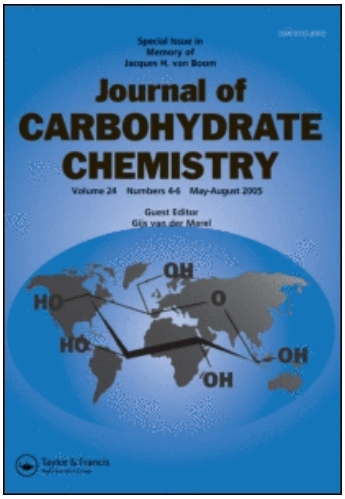

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IMMOBILIZATION OF β-GLUCOSIDASE USING WOOD RESIDUE FOR ENZYMATIC HYDROLYSIS[1]

Shizu Fujishima,* Fumiko Yaku, and Tetsuo Koshijima

Government Industrial Research Institute, Osaka
Midorigaoka, Ikeda, Osaka 563, Japan

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ABSTRACT

A significant amount of β-glucosidase and other cellulase components were adsorbed on the residue remaining after enzymatic hydrolysis of cellulosic materials. The wet wood-residue separated from an enzymatic degradation mixture hydrolyzed cellobiose to glucose in a yield of about 100% and retained the activity even after the 30th treatment. These residues were able to be used as an immobilized β-glucosidase preparation. By drying the wet wood residue, only β-glucosidase was retained on it, and the stability of immobilized β-glucosidase increased, although the specific activity decreased significantly.

INTRODUCTION

Lignocellulosic materials containing cellulose represent one of the largest potential biomass resources for energy and other useful substances. Enzymatic hydrolysis has received much attention because it is very specific and does not involve by-product formation. Cellulolytic enzyme is comprised of endo-glucanase, cellobiohydrolase, and β-glucosidase. In the enzymatic hydrolysis the β-glucosidase activity of the enzyme complex is generally the factor limiting the kinetics of the process, because these enzymes are in fact inhibited by cellobiose which is a reaction product.[2] To obtain a high yield of glucose at a high reaction rate, attempts to immobilize β-glucosidase and use it together with cellulase itself have been made.[3-5]

Adsorption of cellulase on cellulosic materials has been studied but whether β-glucosidase was adsorbed has not been made clear.[2,6-8] Mandels et al.[2] and Ooshima et al.[8] reported that β-glucosidase from *Trichoderma* was not adsorbed on cellulose, but Goel et al.[7] reported that same enzyme was adsorbed on cellulose. In this work, we show that a significant amout of β-glucosidase is adsorbed on the cellulose and wood powder through their hydrolysis[9,10] and the unhydrolyzed wood residue containing enzyme can be used as immobilized β-glucosidase which has high specific activity and stability.[11]

RESULTS AND DISCUSSION

Figure 1(a), (b), (c) and 2 present the adsorption characteristics of β-glucosidase of a (1:1) mixture of two commercial cellulase preparations from *Trichoderma viride* and *Aspergillus niger* on cellulose and wood powder. It became clear that the adsorption of β-glucosidase depends upon substrates, the degradation extent of the substrates, pH and the ionic strength of solution. The degree of adsorption was remarkably affected by pH and maximal adsorption was observed in a region of pH 3.7 to 3.9. It also increased with the degradation extent of the substrates when pH of the solution was kept constant. Hydrolysis was usually performed at pH 4.5 which was the optimum condition for each cellulase component. As shown in Figure 1, a significant amount of β-glucosidase was adsorbed onto the undigested residues through the hydrolysis of cellulose or wood powder.

The recovery and reutilization of the adsorbed β-glucosidase was examined. The wet residue which was separated from the enzymatic degradation mixture of red lauan wood powder and washed, adsorbed β-glucosidase corresponding to 0.12 U per mg of the residue. This residue (300 mg) was suspended in 15 mL of 0.1M acetate buffer (pH 4.5) and incubated at 40 ℃ for 24 h with gentle shaking, and separated from the reaction mixture. The β-glucosidase activities of the recovered residue and the buffer solution were assayed separately. The above experiments were repeated seven times. Although 10% of the adsorbed β-glucosidase desorbed at the first incubation, 90% of it remained on the wood residue. From the results, it was concluded that the β-glucosidase adsorbed on the undigested residue of woods was immobilized.

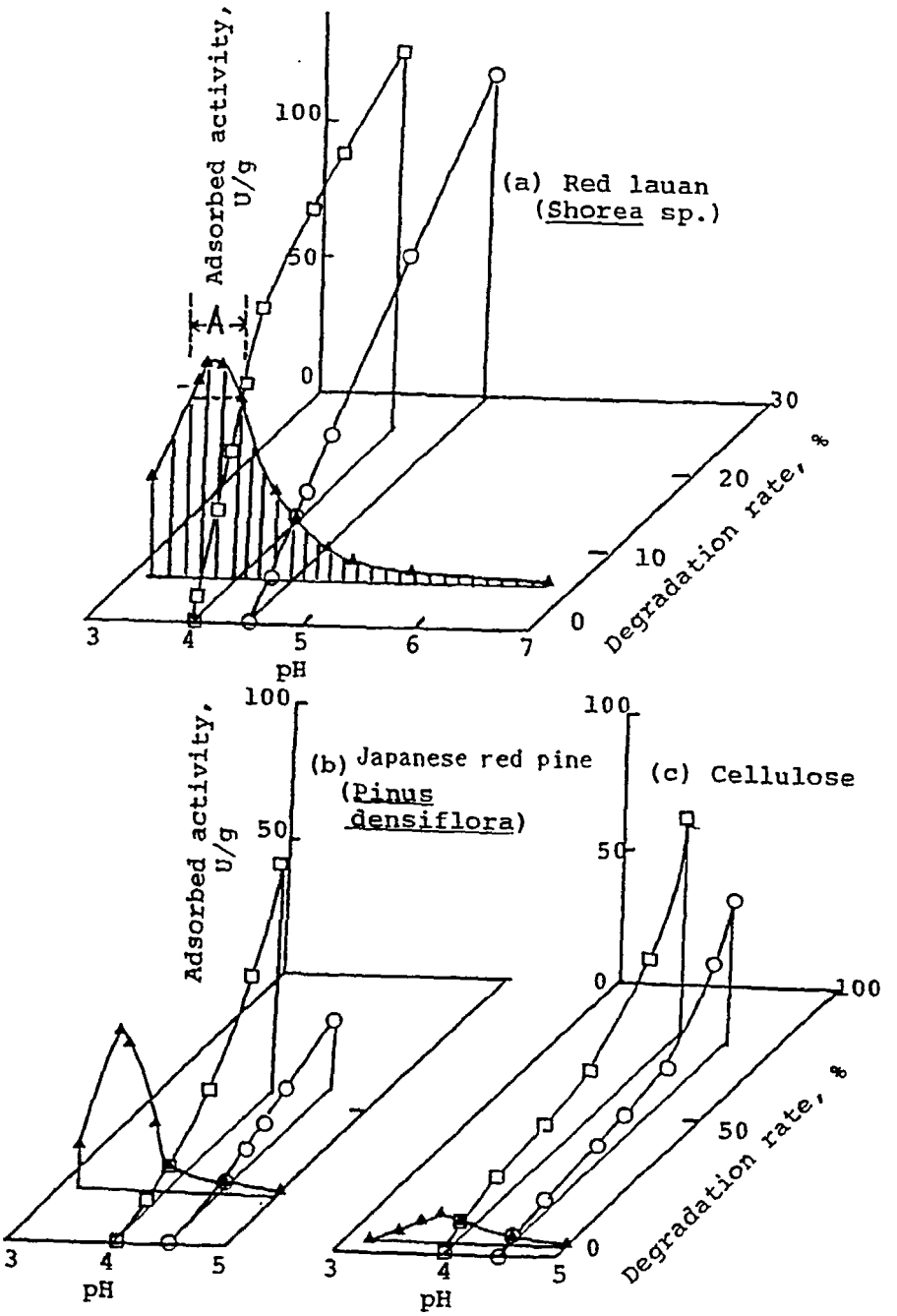

Fig. 1 Adsorption of β-glucosidase on cellulosic materials.

Notes: Substrate 1 g/25mL. Enzyme a (1:1) mixture of Cellulase Onozuka R-10 and Cellulosin AP, 0.6%. Reaction temperature 40 ℃(□○), 5 ℃(▲).

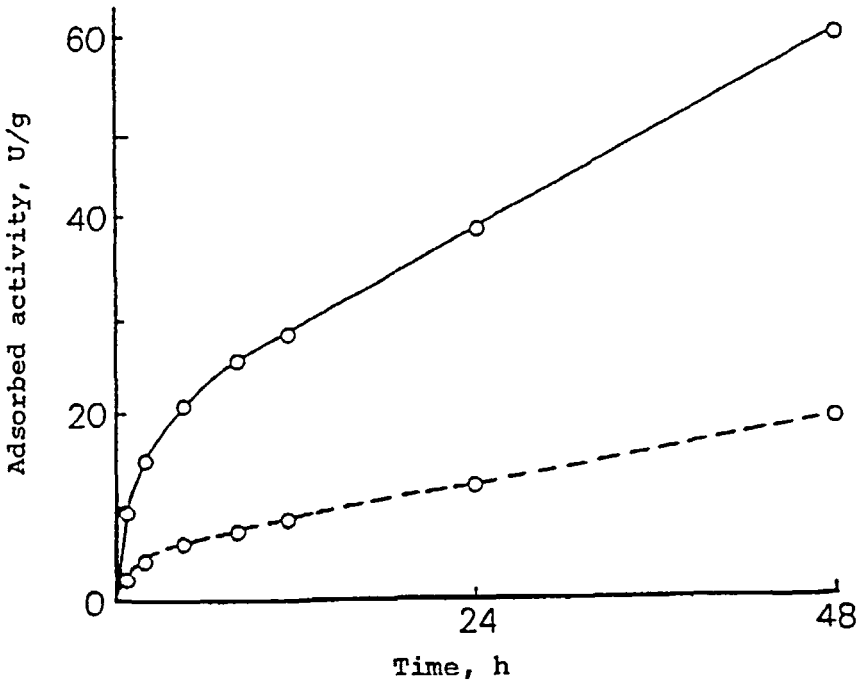

Fig. 2 Effect of ionic strength on the adsorption of β-glucosidase.

Notes: Twenty-five mL of enzyme solution (0.6%, pH 4.0) containing the red lauan wood powder (1 g) was incubated at 5 ℃.

..... , 0.1 M acetate buffer with 0.1 M NaCl.

——— , same buffer without NaCl.

The hydrolysis of cellobiose using this wood residue as enzyme was investigated. Twenty-five mL of acetate buffer (pH 4.5) containing 500 mg of cellobiose and 100 mg of the residue of red lauan was incubated at 40 ℃. The amount of glucose was determined by the Nelson-Somogyi method[12,13] and HPLC. Glucose yield from cellobiose was 100% after 24 h. The substrate was replaced every 24h, and the same glucose yield was obtained even at the 30th reaction (Fig. 3). These results show that the adsorbed β-glucosidase is very stable, and so the undigested wood residue containing enzyme is able to be used as immobilized β-glucosidase. The ac-

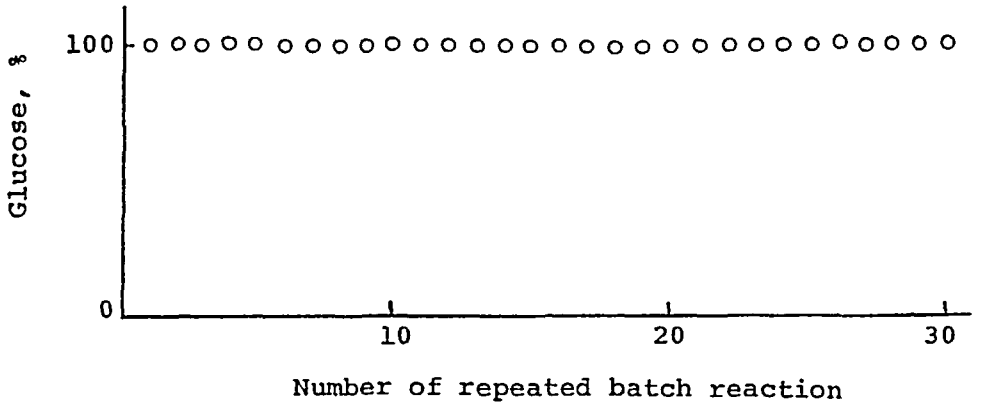

Fig. 3 Enzymatic hydrolysis of cellobiose by immobilized β-glucosidase.
Notes: Twenty-five mL of cellobiose solution (2%, pH 4.5) containing the red lauan wood residue (100 mg) was incubated at 40 ℃ for 24 h. Glucose yield was determined by HPLC (TSK G1000PW column, Tosoh Co.,Ltd.).

tivities of the immobilized β-glucosidase using the undigested residue of several woods are shown in Table 1. These specific activities were more than 0.1 U/mg(carrier) at pH 4.5, 40 ℃. Expressed as units per mL (carrier), the activity of the red lauan wood residue was found to be 18. Thermostability of the immobilized β-glucosidase was the same as the native enzyme. It exhibited high activity after storage in a refrigerator, the decrease in the specific activities was only 5% in a year, and was active even at 60 ℃.

The specific activity of the immobilized β-glucosidase could be enhanced by taking advantage of the differences in adsorption behavior with pH. The red lauan wood residue, which was obtained from hydrolysis at pH 4.5, adsorbed β-glucosidase rapidly by immersing it into a new enzyme solution at pH 4.0 (Fig. 4). The specific activity of the residue separated from the solution was 0.17 U/mg(carrier) and β-glucosidase was entirely retained in this region of pH | ←A→ | as shown in Figure 1(a). On the other hand, there was a region of pH where β-glucosidase immobilized on the undigested residue desorbed, resulting in a decrease of the specific activity of the enzyme. Specific activity of the immobilized β-glucosidase, which was prepared by hydrolysis at pH 4.5, de-

Table 1. β-Glucosidase activity of the enzyme mixture immobilized in wood residue.

Wood species	Specific activity, U/mg(carrier)	Activity retention %*
Red lauan (*Shorea* sp.)	0.11	100
Yellow lauan (*S.* sp)	0.11	98
Japanese red pine (*Pinus densiflora*)	0.10	97
Japanese beech (*Fagus crenata*)	0.10	99

Notes: Twenty-five mL of acetate buffer (pH 4.5) containing 1g of wood powder and 150mg of enzyme was incubated at 40 ℃ for 48h. The undigested residue was separated and washed.

$$* \text{ Activity retention (\%)} = \frac{\text{activity of enzyme on support}}{\text{activity lost from solution}} \times 100$$

creased to 78, and 50% of original at pH 5.0, and 5.5, respectively. Furthermore, 90% of β-glucosidase bound on the undigested residue desorbed by incubating it in a buffer solution of pH 6.8 for 24 h at 40 ℃.

The covalent immobilization of proteins (chymotrypsin, pepsin, and ovomucoid) to a hydrolyzate lignin by means of formaldehyde has been reported.[14] In our method, β-glucosidase can be immobilized by adsorption, presumably with electrostatic interaction between β-glucosidase and substrate in view of the effect of pH and ionic strength on adsorption.

The above immobilized β-glucosidase adsorbed other cellulase components beside β-glucosidase, but it was found that only β-glucosidase was retained and the stability of immobilized β-glucosidase increased by drying the wet wood residue, although the specific activity decreased

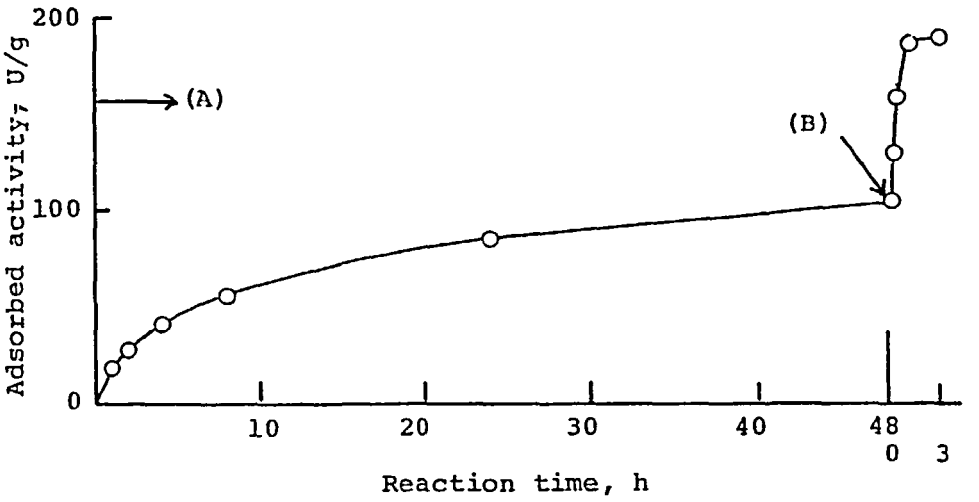

Fig. 4 Effect of pH on adsorption of β-glucosidase.

Notes: (A) One gram of red lauan wood powder in 25 mL of buffer solution (pH 4.5) containing 150 mg of enzyme was incubated at 40 ℃ for 48h.

(B) The unhydrolyzed residue was separated, and added to new enzyme solution (pH 4.0) at 5 ℃.

to 7% of the wet residue. Enzyme was not released from the supports in a region of pH 3 to 7 and activity was completely retained after the storage at room temperature for seven years. The decrease of surface area or porosity of support by drying it resulted in decrease of activity and disappearance of endo-glucanase and cellobiohydrolase, but the specific activity of β-glucosidase increased by pulverizing the dry residue. It is assumed that with low surface area endo-glucanase and cellobiohydrolase could not contact their substrates which are solid or high molecular compounds. Hydrolysis of methyl β-D-glucopyranoside in a column bioreactor with immobilized β-glucosidase on Japanese red pine (dry residue) was very successful yielding glucose in a constant 95% yield (Fig.5). Recently, the synthesis of oligosaccharides using an immobilized β-glucosidase preparation was noted.[15,16] The dry type of immobilized β-glucosidase using undigested wood residue might be employed to polymerize D-glucose.

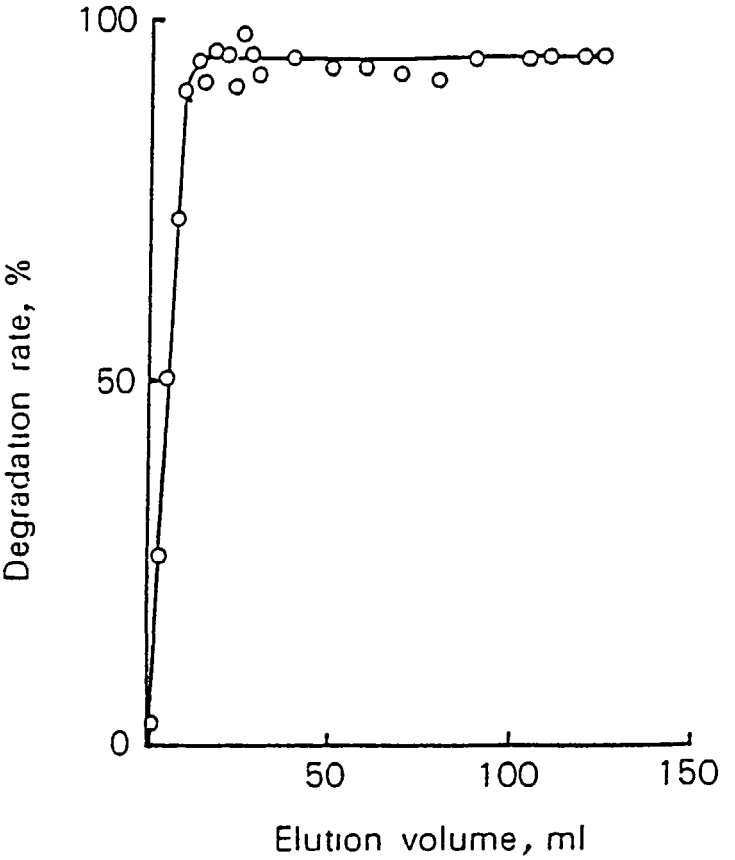

Fig. 5 Hydrolysis of methyl β-D-glucopyranoside by the column reactor with immobilized β-glucosidase.

Notes: A column (7 × 35mm) containing 0.7g of the immobilized enzyme (Japanese red pine wood residue, 50-100mesh, 3.82U) was kept in water bath (40 ℃). Methyl β-D-glucopyranoside solution was fed into the column continuously at a flow rate of 0.1mL/min.

EXPERIMENTAL

Materials. The enzyme used was a (1:1) mixture of two commercial cellulase preparations, Cellulase Onozuka R-10 from *Trichoderma viride* (Yakult Co., Ltd.) and Cellulosin AP from *Aspergillus niger* (Ueda Kagaku Co.,Ltd.). Filter paper manufactured by TOYO ROSHI Co.,Ltd., the fol-

lowing woods and Avicel (Asahi Kasei Co., Ltd.) were used. Filter paper and woods were ball-milled and collected through a sieve (400 mesh). Red lauan (*Shorea* sp.), yellow lauan (*S.* sp.), Japanese beech (*Fagus crenata*), Japanese red pine (*Pinus densiflora*).

Hydrolysis (β-Glucosidase Immobilization). Hydrolysis was performed at 40 ℃ and pH 4.5 (0.1 M acetate buffer) for 48h. The concentration of enzyme and substrate was 0.6% and 1g/25mL, respectively. The undigested residue after the hydrolysis was separated and washed with the same buffer and water.

β-Glucosidase Assay. β-Glucosidase contained in commercial cellulase preparations were immobilized, but the quantity of β-glucosidase was not measured. The amount of enzyme employed was estimated from the measurement activity. Three mg of the undigested residue was incubated in 2.5 mL of a buffer solution (pH 4.5) containing 0.03% of *p*-nitrophenyl β-D-glucopyranoside at 40 ℃ for 10 min, and then *p*-nitrophenol was determined. One unit(U) of enzyme activity was defined as the amount of enzyme required to release 1 μmol of glucose per min at 40 ℃.

Hydrolysis of Cellobiose by Immobilized β-Glucosidase. Twenty-five mL of acetate buffer (pH 4.5) containing 500 mg of cellobiose and the undigested residue of red lauan (11 U) were incubated at 40 ℃. The glucose yield was assayed by the Nelson-Somogyi method and HPLC.[11] The substrate was replaced every 24h.

Continuous hydrolysis of methyl β-D-glucopyranoside. A column reactor (7mm X 35mm) containing 0.7 g of immobilized enzyme (50-100 mesh, 3.82U) was kept in water bath (40 ℃). Methyl β-D-glucopyranoside solution (2.19 μmol/mL) was fed into the column continuously at a flow rate of 0.1 mL/min. Glucose was assayed by the Nelson-Somogyi method.

REFERENCES

1. Presented at the *XVth International Carbohydrate Symposium*, Yokohama, Japan, August 12-17, 1990.

2. M. Mandels, J. E. Medeiros, R. E. Andreotti and F. H. Bissett, *Biotechnol. Bioeng.*, 23, 2009 (1981).

3. M. Tanaka, T. Morita, M. Taniguchi, R. Matsuno and T. Kamikubo, *J. Ferment. Technol.*, 58, 517 (1980).

4. M. B. Fadda, M. R. Dessi, A. Rinaldi and G. Satta, *Biotechnol. Bioeng.*, 33, 777 (1989).

5. M. Mascini, M. Pizzichini, D. Moscone and R. Pilloton, *Biotechnol. Bioeng.*, 33, 262 (1989).

6. B. Philipp, U. kasulke, G. Schulz and W. Hirte, *Acta Biotechnol.*, 5, 309 (1985).

7. S. C. Goel and K. B. Ramachandran, *J. Ferment. Technol.*, 61, 281 (1983).

8. H. Ooshima, D. S. Burns and A. O. Converse, *Biotechnol. Bioeng.*, 36, 446 (1990).

9. S. Fujishima, F. Yaku and T. Koshijima, *Mokuzai Gakkaishi*, 33, 530 (1987).

10. S. Fujishima, F. Yaku and T. Koshijima, *Mokuzai Gakkaishi*, 35, 845 (1989).

11. S. Fujishima, F. Yaku and T. Koshijima, *Mokuzai Gakkaishi*, 35, 1029 (1989).

12. N. Nelson, *J. Biol. Chem.*, 153, 375 (1944).

13. M. Somogyi, *J. Biol. Chem.*, 195, 19 (1952).

14. M. A. Krysteva, T. T. Sokolov, I. D. Dobrev and R. A. Simeonova, *Biotechnol. Bioeng.*, 36, 1128 (1984).

15. T. Tanaka and S. Oi, *Agric. Biol. Chem.*, 49, 1267 (1985).

16. K. Ajisaka, H. Nishida and H. Fujimoto, *Biotechnol. Letters*, 9, 249 (1987).