J. Fac. Appl. Biol. Sci.,
Hiroshima Univ. (1997), **36** : 35~41

Removal of Gaseous Acetic Acid by *Corynebacterium* sp. No. 123 Immobilized onto Ceramic Beads

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Received April 30, 1997

Abstract From the seed culture, thirty bacterial isolates capable of utilizing volatile fatty acids were obtained. An isolate with the highest utilization activity of acetic acid even in the presence of 2% ethanol was selected and identified as *Corynebacterium* sp. The optimum pH and temperature for the growth were 6~9, and 30~45°C, respectively. This strain was immobilized onto the porous ceramic beads *in vacuo*, and cultivated for activation at 37°C, for 24 h. The gaseous acetic acid was passed through the beads in a column at a flow rate of 3.6 M^3 $kg^{-1}day^{-1}$. Under these conditions, about one hundred ppm of acetic acid was removed for 20 days of the operation period.

Key words: acetic acid, biofilter, ceramic beads, immobilization

INTRODUCTION

We have found microorganisms with an ability to deodorize malodorous biological wastes in a short time (OHTA and IKEDA, 1978). From the deodorized wastes, many kinds of actinomycetes and bacteria have been isolated (OHTA and IKEDA, 1978). The latter have been physiologically investigated and classified into three groups according to the utilization of the carbon source (YUN and OHTA, 1997). Most of the isolates are found to utilize volatile fatty acids (VFA) as their sole carbon source.

The evaporated ethanol from brewing factories such as miso, soy sauce, beer, whisky etc., is one of the causes of the environmental pollution around them. Some devices have been made for removal of the ethanol. Among these, the microbial removal of the ethanol is one of the most preferable device. However, some ethanol is oxidized to acetic acid by the microorganisms in the soil of the factories campus. The acid lowers the pH of the biological device which results in the decrease of removal activity of the ethanol (VAN GROENESTIJN and HESSELINK, 1993; SMET *et al.*, 1996; OH and BARTHA, 1994).

This paper is concerned with the preparation of biofilter made of immobilized bacterial cells on ceramic beads for removal of acetic acid even in the presence of ethanol.

MATERIALS AND METHODS

Microorganisms:

Thirty bacterial isolates capable of utilizing volatile fatty acids were used. They were

isolated from the seed culture, which has been used for deodorization of pig feces etc.

Medium and Cultivation:

The artificial medium was used as a basal medium (OHTA and SATO, 1985), which was modified with a substitution of VFA mixture with 1% sodium acetic acid and 2% ethanol as a carbon source. One loopful of culture grown on the slant medium at 37°C, for 2 days was inoculated in 100 ml of the medium in a 500 ml Erlenmyer flask. Cultivation was carried out on a rotary shaker (240 rpm, Takasaki Co., Saitama) at 37°C.

Determination of acetic acid:

The acid was determined with a gas chromatograph equipped with a flame ionization detector. The cultured broth was centrifuged at 15,000×*g*, for 5 min. The supernatant fluid (1 μl) was subjected to gas chromatography (GC) after it was acidified to pH 2.0 with 1 N HCl. The GC conditions were as follows: A glass column (ϕ 3 mm, 2 m) packed with Unisol (30~60 mesh, GL science Co., Tokyo) was employed. Carrier was nitrogen gas at flow rate of 30 ml min^{-1}. Fuel was hydrogen gas at flow rate of 60 ml min^{-1}. The flow rate of air was 30 ml min^{-1}. Injection and oven temperatures were 140°C. The acid was determined by referring to the standard curve.

Immobilization of the bacterial strain onto ceramic beads:

Porous ceramic beads (ϕ 3 mm, Nippon Sharyo Co., Nagoya) were pretreated by washing twice with distilled water and dryed at 80°C. The beads (150 g) were put into a 2 l vacuum bottle sealed with a silicon rubber stopper. A glass tube (ϕ 5 mm, 7 cm long) was fixed through the stopper. The upper side of the tube was connected with a glass funnel (ϕ 7 cm) by rubber tubing. The bottle was evacuated with an aspirator after the rubber tube was pinched with a Hofmann type pinch cock. The cell suspension was poured into the bottle through the funnel by releasing the cock. This procedure enabled the cells to penetrate into the pour of the beads. The cell suspension was prepared as follows: Ten Erlenmyer flasks containing 100 ml of the medium were incubated as mentioned above. The cultured broth from these flasks were combined and centrifuged at 17,000×*g*, for 5 min. The precipitated cells were washed twice with 20 mM phosphate buffer (pH 7.5) by centrifugation. The cells (about 1 g on dry weight) were suspended in 100 ml of the medium. This suspension was used for the immobilization procedure as described above. By repeating this procedure, about 1 kg of the beads was prepared, were piled in the container with holes and this was placed in a larger bowl. The surface of the container was covered with aluminum foil and incubated at 30°C. The incubator was kept aseptic by irradiation of UV light.

An apparatus for removal of gaseous acetic acid:

As shown in Fig. 1, 1% acetic acid solution and 2% ethanol (50 ml) in 2 l glass bottle was bubbled through the air stone with air supplied by a pump, controlled by the air flow meter. Thus, the air containing gaseous acetic acid and ethanol were passing through the beads packed in the glass column (I. D. 5 cm, 40 cm high) equipped with a water jacket. The container was kept in a water bath at 25°C. The exhaust air was conducted into aqueous diluted NaOH solution (pH 8.0) to capture the un-removed acid. The head space air and the exhaust gas were taken for analysis of the acetic acid.

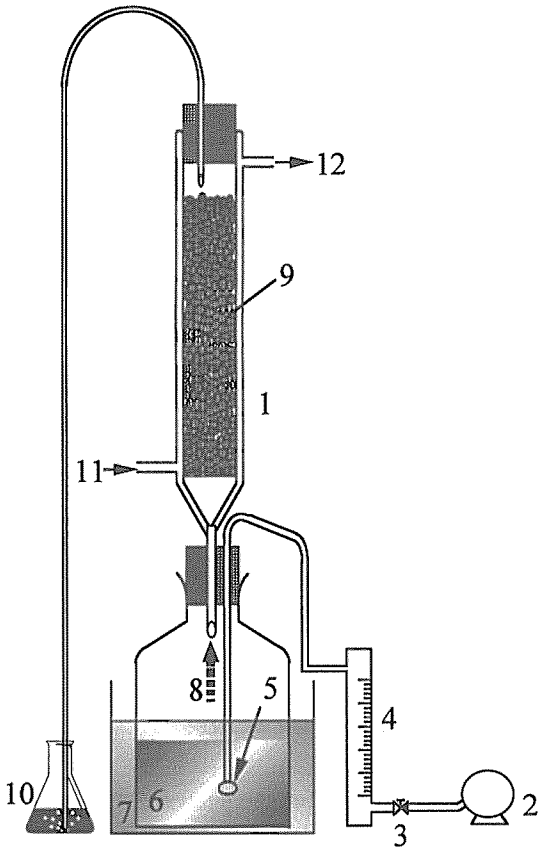

Fig. 1. Biofilter apparatus for removal of vaporized acetic acid.
Symbols: 1, glass column equipped with water jacket; 2, air pump; 3, stop valve; 4, air-flow meter; 5, air ball filter; 6, acetic acid solution (1%); 7, water bath (25°C); 8, inlet; 9, ceramics beads immobilized with *Corynebacterium* sp. No. 123; 10, aquous solution (pH 8.0, 50 ml); 11, water inlet; 12, water outlet.

Determination of pH:

Ten g of the beads were suspended in 90 ml of distilled water. Then, the pH was determined with a pH meter with mixing on a stirrer.

Enumeration of bacteria immobilized onto beads:

The modified medium was used for the enumeration of living cells. One gram of beads taken from different places in the column were disintegrated in a mortar with a pestle. The sample was suspended in 9 ml of saline solution. The suspension was serially diluted and 0.1 ml of dilution was spread on a agar medium, and incubated at 30°C, for 2~3 days. Then, the colonies were counted.

Determination of growth:

The growth was determined routinely by measuring the optical density at 660 nm. The amount was expressed as dry weight determined from the standard correlation curve between OD and dry weight.

RESULTS AND DISCUSSION

Selection of the strain:

Thirty isolates were cultivated in the basal medium with or without 2% ethanol. The ethanol was added to the sterilized medium after cooling. The cultivation was performed at 30°C, for 24 h. The isolates having OD higher than 3.5 (OD_{660}) in the medium with 2% ethanol were shown in Fig. 2. Strains No. 107, 123, and 141 showed the growth of 4.7, 4.9, and 3.7 at OD_{660}, respectively. Two isolates (No. B-88, and No. 22-2) showed higher growth in the medium without acetic acid. Consequently, isolates No. 107, 123, and 141 were able to grow on acetic acid as a sole carbon source even in the presence of 2% (v/v) ethanol. Also, they did not have the ability to grow on ethanol. For better understanding of the consumption of carbon source, the isolate No. 123 was selected for further studies. The isolate No. 123 has the following characteristics: straight to slightly curved short rods, Gram+, non acid-fast, no endospore-formation, facultatively anaerobic, catalase-positive, acid formation (+) from glucose. This isolate was tentatively identified as *Corynebacterium* sp. according to Bergey's manual (SNEATH *et al.*, 1986).

Time course of utilization of acetic acid:

The strain cultivated at 30°C was taken for the analyses of pH, growth amount and acetic acid at appropriate intervals. As shown in Fig. 3, the acetic acid was consumed completely by 36 h of cultivation. The growth amount reached 5.6 at OD_{660} at the same time. The pH of the medium rose from 8.0 to about 10 after 48 h-cultivation. The effects of pH and temperature on the growth of the strain were investigated. The optimum pH for the growth was found to be between 7 and 9. No growth was observed below pH 6 and above

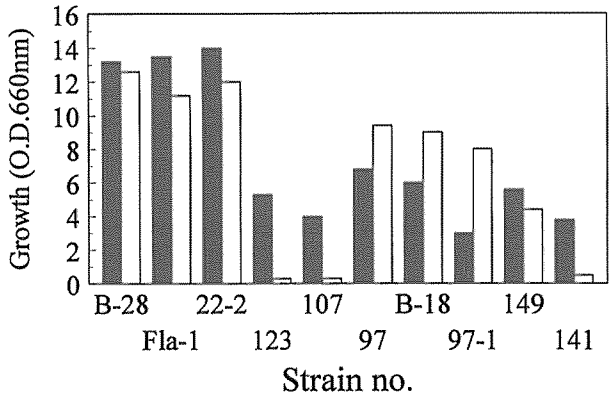

Fig. 2. Growth of various isolates in media containing sodium acetate plus 2% ethanol, and 2% ethanol only.
Symbols: ■, sodium acetate plus 2% ethanol; □, 2% ethanol only.

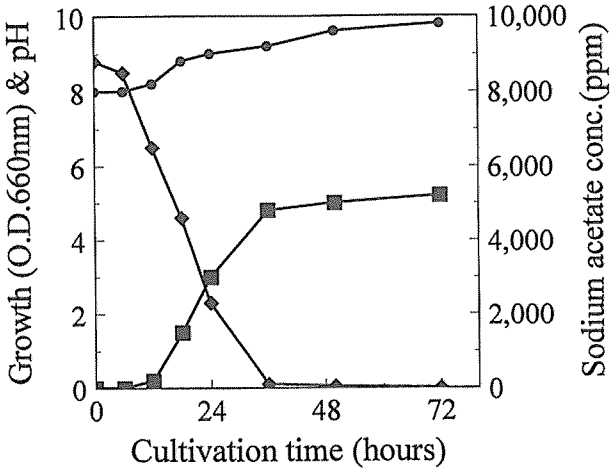

Fig. 3. Growth of *Corynebacterium* sp. No. 123 on sodium acetate.
Symbols: ■, growth; ◆, sodium acetate; ●, pH.

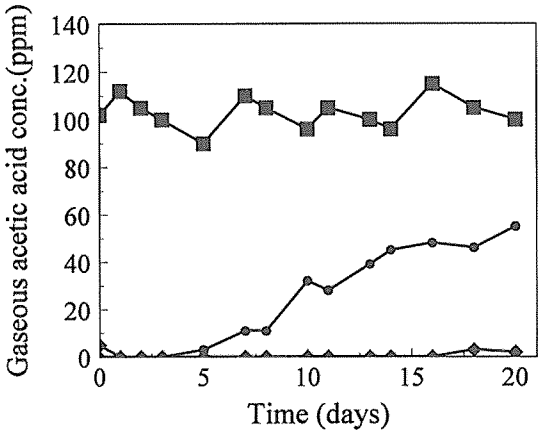

Fig. 4. Continuous removal of vaporized acetic acid by biofilter made of immobilized *Corynebacterium* sp. No. 123.
Symbols: ■, Inlet acetic acid concentration; ◆, Outlet concentration of column No. 1 with cells; ●, column No. 2 without cells.

pH 11. The optimum growth temperature lies between 30 and 45°C. The temperature below 10°C and above 60°C did not allow the growth. This strain could not utilize nitrogen compounds other than peptone as its nitrogen source. Further, it utilizes VFA as its carbon source, but not sugars such as glucose (no data shown). This strain was found to be a typical deodorant microorganism.

Removal of gaseous acetic acid by a biofilter:

The beads (about 500 g) with immobilized cells were packed in the glass column as shown in Fig. 1. The operating conditions were below. Through out the experiment the inlet con-

centration of the acetic acid and ethanol were maintained to 100 ppm and 1,800 ppm, respectively. The flow rate was 3.6 M^3/kg dry-material/day. The initial moisture content was about 40%. The cell number on the beads were about 5.1×10^6 cfu/g. The operation was carried out at 37°C, pH 8.0, for 20 days. As shown in Fig. 4, the acetic acid was removed for 20 days by column No. 1 with immobilized cells. On the other hand, in the column No. 2 without cells, the acetic acid was removed for about 5 days, there after it came out gradually from the outlet. After 20 day-run, only 40% of the acid was removed by the column No. 2. No acetic acid was detected in the dilute NaOH solution from the column No. 1, whereas the acid concentration increased in the solution from the column No. 2. As mentioned above, the acetic acid in the air was removed by the bacterial cells immobilized onto the ceramic beads. The number of the cells increased from 5.1×10^6 cfu/g to 6.9×10^7 cfu/g during the operation (CHO *et al.*, 1991; 1992; ZACHE and REHM, 1989; BALFANZ and REHM, 1991; HINTEREGGER *et al.*, 1992). Larger number of immobilized cells leads to a prolonged removal of acetic acid but not ethanol.

REFERRENCES

BALFANZ, J. and REHM, H. J., 1991. Biodegradation of 4-chlorophenol by adsorptive immobilized *Alcaligenes* sp. A7-2 in soil. *Appl. Mirobiol. Biotechnol.*, 35:662-668.

CHO, K. S., HIRAI, M. and SHODA, M., 1991. Removal of dimethyl disulfide by the peat biofilter seeded with night soil sludge. *J. Ferment Bioeng.*, 71:289-291.

CHO, K. S., HIRAI, M. and SHODA, M., 1992. Enhanced removal efficiency of malodorous gases in a pilot-scale peat biofilter inoculated with *Thiobacillus thioparus* DW 44. *J. Ferment Bioeng.*, 73:46-50.

COLLINS, M. D. and CUMMINS, C. S., 1986. *Corynebacterium:* In Bergey's manual of systematic bacteriology. Vol. 2, SNEATH, P. H. A., MAIR, N. S., SHARPE, M. E. and HOLT, J. G. (Eds), 1266-1283, The Williams & Wilkins Co., Baltimore.

HINTEREGGER, C., LEITNER, R., LOIDL, M., FERSCHL, A. and STREICHSBIER, F., 1992. Degradation of phenol and phenolic compounds by Pseudomonas putida EK 11. *Appl. Microbiol. Biotechnol.*, 37: 252-259.

OH, Y. S. and BARTHA, R., 1994. Design and performance of a trickling air biofilter for chlorobenzene and *o*-dichlorobenzene vapors. *Appl. Environ. Microbiol.* 60:2717-2722.

OHTA, Y. and IKEDA, M., 1978. Deodorization of pig feces by actinomycetes. *Appl. Environ. Microbiol.*, 36:487-491.

OHTA, Y. and SATO, H., 1985. An artificial medium for deodorant microorganisms. *Agric. Biol. Chem.*, 49:1195-1196.

SMET, E., VAN LANGENHOVE, H. and VERSTRAETE, W., 1996. Long-term stability of a biofilter treating dimethy sulphide. *Appl. Microbiol. Biotechnol.* 46:191-196.

VAN GROENESTIJN, J. W. and HESSELINK, P. G. M., 1993. Biotechniques for air pollution control. *Biodegradation.* 4:283-301.

YUN, S. I. and OHTA, Y., 1997. Some physiological properties of microorganisms capable of deodorizing domestic animal feces. *Bioresource Technology* (in press).

ZACHE, G. and REHM, H. J., 1989. Degradation of phenol by a coimmobilized entrapped mixed culture. *Appl. Microbiol. Biotechnol.* 30:426-432.

セラミックビーズに固定した *Corynebacterium* sp. No. 123 によるガス状酢酸の除去

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無臭化細菌群の中から，揮発性脂肪酸利用性の強い菌株を30菌株分離した。これらの菌株について2％エタノール中でも酢酸の利用性の強い菌株を見い出し，これを *Corynebacterium* 属の細菌であると同定した。本菌株の液体培養を行い，その洗浄菌体を真空下で，セラミックビーズに固定した。無菌下で再培養を行って活性化を行った。これをカラムにつめて，バイオフィルターを作り，$3.6 \times M^3\ kg^{-1}\ day^{-1}$ の条件下で約 100 ppm のガス状酢酸を流すと実験の20日間は除去された。

キーワード：酢酸，バイオフィルター，セラミックビーズ，固定化