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Characteristics and Model for Growth of *Rhizopus oryzae* on the Simulated Gas-solid Interface

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Abstract - In order to investigate the effect on morphology of *Rhizopus oryzae* and production of lactic acid, various interface materials were used. Morphology of fungal showed sheet and flock when resin was added. The production of lactic acid was increased dramatically when interface materials were added. Furthermore, the effect of resin was more significant than that of others. It was assumed that interface materials could absorb substrate and microorganism together, so microorganism was not inhibited by substrate. The effect of static electric field on the interface culture was studied. When the exerting potential was 6.78 voltage, the biomass y was obviously higher than that of zero voltage. A simulated gas-solid interface system was developed to study the growth and two phases model for the growth of *Rhizopus oryzae* was build up that depended on the symmetric branching theory. An important parameter F was researched. The results indicated that the value of F had obvious difference at exponential and deceleration period, respectively.

Key words : interface, *rhizopus oryzae*, electric field, two phases model

INTRODUCTION

It is well known that microorganism exist everywhere in the world including the surface of glass, rock and plastic etc. where there almost has no nutrition for growing of them (Shen *et al.* 1991; Zhang *et al.* 1995; Gu *et al.* 1999). How to explain the special phenomena? It cannot be interpreted with the conventional microbe growth conception but with the interface theory (Wang *et al.* 1999). Interface, the boundary between two different phases, shows the special characters as following: Firstly, some interface material such as glass, plastic and steel could absorb microorganism and nutrition to concentrate them; Secondly, interface of liquid-liquid, gas-liquid and solid-liquid take on electric phenomena; Thirdly, interface is not a two dimension conformation but a three dimension structure (Chen *et al.* 2001). Based on these traits the interface takes effect on the growth and metabolism of interface microorganism. So it is necessary to reveal the interaction between microorganism and interface.

Microorganism on the interface held similar characteristics to that of microorganism grew at ordinary condition. However they have special differences. For example, microorganism could grow better on barren interface due to the concentrating function of interface (Chen *et al.* 2001). In additional substrates have different

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transfer mode, which have great effect on the metabolism and production of microorganism. At the hand of life model, the growth model for interface microorganism still was difficult to build since of the complexity of interface system (Han *et al.* 1988; Frame *et al.* 1998).

In this paper, first, in order to investigate the effect on morphology and production of lactic acid from *Rhizopus oryzae*, various interface materials were used. In addition, the effect of static electric field on the interface culture of *Rhizopus oryzae* was studied. Second, a simulated gas-solid interface system was developed to study the growth and two phases model for the growth of *Rhizopus oryzae* was applied successfully that depended on the symmetric branching theory.

MATERIALS AND METHODS

1. Development of a simulated gas-solid interface

Ordinary interface shows irregular shape and various sizes, on which it is very difficult to study microorganism. In order to solve the problem, the ordinary interface was simulated to be artificial plane interface derived from the extreme small area after dividing the former into infinite qualities parts according differential theory. So we make plane as the ideal interface. Depending on the presumption above, a gas-solid interface was simulated in the article (Yano *et al.* 1991; Ogawa *et al.* 1995a, b). Laying a microporous membrane on solid plate, inoculating spore of filamentous microorganism on the center of membrane with microinjector, and cultivated under proper condition in incubator. Mycelium could strike through micropores and absorb the nutrition from medium; the growth of microorganism was restricted within the interface.

2. Microorganisms and Media

Rhizopus oryzae T-1789 was used through this study because of its peculiarity including rapid growing, easily observation and others. The strain was maintained on PDA slant, which contained, per liter: untreated potato, 200 g; agar, 20 g.

3. Culture methods

For submerged culture, spore suspension was inoculated into a 250 mL conical flask bearing 50 mL medium containing 1×10^5 spores per minimum liter and cultured at 35°C for 48 h on a rotary shaker at 180 r min^{-1}.

For simulated interface culture, the plate medium which contained wheat bran, 8 g; κ-carrageenan, 3.5 g; Water 100 mL was autoclaved and then poured into a plate with 11 cm diameter and 5 mm depth. Microporous membrane (0.2 μm) after autoclaved was laid on the solid plate. Spore suspension (0.1 mL) containing 10^7 spores per minimum liter was dropped on the membrane with microinjector and cultured at 35°C for couple days.

4. Analysis

Dry cell weight was determined by harvesting culture samples, filtering through Whatman 4# filter sheets, washing the mycelia a few times with distilled water, and drying until a constant weight was achieved at 80°C.

The lactic acid was measured using EDTA titration. The biomass of cell on the simulated interface was determined by drying mycelium to constant weight at 80°C after being striped from microporous membrane.

RESULTS AND DISCUSSION

1. Influence of different interface materials on the metabolism and morphology of microorganism

Different interface materials have different physical traits such as absorbability, charged capacity and surface area. Active carbon, resin, silica gel and quartzite were added to medium, respectively (Wang *et al.* 1999). Each addition was 0.5 g. Microorganism morphology and production of lactic acid were investigated after being cultured at 35°C and 120 r min^{-1} for 48 h in rotary incubator. The results are shown in Table 1 and Fig. 1. Table 1 showed that morphology of fungal was affected by interface materials obviously. If no interface materials were added, pellet mycelium was observed. When interface materials exist, morphology variation takes place. For example morphology of fungal showed sheet and flock when resin was added.

Also interface materials exert influence on production of lactic acid by fungal. The results are showed in Fig. 1. The production of lactic acid increased dramatically if interface materials were added. Furthermore the effect of resin was more significant than that of others. The reason why interface materials could improve the production of lactic acid was not identified. It was assumed that interface materials could absorb substrate and microorganism together, so microorganism was not inhibited by substrate.

Whether the effect of interface materials on biomass of fungal would be stronger or weaker with the addition increasing of them. A series of experiments were designed to approve the effect. The results were showed in Table 2. Both active carbon and resin were experimented. With the increase of addition, the dry weight of fungal significantly enhanced. It should be noted that active carbon played more evident role than resin did.

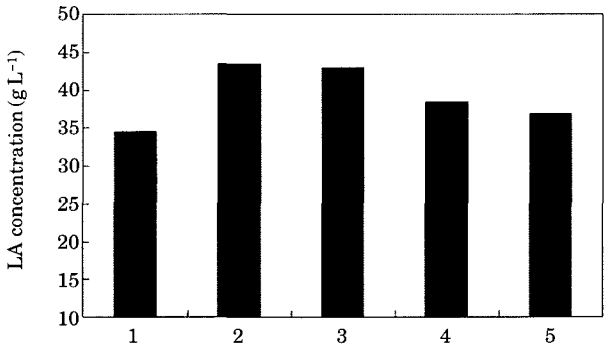

Fig. 1. Effect of interface materials on the Lactic acid concentration. Note: 1. None; 2. Resin; 3. Silica gel; 4. Active carbon; 5. Quartize.

Table 1. Effect of interface materials on the morphology

Material	Morphology
None	Pellet
Active carbon	Sheet and pellet
Resin	Sheet and floc
Quartzite	Sheet and gelatinous
Silica gel	Granular

Table 2. Effect of the interface material concentrations on the biomass

Concentration (%)	Dry weight (g)	
	Active carbon	Resin
0.0	0.27	0.14
0.5	0.38	0.14
1.0	0.37	0.15
1.5	0.40	0.24
2.0	0.53	0.21

2. The determination of *Rhizopus oryzae*' growth profile on the simulated interface

The curve in Fig. 2 reflected the variation of biomass during growth of fungal on the surface of membrane (Flint *et al.* 2001). The process could be divided into such three as spore germination, mycelium expansion and deceleration phase. During the first 20h spore developed into germ tube and further into hyphae, then hyphae continued extending and started to branch.

During the following 20h rapid growing hyphae spread the rest region on the interface. When filamentous microorganism covered the whole membrane, mycelium stopped spreading around. Meanwhile the inhibition of substrate and the accumulation waste from cell made an influence on the growth of cell, so then microorganism began to decelerate.

3. Effect of inoculation amount on the biomass of interface microorganism

Fig. 3 showed that inoculation amount taken a little effect on the growth of interface microorganism. The profile fluctuation was slight. Furthermore the fluctuation was irregular. When spores were inoculated on interface, mycelium first extended horizontally across

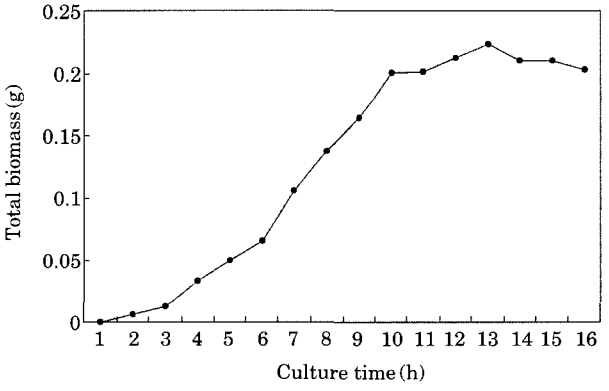

Fig. 2. Effect of culture time on the total biomass in the interface.

the interface, and then turned to vertical growth when hyphae density reached a certain extent. So inoculation amount would probably affect the period of horizontal growth of the fungal. When inoculation amount increase, the period of horizontal growth would be shorten. But fig. 3 shows that the total biomass kept constant whatever the inoculation amount was.

4. Effect of electric field on the growth of *Rhizopus oryzae*

Any biology body was covered by charged electrolyte, meanwhile there exists electric field, which make some effect on natural life including microbe (Fox *et al.* 1996). It was reported that cell membrane potential was induced to increase by electric field and ATP was synthesized greatly. Furthermore metabolism also strengthened. In this experiment interface system was placed under electronic field condition and the multiplying process of microorganism was investigated. The results are shown in Fig. 4. When the exerting potential was 6.78 voltage, biomass obviously was more than that no exerting potential.

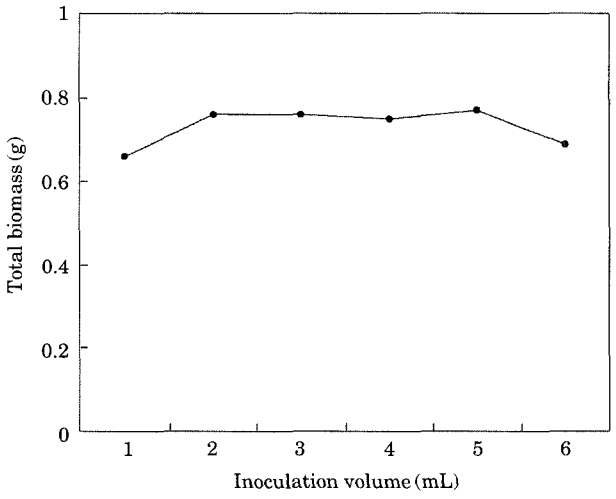

Fig. 3. Effect of inoculation volume on the total biomass in the interface.

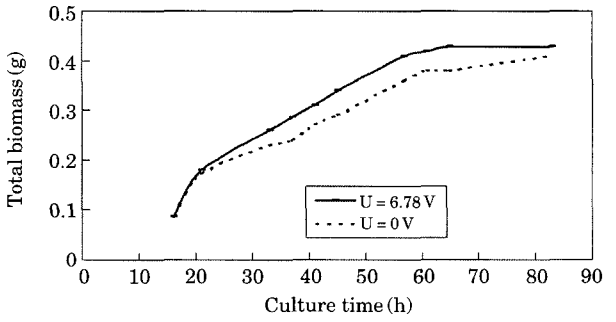

Fig. 4. Effect of electric field on the growth in simulated system.

5. Development of two phases model

Growth kinetics and the mathematical modeling of microbial growth on interface have received little attention due to the difficulties associated with the study of interface (Montira *et al.* 1998). Little is known of the mechanisms controlling the density and structure of fungal biomass above the substrate surface during growth in solid-state fermentation systems. The system is shown in Fig. 1: fungal biomass is spread homogeneously over the surface of a membrane, the underside of which is in contact with a solid medium. Membrane culture was used as a model SSF system (Shen *et al.* 1991).

Their polycarbonate nucleopore membrane had a 0.2 μm pore size, which prevented growth of penetrative hyphae into solid medium. Nutrient diffuses across the membrane and is absorbed into the fungal biomass where it contacts the membrane.

The model proposed in this paper explores possible mechanisms controlling the hyphal concentration as a function of height above surfaces, using equations describing tip generation and movement as the underlying phenomena (Christian *et al.* 2000). However, the model does not aim to describe the growth and branching of individual hyphae rather it describe the collective behavior of the hyphae, which constitute the mycelium.

Depending on the growth characteristics proposed by Mitchall, an experience kinetic model that divides growth process into two parts such as logarithm phase and decelerated phase was proposed in this paper (Robert *et al.* 1997; Lilik *et al.* 2000).

Equations of model was described as following:

1) Logarithm phase

Logarithm phase was defined as beginning with spread of germ tub out of spore. Symmetric branching model was used to describe the growth of microorganism colony. It was assumed that while germ tub reach

to some length, which occur to form two heterogeneous morphological son hyphae and grow at same speed. Since growth rate of mycelium was a constant, the length of every mycelium increased depending on the linearly.

Once the length of mycelium developed into critical value, mycelium branched again. During whole process of culture, the same circle of growth continued.

Mycelium section was defined as the distance between two successive knots. It was assumed that the length of all mycelium sections was identical. These mycelium sections were called non-active sections that not mean there was no metabolism, only mean these sections did not prolonged. While the section was called as active segment since it grew from knot newly. The active segment could transfer into non-active section if the former occurred to branch, then new circle began. During the whole logarithm phase, all active tip spread constantly because of there existing void area on medium.

Since active mycelium had an ability to prolong, the total biomass depended on the increase of active biomass. The general macrocosm growth model of fungal was described as following.

$$\frac{dX_T}{dt} = \mu_g X_A \tag{1}$$

Where X_A is active biomass, X_T is total biomass, μ_g is average growth special rate constant which refer to the growth rate during two successive branching. Although every active segment has its own growth rate, average special growth rate is constant.

The active biomass was obtained through calculating as the quantitative relation between the active biomass and the total biomass as following.

$$X_A = FX_T \tag{2}$$

Where F is ratio between the active biomass and the total biomass.

During logarithm phase active biomass after b times branching could be predicted quantitatively using symmetric branching model.

$$N_A = 2^b \tag{3}$$

Where N_A is the knot number of active segment, b is the number of branch.

The relation between the total number of knot (N_T) and the number of active segments (N_A) was described as the following equation.

$$N_T = 2N_A - 1 = 2(2^b) - 1 = 2^{b+1} - 1 \tag{4}$$

The active segments that exert non-synchronizing and numerable growth circles disperse heterogeneously. For example at any time the average length of active segments is equal to the half of non-active section, furthermore active biomass is equal to the half of non-active biomass too. So F is represented by the following equation.

$$F = \frac{X_A}{X_T} = \frac{X_A}{X_N + X_A} = \frac{0.5X_S N_A}{X_S(N_T - N_A) + 0.5X_S N_A} \tag{5}$$

$$= \frac{0.5X_S 2^b}{X_S(2^{b+1} - 1 - 2^b) + 0.5X_S 2^b}$$

Where X_S is the quality of each hyphae, X_N is the biomass of total non-active segment.

When mycelium grew fast and fast and branched constantly, the value of F was about 0.33. So F was thought as 0.33 and kept constant during the whole logarithm phase.

2) Deceleration phase

Once colonies contact each other, the growth of cell run into deceleration phase. While symmetric branching theory is still fit. However some tip of mycelium stop growing and become non-active segments. The phenomena are called as tip death, which mean not stopping metabolism but prolonging. When tip death occurred, the active sections transfer into non-active segments and the ratio of active segments would decrease.

Here tip death rate was described by tip live factor L that represented the ratio between the active biomass around the cross of logarithm phase and deceleration phase. 1.0 is the maximum value of L, which means that all mycelium tips are live and not been influenced by cell age. The fraction of active biomass relates with 0.33 and live factor.

$$F_i = 0.33L \tag{6}$$

Where is the fraction of active biomass, L is live factor.

6. F value

Fig. 5 showed that F value changed during the process of fungal growth. At the beginning 36 h of culture cell presented logarithm growth. Apparent biomass increasing indicated augment of active biomass. At same time F factor held dominant station. When cell accessed to the decelerate phase, active biomass reduced rapidly and accompanying F value fell fast.

7. Change of active biomass during the schedule of fungal growth

According to equation 5 the profile of active biomass could be obtained. The results were showed in Fig. 6. It was similar to the profile of F that there was rapid reduce after 36 h culture in the profile of active biomass. The reasons for above phenomena might be complained depending on the following ideas. Firstly at the beginning of culture since substrate and surrounding was enough for mycelium to expand and metabolism, active biomass looked increasing fast. However at deceleration phase the growing flatly would stop and transfer to the growing vertically. Since mycelium tangled and competed each other, active biomass would lessen finally. Secondly difficult transportation of substrate on interface is other reason why active biomass would decrease.

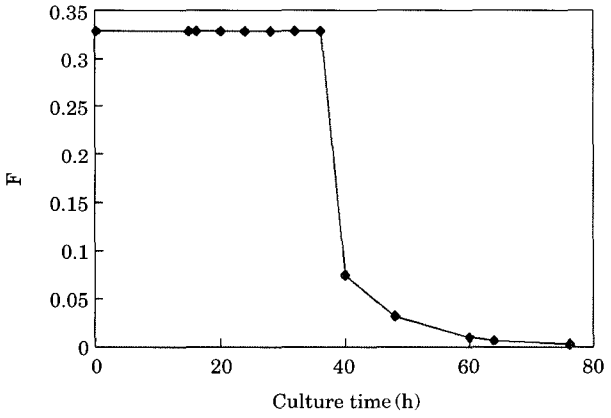

Fig. 5. F-factor change in the growth profile of *Rhizopus oryzae* on the interface.

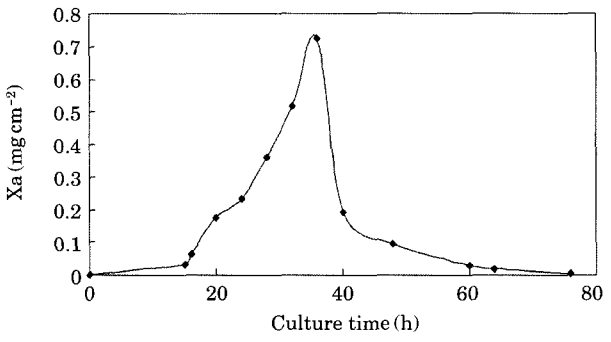

Fig. 6. Active biomass profile of *Rhizopus oryzae* on the interface.

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