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Effect of Insoluble Polymer Particles Added into Culture Liquid on Enzyme Production by Mold

Ryoji MITSUI, Kousuke TAMURA, Toshiro MIYAHARA* and Mitsuo TANAKA
Department of Biochemistry, Faculty of Science,
** Department of Applied Chemistry and Biotechnology, Faculty of Engineering,*
Okayama University of Science, 1-1 Ridai-cho, Kita-ku, Okayama city, Okayama 700-0005, Japan

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We investigated the effect of addition of insoluble polymer particles into culture liquid on enzyme (amylase) production by mold, that is *Aspergillus niger*. The obtainings showed that the highest effect of addition was ca. 0.8 ~ 1.4 (1/cm) of the specific surface area, that is the ratio of total surface area of particles added to total volume of culture liquid and particles added, resulting from the inhibition of flock formation or the fragmentation of flocks in the culture. The effect of addition did not occur in the case of *Bacillus amynoliquefaciens*, that is a bacterium.

Keywords: *Aspergillus niger*, *Bacillus amyloliquefaciens*, amylase production, insoluble polymer particles.

Introduction

The enzymes obtained from the culture of *Aspergillus niger* have been used as ones for a kind of industries since a long time ago. For instance, nowadays, amylase produced by the microorganism is utilized for liquefaction and saccharification of starch. However, although it is well known that a kind of molds forms flocks in liquid culture, and the productivity of enzymes lowers in general, it has almost not reported about the effect of addition of insoluble polymer particles to culture solution to increase the enzyme productivity.

We investigated the effect of the addition of high molecular polymer particles in the culture liquid on productivity of enzymes by using *Aspergillus niger* and *Bacillus amyloliquefaciens* as preliminary test.[1), 2)]

1. Experimental

1.1. Preparation of crude enzymes

Aspergillus niger was inoculated at the cell turbidity, 0.1 (λ = 610 nm), and seed-cultured for 3 days at 28 ℃ under the culture liquid of 10 ml, and main-cultured at the same temperature under the culture liquid of 300 ml at initial pH 4.5 using 500 ml shaking flasks.

The culture of *Bacillus amynoliquefaciens* was also the same conditions, but the pH in main culture was 7.0. Starch (10 g/l) was used as a carbon source. The culture medium for *Aspergillus niger* contained Casamino acid, 1.0; Urea, 0.3; KH_2PO_4, 2.0; $(NH4)_2SO_4$, 1.4; $MgSO_4 \cdot 7H_2O$, 0.3; $CaCl_2 \cdot 2H_2O$, 0.4; $FeSO_4 \cdot 7H_2O$, 0.005; $ZnSO_4 \cdot 7H_2O$, 0.0016; $MnSO_4 \cdot H_2O$, 0.0014; $CoCl_2 \cdot 6H_2O$, 0.004 (g/l). The culture medium for *Bacillus amylo-liquefaciens* consisted of $NaH_2PO_4 \cdot 7H_2O$, 1.56; NH_4Cl, 5.35; KCl, 0.754; $NaSO_4 \cdot 7H_2O$, 0.644; Citric acid, 0.42; $MgCl_2 \cdot 6H_2O$, 0.25; $CaCl_2$, 0.0022; ZnO, 0.0025; $FeCl_3 \cdot 6H_2O$, 0.027; $MnCl_2 \cdot 6H_2O$, 0.010; $CuCl_2 \cdot 2H_2O$, 0.00085; $CoCl_2 \cdot 6H_2O$, 0.0024; $NiCl_3 \cdot 6H_2O$, 0.00025; H_3BO_3, 0.0003; Na_2MoO_4, 0.001; Tryptone, 10; Yeast extract, 5 (g/l).

The mean diameters of high molecular polymer particles added were 2.6 mm and 1.1 mm (polystyrene, density:1.01(g/cm^3)) and 6.3 mm (nylon, density:1.12(g/cm^3)). A part of the main culture liquid was sampled out periodically, centrifuged and the supernatant was used as crude enzyme solution.

1.2. Measurement of enzyme activity

Enzyme reaction was performed under pH 5.0 (acetate buffer) at 30 ℃ using 0.4 % soluble starch (final concentration) as a substrate. Amylase (saccharification type) activity was measured by

Somogyi-Nelson method. Glucose was used as a standard. Optical density was measured as absorbance at λ =650 nm.

1.3. Measurement of sedimentation velocity

The size of sedimentation tube made in glass was 20 mm in inner diameter and 700 mm in height. The main culture liquid of 180 ml at 6 and 12 days of culture time was used. The time course of sedimentation of mixed culture liquid of mycelia and flocks was measured.

2. Results and discussion

2.1. Amylase activity produced by *Aspergillus niger*

As shown in **Figure 1**, in both cases of no addition and addition of particles, the amylase activity was increased with the increase in pH of culture liquid. The maximal activity was obtained at the culture time of ca. 10 days. **Figure 2** and **Figure 3** show the maximal activity. The particle size and quantity used are different.

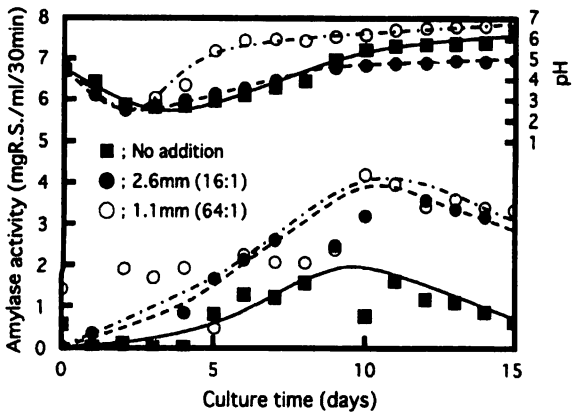

Fig. 1. Time course of amylase production by the culture of *A. niger*.

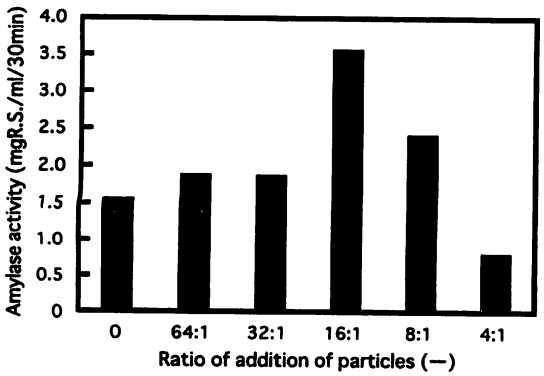

Fig. 2. Comparison of maximal activity (Particle size: 2.6 mm diameter).

The results shown in Fig. 2 and Fig. 3 are the cases that the particle size were 2.6 mm and 1.1 mm, respectively. In the case of the particle size of 2.6 mm, when the ratio of culture liquid (g) to particle quantity (g) was 16 to 1, the maximal activity was obtained. In the case of the particle size of 1.1 mm, when the ratio was 64 to 1, the maximal activity was obtained. In both cases, the maximal activity was 2 times higher than that in the case of no addition of particles. On the basis of the results, the relationship between amylase activity and specific surface area (total surface area of particles added / total volume of culture liquid and particles added) is shown in **Figure 4**.

The maximal activity was obtained under ca. 0.8 to 1.4 (1/cm) of specific surface area, regardless of particle sizes added. The similar result was obtained in the case of the particle size of 6.3 mm (data not shown). These possibly show that the collision frequency

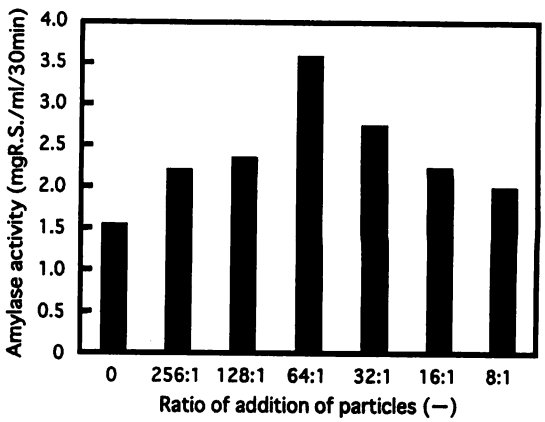

Fig. 3. Comparison of maximal activity (Particle size: 1.1 mm diameter).

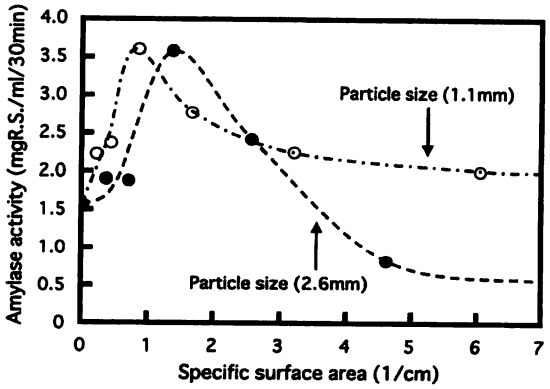

Fig. 4. Influence of specific surface area on amylase production by *A. niger*.

between particles added and mycelia increases, a change of agitation condition occurs, and a kind of force like sheer stress increases.

2.2. Amylase activity produced by *Bacillus amynoliquefaciens*

As shown in **Figure 5**, in the case that particles were not added, in the case that the ratio of culture liquid (g) to particle quantity (g) was 16 to 1 for the particle size of 2.6 mm, and in the case that it was 64 to 1 for the particle size of 1.1 mm, the maximal activity was obtained at the culture time in the vicinity of 3 days in all of the cases.

As shown in **Figure 6**, obviously, the maximal activity was almost no difference. It results from that the microbe used was bacterium, and the bacterium does not form flocks. And also, since the activity was very high in comparison with the case of *Aspergillus niger*, the difference of the activity produced may be not clear.

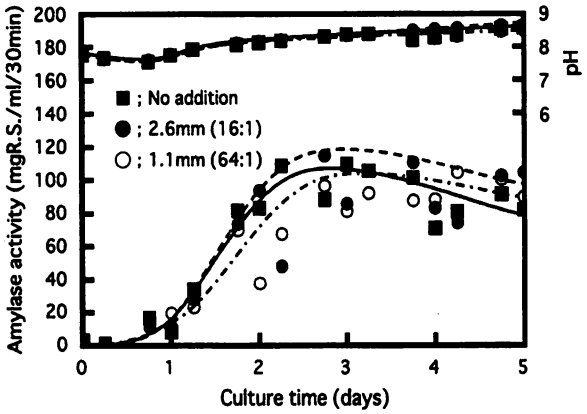

Fig. 5. Time course of amylase production by the culture of *B. amynoliquefaciens*.

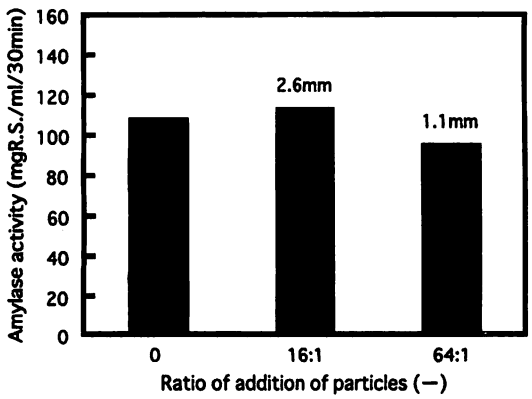

Fig. 6. Comparison of maximal amylase activity on the culture using different size of particles.

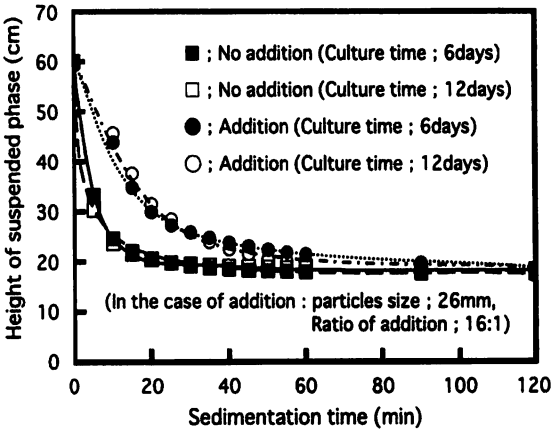

Fig. 7. Sedimentation velocity of mycelia under the culture by using different particle sizes.

2.3. Sedimentation velocity of mycelia of *Aspergillus niger* cultured

Figure 7 shows the sedimentation velocity of mycelia in the culture liquid at the culture time of 6 days and 12 days. The culture was carried out under no addition and addition of the particles (the ratio was 16 to 1 in the case of 2.6 mm). Apparently, the initial sedimentation velocity was lower than that in the case of no addition of particles. This result shows that the flocks formed in culture came loose, and the mycelia were fragmented, or the formation of flocks was inhibited.

Interestingly, when *Aspergillus niger* was cultivated with aeration (superficial gas velocity; 1.06 ~ 2.12 mm/s) on a culture apparatus of towertype, the amylase productivity was ca. 2 times higher than that of the shaking culture method in the case of no addition of particles (data not shown).[3] This probably resulted from an inhibition of formation of flocks and from the fragmentation of flocks by agitation of culture liquid induced by a large number of rising bubbles. On the other hand, although the effect of addition of the above particles on the amylase productivity of *Eupenicillium javanicum* was also investigated, that did not occur (data not shown). The obtaining possibly results from that the high dense and solid flocks were formed, and that the flocks formed cannot be fragmented easily. That is, the effect is more or less dependent on kinds of molds. Therefore, we should investigate in the near future by using *Rhizopus delemar* as another mold, which produces amylase powerfully.

Conclusions

The result obtained by using *Aspergillus niger* showed the highest effect of addition of insoluble particles was ca. 0.8 –1.4 (1/cm) of the specific surface area. The effect occurred resulting from the inhibition of flock formation or the fragmentation of flocks in the culture. Therefore, the effect of addition of particles did not occur in the case of bacterium, which did not form mycelia.

If we carry out the culture under addition of particles, the agitation condition of culture liquid is improved, the formation of flocks is limited or the fragmentation of flocks is caused, and the mold can produce enzymes effectively.

However, because the effect may be dependent on kinds of molds, another molds should be tested. Moreover, we must elucidate the physiological influence to mycelia of agitation of the culture liquid by particles added, for instance the increase in growth activity and/or the increase in enzyme productivity per unit amount of growth.

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