Research article

Bamboo Charcoal as a Lactic Acid Bacteria Carrier for Phosphate Removal

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Abstract A decline in the demand for using bamboo products has led to abandoned bamboo forests in Japan. To reduce the area of abandoned bamboo forests, the use of bamboo as a construction material and bamboo charcoal as an absorbent have gained considerable attention in recent years. In the literature, many studies have described the formation of biofilms on biochar, leading bamboo charcoal to be considered as a microbial carrier. The aim of this study was to examine the potential of bamboo charcoal as the microbial carrier of lactic acid bacteria (LAB) for phosphate removal. Bamboo charcoal was immersed in a LAB solution for 24 h for LAB to adhere to the bamboo charcoal. Then, the bamboo charcoal was placed in a bamboo fermented solution. Two types of bamboo charcoal, i.e., without pretreatment and dissolved in tap water, were used in the experiments. The experiments were also conducted with and without aeration to determine the effects of oxygenation. The bamboo charcoal without pretreatment displayed an increase in the phosphate concentration, indicating that phosphate was released from the bamboo charcoal. LAB-attached bamboo charcoal demonstrated a much smaller increase in phosphate concentration, suggesting phosphate was consumed by LAB. Experiments with dissolved bamboo charcoal also indicated a reduction in the phosphate concentration. The removal rate of phosphate decreased with an increase in the solution pH, suggesting that alkaline conditions limited the activity of the bacteria. Furthermore, the redox potential of the solution became negative in the solution without aeration. Overall, the results demonstrated that bamboo charcoal could be a LAB carrier; however, the bamboo charcoal must be dissolved prior to its use for phosphate removal. Furthermore, aeration and an acidic during phosphate removal are needed to obtain a higher removal rate when using LAB.

Keywords bamboo charcoal, microbial carrier, lactic acid bacteria, phosphate removal

INTRODUCTION

Although bamboo has been used in many different fields in Japan, a decrease in the demand for bamboo products has led to abandoned bamboo forests. According to a report by the Forestry Agency of Japan, the total bamboo forest area in 2007 was approximately 1600 km^2, which was 10% greater than in 1981. In 2007, the bamboo forest area covered approximately 0.6% of the total forest area in Japan, but up to 66% of the bamboo forest area was left unused. To reduce the area of abandoned bamboo forests, several countermeasures have been proposed to increase the utilization rate of bamboo. Historically, bamboo was primarily used as construction material and for household furniture. In recent years, bamboo has been used as a fertilizer in agriculture (Cui and Wu, 2010; Liu et al., 2014), and bamboo biochar has been used as an absorbent for contaminant removal (Liu et al., 2012; Mohamed et al., 2015).

In previous reports, a large number of negative charges was observed on the surface of biochar (Yigit and Mazlum, 2007). These negative charges generate electrostatic repulsion between the biochar surface and anions, making it difficult to adsorb phosphate ions on the surface. Furthermore, some kinds of biochar can release phosphate in aqueous solutions (Yao et al., 2012). For example,

Jung et al. (2015) reported the release of phosphate from bamboo biochar. Therefore, when using biochar for phosphate removal, it is necessary to modify the biochar surface with treatments using acids, alkali, iron oxide, or magnesium ions (Stratful et al., 2001; Tansel et al., 2018). Biochar impregnated with colloidal and nanosized metal oxyhydroxides can enhance the removal rate of phosphate (Zhang and Gao 2013; Zhang et al., 2012). Dai et al. (2020) also reported phosphorus removal in a solution using magnesium-modified biochar from bamboo. Li et al. (2020) reported the enhancement of phosphate adsorption using polyethyleneimine-modified biochar derived from bamboo biomass.

In addition to adsorption, the biological method is one of the various approaches for phosphate removal. As biochar is a porous material, it can serve as a habitat for microorganisms (Egamberdieva et al., 2018). Many studies have examined biofilm formation in biochar and its effect on soil properties (Yang et al., 2019; Ajeng et al., 2020; Gorovtsov et al., 2020). According to the literature, it is believed that organisms-attached biochar can increase the removal rate of phosphate in aqueous solutions. However, to the best of our knowledge, there is a lack of information related to the employment of organisms-attached bamboo charcoal for phosphate removal. In addition, as the efficacy of phosphate removal strongly depends on the properties of the biochar, a study of phosphate removal capabilities is necessary before the widespread use of bamboo charcoal.

OBJECTIVE

This study examines the potential of bamboo charcoal as a microbial carrier for lactic acid bacteria (LAB) and its phosphate removal capabilities. It is believed that LAB-attached bamboo charcoal can increase the removal rate of phosphate in aqueous solutions. Furthermore, this study focuses on deciphering the effects of the pretreatment of bamboo charcoal for phosphate removal. As the release of phosphate from bamboo charcoal has been previously reported in the literature (Yao et al., 2012), and bacteria need oxygen for their activities, the effects of oxygen supply (aeration) and solution pH on the phosphate removal by bamboo charcoal are also examined.

METHODOLOGY

Material Used for the Experiments

Bamboo charcoal (Fig. 1a) and bamboo powder (Fig. 1b) are readily available products. The bamboo charcoal was passed through 3.75 cm and 5.6 mm-mesh sieves. Only the residual on 5.6 mm-mesh sieve was used in experiments, i.e., the diameter range of 0.56-3.75 cm. Two kinds of bamboo charcoal were prepared, i.e., without pretreatment and dissolved in tap water (50 g of bamboo charcoal in 500 mL of water). For the bamboo charcoal dissolved in tap water, the solution was replaced until no further release of phosphate was observed. The bamboo powder was fermented for four days, and then the supernatant (hereafter, called bamboo supernatant) was extracted and used in the experiments.

(a) Bamboo charcoal (b) Bamboo powder

Fig. 1 Bamboo charcoal and bamboo powder used in the experiments

Experimental Procedures and Measurements

First, approximately 50 g of bamboo charcoal was placed in a LAB solution for 24 h to attach LAB to the bamboo charcoal. Then, the bamboo charcoal was placed in the bamboo supernatant to assess the phosphate removal. The LAB solution was produced by mixing 50 mL of LAB beverage with 1 L of deionized water. The experiments were conducted with and without aeration to examine the effects of oxygen on LAB for phosphate removal. The experiments were conducted in a room with the ambient temperature range of 25-30℃.

Temporal measurements of the solution pH, redox potential (ORP), and phosphate concentration were conducted. The pH and ORP were directly measured by placing a pH/ORP meter (Horiba, D-73) into the solution. The solution in the bamboo charcoal layer was extracted using a syringe, and the phosphate concentration in the extracted solution was measured using a digital Packtest (Kyoritsu, DPM2-PO4-D). A summary of these experimental procedures is shown below in Fig. 2. A duplicate measurement was conducted, and the average value was used for discussion. Phosphate concentration, pH and ORP were measured with relative errors of 0.1 mg/L, 0.1, and 1 mV, respectively.

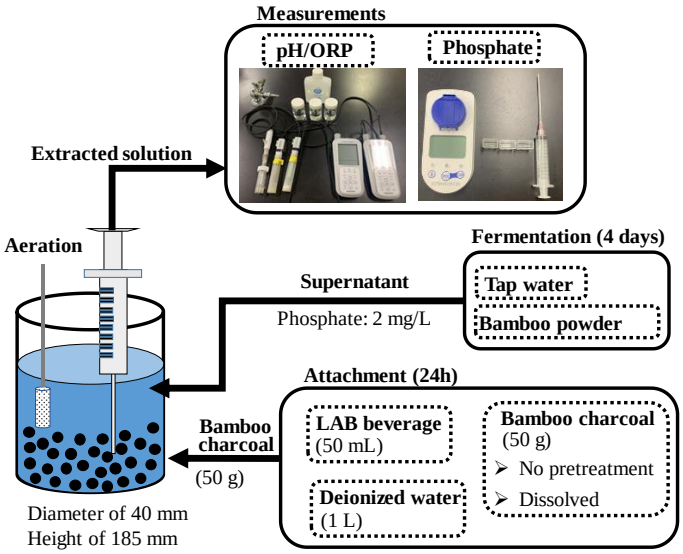


Fig. 2 Summary of experimental procedures

RESULTS AND DISCUSSION

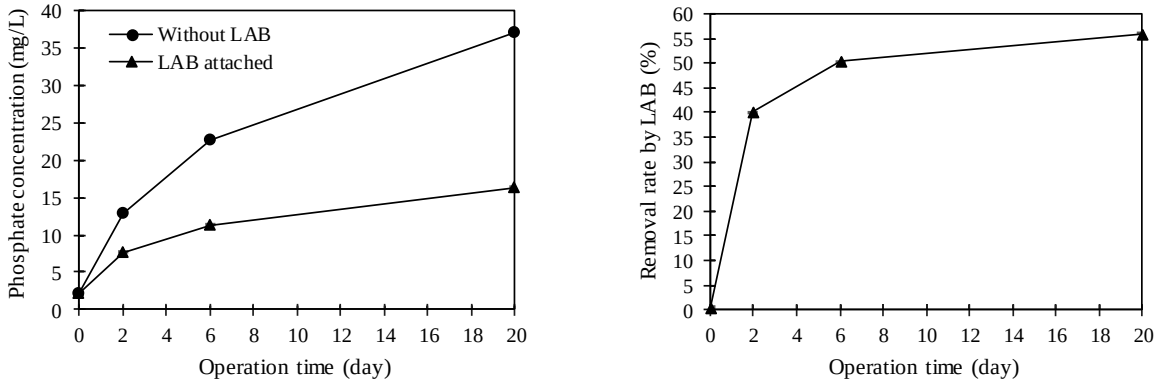
Temporal Changes in Phosphate Concentrations (Bamboo Charcoal Without Pretreatment)

Figure 3 shows the temporal changes of phosphate concentration when using bamboo charcoal (no pretreatment) without aeration. The phosphate concentration in the bamboo supernatant was approximately 2 mg/L, and increased to 37 mg/L at day 20 after the experiment started (Fig. 3a). This increase indicates a release of phosphate from the bamboo charcoal, which is in good agreement with the findings by Jung et al. (2015).

When LAB-attached bamboo charcoal was used, the increase in the phosphate concentration was smaller compared to the bamboo charcoal without LAB. This indicates phosphate removal by LAB. Even though the LAB is attached to the bamboo charcoal, they effectively remove phosphate from the aqueous solution. These results suggest that LAB-attached bamboo charcoal can be an absorbent for phosphate removal.

The removal rate of phosphate by LAB was 40% at day 2, 50% at day 6, and 56% at day 20, see Fig. 3b. These removal rates are on the same scale as conventional biological methods, which remove 20-40% of phosphorus (Ruzhitskaya and Gogina, 2017). Furthermore, it can be inferred from Fig. 3b that the removal capacity rapidly decreased, as it was 40% at day 2, but only increased by

10% from day 2 to day 6 and by 6% from day 6 to day 20. It is likely that the metabolic activity or the bacteria growth was limited due to the lack of oxygen. Without aeration, the ORP decreased significantly at day 20 when using LAB-attached bamboo charcoal to -300 mV vs. Ag/AgCl (no oxygen in water), while the ORP was maintained at 190 mV when using bamboo without LAB. Moreover, the solution pH was 7 when using LAB-attached bamboo charcoal but climbed to 9 when using bamboo charcoal, indicating that the dissolution of bamboo charcoal increases the solution pH. In summary, these results indicated that pretreatment and aeration are necessary when using LAB-attached bamboo charcoal for phosphate removal.



(a) Phosphate concentration (b) Removal rate of phosphate by LAB

Fig. 3 Changes over time of phosphate concentration and removal rate of phosphate by LAB using bamboo charcoal (no pretreatment) without aeration

Temporal Changes in Phosphate Concentrations (Dissolved Bamboo Charcoal)

Based on the findings shown in Fig. 3, pretreatment of the bamboo charcoal and aeration are both needed for phosphate removal. Figure 4 shows the temporal changes of phosphate concentration when using dissolved bamboo charcoal with aeration. The phosphate concentration of the bamboo supernatant was approximately 2 mg/L and slowly increased to 12 mg/L on day 13 after the experiment began (Fig. 4). In comparison with Fig. 3a, the release of phosphate from the bamboo charcoal was strongly suppressed due to the dissolution of bamboo charcoal before its use in the experiments. This indicates that bamboo charcoal without pretreatment is not an effective absorbent for phosphate removal.

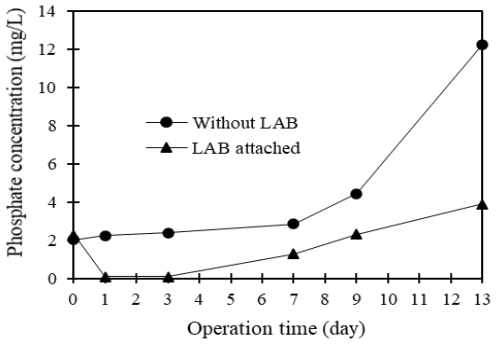


Fig. 4 Temporal changes of phosphate concentration using dissolved bamboo charcoal with aeration

When using LAB-attached bamboo charcoal, a decrease in the phosphate concentration was observed, indicating phosphate removal by LAB. Up to 95% of phosphate was removed during the first 3 days due to a low initial concentration of phosphate. According to Fig. 3a, LAB removed 5 mg/L of phosphate on day 2 and 11.5 mg/L on day 6. Interestingly, according to Fig. 4, LAB could

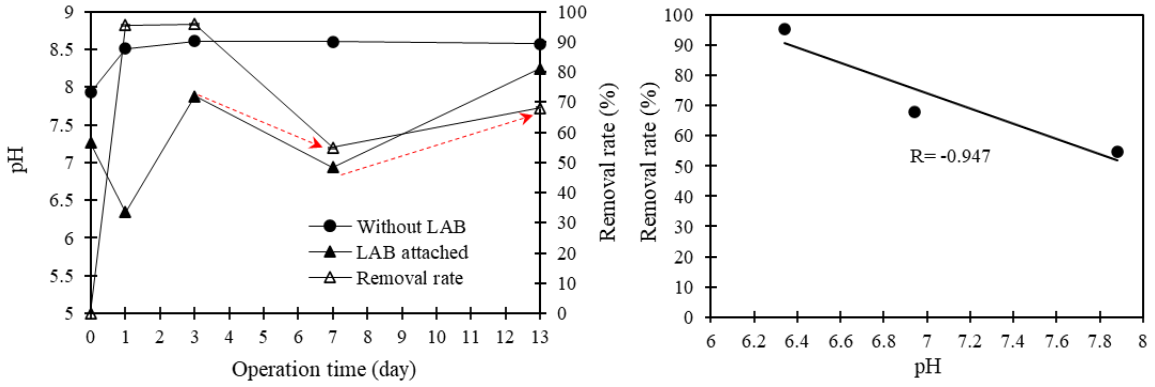
not completely remove the 2.8 mg/L of phosphate produced on day 7; however, it was able to remove 8.3 mg/L of phosphate on day 13. As bacteria are involved in this reaction, it is believed from these results that pH and dissolved oxygen (DO) concentration may also affect phosphate removal.

Effects of DO Concentration and pH on Phosphate Removal

By providing aeration, the ORP was maintained at an oxidizing potential of 275 mV when using bamboo charcoal without LAB, and the ORP was 245 mV with LAB-attached charcoal on day 13. The slightly lower ORP was observed for LAB-attached charcoal, indicating a small oxygen consumption by LAB. In other words, DO was enough for bacteria respiration when aeration was provided, which was considered to be less effect on the bacteria's activity. In addition, it is known that LAB is more active in acidic conditions; however, the dissolution of bamboo charcoal increases the solution pH, potentially influencing the bacteria's activity.

Figure 5 depicts the temporal changes in the solution pH when using dissolved bamboo charcoal with aeration. The dissolution of the bamboo charcoal increased the solution pH with and without LAB; however, the pH was lower with LAB-attached bamboo charcoal (Fig. 5a). This was attributed to the activities of LAB which released lactic acid. The data from Fig. 5a indicate that the solution pH was 6.3 at day 1, which induced the high phosphate removal rate of 95% on day 3. The solution pH increased from 6.3 on day 1 to 7.9 on day 3, and then decreased to 6.9 on day 7. Although the solution pH decreased from day 3 to 7, the pH value of 7.9 influenced the removal rate from day 3 to 7, i.e., a decrease in the removal rate to 55% was observed on day 7.

Similarly, a pH value of 6.9 on day 7 led to an increase in the removal rate to 68% on day 13. It can be understood from these results that the removal rate is dependent on the solution pH. By plotting the removal rate versus the solution pH (Fig. 5b), it can be observed that the removal rate and pH were strongly correlated (R=-0.947), as expected. These results indicate that the solution pH should be maintained in an acidic state (lower than 6.5) to achieve a higher removal rate.



(a) Temporal changes in pH and removal rate (b) Relationship between pH and removal rate

Fig. 5 Relationship between pH and removal rate

CONCLUSION

In this study, the potential of bamboo charcoal as a microbial carrier for LAB was evaluated, and its ability to remove phosphate was investigated. The effects of oxygen supply and solution pH on the phosphate removal by bamboo charcoal were also examined. Without aeration, a negative redox potential (ORP) was observed, indicating a large oxygen consumption by LAB. Thus, aeration is needed when using LAB. Even with dissolved bamboo charcoal, the release of phosphate was observed, indicating that bamboo charcoal alone is not an effective absorbent for phosphate removal. When LAB-attached bamboo charcoal was used, a decrease in the phosphate concentration was observed. This suggests that LAB-attached bamboo charcoal is an effective adsorbent for phosphate removal. With aeration, the ORP was maintained in an oxidizing state, which had no impact on

phosphate removal. However, the removal rate was found to depend on pH, where the removal rate decreased with an increase in the solution pH. Therefore, the phosphate removal should be conducted under acidic conditions (pH lower than 6.5) to obtain a higher removal rate.

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