Treatment of White Water with Combined Predominant Bacteria and Immobilized Enzyme

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White water treatment with combined predominant bacterial species and immobilized enzyme was investigated. The use of the single predominant bacteria of *Brevundimonas diminuta* or *Virgibacillus pantothenticus* resulted in poor treatment responses. With the combined bacterial species, the treatment effect was clearly improved. When the dosage ratio of *Brevundimonas diminuta* to *Virgibacillus pantothenticus* was 1:2, the chemical oxygen demand (COD_{Cr}) removal rate reached 70.5%, the cationic demand decreased 46.0%, and the electrical conductivity decreased 18.6% after 16 h of treatment. When mixed with the immobilized enzyme, the treatment efficiency increased with the immobilized pectinase dosage. When 8 g/L immobilized pectinase was added, the treatment time was shortened from 16 h to 4 h, the highest removal rate of COD_{Cr} was 74.1%, the cationic demand decreased 68.7%, and the electrical conductivity in the white water decreased 30.1%. The results indicated that the combination of predominant bacterial species and immobilized pectinase could greatly improve the treatment efficiency of white water.

Keywords: White water; Predominant bacteria; Immobilized enzyme

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INTRODUCTION

White water mainly contains fines, fibers, fillers, adhesives, and appreciable amounts of dissolved and colloidal substances, which have a considerable adverse influence on the wet-end chemistry of the papermachine (Latorre *et al.* 2007). The recycling of white water could greatly reduce the papermaking water consumption, which is one of the important ways towards cleaner production, energy conservation, and pollution emissions reduction (Toczyłowska-Mamińska 2017).

Although traditional biological processes for white water treatment have certain effects on the dissolution and removal of colloidal substances in white water, these methods have disadvantages, such as high treatment cost and break the electric charge balance (Julio-González *et al.* 2018). Addition of predominant bacterial species in the conventional activated sludge method has the potential to enhance or improve the degradation of specific pollutants, improve the treatment effect, and stabilize effluent quality (Sun *et al.* 2015). Hence, the selection and application of efficient predominant bacterial species have become both an effective measure to solve such problems and an important research direction in biological treatment. In the study of Sonkar *et al.* (2019), the chemical oxygen demand (COD) removal rate reached as high as 89.5% with a new bacillus for paper wastewater treatment. Lan *et al.* (2019) used three dominant bacteria, which included *Virgibacillus pantothenticus*, *Bacillus cereus*, and *Bacillus subtilis*, screened from a domesticated activated sludge to treat white water. The COD_{Cr} removal rate reached 77%, the electrical conductivity decreased 0.86 mS/cm, and the cationic demand decreased 73.7%.

Although predominant bacterial species are good at degrading certain substances, the adaptability of a single colony to the environment is relatively weak, and the adaptability of multiple strains could be greatly improved and could have a good treatment effect (Liu *et al*. 2018). However, the degradation rate of cellulose by the predominant bacterial species is slow, whereas some enzymes could be used to degrade the cellulose polymer rapidly.

Pectinase can cleave the glycoside bonds in the polymeric cellulose, promote the degradation of cellulose, and reduce the cationic demand (CD) of the white water (Fu *et al*. 2019). Liu *et al.* (2012) treated white water with immobilized pectinase and lipase for 15 min, and the CD value decreased 58%. Wu *et al*. (2014) showed that the fixation of pectinase onto pulp fibers could effectively reduce the cationic demand of white water for papermaking (Wu *et al*. 2014). However, in the process of white-water treatment, the activities of free enzymes remained relatively short, and the intermediate products cannot be degraded further, which made it impossible to use the enzymes alone (Elazzazy *et al*. 2015).

In the treatment of white water, there have been few studies that used predominant bacterial combined with immobilized enzyme. Through the rapid degradation of polymeric substances, such as cellulose with pectinase enzyme, the intermediate degradation products were then degraded by the predominant bacteria; thus the inhibition of the enzymatic reaction was relieved. The immobilized pectinase and predominant bacterial species worked together to mineralize pollutants in the white water, and to improve the performance of papermaking white water recycling. The present study not only has important practical application and theoretical values for white water closed circulation in paper mill, but it also can provide a new process for final treatment of white water in paper mills.

EXPERIMENTAL

Materials

White water was taken from the no. 2 white water chest of a paper factory in Shouguang City, China. The total solids content and ash content were 0.01% and 0.001‰. The water used for the experiments was obtained by the centrifugation of white water for 20 min. The cationic demand (CD) value was 425 μeq/L, the electrical conductivity was 1926 μs/cm, the COD_{Cr} and BOD_5 of white water were approximately 950 mg/L and 450 mg/L, and the ratio of BOD_5/COD_{Cr} was 0.47, indicating a good biodegradability. The pH value was 6.94.

The method to isolate and purify the strains was proceeded according to the reference of Lan *et al*. (2018).

Two kinds of predominant bacterial species (*Virgibacillus pantothenticus* and *Brevundimonas diminuta*) were isolated and purified from the activated sludge domesticated by the midcourse pulping wastewater and were dried to make bacterial powders. Micrographs of the two colonies were provided in a previous publication by the authors (Lan *et al*. 2018).

The procedure of immobilized pectinase was according to the reference of Dai *et al.* (2018); 2.0 g chitosan beads and 10.0 mL of 0.005% glutaraldehyde solution were added to the conical flask and mixed for 30 min. A certain amount of crude enzyme solution was added and mixed for 3 h at 25 °C and pH=8. Then the chitosan beads were filtered out and washed with deionized water for 3 times.

Methods

The concentration of COD_{Cr} was determined with a COD detector (DR1010; Hach Co., Loveland, CO, USA) that used potassium dichromate. The electrical conductivity of the water sample was measured using a conductivity meter (DDS-11C; Shanghai San-Xin Instrumentation Inc., Shanghai, China).

The CD value was measured using the Particle Charge Detector (PCD-03; BTG Instruments GmbH, Bayern, Germany) with poly-diallyldimethylammonium chloride (poly-DADMAC) as the standard cationic titrant (10^6 μeq/L). The CD value of the white water was calculated with Eq. 1,

$$CD = V \times C \times 10^3 \ (\mu eq/L) \qquad (1)$$

where V is the standard cationic titrant consumed by the sample (μL), and C is the charge density of the standard cationic titration solution (10^3 μeq/L).

Each experiment was repeated three times.

RESULTS AND DISCUSSION

Treatment of White Water with the Single Predominant Bacteria

The treatment effects of pH and turbidity with the single bacterial specimen are shown in Table 1.

Table 1. Treatment Effects on pH and Turbidity with the Single Bacterium

	Brevundimonas diminuta		*Virgibacillus pantothenticus*	
	pH	Turbidity (NTU)	pH	Turbidity (NTU)
Before treatment	6.94	124	6.93	126
After treatment	5.7	98	5.6	94

As can be seen from Table 1, after the treatment, the pH values of white water with both the predominant bacteria dropped to about 5.7 and the turbidity removal rates were both about 20%. The turbidity removal rates were relatively low due to the increasing turbidity by the newly reproductive free bacteria.

The treatment effects with the single bacterial specimen are shown in Fig. 1.

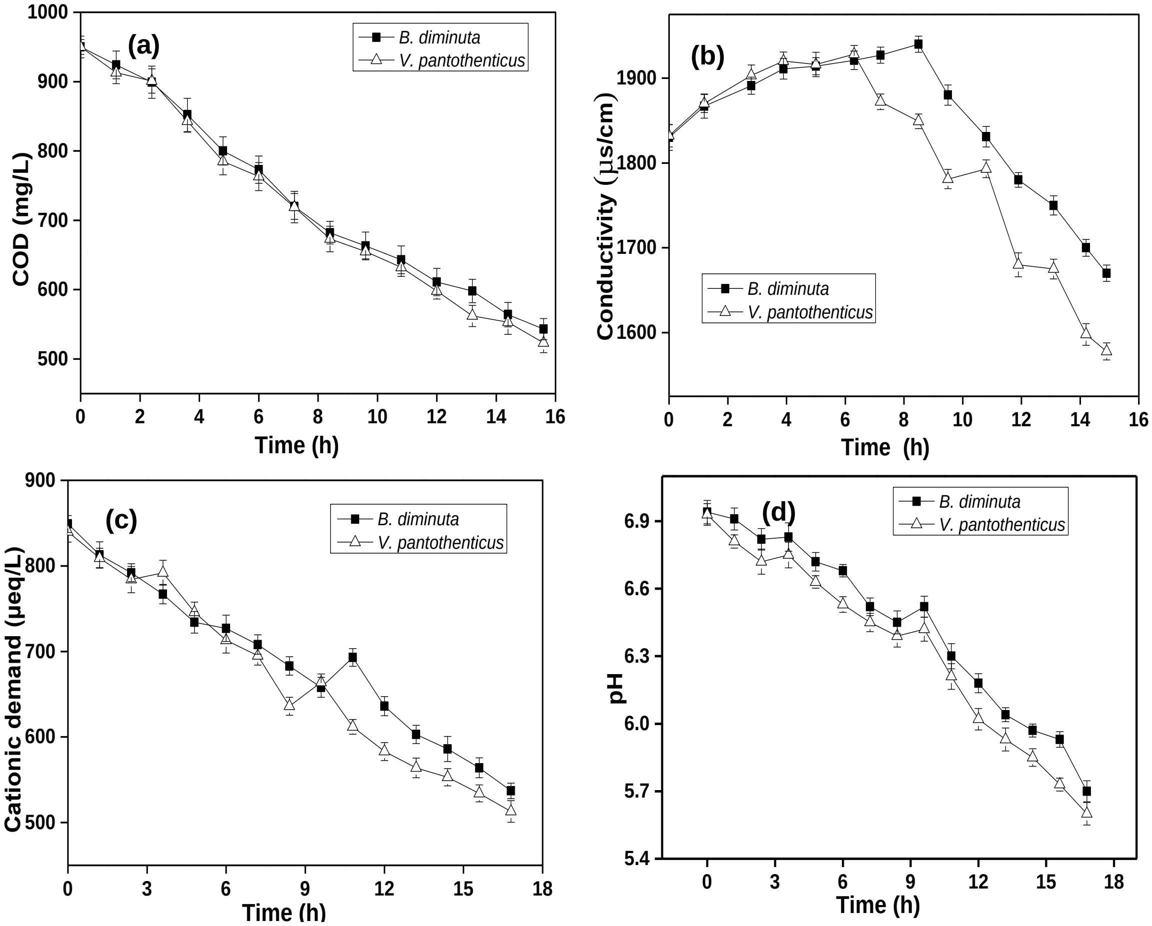

Fig. 1. Treatment effects of the single bacterium specimen: (a) COD$_{Cr}$, (b) electrical conductivity, and (c) CD (d) pH

The *Brevundimonas diminuta* or *Virgibacillus pantothenticus* was dosed at 0.5 g/L. The results can be seen in Fig. 1a. The COD$_{Cr}$ concentration decreased with time for each species alone. This was because the pectins, fatty acids, lignin derivatives, and other organic compounds in the white water were consumed by *Virgibacillus pantothenticus* or *Brevundimonas diminuta*, which led to a decline in effluent COD$_{Cr}$ concentration (Triolo *et al.* 2011).

Electrical conductivity is an important index to evaluate the reuse performance of papermaking white water (Li *et al.* 2014). With each reuse of the white water, the inorganic salt electrolytes and anionic wastes continuously accumulate, which has a great influence on the operation performance of the papermachine and the quality of the paper made (Yates and Smotzer 2007). Excessive inorganic salt electrolytes in the white water not only changes the adsorption capacity of the pulp to functional chemical auxiliaries, but it also affects the retention and filtration performance of cationic polymer, and the strength of the paper made (Zhu *et al.* 2012). Therefore, whether the reuse performance of white water was improved or not could be judged by measuring the change of electrical conductivity. It can be seen from Fig. 1(b) that the electrical conductivity first increased and then decreased with time. The electrical conductivity decreased 13.37% with *Brevundimonas diminuta* (increased from 1832 µS/cm to 1920 µS/cm and then decreased to 1587 µS/cm), and decreased 12.46% (increased from 1830 µS/cm to 1940 µS/cm and then decreased to

1602 μS/cm) with *Virgibacillus pantothenticus*. This was because the microorganisms decomposed the polymeric substances into smaller molecular substances that led to an increase in electrical conductivity (Sun *et al.* 2015). For example, polygalactouronic acid was decomposed into beta-galactouronic acid, which increased the electrical conductivity of the water. Then, the small molecular substances, such as beta-galactouronic acid, turpentine acid, and dehydroacetic acid, were mineralized, which led to a decrease in electrical conductivity.

The CD is another important index to evaluate the reuse performance of white water (Liao and Qian 2006). The reduction of CD meant that the dosage of cationic auxiliaries could be reduced in the reuse process, and the quality of the paper and the stability of the papermachine system could be improved (Zhuang *et al.* 2016). As shown in Fig. 1c, the CD decreased with time. The CD decreased from 840 μeq/L to 513 μeq/L, which was a decrease of 38.9%, with *Virgibacillus pantothenticus*; a decrease of 36.8% (from 849 μeq/L to 537 μeq/L) was seen with *Brevundimonas diminuta*. This was because the colloid materials with negative charges in white water were decomposed into small molecules by the bacteria. Decomposition products could include glycerol, propane, and other smaller molecular species (Kansal *et al.* 2011; Yang *et al.* 2014), which made the CD continuously decrease.

The results indicated that the treatment effect of the single bacterial specimen was not ideal. The treatment efficiency of *Virgibacillus pantothenticus* was slightly higher than that of *Brevundimonas diminuta*. The two microbes belong to the genera bacillus and pseudomonas, which have different growth and metabolism rates that led to different treatment efficiency.

Treatment of White Water with the Combined Predominant Bacteria

The effect of the single bacterial specimen was poor, so the combination of the two was used to treat the white water. The dosage of *Brevundimonas diminuta* was 0.25 g/L, 0.50 g/L, 0.75 g/L, 1.00 g/L, or 1.25 g/L, and the dosage of *Virgibacillus pantothenticus* was 1.25 g/L, 1.00 g/L, 0.75 g/L, 0.50 g/L, or 0.25 g/L. The ratio of the two bacteria species was either 1:5, 1:2, 1:1, 2:1, or 5:1. Results when using both bacteria are shown in Fig. 2.

It can be seen from Fig. 2 that when the ratio of the two bacterial powders was 1:2, the white water had the highest removal rate of COD_{Cr}, the highest reduction in CD value, and the highest reduction in electrical conductivity. At this ratio, the removal rate of COD_{Cr} was 70.5% (from 950 mg/L to 280 mg/L), the electrical conductivity decreased 18.6% (from 1840 μS/cm to 1498 μS/cm), and the CD value decreased 46.0% (from 845 μeq/L to 460 μeq/L), with a higher efficiency than that of the single bacteria (COD_{Cr} removal rate of 44.9%, a decrease of 38.9% of CD value and 13.8% of electrical conductivity). The two kinds of bacteria were selected from the activated sludge that was domesticated by mid-stage pulping effluent, and they had the same growing and metabolic conditions. A co-metabolism may be formed with the combined bacterial species, and the synergy between the microorganisms improved their metabolic activity, which led to a better treatment effect than either bacterial specimen alone (Chang *et al.* 2015).

The CD value finally decreased 46.0% with the two bacteria species at a ratio of 1:2, while the electrical conductivity only decreased 18.6%. This was because the colloidal substances were the major contributors to anionic trash in the white water. Macromolecular substances in the white water were degraded by the bacteria, which led to a great decrease in the CD value. The electrical conductivity was mainly caused by soluble charged substances, which included inorganic salts that were not consumed by the growth of the microorganisms. Therefore, the electrical conductivity decrease was less after the treatment.

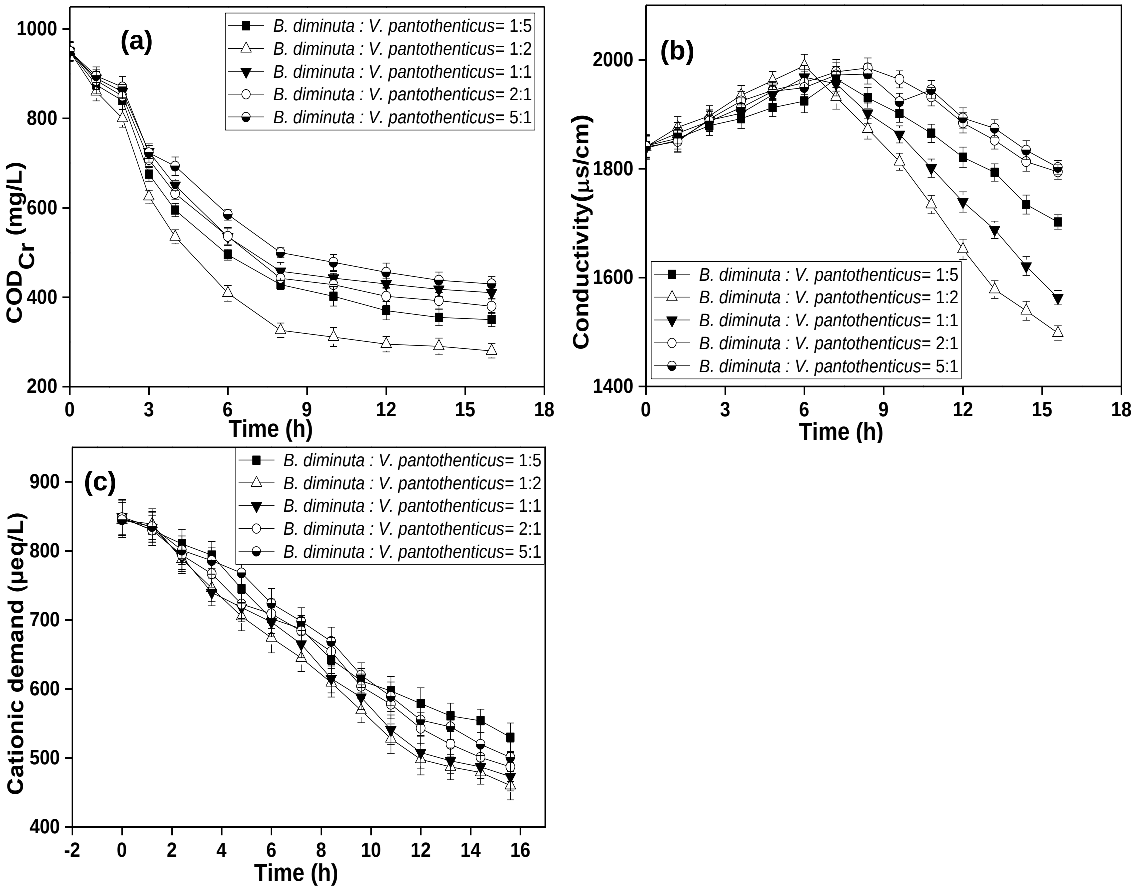

Fig. 2. The treatment effect of the combined bacterial species: (a) COD$_{Cr}$, (b) electrical conductivity, and (c) CD

Treatment of White Water with Combined Predominant Bacteria and Immobilized Enzyme

Although the reuse performance of the white water was clearly improved with the combined bacterial treatment, the treatment time was too long because the colloidal substances were degraded slowly by the bacteria. Hence, a specific enzyme was introduced with the combined bacterial species to shorten the treatment time. On the premise of ensuring the dosing ratio of *Brevundimonas diminuta* to *Virgibacillus pantothenticus* was 1:2 (0.50 g/L and 1.00 g/L, respectively), immobilized pectinase of 4 g/L, 5 g/L, 6 g/L, 7 g/L, or 8 g/L was added. The results are shown in Fig. 3.

It can be seen from Fig. 3a that the COD$_{Cr}$ removal rate with 8 g/L immobilized pectinase was the highest (74.1%), which was higher than that in the system with only predominant bacteria. Moreover, within a certain range, the more immobilized pectinase that was added, resulted in a higher removal rate of COD$_{Cr}$. The combination of predominant bacterial species and immobilized pectinase effectively improved the treatment effect. After adding the immobilized enzyme, the treatment time was reduced from 16 h to 4 h, which greatly improved the treatment efficiency and shortened the treatment time.

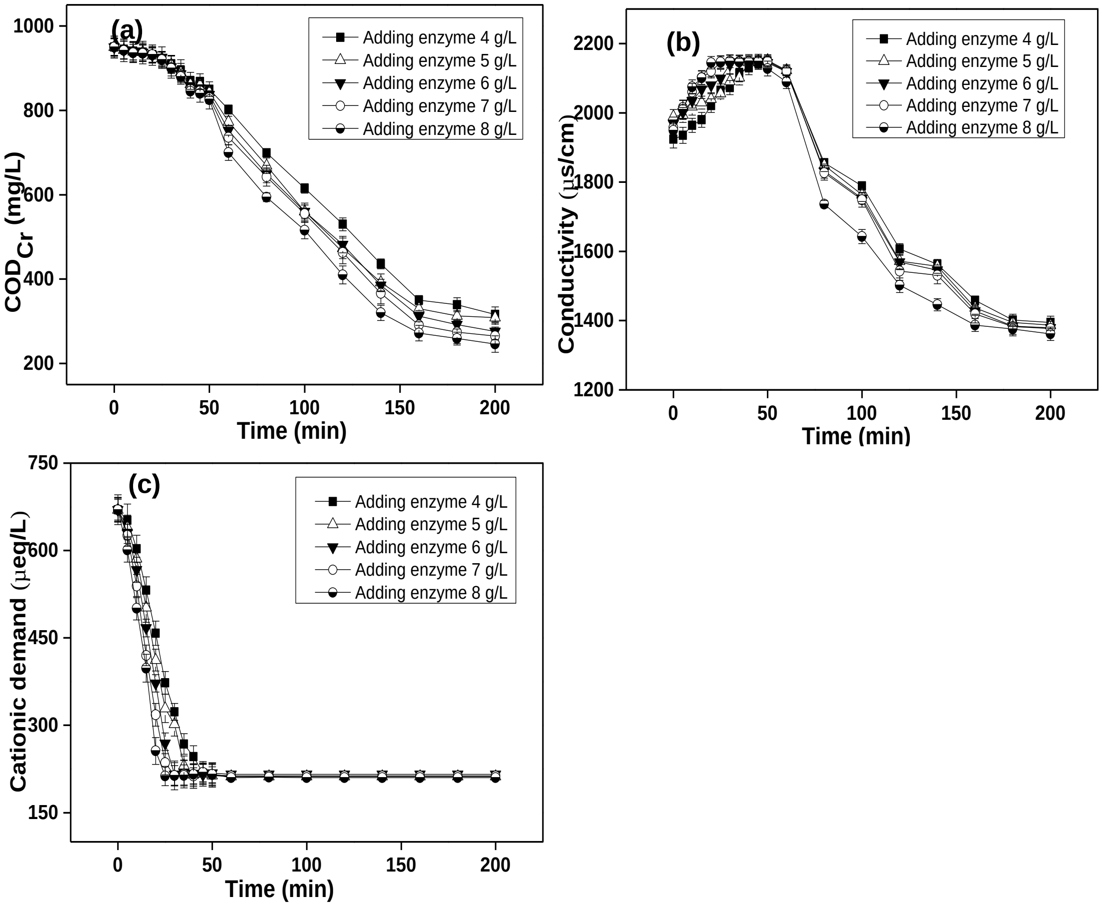

Fig. 3. The treatment effect of the combined immobilized pectinase and two bacterial species: (a) COD$_{Cr}$, (b) electrical conductivity, and (c) CD

It can be seen from Fig. 3b that when different dosages of immobilized pectinase were added, the electrical conductivity initially increased and then decreased. Moreover, the higher the amount of immobilized pectinase that was added resulted in a faster rate. The electrical conductivity with 8 g/L immobilized pectinase reached a maximum value of 2149 μS/cm after approximately 20 min treatment and decreased to 1362 μS/cm after 180 min (a 30.12% reduction). This was notably higher than the system that added only the single predominant bacteria (a 13.4% and a 12.5% reduction, respectively) and the combined predominant bacteria system (an 18.6% reduction). In addition, Fig. 3c shows that the CD value decreased increasingly fast with the increase of enzyme dosage. When the immobilized pectinase was 8 g/L, the CD value decreased from 670 μeq/L to 218 μeq/L at approximately 25 min, and then it stayed the same. The CD value decreased 68.7%, which was much higher than that of the system that only added the predominant bacteria (a decrease of 46.0%). This was because the immobilized pectinase rapidly broke the glycosidic bonds in the pectins, which promoted the galactouronic acid to degradate into single molecule galactouronic acid. Because pectinase has high efficiency and specificity, this process can be completed in a short time. CD value decreased significantly after combination of predominant bacterial species and immobilized pectinase compared with the system that only added the predominant bacteria. This observation is also consistent with the noted results of COD$_{Cr}$ and electrical conductivity, which indicated that the combined immobilized pectinase with the predominant bacterial species effectively improved the efficiency of white-water treatment.

The rapid degradation of polymeric substances like cellulose with pectin enzyme occurred, and the intermediate degradation products were then degraded by the predominant bacteria more easily. Thus, the treatment of white water was significantly more effective by combined predominant bacteria with immobilized pectinase than the single bacteria, and the treatment cycle was sharply shortened. As can be seen from Fig. 2 and Fig. 3, COD_{Cr} concentration decreased to 523 mg/L with *Virgibacillus pantothenticus* for 16 h treatment, but the same effect was achieved with combined predominant bacteria only for 4 h. The treatment effect of the electrical conductivity and CD value with combined predominant bacteria were also apparently higher than that with *Virgibacillus pantothenticus*.

CONCLUSIONS

1. The treatment efficiency with the single bacterial species of either *Virgibacillus pantothenticus* or *Brevundimonas diminuta* was poor. The combination of the two bacterial species achieved a higher removal efficiency (70.5% COD_{Cr}, 46.0% CD value, and 18.6% electrical conductivity) when the ratio of *Brevundimonas diminuta* to *Virgibacillus pantothenticus* was 1:2. However, the treatment time was as long as 16 h.
2. The treatment efficiency greatly improved when the combined predominant bacteria and immobilized enzyme were used. When 8 g/L immobilized pectinase was added, the treatment time was shortened from 16 h to 4 h. The highest removal rate of COD_{Cr} was 74.1%, the CD value decreased 68.7%, and the electrical conductivity decreased 30.1%; this was much higher than the system that added only the predominant bacteria.

ACKNOWLEDGMENTS

The authors are grateful for the support of the Fujian Provincial Key Laboratory of Ecology-Toxicological Effects & Control for Emerging Contaminants (PY18002) and the National Natural Science Foundation of China (NSFC) (Project No. 21607089).

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Article submitted: December 17, 2019; Peer review completed: February 22, 2020;
Revised version received and accepted: April 6, 2020; Published: April 9, 2020.
DOI: 10.15376/biores.15.2.4016-4025