Enhanced Degradation of Polyvinyl Alcohol by *Pycnoporus cinnabarinus* after Pretreatment with Fenton's Reagent

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Degradation of polyvinyl alcohol (PVA) was investigated by using a combination of chemical treatment with Fenton's reagent and biological degradation with the white rot fungus *Pycnoporus cinnabarinus*. Inclusion of the chemical pretreatment resulted in greater degradation of PVA than the degradation observed when biological degradation alone was used.

Polyvinyl alcohol (PVA) has commercial applications in the adhesive, paper coating, and textile industries (10, 18). Due to its biodegradability (14, 15), this synthetic polymer is now being used in the production of biodegradable polymers (5). PVA biodegradation is believed to be due to a random chain cleavage process in which a two-enzyme catalyzed oxidation process breaks the carbon backbone of the polymer (25). The enzymes responsible for cleavage of the polymer have been shown to be oxidases (5, 21, 22, 25) and/or hydrolases (9). A range of organisms that utilize these enzyme systems have been shown to degrade PVA in a variety of environments (9); however, the degradation process is slow and limited in most cases (6, 7, 9).

The rate and extent of PVA biodegradation can be increased by including a chemical oxidative agent, such as Fenton's reagent (FR), which has been used in the treatment of aromatic hydrocarbons (2, 20). For example, Martens and Frankenberger (13) used treatment with FR as a preoxidation step to degrade microbially resistant polycyclic aromatic hydrocarbons. This process was shown to result in partial hydrolysis of polycyclic aromatic hydrocarbons and thus in higher levels of biological degradation. Huang et al. (8) reported that partial chemical oxidation of synthetic polymers resulted in the formation of low-molecular-mass carboxylic acids. These acids proved to be more readily utilized by the microbes than the untreated polymers were. The use of FR as a preoxidative treatment for PVA may therefore result in greater accessibility to biological degradation. The use of FR for PVA degradation does not appear to have been described previously; however, PVA-containing wastes have been studied. Lin and Peng (11), for example, studied treatment of textile wastewater containing PVA with FR, although the purpose of the study was to decolorize the water, not degrade PVA.

In the current study we investigated preoxidation of PVA by treatment with FR as a precursor to enhance biological degradation by the white rot fungus *Pycnoporus cinnabarinus*. This oxidase-secreting fungus has been shown to be effective in decolorizing industrial dyes (12) and treating toxic pulp effluents (19).

PVA samples. A solution containing PVA powder (DuPont Elvanol 71/30; average degree of polymerization, 1,800) and all other solutions were prepared with distilled water and auto-

claved at 90°C for 30 min. FR was prepared by mixing equal volumes of H₂O₂ (2.8 M) with FeSO₄ (0.10 M) in the presence of PVA (13); the excess FeSO₄ ensured that negligible H₂O₂ was present after the reaction. This reaction led to the formation of hydroxyl radicals (24).

The concentration of PVA was determined by using a modification of the colorimetric technique described by Bugada and Rudin (3). A 100- μ l sample of the PVA solution was diluted to a volume of 10 ml, and then 5 ml of 4% boric acid and 2 ml of I₂-KI (1.27 g of I₂ and 25 g of KI in 1 liter) were added. The solutions were equilibrated for 5 min; then they were diluted to a volume of 25 ml and analyzed at a wavelength of 690 nm. All measurements were determined in triplicate.

Preoxidation of PVA with FR. Either 0.1 or 0.2 ml of FR was added to Erlenmeyer flasks (250 ml) containing 20 ml of a sterile PVA solution (0.5%, wt/vol), and the preparations were incubated for 24 h. The PVA could be completely degraded by adding more than 1 ml of FR; however, the volumes mentioned above were selected so that we could study the combined chemical-biological degradation process. The pH of each solution was then adjusted to 4.2, after which the PVA concentration was determined.

Inoculation with *P. cinnabarinus*. Sterile solutions of PVA were preoxidized with FR and supplemented with the nutrient medium described by Tien and Kirk (23). This medium was modified by adding 1 ml of 0.043 M ammonium tartrate, 2 ml of 0.10 M 2,2-dimethyl succinate, and 1 ml of a trace element solution. No glucose was included in this medium unless otherwise stated. The flasks were inoculated with 5-mm-diameter plugs of *P. cinnabarinus* CBS 101046 subcultured on 2% malt extract agar. The flasks were prepared in triplicate and spiked with 0.10% (wt/vol) glucose. The flasks were weighed and then incubated in a sterile environment with a humidity of 95% at 37°C for 30 days; adjustments for moisture loss were made.

Oxidase activity and pH. Total extracellular oxidase, laccase, manganese peroxidase, and lignin peroxidase activities were measured by the methods described by Niku-Paavola et al. (17), Coll et al. (4), Aitken and Irvine (1), and Tien and Kirk (23), respectively. Positive controls were included in each case to validate the assays. The solution pH was measured throughout the study.

FR treatment. The degradation of PVA after FR preoxidation is shown in Fig. 1. Greater overall degradation was observed in the presence of FR treatment than in the absence of FR treatment (and there was an accompanying increase in biomass); however, the rate of degradation appeared to be

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FIG. 1. Degradation of PVA after preoxidation with FR, followed by biological degradation in nutrient-supplemented solutions by *P. cinnabarinus*. The oxidase activity and solution pH were measured on the same days. All of the data are means of values from three replicates; the bars indicate standard errors.

independent of the preoxidation step. The oxidase activity was found to be greater for the samples subjected to preoxidation than for the samples that were not preoxidized, particularly after day 10. PVA preparations not subjected to FR treatment exhibited very low levels of oxidase activity between days 5 and 20. In all cases, however, neither lignin peroxidase nor Mn peroxidase was detected. The total oxidase activity was found to be equivalent to the laccase activity, suggesting that the predominant enzyme secreted by *P. cinnabarinus* under these conditions was laccase.

The pH increased initially with time and then decreased until it reached a plateau. The initial increases in pH were consistent with utilization of carboxylic acids resulting from PVA cleavage by the fungus. The greatest change in pH was observed with the samples treated with FR, and the greatest increase in pH was observed when 0.2 ml of FR was added. The subsequent decreases in pH suggested that the carboxylic acid concentration increased due to decreased utilization of this compound.

FR treatment with glucose addition. The results of PVA degradation in the presence of glucose after FR treatment are shown in Fig. 2. Preoxidation of the PVA resulted in greater overall degradation compared to the degradation obtained without an FR treatment. The degree of degradation was also greater than the degree of degradation observed in the absence of glucose. The rate of degradation was greater in samples subjected to FR pretreatment than in samples not subjected to FR pretreatment. The levels of oxidase activity were elevated in these cases (which was consistent with the observed higher levels of biomass and PVA degradation); however, samples treated with FR maintained their activity after day 10, when the activity of the untreated samples began to decrease. Again, neither lignin peroxidase nor Mn peroxidase was detected.

FIG. 2. Degradation of PVA after preoxidation with FR, followed by biological degradation in glucose- and nutrient-supplemented solutions by *P. cinnabarinus*. The oxidase activity and solution pH were measured on the same days. All of the data are means of values from three replicates; the bars indicate standard errors.

The pH behavior of each sample was different from the behaviors shown in Fig. 1. The pH effectively remained constant for the samples treated with FR for the entire incubation period; there was no substantial increase in pH. The PVA sample without FR pretreatment exhibited a small increase in pH over a 30-day period. This result is consistent with the decrease in carboxylic acid concentration resulting from utilization of this compound by the fungus.

Conclusion. PVA was degraded by using a combination of chemical and biological treatments, which resulted in greater levels of degradation than the levels of degradation obtained when biological treatment alone was used. Inclusion of glucose as a carbon source resulted in higher oxidase activity and an increase in the degree of PVA degradation. The absence of Mn peroxidase and lignin peroxidase indicated that the ligninolytic enzyme secreted by the fungus was laccase. Higher levels of PVA degradation occurred when increased oxidase levels were detected.

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