J. Microbiol. Biotechnol. (2004), 14(5), 1009–1013

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Polyvinyl Alcohol Degradation by *Microbacterium barkeri* KCCM 10507 and *Paenibacillus amylolyticus* KCCM 10508 in Dyeing Wastewater

CHOI, KWANGKEUN, CHULHWAN PARK[1], SANGYONG KIM[1], WONSEOK LYOO[2], SANG HUN LEE[3], AND JINWON LEE*

Department of Chemical Engineering, Kwangwoon University, Seoul 139-701, Korea
[1]Korea Institute of Industrial Technology, Chonan, Chungnam 330-825, Korea
[2]School of Textile, Yeungnam University, Gyeongsan, Gyeongbuk 712-749, Korea
[3]Department of Electronic Engineering, Kwangwoon University, Seoul 139-701, Korea

Received: February 2, 2004
Accepted: April 20, 2004

Abstract The purpose of this study was to investigate the degradation of PVA (polyvinyl alcohol) contained in dyeing wastewater by a mixed culture of *Microbacterium barkeri* KCCM 10507 and *Paenibacillus amylolyticus* KCCM 10508. Firstly, synthetic wastewater which contained different initial concentrations of PVA varying from 50 to 3,500 mg/l were tested to obtain optimal PVA biodegradation activity of isolated strains, and the above two strains were found to degrade PVA up to 90%, when the initial concentration of PVA was 750 mg/l and below. Next, dyeing wastewater was tested by a mixed culture of the two isolated strains, and 42% and 55% of the initial concentrations of PVA and COD, respectively, was removed after five days. MLSS was gradually increased from an initial 1,400 to 2,500 mg/l, and the pH was also increased from 5.1 to 7.8. Sterilized dyeing wastewater was tested to find the effect of strains only on the biodegradation of PVA, and PVA degradation ratio and COD removal ratio were 50% and 72.8%, respectively. Thus, the results indicated that these two strains have good ability to degrade PVA and remove COD in dyeing wastewater. Finally, it is expected that if these two strains were used in the dyeing wastewater treatment, good efficiency for PVA degradation and COD removal could be achieved.

Key words: *Microbacterium barkeri*, *Paenibacillus amylolyticus*, polyvinyl alcohol, biodegradation

In the previous study [3], two strains (*Microbacterium barkeri* KCCM 10507 and *Paenibacillus amylolyticus* KCCM 10508), which have characteristics including little effect on polymerization degree, and efficient and fast degradation activity of PVA (polyvinyl alcohol), were isolated from dyeing wastewater, and conditions for microbial growth and PVA degradation were optimized. The PVA degrading activities of the two strains were confirmed by using PVA containing synthetic wastewater. Furthermore, the two strains showed that over 90% of PVA was degraded. Therefore, it was considered that if they were used to treat dyeing wastewater, they would be highly efficient for PVA degradation [6].

Furthermore, many researches on biological treatment of PVA have been performed since the report by Nord in 1936 [12]. In 1976, Suzuki [18] isolated *Pseudomonas* O-3 from soil, using PVA as the sole carbon source, and showed that 0.5% PVA was fully degraded after a week, and TOC was changed from 2,700 mg/l to 250–300 mg/l. Suzuki also observed the presence of PVA-degrading enzyme secreted from the strains and obtained it by using ion-exchange chromatography and gel filtration processes [19]. According to this report, PVA was first oxidized by secondary alcohol oxidase and then completely degraded by diketone hydrolase. In 1981, Sakazawa and Shimao [17] reported that PVA was degraded by symbiosis between two different strains. Characteristics of the symbiotic strains were determined by Shimao *et al.*, and the oxidase was found to be secreted from these symbiotic strains [15, 16]. Subsequently, different PVA-degrading strains were reported by Jo *et al.* [7] in 1992 and they found that PVA was able to be degraded by only symbiotic correlation between PVA-degrading strains. In 1994, Ryu *et al.* [14] reported that the strains which have the PVA-degrading properties under high temperature were isolated. Up to the present, many PVA-degrading microorganisms have been isolated, and these microorganisms are capable of degrading PVA only under symbiotic condition between two different strains [1, 11]. Many strains were isolated to treat wastewater.

*Corresponding author
Phone: 82-2-940-5172; Fax: 82-2-909-0701;
E-mail: jwlee@daisy.kw.ac.kr

However, they reported that strains isolated for degrading PVA, and wastewater treatment require symbiotic relationship and longer degrading time [2, 8, 9, 10, 13]. Furthermore, few studies reported that isolated strains were successfully applied to the wastewater treatment.

Therefore, the purpose of this study was to examine the biodegradability of PVA by PVA-degrading strains, which were isolated in the previous study [3], when applied to the dyeing wastewater. Through PVA-degrading experiments with synthetic wastewater and industrial dyeing wastewater, we tried to find optimal biodegradation conditions of PVA, when PVA-containing dyeing wastewater was treated.

MATERIALS AND METHODS

Microorganisms and Media

Strains used in this study were *M. barkeri* KCCM 10507 and *P. amylolyticus* KCCM 10508, as reported in the previous paper [3]. Media for the PVA-degrading test were prepared by following the composition used by Suzuki [18]. PVA was used as the sole carbon and energy sources, and its polymerization degree was 500. Other components were $(NH_4)_2SO_4$ 1 g, KH_2PO_4 0.4 g, K_2HPO_4 3.2 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, NaCl 0.1 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, and yeast extract 1 g per 1 l of distilled water. The pH was adjusted to 7 with 0.1 N NaOH and 0.1 N HCl. The solid media were prepared by adding 1.5% (w/v) agar.

Quantitative Analysis of PVA

PVA in wastewater was quantitatively analyzed by Finley's method [5]. Before PVA was analyzed, pre-treatment to remove some effectors like starch was required, since, when PVA was spectrophotometrically analyzed, starch gives an error due to similar absorbance with PVA. Pre-treatment for removal of starch was performed by acid hydrolysis with 0.2 to 0.5 N HCl solutions for 90 min at 100°C [5].

Dyeing Wastewater Treatment

Dyeing wastewater was sampled from the S industrial complex located in Daegu, Korea. In the process of the S industrial complex, PVA and starch as a sizing agent were used together. The characteristics of dyeing wastewater were as follows: concentrations of PVA 950 mg/l, pH 5.1, MLSS 1,400 mg/l, and COD 2,250 mg/l. In this study, dyeing wastewater was tested for degrading PVA by using a mixed culture of *M. barkeri* and *P. amylolyticus*. Experimental conditions for the PVA degradation test were at pH 7, 30°C, and 150 rpm during five days under aerobic condition, and a mixed culture as inoculate was used as 10% (v/v) of dyeing wastewater. First, starch has to be removed before the analysis of PVA, because starch gives an error due to similar absorbance with PVA. COD was measured by HACH spectrophotometer DR/2010 using the COD Reactor Digestion Method, and MLSS (mixed liquor suspended solid) was measured using the 2540 D standard methods used for the examination of water and wastewater [4].

RESULTS AND DISCUSSION

As shown in Fig. 1, PVA-containing synthetic wastewater was tested to compare the PVA degradation rate by a mixed culture of two isolated strains or single species.

The mixed cultures had a good ability for degrading PVA, and the degradation rate was also high even if a single strain was used. From Fig. 1, the PVA-degrading percentages by *M. barkeri*, *P. amylolyticus*, and their mixed culture were obtained as 98, 96, and 99%, respectively. Therefore, it was concluded that these two strains have an excellent ability to degrade PVA, whether a single strain or a mixed culture was used.

The effect of the polymerization degree of PVA on its degradation was tested by using a mixed culture of the two strains. It has generally been reported that the PVA degradation rate decreases with increase in polymerization degree. Therefore, three types of PVA, 500, 1,700, and 2,000 of the polymerization degree, were used in this experiment, and the results are shown in Fig. 2.

PVA degradation in the cases of 500 and 1,700 polymerization degrees rose quickly and reached over 90% degradation after four days. On the other hand, the degradation in the case of 2,000 polymerization degree rose slower than the above case and reached about 80% degradation in five days. Although less PVA degradation (80%) was observed in the case of 2,000 polymerization

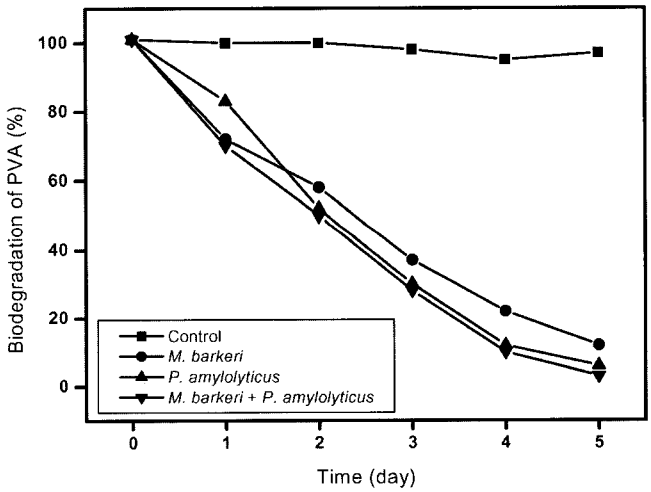

Fig. 1. Biodegradation of PVA contained in the synthetic wastewater by a single culture and a mixed culture of *Microbacterium barkeri* KCCM 10507 and *Paenibacillus amylolyticus* KCCM 10508.
(■: Control; ●: *M. barkeri*; ▲: *P. amylolyticus*; ▼: a mixed culture of *M. barkeri* and *P. amylolyticus*).

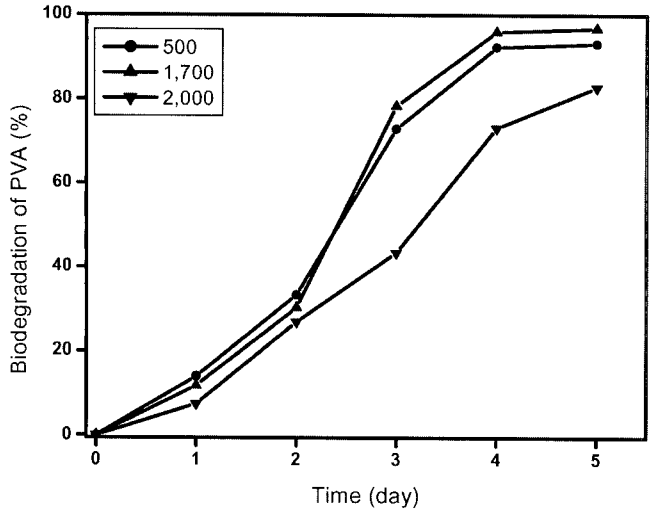

Fig. 2. Effect of polymerization degree on PVA degradation by using a mixed culture of *Microbacterium barkeri* KCCM 10507 and *Paenibacillus amylolyticus* KCCM 10508.
●: PZD 500; ▲: PZD 1,700; ▼: PZD 2,000). PZD: Polymerization degree.

degree, the polymerization degree does not seem to have a significantly large effect on the degradation ability of *M. barkeri* and *P. amylolyticus* in the wastewater treatment processes. This result is similar to the study by Suzuki [18] that there was no effect of polymerization degree on the PVA degradation when using *Pseudomonas* sp. Therefore, it is concluded that the PVA degradation rate and microbial growth were significantly influenced by polymerization degree.

Various initial concentrations of PVA were tested to find how much PVA could be degraded by *M. barkeri* and *P. amylolyticus*. The polymerization degree of PVA used was

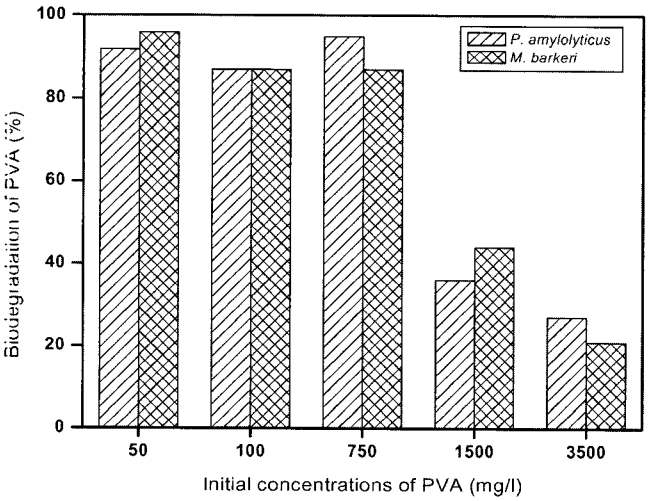

Fig. 3. Effect of different initial concentrations of PVA on the biodegradation of PVA (●, *Microbacterium barkeri* KCCM 10507; ■: *Paenibacillus amylolyticus* KCCM 10508).

500 because it was used most extensively in the dyeing industries, and the results of the initial PVA concentrations ranging between 50 and 3,500 mg/l are shown in Fig. 3.

As shown in the figure, if the initial concentration of PVA in the dyeing wastewater was lower than 750 mg/l, about 90% of PVA could be degraded. With more than 1,500 mg/l PVA concentration, *M. barkeri* and *P. amylolyticus* showed much lower degradation, however, such high concentration is seldom found in the dyeing industries.

Dyeing wastewater was taken from the dyeing industry and was tested for degradation of PVA contained in the wastewater. The characteristics of the dyeing wastewater were as follows: PVA concentrations, 950 mg/l; COD, 2,250 mg/l; MLSS, 1,400 mg/l; and pH, 5.1. A mixed culture of *M. barkeri* and *P. amylolyticus* was inoculated into the dyeing wastewater, and the results of PVA degradation are shown in Fig. 4.

Figure 4 shows the results of PVA degradation in the two cases: (a) sterilized wastewater and (b) nonsterilized wastewater. This classification was made in order to observe whether there are some interactions between inoculated species and original species present in the dyeing wastewater, or not. In the case of the sterilized wastewater, as shown in Fig. 4(a), 50% of PVA was degraded and 72.8% of COD was removed within five days. During this process, MLSS increased from 1,400 mg/l to 2,500 mg/l, and the pH was changed from 5.1 to 7.8. From the result of Fig. 4(a), it was concluded that *M. barkeri* and *P. amylolyticus* seemed to have excellent ability for PVA degradation and COD removal.

On the contrary, the results in Fig. 4(b) show less degradation rates, compared to the results in Fig. 4(a). Namely, just 30% of PVA was degraded, and 55% of COD was removed after five days. Changes of pH, however, were similar to the case of using sterilized wastewater. This result demonstrates that the process stability was increased by inoculating a mixed culture of the two strains, and these two strains grew well in the dyeing wastewater. Therefore, it was concluded that the two strains have a symbiotic correlation with other strains in dyeing wastewater. According to this result, *M. barkeri* and *P. amylolyticus* seemed to degrade PVA well without any other species, but there seemed to be some inhibitory effects by other species when applied to the industrial wastewater directly. Therefore, process development for the avoidance of these inhibitory effects needs to be pursued in future.

The results on the PVA-degrading rate were also different from the test results when using synthetic wastewater: That is, when the initial concentration of PVA was 750 mg/l in synthetic wastewater, PVA degradation rate was about 90% (Fig. 3). Thirty percent of PVA, however, was degraded by using industrial wastewater. It is quite likely that many materials including strains that inhibit degradation of PVA seemed to exist in the wastewater. Therefore, suitable pre-

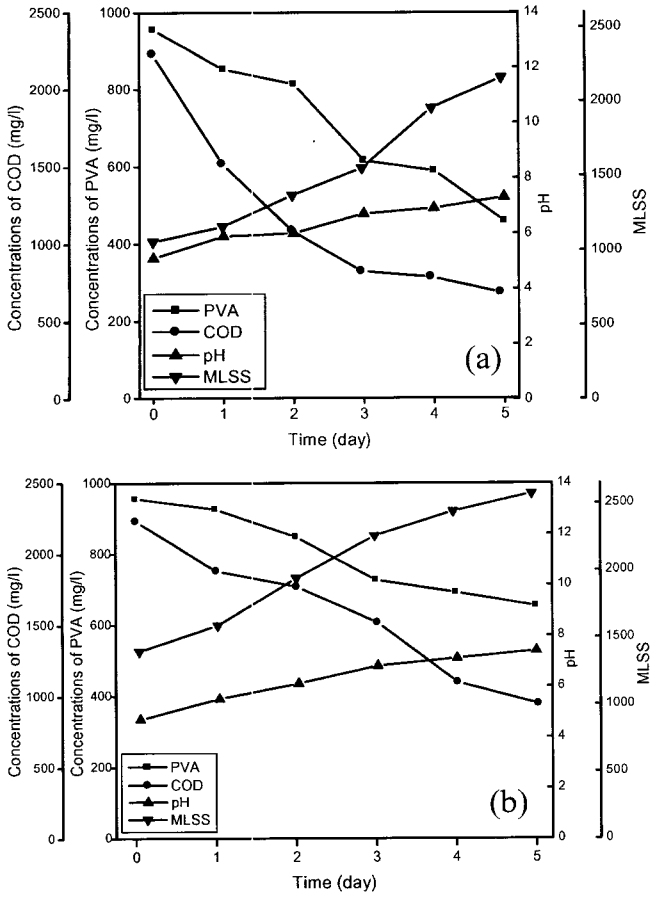

Fig. 4. Treatment efficiency of PVA and COD contained in (a) sterilized wastewater and (b) dyeing wastewater by the mixed culture of *Microbacterium barkeri* KCCM 10507 and *Paenibacillus amylolyticus* KCCM 10508.
(■ : concentrations of PVA; ● : concentrations of COD; ▲ : pH change; ▼ : MLSS change).

treatment for removing inhibitors and adaptation of *M. barkeri* and *P. amylolyticus* seemed to be needed for increasing the PVA degradation rate.

In summary, when these two strains were used, the degradation rate was 90% with initial PVA concentration ranging 750 mg/l and the upper concentration, and the degradation rate was gradually decreased to 20%. When these strains were inoculated to dyeing wastewater, MLSS was changed from 1,400 mg/l to 2,500 mg/l, and COD and PVA were removed by 55% and 30%, respectively. Thus, these two strains seem to be useful for removing COD in dyeing wastewater.

CONCLUSION

Two strains (*M. barkeri* KCCM 10507 and *P. amylolyticus* KCCM 10508), which have a good ability for degrading PVA, were isolated from dyeing wastewater in the previous study. The result showed that these strains degraded PVA, which was contained in synthetic wastewater, up to 96% and 98% within three days, respectively. These degradation ratios were obtained within three days in the present study. It should be noted, however, that no other study has yet reported such a high ratio in the same treatment period.

A mixed culture of the above two strains was inoculated to treat PVA contained in dyeing wastewater, and the results showed that 42% of PVA and 55% of COD were removed. Therefore, these two species appear to have the ability to remove COD, as well as having PVA-degrading activity. Furthermore, the pH of dyeing wastewater was changed from an initial pH of 5.1 to 7.8, indicating that a mixed culture of the two strains grew well under low pH conditions, and that these two strains have symbiosis with other strains existed in the dyeing wastewater: Single strains did not grow well under low pH conditions, for example pH 5.

To search the effect of two strains isolated in this study on PVA degradation and COD removal, dyeing wastewater was sterilized and used. The results showed that the PVA degradation ratio and COD removal ratio were 50% and 72.8%, respectively, thus demonstrating that these two strains have good PVA-degrading activity as well as efficient COD removal activity of dyeing wastewater.

Acknowledgments

This work was supported by the Ministry of Environment (Eco-Technopia 21 program) and the National Research Laboratory Program of Korea Ministry of Science and Technology, Republic of Korea. The authors appreciate their financial supports.

REFERENCES

1. Chiellim, E., A. Corti, S. D'Antone, and R. Solaro. 2003. Biodegradation of poly(vinyl alcohol) based materials. *Prog. Polym. Sci.* **28:** 963-1014.
2. Choi, H. P., H. J. Kang, H. C. Seo, and H. C. Sung. 2002. Isolation and identification of photosynthetic bacterium useful for wastewater treatment. *J. Microbiol. Biotechnol.* **12:** 643-648.
3. Choi, K. K., J. C. Shin, H. H. Jeon, S. Y. Kim, and J. W. Lee. 2003. Isolation and characterization of *Microbacterium barkeri* LCa and *Paenibacillus amylolyticus* LCb for PVA (polyvinyl alcohol) degradation. *Korean J. Biotechnol. Bioeng.* **18:** 479-484.
4. Clescerl, L. S., A. E. Greenberg, and A. D. Eaton. 1998. *Standard Methods for the Examination of Water and Wastewater.* 20th Ed., pp. 2-57. APHA, Washington U.S.A.

5. Finley, J. H. 1961. Spectrophotometric determination of polyvinyl alcohol in paper coatings. *Anal. Chem.* **33:** 1925-1927.
6. Hashimoto, S. and M. Fujita. 1985. Isolation of a bacterium requiring three amino acids for polyvinyl alcohol degradation. *J. Ferment. Technol.* **63:** 471-474.
7. Jo, Y.-L., S. Y. Jeong, and M. W. Cho. 1992. Isolation and characteristics of polyvinyl alcohol degrading bacteria. *Kor. J. Appl. Microbiol. Biotechnol.* **20:** 96-101.
8. Ka, J. O. and I. H. Park. 2003. Isolation and characterization of 4-(2,4-dichlorophenoxy)butyric acid-degrading bacteria from agricultural soils. *J. Microbiol. Biotechnol.* **13:** 243-250.
9. Lee, K. Y. and C. G. Lee. 2002. Nitrogen removal from wastewaters by microalgae without consuming organic carbon sources. *J. Microbiol. Biotechnol.* **12:** 979-985.
10. Lee, W. S., C. S. Park, J. E. Kim, B. D. Yoon, and H. M. Oh. 2002. Characterization of TCE-degrading bacteria and their application to wastewater treatment. *J. Microbiol. Biotechnol.* **12:** 569-575.
11. Mori, T., M. Sakimoto, T. Kagi, and T. Sakai. 1996. Isolation and characterization of a strain of *Bacillus megaterium* that degrades poly(vinyl alcohol). *Biosci. Biotech. Biochem.* **60:** 330-332.
12. Nord, F. F. 1936. Dehydrogenation activity of *Fusarium lini* B. *Naturwissenschaften* **24:** 763.
13. Park, C. H., T. H. Kim, S. Y. Kim, S. W. Kim, and J. W. Lee. 2002. Enhanced biodegradation of 2,4,6-trinitrotoluene (TNT) with various supplemental energy sources. *J. Microbiol. Biotechnol.* **12:** 695-698.
14. Ryu, J. K., Y. J. Choi, and C. W. Lee. 1994. Studies on the optimal conditions for symbiotic utilization of polyvinyl alcohol by the KMG1 and KMG5 mixed culture and isolation of a thermophilic mutant of the PVA degrader KMG5. *J. Kor. Soc. Water Quality* **10:** 112-119.
15. Shimao, M., K. Ninomiya, N. Kato, and C. Sakazawa. 1986. Existence of a novel enzyme, pyrroloquinoline quinone-dependent polyvinyl alcohol dehydrogenase, in a bacterial symbiont, *Pseudomonas* sp. strain VM15C. *Appl. Environ. Microbiol.* **51:** 268-275.
16. Shimao, M., Y. Taniguchi, S. Shikata, N. Kato, and C. Sakazawa. 1982. Production of polyvinyl alcohol oxidase by a symbiotic mixed culture. *Appl. Environ. Microbiol.* **44:** 28-32.
17. Sakazawa, C. and M. Shimao. 1981. Symbiotic utilization of polyvinyl alcohol by mixed cultures. *Appl. Environ. Microbiol.* **41:** 261-267.
18. Suzuki, T. 1976. Purification and some properties of polyvinyl alcohol degrading enzyme produced by *Pseudomonas* O-3. *Agric. Biol. Chem.* **40:** 497-504.
19. Suzuki, T. 1977. Oxidation of secondary alcohols by polyvinyl alcohol-degrading enzyme produced by *Pseudomonas* O-3. *Agric. Biol. Chem.* **42:** 1187-1194.