

## Degradation of a Sodium Acrylate Oligomer by an *Arthrobacter* sp.

TAKAYA HAYASHI,<sup>1\*</sup> MASAHARU MUKOUYAMA,<sup>1</sup> KOUICHI SAKANO,<sup>1</sup> AND YOSHIKI TANI<sup>2</sup>

*Tsukuba Research Laboratory, Nippon Shokubai Co., Ltd., 1-25-12, Kan-nondai, Tsukuba, Ibaraki 305,<sup>1</sup> and Graduate School of Biological Sciences, Advanced Institute of Science and Technology, Nara 630,<sup>2</sup> Japan*

Received 20 October 1992/Accepted 1 March 1993

*Arthrobacter* sp. strain NO-18 was first isolated from soil as a bacterium which could degrade the sodium acrylate oligomer and utilize it as the sole source of carbon. When 0.2% (wt/wt) oligomer was added to the culture medium, the acrylate oligomer was found to be degraded by 70 to 80% in 2 weeks, using gel permeation chromatography. To determine the maximum molecular weight for biodegradation, the degradation test was done with the hexamer, heptamer, and octamer, which were separated from the oligomer mixture by fractional gel permeation chromatography. The hexamer and heptamer were consumed to the extents of 58 and 36%, respectively, in 2 weeks, but the octamer was not degraded. Oligomers with three different terminal groups were synthesized to examine the effect of the different terminal groups on biodegradation, but few differences were found. *Arthrobacter* sp. NO-18 assimilated acrylic acid, propionic acid, glutaric acid, 2-methylglutaric acid, and 1,3,5-pentanetricarboxylic acid. Degradation of the acrylic unit structure by this strain is discussed.

Poly(sodium acrylate) is a water-soluble and harmless synthetic polymer widely used as a functional polymer in various fields. Although microbial degradation of synthetic polymers has attracted much interest as a means of mild and effective waste treatment, little information is available on biodegradation of poly(sodium acrylate), which is considered highly resistant to microbial degradation.

Among water-soluble synthetic polymers, the biodegradability of polyvinyl alcohol (3, 11, 12, 15, 17) and polyethylene glycol (2, 4, 5, 10, 13) has been studied in detail by using microorganisms. However, there has been no report on microorganisms capable of degrading the sodium acrylate oligomer or the polymer.

In screening investigations, a bacterium identified as *Arthrobacter* sp. strain NO-18 was first isolated from soil as a sodium acrylate oligomer-degrading microorganism. This bacterium could utilize the sodium acrylate oligomer as the sole source of carbon and was found to achieve 70 to 80% degradation of the oligomer.

In this report, we present the characteristics of microbial degradation of the sodium acrylate oligomer by *Arthrobacter* sp. strain NO-18.

### MATERIALS AND METHODS

**Isolation of sodium acrylate-degrading microorganisms.** Screening was carried out at 30°C by a conventional enrichment culture technique, using activated sludge and soil samples as the microbial source. The composition of the culture medium was as follows: sodium acrylate oligomer with butylthioether, 2.0 g; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1 g; K<sub>2</sub>HPO<sub>4</sub>, 0.5 g; KH<sub>2</sub>PO<sub>4</sub>, 0.5 g; MgSO<sub>4</sub> · 7H<sub>2</sub>O, 0.2 g; NaCl, 0.1 g; yeast extract, 0.1 g; CaCl<sub>2</sub> · 2H<sub>2</sub>O, 2 mg; FeSO<sub>4</sub> · 7H<sub>2</sub>O, 2 mg; ZnSO<sub>4</sub> · 7H<sub>2</sub>O, 7 mg; MnSO<sub>4</sub> · 4H<sub>2</sub>O, 2 mg, in 1 liter of distilled water. The pH of the medium was adjusted to 7.0 with 1 N NaOH solution. After cultivation aerobically at 30°C for several days, the culture broth was streaked on an agar plate containing the same medium and incubated at

30°C to develop colonies. Colonies appearing were picked up independently and purified on the same agar plate.

**Identification of microorganisms.** Morphological, physiological, and biochemical studies on the isolated microorganism were carried out by conventional methods (6). Identification was made by using *Bergey's Manual of Systematic Bacteriology* (14).

**Degradation test.** The degradation test with *Arthrobacter* sp. strain NO-18 was done aerobically at 30°C in a 300-ml Erlenmeyer flask in 50 ml of culture screening medium with a sodium acrylate oligomer of different terminal groups.

Cell growth was measured by reading the *A*<sub>660</sub>.

The amount of oligomer was determined by gel permeation chromatography (GPC) and total organic carbon, using Shimadzu model LC-6AD and Shimadzu model TOC-500 apparatuses, respectively, after bacterial cells were removed by centrifugation and filtration.

**Synthesis of sodium acrylate oligomers.** Three kinds of sodium acrylate oligomers containing butylthioether (—SC<sub>4</sub>H<sub>9</sub>), butanol [—CH(OH)C<sub>3</sub>H<sub>7</sub>], or sodium sulfate (—SO<sub>3</sub>Na) in a terminal position were synthesized. The scheme of polymerization is illustrated in Fig. 1a to c.

The oligomers with a terminal butylthioether and a terminal butanol were prepared by radical polymerization of methyl acrylate followed by alkaline hydrolysis, using the procedure of Abe et al. (1) with minor modifications.

To prepare the oligomer with a butylthioether (Fig. 1a), 80 g (0.93 mol) of methylacrylate was mixed with 320 g of benzene. Benzoyl peroxide, 2.256 g (9.3 mmol), as the initiator and 16.8 g (0.186 mol) of 1-butanethiol as the chain transfer reagent were added to the mixture, and under a nitrogen atmosphere the temperature was increased to 80°C. After a 2-h reaction, the methyl acrylate oligomers obtained were added to methanol, purified by repeated ion-exchanger resin (Amberlite IRA 93 ZU) treatment, and then reprecipitated into *n*-hexane. Alkaline hydrolysis of the methyl acrylate oligomers was performed with an excess of 5 N NaOH solution to prepare the corresponding sodium salt.

To prepare the oligomer with butanol in a terminal position (Fig. 1b), 182.0 g (2.46 mol) of 1-butanol was mixed with 8.46 g (98.4 mmol) of methyl acrylate, and 1.73 g (7.1 mmol)

\* Corresponding author.

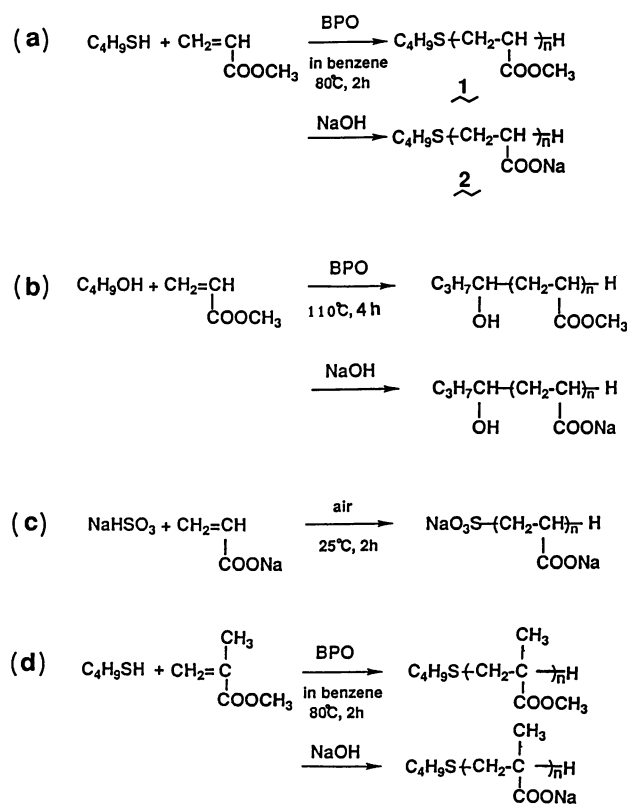


FIG. 1. Scheme of polymerization of sodium (meth)acrylate oligomers. (a) Sodium acrylate oligomer with butylthioether; (b) sodium acrylate oligomer with butanol; (c) sodium acrylate oligomer with sodium sulfonate; (d) sodium methacrylate oligomer with butylthioether. BPO, benzoyl peroxide.

of benzoyl peroxide was added to the mixture. The reaction was done at 110°C for 4 h. Purification and alkaline hydrolysis of the oligomer were carried out in the manner described above.

The oligomer with a terminal sulfonic group (Fig. 1c) was prepared by the procedure of Tsubakimoto et al. (16). Polymerization was carried out in the presence of oxygen. Sodium acrylate monomer (540.5 g, 2.13 mol), 37% (wt/wt), and 35% (wt/wt) sodium hydrogen sulfite (252.9 g, 0.85 mol) solution were mixed with 206.6 g of the deionized water, and the mixture was allowed to react at 25°C under gentle stirring. After a 2.5-h reaction, the oligomer obtained was purified by dialysis against an excess of running water.

**Synthesis of the sodium methacrylate oligomer.** The sodium methacrylate oligomer was prepared by the same procedure used for the sodium acrylate oligomer (Fig. 1d). Methyl methacrylate, 93.2 g (0.93 mol), was reacted with 2.256 g (9.3 mol) of benzoyl peroxide and 16.8 g (0.186 mol) of 1-butane-thiol in 320 g of benzene at 80°C for 2 h. Purification and alkaline hydrolysis were carried out as described above to obtain the sodium salt.

**Fractionation of methyl acrylate oligomers.** The fractionation of methyl acrylate oligomers was carried out in order to obtain the monodispersed oligomer fractions. The fractional GPC system used was as follows: two columns, G 2000H6 (2 in. by 2 ft [ca. 5 by 60 cm]) (Tohso, Tokyo, Japan); column temperature, 25°C; eluent, tetrahydrofuran; flow rate, 20 ml/min; detector, UV (254 nm) and refractive index.

**Analysis of oligomers.** The methyl acrylate oligomer was analyzed by analytical GPC as follows: columns, TSK-GEL G 2000 plus G 4000 (7.8 by 300 mm) (Tohso); column temperature, 40°C; eluent, tetrahydrofuran; flow rate, 1.0 ml/min; detector, UV (254 nm) and refractive index.

The sodium acrylate oligomer was analyzed by an aqueous GPC system as follows: columns, Shodex OH pak KB-804 plus KB-803 plus KB-802.5 plus KB-802.5 (8.0 by 300 mm) (Showa Denko Co., Ltd., Tokyo, Japan); column temperature, 40°C; eluent, potassium phosphate buffer, pH 7.0, containing 100 mM  $\text{KH}_2\text{PO}_4$  and 200 mM NaCl; flow rate, 1.0 ml/min; detector, UV (210 nm).

Nuclear magnetic resonance spectra of oligomers were recorded on a Gemini-200 spectrometer (Varian Instrument Division).

Gas chromatography-mass spectrometry spectra of oligomers were recorded on an INCOS 50 spectrometer (Finnigan Mat Instruments, Inc.).

**Materials.** The sodium acrylate monomer was made by the Nippon Shokubai Co., Ltd., Osaka, Japan. Other materials used in these studies were the usual commercial products of reagent grade.

## RESULTS

**Molecular weight distribution of the acrylate oligomer with butylthioether.** The methyl acrylate oligomer with butylthioether as a terminal group (Fig. 1a1) was observed to have nine peaks on a GPC chromatogram. Each peak was isolated with the fractional GPC system and identified as a dimer (degree of polymerization [DP] = 2) to decamer (DP = 10) of methyl acrylate by gas chromatography-mass spectrometry and nuclear magnetic resonance analyses.

The sodium acrylate oligomer mixture (Fig. 1a2) with a dimer (DP = 2) to decamer (DP = 10) was obtained after alkaline hydrolysis and used as a carbon source in culture medium for screenings.

**Characteristics of a sodium acrylate oligomer-degrading microorganism.** Several bacteria having the ability to assimilate the sodium acrylate oligomer have been isolated from soils. Among them, the most active strain, NO-18, was selected as an oligomer-degrading strain and used throughout the following study.

Strain NO-18 was identified by its morphological and biochemical characteristics. Taxonomic examinations showed that this strain was an aerobic, motile, non-spore-forming, and gram-positive bacterium. The cells (colony color, orange) were irregular rods (~0.6 by ~1.4  $\mu\text{m}$ ), but eventually presented as coccoid forms as growth continued. The pH for growth was 5 to 9, and growth in NaCl was up to 7%. Tests for catalase and utilization of citrate were positive. The following tests were negative: oxidase, urease, indole production, methyl red-Voges-Proskauer, and nitrate reduction. No acid was produced from glucose, arabinose, lactose, glycerol, fructose, or starch. These results suggest that the isolate belongs to the genus *Arthrobacter*.

**Degradation of the sodium acrylate oligomer with butylthioether by *Arthrobacter* sp. strain NO-18.** A time course study of the degradation of the sodium acrylate oligomer with butylthioether (Fig. 1a2) by *Arthrobacter* sp. strain NO-18 is shown in Fig. 2. Degradation of the oligomer in the medium began after 70 h of cultivation, and about 80% of the oligomer was found to be degraded in 2 weeks. The total organic carbon value of the culture filtrate was also determined. A 69% decrease in total organic carbon was observed after 2 weeks. The pH of the medium increased gradually

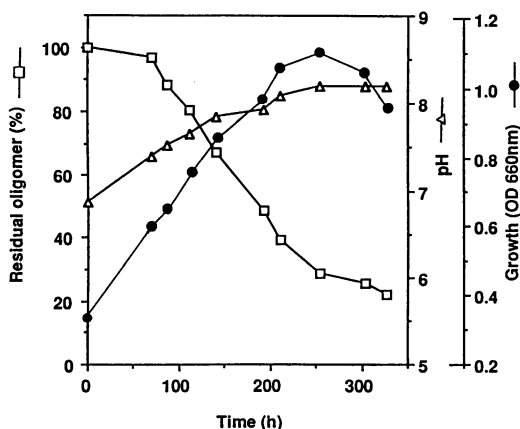


FIG. 2. Time course of sodium acrylate oligomer degradation by *Arthrobacter* sp. strain NO-18. Degradation of the oligomer is expressed in terms of the residual percentage of the GPC peak area. OD, optical density.

from 6.9 to 8.2, possibly due to the accumulation of Na<sup>+</sup> in the culture broth by the degradation of the carboxylic structure.

A GPC chromatogram of the residual sodium acrylate oligomer after degradation by *Arthrobacter* sp. strain NO-18 was compared with the control sample (Fig. 3). Peaks 2, 3, 4, and 5 corresponded to the dimer, trimer, tetramer, and pentamer, respectively, and peak 6 was composed of the hexamer to decamer. Figure 3 shows that biodegradation of the acrylate oligomer undoubtedly occurs, and a peak fraction of a higher degree of polymerization than the hexamer seems to remain without degradation.

To determine the maximum molecular weight for biodegradation, the degradation test was also done with the hexamer, heptamer, and octamer of sodium acrylate, which were prepared by alkaline hydrolysis of the methyl acrylate oligomer separated by fractional GPC. A time course of degradation, which was calculated from the peak area, is shown in Fig. 4. The hexamer and heptamer were consumed to the extents of 58 and 36%, respectively, in 2 weeks, but the octamer was not degraded. *Arthrobacter* sp. strain NO-18 had the molecular weight limitation for biodegradation on a heptamer. Hexamer and heptamer peaks were observed to reduce gradually without any significant by-product from GPC. The sodium acrylate oligomer was thought to be metabolized finally to CO<sub>2</sub> in the bacterial cells.

**Effect of acrylate oligomer terminal group on degradation.**

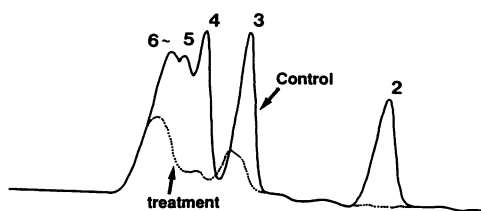


FIG. 3. GPC chromatogram of the sodium acrylate oligomer after 4 weeks of cultivation of *Arthrobacter* sp. strain NO-18. Peaks 2 to 5 correspond to the dimer to pentamer. Peak 6 contains the hexamer to decamer. - - - - -, residual oligomer after cultivation; —, control without inoculation.

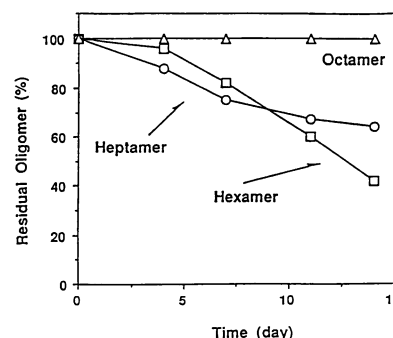


FIG. 4. Time course of degradation of the hexamer, heptamer, and octamer by *Arthrobacter* sp. strain NO-18. Degradation of the oligomer is expressed in the same terms as given in the legend to Fig. 2.

The degradation test with *Arthrobacter* sp. strain NO-18 was carried out in the same way as described above, using three kinds of sodium acrylate oligomer with different terminal groups. Since each oligomer had a different molecular weight distribution, the comparison of biodegradability of these three oligomers was made by determining the reduction of the pentamer peak. The time course of pentamer peak reduction is shown in Fig. 5. The results indicate that the structure of the terminal group has little influence on biodegradation by *Arthrobacter* sp. strain NO-18.

**Effect of cell amount as seed inoculum on degradation.** To examine the effect of cell amount as seed inoculum for culture on biodegradation speed, *Arthrobacter* sp. strain NO-18 was inoculated into 50 ml of the acrylate oligomer medium in the range of 0.56 to 5.6 g (wet weight) of cells per liter. The increase in cell amount led to a remarkable reduction of culture time. Eighty percent degradation of the oligomer was achieved in 4 days with 5.6 g of cells per liter and in 20 days with 0.56 g of cells per liter.

**Degradation test of the sodium methacrylate oligomer by Arthrobacter sp. strain NO-18.** To examine the effect of the methyl group at the α-position on biodegradation, the degradation test was carried out with the sodium methacrylate oligomer with a terminal butylthioether. *Arthrobacter* sp. strain NO-18 showed no ability to degrade the sodium methacrylate oligomer upon analysis of GPC chromato-

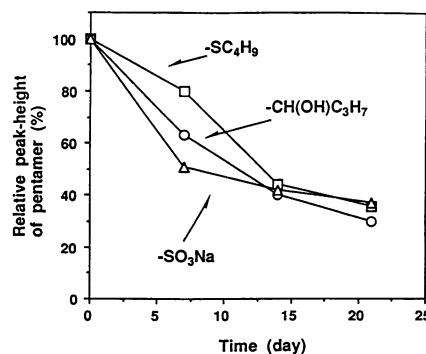


FIG. 5. Effect of terminal groups of the acrylate oligomer on degradation by *Arthrobacter* sp. strain NO-18. The degradation is compared in terms of the residual percentage of pentamer peak height.

TABLE 1. Growth of *Arthrobacter* sp. strain NO-18 on various carboxylic acids

Carboxylic acid (0.2%, wt/wt)	Time (days)	Growth (optical density at 660 nm)
Acetic acid	2	1.11
Lactic acid	2	0.972
Pyruvic acid	2	1.02
Acrylic acid	2	1.29
Methacrylic acid	2	0.920
Succinic acid	2	0.610
Fumaric acid	2	1.12
Propionic acid	2	1.33
Glutaric acid	6	1.05
2-Methylglutaric acid	6	0.494
1,3,5-Pentanetricarboxylic acid	6	0.342
Maleic acid	6	0.093
1,2,3-Propanetricarboxylic acid	6	0.110
1,2,3,4-Butanetetracarboxylic acid	6	0.023
2,3-Dimethylsuccinic acid	6	0.050
None	6	0.064

grams. This indicates that the main chain structure affects biodegradation by *Arthrobacter* sp. strain NO-18.

**Substrate specificity on various carboxylic acids.** The growth of *Arthrobacter* sp. strain NO-18 on various carboxylic acids was tested with the 15 compounds listed in Table 1. This strain grew on acrylic acid, propionic acid, methacrylic acid, fumaric acid, succinic acid, glutaric acid, 2-methylglutaric acid, and 1,3,5-pentanetricarboxylic acid. However, it grew poorly on maleic acid, 1,2,3-propanetricarboxylic acid, butane-1,2,3,4-tetracarboxylic acid, and 2,3-dimethylsuccinic acid.

## DISCUSSION

Our results demonstrate that *Arthrobacter* sp. strain NO-18, which was isolated from soil, has a remarkable ability to degrade the sodium acrylate oligomer. This bacterium is the first strain isolated as a sodium acrylate oligomer-degrading microorganism.

Studies by Matsumura et al. (7) on the biodegradability of poly(sodium acrylate) using activated sludge showed that the dimer and trimer were consumed by 27.9 and 30.1%, respectively, after 5 days, but the polymer (average molecular weight, >500) was degraded to a limited extent (<8%).

*Arthrobacter* sp. strain NO-18 could utilize the sodium acrylate oligomer as the sole source of carbon and degrade it by 70 to 80% in 2 weeks. Microbial degradation was proved by GPC chromatograms and total organic carbon. A degradation test with the fractionated oligomer showed that the strain degraded the heptamer (DP = 7) but not the octamer (DP = 8).

Few differences were found in biodegradation among three oligomers with different terminal groups. How the C—C linkage in the oligomer is cleaved by *Arthrobacter* sp. strain NO-18 is not yet clear, but all three of the terminal groups, which were introduced in this study, may be easily cleaved from the main chain of the oligomer. The sodium methacrylate oligomer was not degradable. Substituent groups seemed to have a great influence on biodegradation by this strain.

The growth pattern of *Arthrobacter* sp. strain NO-18 on carboxylic acids suggested that the acrylic unit was susceptible to attack by this strain, but the neighboring carboxylic groups seriously interfered with degradation.

The pyrolysis of poly(sodium acrylate) has been investigated for many years, and degradation is proposed to commence with a dehydration reaction that occurs by intramolecular cyclization of an adjacent monomer which is followed by decarboxylation and chain scission (8, 9). However, it would be difficult for such a degradation, which involves the formation of cyclic anhydrates, to occur in a microbial metabolism pathway.

Polyvinyl alcohol is known to be biodegradable in vinyl-type polymers, and its degradative pathway has been investigated with the 1,4-nonanediol as a model material. The biodegradation is suggested to occur by oxidation of the polyvinyl alcohol with secondary alcohol oxidase to form partially oxidized polyvinyl alcohol; this is followed by hydrolytic cleavage (11).

It is well known that fatty acids are biodegraded by  $\beta$ -oxidation. In view of the chemical structure, the successive  $\beta$ -oxidation might take place during the biodegradation of the sodium acrylate oligomer.

In any event, our experimental results strongly suggested that the cleavage of C—C linkage in the main chain of the oligomer was caused by *Arthrobacter* sp. strain NO-18. In spite of the limitation to biodegradation by molecular weight, it appears possible to find microorganisms capable of degrading poly(sodium acrylate) of higher molecular weights in nature.

## ACKNOWLEDGMENTS

We thank Shigeru Hattori, National Chemical Laboratory for Industry, Tsukuba, Japan, for help with part of the fractionation of the methyl acrylate oligomer. We are also grateful to Tsuneaki Tsubakimoto, R&D Section of Nippon Shokubai, Co., Ltd., and Kunihiro Kubota, Tsukuba Laboratory of Nippon Shokubai Co., Ltd., for their encouragement and support.

## REFERENCES

- Abe, Y., S. Matsumura, H. Yajima, Y. Masago, T. Miura, and K. Sakao. 1984. Telomers from acrylic acid and methacrylic acid. *Yukagaku* 33:219-227.
- Haines, J. R., and M. Alexander. 1975. Microbial degradation of polyethylene glycols. *Appl. Microbiol.* 29:621-625.
- Hashimoto, S., and M. Fujita. 1985. Isolation of a bacterium requiring three amino acids for polyvinyl alcohol degradation. *J. Ferment. Technol.* 63:471-474.
- Kawai, F., M. Fukaya, Y. Tani, and K. Ogata. 1977. Identification of polyethylene glycol-assimilable bacteria and culture characteristics of PEG 6,000 degradation by a mixed culture. *J. Ferment. Technol.* 55:429-435.
- Kawai, F., T. Kimura, M. Fukaya, Y. Tani, K. Ogata, T. Ueno, and H. Fukami. 1978. Bacterial oxidation of polyethylene glycol. *Appl. Environ. Microbiol.* 35:679-684.
- Komagata, K. *Biseibutu no bunri to dootei*. Gekken Shuppan Center. Tokyo.
- Matsumura, S., S. Maeda, J. Takahashi, and S. Yoshikawa. 1988. Biodegradation of poly(vinyl alcohol) and poly((sodium acrylate)-co-(vinyl alcohol)). *Kobunshi Ronbunshu* 45:317-324.
- McNeill, I. C., and M. T. Sadeghi. 1990. Thermal stability and degradation mechanisms of poly(acrylic acid) and its salts. I. Poly(acrylic acid). *Polym. Degrad. Stabil.* 29:233-246.
- McNeill, I. C., and M. T. Sadeghi. 1990. Thermal stability and degradation mechanisms of poly(acrylic acid) and its salts. II. Sodium and potassium salts. *Polym. Degrad. Stabil.* 30:213-230.
- Ogata, K., F. Kawai, M. Fukaya, and Y. Tani. 1975. Isolation of polyethylene glycol-assimilable bacteria. *J. Ferment. Technol.* 53:757-761.
- Sakai, K., N. Hamada, and Y. Watanabe. 1986. Degradation mechanism of poly(vinyl alcohol) by successive reactions of secondary alcohol oxidase and  $\beta$ -diketone hydrolase from *Pseudomonas* sp. *Agric. Biol. Chem.* 50:989-996.

12. Sakazawa, C., M. Shima, Y. Taniguchi, and N. Kato. 1981. Utilization of polyvinyl alcohol by mixed cultures. *Appl. Environ. Microbiol.* **41**:261-267.
13. Schink, B., and M. Stieb. 1983. Fermentative degradation of polyethylene glycol by a strictly anaerobic, gram-negative, non-spore-forming bacterium, *Pelobacter venetianus* sp. nov. *Appl. Environ. Microbiol.* **47**:850-857.
14. Sneath, P. H. A., N. S. Mair, M. E. Sharpe, and J. G. Holt. 1986. *Bergey's manual of systematic bacteriology*, vol. 2. The Williams & Wilkins Co., Baltimore.
15. Suzuki, T., Y. Ichihara, M. Yamada, and K. Tonomura. 1973. Some characteristics of *Pseudomonas* O-3 which utilizes polyvinyl alcohol. *Agric. Biol. Chem.* **37**:747-756.
16. Tsubakimoto, T., T. Shimomura, and Y. Irie. October 1979. Preparation of oligo(acrylate salts). Japanese patent (Japan Kokai Tokkyo Koho) 81-55407.
17. Watanabe, Y., N. Hamada, M. Morita, and Y. Tsujisaka. 1975. Hydrogen peroxide by a polyvinyl alcohol degrading enzyme. *Agric. Biol. Chem.* **39**:2447-2448.