpful in understanding the dynamics of the system. An appropriate operating condition can also be obtained via a study in batch culture.

In the present study, a novel strategy for scaling-up a fermentation system is introduced. Transport phenomena are considered separately from the cell kinetics of the culture. The cultivation of

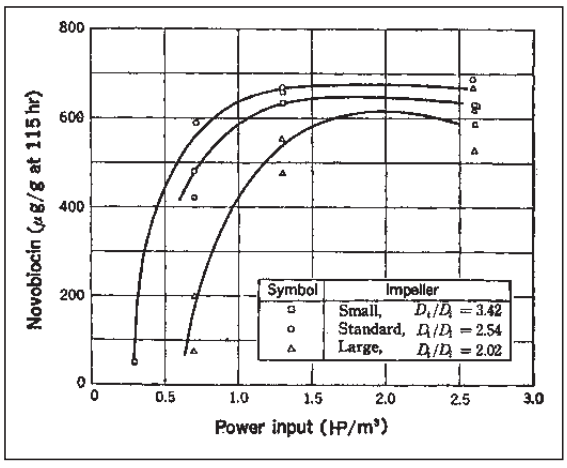

Figure 4 – Effect of different values of power input on the yield of novobiocin in fermentors fitted with different-size impellers (12)

Bacillus thuringiensis for thuringiensin production with pH as the environmental state variable was demonstrated. The other environmental variables that are important in scale-up, such as dissolved oxygen (DO) concentration and shear stress due to agitation, are not investigated in this study. Thuringiensin, produced by some serotypes of *B. thuringiensis*, is a heat-stable biopesticide that has a broader spectrum of affectivity against many insects as compared to α-endotoxin. The production of thuringiensin occurs in a mixed-growth-associated system that provides an appropriate example in demonstrating the proposed method of scale-up

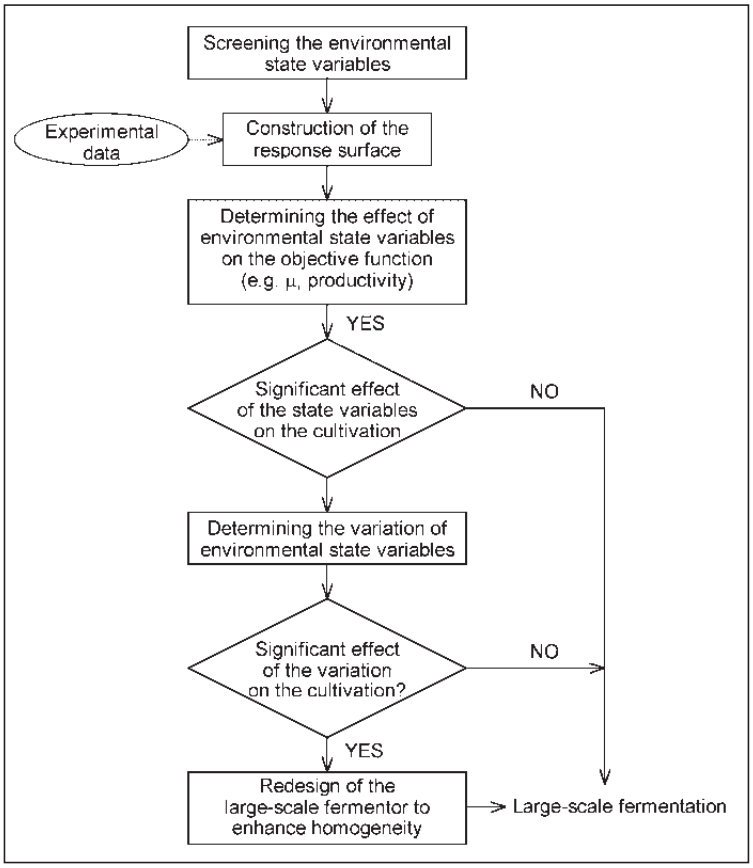

Figure 5 – The flow chart of the proposed method of scaling-up a fermentation system

MATERIALS AND METHODS

The Microorganism

The microorganism used in this study was *B. thuringiensis* sub sp. *darmstadiensis* (HD-199). Stock cultures of the microorganisms were maintained in 20% glycerol (v/v) at -20°C.

The Cultivation

Pre-culture procedures were carried out for preparing the inoculum. The microorganisms were cultivated in a 1 l Erlenmyer flask containing 250 ml of complex medium (5 g/l-yeast extract, 8 g/l-nutrient broth) on a rotary shaker at 200 rpm and 30°C for 12 hrs. The pre-culture broth of 200 ml was then inoculated to the main culture with 2 l of fresh medium in the fermentor. The medium used for the main culture contained (g/l): soybean protein, 45; glucose, 30; KH_2PO_4, 5; K_2HPO_4, 5; $MnSO_4{\cdot}4H_2O$, 0.03; $MgSO_4{\cdot}7H_2O$, 0.05; $CaCl_2{\cdot}7H_2O$, 0.05; $FeSO_4{\cdot}7H_2O$, 0.01; $NaNH_4HPO_4{\cdot}4H_2O$, 1.5. A 5 l stirred tank fermentor was employed for the main culture, which was operated at 30°C with an agitation speed of 500 rpm and an aeration of 1.5 vvm. The pH value was manipulated between 5.8 and 8.4 for variation study. The working volume was 2.2 l.

Assay

The method of analyzing thuringiensin was modified from that of Campbell et al. and Levinson et al. High performance liquid chromatography (HPLC) was employed for determining the concentration of thuringiensin. Glucose concentration was measured using a glucostat reagent (Glucostat, BGH). Detection was carried out with a spectrophotometer at a wavelength of 505 nm. The number of cells was counted directly using a microscope with a counting chamber. The concentration of DO in the broth was monitored by a polarographic oxygen electrode placed in the fermentor.

The Algorithm

In a large-scale fermentor, both transport phenomena and cell kinetics have significant effects on the outcome of the cultivation. The proposed algorithm is based on a series of special operations in a small-scale fermentor to obtain the information for scale-up. The operations include the determination of the effect of the environmental variables and the variation of the variables. The flow chart is shown in Figure 5.

Effect of the Environmental Variables

In a fermentation system, environmental variables such as pH value, temperature, medium composition and the DO concentration have substantial effects on cell growth and/or product formation. In many cases, these environmental variables should be considered simultaneously in the fermentation system. A common and powerful approach for optimizing a multivariable system is the response surface method (RSM). The interactions between key factors as well as curvature effects are taken into account and quantified using the RSM. An empirical equation in the form of a second-order polynomial is obtained with specified and limited data. Typically, a central composite design (CCD) is employed for fitting the second-order polynomial. Besides, other optimization approaches such as neurofuzzy methods or genetic algorithms (GAs) can be applied to obtain the response surface. The RSM has been successfully applied to various fermentation processes.

In the present study, the response surface is employed to determine the effect of the environmental variables on the cultivation. If the environmental variables have no significant effect on the objective function, such as specific growth rate or productivity, the response surface has a flat zone with respect to the variables. A wide range of the flat zone implies that the environmental variable can be controlled within the desired range in the large-scale fermentor. Hence, scaling-up the fermentation system is not a problem.

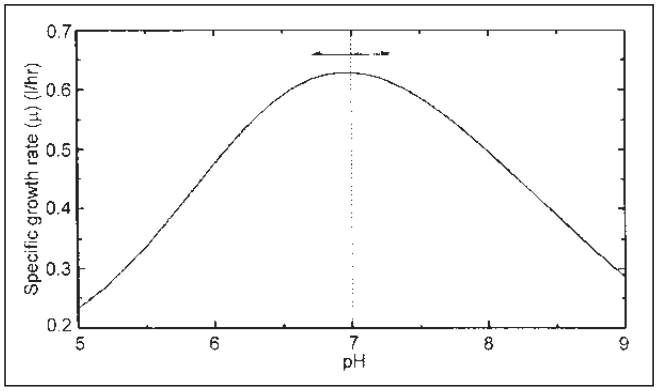

Figure 6 – The pH effect on the specific growth rate in the cultivation of B. thuringiensis

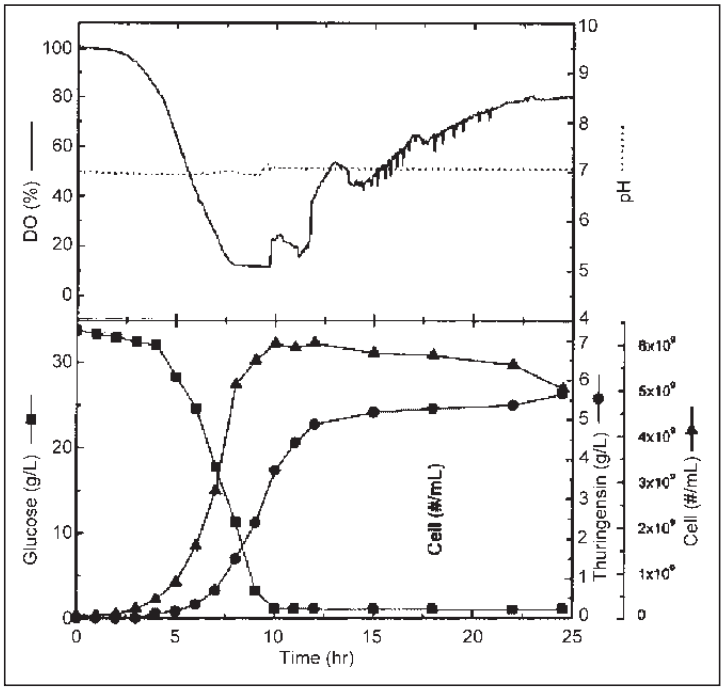

Figure 7 – The time course of the cultivation with the pH control at 7.0 throughout the cultivation

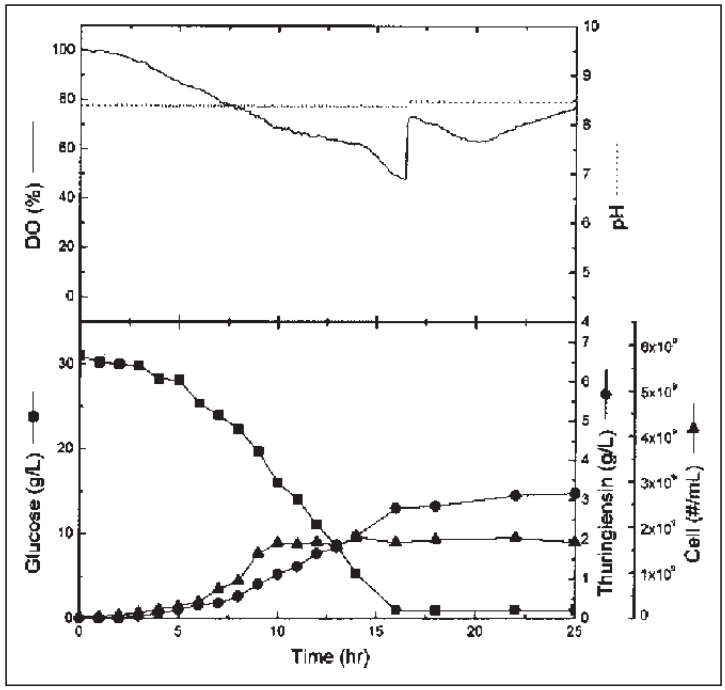

Figure 8 – The time course of the cultivation with the pH control at 8.4 throughout the cultivation

On the other hand, if the environmental variables significantly affect the cultivation outside of a small range, this means the state variables in the large-scale fermentor cannot be manipulated within the required range. It is then necessary to proceed to the second step.

Variation of the Environmental Variables

Incomplete mixing in a large-scale fermentor may cause a gradient of the environmental variables in the fermentor, especially during the period of feeding for a fed-batch culture or adding alkali/acid for pH control. This incomplete mixing is simulated by using pulse or periodical change of the environmental state variables in a well-mixed small-scale fermentor.

A fermentation process with growth-associated, non-growth-associated or mixed-growth-associated product requires different cultivation modes. For the fermentation with a growth-associated product, cultivation is carried out by extending the exponential growth phase. On the other hand, for the case with a non-growth-associated product, the cultivation strategy is to prolong the period of the stationary phase. Therefore, simulation of the incomplete mixing in a large-scale fermentor using pulse or periodical change in a small-scale fermentor is employed in both the exponential growth and the stationary phases.

If variation of the environmental variables has no significant effect on the cultivation, scaling-up the fermentation system is not a problem. In contrast, if the variation has a substantial effect on the cultivation, scaling-up is a problem and careful consideration of the fermentor, such as utilizing more positions for the inputs, is required to increase homogeneity in the large-scale fermentor.

RESULTS

Cultivation of *B. thuringiensis* for thuringiensin production was carried out using the pH value as the environmental variable to elucidate the methods of scale-up.

Effect of pH on the Cultivation

Figure 6 shows the effect of pH on specific growth rate, μ, under the condition of the aeration of 1.5 vvm and the agitation speed of 500 rpm. The optimum pH value was found to be around 7.0. The time courses of batch cultures of *B. thuringiensis* at pH 7.0 and 8.4 are shown in Figure 7 and Figure 8, respectively. Obviously, the cell number and thuringiensin concentration at pH 8.4 were much lower than those at pH 7.0. In a large-scale fermentor, if the fluctuation of pH value could be controlled within 7.0±0.3 (as the range marked in Figure 6) throughout the cultivation, the growth of microorganisms would be almost the same as the case with pH control at 7.0 in a small-scale fermentor. If the pH value in the large-scale fermentor could not be well controlled within the required range, the transient response of pH variation would need to be investigated.

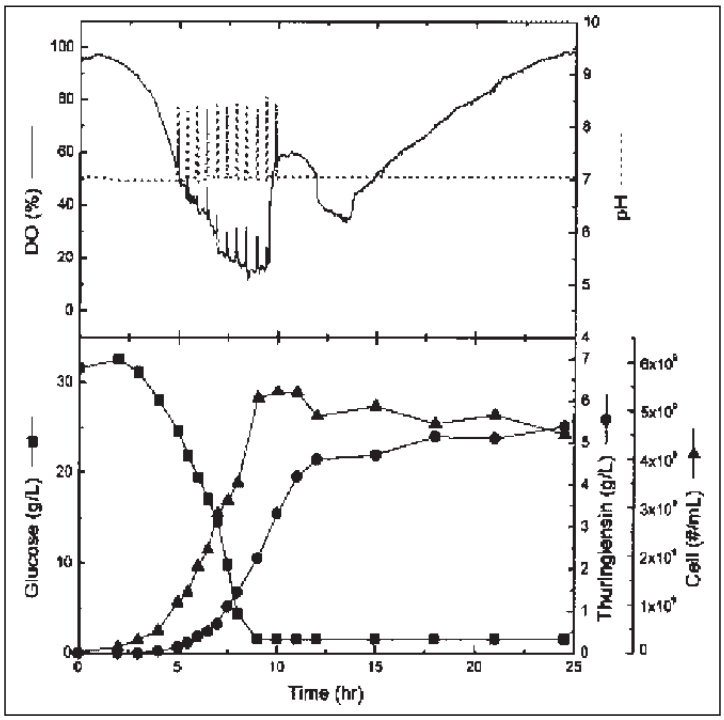

Figure 9 – The time course of the cultivation with the periodical variation of pH value during the exponential growth phase (from 5 to 10 h). The period was 30 min with 2 min at pH 8.4

Variation of pH Value

Since thuringiensin is a mixed-growth-associated

product in the cultivation of *B. thuringiensis*, variation of pH value in the exponential growth and stationary phases was carried out. All cultures were maintained at pH 7.0 as a standard cultivating condition for the *B. thuringiensis* before subsequent pH manipulation. The addition of acid and alkali were employed for pH control. A certain amount of metabolic acids were found to be excreted into the broth during the exponential growth phase. These acids were utilized by the microorganisms as a carbon source after the glucose was depleted during the stationary phase. Thus, pH varied between 7.0 and 8.4 during the exponential growth phase, and between 5.8 and 7.0 during the stationary phase to mimic the additions of NaOH and H_2SO_4 solutions during the exponential growth phase and the stationary phase, respectively. Good mixing is essential for a large-scale fermentor in order to prevent the cells from being subjected to pH perturbation due to the intermittent action of pH control. Variation of the fermentation system from pH 7.0 will lead to a decrease in the biomass and product concentration.

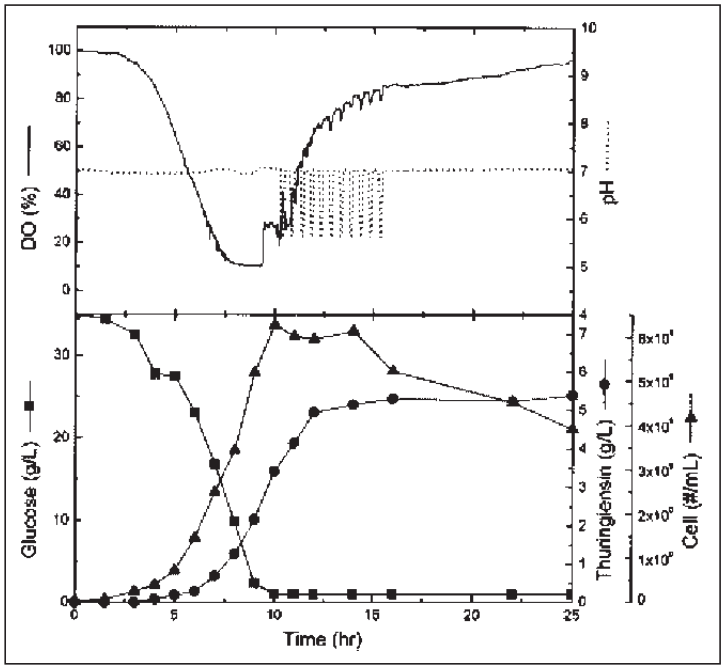

Figure 10 – The time course of the cultivation with the periodical variation of pH value during the stationary phase (from 10.5 to 15.5 h of cultivation).

Variation in the Exponential Growth Phase

Figure 9 shows the time course of pH change during the period between 5 and 10 h of cultivation. The period was 30 min with 2 min at pH 8.4. The final concentration of thuringiensin was about 92% of that at pH 7.0. As demonstrated in Figure 9, periodical variation with a short duration time (e.g. less than 2 min) has a minimal effect on thuringiensin production.

Variation in the Stationary Phase

Figure 10 shows the time course of pH change during the stationary phase (from 10.5 to 15.5 h of cultivation). There was no difference in the thuringiensin production throughout the stationary phase regardless of pH variation. An experiment without pH control during the stationary phase was also carried out for comparison. As shown in Figure 11, the final concentrations of biomass and thuringiensin were similar to those in Figure 7.

In a large-scale fermentor, variation in pH value is possible due to the fact that the reactor does not provide a completely well-mixed environment. The experimental results demonstrate that a short period of pH variation has little influence on thuringiensin production as well as biomass concentration, especially during the stationary phase.

CONCLUSIONS

The proposed method of scale-up is based on the evaluation of: i) the effect of the environmental state variables on the cultivation; and ii) the variation of the environmental state variables. If the fermentation system, which is tested in a small-scale fermentor, is insensitive to a wide range of the environmental state variables, scale-up is not a problem. If the fermentation system is sensitive to the environmental state variables but insensitive to a short period variation of the state variables, scale-up is still not a problem. If the fermentation system is sensitive to both the environmental variables and the variation of the state variables, scale-up is a problem. Design of a large-scale fermentor should be done carefully. Utilizing more positions for the inputs to increase homogeneity in the fermentor could be employed. In the illustrative example of the cultivation of *B. thuringiensis* for thuringiensin production, different pH values had a substantial effect on cultivation. However, variation of pH value for a short period of time did not have significant effect on either cell growth or product formation. Therefore, if a large-scale fermentor had a short mixing time to guarantee that the variation of pH value remained within the limit, the environmental state variable, pH, would not cause a problem in a large-scale fermentor.

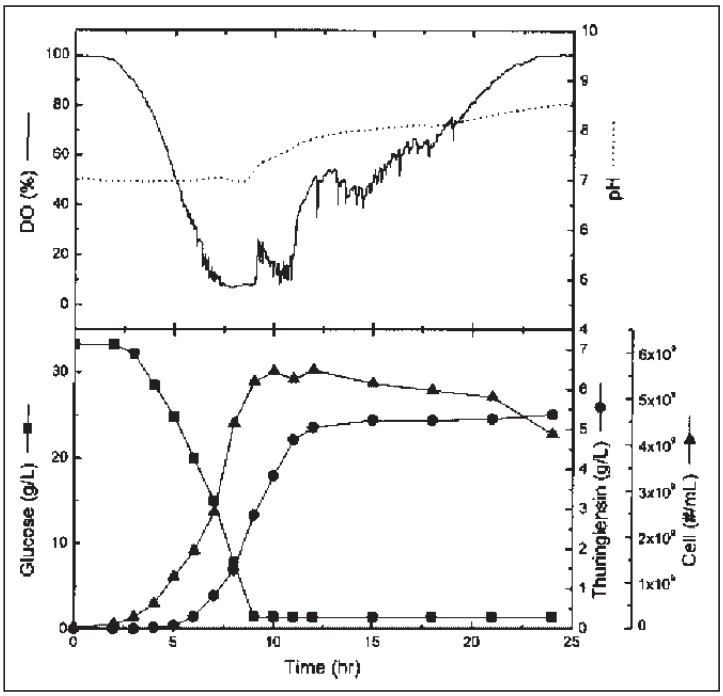

Figure 11 – The time course of the cultivation without pH control during the stationary phase

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