Effects of pH on Biological Treatment of Paper Mill White Water with the Addition of Dominant Bacteria

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Virgibacillus pantothenticus, *Bacillus cereus*, and *B. subtilis* were screened from an activated sludge that had become acclimated to white water in the laboratory. The effects of the pH on the ability of biological treatment to reduce the biological oxygen demand of white water was studied for three dominant bacteria. The pH ranged from 4 to 8, and the optimum treatment efficiencies for white water treatments with the single dominant bacterium *V. pantothenticus*, *B. cereus*, and *B. subtilis* occurred at pH values of 5, 6, and 6, respectively. The results also indicated that the best treatment effect was achieved at a hydraulic retention time of 14 h. When each dominate species was tested under their optimum pH value and hydraulic retention time, the chemical oxygen demand removal rate was 67.7%, 77%, and 75.4%; the electrical conductivity decreased by 0.18 mS/cm, 0.93 mS/cm, and 0.51 mS/cm; and the cationic demand decreased by 69.7%, 70.0%, and 70.9% for *V. pantothenticus*, *B. cereus*, and *B. subtilis*, respectively. These results are helpful for promoting the practical application of dominant bacteria in white water treatment.

Keywords: White water; pH; Dominant bacteria; Virgibacillus pantothenticus; Bacillus cereus; Bacillus subtilis

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INTRODUCTION

White water mainly contains fines, fibers, fillers, adhesives, dry strength agents, sizing agents, and non-soluble substances. The complex components lead to poor biodegradability of white water (Pokhrel and Viraraghavan 2004; Latorre *et al*. 2007; Toczyłowska-Mamińska 2017). Considering economic and environmental protections, the closed circulation of white water in papermaking is an ideal way for water source conservation. However, it can generate adverse impacts. For example, the anionic waste and lipophilic extractives can increase remarkably (Bengtsson *et al*. 2008), a large amount of foam can be formed (Karikallio *et al*. 2011), and toxic and inflammable gases can be produced. Therefore, to achieve zero wastewater discharge, an efficient white water purification technology is important. Commonly used white water recycling technologies include physicochemical and biological methods, with the biological method being an important white water treatment technology (Buyukkamaci and Koken 2010; Mänttäri *et al*. 2015; Kamali *et al*. 2016).

Currently, the dominant bacteria have aroused wide concern of researchers, because they have special degradation ability to the refractory organics. The addition of dominant bacteria into an aerobic activated sludge system makes efficient utilization and degradation of organic pollutants in the industrial wastewater possible, and thus the quality of the effluent is enhanced and the concentration of the suspended matter in the effluent is reduced. Therefore, the effect of treatment on white water can be improved to a certain extent. Studies on dominant bacteria technology have been done. Shi *et al* (2012) introduced marine halophilic bacteria into an intermittently aerated biological filter (IABF) to enhance the bioaugmentation. Their findings demonstrated that the removal efficiencies of chemical oxygen demand (COD), total nitrogen, and total phosphorus were increased by about 8.6%, 15.7%, and 17.3%, respectively. So the introduction of marine halophilic bacteria can enhance the activity of biofilm.

The pH is one of the most important control parameters for sewage treatment. It directly affects the growth and metabolism of microorganisms and the activity of biological enzymes. The pH values required for the treatment of wastewater differ with the microorganism (Maspolim *et al*. 2015; Yuan *et al*. 2016). Kikot *et al.* (2010) studied the effect of the pH on the growth of a mesophilic sulfate-reducing strain isolated from the effluent from a tannery. It was found that the pH had a noticeable effect on the sulfate reduction. When the pH decreased from 7 to 5, the activity of the sulfate reducing strains and communities were inhibited. Liang *et al.* (2013) found that controlling the pH was crucial for removing nutrients during wastewater treatment *via* a combination of *Chlorella vulgaris* and *Bacillus licheniformis*. When the pH was 7, higher removal efficiencies of NH_4^+ (86%) and total phosphorus (TP) (93%) were achieved. However, algal cells were severely damaged by a low pH value (3.5). It has been reported that maintaining the pH value between 6.4 and 7.0 inhibited the activity of ammonia-oxidizing bacteria and mitigated N_2O production (Law *et al*. 2011). However, a maximum N_2O production was achieved at a pH of 8.0. Therefore, the pH is vitally important in the wastewater treatment process *via* dominant bacteria. Therefore, even if the dominant bacteria play an important role in the biochemical treatment system, once the pH value is not adjusted properly, it will cause negative effects and waste resources.

In this study, *Virgibacillus pantothenticus*, *B. cereus*, and *B. subtilis* were isolated and purified in a laboratory. They exerted their functions in the degradation of lignin and fiber in white water. To maximize the effect of the treatment, the effect of the pH on white water treatment *via* a single dominant bacterium was studied.

EXPERIMENTAL

Materials

White water was obtained from alkaline hydrogen peroxide mechanical pulp obtained from a paper mill in Shandong Province, China. The COD and BOD_5 of the white water were approximately 1320 mg/L and 450 to 500 mg/L. The cationic demand was approximately 510 µeq/L. The electrical conductivity was approximately 1894 µs/cm, and the pH was 7.64. The aerobic activated sludge comes from secondary settling tank of sewage treatment station in paper mill. The dominant bacteria were screened in the laboratory from aerobic activated sludge domesticated with the white water.

Methods

Analysis methods

The conductivity was measured with a DDS-11C type conductivity meter (Shengke Instrument Equipment Co. Ltd., Shanghai, China). The oxygen concentration for the calculation of the chemical oxygen demand (COD_{Cr}) was determined with a COD analyzer (DR1010, HACH, Loveland, CO, U.S.). The pH was determined with a pH meter (PHS-3C type, Yoke Instrument Co. Ltd., Shanghai, China). The cationic demand was measured with a streaming current detector (PCD-03, BTG Ltd., Eclépens, Switzerland). The standard cation titration solution was polydiallyldimethylammonium chloride (pDADAMC), after diluting 100 times, with a charge density of 10^4 µeq/L. White water was diluted 10 times, and the volume of water sample was 10 mL. The cationic demand was obtained *via* Eq. 1,

$$CD = \frac{V_1 \times C \times 10}{V_2} \tag{1}$$

where CD is the cationic demand (µeq/L), V_1 is the volume of the standard cation liquid used as the titrant (L), C is the charge density of the diluted standard cation titration solution (10^4 µeq/L), and V_2 is the volume of water sample (L). 10 is the dilution multiple.

Experimental methods

Five groups with 60 mg of pure microbial inoculum and 200 mL of white water were added to 250-mL conical bottles. The pH of the bottles was adjusted to 4, 5, 6, 7, and 8 *via* 2 M sulfuric acid. Then, the five reactions were simultaneously placed into a 30 °C water bath. Samples were collected every 1 h or 2 h, and the COD_{Cr}, conductivity, and cationic demand were measured for each sample.

RESULTS AND DISCUSSION

Effect of the pH on the COD Removal Rate

For different pH values (4 to 8), the COD removal rates of *V. pantothenticus*, *B. cereus*, and *B. subtilis* are shown in Fig. 1.

Figure 1 shows that the maximum COD removal rate was achieved at a pH of 5 and 6 for *V. pantothenticus*, with COD removal rates of 28.2% and 26.2% after 2 h, and 67.7% and 66.0% after 14 h, respectively. The treatment was effective for the reaction with a pH of 6 for *B. cereus* and *B. subtilis*, as well. The COD removal rates after 2 h were 24.1% and 30.5%, and the COD removal rates after 14 h were 77% and 75.4% for *B. cereus* and *B. subtilis*, respectively.

During the primary treatment stage, the low amounts of molecular organic acids, alcohols, phenols, and other compounds in the white water were decomposed *via* the dominant bacteria. However, over a short period of time, the microbes did not rapidly decompose the polycyclic aromatic compounds and other long chain macromolecular organic compounds in the white water. As the reaction time increased, the macromolecular compounds were slowly decomposed into smaller molecules *via* the microbes and a series of reactions, such as ring-opening and breaking. The COD removal rate gradually stabilized at 14 h. Figure 1 shows that the COD removal rates for the extreme pH conditions (pH values of 4 and 8) were remarkably lower than those for the median pH values. This was because the components of the bacteria had different sensitivities to acidic and alkaline conditions. For example, DNA and adenine ribonucleotides in bacteria are more sensitive to acid (Huang *et al*. 2016; Kunacheva *et al*. 2017). Under acidic conditions, the chemical bonds of the DNA were broken, the spatial conformation of the double helix was destroyed, the genetic information was changed, the synthesis of gene expression was blocked, and the life activity of the microorganism was disturbed or even stopped. Under alkaline conditions, the phospholipids, an important component of cellular membranes, were easily hydrolyzed, which changed the structure, fluidity, and selective permeability of the membrane. This impeded the transportation of substances and hindered the normal life activities of the microbes. The structure of the bacterial RNA was also easily destroyed under alkaline conditions, which affected the growth and metabolism of the microorganisms and made the COD removal rate worse.

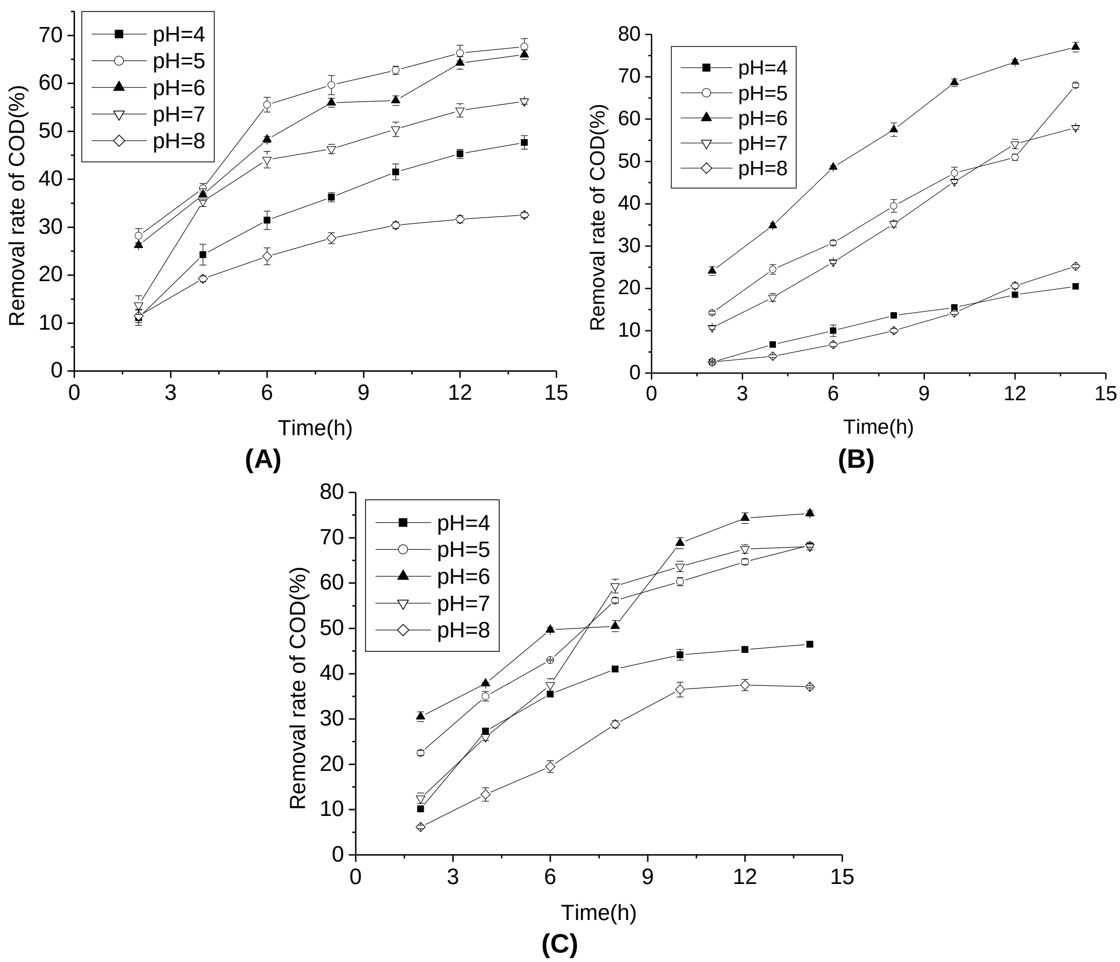

Fig. 1. Effect of the pH on the COD removal rates of (A) *V. pantothenticus*, (B) *B. cereus*, and (C) *B. subtilis*

On the other hand, microbial treatment of white water was essentially due to enzyme produced by microorganisms. *Virgibacillus pantothenticus*, *Bacillus cereus*, and *B. subtilis* could synthesize laccase and pectinase *in vivo* and degrade the pollutants in white water by bioenzyme. However, under the condition of peracid or peralkali, the activity of cell membrane proteins would be inhibited, thus affecting the normal absorption and transport of nutrients, which was not conducive to the synthesis of enzymes and COD removal. Consequently, it had been shown by experiment that adding three dominant bacteria to activated sludge and adjusting pH value of 5 to 6 in actual white water treatment can obviously increase the treatment effect.

Effect of pH on the Conductivity

The effects of the pH on the conductivity of the effluent treated *via* the dominant bacteria are shown in Fig. 2.

Within the range of investigated pH values, the conductivity increased at first and then decreased over time. When the pH values were 5 and 6, the conductivity reached maximum values of 6.46 mS/cm and 6.34 mS/cm, respectively, at 5 h with the dominant bacterium *V. pantothenticus*. The conductivity increased by 24.2% and 21.9%, respectively, compared with the initial conductivity (5.20 mS/cm). Subsequently, the conductivity showed a downward trend. The conductivity values decreased to 5.02 mS/cm and 5.08 mS/cm at a pH of 5 and 6 at 14 h, which was a decrease of 22.3% and 19.9%, respectively, compared with the peak conductivity. Compared with the initial conductivity, the values decreased by 0.18 mS/cm and 0.12 mS/cm, respectively. The conductivity variation with *B. cereus* was the largest at pH values of 5 and 6.

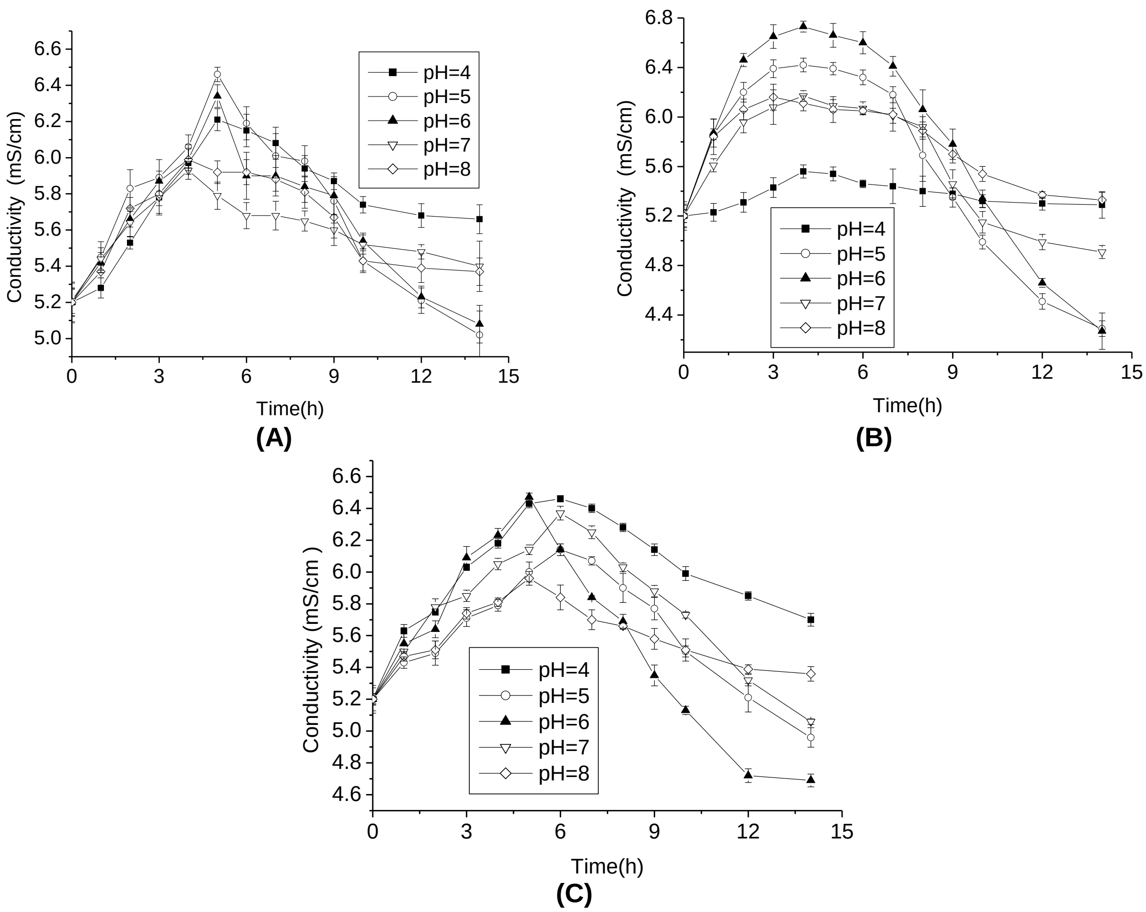

Fig. 2. Effect of the pH on the conductivity of the effluent treated by (A) *V. pantothenticus*, (B) *B. cereus*, and (C) *B. subtilis*

In the initial stage, the polycyclic aromatic compounds and other long chain macromolecular substances in the white water were converted into small molecules *via* ring opening and chain scission reactions through the action of the dominant bacteria (Li *et al*. 2017). For example, some lignin derivatives were decomposed into short chain fatty acids, phenols, and alcohols, which could produce hydrogen ions and negative ions, contributing to the increase in the conductivity value. As the treatment time increased, a portion of these small molecules were consumed by the dominant bacteria and converted into large molecular components of the cells, which led to bacterial growth. The other portion of the small molecules were completely decomposed into water and CO_2, which generated energy for bacterial growth. The CO_2 entered the air as a gas, and this led to a decrease in the conductivity. Therefore, the degradation efficiency of the macromolecular organic compounds in white water could be reflected by an increasing conductivity in the initial stage; meanwhile, the conductivity decreased in subsequent stages, which reflected the utilization and mineralization efficiency of the intermediate products of the small molecules *via* the dominant bacteria. For *V. pantothenticus* and *B. cereus*, when the pH values were 5 and 6, respectively, the mineralization efficiencies of the compounds were the highest. For *B. subtilis*, the treatment effect was the highest when the pH was 6.

Effect of the pH on the Cationic Demand

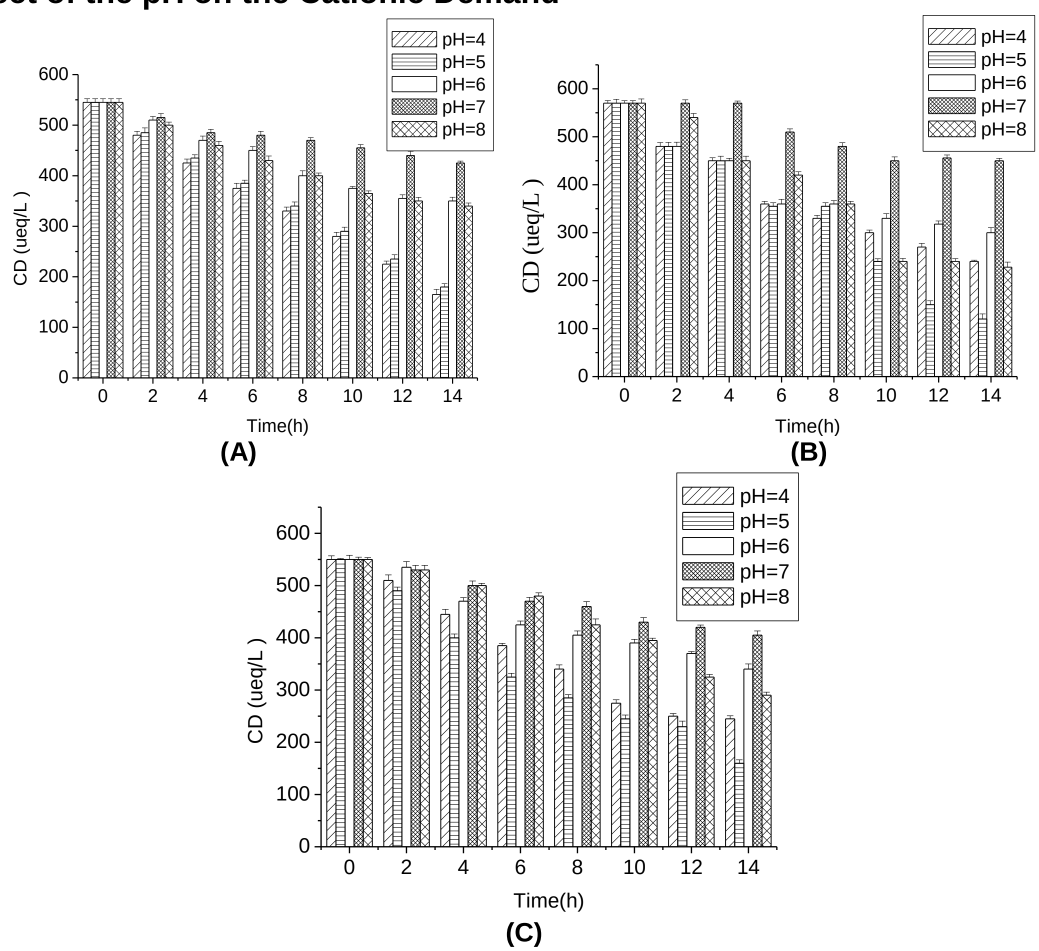

Fig. 3. Effect of the pH on the cationic demand of the effluent treated by (A) *V. pantothenticus*, (B) *B. cereus*, and (C) *B. subtilis*

The effects of the pH on the cationic demand of the effluent treated by *V. pantothenticus*, *B. cereus*, and *B. subtilis* are shown in Fig. 3. The cationic demand decreased with time. With an increase in the pH, the cationic demand of the effluent decreased at first and then increased. For *V. pantothenticus*, when the pH was 5 and 6, the cationic demand values were the smallest. The cationic demand values were 165 µeq/L and 180 µeq/L at 14 h and decreased by 69.7% and 67.0% at pH values of 5 and 6, respectively, when compared with the initial value (545 µeq/L). When the pH value was 6 for *B. cereus* and *B. subtilis*, the cationic demand reduction rates were the fastest and decreased to 120 µeq/L and 160 µeq/L at 14 h, respectively. Compared with the initial values (570 µeq/L and 550 µeq/L), the cationic demand values were decreased by 79.0% and 70.9% for *B. cereus* and *B. subtilis*, respectively.

In the treatment process, the negative colloidal substances in the waste water were decomposed into small molecular substances by the microorganisms. The anionic waste and cationic demand were continuously reduced. In this experiment, when the pH values were 5 and 6, the treatment efficiency of the anionic waste was the best for all three dominant bacteria with a minimum cationic demand value. Under peracid or peralkaline conditions, the structure of the protein is broken and the enzyme in the microorganism is deactivated, which leads to a halt of the normal life activity and death of a microorganism. Hence, for the three dominant bacteria, the microbial activity and treatment effects decreased when the pH was 4 and 8. Consequently, the optimum pH values of the three dominant bacteria were basically the same, which is beneficial for the growth of flora. Therefore, in the actual white water treatment, the pH value should be adjusted to the range of 5 to 6, so that the dominant bacteria can grow and metabolize better and exert the function of co-metabolism greatly, making the treatment effect of mixed bacteria be much higher than that of single strain.

CONCLUSIONS

1. In this experiment, white water was treated *via* a single dominant bacterium and the results indicated that the optimum metabolic pH values for *V. pantothenticus*, *B. cereus*, and *B. subtilis* were 5, 6, and 6, respectively.
2. Under these conditions, the treatment effect was the best with maximum variation in the conductivity and a minimum cationic demand. After 14 h of treatment, the COD_{Cr} removal rate was 67.7%, 77.0%, and 75.4%, the electrical conductivity decreased by 0.18 mS/cm, 0.93 mS/cm, and 0.51 mS/cm, and the cationic demand decreased by 69.7%, 79.0%, and 70.9% for *V. pantothenticus*, *B. cereus*, and *B. subtilis*, respectively.
3. The optimum pH values of the three dominant bacteria were basically the same, which is beneficial for the growth of flora in the actual white water treatment. Thus, it was advantageous to maximize the effect of treatment.

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