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Biological and chemical phosphorus solubilization from pyrolytical biochar in aqueous solution

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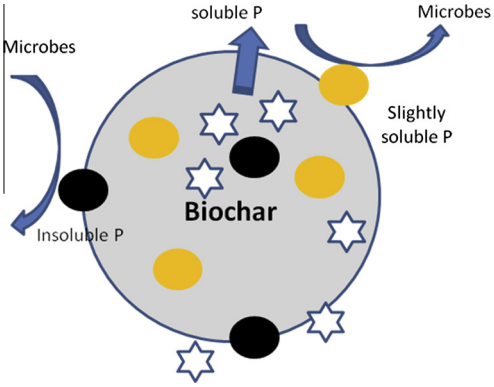
HIGHLIGHTS

- The use of pyrolytical biochar as a P source was proposed for the first time.
- The biological and chemical P solubilization from biochar was demonstrated.
- Biological effects on P release of biochar was more notable than the chemical ones.

GRAPHICAL ABSTRACT



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ABSTRACT

Biochar, a massive byproduct of biomass pyrolysis during biofuel generation, is a potential P source for the mitigation of P depletion. However, the chemical and biological effect of the release of P from biochar is still unclear. In this study, two types of Lysinibacillus strains (*Lysinibacillus sphaericus* D-8 and *Lysinibacillus fusiformis* A-5) were separated from a sediment and their P-solubilizing characteristics to biochar was first reported. Compared with the bacterial mixture W-1 obtained from a bioreactor, the introduction of A-5 and D-8 significantly improved P solubilization. The release of P from biochar by A-5 and D-8 reached 54% and 47%, respectively, which is comparable to that under rigorous chemical conditions. SEM images and XPS spectra demonstrated that the physicochemical properties of the biochar surface have changed in the process which may be caused by the activities of the microbes.

1. Introduction

As one of the means to resolving the growing global energy crisis, waste biomass such as rice husk, crop straw, and sawdust are being utilized to produce renewable liquid fuel or biopower through fast pyrolysis technique, from which about 40–50% bio-oil and 30–40% biochar can be obtained (Czernik and Bridgwater, 2004; Huber et al., 2006; Vispute et al., 2010). Waste biomass was estimated to supply 12% of the United State's renewable energy generation capacity in 2010 (Jeffers et al., 2013). Along with the ever-increasing demand for biofuel, a large amount of biochar is also produced in the process. An emerging application of biochar is in soil conditioning and CO_2 sequestration (Manya, 2012; Meyer et al., 2012; Yao et al., 2012; Delwiche et al., 2014). The properties of biochar such as water retention, pollution

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remediation, heavy metal sequestration, and carbon sequestration are well documented (Toupin and Belanger, 2008; Uchimiya et al., 2011; Yuan et al., 2011; Lehmann et al., 2011; Ennis et al., 2012; Harvey et al., 2012; Oleszczuk et al., 2012).

Phosphorus, an essential nutrient element for agricultural plants, would be used up with the ever-increasing of population. Biomass wastes such as rice straw, rice husk, and sawdust usually contain 0.15–0.95% P (Tan and Lagerkvist, 2011). The P content of biochar can increase by 2- or 3-fold during the pyrolysis process because of the P in the biomass is enriched with inorganic phosphates and pyrophosphates (Liu et al., 2011). Given the potential application of the mass production of biochar on soil, focus must be given on the fate of P in the environment.

The behavior of biochar P in soil is influenced by a number of factors, such as pH and ionic strength, but the neutral pH (pH 6.0–9.0) and low ionic strength of conventional soil can hardly influence the release of P (Silber et al., 2010). Also, biological factors should be considered so as to promote the release of P from biochar. Some microbes such as *Aspergillus niger*, *Saccharomyces cerevisiae*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Citrobacter* sp., *Burkholderia vietnamiensis*, *Acinetobacter rhizosphaera*, and *Pantoea agglomerans* can convert the insoluble P in soil to a liable form (such as HPO_4^{2-}) that plants can use (Son et al., 2006; Patel et al., 2008; Gulati et al., 2009; Ahemad and Khan, 2010; Park et al., 2010; Vassilev et al., 2012). However, no reports were found about the P solubilization in biochar by these microbes. To find new microbes that can enhance the solubilization of slightly soluble or insoluble P, such as $CaHPO_4$, Ca_3PO_4, $FePO_4$, and $AlPO_4$, as well as their corresponding pyrophosphates, in pyrolytical biochar should be practically important.

In our previous work, we studied the effect of chemical factors such as time, nutrient cations, and anions on the release of P from biochar. We found that <50% P in biochar can be released under natural environmental conditions (Qian et al., 2013). The present study aimed to isolate P-solubilizing microbes from a sediment sample and investigate their effectiveness in solubilizing P in biochar. The biochar was prepared from readily available rice husk, and a simplified biochar–water system was adopted to exclude the effect of complicated soil conditions. Two types of microbes (i.e., *Lysinibacillus sphaericus* D-8 and *Lysinibacillus fusiformis* A-5) were isolated from the sediment. The bacterial mixture W-1 which is compose of nitrifiers, polyphosphate accumulating organisms, and other bacteria was isolated from an enhanced biological phosphorus removal bioreactor. Lastly, the biological effects of the release of P from biochar were analyzed.

2. Materials and methods

2.1. Materials

Biochar obtained from pyrolysis of rice husk was supplied by Anhui Yineng. (China). It was rinsed with deionized water and dried prior to use. All chemicals were analytical grade and purchased from Sinopharm Chemical Reagent, China.

The composition of mineral medium is referred to the literature (Lovley and Phillips, 1988; Baron et al., 2009). The mineral medium (in g L^{-1}) contained 10 mM of 4-(2-Hydroxyethyl)-1-Piperazineethane sulfonic acid (Hepes), 0.46 of NH_4Cl, 0.117 of $MgSO_4{\cdot}7H_2O$, 0.225 of $(NH_4)_2SO_4$ and 10.0 of biochar (substituting for inorganic P source) with an initial pH 7.0. Prior to autoclaving, 10 mL of trace mineral mix (containing (in g L^{-1}) 1.5 of nitrilotriacetic acid, 0.1 of $MnCl_2{\cdot}4H_2O$, 0.3 of $FeSO_4{\cdot}7H_2O$, 0.17 of $CoCl_2{\cdot}6H_2O$, 0.1 of $ZnCl_2$, 0.04 of $CuSO_4{\cdot}5H_2O$, 0.005 of $AlK(SO_4)_2{\cdot}12H_2O$, 0.005 of H_3BO_3, 0.09 of Na_2MoO_4, 0.12 of $NiCl_2$, 0.02 of $NaWO_4{\cdot}2H_2O$, and 0.10 of Na_2SeO_4) was added (Baron et al., 2009). Vitamins (1 mL L^{-1}) (Lovley and Phillips, 1988) filtered through 0.22 μm membrane were added after autoclaving. 20 mM lactate was the sole carbon source in this study.

Two facultative anaerobic, Gram-positive bacterial strains were isolated using the repeated streak plate method on Luria–Bertani (LB) solid plates from the sediment of Shiwulihe River located in Hefei City, Anhui Province, China. Genomic DNA was extracted according to the manufacturer's instructions using a 3S DNA isolation kit for environmental samples (Shenergy Biocolor, Shanghai, China). The 16S rRNA gene fragment of the extracted DNA was amplified by PCR using a pair of universal primers: 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). Each 50 μL of PCR reaction solution contained 1 μL each of 25 mM primers, 2.5 ng of DNA template, 4 μL of 20 mM dNTPs, 5 μL of 10×Ex Taq buffer with Mg^{2+}, and 0.25 μL of Ex Taq polymerase (TaKaRa, Dalian). PCR was carried out on a DNA amplification machine (Bio-Rad Laboratories, Hercules, CA, USA) with the following profile: initial DNA denaturation at 94 °C for 5 min; followed by 30 cycles at 94 °C for 1 min, 55 °C for 0.5 min, and 72 °C for 1 min; and a final extension at 72 °C for 10 min. The PCR products were purified and sequenced by Invitrogen. *L. sphaericus* D-8 and *L. fusiformis* A-5 were identified by comparing the amplified 16S rRNA gene sequences with those sequences most similar to it in the GenBank database as suggested by BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). 5 mL liquid culture containing mixture microbes from a bioreactor was harvested (denoted as W-1), then centrifuged and washed with modified mineral medium (2.1 Materials) for three times; the last resuspended culture (1 mL) is the inoculums.

2.2. Characterization of biochar

The textural features (surface area and pore volume) of the biochar were analyzed by N_2 adsorption–desorption isotherms using a Micromeritics Gemini apparatus (ASAP 2020 M+C, Micromeritics. USA) at 77 K. The specific surface areas of the biochars were determined by the BET method, in which the volumes of the pores were approximated by the amount of nitrogen adsorbed at a relative pressure of 0.99 atm. The functional groups of the biochar were analyzed by Fourier-transform infrared (FTIR) spectroscopy on an EQUINOX55 IR (Bruker, Germany). The samples were mixed with KBr at a ratio of 1:100, compressed into films, and then scanned by FTIR spectrophotometry within the wavenumber range of 4000–400 cm^{-1}. The surface morphology of the biochar was analyzed by SEM on Sirion 200 (FEI Electron Optics. USA). XPS was used to determine the surface compositions and chemical states of the biochar. XPS spectra were recorded with an X-ray photoelectron spectrometer (ESCALAB250, Thermo-VG Scientific, UK) using monochromatized Al Kα radiation (1486 eV). The XPS peaks were deconvoluted into subcomponents using a Gaussian (80%)–Lorentzian (20%) curve-fitting program (XPSPEAK 4.1) with a Shirley-type background.

2.3. Phylogenetic analysis

The 16S rRNA gene sequences of related P-solubilizing bacteria were acquired from GenBank and Ribosomal Database Project using NCBI. Phylogenetic relationships were analyzed with the evolutionary distance matrix generated from the neighbor-joining model based on Kimura's two-parameter method (Kimura, 1980). A neighbor-joining tree was constructed with the program MEGA5. Confidence estimates of the branching order were determined by bootstrap resampling analysis with 1000 replicates.