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Optimal Criterion for the Scale-Up Production of Schizophyllan in the Stirred Tank Reactor

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Abstract Optimal criterion for the scale-up production of schizophyllan, a fungal polysaccharide secreted by *Schizophyllum commune*, was investigated. For the production of the polysaccharide in a 150-l bioreactor, the culture conditions optimized in a 15-l bioreactor were applied to a 150-l bioreactor with scale-up process, by changing impeller speed and airflow rate. The optimized impeller speed in the 15-l bioreactor was 50 rpm in a technical medium based on barley. For establishment of the scale-up process, 3 kinds of criteria were used while the gas throughput number was kept constant, as follows; constant volume-related power input, constant tip speed of stirrer, and constant Reynolds number. In the 150-l bioreactor, the highest values for the maximum specific growth rate (1.17/day) and productivity (0.63 g/L·day) were achieved in the culture condition from constant volume-related power input criterion.

Key words: Scale-up process, schizophyllan, gas throughput number, volume related power input, tip speed of stirrer, Reynolds number

Functional materials, including various polysaccharides, have been studied in biotechnological fields such as pharmaceutical and food industries, and these materials have potential useful properties such as antitumoral, antiviral, and antimicrobial activities, as well as being a source of dietary fiber [6, 7, 9, 17, 20]. Of these functional materials, β-glucan is the component of some cereals [2] and composed of a β-(1,3)-glucan, having a single β-(1,6)-glucosyl side chain on every second glucose unit [14]. They could stimulate the activity of macrophage, regulate the aging caused by ultraviolet light, and scavenge the free radical in human body [19]. It has been reported that macrophage produced in the medulla can selectively recognize normal and infected cells by tumor, but nonselectivity for the shape of tumor cells *in vitro*, therefore macrophage is an important intracellular communicator in the human immune system. Moreover, β-(1,3)-(1,6)-glucan from cell wall of *Saccharomyces cerevisiae* has been used in aquaculture and dietary fiber [1, 13, 16]. Among the group of β-1,3-D-glucans, schizophyllan is a neutral exopolysaccharide from *Schizophyllum commune*, and its repeat unit also consists of linearly linked β-(1,3)-D-glucose residues with one of every three laterally substituted with a β-(1,6)-D-glucose residue [4]. Until now, there has been little information available concerning cultivation conditions and parameters, especially scale-up process for the mass production of fungal polysaccharides, even though culture conditions for the formation of fungal exopolysaccharide were investigated; scleroglucan by *Sclerotium glucanicum* [19] and schizophyllan by *Schizophyllum commune* [12]. In this study, based on barley in the stirred tank reactors, the optimal scale-up condition was investigated for the production of immuno-stimulative polysaccharide with the technical medium.

Materials and Methods

Microorganism

Schizophyllum commune 1025 (DSMZ Co., Germany) was used for the production of polysaccharide.

Technical Medium Based on Barley

Barley was selected as a nutrient, since it contains β-glucan which is the object product in this study, and it might also work as an inducer [8]. Barley medium was

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prepared as follows; ground barley was first boiled at 121°C for 30 min (1.5 bar) and then cooled down to room temperature. Then, the extracts of barley were filtered with 300 μm mesh to separate the extracted solution. pH of the liquid extracts was then adjusted to 5.6 with 1 M HCl and NaOH. Finally, the culture medium was sterilized again at 121°C and 1.5 bar for 20 min, and then cooled down to 30°C before use.

Impeller Speed and Airflow Rate in the 15-l Bioreactor

For investigating the production of the polysaccharide with different impeller speeds, *S. commune* was cultivated in 25, 50, and 100 rpm at fixed aeration rate of 0.3 vvm. Temperature and initial pH were adjusted to 30°C and 5.6, respectively. All cultivations were performed without pH control.

Bioreactor

Scale-up process was performed in 15-l and 150-l bioreactors (Fig. 1). The culture solution in the 15-l bioreactor was used as an inoculum in the 150-l bioreactor. The geometrical parameters of both bioreactors are shown in Table 1.

Design of Scale-Up Process

For the production of schizophyllan in 150-l bioreactor, the optimized impeller speed in the 15-l bioreactor was applied to a 150-l bioreactor with scale-up process by changing impeller speed and airflow rate. Three kinds of scale-up processes are indicated as follows; impeller speed (n_2) in 150-l bioreactor can be calculated with impeller speed (n_1) and diameter of stirrer (d_1) in 15-l bioreactor and diameter of stirrer (d_2) in 150-l bioreactor: ① constant volume-related power input (P/V), $n_2=n_1*(d_1/d_2)^{2/3}$; ② constant tip speed of stirrer ($n \cdot d_s$), $n_2=n_1*(d_1/d_2)^1$; and ③ constant Reynolds number (Re), $n_2=n_1*(d_1/d_2)^2$. Gas throughput number Q_1 in the 15-l bioreactor corresponded to Q_2 in the 150-l bioreactor with the equation of $Q=q/d^3*n$; q was airflow rate [vvm], d was the diameter of stirrer in bioreactor, and n was impeller speed. Q_1 was calculated with optimal impeller speed (n_1), airflow rate (q_1), and diameter of stirrer (d_1) in the 15-l bioreactor. Therefore, airflow rate (q_2) in the 150-l bioreactor could be determined with the identical Q value, impeller speed (n_2), and diameter of stirrer (d_2). Table 2 shows the 3 kinds of culture conditions in the 150-l bioreactor by a scale-up process.

Table 1. Geometrical parameters of 15-l and 150-l bioreactors.

	Stirrer	Bioreactor		Geometrical dimensionless number [-]	
	d_s [m]	d_R [m]	H_R [m]	d_s/d_R	d_R/H_R
15-l	0.09	0.21	0.46	0.43	0.45
150-l	0.20	0.45	1.00	0.44	0.45

Verification of Optimal Culture Condition with Glucose Medium

For the verification of optimal culture condition of *S. commune* from scale-up process, the fermentation was carried out with the glucose medium [3]. Since the glucose medium contains the optimal composition for the production of polysaccharide of *S. commune*, cultivation results such as growth pattern and polysaccharide concentration were

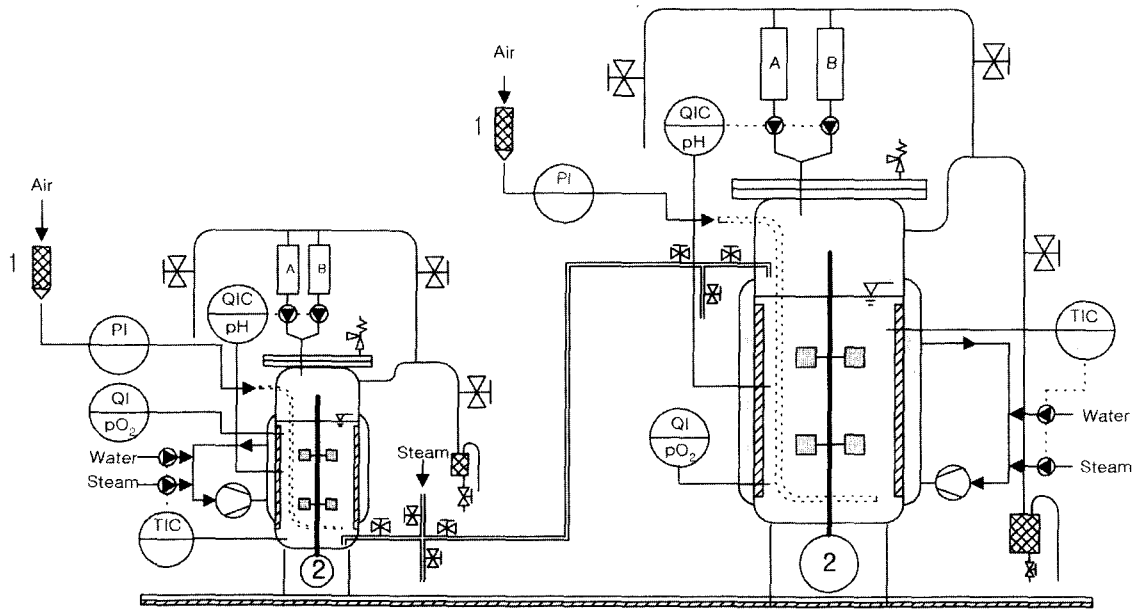

Fig. 1. Schematic of 15-l and 150-l bioreactors for scale-up process (1, air filter; 2, agitation motor; QIC/pH, quantity indicator and controller of pH; QI/pO_2, quantity indicator of pO_2; TIC, temperature indicator and controller; A, acid chamber; B, base chamber).

Table 2. Impeller speed and airflow rate in 150-l bioreactor by the scale-up process.

	Impeller speed [rpm]	Airflow rate [vvm]	Q [-]
Constant P/V	30.0	0.18	0.055
Constant n·d_s	22.5	0.14	0.055
Constant Re	10.0	0.06	0.055

compared between 15-l and 150-l bioreactors. Therefore, the maximal concentration of polysaccharide, productivity, and maximum specific growth rate were evaluated in 15-l and 150-l bioreactors.

Analysis of the Amount of Biomass and Polysaccharide

The culture solution was diluted by adding twice the volumes of water, and then the culture mixture was crushed with a high-speed blender (IKA-Labortechnik Co.) for 2 min. After centrifugation (7,500 ×*g*, 15 min) of culture solution, the precipitate, designated as biomass, was dried to constant weight at 60°C. The polysaccharide in the supernatant was precipitated by adding an equal volume of ethyl alcohol, and the precipitate was dried to constant weight at 60°C.

Results and Discussion

Optimal Impeller Speed and Airflow Rate in 15-l Bioreactor

Viscous fermentation fluids, such as those resulting from polysaccharide formation, present the operator with significant problems of ensuring adequate mass, heat, and momentum transfer [10]. A number of studies have indicated that glucan formation may be stimulated by oxygen limitation in some fungi [11], and that impeller speed may affect all of these factors such as formation of biomass and polysaccharide. In order to obtain the optimal impeller speed, stirrer speeds were regulated as 25, 50, and 100 rpm. Other fermentation conditions were as follows: initial inoculation was 7%, working volume 7 l in 15-l bioreactor, and airflow rate 0.3 vvm. After 3 days, biomass of *S. commune* increased and significant formation of polysaccharide occurred at the beginning of the stationary phase. As the concentration of polysaccharide produced by *Monilinia fructigena* increased, the viscosity of culture solution also increased, whereas pO_2 value [%] decreased by low mass transfer [8]. In this experiment, it seems that the oxygen limitation condition by increasing viscosity of polysaccharide solution may also lead to high secretion of fungal polysaccharide. In Table 3, maximum specific growth rate (μ_{max}) and productivity (PD) are compared with each condition. Optimized impeller speed of 50 rpm and 0.3 vvm of airflow rate in the 15-l bioreactor were applied to scale-up process in the 150-l bioreactor.

Table 3. Maximum specific growth rate (μ_{max}) and productivity (PR) of *S. commune* in the barley medium.

	25 rpm	50 rpm	100 rpm
μ_{max} [1/day]	1.5	1.76	1.4
PR [g/L·day]	0.110	0.282	0.126

Establishment of Optimal Scale-Up Criterion

For the production of the polysaccharide in 150-l bioreactor, optimal culture conditions from the 15-l bioreactor (50 rpm and 2 l/min) were used to calculate impeller speed and airflow rate in the 150-l bioreactor. For the inoculation to 150-l bioreactor, *S. commune* was cultivated in a 15-l bioreactor under optimized culture conditions such as 50 rpm and 0.3 vvm. The cultures including biomass and polysaccharide from the 15-l bioreactor were transferred to the 150-l bioreactor as an inoculum. Gas throughput number (Q) in the 15-l bioreactor was calculated as 0.055 [-], therefore, impeller speed and airflow rate in the 150-l bioreactor were regulated to correspond with 0.055 of Q number in the 15-l bioreactor. Preculture (400 ml in 7 l working volume) was inoculated to the 15-l bioreactor, when the biomass of *S. commune* reached the maximum content. The inoculation concentration (4 l in 70 l working volume) in the 150-l bioreactor was corresponded with the preculture content in the 15-l bioreactor. Until today, a standard bioreactor for aerobic process is based on its application to the aerating mixing vessel. If a dimension analytical viewing is used, it is easily recognized that, in the point on the total problem of the scale-up (i.e. upholding of all relevant factors like the mixing time), the mass transfer, the mechanical load, and the entry of power have itself derived. For this reason, a scale-up can be carried out only after the principle of partial similarity. This principle is represented in Table 4. The values were changed from 15 l to 150 l under another constant value in a scale-up process. This table explains how one value could be changed from 15 l to 150 l under another constant value in a scale-up process. The task of the regime analysis is now to determine the significance of each value, in order to be able to encounter the statement, about which the observed values must be constant for an upholding of the operating condition.

Table 4. Comparison of the values under the various scale-up factors with the principle of the partial similarity.

Scale-up factor	Term	Bioreactor			
		15-l		150-l	
Volume related power input	P/V	1	1	0.455	0.043
Tip speed of stirrer	n·d_s	1	1.3	1	0.455
Reynolds number	Re	1	2.86	2.2	1
Impeller speed	Rpm	1	0.6	0.455	0.2
Diameter of stirrer	d_s	1	2.2	2.2	2.2

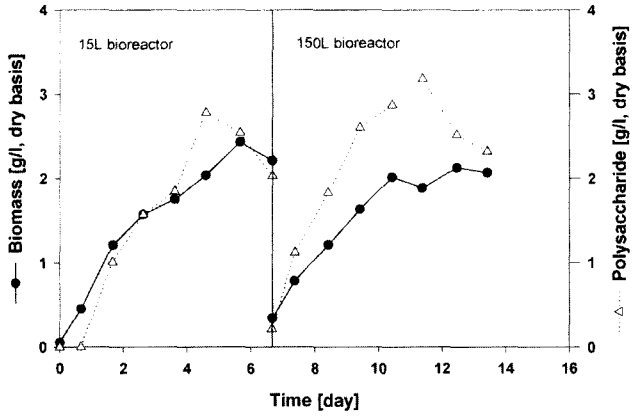

Fig. 2. Changes in biomass of *S. commune* and concentration of polysaccharide in the scale-up process with constant volume-related power input and gas throughput number.

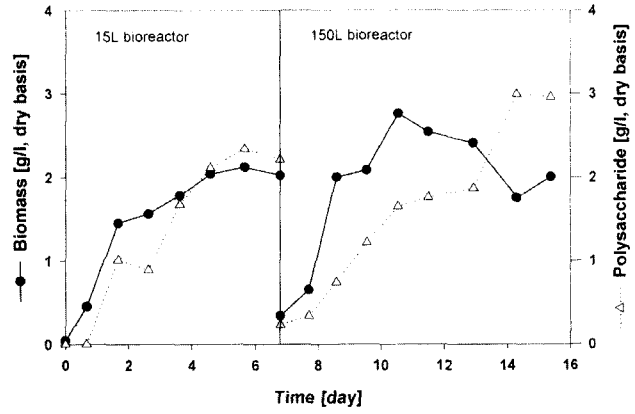

Fig. 3. Changes in biomass of *S. commune* and concentration of polysaccharide in the scale-up process with constant tip speed of stirrer and gas throughput number.

The impeller speed and airflow rate in the 150-l bioreactor by constant P/V and gas throughput number were maintained to 30 rpm and 0.19 vvm, respectively. The maximal concentration of biomass and polysaccharide in 15 l bioreactor were 2.4 g/l and 2.8 g/l, respectively. The concentration of polysaccharide reached to maximal point at 4.6 days. The culture in the 15-l bioreactor was used as an inoculum to the 150 l bioreactor. The maximal concentration of biomass in the 150-l bioreactor was 2.1 g/l. After 4.7 days, the content of polysaccharide reached the maximal point (3.2 g/l), implying that the production period of the polysaccharide to reach the maximal concentration was almost the same in both 15-l and 150-l bioreactors. Figure 2 shows the time course of a scale-up fermentation with constant P/V and gas throughput number.

The impeller speed and airflow rate in the 150-l bioreactor by constant $n \cdot d_s$ and gas throughput number were regulated to 22.5 rpm and 0.14 vvm, respectively. The maximal concentration of biomass and polysaccharide in the 15-l bioreactor were 2.1 g/l and 2.3 g/l, respectively. The concentration of polysaccharide reached the maximal point at 5.7 days. The culture in the 15-l bioreactor was used as an inoculum to the 150-l bioreactor. The maximal concentration of biomass in the 150-l bioreactor was 2.8 g/l. After 7.5 days, the content of polysaccharide reached the maximal point (3 g/l). Even though the concentration of biomass in the 150-l bioreactor was higher than that in the 15-l bioreactor, the production period of the polysaccharide to reach the maximal concentration needed 2 more days. Figure 3 shows the time course of a scale-up fermentation with constant $n \cdot d_s$ and gas throughput number.

The impeller speed and airflow rate in the 150-l bioreactor by constant Re and gas throughput number were regulated to 10 rpm and 0.063 vvm, respectively. The maximal concentration of biomass and polysaccharide in the 15-l bioreactor were 2.2 g/l and 2.5 g/l, respectively. The concentration of polysaccharide reached the maximal point at 5.7 days. The culture in the 15-l bioreactor was used as an inoculum to the 150-l bioreactor. The maximal concentration of biomass in the 150-l bioreactor was 1.7 g/l. After 6.5 days, the content of polysaccharide reached the maximal point (1.6 g/l). Figure 4 shows the time course of a scale-up fermentation with constant Re and gas throughput number. At around 6 days, the culture in the 15-l bioreactor was transported to the 150-l bioreactor. In the case of constant P/V and constant $n \cdot d_s$, the growth pattern and polysaccharide formation by *S. commune* in the 150-l bioreactor was similar to that in the 15-l bioreactor. In the case of constant Re, however, biomass and polysaccharide were lower than those in the other conditions. The low efficiency of constant Re was due to the extremely low impeller speed and airflow rate. The production period of the polysaccharide to reach the maximal concentration with constant P/V and gas throughput number was almost

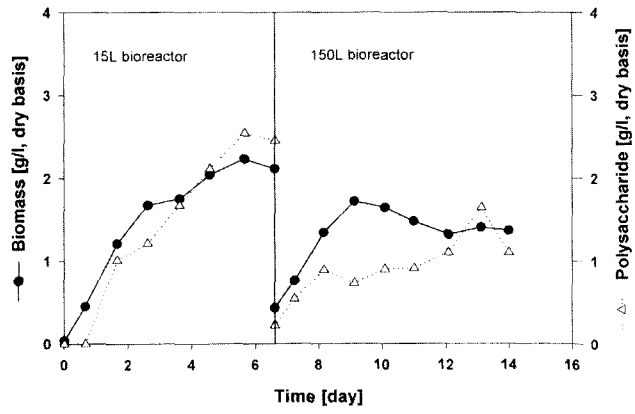

Fig. 4. Changes in biomass of *S. commune* and concentration of polysaccharide in the scale-up process with constant Reynolds number and gas throughput number.

Table 5. Comparison of the maximum specific growth rate (μ_{max}), productivity (PR), and maximal concentration of polysaccharide (MCP) with different scale-up criteria in a 150-l bioreactor.

	μ_{max} [1/day]	PR [g/L·day]	MCP [g/l]
Constant P/V	1.17	0.63	3.18
Constant nd_s	0.99	0.37	3.00
Constant Re	0.74	0.21	1.64

the same in both 15-l and 150-l bioreactors. In the case of constant $n{\cdot}d_s$ and Re, the production period of the polysaccharide to reach the maximal concentration needed one more day. The long period for the maximal concentration of polysaccharide caused low productivity of the polysaccharide. Constant P/V and gas throughput number, therefore, were the optimal criterion for the scale-up process in the barley medium. Optimal impeller speed of 30 rpm and airflow rate of 0.19 vvm were applied to further fermentation in the 150-l bioreactor. In Table 5, the maximum specific growth rate (μ_{max}) and productivity (PR) are compared with each scale-up condition.

Verification of Optimal Culture Condition with Glucose Medium

For the verification of the optimal scale-up criterion (constant P/V and Q) derived from the barley medium used, a glucose medium [3] was used. Optimal impeller speed in the 15-l bioreactor was maintained at 50 rpm, and other fermentation conditions were as follows: initial inoculation was 7%, working volume 7 l in the 15-l bioreactor, and airflow rate of 0.3 vvm. The impeller speed and airflow rate in the 150-l bioreactor at constant P/V and gas throughput number were set to 30 rpm and 0.19 vvm, respectively. Figure 5 shows the time course of a fermentation scale-up with constant P/V and gas throughput number for the glucose medium. The maximal concentration of biomass and polysaccharide in the 15-l bioreactor were 9.9 g/l and 8.1 g/l, respectively. The concentration of polysaccharide reached a maximal point after 8 days. The culture in the 15-l bioreactor was used as an inoculum to the 150-l bioreactor. The maximal concentration of biomass in the 150-l bioreactor was 9.1 g/l. After 6 days, the content of polysaccharide again reached a maximal point (7.8 g/l) in the 150-l bioreactor. It was reported that the maximum concentration of schizophyllan produced by *S. commune* was about 8 g/l in glucose medium [5; also our unpublished result]. The scale-up criterion (constant volume-related power input and gas throughput number) optimized in the barley medium, therefore, could be used as a general criterion for *S. commune*.

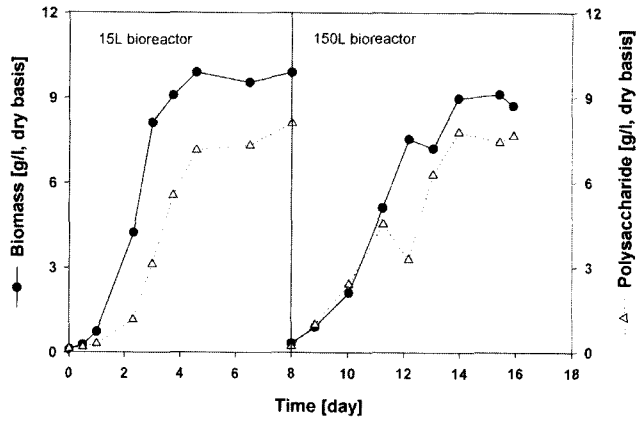

Fig. 5. Changes in biomass of *S. commune* and concentration of polysaccharide for scale-up process with constant volume-related power input and gas throughput number for the glucose medium.

There are various scale-up criteria such as power input, volume-related power input, pump rate of stirrer, pump rate of stirrer per volume, tip speed of stirrer, and Reynolds number for the enhancement of the production yield. These scale-up factors were designed from the agitation ($n{\cdot}d_s$) because all factors contain the rpm (n) and diameter of stirrer (d_s), except a factor which is the pump rate of stirrer per volume. The exponents (x, y) of rpm (n) and diameter (d), $n^x d^y$, therefore, were changed with the factors. The ratio of x and y was 1:2/3 (constant volume-related power input), 1:1 (constant tip speed of stirrer), and 1:2 (constant Reynolds number) in this scale-up process. Impeller speed was a key factor in the scale-up process, since *S. commune* is a filamentous basidiomycetes, which could be damaged during agitation. Gas throughput number (Q), which contains the impeller speed and airflow rate in the equation, was introduced for the low airflow rate in the 150-l bioreactor. After about 6 days, the culture from the 15-l bioreactor was transferred to the 150-l bioreactor. In the case of constant P/V and nd_s, the growth pattern and polysaccharide formation by *S. commune* in the 150-l bioreactor was similar to that in the 15-l bioreactor. In the case of constant Re, however, biomass and polysaccharide were low within the whole criteria. Several researchers reported that glucan formation by some fungi might be stimulated by oxygen limitation [12, 15]. In this experiment, the oxygen limitation by low mass transfer might occur in constant Re and gas throughput number criterion. Therefore, the concentration of biomass and polysaccharide were lower than that in the other scale-up conditions (constant P/V or constant $n{\cdot}d_s$). Extremely low impeller speed (10 rpm) and the airflow rate (0.064 vvm) in the constant Re criterion influenced the growth of *S. commune* and the production of polysaccharide by low mixing rate, indicating that the impeller speed and the airflow rate, which influence the oxygen limitation, must not be simultaneously maintained at low value. Therefore, the proper impeller speed and airflow rate, by which the high concentration of polysaccharide can be obtained, are likely to require optimal fermentation. It was reported that

0.083 vvm of airflow rate was applied for the filamentous fungi, but the impeller speed was controlled to 250 rpm with the Rushton-turbine agitator [12], and that 0.067 vvm of airflow rate was used for the production of scleroglucan and the impeller speed was controlled to 200 rpm with a three-fan agitator [15]. However, the impact of an increased stirrer tip speed on the microbial glucan formation remains unclear. In the present research, constant volume-related power input and gas throughput number was found to be the optimal criterion for the production of schizophyllan by scale-up process in 15-l and 150-l bioreactors.

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