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Degradation of Polyvinyl Alcohol by a Mixed Microbial Culture Isolated from Paper Mill Treatment

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ABSTRACT

Mixed microbial culture from paper mill treatment system was enriched on polyvinyl alcohol (PVA) medium and its PVA biodegradation capacity was investigated as a function of change in the initial pH values and PVA concentrations. The optimum pH value at 0.75 gl^{-1} initial PVA concentration was determined to be 8. PVA biodegradation by mixed microbial culture was investigated at the range of 0.68 – 15.5 $g1^{-1}$ initial PVA concentrations. The mixed microbial culture was found to degrade within 5 days 98.6–88.5% of the initial PVA levels which ranged from 0.68 to 1.14 gl^{-1}. The mixed microbial culture was found to remove more than 90% of the initial PVA concentrations, within the range of 3.13 to 6.64 $g1^{-1}$ in 16–25 days of incubation at 35 °C.

Key Words: *polyvinyl alcohol, PVA, mixed culture, biodegradation*

1. INTRODUCTION

Widespread production and use of synthetic polymers worldwide brings the introduction of increasing amounts of these polymers into the ecosystem as industrial waste products. For this reason, there is increasing attention for the use of environmentally benign, biodegradable plastic items. Polyvinyl alcohol (PVA) is a water-soluble synthetic polymer, with excellent physical properties. PVA, which is produced and consumed in great quantities by China, Japan and the United States, has various commercial applications in pure or blended forms [1, 2]. For example it is used in the textile industry as synthetic sizing agent to protect fibers from being damaged during weaving. It is an indispensable material in adhesive and paper production industry as well as in the manufacture of air-tight packaging films and water soluble coatings [3-6]. Large amounts of PVA are discarded with waste waters from these industries and contaminate the environment.

Although known as heavily degradable, PVA can be completely biodegraded in suitable process conditions and in the presence of acclimatized microorganisms [7, 8]. PVA utilization in pure cultures is not very common [4]. So far, a number of microorganisms have been reported to degrade PVA partially or completely [2] such as *Pseudomonas* sp. [9, 10], *Alcaligenes faecalis* [11], *Bacillus megaterium* [12], *Penicillium* sp. [13], *Sphingopyxis* sp. [14], *Phanerochaete chrysosporium* [3, 15]. Symbiotic and mixed cultures capable of degrading PVA have also been isolated from PVA contaminated sites [16-19]. In the present paper, we report the biodegradation of PVA in the presence of an acclimated

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mixed PVA-degrading microbial culture, enriched from activated sludge of wastewater treatment system of a paper mill. The major objective of this study was to investigate the biodegradation properties of the mixed microbial culture in the media including PVA as the sole source of the carbon.

2. EXPERIMENTAL

2.1. Screening of PVA-Degrading Microorganisms and Medium

PVA-degrading mixed microbial culture obtained from the activated sludge collected from the aerobic wastewater treatment system of the Meteksan Pulp and Paper Mill (Ankara, Turkey) was used in this study. Initial examination of samples about the presence of PVA degrading cultures was made on solid PVA medium after 48 h of incubation period. PVA-degrading mixed microbial culture obtained from samples enriched by the following procedure consisting of periodic subculturing of samples taken from wastewater in liquid PVA medium containing PVA as a sole carbon source. The composition of the PVA medium is as follows: 0.05 g yeast extract, 2.2 g K_2HPO_4, 0.8 g KH_2PO_4, 0.1 g NaCl, 2.5 g NH_4NO_3, 0.02 g $CaCl_2$. $2H_2O$, 0.7 g $MgSO_4.7H_2O$, 0.01 g $FeSO_4.7H_20$, 0.001 g $MnSO_4$, 0.75 g PVA in 1 l distilled water [20]. PVA 22000 (Merck) was used through the study. The initial pH of the medium was adjusted to 5-9 with 0.1M NaOH and 0.1M HCl. The cultures developing on different pH values were kept at 4 °C. Morphology of mixed microbial culture used in the study was examined by scanning electron microscope (SEM). The preparation of microorganisms for SEM was according to Cetin [21] with some modifications. Sample of mixed microbial culture was fixed for 3 h in 2.5% glutaraldehyde. Then, it was rinsed with distilled water. This was followed by dehydration in a graded series of ethanol. It was then passed through amyl acetate. The sample was next dried at the critical point with CO_2 (Polaron, CPD 7501) and coated with gold (Polaron SC 502 sputter coater) for SEM examination. Scanning electron micrographs of the mixed microbial culture was taken digitally at 20 kV with Jeol JSM 6060. In order to determine the bacterial composition of PVA degrading mixed culture, samples were streaked on agar plates and 5 bacterial isolates were obtained. Isolates were identified with sequencing of 16S rRNA genes at Gazi University, Molecular Biology Research and Application Center (MOBAM). The 16S rRNA sequence of isolates (approximately 1400 bps) were compared with all deposited nucleotide sequences in the GenBank database using the BLAST N program provided online.

2.2 Culture Conditions

The mixed cultures were transferred into 100 ml of the PVA medium. The cultivation was carried out at 35 °C for 30 days on a rotary shaker (Finepcr SH 30, Korean) at 100 rpm, mounted in incubator (Sanyo MIR-162, Japan).

2.3 PVA Degradation Experiments

To examine the effect of initial pH on the degradation, PVA medium was prepared at pH 5, 6, 7, 8 and 9. To acclimatize the mixed cultures to various pH values, repeated transfers of cultures were done into fresh PVA medium prepared at pH 5-9. For the experiments, 100 ml of PVA media were inoculated with 1 ml of these acclimatized cultures. At 1, 3, 5, 7, 9, 13, 16, 20, 25 and 30^{th} days samples were taken and analyzed. In order to examine the effect of initial PVA concentration on the degradation PVA medium was prepared with 0.75, 1, 1.5, 3, 7 and 15 g l^{-1} of PVA at optimum pH value. To acclimatize the mixed cultures to increasing concentrations of PVA, the cultures were gradually exposed to increasing concentrations of PVA. This was achieved through successive transfers of cultures into fresh PVA medium containing 0.75, 1, 1.5, 3, 7 and 15 g l^{-1} of the PVA. For the experiments, 100 ml of PVA media were inoculated with 1 ml of these acclimatized cultures. At 1, 2, 3, 5, 7, 9, 13, 16, 20, 25 and 30^{th} days samples were taken and analyzed. In addition, control media containing PVA without inoculation of culture were prepared to observe any reaction of the medium with PVA.

2.4 Analysis

The PVA concentration in liquid culture was measured spectrophotometrically, following to the procedure described by Finley [22]. For determination of PVA concentration, boric acid and iodine solution (I_2–KI) were added on appropriately diluted culture samples. The concentration of PVA was determined by reading absorbance of mixture at 660 nm [23]. Absorbance measurements were done by using a spectrophotometer (Shimadzu UV 1700, Kyoto, Japan). The mixture prepared with PVA free medium was used as the blank. The standard curve for PVA was linear over 0–20 μg/ml. All measurements were determined in triplicate and average of the three values was given. At the end of incubation period, culture bottles were opened and centrifuged to precipitate suspended biomass at 5000 rpm for 10 min. Cell growth was determined by measuring dry weight of washed biomass. To examine the PVA degradation by culture on solid PVA medium mixure of boric acid and iodine solution was poured on plates [13].

3. RESULTS AND DISCUSSION

Activated sludge of wastewater treatment system of a paper mill was used as a source for isolating PVA-degrading microorganisms in this study. PVA degrading cultures formed a halo after the reaction of PVA with boric acid-iodine mixture as shown in Figure 1. Scanning electron micrograph of PVA degrading mixed microbial culture enriched by culturing in liquid PVA medium is shown in Figure 2.

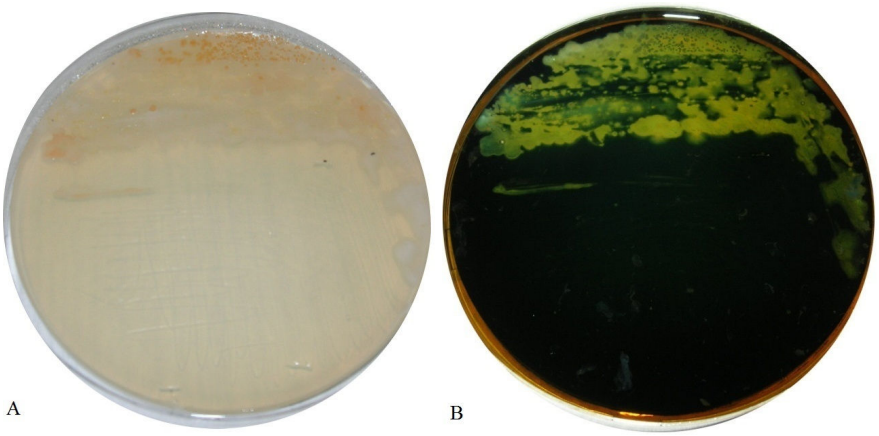

Fig 1. Photographs of PVA plates inoculated with a sample from wastewater treatment system, before (A) and after (B) the addition of boric acid-iodine mixture.

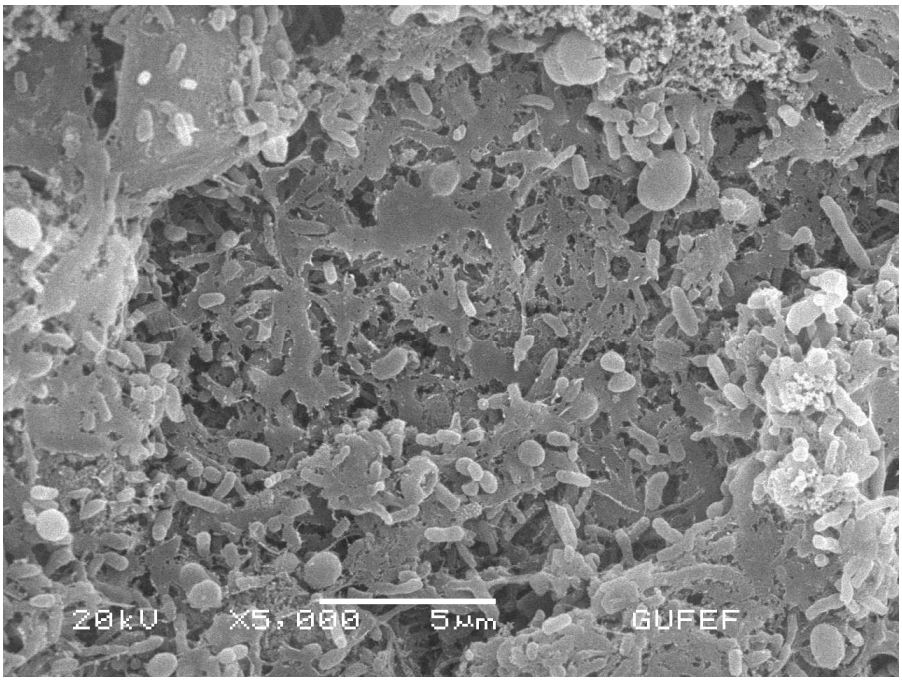

Fig 2. A scanning electron micrograph of mixed microbial culture.

PVA degradation by the mixed microbial culture was investigated as a function of changes in the initial pH values and PVA concentrations. The results are given as degraded PVA concentrations at the end of the growth period (C_{deg} : g l^{-1}). The degradation yield (degradation %) is defined as the ratio of degraded concentration of PVA at the end of the microbial growth period to the initial PVA concentrations (C_0 : g l^{-1}).

The effect of different pH values of the medium on PVA degradation at the end of the incubation period was determined in the mixed microbial culture samples obtained from the enrichment procedure. Experiments were performed at 5 – 9 initial pH values and about 0.75 g l^{-1} initial PVA concentrations. As shown in Table 1, the removal yield of the mixed cultures reached 100 % at pH 7 and 8. In the experiments with pH 7 and 8, the shortest incubation period for complete degradation was obtained as 13 and 7 days, respectively. At the end of these experiments, the optimum pH value for PVA degradation was determined as pH 8, because the shortest incubation period for complete degradation was obtained at this pH.

Table 1. The effect of initial pH on the maximum degraded PVA concentrations (C_{deg}) and degradation yields of mixed cultures at about 0.75 g l^{-1} initial PVA concentration (C_0) (T: 35 °C)

pH	C_0 (g l^{-1})	C_{deg} (g l^{-1})	Degradation (%)	Exposure time (days)
5	0.77	0	0	30
6	0.64	0	0	30
7	0.68	0.68	100	13
8	0.66	0.66	100	7
9	0.65	0.64	97.5	30

PVA degradation by mixed microbial culture was investigated at different initial PVA concentrations varying between 0.68 and 15.5 g l^{-1} (Fig. 3A). At 0.68 – 1.14 g l^{-1} PVA concentrations 98.6 – 88.5 % of the initial PVA is removed at the end of 5 days incubation period. At 1.62 g l^{-1} initial PVA concentration, 92 % of polymer is degraded after 13 days incubation period. The mixed cultures were found to remove within 16 – 25 days more than 90 % of the initial PVA concentrations, which ranged from 3.13 to 6.64 g l^{-1}. At higher PVA concentration (15.5 g l^{-1}), in spite of longer incubation periods no degradation is observed. At 0.68 – 6.64 g l^{-1} PVA concentrations pH of the medium is decreased for the first few days, and then pH values varied between 7.25 – 7.92 at the end of incubation periods (Fig. 3B). This shows that there were some acidic substances produced during the beggining of PVA degradation process. The pH value of the medium increased when the PVA was completely degraded, which shows that these acidic metabolites should be used by mixed culture after PVA degradation.

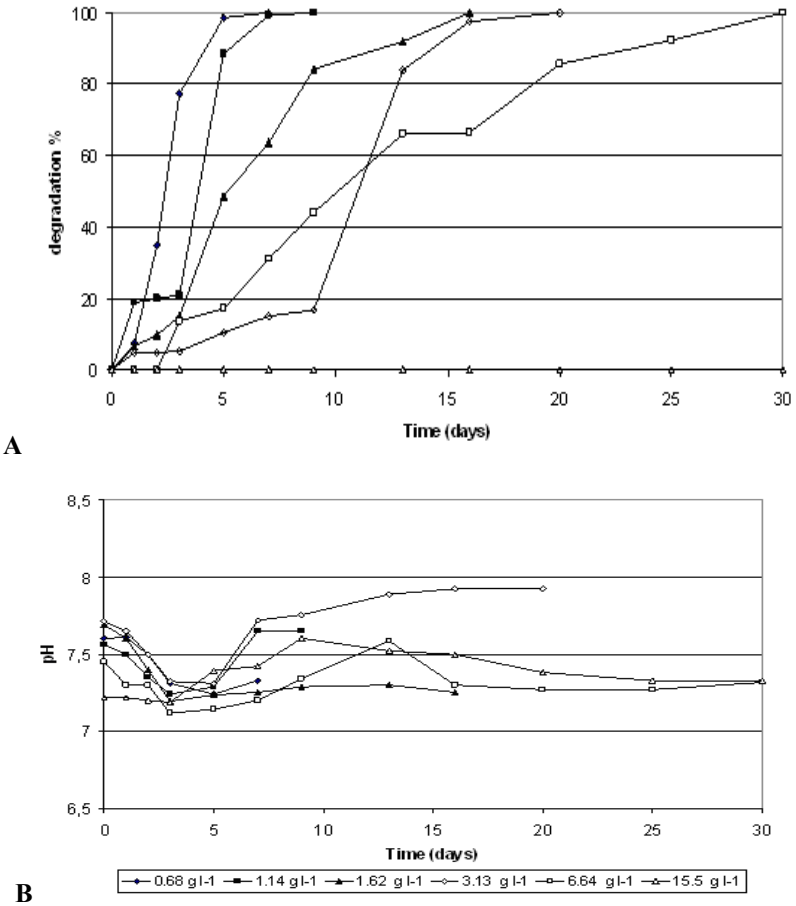

Fig. 3. PVA degradation (A) and pH change through the incubation period (B) of the mixed microbial culture.

At the end of 7 – 30 days incubation period, the variation of degradation yields and the degraded PVA quantities per grams of dry weight of mixed cultures, obtained at different initial PVA concentrations are given in Table 2. As it can be seen from the table, at the increasing initial PVA concentrations, longer incubation times necessary to achieve complete degradation. The highest amount of degraded PVA per grams of dry weight of mixed microbial culture (q) was 4.11 g g^{-1} which is obtained at 3.13 g l^{-1} initial PVA concentration. This value decreased at higher initial PVA concentrations.

Table 2. The comparison of the maximum degraded PVA concentration (C_{deg}) and degradation yields of mixed cultures and amount of degraded PVA per grams of dry weight of mixed microbial culture (q) at the end of incubation times at different initial PVA concentrations (C_0) (T: 35 °C; pH 8)

C_0 (g l^{-1})	C_{deg} (g l^{-1})	Degradation (%)	q (g g^{-1})	Exposure time (days)
0.68	0.68	100	0.75	7
1.14	1.14	100	1.03	9
1.62	1.62	100	2.65	16
3.13	3.13	100	4.11	20
6.64	6.64	100	1.36	30
15.5	0	0	0	30

The present paper reports the potential of a simple consortium of microorganisms for treating wastewater containing PVA. Phylogenetic analysis of the partial sequences of the 16S rRNA genes of isolates from mixed microbial culture revealed that isolate 1 was a relative of *Chelatococcus* sp., isolates 2 and 3 were relatives of *Stenotrophomonas* sp., isolates 4 and 5 were relatives of *Microbacterium* species (similarity 99%). Although mixed microbial culture obtained in this study could degrade PVA, bacterial pure cultures isolated from mixed culture do not have the ability to degrade PVA effectively. This shows that these bacteria can degrade PVA symbiotically. PVA degradation by pure, symbiotic or mixed cultures was also reported in the previous studies. In these previous experiments, it was shown that the PVA removal capacities of the microbial biomasses varied with the pH values of the culture media, where the preferred pH values were found to be around 7 – 8 in most of the studies [23-25]. In the present study, the optimum value for PVA degradation at about 0.75 g l^{-1} initial PVA concentration was found to be pH 8. Thus our data indicate that the enriched mixed microbial culture is suitable for use in biological treatment of alkaline wastewater effluents.

In this study, it is apparent from Table 2 that complete PVA removal was attained within a period of 7 – 30 days, depending on the PVA concentrations. Degrading rates of other identified PVA-degrading strains and mixed cultures vary considerably. For example it is reported that about 90 % of PVA, at an initial concentration of 0.1%, was degraded within 6-days cultivation with *Sphingopyxis* sp. [14]. Chen et al. [18] demonstrated that complete degradation of four types of PVA at 1 g l^{-1} concentration was achieved with mixed microbial culture at 3 to 5 days according to polymers' saponification and polymerization properties. Mori et al. [12] showed that all of PVA with initial concentration of 0.1 % was degraded after 7 days incubation with mixed microbial culture obtained from activated sludge of textile factory. Mixed cultures of *Sphingomonas* sp. SA3 with symbiotic strain, SA2, degraded about 95% of the PVA with an initial concentration of 5 g l^{-1} after 4 days incubation [23]. In a recent study, Guo et al. obtained PVA degrading mixed strains from a textile factory [26]. They showed that PVA with the initial average molecular weight of 51,260 Da, and an initial concentration of 1 g/l could be degraded by mixed strains in 5 days [26]. They also observed a decrease at the pH value of the fermentation broth at first, and then an increase during the degradation process of PVA similar to our findings. They showed that PVA is first degraded into aldehydes and ketones and then into acid substances, which are all broken down eventually [26]. It is possible to say the degrading rates of our mixed microbial culture under the conditions described here were similar to those of most identified PVA-degrading cultures. However, properties of PVA samples, culture media, inoculums' size and culture conditions as well as microbial composition vary considerably in different studies. Therefore, direct comparison of the PVA-degrading effectiveness of cultures may not be accurately informative about their capacities.

The use of living biomass in wastewater treatment systems specially developed for effluents containing PVA is of paramount importance. It should be remembered that these waste waters did not solely composed of PVA. They also contain dyes, heavy metals and other pollutants. For this reason the selection of microbial consortium which is able to withstand high levels of PVA

and toxic compounds, is essential for the successful treatment systems. In conclusion our mixed microbial culture could perform degradation of PVA present at high concentrations and has the advantage of being used for the pollutants of paper mill wastes.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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