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Some Characteristics of *Pseudomonas* O-3 which Utilizes Polyvinyl Alcohol†

Tomoo SUZUKI, Yoshihiro ICHIHARA*, Masaru YAMADA and Kenzo TONOMURA

Fermentation Research Institute, Inage, Chiba, Japan,
**Central Research Laboratories, Kuraray Co., Ltd., Kurashiki*

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Pseudomonas O-3 strain which was isolated from soil can grow on polyvinyl alcohol (PVA) as a sole carbon source. When 0.5 per cent of PVA (500, 1500 or 2000) was employed as the carbon source in the culture medium, PVA was almost completely lost from the culture fluid after a week and the concentration of total organic carbon measured by a TOC analyzer decreased from the initial value of about 2700 ppm to 250~300 ppm after 7~10 days culture. This bacterium was found to produce and secrete an inducible enzyme which degrade PVA. The way by which this enzyme degrades PVA was examined and the results were obtained which suggested that PVA was broken down oxidatively in a way of endowise splitting. However, the mechanism of PVA degradation has not been clarified yet. The optimum pH and temperature for enzyme activity were examined and they were 7.5~8.5 and 35~45°C, respectively. Morphological and biological characteristics of this bacterium were examined and it was similar to a strain of *Pseudomonas boreopolis*.

In recent years, various kinds of synthetic polymers are produced and used widely. However, the public problem of disposal of these polymers has arisen because of difficulties of their decomposition in nature.

Among them, the decomposition of water-soluble polymers are thought to be easier than that of water-insoluble ones. Polyethyleneglycol (PEG), for example, is known to be attacked by microorganisms.[1] We isolated a bacterium from soil which can grow on polyvinyl alcohol (PVA), another water-soluble synthetic polymer, as the sole source of carbon. Some aspects of breakdown of PVA by this organism were examined and it was found that this bacterium produced an inducible enzyme which degraded PVA and grew assimilating degraded PVA. Some characteristics of this bacterium and PVA-degrading enzyme are reported here.

MATERIALS AND METHODS

Microorganism and culture. *Pseudomonas* O-3 strain which was isolated from soil by enrichment culture and repeated isolations on plate culture was used throughout this study. The composition of culture medium was as follows; carbon source, 5.0 g; $(NH_4)_2SO_4$, 1.0 g; KH_2PO_4, 1.0 g; K_2HPO_4, 8.0 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; NaCl, 0.1 g; $CaCl_2 \cdot 2H_2O$, 0.02 g; $FeSO_4$, 0.01 g; $Na_2MoO_4 \cdot 2H_2O$, 0.5 mg; $Na_2WO_4 \cdot 2H_2O$, 0.5 mg; $MnSO_4$, 0.5 mg; Ca-pantothenate, 0.4 mg; inositol, 0.2 mg; niacin, 0.4 mg; *p*-aminobenzoate, 0.2 mg; pyridoxine, 0.4 mg; thiamine, 0.4 mg; biotin, 2 μg, vitamin B_{12}, 0.5 μg in 1 liter of distilled water, pH was adjusted to 7.5. The culture of organism was carried out on a rotary shaker of 180 rpm at 30°C. The organism was inoculated to 30 ml of culture medium in 100 ml-Erlenmeyer flask and cultured for 7 days. Ten ml of the culture broth was seeded to 500 ml-Erlenmeyer flask containing 200 ml of medium.

Measurement of cell growth. Cell growth was measured by reading the absorbance at 660 mμ.

Estimation of polyvinyl alcohol. Estimation of PVA was made by the method of Finley.[2] The method is based on the green color produced by the reaction of PVA with iodine in the presence of boric acid. Colorimetric determination was made by reading the optical density at 660 mμ. Estimation of PVA and degraded PVA concentration was also made by total organic carbon (TOC) measurement. When only PVA or degraded PVA was present in the solution, TOC concentration was measured from which

† Degradation of Polyvinyl Alcohol by Microorganism. Part I.

PVA or degraded PVA concentration was calculated being multiplied by the factor of 1.833 because PVA contains 54 per cent carbon in the molecule.

Estimation of total organic carbon (TOC) *concentration.* TOC concentration was estimated by Beckmann model 915 TOC analyzer which was consisted of two systems, total carbon (TC) and inorganic carbon (IC) measuring systems. Twenty μl of samples was injected by a microsyringe into the analyzer. TC was measured by combusting samples at 950°C with cupric oxide as the catalyst. IC was measured by combusting samples at 150°C with phosphoric acid as the catalyst. Concentration of carbon dioxide which was formed by combustion was measured by an infrared spectrophotometer. The difference of TC and IC was expressed as TOC.

Preparation of resting cells and crude enzyme solution. After the organism was cultured, cells were harvested by centrifugation at 11,000$\times g$ for 10 min, washed twice with 0.01 M phosphate buffer, pH 7.5, suspended in the same buffer and employed as the resting cell suspension. The culture supernatant solution was filtered through millipore filter (0.45 μ in pore size, 47 mm in diameter). The filtered solution was dialyzed against 0.01 M phosphate buffer (pH 7.5) overnight and was employed as the crude enzyme solution.

Preparation of degraded PVA solution. Incubation medium which consisted of 20 ml of crude enzyme solution, 10 ml of 0.2 M phosphate buffer, pH 7.5, 10 ml of 2% PVA solution; total volume, 40 ml was incubated on a rotary shaker of 180 rpm at 30°C for 7 days.

Assay of PVA-degrading activity. Incubation medium which consisted of resting cell suspension or supernatant solution, 2 parts; 0.2 M phosphate buffer, pH 7.5, 1 part; and 1 part of 2% PVA 500 solution was incubated on a reciprocal shaker of 120 rpm at 30°C and colorimetric determination of PVA was carried out. PVA-degrading activity was expressed as the difference of $E^{1\,cm}_{660\,m\mu}$ before and after incubation.

Gel filtration of PVA and degraded PVA on Sephadex G–50. Gel filtration of PVA and degraded PVA was carried out on a column (2.5$\times$97 cm) of Sephadex G–50 which had been equilibrated with 0.01 M phosphate buffer, pH 7.5. Column was warmed to 70°C to prevent the gelification of PVA.[3] Samples were previously boiled for a while, cooled to about 70°C and placed on the column. Column was eluted with the same buffer at a flow rate of 40 ml per hr.

Measurement of the uptake of degraded PVA by organism. The orgamism was cultured in the medium which contained glucose for 18 hr. Cells were harvested by centrifugation at 11,000$\times g$ for 10 min, washed twice with 0.01 M phosphate buffer (pH 7.5) and suspended in the culture medium without carbon source at a concentration of about 1 mg per ml. Twenty ml of 0.5% PVA 500 or degraded PVA solution was added to 20 ml of cell suspension and cultured on a rotary shaker as usual. PVA concentration was measured by iodometry or TOC measurement.

Determination of protein. Protein was determined by the method of sodium carbonate-Folin using bovine serum albumin as a standard or by reading the absorbance at 280 mμ.

Measurement of viscosity. Viscosity of culture filtrate was measured with Ostwald-type viscosimeters for PVA as indicated in JIS K6726–1965 and expressed as the relative viscosity to water at 30°C.

Measurement of oxygen uptake. Oxygen uptake was measured by a Mitamura 6–30R15 Warburg manometer at 30°C with reciprocal shaking of 100 rpm in 2 cm width. Composition of vessels was as follows; main room contained resting cell suspension (about 1 mg per ml) or crude enzyme solution, 1 ml and 0.1 M phosphate buffer, pH 7.5, 0.5 ml; side arm contained substrate (2% in concentration), 0.5 ml; central well contained 20% KOH, 0.3 ml; total volume, 2.3 ml.

Materials. Three kinds of polyvinyl alcohol (PVA 500, 1500, and 2000) were purchased from Wako Pure Chemical Industry, Ltd. PVA H17, 117 and 217 (degree of polymerization is about 1700 and degree of saponification are 99.9, 99 and 88%, respectively) were products of Kuraray Company. Degrees of polymerization, degrees of saponification and contents of sodium acetate of PVA were determined by the method described in JIS K6726–1965. Contents of 1,2 glycol bond in PVA were determined by the method of Flory *et al.*[4] These values for PVA 500, 1500 and 2000 were summarized in Table I.

RESULTS

Growth of Pseudomonas O–3 on PVA

Growth of *Pseudomonas* O–3 was compared on PVA 500, 1500 and 2000 (Fig. 1). With the growth PVA concentration measured by iodometry decreased rapidly and completely lost after 7 days. TOC value of the culture filtrate was about 2700 ppm at first and gradually decreased reaching to the value of about 250~300 ppm after 10 days. pH

TABLE I. PROPERTIES OF PVA 500, 1500 AND 2000

	Degrees of polymerization	Degrees of saponification %	Contents of 1,2-glycol bond, mole %	Contents of sodium acetate %
PVA 500	533	88.0	1.63	0.46
PVA 1500	1163	88.9	1.90	2.29
PVA 2000	2026	99.3	1.64	0.46

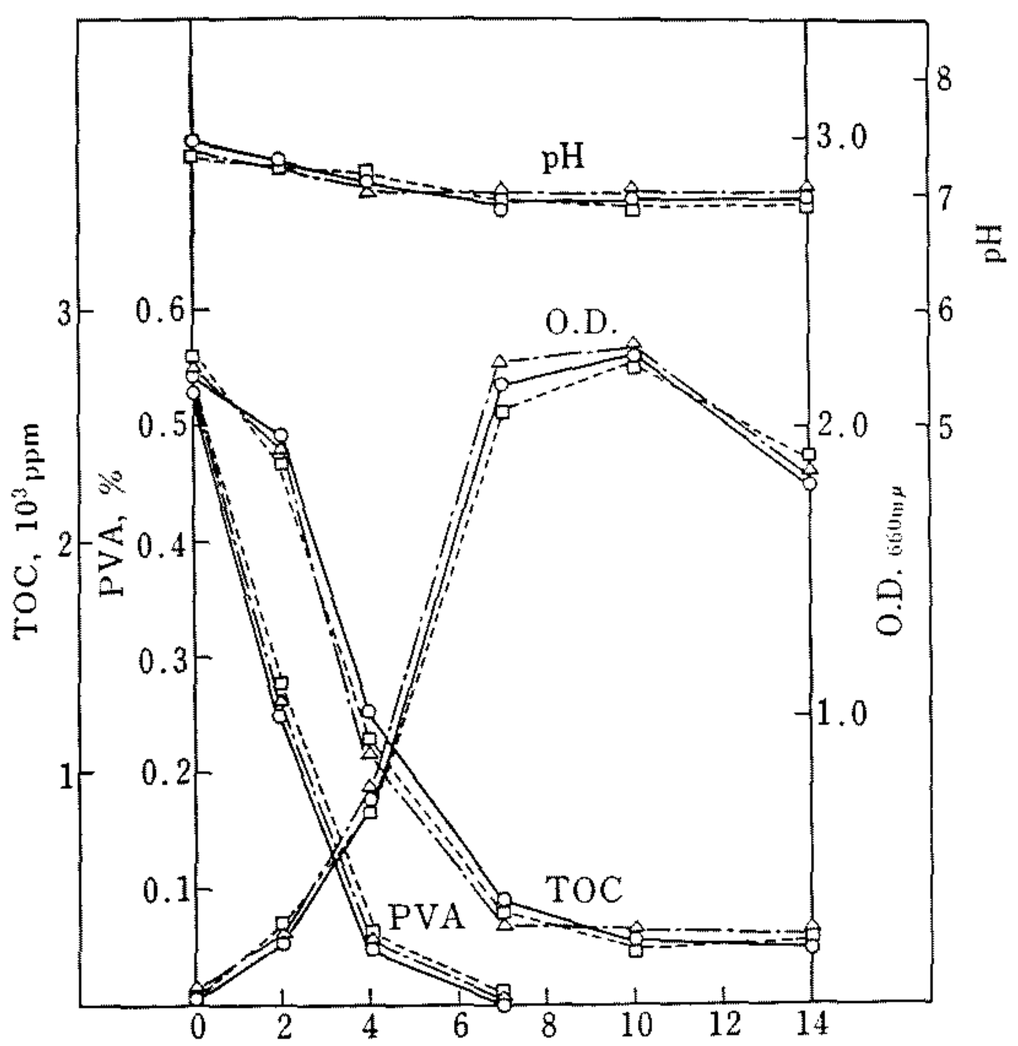

FIG. 1. Growth of *Pseudomonas* O–3 on PVA.
Pseudomonas O–3 was cultured in the medium containing PVA 500, 1500, 2000 as the carbon source. Cell growth, PVA concentration, TOC and pH were measured. Symbols are, circles for PVA 500, triangle for PVA 1500 and squares for PVA 2000.

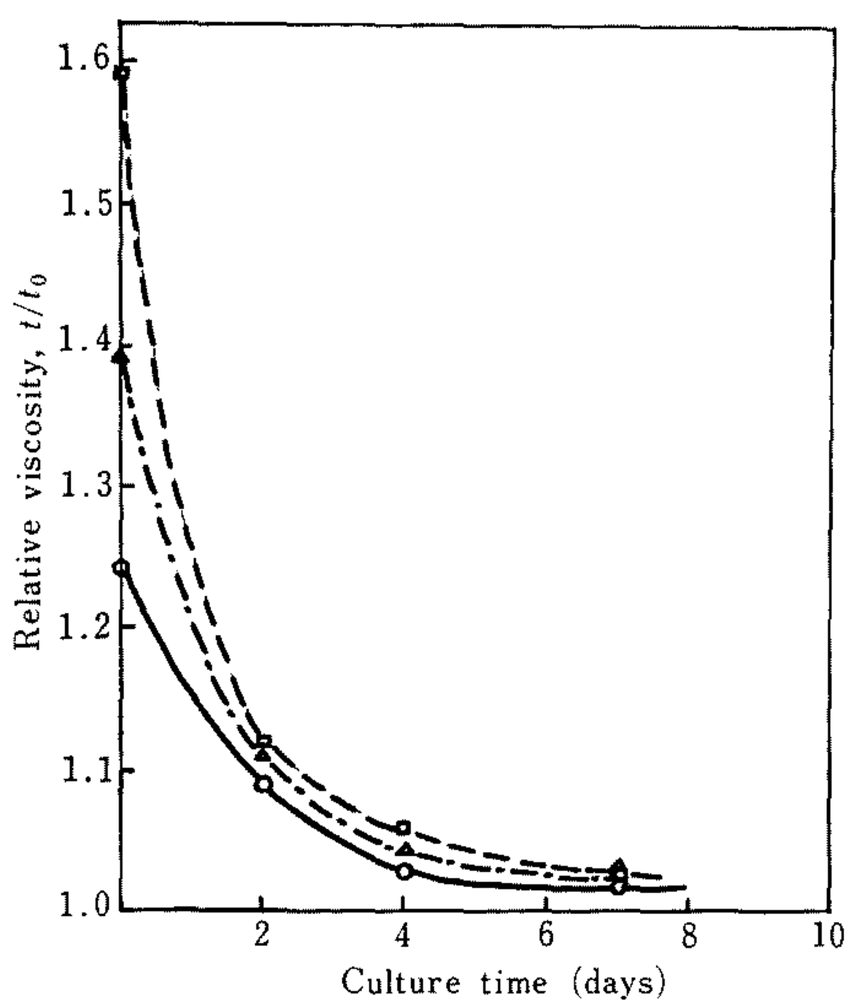

FIG. 2. Viscosity of Culture Filtrate.
Pseudomonas O–3 was grown in the medium containing PVA 500, 1500 and 2000 as the carbon source and relative viscosity of culture filtrate was measured. Circles, PVA 500, triangles, PVA 1500, squares, PVA 2000.

of the medium dropped gradually from 7.5 to about 7.0. There was no great difference between PVA 500, 1500 and 2000 as the substrates. Viscosity of the culture filtrate is shown in Fig. 2. The higher the degree of polymerization of PVA used, the higher was the viscosity of initial culture medium, but viscosity of culture filtrate decreased remarkably with the culture period and approached to the value of water after 7 days.

Effect of substrate of culture on PVA degradation

The organism was cultured on glucose, ethylene glycol or PVA and the ability of the cells grown on glucose and ethylene glycol to degrade PVA was compared with that of the cells grown on PVA (Fig. 3). As can be seen from Fig. 3, only cells grown on PVA could degrade PVA and those grown on glucose and ethylene glycol could not. It is presumed that PVA-degrading enzyme is inducibly produced in the cell grown on PVA.

Oxygen uptake by resting cells

Oxygen uptake by resting cells was measured using PVA-, glucose- and ethylene glycol-grown cells (Table II). From Table II, it is clear that only PVA-grown cells can oxidize PVA. This fact coincides with the result of effect of substrate of culture on PVA degradation and suggests that PVA is oxidatively degraded by PVA-grown cells.

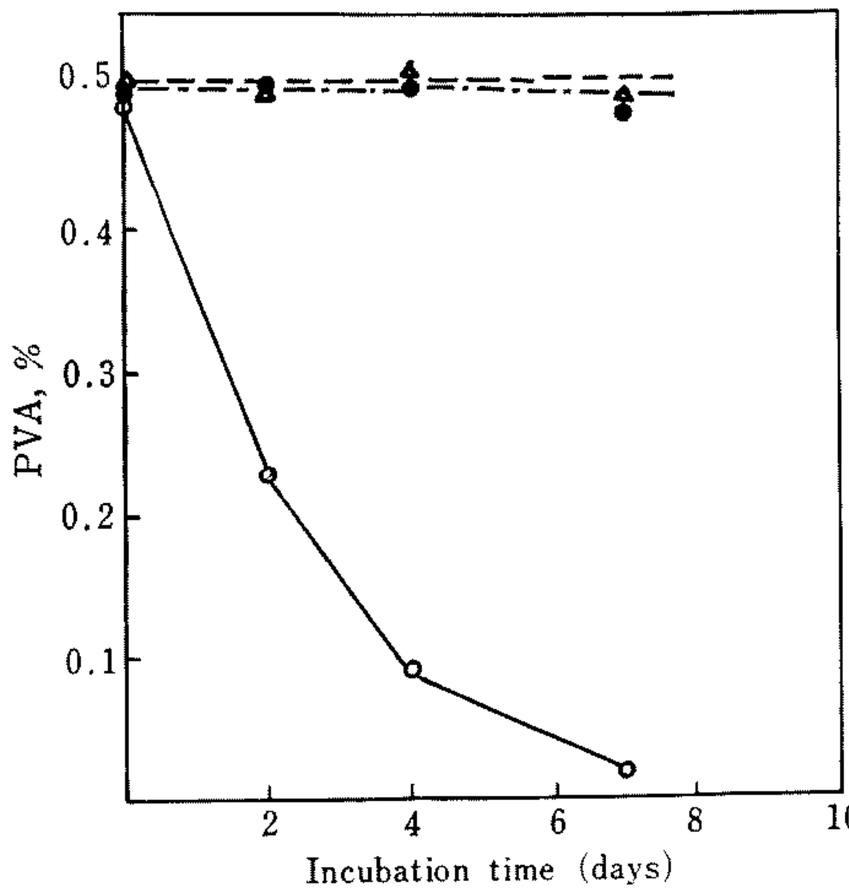

FIG. 3. Effect of Substrate of Culture on PVA Degradation.

Pseudomonas O–3 was cultured in the medium containing PVA, glucose and ethyleneglycol as the carbon source for 5 days, 2 days and 5 days respectively. Cells were harvested and incubated in the medium containing PVA 500. Degradation of PVA was measured until after 7 days. ○—○, cells grown with PVA; ●-·-●, cells grown with glucose; △—△, cells grown with ethylene glycol.

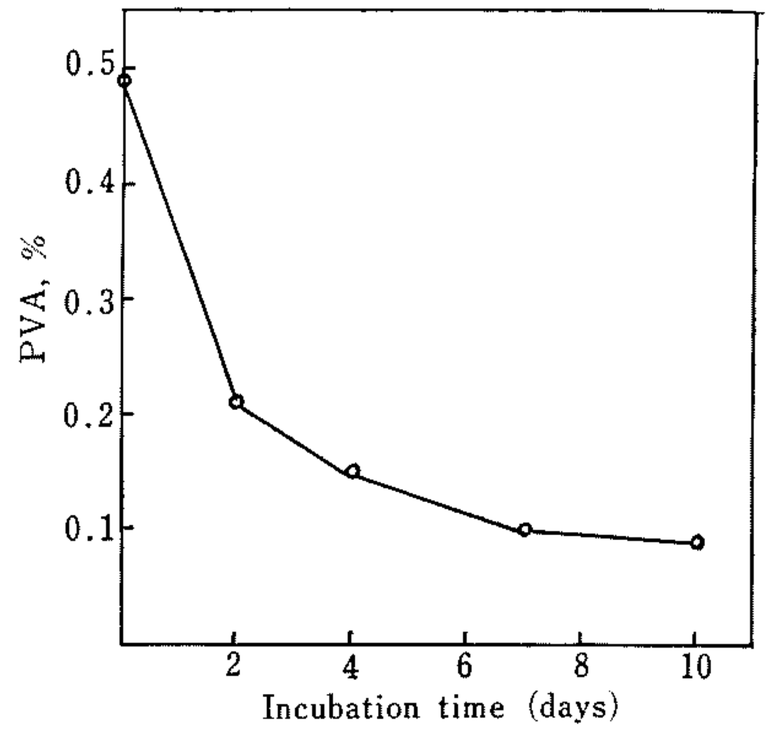

FIG. 4. Degradation of PVA by Culture Supernatant.

Pseudomonas O–3 was cultured in the medium containing PVA 500 for 7 days. Culture fluid was incubated with PVA 500. Degradation of PVA was measured for 10 days.

TABLE II. OXYGEN UPTAKE BY RESTING CELLS

Oxygen uptake by resting cells was measured as described in the methods. Cells grown on glucose, ethyleneglycol and PVA for 2 days, 3 days and 7 days respectively were harvested and employed for oxygen uptake measurement with PVA, glucose and ethyleneglycol as the substrates.

Substrates for culture	PVA 500	Qo_2 with Glucose	Ethylene-glycol	Endo-genous
Glucose	0.78	2.71	0.74	0.93
Ethylene-glycol	0.40	0.54	1.28	0.37
PVA 500	1.32	0.85	0.39	0.35

Degradation of PVA by the culture fluid

In Fig. 4, degradation of PVA by culture fluid is shown. PVA was broken down by the culture fluid, indicating that PVA-degrading enzyme is secreted from cells into the culture medium.

PVA-degrading activity in the cells and the culture fluid

Pseudomonas O–3 was cultured in the medium containing PVA and PVA-degrading activity in the cells and the culture fluid was compared along with the culture period. Cells were harvested by centrifugation, washed and suspended in the same volume of phosphate buffer, pH 7.5 to the original culture medium. PVA-degrading activity of cells and the supernatant was measured as described in the methods. As can be seen in Fig. 5, activity in the supernatant was stronger than that of cells through the culture period, reaching to its maximum after 7 days in contrast to the maximum activity for the cells which was brought about after 5 days. From the result, PVA-degrading enzyme is thought to be an exogenous enzyme. Protein concentration of the culture medium was also measured. The period of its maximum concentration coincided with the time of maximum cell growth.

Effect of degree of polymerization on the PVA degradation by the crude enzyme

Effect of degree of polymerization on the PVA degradation by crude enzyme was examined using PVA 500, 1500 and 2000 as the substrates. The crude enzyme solution was obtained from the culture filtrate of PVA 500. Three kinds of PVA were equally degraded by the crude enzyme.

Effect of degree of saponification on the PVA degradation by crude enzyme

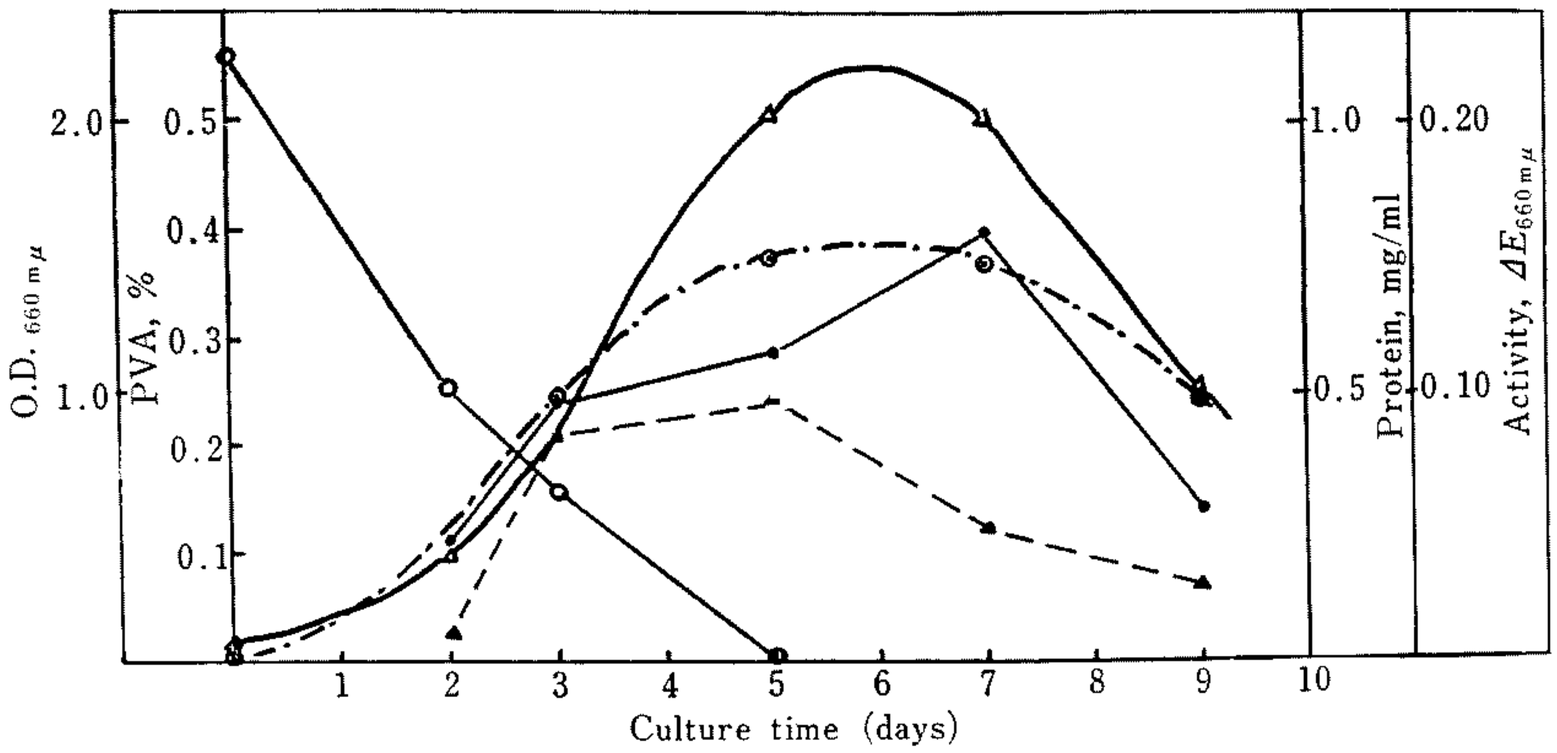

FIG. 5. Relation between Cell Growth and Enzyme Activity.

Pseudomonas O–3 was cultured in the medium containing PVA 500. Occassionally 10 ml of culture broth was taken and cell growth, PVA concentration, protein concentration in culture filtrate and enzyme activity both in cells and culture fluid were measured. Cells were harvested by centrifugation, washed and suspended to the 10 ml of 0.05 M phosphate buffer, pH 7.5. To 5 ml of resting cell suspension or culture fluid added 5 ml of 1% PVA 500 solution and incubated at 30°C for 24 hr. Decrease of $E_{660m\mu}$ was expressed as enzyme activity. △—△, cell growth; ○—○, PVA concentration; ⊙–·–⊙, protein concentration; ●—●, enzyme activity in culture supernatant; ▲—▲, enzyme activity in cells.

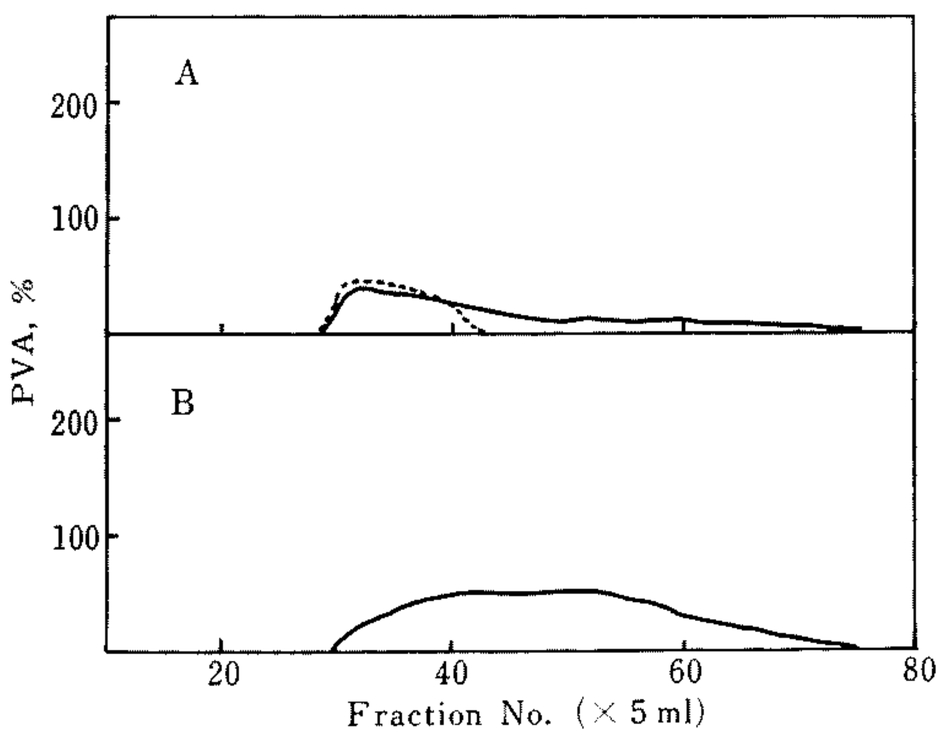

FIG. 6. Gel Filtration of Culture Filtrate (1).

1 ml of 2 days culture filtrate (A) and 2 ml of 4 days culture filtrate (B) were applied to the column of Sephadex G–50. Fractions of 2 days culture filtrate were estimated for PVA by iodometry (------) and TOC measurement (——). Fractions of 4 days culture filtrate were estimated for PVA by TOC measurement.

Effect of degree of saponification on PVA degradation by crude enzyme was examined using three kinds of PVA 1700 with different degree of saponification (88, 99 and 99.9%, respectively). Sample of PVA 1700 with degree of saponification of 99 per cent was degraded a lettle faster than the other two, but there was no remarkable difference among them.

Optimum pH for the PVA degradation by the crude enzyme

Optimum pH for PVA degradation by the crude enzyme was measured changing the pH of incubation medium with phosphate buffer. Optimum pH was between 7.5 and 8.5.

Optimum temperature for the PVA degradation by the crude enzyme

Temperature of incubation was varied from 30°C to 50°C and activity of the PVA degrading enzyme was measured. Optimum temperature was between 35°C and 45°C.

Gel filtration of culture filtrate

Gel filtration of culture filtrate was carried out as described in the methods. PVA 500 was eluted as a single peak around No. 30. Figure 6 shows the result of gel filtration of 2 days and 4 days culture filtrates. It was shown that the peak of PVA was lowered by the culture and broad elution pattern between No. 30 to 75 was shown. Color for iodometry did not develop any more for fractions later than No. 40. This means that once PVA was degraded, degraded PVA did not develop

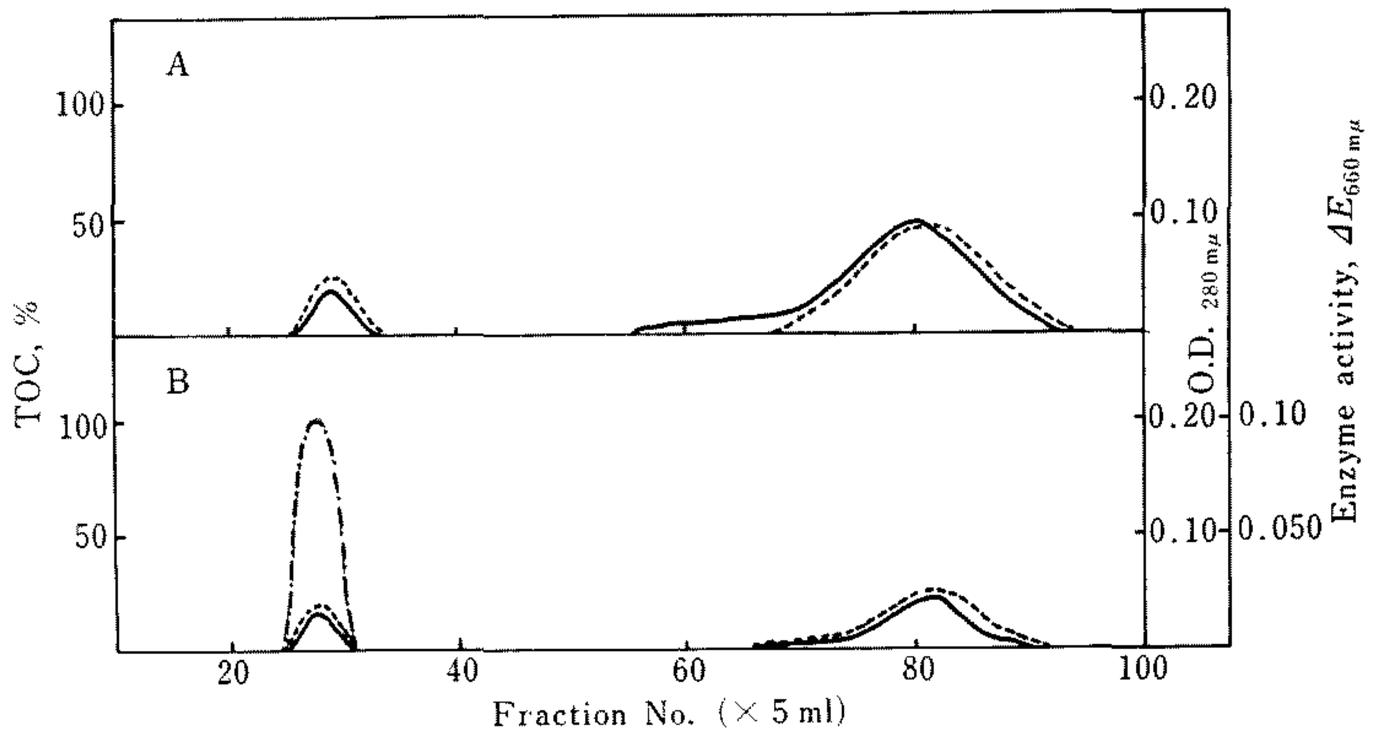

FIG. 7. Gel Filtration of Culture Filtrate (2).

5 ml of 8 days culture filtrate (A) and 10 days culture filtrate (B) were applied to the column of Sephadex G–50. Fractions of 8 days culture filtrate were estimated for TOC concentration (——) and the absorbance at 280 mμ (------). Fractions of 10 days culture filtrate which were gel-filtrated at room temperature were estimated for TOC concentration (——), absorbance at 280 mμ (------) and enzyme activity (ΔE) (—·—) was also measured for 24 hr.

color with iodine. A broad distribution of degraded PVA means that PVA was broken down by the PVA-degrading enzyme at random in the polymer chain resulting in degraded PVA compounds with a wide variety of molecular weight. Figure 7 shows the result of gel filtration of 8 days and 10 days culture filtrates. Both TOC values and absorbance at 280 mμ were measured for each fraction. It is clear PVA was almost completely lost from the culture filtrate and two peaks of protein appeared instead. The lower part of Fig. 7 shows the result of gel filtration of 10 days culture filtrate which was carried out at room temperature. In this case, PVA-degrading activities were also measured for each peak. It was shown that the earlier peak had PVA-degrading activity whereas the latter peak had not. Therefore, the earlier peak was assumed to be PVA–degrading enzyme protein.

Gel filtration of degraded PVA by the crude enzyme

PVA 500 was degraded by the crude enzyme solution for 7 days and the degraded PVA was gel-filtrated on Sephadex G–50. The result is shown in Fig. 8. It showed a broad elution pattern from No. 30 to 95 indicating degraded

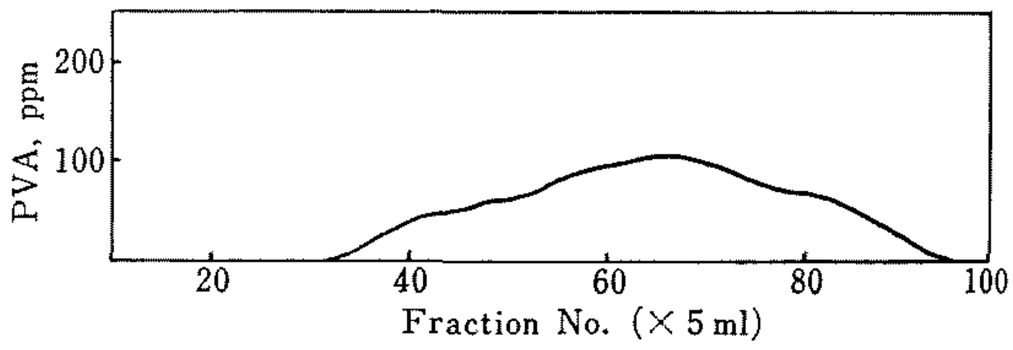

FIG. 8. Gel Filtration of PVA Degraded by Crude Enzyme.

PVA 500 was degraded by crude enzyme for 7 days. 5 ml of degraded PVA solution was applied to the column of Sephadex G–50. TOC concentration of each fraction was measured and expressed as PVA concentration.

PVA had a very wide distribution of molecular weight.

Uptake of degraded PVA by glucose-grown cells

Uptake of degraded PVA by glucose-grown cells were examined as described in the methods. Glucose-grown cells were incubated in the medium containing PVA or degraded PVA as a carbon source. PVA concentration was measured by both TOC measurement and iodometry. Degraded PVA concentration was measured by TOC measurement. TOC value of degraded PVA was unchanged for the first two days and then gradually decreased until 7 days indicating

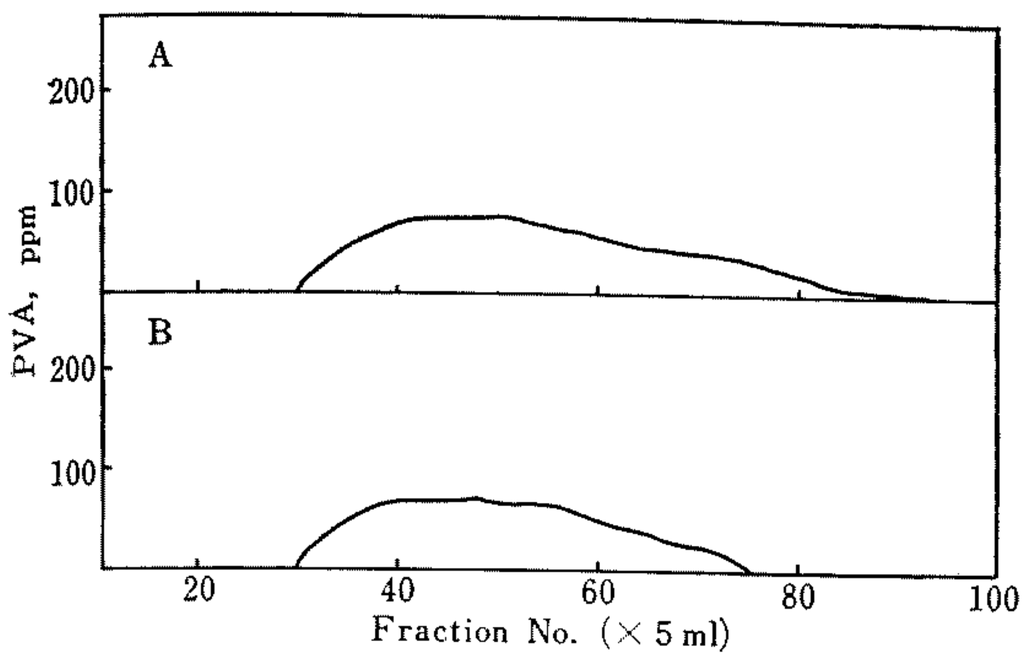

FIG. 9. Gel Filtration of Degraded PVA before and after Uptake.

4 ml of culture filtrate before (A) and after (B) uptake of degraded PVA by glucose-grown cells was applied to the column of Sephadex G–50. Each fraction was estimated for TOC concentration and expressed as PVA concentration.

degraded PVA was assimilated by glucose-grown cells. On the contrary, TOC value and iodometric value for PVA were almost unchanged throughout the culture. This indicates that glucose-grown cells cannot degrade nor assimilate PVA, but once PVA is degraded they can assimilate at least a part of degraded PVA.

Gel filtration of degraded PVA in culture filtrate before and after uptake by glucose-grown cells was carried out and the results are shown in Fig. 9. Distribution of degraded PVA before uptake was from No. 30 to 95. After uptake, it was from No. 30 to about No. 75 and the peak height between No. 30 to 75 was almost unchanged. This suggests glucose-grown cells take up degraded PVA with small molecular weight distributed between No. 75 and 95.

Molecular weight estimation by gel filtration

Molecular weight estimation by gel filtration was carried out using ethylene glycol, polyethylene glycol 200, 600 and 1000 as the standard substances. Ratios of their elution volume to void volume for each substance were plotted against their molecular weight. Except ethylene glycol, PEG 200, 600 and 1000 lay on a straight line (Fig. 10). From this line, the molecular weight of No. 75 was estimated to be 400.

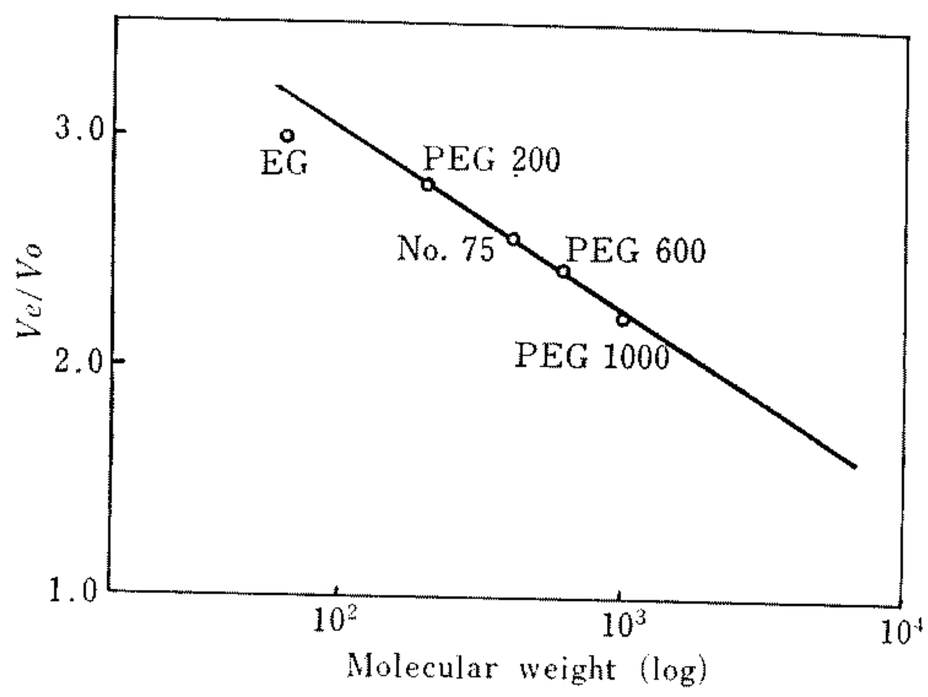

FIG. 10. Estimation of Molecular Weight by Gel Filtration.

Ethylene glycol, polyethylene glycol 200, 600 and 1000 were applied to the column of Sephadex G–50. γ-Glubulin was used as a void volume marker. Ratios of elution volume to void volume were calculated and plotted against molecular weights.

TABLE III. EFFECT OF OXYGEN ON PVA DEGRADATION

Contents of Thunberg tubes were as follows; 5 ml of crude enzyme solution, 2.5 ml of 0.2 M phosphate buffer (pH 7.5) and 2.5 ml of 2% PVA 500 solution. One tube was incubated aerobically and the other two statically one of which had previously been filled with nitrogen gas. After 15 hr $E^{1cm}_{660m\mu}$ was measured.

Conditions of incubation	Initial $E_{660m\mu}$	After 15 hr $E_{660m\mu}$	$\Delta E_{660m\mu}$
Shaking	0.290	0.125	0.165
Standing	0.290	0.210	0.080
Standing in N_2	0.290	0.293	—

Effect of oxygen on degradation of PVA

Effect of oxygen on degradation of PVA by the crude enzyme was examined changing the condition of incubation. Three Thunberg tubes were prepared, each containing 5 ml of the crude enzyme solution, 2.5 ml of 0.2 M phosphate buffer, pH 7.5 and 2.5 ml of 2% of PVA 500 solution. One tube was incubated shaking as usual, the second one was incubated standing and for the third one air was replaced by nitrogen gas and the tube was also incubated standing. Incubation was carried out at 30°C and the absorbance at 660 mμ was compared with the initial values (Table III). In Table III it is shown that ΔE in shaking tube was

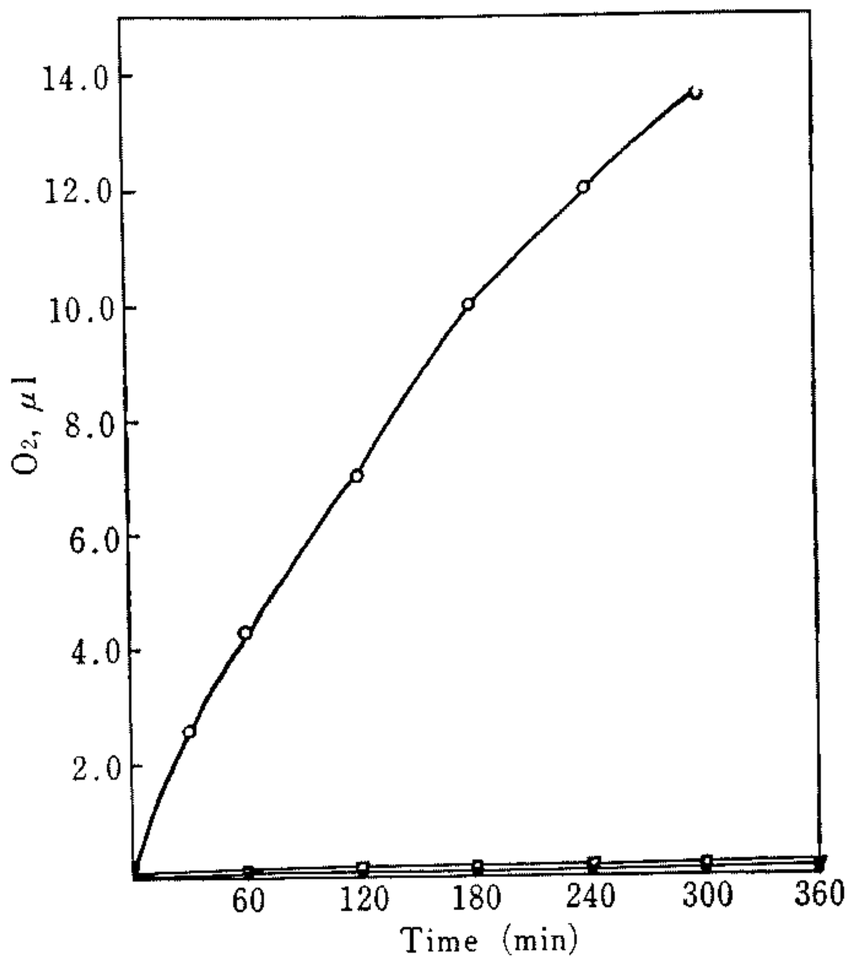

FIG. 11. Oxygen Uptake by Crude Enzyme.

Crude enzyme was prepared from 7 days culture broth with PVA 500 and oxygen uptake by crude enzyme was measured in a Warburg manometer with PVA, 500 as the substrate. ○—○, PVA 500; □—□, without substrate; ●—●, without enzyme.

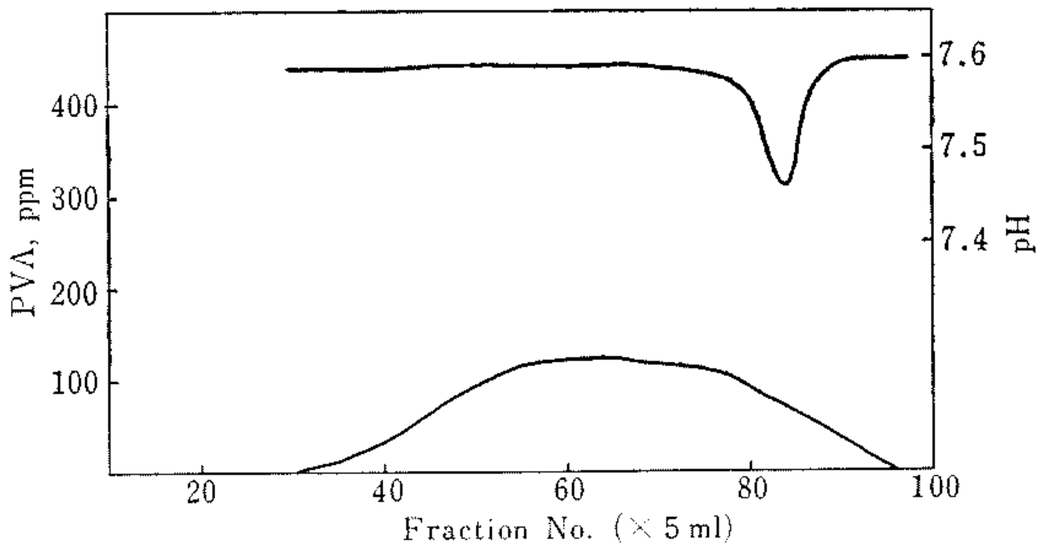

FIG. 12. pH of Gel-filtrated Solution.

PVA 500 was degraded by crude enzyme for 7 days and 5 ml of the degraded solution was applied to the column of Sephadex G–50. pH (upper line) and TOC concentration of each fraction was measured and expressed as PVA concentration (lower line).

0.165 whereas that of static incubation was 0.080. Absorbance of the third N2-purged tube was unchanged. These facts indicate that oxygen is required for enzymatic degradation of PVA. Oxygen uptake by the crude enzyme was measured manometrically. Two kinds of control vessels, one of which contained distilled water instead of enzyme solution and the other of which contained distilled water instead of PVA respectively were run together. It was shown that oxygen uptake took place only when both enzyme solution and substrate were present and when either enzyme solution or PVA was omitted from the system no oxygen uptake took place (Fig. 11).

pH of gel-filtrated solution

To know the property of the terminal group of degraded PVA, pH of gel-filtrated solution was measured by a pH meter (Fig. 12). PVA was degraded by the crude enzyme for 7 days and degraded solution was gel-filtrated as usual. pH of elution buffer was 7.58. pH of filtrated solution was about 7.58 initially and it gradually dropped as the number of fractions increased. pH dropped greatly at about No. 75 reaching to 7.45 at No. 84. Then it got higher and reached to about 7.6 at No. 90. This indicates that terminal group of degraded PVA located from No. 75 to 90 might be carboxyl group. Aldehyde tests by Shiff base and 2,4-dinitrophenyl hydrazine were negative throughout fractions.

Morphological and biological characteristics of Pseudomonas O–3 strain

Morphological and biological characteristics.

TABLE IV. TAXONOMIC DESCRIPTIONS OF *Pseudomonas* 0–3

1.	Morphological characteristics	
	Form	Rods
	Size	0.8—1.0×1.6—1.8 μ
	Flagellum	Polar flagellum
	Gram staining	Negative
	Nutrient agar colony	Circular, smooth, entire, convex, pale yellow, translucent
2.	Biological characteristics	
	Glucose acidified	gas (−)
	Fructose acidified	gas (−)
	Hydrolysis of starch	Negative
	Utilization of cellulose	Negative
	Liquefaction of gelatin	Positive
	Reduction of nitrate to nitrite	Positive
	Production of indole	Negative
	Production of acetyl methyl carbinol	Negative
	BCP milk	pH changed (acidified)
	Temperature of growth	Grow poorly or not at all at 42°C Grow readily at 37°C

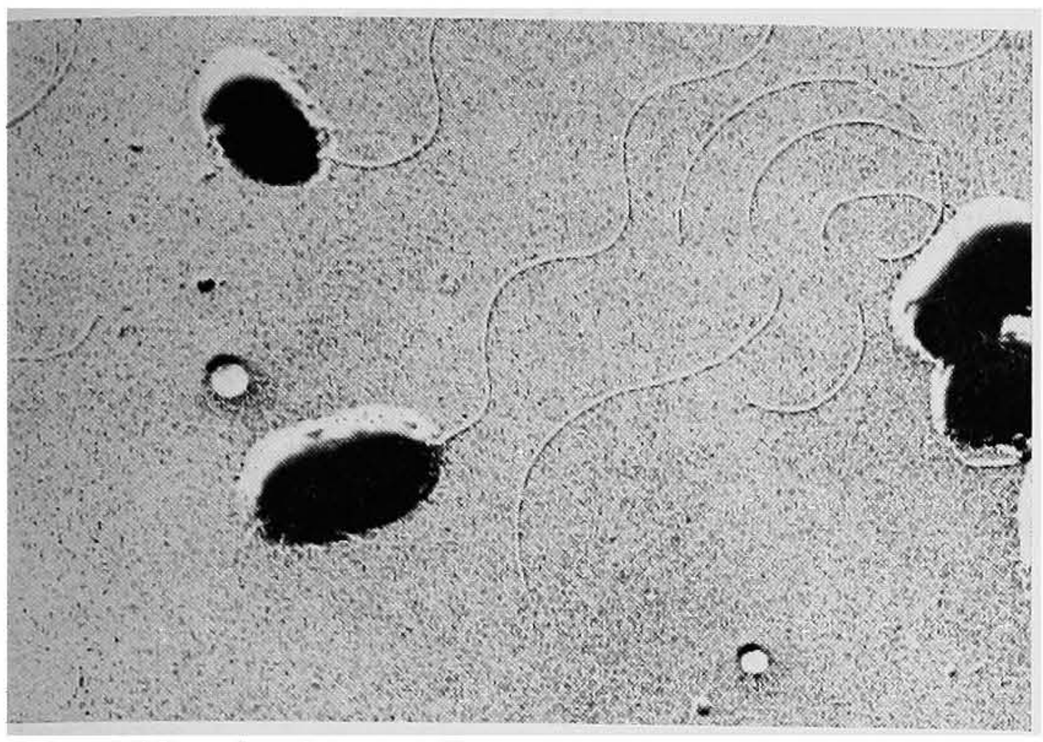

FIG. 13. A picture of *Pseudomonas* O–3 Taken by Electronmicroscope.

of this organism were examined. Results are summarized in Table IV. This bacterium is an aerobic, non-spore forming, gram-negative short-rod and has a polar flagellum (Fig. 13). It produced acid from glucose and fructose, did not hydrolyze starch and cellulose, liquefied gelatin, reduced nitrate to nitrite and did not form indole or acethyl methyl carbinol. BCP milk was acidified. Optimum temperature for the growth of this bacterium was from 30°C to 37°C and did not grow at 42°C. From these results, this bacterium seemed to be similar to a strain which belonged to *Pseudomonas boreopolis* from 7th edition of Bergey's Manual of Determinative Bacteriology.

DISCUSSION

There are many literatures on the microbial deterioration of synthetic polymers. However, these are the deterioration of polymers caused by, for example, microbial assimilation of plastisizer included in polymers and not the degradation of polymers by microorganisms. Literatures on the microbial degradation of synthetic polymers are very few. It is reported that polyurethanes of polyester type are degraded by fungi[5,6] but the mechanism of degradation of polymer is not clear. *Pseudomonas* O–3 strain which was isolated from soil grew well in the medium containing PVA as the sole source of carbon. When 0.5% of PVA was used for culture, PVA measured by iodometry was almost completely lost from the medium after about a week, and TOC value measured by TOC analyzer was decreased from the initial value of about 2700 ppm to 250~300 ppm after 10 days. The remaining TOC was revealed by gel filtration to be that of protein produced and excreted into the culture medium by the organism. *Pseudomonas* O–3 was shown to produce and secrete into the culture medium an enzyme which degraded PVA and grew by assimilating degraded PVA. The formation of this enzyme was inducible, because the organism grown with other carbon sources than PVA exhibited no enzyme activity.

How the C–C linkage in PVA molecule is splitted by this enzyme is not clear yet. C–C linkages are in general very stable and not labile to enzymatic cleavage. One example of enzymatic cleavage of C–C linkage is that of fructose-1,6-diphosphate by aldolase yielding two 3 carbon compounds. Another example is oxidative cleavage of benzene ring. It is known that such aromatic compounds as benzene and phenol are oxidized to form catechol and benzene ring in the catechol molecule is oxidatively cleaved producing *cis, cis*-muconic acid. β-Oxidation of fatty acids is also well known. But these are the cases with rather simple compounds with low molecular weight and not with the polymer.

R. A. Johnson[7] studied about oxidative break-down of acyclic N-alkyl benzamides and revealed alkyl chain of benzamide having an alcohol group at the end was oxidatively splitted to an alcohol with alkyl chain of two less carbons by *Sporotrichum sulfurescens*. He assumed an acetate as an intermediate in this oxidative breakdown. However, this is exotype breakdown similar to β-oxidation of fatty acids and not endotype one.

Cleavage of C–C linkage in vinyl polymer has not been known thus far. PVA-degrading enzyme produced by *Pseudomonas* O–3 is throught to be an liquefying-type enzyme such as α-amylase, endo-type cellulase and endo-type pectinase. But these liquefying enzymes so far known hydrolyze polymer chains and not

oxidize them being different from PVA-degrading enzyme of *Pseudomonas* O–3. Whether this enzyme is an oxidase type or an oxygenase type enzyme cannot be decided until it is purified and the mechanism of action of this enzyme is clarified. Purification of this enzyme is now under investigation.

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