Biomass Evaluation of Fungi Pellets by Respiratory Activity

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Abstract

A theoretical discussion on the biomass evaluation of pellet based on the respiratory activity was made and its experimental investigations were carried out using pellets formed by *Aspergillus niger*, of which average diameters were 2.0 mm, 5.0 mm and 10.0 mm, respectively. The maximum respiration rate (Rr) was recognized when the initial TOC concentration exceeded about 5,000 mg/L. The biomass concentration is linearly related to the Rr value in case of the same pellet diameter. However, the Rr value decreased as the pellet diameter became larger in case of the same biomass concentration, because of the oxygen limitation. Comparing the theory based on the oxygen limitation with the experimental results, it was suggested that the area in 0.5–0.7 mm from the pellet surface was an aerobic and active area not susceptible to the oxygen limitation. It was shown by the proposed theory that the maximum respiration rate was effective as an index of biomass evaluation of pellets, which were formed by the five-day culture and of which diameter was more than 5.0 mm.

Key words : biomass evaluation, pellet, *Aspergillus niger*, respiratory activity, maximum respiration rate, oxygen limitation

INTRODUCTION

Production of various materials and their use have been recently accelerated by rapid progress in the technology and improvement of the life style. These materials are often discharged into a wastewater, resulting in its quantitative and qualitative changes. It has been indicated that conventional wastewater treatment process, such as the activated sludge process, tends to be difficult to treat these materials sufficiently. Therefore, application of the entrapped immobilization method using the specified microorganisms, i.e. phenol degrading bacterium¹⁾,

recombinant bacterium²⁾, and use of fungi^{3)–6)} which have the peculiar enzyme activities have been developed.

We have experimentally investigated starch wastewater treatment using pellets of *Aspergillus niger*⁶⁾. The results suggested that the pellets were applicable to the starch wastewater treatment, because of the excellent solid-liquid separation and the strong amylase activities. However, the kinetic analysis and the model simulation for design, operation and control of the wastewater treatment plant using fungi pellets are not applicable, because the biomass evaluation of pellets has not been developed. In

this study, applying the biomass evaluation of the entrapped-immobilization method proposed by Hashimoto *et al.*⁷⁾, a theoretical discussion on the biomass evaluation based on the respiratory activity is made and its experimental investigations are carried out using pellets formed by *Asp. niger*.

THEORETICAL BACKGROUND

The respiration rate of *Asp. niger* associated with glucose decomposition is generally expressed by the following equation:

$$\frac{dO_2}{dt} = Y' \cdot \left(-\frac{dG}{dt} \right) + b' \cdot X \quad (1)$$

where dO_2/dt is the respiration rate of *Asp. niger* and $-dG/dt$ is the glucose decomposition rate. Y' , b' , G and X are the ratio of oxygen consumed to glucose decomposed, an endogenous respiration rate constant, the glucose and the biomass concentrations, respectively.

The glucose decomposition rate can be expressed by the following equation, taking into consideration both growth of *Asp. niger* and production of citric acid:

$$-\frac{dG}{dt} = Y_{GX} \cdot \frac{dX}{dt} + Y_{GC} \cdot \frac{dC}{dt} \quad (2)$$

where dX/dt , dC/dt , Y_{GX} and Y_{GC} are the growth rate of *Asp. niger*, the production rate of citric acid, the inverse of yield coefficient on *Asp. niger* growth and stoichiometric coefficient of glucose to citric acid, respectively. C is the citric acid concentration.

The growth rate, dX/dt , is expressed as Monod type equation taking into account the autolysis of *Asp. niger* and the production rate, dC/dt , is as Luedeking-Piret type equation⁸⁾, respectively.

$$\frac{dX}{dt} = \frac{k_1 \cdot G}{K_X + G} \cdot X - b \cdot X \quad (3)$$

$$\frac{dC}{dt} = k_2 \cdot \frac{dX}{dt} + \frac{k_3 \cdot G}{K_C + G} \cdot X \quad (4)$$

where k_1 , k_2 and k_3 are the rate constants. K_X and K_C are the saturation constants on growth

and production, respectively. b is the decay coefficient.

When the respiration rate of *Asp. niger* is measured using glucose as a substrate, dX/dt may be assumed to be equal to zero, because of the short measurement period. Therefore, Eqs. (2) and (4) can be approximated as follows:

$$-\frac{dG}{dt} = Y_{GC} \cdot \frac{dC}{dt} \quad (5)$$

$$\frac{dC}{dt} = \frac{k_3 \cdot G}{K_C + G} \cdot X \quad (6)$$

Substituting Eqs.(5) and (6) into Eq.(1) yields Eq. (7).

$$\frac{dO_2}{dt} = [Y' \cdot Y_{GC} \cdot \frac{k_3 \cdot G}{K_C + G} + b'] \cdot X \quad (7)$$

If high strength glucose concentration is used for the respiration rate measurement, Eq.(7) can be approximated as the following equation because of $K_C \ll G$:

$$\frac{dO_2}{dt} = (Y' \cdot Y_{GC} \cdot k_3 + b') \cdot X \quad (8)$$

Under the conditions described above, the value of dO_2/dt in Eq.(8) may be considered the maximum respiration rate (R_r) and Eq.(8) can be rewritten as follows:

$$X = \frac{R_r}{Y' \cdot Y_{GC} \cdot k_3 + b'} \quad (9)$$

As Y' , Y_{GC} , k_3 and b' in Eq.(9) are constant, X is proportional to R_r . Therefore, the maximum respiration rate allows the evaluation of the biomass concentration.

MATERIALS AND METHODS

Microorganism *Aspergillus niger* KCC F-0086 (ATCC6275) subcultured on a Czapek-Dox agar medium, as shown in Table 1, was used in the experiments.

Preparation of spore suspension Sterilized water was added to a slant of *Asp. niger* and mixed to form a suitable solution of suspended spores. The optical density of this suspension was then adjusted to 0.5 at the wave length of 600 nm (equivalent to 10^6 spores per ml).

Table 1 Composition of Czapek-Dox agar medium

Glucose	25.0 g/l
NaNO ₃	3.0 g/l
K ₂ HPO ₄	1.0 g/l
KCl	0.5 g/l
MgSO ₄ ·7H ₂ O	0.5 g/l
FeSO ₄ ·7H ₂ O	0.01g/l
Agar	15.0 g/l
TOC	1000mg/l
pH ^a	5.6

^a pH was adjusted by the sterilized 2N HCl.

Table 2 Average diameter and number of pellets used in each experiment

Run No.	Pellet	
	Average diameter (mm)	Number (N)
1	2.0 ± 0.5	300
		600
		900
2	5.0 ± 0.5	19
		38
		58
3	10.0 ± 0.5	2
		5
		7

Medium A Czapek-Dox medium without agar was used for experiments. This medium was diluted to have a prescribed TOC concentration by deionized water.

Preparation of pellets Three hundred ml Erlenmeyer flasks were filled with 100 ml of Czapek-Dox medium respectively. Then, each flask was inoculated with a fixed G value by a rotational shaking the same as that of Fujita *et al.*⁹⁾ From the pellets formed by five-day culture, those of 2.0 mm, 5.0 mm, and 10.0 mm in diameter were chosen, as shown in Table 2, and thoroughly washed with deionized water before being used in the experiments. It is obvious from

Fig.1 Electron microscopic photograph of a pellet. (5.0 mm in diameter)

fig.1 that these pellets have a highly porous structure.

Methods A medium of predetermined TOC concentration (oxygen enriched by aeration) and the pellets shown in Table 2 were placed into a 200 ml BOD incubation bottle. This was kept in a constant-temperature water bath at 20°C and the respiration rate was measured according to the standard method¹⁰⁾. At the end of the experiment, the dry weight of pellet was measured. The TOC concentrations before and after experiment were measured to confirm that there was no growth of biomass.

RESULTS AND DISCUSSION

Initial TOC concentration affecting respiration rate of pellets Figure 2 shows the relationship between the respiration rate and initial TOC concentration as the representative. It is found that the respiration rate rises as the TOC concentration is higher, regardless of the

Fig. 2 Relationship between initial TOC concentration of medium and respiration rate of pellet.
 Symbols: ○, Pellet No. is 300 in Run 1; ●, Pellet No. is 19 in Run 2; △, Pellet No. is 2 in Run 3.

number or diameter of pellets. However, the respiration rate becomes almost constant when the TOC concentration exceeds about 5,000 mg/l. Results of other experimental conditions are similar to those in this figure. Therefore, the respiration rate at this steady state can be regarded as the maximum respiration rate (R_r).

The relationship between R_r as measured for each run and the biomass concentration (X) is shown in Fig. 3. The relationships of $X = 27.62 \cdot R_r$ for Run 1, $X = 31.73 \cdot R_r$ for Run 2 and $X = 51.42 \cdot R_r$ for Run 3 were obtained, respectively. It is obvious that the relationship is given by the theoretical equation, Eq.(9), in case of the same pellet diameter. However, the coefficient of Eq. (9), $1/(Y \cdot Y_{GC} \cdot k_3 + b')$, tends to be lower as the pellet diameter becomes smaller. This result shows that, in case of the same biomass concentration, R_r decreases as the pellet diameter becomes larger. Therefore, it was suggested that oxygen was limited inside the pellet

and its respiratory activity was degraded as the pellet diameter became larger.

Respiratory activity of pellet based on oxygen limitation A theoretical equation describing the relationship between R_r and the biomass concentration for the same diameter of pellet was developed and verified by the experimental investigation. This result was similar to that immobilized by PVA-boric acid method⁷⁾. However, the same biomass evaluation of fungi pellets as that of the entrapped-immobilized microorganisms is not suitable, because of the possibility of oxygen supply being limited inside the pellets as they grow. Therefore, the biomass evaluation of pellets by the respiratory activity based on the oxygen limitation was investigated as the following assumptions:

- The DO concentration decreases linearly from the pellet surface to the interior, as shown in Fig.4.
- The respiration rate is constant until

Fig. 3 Relationship between maximum respiration rate and biomass concentration.
Symbols: ○, Run 1; ●, Run 2; △, Run 3.

restricted by the lack of oxygen.

- (c) The respiration rate decreases linearly below A^* mg/l of DO concentration.

The respiration rate per one pellet, Rr' , can be calculated using the following equation:

$$Rr' = \frac{1}{V} \cdot \int_0^\alpha \{ -h(\alpha - \beta) \cdot 4\pi(r-x)^2 \} dx + \frac{1}{V} \cdot \int_\alpha^\beta \{ -h(x - \beta) \cdot 4\pi(r-x)^2 \} dx \quad (10)$$

where V and r are the volume of medium and the diameter of pellet, respectively. The following equation is obtained by solving Eq. (10):

$$Rr' = -\frac{4\pi h}{V} \cdot \left(\frac{\alpha^4}{12} - r \cdot \frac{\alpha^3}{3} + r^2 \cdot \frac{\alpha^2}{2} - r^2 \cdot \frac{\beta^2}{2} + r \cdot \frac{\beta^3}{3} - \frac{\beta^4}{12} \right) \quad (11)$$

If the pellet diameter is sufficiently large, the high-order terms of α and β may be neglected and Eq.(11) is approximated as the following:

$$Rr' = -\frac{4\pi h}{V} \left(r^2 \frac{\alpha^2}{2} - r^2 \cdot \frac{\beta^2}{2} \right) \quad (12)$$

As the initial DO concentration, A_0 , at the Rr measurement is about 4.0 mg/l and the DO concentration, A^* , at which microorganisms are not generally limited by the oxygen is about 0.5 mg/l⁽¹¹⁾, the relationship of $\alpha = 7\beta/8$ can be established and Rr' is expressed as follows:

$$Rr' = \frac{15\pi \cdot h \cdot r^2 \cdot \beta^2}{32 \cdot V} \quad (13)$$

Trial and error method was used to calculate a value corresponding to the measured Rr' value by changing β as shown in Eq. (13). β was presumed to be 0.5–0.7 mm as a result. In other words, it was suggested that the area in 0.5–0.7 mm from the pellet surface was an aerobic and active area not susceptible to the oxygen limitation.

Assuming that the density of pellet is ρ , the biomass concentration per pellet, X' , can be expressed by the following equation:

$$X' = \frac{4 \cdot \pi \cdot r^3}{3 \cdot V \cdot \rho} \quad (14)$$

Fig. 4 Schematic diagram of both DO concentration and respiration rate profiles in pellet.
 $Rr' = -h \cdot (\alpha - \beta)$ when $x < \alpha$, $Rr' = -h(x - \beta)$ when $\alpha < x < \beta$, $Rr' = 0$ when $\beta < x < r$.

If the number of pellets used in the experiments is n , the biomass concentration (X) and the maximum respiration rate (Rr) are expressed as $Rr = n \cdot Rr'$ and $X = n \cdot X'$, respectively. Therefore, the biomass concentration can be derived combining with Eqs. (13) and (14) as follows:

$$X = \frac{128 \cdot \rho \cdot r}{45 \cdot h \cdot \beta^2} \cdot Rr \quad (15)$$

If h , β and ρ are constant regardless of pellet diameter, the biomass concentration may be expressed as a function of the Rr value and the pellet diameter. The relationship between the coefficient obtained in Fig. 3, i.e. $128 \cdot \rho \cdot r / (45 \cdot h \cdot \beta^2)$ and the pellet diameter is shown in Fig. 5. It is obvious that the coefficient is linearly related to the pellet diameter in case of Runs 2 and 3. However, Run 1 seems to be distinctly different from Runs 2 and 3. This result was thought that h or β value in Run 1 was lower than those in Runs 2 and 3 because of the shorter diameter. Therefore, it was concluded

that the maximum respiration rate was effective as an index of biomass evaluation of pellets, which were formed by the five-day culture and of which diameter was more than 5.0 mm.

CONCLUSIONS

A theoretical discussion on the biomass evaluation of pellet based on the respiratory activity was made and its experimental investigations were carried out. The results obtained are summarized as the following:

- (1) The respiration rate of pellets, of which average diameters were 2.0 mm, 5.0 mm and 10.0 mm, respectively, became almost constant when the initial TOC concentration exceeded about 5,000 mg/l. This respiration rate was recognized as the maximum respiration rate of pellets (Rr).
- (2) The biomass concentration is linearly related to the Rr value in case of the same pellet diameter. However, the Rr value decreased as

Fig. 5 Relationship between pellet diameter and coefficient in Fig. 3. The coefficient is the same as the proportional constant of Eq. (15). Symbols: ○, Run 1; ●, Run 2; △, Run 3.

the pellet diameter became larger in case of the same biomass concentration, because of the oxygen limitation.

(3) The theory of biomass evaluation of pellets based on the oxygen limitation was developed. Comparing with the experimental results, it was suggested that the area in 0.5–0.7 mm from the pellet surface was an aerobic and active area not susceptible to the oxygen limitation. Moreover, using the proposed theory, it was concluded that the maximum respiration rate was effective as an index of biomass evaluation of pellets, which were formed by the five-day culture and of which diameter was more than 5.0 mm.

NOMENCLATURE

A_0 : Initial DO concentration of medium, mg/l
 A^* : DO concentration at which microorganisms are not limited by oxygen,

mg/l
 b : Decay coefficient, 1/hr
 b' : Endogenous respiration rate constant, 1/hr
 C : Citric acid concentration, mg/l
 dC/dt : Production rate of citric acid, mg/l·hr
 $-dG/dt$: Glucose decomposition rate, mg/l·hr
 dO_2/dt : Respiration rate of pellets, mg/l·hr
 dX/dt : Growth rate of pellets, mg/l·hr
 G : Glucose concentration, mg/l
 h : Consumed oxygen per unit of distance, mg/mm
 k_1 : Rate constant, 1/hr
 k_2 : Rate constant, —
 k_3 : Rate constant, 1/hr
 K_X : Saturation constant on growth, mg/l
 K_C : Saturation constant on production, mg/l
 n : Number of pellets, N
 r : Diameter of pellet, mm
 R_r : Maximum respiration rate, mg/l·hr

Rr'	: Respiration rate per pellet, mg /l·hr·N
V	: Volume of medium, ml
X	: Biomass concentration of pellets, mg /l
X'	: Biomass concentration per pellet, mg /l·N
Y_{GX}	: Inverse of yield coefficient, —
Y_{GC}	: Stoichiometric coefficient of glucose to citric acid, —
Y'	: Ratio of oxygen consumed to glucose decomposed, —
α	: Critical point of rate limitation, mm
β	: Critical point of anaerobic area, mm
ρ	: Density of pellet, mg/l

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