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Biodegradation of PVAs with Various Stereoregularities

*Ryohei Fukae^{*1}, Koji Nakata^{*2}, Masahiro Takeo^{*3},
Tohei Yamamoto^{*3} and Osamu Sangen^{*4}*

*1 School of Environmental Policy and Technology, Himeji Institute of Technology, Shinzaikehoncho, Himeji 670-0092, Japan

^{*2, *3} Faculty of Engineering, Himeji Institute of Technology, Shosha, Himeji 671-2201, Japan

*4 Tajima Technical Institute, Kokonokaichi, Toyooka 668-0051, Japan

*2 Present Address : Daicel Chemical Industries, Ltd. Aboshi-ku, Himeji 671-1283, Japan

Abstract : Biodegradation of poly(vinyl alcohol) with high syndiotacticity (*s*-PVA) was studied under the presence of *Pseudomonas* sp. as a bacterium. The effects of stereoregularity of *s*-PVA on its biodegradability were evaluated compared to PVA homologs, *i.e.* atactic PVA (*a*-PVA) and isotactic PVA (*i*-PVA). Changes in total organic carbon concentration (TOC) in PVA cultures with incubation time were checked, and pentad tacticity was estimated by ^{13}C NMR analysis for tacticity contents of degraded PVA.

TOC in *i*-PVA culture decreased rapidly in early stage of the incubation. Sixty-two percent of initial amounts of *i*-PVA was assimilated in 12 days of incubation. On the contrary, only 13% of the initial amounts of *s*-PVA was assimilated even after 20 days of incubation. Little changes were observed in ^1H NMR spectrum of *s*-PVA compared with that of *a*-PVA or *i*-PVA after the incubations. It was obviously confirmed in the present work that *mmmm* sequence is especially subject to degradation in meso sequences on the basis of ^{13}C NMR analysis and the enzyme engaged in this degradation should have a sequence selectivity at pentad level. Experimental results supports the view that *i*-PVA having a number of long meso sequences should be susceptible to biodegradation, and *s*-PVA should not so.

1. Introduction

In the course of our investigation for the synthesis and physical properties of poly(vinyl alcohol) (PVA) with various stereoregularities, it has been found that the isotactic sequences in PVA skeletal linkages are susceptible to biodegradation preferentially in the presence of a strain of *Pseudomonas* sp. as a bacterium [1,2]. It is well known that the change in stereoregularity of PVA remarkably affects the polymer property. The PVA with high syndiotacticity shows more highly

crystallinity than the PVA with atacticity or isotacticity. Its crystal melting point is extremely high [3,4]. In the connection, it would be interesting to study the biodegradation of PVA with high syndiotacticity as compared to that in PVA with high isotacticity, where such biodegradable specificity by the syndiotactic stereoregularity should be present. In the previous report, no satisfactory experiment for biodegradation using the PVA with high syndiotacticity had been done independently. Detailed reports mentioning the influence of stereoregularity of OH groups in PVA on the

biodegradability had not been published except the work by Matsumura *et al.* [5]. The present report deals with the results of biodegradation of PVA with high syndiotacticity using a strain of *Pseudomonas* sp. Especially in the present experiment, further detailed information concerning the changes in tacticity could be obtained by the evaluation of a pentad tacticity on the basis of ^{13}C NMR analysis.

2. Experimental

2.1 PVA Samples

PVA samples used for biodegradation are listed in Table 1. PVA with diad syndiotacticity of 62% (*s*-PVA) was derived from poly(vinyl pivalate) being radically polymerized in methanol at 60°C using α, α' -azobisisobutyronitrile [6,7]. It had a degree of saponification of not less than 99.9%. PVA having high isotacticity (*i*-PVA) was prepared according to the procedure described in the previous report [1]. Obtained *i*-PVA had residual ether groups at less than 0.1 mol%. *a*-PVA was a product of Kuraray Co., Ltd. and had a degree of saponification of not less than 99.9%. It was used after purification by the reprecipitation. Weight-average molecular weight (M_w) and number-average molecular weight (M_n) of PVA were estimated from gel-permeation chromatogram of acetylated PVA. Tacticity and the content of 1,2-glycol linkage were determined from ^1H NMR spectrum.

Table 1 PVA samples used for biodegradation

Sample No.	M_n ($\times 10^{-4}$)	M_w/M_n	C_{gl}^a (mol%)	Tacticities (%) ^{b)}		
				<i>I</i>	<i>H</i>	<i>S</i>
<i>s</i> -PVA	3.4	2.5	1.7	14	49	37
<i>a</i> -PVA	3.1	2.5	1.6	21	50	29
<i>i</i> -PVA	6.5	4.7	< 0.1	72	24	4

^{a)} Content of 1,2-glycol linkage.

^{b)} *I*, Triad-isotacticity; *H*, heterotacticity; *S*, syndiotacticity.

2.2 PVA Degradation Test

The same PVA-degrading bacterium, *Pseudomonas* sp. strain A-41, was used for the degradation

test as described previously [1]. Preparations of culture media and conditions of degradation test were also carried out on the basis of the above paper. The concentrations of PVA in culture media were adjusted to 0.5g/l of equal portion taking into account the poor solubility of *s*-PVA in water and its easy gel-formability.

2.3 TOC and NMR spectrum

Total organic carbon concentration (TOC) of a supernatant solution of the culture was measured by Shimadzu model TOC-500. The supernatant solution was prepared by refrigerated centrifuging at 12,000 rpm for 10 min.

Residual PVA in a culture was recovered by dialyzing the supernatant solution for a week against water. The dialyzed solution was condensed and poured into methanol. The deposits were purified by the reprecipitations of its dimethyl sulfoxide solution, followed by 1,1,1,3,3,3-hexafluoro-2-propanol solution with methanol.

^1H NMR and ^{13}C NMR spectroscopies were carried out with a Bruker DRX500 using 5% dimethyl sulfoxide- d_6 solution of PVA. It is necessary to keep enough relaxation time and to suppress Overhauser effects when pentad tacticity is evaluated accurately by ^{13}C NMR analysis. An additional delay of 3 seconds was applied without decoupling (non-NOE method).

3. Results and Discussion

3.1 Biodegradation of PVAs

Figure 1 shows changes of TOC in PVA cultures with incubation time. There are considerable differences in TOC changes between three kinds of PVAs. TOC in *a*-PVA culture decreased to 70% of the initial amount after 12 days of incubation and thereafter remained approximately constant. Under the incubation condition of this work, the further assimilation is not considered to proceed. TOC in *i*-PVA culture decreased rapidly in early stage of the incubation, and the decrease of TOC was larger than that in *a*-PVA culture, as described in our previous paper [1]. Sixty-two per cent of initial amounts of *i*-PVA was assimilated in 12 days of incubation. On the contrary, the decrease of TOC in *s*-PVA culture is small,

Fig. 1 Changes in TOC of culture media with incubation time: $\square$, *s*-PVA; $\circ$, *a*-PVA; $\triangle$, *i*-PVA.

Fig. 2 ^1H NMR spectrum of recovered *a*-PVA: (a), control; (b), after 5 days of incubation; (c), after 10 days of incubation.

and only 13% of the initial amount of *s*-PVA is assimilated even after 20 days of incubation.

Figure 2 shows ^1H NMR spectrum of *a*-PVA recovered from the culture after a given incubation period and that of *a*-PVA from a control medium without bacterial inoculation. ^{13}C NMR spectrum of the recovered *a*-PVA after 5 days of incubation is shown in Figure 3. New peaks appeared at 2.2 ppm on the ^1H NMR spectrum and at 177 ppm and 210 ppm on the ^{13}C NMR spectrum after 5 days of incubation.

Biodegradation of PVA is proposed to proceed by the oxidation of the hydroxyl groups of PVA and by the hydrolysis of the resulting β -diketone due to the subsequent oxidation [8]. According to this process, the groups of $-\text{CH}_2\text{COCH}_3$ and $-\text{CH}_2\text{COOH}$ must be newly generated in terminal parts of the cloven chains. The new peaks found newly in the NMR spectra are considered to be resulted in these terminal structures [9]. Three peaks in the hydroxyl proton regions, which are assigned to *mm*, *mr*, *rr* sequences from lower magnetic field, became somewhat broad, respectively, after 5 days of incubation. They changed approximately one broad peak in 10 days due to the complication of the structural change in the vicinity of hydroxyl groups.

Fig. 3 ^{13}C NMR spectrum of *a*-PVA after 5 days of incubation.

Figure 4 shows the ^1H NMR spectrum recovered from *i*-PVA culture after 5 days of incubation.

Fig. 4 ^1H NMR spectrum of *i*-PVA recovered after 5 days of incubation: (a), control; (b), sample.

Fig. 5 ^1H NMR spectrum of *s*-PVA recovered after 20 days of incubation: (a), control; (b), sample.

The spectrum has become more complicated as a whole than that of *a*-PVA after 10 days of incubation. Three peaks in hydroxyl proton regions have already become approximately one-broad peak after 5 days of incubation. On the other hand, little changes are observed in spectrum of *s*-PVA compared with that of *a*-PVA or *i*-PVA, as shown in Figure 5. Even after 20 days of incubation, the peaks assigned to *mr*, *rr* can be still observed clearly, and even the peak of *mm* is recognized as a shoulder. *s*-PVA is less assimilated as compared to *a*-PVA, although it has the same molecular weight and the same content of 1, 2-glycol linkage as *a*-PVA.

3.2 Changes of Tacticity Contents of PVAs by Biodegradation

Figure 6 shows methine carbon atom regions of resolution-enhanced ^{13}C NMR spectra of *a*-PVA which is recovered from the culture. This was done under non-NOE condition. Each peak in these spectra is assigned as follows [10-12]. The *rr* triad feature is clearly split into the three

Fig. 6 Methine carbon atom regions of ^{13}C NMR spectrum of *a*-PVA recovered after 5 days of incubation: (a), control; (b), sample.

possible pentads, *rrrr*, *mrrr* and *mrrm* from lower magnetic fields. The *mm* triad feature can be composed of three portions. In order of decreasing frequency, the first three peaks are assigned to the three possible heptads from the *rmnr* pentad. The next two peaks are arising from partial overlap of the four heptads derived from the *mmmr* pentads. Finally, there is a peak from the *mmmm* pentad. As the *mr* triad appears as overlapping peaks, no detailed assignments for this triad are reported. Only the fractions of pentads are determined in *mm* sequences.

The relative intensities between three peaks assigned to *rrrr*, *mrrr* and *mrrm* in *rr* sequences hardly changed before and after biodegradation, though each of the peaks became broad. These sequences are suggested to remain without the reception of degradation. As for *mm* sequences, the peak of *rmnr* became broad, however its intensity did not change appreciably. The intensity of peak assigned to *mmmr* somewhat lowered, however its peak remained with fair intensity after degradation. Whereas, the peak assigned to *mmmm* sequence characteristically disappeared unlike the peaks of *rmnr* and *mmmr*.

The *mm* triad sequence are subject to biodegradation in PVA [1,2,5]. Furthermore, it was obviously confirmed in the present work that *mmmm* sequence is especially subject to degradation in meso sequences and the enzyme engaged in this degradation should have a sequence selectivity at pentad level. Therefore, *i*-PVA having a number of long meso sequences should be more subject to biodegradation compared with *a*-PVA. Thus, in particular, *s*-PVA is not susceptible to biodegradation. Sakai *et al.* investigated the substrate-selectivity of second alcohol oxidase concerned in the biodegradation of PVA using suitable model substances [13]. They reported that the oxidase required a sequence joining more than each three carbon atoms of

both sides of the α -carbon as a substrate, which is attached to a hydroxyl group. The strain, *Pseudomonas* sp., used in the present work was also confirmed to have a long sequence selectivity, and this should be connected with stereostructure of the active site of the enzyme.

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