Digestion of Crosslinked Poly(vinylpyridinium Halide) by Activated Sludge, and Application to Make Poly(methyl Methacrylate) Biodegradable by Incorporation of a Pyridinium Group into the Main Chain

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SYNOPSIS

Crosslinked poly(*N*-benzyl-4-vinylpyridinium bromide) has been found to be digested by activated sludge obtained from sewage works. As an extension of this work, the authors attempted to make poly(methyl methacrylate) biodegradable by incorporation of a pyridinium group. Poly(methyl methacrylate) containing a small amount of *N*-benzyl-4-vinylpyridinium chloride in the main chain showed remarkable reduction in molecular weight and gravimetric weight when placed in an aeration tank of a sewage works. Molecular weight reduction by activated sludge was more conspicuous when content of the pyridinium group was larger and the original molecular weight before the biological treatment was lower. Since insoluble pyridinium-type polymer captures microbial cells alive, this biodegradation appears to be facilitated by enhancement of affinity of the synthetic hydrophobic polymer with cells of microorganisms. © 1994 John Wiley & Sons, Inc.

INTRODUCTION

For decades, the chemical industry has worked on polymeric materials that are durable, long lasting, and resistant to environmental factors. As the use of synthetic polymers has become ever more prevalent, however, considerable attention has recently been focused on their disposal, and many groups have looked for solutions to the waste problem. Biodegradability should be given to all synthetic polymers. If not, all used synthetic polymers should be recovered and decomposed completely. Reports available in the literature on the development of biodegradable polymers can be classified into three categories: (i) utilization of polymers produced by microorganisms; (ii) utilization of natural polymers and their derivatives; (iii) development of biodegradable synthetic polymers. The third category would be most important for the chemical industry. However, synthetic polymers, especially those with exclusively carbon–carbon bonds in the main chain, are highly resistant to microbial degradation or deterioration, although oligomers of these polymers exhibit biodegradability to some extent.[1,2]

Fortunately, however, some synthetic polymers such as poly(vinyl alcohol)[3,4] and poly(ethylene glycol)[5,6] are known to be biodegradable. These reports encourage us to consider that it is not always hopeless to seek biodegradability for hydrophobic synthetic polymers with exclusively carbon–carbon bonds in the main chain. In this work, we have attempted to improve biodegradability of hydrophobic synthetic carbon–chain polymers based on a new concept.

A significant defect of synthetic hydrophobic polymers responsible for this poor biodegradability would be the poor affinity for microorganisms. Biodegradation of synthetic polymers in the environment has to be entrusted to microorganisms. Therefore, enhancement of the affinity of synthetic poly-

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mers with microorganisms would be effective in making them biodegradable.

A recent publication from our laboratory[7] describes the ability of crosslinked poly(*N*-benzyl-4-vinylpyridinium halide) to remove bacteria from water. This crosslinked pyridinium-type polymer can capture many bacterial cells alive on its surface. As an extension of this work, we attempted to use this crosslinked pyridinium-type polymer as a filter medium for the biofilm process of the aerobic treatment of sewage,[8-10] i.e., a supporting material for fixation of biomass in the biological treatment system. Stable fixation of biomass on the supporting material is an important subject in the biofilm process. Unexpectedly, however, we found that the crosslinked pyridinium-type polymer was digested by activated sludge during the aerobic treatment. This observation led us to expect that incorporation of a pyridinium group into synthetic polymers may enhance the affinity of the polymers for cells of microorganisms, and could improve biodegradability of the synthetic polymers. In this work, therefore, we attempted to make synthetic hydrophobic polymers biodegradable by incorporation of a pyridinium group into the main chain. Poly(methyl methacrylate) was selected as a synthetic hydrophobic polymer having carbon–carbon bonds in the main chain, and *N*-benzyl-4-vinylpyridinium chloride was used as a pyridinium group.

EXPERIMENTAL

Chemicals

Methyl methacrylate was purified by washing with aqueous sodium hydroxide solution followed by drying. 4-Vinylpyridine and divinylbenzene were purified before polymerization as reported previously.[11] Other chemicals were used without further purification.

Crosslinked Poly(*N*-Benzyl-4-Vinylpyridinium Bromide)

We prepared crosslinked poly(*N*-benzyl-4-vinylpyridinium bromide) in the form of Raschig ring. A commercial sample of transparent tubing made of poly(vinyl chloride) with inner and outer diameters of 6 and 8 mm, respectively, was cut into 10 mm length. The cut tubes were soaked in acetone for 1 day at room temperature to eliminate the plasticizer, and were dried at room temperature under atmospheric pressure. Eight grams of poly(vinyl alcohol) at a polymerization degree of 2000 was dissolved into 2.0 L deionized water in a 3-L three-necked flask equipped with a mechanical stirrer and a nitrogen inlet. The solution was stirred at 80°C under a nitrogen atmosphere. In the meantime, dried pieces of cut tubes mentioned above were soaked in a monomer mixture containing 4-vinylpyridine (168 g), 55% divinylbenzene (12 g), toluene (536 g), cyclohexanone (144 g), and 2,2′-azobisisobutyronitrile (1.4 g) for exactly 40 min under a nitrogen atmosphere in a refrigerator, and the swollen tubes were added to the aqueous solution of poly(vinyl alcohol) at 80°C. The mixture was stirred at the same temperature under a nitrogen atmosphere for 4 h. At the end of this procedure, the cut tubes were removed and were washed with deionized water, soaked in acetone for 2 h, and dried at room temperature under atmospheric pressure.

The dried cut tubes were soaked again in the above-mentioned monomer mixture overnight in a refrigerator under a nitrogen atmosphere. The swollen cut tubes were added to 2.0 L deionized water in a 3-L three-necked flask and were stirred at 80°C for 5 h under a nitrogen atmosphere. This repolymerization procedure was repeated four more times. After these polymerization procedures, crosslinked poly(4-vinylpyridine) in the form of Raschig ring was allowed to react with an equimolar amount of benzyl bromide in toluene at 60–65°C for 5 h. At the end of these procedures, crosslinked poly(*N*-benzyl-4-vinylpyridinium bromide) in the form of Raschig ring 14 mm long with inner and outer diameters of 10 and 20 mm, respectively, was obtained. The polymer was dried to constant weight. At this final stage, about 90% (w/w) of the Raschig ring was crosslinked poly(*N*-benzyl-4-vinylpyridinium bromide).

Poly(methyl Methacrylate) Containing a Pyridinium Group

Poly(methyl methacrylate) containing a small amount of *N*-benzyl-4-vinylpyridinium chloride was prepared as follows. Free radical copolymerization of methyl methacrylate with a prescribed small amount of 4-vinylpyridine was performed using 2,2′-azobisisobutyronitrile as an initiator in toluene at 90°C for 6 h. The copolymer was allowed to react with benzyl chloride in toluene at 80°C for 4 h. The amount of benzyl chloride was set to be equimolar to that of 4-vinylpyridine contained in the copolymer. Poly(methyl methacrylate) containing the

pyridinium group was isolated by pouring the reaction mixture into ethanol, and was dried to constant weight. Composition of the polymer, as well as the amount of the pyridinium group contained in the polymer, were ascertained based on the contents of nitrogen and chlorine determined by elemental analyses of the polymer. Since the pyridinium group was introduced by the reaction of benzyl chloride with the pyridyl group of the copolymer of methyl methacrylate and 4-vinylpyridine, the pyridinium group was not bonded to the side group of poly(methyl methacrylate), but was directly bonded to the carbon–carbon bond of the main chain. Films of the poly(methyl methacrylate) containing a small amount of the pyridinium group were prepared by the conventional casting method using acetone as the solvent.

Digestion of Crosslinked Poly(*N*-Benzyl-4-Vinylpyridinium Bromide) by Activated Sludge

The test apparatus used in this work was a cylindrical reactor 15 cm in diameter and 30 cm deep equipped with an aeration unit. The reactor was made of poly(vinyl chloride) resin with a perforated plate at the bottom to distribute the air supplied from outside. The working reactor volume was 3.9 L. Artificial sewage was used as a model wastewater for this study, according to a literature recipe.[9] Peptone (6.0 g), meat extract (4.0 g), urea (1.0 g), sodium chloride (0.30 g), potassium chloride (0.14 g), calcium chloride (0.14 g), magnesium sulfate (0.10 g), and disodium hydrogenphosphate (1.0 g) were dissolved in 1.0 L deionized water. The chemical oxygen demand (COD) of this undiluted solution was about 10,000 mg/L. This solution was diluted with an appropriate amount of deionized water to prepare synthetic sewage of prescribed COD concentration, i.e., 100, 200, 300, or 500 mg/L. Activated sludge used in this work was obtained from sewage works.

The test apparatus was filled with the cross-linked pyridinium-type polymer prepared in the form of Raschig ring, and was placed in a water bath maintained at 30 ± 1°C. The artificial sewage was supplied by means of a peristaltic pump. The amount of innoculated activated sludge was 1000 mg/L as mixed liquor suspended solid (MLSS). During the early stage of the treatment, aeration was controlled at 0.1 volume of air per volume of medium per minute (vvm) (based on the empty-bed volume of the reactor). After 4 days, aeration was increased to 0.4 vvm.

Degradation of Poly(methyl Methacrylate) Containing a Pyridinium Group in the Main Chain during Placing in an Aeration Tank of Sewage Works

Degradation of poly(methyl methacrylate) containing a pyridinium group in the main chain by activated sludge was carried out by placing films of the polymer in an aeration tank of Kawamata Sewage Works of Osaka Prefecture. The degradation experiments were carried out under the following three conditions: (i) polymer films were directly placed in the aeration tank by hanging via a nylon fishing line and a weight made of metal; (ii) polymer films were placed in a cage made of 8-mesh saran net, and the cage was placed in the aeration tank by hanging with a rope; (iii) polymer films were placed in a bag made of 100-mesh nylon net, and the bag was placed in the aeration tank by hanging with a rope. After the prescribed time, the polymer samples were taken from the aeration tank, washed with deionized water, and dried to constant weight.

Analytical Methods

Determination of COD was performed according to the dichromate method.[12] The amount of MLSS was determined according to the standard method[12] using Whatmann GF/B glass fiber paper. Measurement of pH was performed with a Toa model HM-1F pH meter. Determination of dissolved oxygen (DO) was carried out with a Horiba BOD-1 simplified BOD monitor.

Intrinsic viscosity of poly(methyl methacrylate) containing a pyridinium group was measured using chloroform or toluene as the solvent at 30°C.

Gel permeation chromatogram of poly(methyl methacrylate) containing a pyridinium group was obtained using Shimadzu LC-10AD high performance liquid chromatograph system with RID-6A refractive index detector, and Shimpack GPC-802 as the column at 30°C. Chloroform was used as the eluate.

RESULTS AND DISCUSSION

Digestion of Crosslinked Poly(*N*-Benzyl-4-Vinylpyridinium Halide) by Activated Sludge

Continuous aerobic treatment of artificial sewage by activated sludge was carried out at 30°C and at a flow rate of 1.0 L/h using a test apparatus filled with the crosslinked pyridinium-type polymer prepared for this work in the form of Raschig ring. The aeration was controlled at 0.1 vvm during the first

4 days, and was then increased to 0.4 vvm. Concentration of COD of the influent sewage was controlled to be 100, 200, 300, or 500 mg/L. Since the working volume was 3.9 L, the substrate loads were 130, 260, 390, or 650 mg COD/h, respectively. In other words, the substrate loads were 0.80, 1.60, 2.40, or 4.00 kg COD/m^3 day.

Time course of COD of the effluent solution during the biological treatment is shown in Figure 1. We had expected removal of COD by the aerobic treatment of the artificial sewage. Contrary to this expectation, however, COD of the effluent solution was larger than that of the influent sewage during the early 2–3 weeks, as can be seen in Figure 1. This increase in COD during the biological treatment was attributed to the crosslinked pyridinium-type polymer, because no other organic materials existed in the treatment system than the organic components of the artificial sewage. Since the crosslinked polymer was insoluble in water, increase of COD during the aerobic treatment was explained in terms of increase of microbial cell population in the treatment system. In other words, the increase of COD was explained in terms of the increase of microbial cells during the aerobic treatment due to multiplication of the microorganisms of activated sludge digesting the crosslinked pyridinium-type polymer as an organic nutrient.

In Figure 2 is shown the change in DO during the biological treatment. Concentration of DO was low in the case where the influent COD was high. A larger amount of dissolved oxygen would be required under the condition of higher substrate load.

Since we have conjectured digestion of the crosslinked pyridinium-type polymer by activated sludge, gravimetric weight of the polymer was pursued during the biological treatment. After a prescribed time of the treatment, the polymer samples were taken out from the treatment system, washed with deionized water, and were dried to constant weight. Dry weight of the crosslinked pyridinium-type polymer was found to reduce during the biological treatment as can be seen in Figure 3. Weight reduction of the

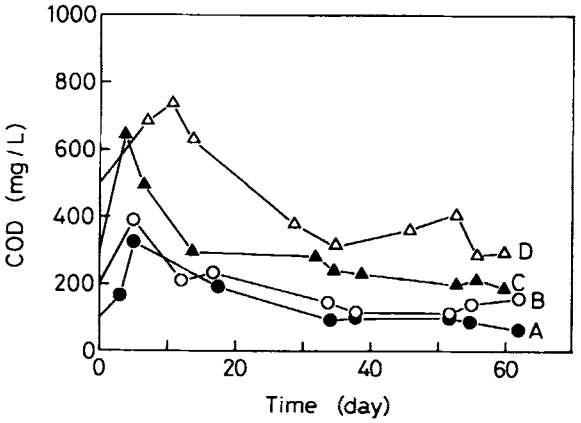

Figure 1 Course of COD in continuous aerobic treatment of artificial sewage by activated sludge in the presence of crosslinked poly(*N*-benzyl-4-vinylpyridinium bromide) prepared in the form of Raschig ring. Influent COD (mg/L): (A) 100; (B) 200; (C) 300; (D) 500.

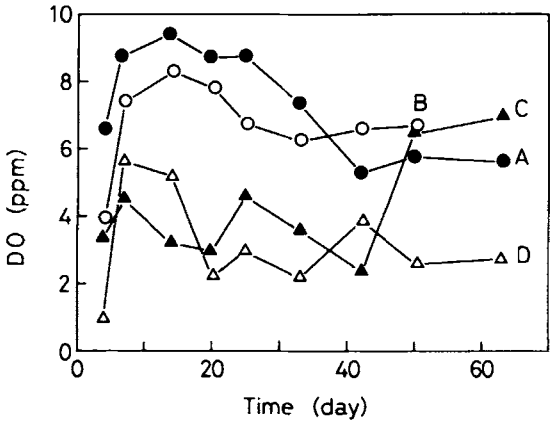

Figure 2 Course of DO in continuous aerobic treatment of artificial sewage by activated sludge in the presence of crosslinked poly(*N*-benzyl-4-vinylpyridinium bromide) prepared in the form of Raschig ring. Influent COD (mg/L): (A) 100; (B) 200; (C) 300; (D) 500.

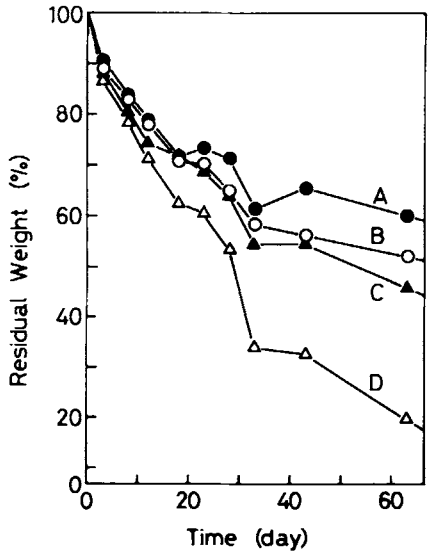

Figure 3 Change of weight of crosslinked poly(*N*-benzyl-4-vinylpyridinium bromide) prepared in the form of Raschig ring in continuous aerobic treatment of artificial sewage by activated sludge in the presence of the polymer. Influent COD (mg/L): (A) 100; (B) 200; (C) 300; (D) 500.

polymer during the aerobic treatment would be evidence of digestion of the polymer by activated sludge. Extent of the weight reduction was obviously increased with substrate load of the influent sewage. This result suggests that digestion of the crosslinked pyridinium-type polymer by activated sludge was accompanied by digestion of organic substrates in the influent sewage.

This observation is a negative result when aimed at developing a new material useful for the biofilm process. Values of COD increased during the aerobic treatment, contrary to our expectation. However, digestion of a synthetic crosslinked polymer by activated sludge is an exciting discovery, and would give us promising and potentially breakthrough methodology for making synthetic high molecular weight polymers biodegradable. We assumed that digestion of the crosslinked pyridinium-type polymer was attributed, at least partly, to the presence of *N*-benzyl-4-vinylpyridinium group in the main chain of the synthetic polymer. Therefore, we attempted to give biodegradability to synthetic and hydrophobic high molecular weight polymer with exclusively carbon–carbon bonds in the main chain by incorporation of the pyridinium group into the main chain.

Degradation of Poly(methyl Methacrylate) Containing a Pyridinium Group in the Main Chain During Placing in an Aeration Tank of Sewage Works

Degradation of poly(methyl methacrylate) containing a little *N*-benzyl-4-vinylpyridinium chloride in the main chain by activated sludge was investigated. In the first series of experiments, films of the poly(methyl methacrylate) of 1 cm wide, 2 cm long, and 0.5 mm thick were prepared by the conventional casting method, and were directly placed in an aeration tank of sewage works by hanging via a nylon fishing line and a weight made of metal. After a prescribed time, samples of the polymer film were taken from the aeration tank, washed with deionized water, and dried to constant weight. Gravimetric weight and intrinsic viscosity of the recovered polymer samples were measured. Weight reduction was not significant within 70 days of the treatment time. However, intrinsic viscosity of the recovered polymer was obviously smaller than before.

Before the biological treatment, intrinsic viscosity determined in toluene at 30°C was in the range 0.18–0.24 dL/g. Viscosity average molecular weight was estimated to be in the range 34,000–48,000 based on the relationship between molecular weight and intrinsic viscosity.[13,14] In Figure 4 is shown the ratio of intrinsic viscosity of the recovered polymer sample to that before the biological treatment as a function of the treatment time. Homopolymer of methyl methacrylate did not exhibit significant change of intrinsic viscosity [Fig. 4(A)]. However, intrinsic viscosity of the recovered polymer was obviously lower than that before the biological treatment in the case where the polymer contained the pyridinium group in the main chain [Figs. 4(B), 4(C), and 4(D)]. Extent of the reduction of intrinsic viscosity was in the order: Figure 4(D) > Figure 4(C) > Figure 4(B) and was more significant where content of the pyridinium group was larger. Poly(methyl methacrylate) containing the pyridinium group was prepared by the reaction of benzyl chloride with pyridyl groups of the copolymer of methyl methacrylate with 4-vinylpyridine, and main chain of the polymer was exclusively carbon–carbon bond. The pyridinium group was not bonded to the side group of poly(methyl methacrylate), but was directly bonded to the carbon–carbon bond of the main chain. Therefore, considerable reduction of the intrinsic viscosity of poly(methyl methacrylate) containing the pyridinium group must be attributed to the scission of carbon–carbon bond of the main chain. These experimental results indicate that incorporation of the pyridinium group into the main chain enabled scission of the carbon–carbon bond of the main chain during the biological treatment. In other words, the hydrophobic carbon-chain poly-

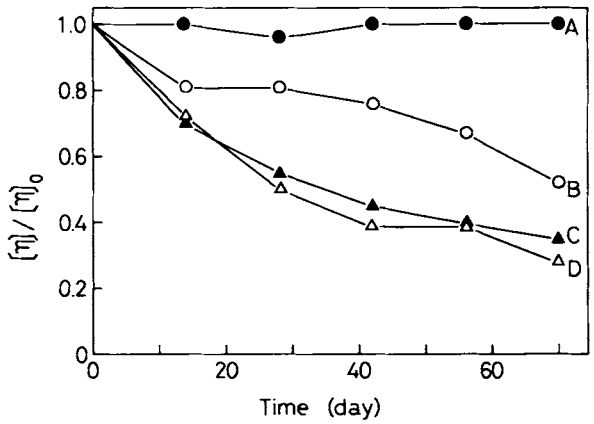

Figure 4 Ratio of intrinsic viscosity of poly(methyl methacrylate) containing *N*-benzyl-4-vinylpyridinium chloride in the main chain recovered after placing in an aeration tank of sewage works to that before the biological treatment. Intrinsic viscosity of the polymer determined in toluene at 30°C before the treatment (dL/g): (A) 0.24; (B) 0.21; (C) 0.20; (D) 0.18. Content of the pyridinium group (mol %): (A) 0; (B) 1; (C) 3; (D) 5.

mer turned biodegradable by incorporation of the pyridinium group into the main chain.

In the second series of experiments, polymer samples were prepared as films of 3 cm wide, 3 cm long, and 0.13 mm thick. The polymer films were placed in a cage made of 8-mesh saran net, and the cage was placed in an aeration tank of a sewage works by hanging with a rope. Before the biological treatment, intrinsic viscosity determined in chloroform at 30°C was in the range 0.41–0.55 dL/g. Viscosity average molecular weight was estimated to be in the range 94,000–136,000.

In Figure 5 is plotted the ratio of intrinsic viscosity of the recovered polymer sample to that before the biological treatment versus the treatment time. Change of molecular weight was not very significant in the case of homopolymer [Fig. 5(A)]. Extent of the molecular weight reduction was in the order: Figure 5(D) > Figure 5(C) > Figure 5(B), and became more significant when the polymer contained more amount of the pyridinium group. Figure 5(B) shows negligible reduction of the molecular weight of poly(methyl methacrylate) containing 1 mol % of the pyridinium group during the treatment with activated sludge. This observation indicates that incorporation of 1 mol % of the pyridinium group is insufficient for making poly(methyl methacrylate) biodegradable. However, the molecular weight reduction appeared to reach the uppermost limit after about 30 days of the treatment. The intrinsic viscosity was measured for the recovered polymer samples, and degraded polymers having molecular weight lower than this level appeared to be lost during the treatment.

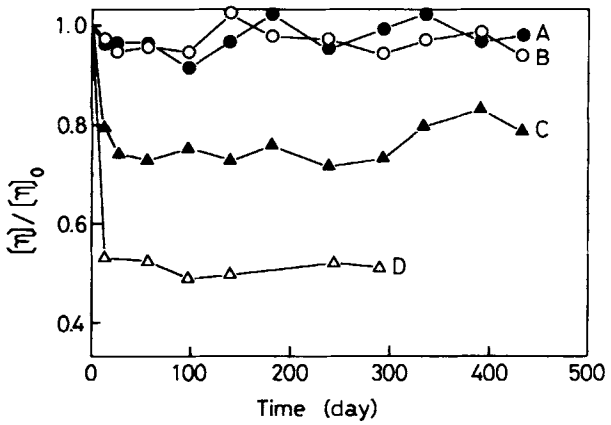

Figure 5 Ratio of intrinsic viscosity of poly(methyl methacrylate) containing *N*-benzyl-4-vinylpyridinium chloride in the main chain recovered after placing in an aeration tank of sewage works to that before the biological treatment. Intrinsic viscosity of the polymer determined in chloroform at 30°C before the treatment (dL/g): (A) 0.54; (B) 0.41; (C) 0.50; (D) 0.55. Content of the pyridinium group (mol %): (A) 0; (B) 1; (C) 5; (D) 10.

When Figure 4 was compared with Figure 5, the extent of the molecular weight reduction in the first series of experiments (Fig. 4) was notably larger than that in the second series of experiments (Fig. 5), in spite of the fact that the polymer films were thicker in the former experiments. In the case of poly(methyl methacrylate) containing 1 mol % of the pyridinium group, molecular weight reduction was more significant in the first series of experiments (Fig. 4B) when compared with that in the second series of experiments (Fig. 5B). With respect to the polymer containing 5 mol % of the pyridinium group, extent of the main chain scission was more conspicuous in the first series of experiments (Fig. 4D) when compared with that in the second series of experiments (Fig. 5C).

This difference in the extent of molecular weight reduction between the two series of experiments can be attributed to the influence of molecular weight of the original polymer before the biological treatment. Intrinsic viscosity of the polymer containing 1 mol % of the pyridinium group before the biological treatment was 0.21 and 0.41 dL/g for Figures 4(B) and 5(B), respectively. Intrinsic viscosity of the polymer containing 5 mol % of the pyridinium group was 0.18 and 0.55 dL/g for Figures 4(D) and 5(C), respectively. Thus, biodegradation of higher molecular weight polymer was suggested to be more difficult.

In Figure 6 is shown weight reduction of the polymer during the biological treatment. Although the data fluctuated widely, weight of the polymer samples reduced during the biological treatment. Weight reduction was negligible when the polymer samples were placed in deionized water in the absence of activated sludge. Extent of the weight reduction was more significant in the case where content of the pyridinium group was higher.

In the first series of experiments, weight reduction was negligible within 70 days of the treatment time. However, Figure 6 indicates significant weight reduction in the second series of experiments. This result could be attributed to the difference in thickness of the polymer samples. In the first series of experiments, polymer films 0.5 mm thick were used, whereas polymer films 0.13 mm thick were used in the second series of experiments. Weight reduction would be more significant in the case where thinner samples were used.

In Figures 7–10 are shown gel permeation chromatograms of poly(methyl methacrylate) containing the pyridinium group in the main chain. The molecular weight was based on standard polystyrene.

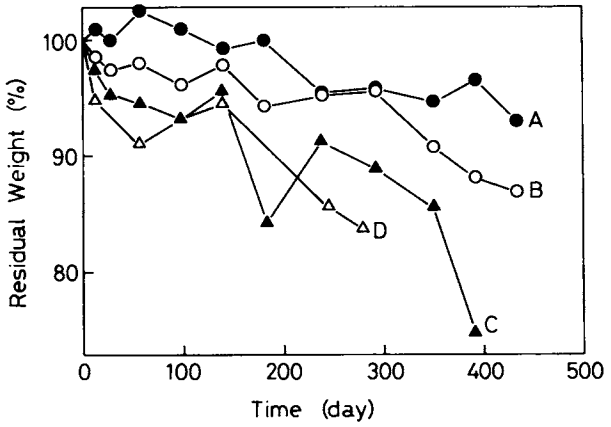

Figure 6 Weight reduction of poly(methyl methacrylate) containing *N*-benzyl-4-vinylpyridinium chloride during placing in an aeration tank of sewage works. Intrinsic viscosity of the polymer determined in chloroform at 30°C before the treatment (dL/g): (A) 0.54; (B) 0.41; (C) 0.50; (D) 0.55. Content of the pyridinium group (mol %): (A) 0; (B) 1; (C) 5; (D) 10.

In the case of homopolymer of methyl methacrylate, change of intrinsic viscosity was negligible during the biological treatment [Figs. 4(A) and 5(A)]. However, Figure 7 shows that content of low

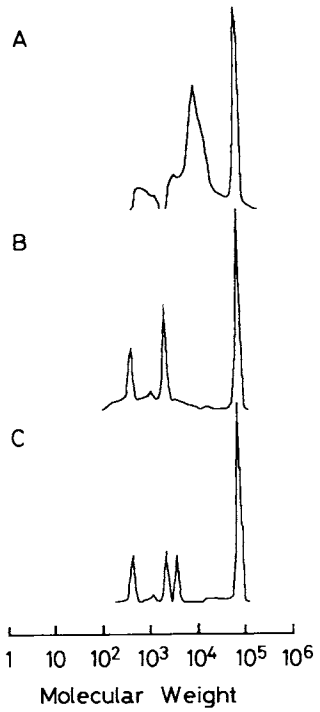

Figure 7 Gel permeation chromatogram of homopolymer of methyl methacrylate after placing in an aeration tank of sewage works: (A) before the biological treatment; (B) after 140 days; (C) after 350 days. Intrinsic viscosity of the polymer determined in chloroform at 30°C before the biological treatment was 0.54 dL/g.

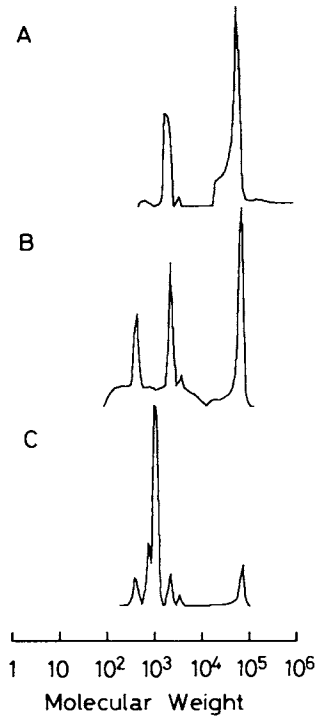

Figure 8 Gel permeation chromatogram of poly(methyl methacrylate) containing 1 mol % of the pyridinium group after placing in an aeration tank of sewage works: (A) before the biological treatment; (B) after 140 days; (C) after 350 days. Intrinsic viscosity of the polymer determined in chloroform at 30°C before the biological treatment was 0.41 dL/g.

molecular weight fraction reduced during the treatment, although change of the peak due to high molecular weight fraction appeared to be insignificant. Since it is quite inconceivable that this low molecular weight fraction of homopolymer of methyl methacrylate was lost by extraction with water during the biological treatment, Figure 7 suggests that degradation of poly(methyl methacrylate) by activated sludge is possible even in the absence of the pyridinium group in the case where molecular weight was less than 10,000.

Figures 8–10 show considerable enlargement of the content of low molecular weight fraction and reduction of that of high molecular weight fraction during the biological treatment. Thus, GPC analysis clearly indicated that incorporation of the pyridinium group into the main chain exerted scission of the carbon–carbon bonds of the main chain during the biological treatment. As can be seen in Figures 8–10, lower molecular weight fractions predominantly underwent degradation by activated sludge. Molecular weight of the remained high molecular fraction tended to increase with progress of the biological treatment. Degradation of higher molecular

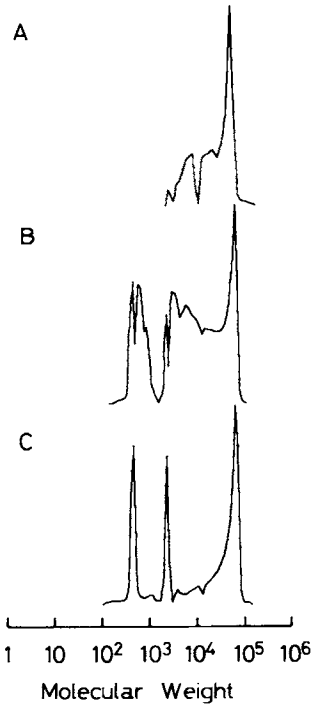

Figure 9 Gel permeation chromatogram of poly(methyl methacrylate) containing 5 mol % of the pyridinium group after placing in an aeration tank of sewage works: (A) before the biological treatment; (B) after 140 days; (C) after 350 days. Intrinsic viscosity of the polymer determined in chloroform at 30°C before the biological treatment was 0.50 dL/g.

weight polymers by activated sludge seems to be more difficult.

Comparison of chromatograms of the polymer containing 1 mol % of the pyridinium group (Fig. 8) with that of the polymer containing 5 mol % of the pyridinium group (Fig. 9) appears to show easier degradation of the former polymer. However, intrinsic viscosity of the former polymer before the biological treatment was 0.41 dL/g whereas that of the latter polymer was 0.50 dL/g. Degradation of lower molecular weight polymers appears to be easier. We should take not only the content of the pyridinium group but also original molecular weight into consideration.

In the third series of experiments of the biological treatment, polymer samples were prepared as films 3 cm wide, 3 cm long, and 0.13 mm thick. The polymer films were placed in a bag made of 100-mesh nylon net, and the bag was placed in the aeration tank by hanging with a rope. Intrinsic viscosity of the original polymer determined in chloroform at 30°C was in the range 0.50–0.54 dL/g before the biological treatment. Viscosity average molecular weight was estimated to be in the range 121,000–133,000.

In Figure 11 is shown the ratio of intrinsic viscosity of the recovered polymer sample to that before the biological treatment as a function of the treatment time. In Figure 12 is shown weight reduction of the polymer during the treatment. Both molecular weight and gravimetric weight of poly(methyl methacrylate) containing the pyridinium group reduced during the biological treatment. The extent of the reduction increased with content of the pyridinium group. However, when Figures 11 and 12 were compared with Figures 5 and 6, respectively, the extent of the biodegradation was much the same for the two series of experiments.

A Consideration on the Mechanism of Degradation of Poly(methyl Methacrylate) Containing the Pyridinium Group in the Main Chain by Activated Sludge

Based on the above observations, we have considered a mechanism for degradation of poly(methyl meth-

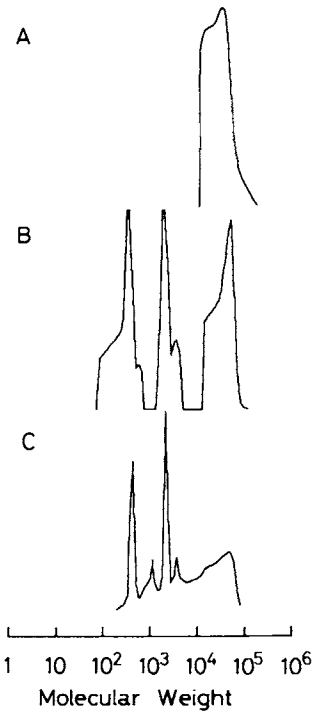

Figure 10 Gel permeation chromatogram of poly(methyl methacrylate) containing 10 mol % of the pyridinium group after placing in an aeration tank of sewage works: (A) before the biological treatment; (B) after 14 days; (C) after 140 days. Intrinsic viscosity of the polymer determined in chloroform at 30°C before the biological treatment was 0.55 dL/g.

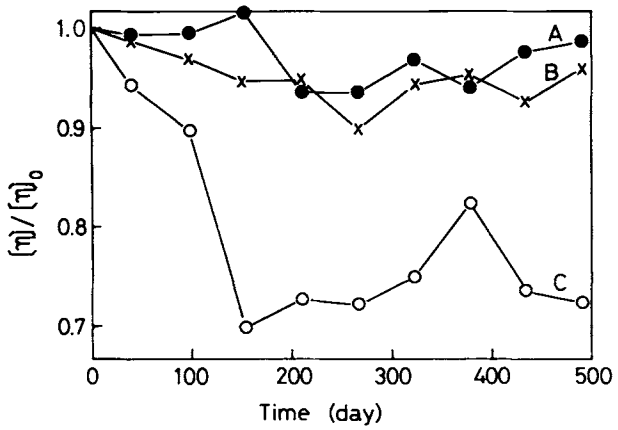

Figure 11 Ratio of intrinsic viscosity of poly(methyl methacrylate) containing *N*-benzyl-4-vinylpyridinium chloride in the main chain recovered after placing in an aeration tank of sewage works to that before the biological treatment. Intrinsic viscosity of the polymer before the treatment (dL/g): (A) 0.54; (B) 0.50; (C) 0.50. Content of the pyridinium group (mol %): (A) 0; (B) 3; (C) 5.

acrylate) containing the pyridinium group in the main chain by activated sludge. The degradation is probably performed by microorganisms, and must be catalyzed by some enzymes. Enzymes produced by microorganisms are classified into two categories, i.e., intracellular and extracellular enzymes. Degradation of the polymer must be catalyzed by extracellular enzymes, because the polymer is insoluble in water and cannot penetrate through the cell wall of microorganisms.

In this work, the biological treatment was performed using films of the polymer. Only the surface part of the film would be exposed to activated sludge. The inside was perhaps not in contact with activated sludge. Determination of intrinsic viscosity, as well as measurement of gel permeation chromatogram, of the recovered polymer film were performed for whole sample, and were not limited to the surface part. Nevertheless, Figures 8–10 clearly indicate that content of higher molecular weight fraction considerably reduced during the biological treatment. In other words, Figures 8–10 strongly suggest that the biodegradation is not limited to the surface part of the polymer film. This result would suggest that the enzymes permeated the polymer film and catalyzed the degradation.

Pyridinium-type polymer is strongly hydrophilic. For example, noncrosslinked poly(*N*-benzyl-4-vinypyridinium chloride) is hygroscopic.[15] As a result, poly(methyl methacrylate) containing the pyridinium group swells in water. For example, in the second and third series of experiments, films of poly(methyl methacrylate) containing 1, 3, 5, and 10 mol % of the pyridinium group contained 4, 5, 7, and 10 wt % of water, respectively, in the wet state. Incorporation of the pyridinium group into poly(methyl methacrylate) thus enhances hydrophilicity of the polymer, and would make the enzymes permeate the polymer films, and facilitate the biodegradation. Therefore, it is quite conceivable that extracellular enzymes produced by microorganisms permeate the polymer films, and catalyze the degradation.

Scission of the main chain of polymers can be classified into two categories, i.e., a systematic depolymerization from the end of the polymer chain (exo type), and a random degradation of the polymer chain (endo type). If the exo-type degradation were predominant, molecular weight reduction of the high molecular weight fraction would occur gradually. On the other hand, if the endo-type degradation were predominant, molecular weight reduction of high molecular weight fraction would occur vigorously even at the early stage of the degradation. Figures 8–10 suggest that the endo-type scission is predominant in the degradation of poly(methyl methacrylate) containing the pyridinium group in the main chain by activated sludge.

Extracellular enzymes that catalyze degradation of the hydrophobic synthetic polymer are probably secreted by microorganisms contained in activated sludge. It is quite inconceivable that microorganisms existed in the aeration tank of sewage works are familiar with such hydrophobic synthetic polymer as poly(methyl methacrylate). Therefore, produc-

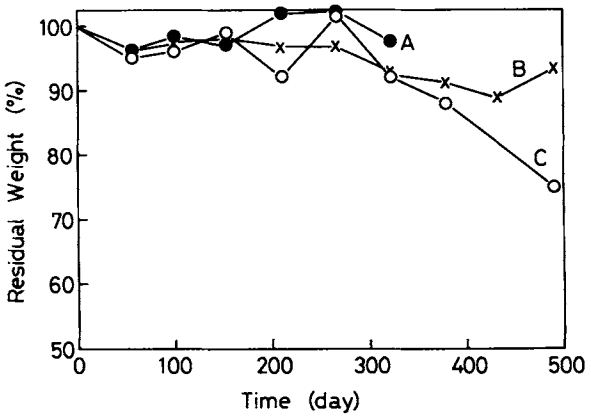

Figure 12 Weight reduction of poly(methyl methacrylate) containing the pyridinium group during placing in an aeration tank of sewage works. Intrinsic viscosity of the polymer before the treatment (dL/g): (A) 0.54; (B) 0.50; (C) 0.50. Content of the pyridinium group (mol %): (A) 0; (B) 3; (C) 5.

tion of the enzymes must be induced in the cells of acclimatized microorganisms. Acclimatization of microorganisms to the hydrophobic synthetic polymers, as well as induced production of such enzymes in the acclimatized microbial cells, is probably stimulated by the polymer, and would be facilitated by capture of microbial cells by the polymer or contact of the cells with the polymer. Therefore, enhancement of the affinity of the synthetic hydrophobic polymer with microorganisms would facilitate the acclimatization of microorganisms to the polymer as well as the induced production of the enzymes for the degradation. Since insoluble pyridinium-type polymer captures cells of microorganisms alive,[16] incorporation of the pyridinium group into the hydrophobic synthetic polymer would enhance the affinity of the polymer with microorganisms. As a result, incorporation of the pyridinium group into poly(methyl methacrylate) would make the polymer biodegradable.

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