Characterization of Biochar Prepared from Cotton Stalks as Efficient Inoculum Carriers for *Bacillus subtilis SL-13*

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Biochar has the potential for use as a carrier of plant growth promoting rhizobacteria. The biochar in this experiment was produced by the pyrolysis of cotton stalks at high temperatures. Fourier transform infrared spectroscopy (FTIR) and an elemental analyzer characterized the structure and composition of the biochar, while scanning electron microscopy (SEM) observed the relationship between the bacteria and biochar. This study investigated the effects of biochar on the growth of *Bacillus subtilis SL-13*, a plant growth promoting rhizobacteria, as well as the adsorption of *B. subtilis SL-13* to biochar under different conditions. The addition of biochar in a liquid nutrient broth medium was thought to promote the growth of *B. subtilis SL-13* bacteria. The SEM images showed that the bacteria entered into the tubular structure of biochar. The adsorption of bacteria onto biochar increased with decreasing biochar particle size. The *B. subtilis SL-13* population in biochar was maintained at 10^6 colony forming units g^{-1} (CFU) of biochar for up to 120 d. Therefore, the biochar could provide a beneficial microenvironment for the slow release and prolonged survival of bacteria as a carrier in future practical applications.

Keywords: Biochar; Plant growth promoting rhizobacteria; Carrier; Environmentally friendly

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INTRODUCTION

Chemical fertilizers and pesticides are the most widely used fertilizers and disease control preparations in agriculture today. However, there are many environmental and health problems associated with their long-term application (Rashid and Romshoo 2013; Ozekeke *et al*. 2015). The continuous application of chemical fertilizer causes soil compaction and the reduction of organic matter and other nutrients, leading to the deterioration of soil quality. The excessive use of pesticides causes pesticide residues to remain on the surface of agricultural products. When pesticides are in soil, they can infiltrate into the groundwater through soil leaching, causing serious pollution to water bodies and posing a great threat to human health (Clevo and Clem 2001; Mahboob *et al*. 2015).

The introduction of plant growth-promoting rhizobacteria (PGPR) in agriculture not only can alleviate the harm caused by chemical fertilizers and pesticides, but also it can improve the soil and the environment for plants (Holguin *et al*. 2001). The PGPR produces substances that either promote crop growth or prevent diseases and insect pests, such as 3-indoleacetic acid (a growth promoting agent), chitinase (pathogen inhibition), and others (MacMillan 2001; Spaepen *et al*. 2008).

Continuously planting field crops can result in a large number of plant pathogens in plant residues and cultivated soil, and the presence of those bacteria can seriously affect healthy plant growth. The introduction of PGPR can antagonize pathogens in soil (Mills and Bever 1998), inhibit the growth of soil pathogens, and greatly improve the plant growth conditions of the soil. Soil organic matter content is an important indicator of soil fertility. While most organic matter relates to soil microbial activity and metabolism, the growth and metabolism of PGPR in soil can increase the organic matter content (Cervantes *et al.* 2003). Many studies of PGPR have been conducted on *Trichoderma* (Koch 1999), *Streptomyces* (Beyer and Diekmann 1985), *Agrobacterium* (Kerr and Htay 1974), *Bacillus* (Boer *et al.* 2003), and *Pseudomonas* (Schippers 1993).

In this paper, *B. subtilis SL-13* was used, which was isolated from the root of tomatoes. The strain *SL-13* promotes plant growth and disease control in pot field experiments, and the chitinase produced by *B. subtilis SL-13* plays an important role in pathogen inhibition (Liu *et al.* 2011). Therefore, *B. subtilis SL-13* might have wide prospects for field application. However, the response of inoculated bacteria to environmental change is very sensitive. The bacteria are vulnerable to environmental changes such as temperature, humidity, complex soil composition, acidity, alkalinity, competition between soil-borne microorganisms, and protozoan predation (He *et al.* 2016). Thus, problems, such as unstable growth of bacteria, unstable metabolism, short survival period, low number of viable bacteria, and short application time, have been observed during the production and application of microbial inoculants, greatly limiting the actual popularization and application of microbial inoculants (Wu *et al.* 2014a, 2014b). Therefore, maintaining high bacterial content in soil is a major premise for the application of PGPR.

Biochar is a pyrolysis product of biomass produced at a low oxygen content and a high temperature, and it generally has a high carbon content, a tubular structure similar to graphene, a good adsorption effect, and high cation exchange capacity (CEC) properties (Gul *et al.* 2015; Xu *et al.* 2016; Waqas *et al.* 2017). Biochar can promote carbon fixation in soil and reduce the emissions of greenhouse gases in the atmosphere (Fang *et al.* 2014). The special structure of biochar can also provide the living space for soil microorganisms (Waqas *et al.* 2017). Biochar has been reported as one of the better carriers for the inoculation of PGPR (Hale *et al.* 2015; Hansen *et al.* 2017; Nguyen *et al.* 2017), as it can stimulate growth and activity in microorganisms. The inoculation of PGPR into the carrier creates favorable planting conditions for agricultural products, and the slow release effect of the inoculated carrier can also be valuable for long-term biological control (Abujabhah *et al.* 2016).

In this paper, two samples of biochar were prepared from the pyrolysis of cotton stalks at 400 °C and 600 °C; the structure and elemental content of the biochars were analyzed by scanning electron microscopy (SEM), elemental analysis, and a Brunauer-Emmett-Teller (BET) analysis of the specific surface area. The relationship between biochar and microorganisms and the effects of biochar on the growth and reproduction of microorganisms were also studied. At the same time, different biochars were used as inoculation carriers of *B. subtilis SL-13* to study the loading effect of biochar on bacteria under varied conditions, as well as the release effect and their preservation after inoculation. The optimum conditions and raw materials for the preparation of biochar-based microbial agents were obtained.

EXPERIMENTAL

Materials

Bacterial strain and medium

B. subtilis SL-13 (GenBank accession number EF508705) was isolated from processed tomato fields (Xinjiang, China). The strain was cultivated at 30 °C, 170 rpm in 50 mL of nutrient broth (NB) consisting of 10 g L^{-1} of tryptone (Aoxing Bio-Tech Ltd., Beijing, China), 5 g L^{-1} of beef extract (Aoxing Bio-Tech Ltd., Beijing, China), and 5 g L^{-1} of sodium chloride (Yongshen Ltd., Tianjin, China). The pH of the medium was maintained between 7.0 and 7.2. The obtained culture suspension was used as the seed broth of *B. subtilis SL-13*.

Two percent of the seed broth was cultivated in 50 mL of NB medium in shaking flasks at 170 rpm and 30 °C for 24 h. The cells were then washed twice with sterile 0.85% NaCl solution using centrifugation at 6000 rpm and 4 °C for 30 min. The *B. subtilis SL-13* suspension obtained by suspending washed cells in 10 mL of sterile 0.85% NaCl solution was used for subsequent inoculation onto biochar. The viable cell count of this cell suspension was determined by dilution plating onto nutrient agar (NA) plates for counting colony forming units (CFU). The viable cell counts of bacterial suspensions prepared in this way were in the range of 8 $\log_{10}$CFU mL^{-1} to 9 $\log_{10}$CFU mL^{-1}.

Pulverized cotton stalks were used to prepare biochar materials *via* slow pyrolysis in a tube furnace with N_2 gas. The biochars were pyrolyzed for 2 h at temperatures of 400 °C and 600 °C, and once prepared they were marked as BC400 and BC600.

Preparation of biochars

Cotton stalks, the feedstocks of the biochar, were provided by the College of Agriculture of Shihezi University (Shihezi, China) and were pulverized by universal high-speed smashing machines. The pulverized cotton stalks were used to prepare biochar materials *via* slow pyrolysis in a tube furnace (LTKC-4-10A, Lantian, Hangzhuo, China) while being passed over N_2 gas. The prepared biochars were placed for 2 h at a time at a temperature of either 400 ºC or 600 ºC and were marked as either BC400 or BC600 accordingly.

Methods

Characterization of biochar

The surface and cross-section morphologies of the biochar samples were studied using a scanning electron microscope (JSM-6490LV, JEOL Ltd., Tokyo, Japan). The samples were dried and adhered to sample holders with carbon LIT-C glue, and their surfaces were covered with a thin layer of gold to prevent the collapse of the biochar porous structure. The specific surface areas were determined using the BET N_2 method on an ASAP 2020 Physisorption Analyzer (Micromeritics, Atlanta, GA, USA). The FTIR characteristic peaks of the biochar before and after adsorption of bacteria were determined by an infrared spectroscopy analyzer (IS10, Thermo Fisher Scientific, Waltham, MA, USA). The components of the biochar were determined by an elemental analyzer (Vario EL Cube, Elementar, Hanau, Germany).

The effects of biochar on bacterial growth curve

B. subtilis SL-13 cultures were grown in 50 mL of NB with 0.02 g of BC400 or BC600 at 30 °C, 170 rpm for 60 h. Liu and researchers obtained the best culture method for *B. subtilis SL-13* at 30 °C, 170 rpm for 1 d to 2 d (Liu *et al.* 2011). Thus, to further study the effect of biochar on the growth of *B. subtilis SL-13*, the authors extended the incubation time to 60 h for *B. subtilis SL-13*. Viable counts of growing bacteria were measured by a dilution plate counting on NA plates at different time intervals. *B. subtilis SL-13* cultures were also grown in NB without biochar as a control.

Inoculation procedures of biochar in different inoculation conditions

A-50 mL conical flask containing 10 mL of 0.85% NaCl cell suspension was incubated by shaking at 30 °C for 1 h, 6 h, 12 h, and 24 h with 1 g biochar (40-mesh to 60-mesh). Aliquots (100 µL) of the cell-biochar mixture were serially diluted, inoculated onto NA plates, and incubated at 28 °C for 24 h to 48 h to determine cell counts. In all experiments, the cell numbers were determined by plate count methods, and the number of adsorbed *B. subtilis SL-13* cells in biochar samples was calculated by subtracting the number of bacteria remaining after adsorption from the initial number of bacteria in suspension. All experiments were conducted with three replicates.

To determine the effect of different biochar particle size (40- to 60-mesh, 100- to 120-mesh, and 160- to 180-mesh) on its adsorption capacity, 10 mL of bacterial suspension was incubated with 1 g of biochar of different particle sizes at 170 rpm and 30 °C for 24 h on a rotary shaker.

To explore the effect of different mixing ratios of biochar and suspension on the inoculation of biochar, ratios of 20:1, 10:1, and 5:1 of suspension:biochar (v/w) with 40-mesh to 60-mesh biochar were shaken at 170 rpm.

The effect of the shaking speed on adsorption was determined. A total of 10 mL aliquots of bacterial suspension with 1 g biochar (40-mesh to 60-mesh) were shaken at 80 rpm, 130 rpm, and 180 rpm for 24 h.

The optimal adsorption conditions of *B. subtilis SL-13* to biochar were determined from the results of all of the above experiments.

Shelf life determination of biochar inoculum

Glass test tubes containing 10 g of the inoculated biochar were incubated at 4 °C for 120 d in triplicate. The survival of *B. subtilis SL-13* was measured by suspending 1 g of biochar inoculum in 9 mL of sterile distilled water and mixing thoroughly to ensure complete separation of the bacteria from the biochar. Bacterial survival in the biochar inoculum was measured at several time intervals. Bacterial cell counts in the distilled water suspension were serially diluted and inoculated onto NA plates in triplicate for enumeration of colony counts. The plates were then incubated at 28 °C for 24 h to 48 h, and the colony numbers were counted and recorded.

Release of biochar inoculum

A total of 2 g of biochar inoculum were added into a 100-mL conical flask with 20 mL sterile 0.85% NaCl and the mixture was shaken at 20 °C for 60 d. Aliquots of solution were taken out at different time intervals to determine the population of *B. subtilis SL-13*. The cell numbers were determined by plate count methods as described above, and all of the experiments were conducted with three replicates.

RESULTS AND DISCUSSION

Elemental Analysis and BET Surface Analysis of the Biochar

There were considerable differences in the carbon contents of BC400 and BC600, with BC600 having a higher content (Table 1). The biomass of cotton stalks possessed a higher degree of carbonization at 600 °C than at 400 °C because most of the aliphatic chains and carbohydrates in the cotton stalks were cracked at the higher temperature. This finding was in good agreement with previous reports that the carbon content of biochar was further concentrated and the removal of water was more intense with increasing temperatures of pyrolysis (Mohan *et al.* 2014). Keiluweit and researchers reported that the oxygen and hydrogen contents decreased with increasing pyrolysis temperature, and a series of carbon chain reactions, including dehydrogenation, dehydration, and decarboxylation reactions, occurred during the process of biomass pyrolysis (Keiluweit *et al.* 2010).

The average pore size of BC600 was greater than that of BC400, while the BET specific surface area of BC600 was lower than that of BC400 (Fig. 1). This may be due to the further crystallization of certain components in the biomass at higher temperature, resulting in the blockage of pores and a decrease in the specific surface area (Antal, Jr. and Grønli 2003). Therefore, while it was concluded that the surface structure of biochar was beneficial for adsorption, the pore width distribution of BC400 and BC600 indicated that the adsorption effect of BC400 might have been better than that of BC600.

Table 1. Elemental Composition and BET Area of BC400 and BC600

Materials	C (%)	N (%)	H (%)	S (%)	O (%)	BET Surface Area (m^2 g^{-1})	Pore Opening Diameter (nm)	Total Pore Volume (m^2 g^{-1})
BC400	72.25	00.97	30.39	00.074	23.32	39.515	3.417	0.0351
BC600	78.25	00.63	20.12	00.09	18.91	24.864	6.289	0.0189

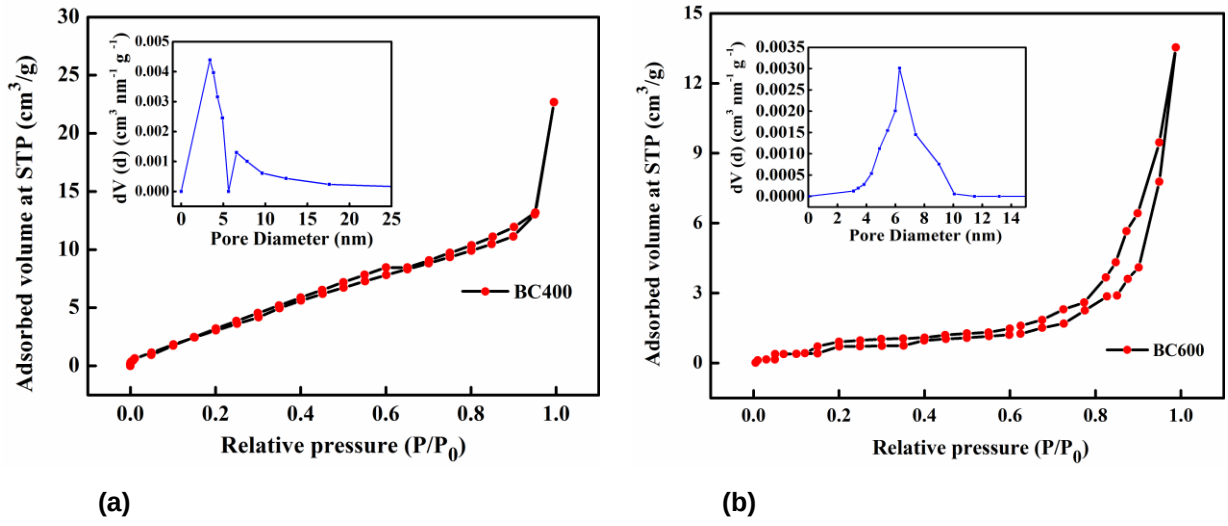


Fig. 1. Nitrogen adsorption–desorption isotherm and pore size distribution curves (inset) of BC400 (a) and BC600 (b); STP- the nitrogen adsorption and desorption process of biochar at standard temperature and pressure

Biochar Effects on Bacterial Growth Curve

To investigate the effects of biochar on the growth of *B. subtilis SL-13*, the bacterial growth was measured by adding biochar into the bacterial suspension. As shown in Fig. 2, bacteria in the broth inoculated with biochar grew within 6 h, which was much quicker when compared to the bacteria without the addition of biochar. Both the bacterial logarithmic growth periods of the culture solutions with BC400 and BC600 addition were accelerated, and the growth rates of bacteria in both experimental solutions were greater than that of the control. The maximum concentration of bacteria in the broth with BC400 and BC600 was slightly higher than in the treatment without biochar. Biochar positively influenced microbial growth and reproduction in the logarithmic phase. These results demonstrated that biochar was not harmful to bacteria and instead increased bacterial growth rates in the culture solution, indicating that biochar provided nutrients for bacterial growth (Sun *et al.* 2015). The growth curves of *B. subtilis SL-13* with either BC400 or BC600 addition were similar, which suggested that both of these biochar materials were suitable carriers for the inoculation of *B. subtilis SL-13*.

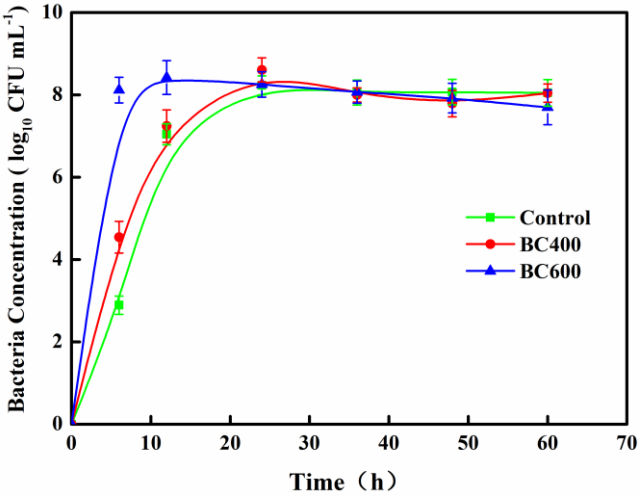
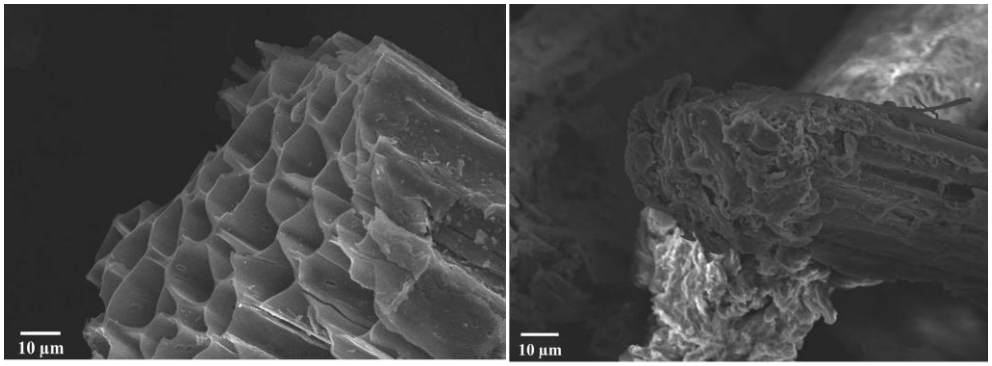


Fig. 2. Effect of BC400 and BC600 samples on growth curve of *B. subtilis SL-13*

SEM of Biochar With and Without *B. Subtilis SL-13*

The appearance of biochar was observed by SEM, which showed that the tubular structures were densely distributed in the biochar, and the average tube diameter was approximately 10 µm. For comparison, the size of a single cell of *Bacillus subtilis* is 0.8 µm × 1.5 µm to 1.8 µm (Buchanan and Gibbons 1984) and the tubular diameter of biochar was far greater than the bacterial length. In addition, the side of the surface structure of biochar can adsorb *B. subtilis SL-13* cells and provide a space for microorganisms to grow and reproduce (Lehmann *et al.* 2011).

The bacteria might be gathered in the biochar tubular structure and on the lateral folds, which is conducive to the release of bacteria into the soil environment (Lehmann *et al.* 2011). The bacteria were mostly attached in the tubular structure of biochar, with some being attached on the surface of biochar (Fig. 3). The tubular structure and lateral folds of biochar were suitable for the adsorption of bacteria, therefore biochar is potentially a suitable carrier of PGPR for agricultural applications.



(a) (b)

Fig. 3. SEM of biochar BC400 (a) and BC400 loaded with *B. subtilis SL-13* (b)

FTIR of Biochar With and Without *B. Subtilis SL-13*

The FTIR spectrum of biochar and biochar-loaded bacteria is demonstrated in Fig. 4, with characteristic peaks for the radical induced stretching vibration of hydroxyl appearing at 3431 cm^{-1} and 3427 cm^{-1} in BC400 and BC600, respectively. Another two peaks appeared at 1637 cm^{-1} and 1380 cm^{-1} in BC400 and at 1633 cm^{-1} and 1380 cm^{-1} in BC600 for the stretching vibrations of carbonyl and carboxylate, respectively. A new vibration peak arose at 1560 cm^{-1} and 1562 cm^{-1} with *B. subtilis SL-13* inoculation onto BC400 and BC600, respectively, corresponding with the in-plane bending vibration of amide II and the amino acid substance that usually forms a characteristic peak around this location (McWhirter *et al.* 2002). The peak around 2920 cm^{-1} in BC400 and BC600 was the antisymmetric stretching vibration induced by methylene. Different peaks appeared at 828 cm^{-1} and 829 cm^{-1} for the stretching vibration of Si-O in BC400 and BC600, respectively.

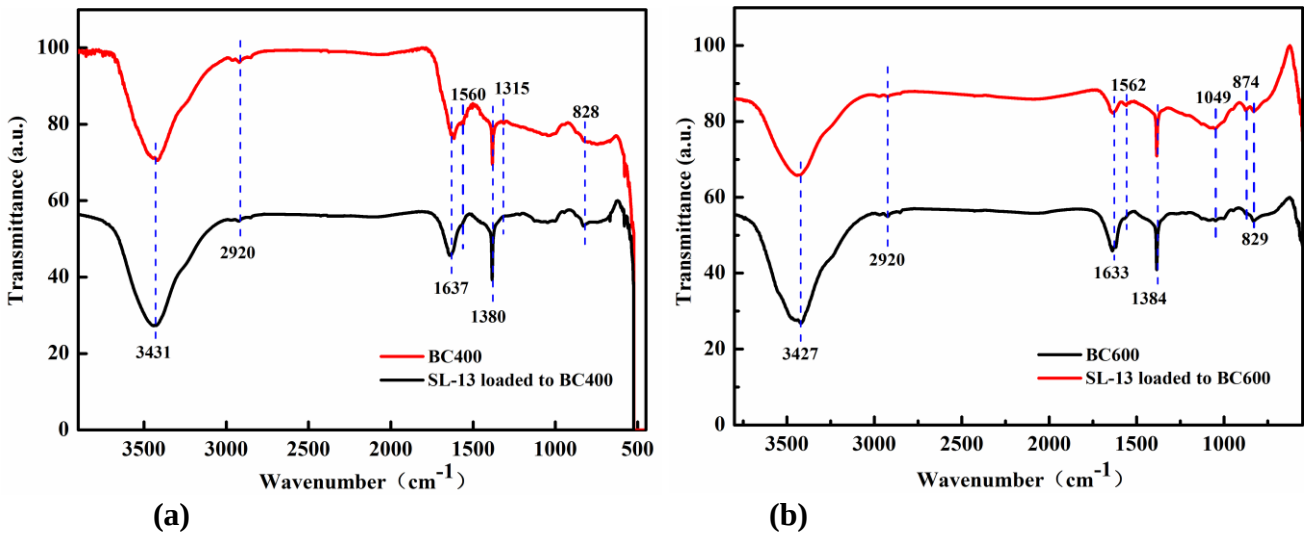


Fig. 4. FTIR analysis of biochar and *B. subtilis SL-13* inoculated to BC400 (a) and BC600 (b)

Substances containing Si-O bonds increased with the increase in pyrolysis temperature of biomass. However, these substances might have blocked the pores and led to a decrease in the surface area of biochar, which explained why the peak in BC600 was more prominent when compared with that in BC400. The peak of BC600 at 1315 cm^{-1}

corresponded with the symmetrical stretching vibration of carboxylic acid radical. A peak appeared at 1049 cm^{-1} in BC600, which corresponded with the stretching vibration produced by the phosphate group in the cell wall. *B. subtilis SL-13* is a Gram positive bacteria, and the cell wall is composed of abundant peptidoglycan and certain proportions of phosphoric acid, uronic acid, and protein (Hammond *et al.* 1984). The characteristic peaks of carboxyl groups, phosphate groups, and amino groups still existed in the biochar loaded bacteria as they did in the original biochar. In summary, the characteristic peaks of biochar loaded with bacteria did not substantially change when compared with the original biochar.

The Colony Numbers of *B. Subtilis SL-13* on Biochar Under Different Inoculation Times

The number of bacteria adsorbed into biochar increased over the 24 h period, with most of the *B. subtilis SL-13* cells adsorbing onto biochar within the first hour. The adsorption rate for *B. subtilis SL-13* on biochar slightly decreased after 1 h (Fig. 5). However, some bacteria were still adsorbed onto biochar in the period from 1 h to 24 h, and the adsorption rate for bacteria was relatively stable. The optimal absorption of *B. subtilis SL-13* in suspension onto biochar was obtained with the incubation time of 24 h.

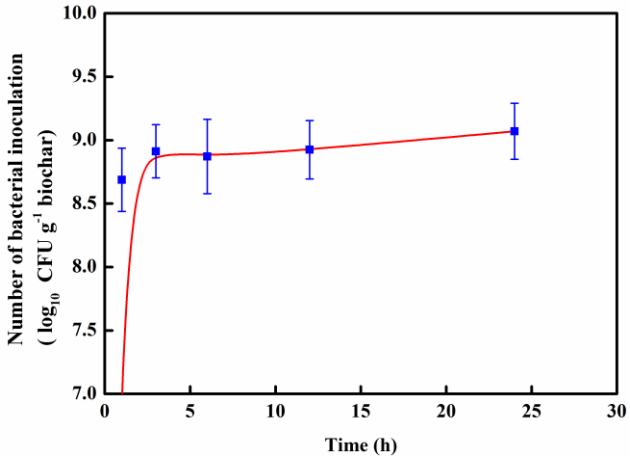


Fig. 5. The amounts of bacteria loaded on BC400 at different shaking times

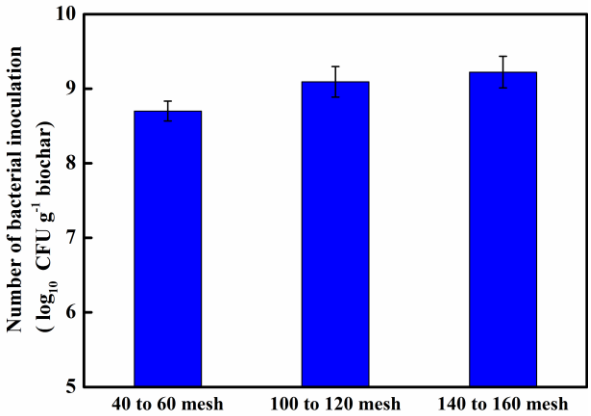


Fig. 6. The adsorption amount of biochar BC400 with different particle sizes (initial bacterial concentration: log_{10} CFU mL^{-1} = 9.25)

Effect of Different Particle Size of Biochar on its Adsorption Capacity

Biochar screened with sizes of 46-mesh to 60-mesh, 100-mesh to 120-mesh, and 140-mesh to 160-mesh were used to adsorb *B. subtilis SL-13* bacteria. The relationship between biochar particle size and the adsorption efficiency of biochar is shown in Fig. 6. The numbers of bacteria adsorbed onto biochar increased as the particle size decreased. This might have been because smaller particles increased the contact area between the biochar and bacterial cells, which further increased the adsorption capacity of bacteria onto biochar. This result indicated that the contact area between the biochar and the bacterial cells was one of the most important factors in the adsorption process.

Effect of Different Ratios of Bacterial Suspension and Biochar for Adsorption Capacity

The adsorption amount of *B. subtilis SL-13* onto biochar was the highest at the ratio of 20:1. This was because the dispersion of biochar in bacterial suspension was better when smaller quantities of biochar were added. The adsorption amount of biochar increased with decreasing amounts of biochar in bacterial suspension, and this was consistent for both BC400 and BC600. The differences in absorption between ratios were not noticeably different. The optimal absorption of bacteria onto biochar was obtained with a bacterial suspension to biochar (v/w) ratio of 5:1.

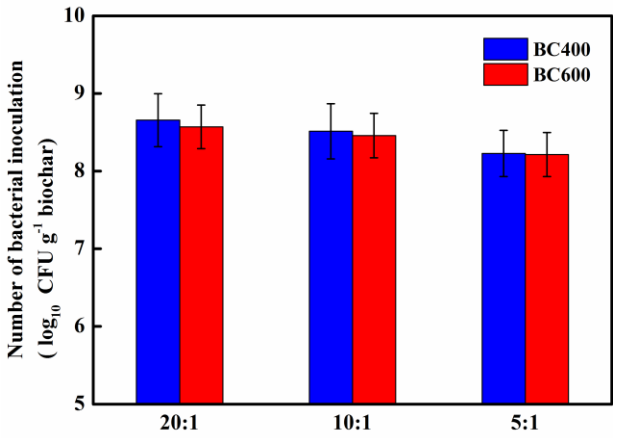


Fig. 7. Effect of ratio of different bacterial suspension and biochar on adsorption (initial bacterial concentration: $\log_{10}$ CFU mL^{-1} = 8.67)

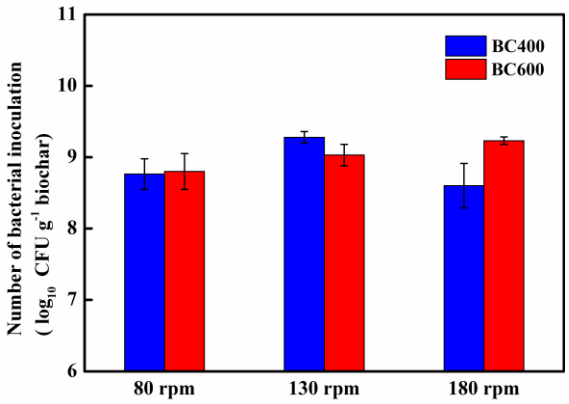


Fig. 8. Effect of different rotating speed on adsorption (initial bacterial concentration: $\log_{10}$ CFU mL^{-1} = 9.36)

Influence of Different Mixing Speed on Loading of Biochar for *SL-13*

The adsorption of bacteria in suspension onto biochar under three different shaking speeds is shown in Fig. 8. Increasing the shaking speed from 80 rpm to 130 rpm led to increased absorption for both biochars, whereas the increase from 130 rpm to 180 rpm did not lead to any further increases in adsorption. This might have been because at low speeds (*i.e.*, 80 rpm), there was insufficient mixing of biochar and bacteria for optimal adsorption, whereas at higher speeds the bacteria were unable to effectively attach to the biochar. The optimal bacterial adsorption for both biochars was obtained at 130 rpm.

Survival and Release of the Inoculum

The release of *B. subtilis SL-13* from BC400 and BC600 inoculated biochar in physiological saline solution over 60 d is shown in Fig. 9. The release curves were different for the different biochar inoculums. The surface-attached bacteria of the inoculated biochar quickly entered into suspension, which led to a certain bacterial concentration in the early release phase. Bacteria released from the BC400 inoculum decreased to the lowest value at 5 d, a phenomenon that might have been caused by the death of the surface-attached bacteria after entering into suspension. Thereafter, bacteria from the tubular structures of BC400 entered into suspension and reached the maximum number of bacteria in suspension at 30 d. The number of bacteria in suspension decreased during the time period of 30 d to 60 d, possibly because the death rate of bacteria in suspension was greater than the release rate of bacteria from the BC400 inoculum. In contrast, the number of bacteria in suspension with the addition of the BC600 inoculum increased up to the maximum at 5 d ($\log_{10}$ CFU mL^{-1} = 7.9). This suggested that the bacteria on the surface and in the tubular structures of BC600 were released into solution at the same time. Thereafter, the number of bacteria in suspension steadily decreased during the period of 5 d to 16 d, and then slightly increased in the period 16 d to 30 d as there was still a small number of bacteria released from BC600. The rate of bacterial decline in suspension with the BC600 inoculum began to increase and was greater than that of BC400 in the period 30 d to 60 d. Therefore, both BC400 and BC600 are suitable inoculation carriers for bacteria. The release rate of bacteria from BC600 was faster than that from BC400, which indicated that BC400 is more useful as a slow release carrier for bacteria.

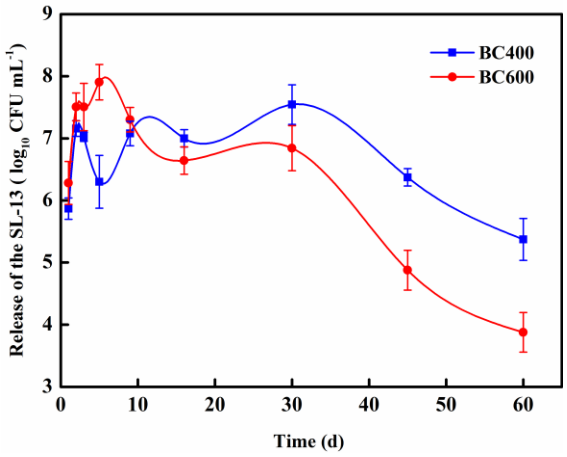


Fig. 9. The release of two kinds of biochar-based microbial formulations within 60 d (the number of *B. subtilis SL-13* inoculated to biochar: $\log_{10}$ CFU g^{-1} BC400 = 9.10, $\log_{10}$ CFU g^{-1} BC600 = 8.96)

Preservation of Microbial Biochar Formulations

The survival of *B. subtilis SL-13* in BC400 and BC600 was determined over 120 d. The number of viable bacteria in biochar was relatively higher than that in bacterial suspension, with viable counts of 10^7 CFU mL^{-1} to 10^8 CFU mL^{-1} of free *B. subtilis SL-13* in the bacterial suspension without biochar. The survival of *B. subtilis SL-13* in biochar and free bacterial suspension consistently decreased over 120 d. The rate of decline of free *B. subtilis SL-13* in bacterial suspension was faster than those in the BC400 and BC600 inoculants after 10 d. Moreover, the total amounts of bacteria in biochar were always larger than the free bacteria in bacterial suspension over the 120 d period (Fig. 10). The results showed that *B. subtilis SL-13* died more slowly after inoculation onto BC400 and BC600 than with *B. subtilis SL-13* in bacterial suspension. The reason for this might have been a lack of nutrients in suspension because biochar might have provided nutrients and a habitat to prolong bacterial survival. Bacterial survival was enhanced by inoculation onto biochar when compared with survival in suspension.

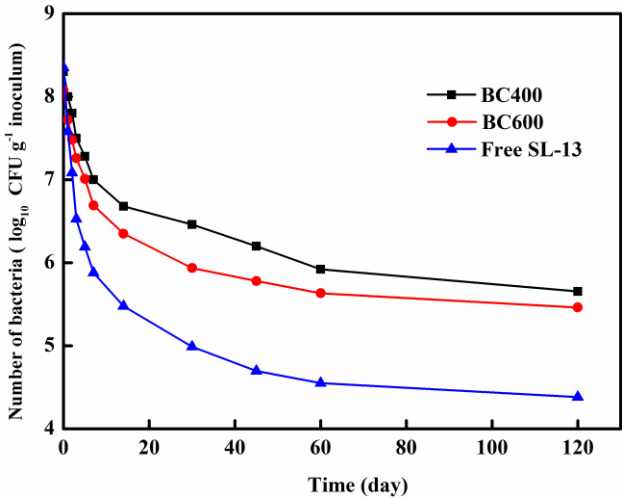


Fig. 10. The population of bacteria survivals in BC400 and BC600

CONCLUSIONS

1. The growth rate of *B. subtilis SL-13* in NB medium increased with the addition of biochar, because biochar contains nutrients for the growth of *B. subtilis SL-13*. The special porous structure of the biochar had a positive effect on the adsorption of bacteria.
2. The optimal adsorption time of biochar to *B. subtilis SL-13* in a bacterial suspension was achieved at 24 h. Biochar with smaller particle sizes and larger specific surface areas were more effective in the adsorption of bacteria. The adsorption efficiency of biochar for *B. subtilis SL-13* was highest when the rotational speed was 130 rpm. To fully utilize *B. subtilis SL-13* in the bacterial suspension, the optimum ratio value of bacterial suspension to biochar (v/w) was 5:1.
3. The FTIR analysis showed that the adsorption process may not be chemisorption. Therefore, biochar were more likely to create a beneficial microenvironment for the slow release and prolonged survival of *B. subtilis SL-13*. The results of this study

provide a theoretical basis for the use of biochar as a carrier of PGPR for future practical application.

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