Transformation of phosphorus in sewage sludge biochar mediated by a phosphate-solubilizing microorganism

Tingting Qian [a, b], Qin Yang [a, c], Desmond Chua Feng Jun [a, b], Feng Dong [a, b], and Yan Zhou [a, b, *]

[a] Advanced Environmental Biotechnology Centre, Nanyang Environment and Water Research Institute, Nanyang Technological University, 1 Cleantech Loop, Singapore 637141, Singapore

[b] School of Civil and Environmental Engineering, Nanyang Technological University, Singapore 639798, Singapore

[c] Interdisciplinary Graduate School, Nanyang Technological University, 1 Cleantech Loop, Singapore 637141, Singapore

*Corresponding Author: Yan Zhou

Address: 50 Nanyang Avenue, Singapore 639798

E-mail: ZhouYan@ntu.edu.sg

Tel: (+65) 67906103

Fax: (+65) 67910676

Abstract

Producing sewage sludge biochar followed by applying it as a P fertilizer is considered to be a competitive way to recover and reuse P resources. To apply sewage sludge biochar as a P fertilizer, it is essential to understand the transformation pathway of different P species in biochar mediated by soil microorganisms, as soil microorganisms are closely related to the mineralization, immobilization, redox reaction, and solubilization of various nutrients in the soil. However, this process has largely been ignored by previous studies. Here, two sludge biochars produced at different temperature (400 and 700 ^{o}C, denoted as B400 and B700) were incubated with a phosphate-solubilizing microorganism (i.e., *Pseudomonas putida* (*P. putida*)) that is closely involved in soil P turnover. The release pattern and transformation of biochar-P were investigated in both abiotic and biotic conditions. The results demonstrate that the release of biochar-P could be enhanced by *P. putida*. The P species in B400 were more vulnerable to *P. putida* than those in B700, as the P species in B400 had lower polymerization degree and poorer crystal structure than those in B700 did. The Pyro-P released from biochar could be easily transformed into Ortho-P by *P. putida*, which can further benefit other species in soil. This study provides insights on the release and transformation of sludge biochar-P mediated by PSM and reveals the effect of the pyrolysis temperature on biochar-P release and transformation which is essential for the production and application of sewage sludge biochar.

Keywords: Sewage sludge; Phosphorus fertilizer; Pyrolysis; Biochar; Availability;

1. Introduction

The fast consumption of limited phosphate rock could lead to the phosphorus (P) scarcity in the next hundreds of years [1, 2], this predicament has been prompting people to seek alternative P sources. Biochar derived from sewage sludge in wastewater treatment plants could be a potential P fertilizer [3-6], as the sewage sludge contains high amount of P (1- 4 % [5, 7, 8]) and most of the P in sewage sludge can be concentrated in the biochar [5, 6].

As a fertilizer, the availability of P is very critical for its application. To evaluate the availability of P in biochar, the release behavior/pattern of biochar-P was studied in various systems [6, 9-12]. It was found that the availability of biochar-P can be altered by the abiotic environmental conditions. For example, low environmental pH, high ambient temperature, and the co-existing anions can enhance the release of biochar-P; while high environment pH and co-existing cations can inhibit it [6, 11, 12]. In addition to the effect of the abiotic environmental conditions, the effect of microbial activities may also play an indispensable role on the release and transformation of biochar-P in soil, as soil microorganisms are closely related to the mineralization, immobilization, redox reaction, and solubilization of various nutrients in the soil [13]. Among the soil microorganisms, phosphate-solubilizing microorganisms (PSMs), such as the strains from the genera *Pseudomonas*, *Bacillus*, and *Rhizobium*, can play a special role on the P cycling in soil. This is because they are accounted for 1-50 % of the microbial population [14] and can promote the transformation of some insoluble P minerals and organic P into soluble reactive P [15], which has a positive effect on the full use of the P fertilizer.

Biochar-P is heterogeneous, in addition to liable-P such as water extractable P and $NaHCO_3$ extractable P (P adsorbed on organic matters in biochar), biochar also contains less bioavailable-P, such as NaOH extractable P (Al/Fe phosphates and P bonds to Al/Fe-containing compounds) and HCl extractable P (insoluble P minerals) F [3, 5]. If the less available-P could be activated by PSMs, the quality and sustainability of biochar-P can be re-evaluated. Thus, understanding the transformation of different P species with the presence of the PSMs is crucial for biochar-P application. Although a few studies have confirmed the enhancement behavior of PSMs on the release biochar-P, the aim of these studies was PSMs screening [16, 17]. The specific transformation of various P species in biochar mediated by PSMs is still unclear.

In this study, biochar was derived from a waste activated sewage sludge, and a highly efficient PSM [18], *Pseudomonas putida (P. putida)*, was chosen as a representative soil microorganism to incubate with biochar [19]. As the pyrolysis temperature is a determining factor on the polymerization degree and solubility of P-containing compounds in biochar [4, 5], two biochars with different pyrolysis temperature, i.e. 400 and 700 ^{o}C, were subjected to the incubation experiments. The objectives of this study are 1) to investigate the release pattern of sludge biochar-P with the presence of *P. putida*; 2) to reveal the transformation pathway of different P species in sludge biochar mediated by *P. putida*; 3) to explore the role of pyrolysis temperature on the release efficiency of biochar-P by the action of *P. putida*. The results of this study can provide some basic knowledge on the production and application of P-rich sludge biochar.

2. Materials and Methods

2.1 Biochar characterization

Biochars were produced through pyrolysis process at 400 ^{o}C (B400) and 700 ^{o}C (B700) according to the preparation method in supporting information (SM - S1). The contents of P, K, Ca, Mg, Al, and Fe in the raw sludge and some heavy metals (HMs) (Cr, Mn, Ni, As, Mo, Cd, Pb and Zn) in biochar were determined (method detailed in SM - S2).

X-ray photoelectron spectroscopy (XPS) analysis of the biochar was performed by using a Kratos Axis Supra Spectrometer with an Al Kα excitation radiation (1486.6 eV). X-ray powder diffraction (XRD) analysis of the biochar was conducted using Bruker AXS D8 Advance diffractometer (Cu-Ka radiation operated at 40kV and 40mA). The liquid state ^{31}P NMR analysis was used to identify the P species in NaOH-EDTA extract of biochar. The preparation method of the NaOH-EDTA extract is shown in SM (S3). The ^{31}P NMR spectrum was obtained using a Bruker 500 UltraShield NMR spectrometer operating at 500 MHz at 24 °C. The details of testing parameters and data analysis of these characterization methods can be found in SM (S4).

2.2 Cultivation media

The growth media of *P. putida* in this study was mineral media (MM) in which the biochar (B400 or B700) was used as the sole P source. The mineral media without the addition of P source (denoted MM*) was prepared according to Topolska [19] with some modification and the composition is listed in Table S1. Prior to autoclaving, the pH of MM* was adjusted to 7.0. To minimize the change of the P species in biochar, the dry biochar powder and MM* were autoclaved separately and then mixed together (0.5 g biochar/500 mL MM*) to form MM before addition of *P. putida*.

2.3 Incubation of *P. putida* in MM media

P. putida was firstly pre-incubated in LB broth until the bacteria suspension optical density at 600 nm (OD_{600}) reached 0.8~1 (lag-log phase in the growth curve). Then, the suspension was centrifuged, and the supernatant was discarded. The bacteria pallet was washed with MM* and resuspended in MM* to prepare the inoculum. Five milliliter of the inoculum was transferred into a 1.5 L-Erlenmeyer flask containing 0.5 L of MM. The incubation was performed on a shaker at 25±3 °C and 130 rpm. The biochar in experimental group was denoted as $B400_{P.}$

$_{putida}$/B700$_{P. putida}$. For control group (B400$_{control}$ and B700$_{control}$), *P. putida* was absent.

During the incubation, MM* was supplemented to the mixture every 10 to 14 days (one cycle). Briefly, mixing was stopped and the mixture in the flasks was allowed to settle to separate the culture suspension and biochar. 400 mL of suspension that contained *P. putida* mainly was replaced by 400 mL of MM*. Twenty five milliliter suspension was stored at 4 ^{o}C for analysis and the rest was discarded. The newly replenished mixture was incubated in the shaker for another 10 to 14 days before the media was changed again. MM* was changed for six times (six cycles) during the incubation period.

In the first cycle (10 days), 25 ml of suspension was withdrawn on the 2nd, 4th, and 7th day of the incubation. On the 10th day, 325 mL of suspension was pipetted out, 25 mL of which was used for analysis and the remaining 300 mL suspension was transferred to a new flask and incubated at 25±3 ^{o}C and 130 rpm without supply of MM* and P source (biochar). This was to investigate the P-transformation under *P. putida* starvation conditions. The *P. putida* suspension incubated in the new flask was denoted as Starvation-B400$_{P. putida}$/Starvation-B700$_{P. putida}$. P concentration and other relevant parameters in this new *P. putida* suspension were also monitored. For other 5 cycles, suspension was only sampled at the end of the cycle and no starvation experiment was carried out. To avoid contamination, each flask was loosely covered with a lid. All the sampling was conducted in a biosafety cabinet and all the experimental containers were sterilized. For experimental groups, triplicate samples were prepared, while for control groups, duplicated samples were applied.

2.4 Sample analysis

The sampling flow is shown in Fig. S1. The 25 mL of the suspension was divided into three subsamples, i.e. sample A, B, and C. Sample A was digested with HNO_3-H_2SO_4 for total P (TP) determination (SM - S5). Sample B was centrifuged, and the supernatant was filtered and divided into samples B1, B2, and B3. Sample B1 and B2 were used to determine orthophosphate (Ortho-P) and total dissolved phosphate (TDP) (SM - S5), respectively. Sample B3 was used for the pH and volatile fatty acids (VFA) analysis (SM - S6). Sample C was used for measuring the density of viable bacteria (Colony-Forming Unit (CFU)) as well as the identification of the poly-P in *P. putida* cell. The staining and observation protocol of poly-P can be found in SM - S7. As *P. putida* was not in the control group samples, the concentration of TP in the liquid phase of control group was the same as TDP, thus, only Ortho-P and TDP were determined. The amount of released P in different cycles was calculated by the methods shown in SM - S8**.** TP, TDP, and Ortho-P, released pyrophosphates from the biochar (Pyro-P) and phosphorus absorbed or adsorbed by *P. putida* (Microbe-P) can be obtained by using the relationship among different P species (SM - Fig. S1). Briefly, the amount of Pyro-P is the difference between those of TDP and Ortho-P, and the amount of Microbe-P is the difference between those of TP and TDP. The release of the HMs from biochar in 0.01 M NaCl solution, of which the ionic strength (IS) was similar to that of the pore water in many soils [20], was also studied. The detailed method of HMs determination can be found in SM - S9.

2.5 Sequential extraction of biochar

The raw biochar (B400 and B700), biochar incubated with MM ($B400_{control}$ and $B700_{control}$), as well as those incubated with *P. putida* ($B400_{P.\ putida}$ and $B700_{P.\ putida}$) were subjected to a sequential extraction according to the *Handbook of Soil Analysis* [21]. Briefly, the biochar was sequentially extracted by water, 0.5 M $NaHCO_3$, 0.1 M NaOH, and 1 M HCl solution at the solid to liquid volume ratio of 1:100. The shaking speed, reaction temperature, and reaction time were respectively 200 rpm, 25±3 ^{o}C and 16 h. Solution of each extracting step was obtained by centrifuge and filtration. The concentration of TP in each extraction solution was measured. The biochar subjected to the sequential extraction was collected and digested for residual P determination (SM - S3).

2.6 Statistical analysis

The paired sample *t*-test was applied to compare the amount of TP released from the experimental group (with the presence of *P. putida*) and that released from control group (without the presence of *P. putida*). The statistical analyses were conducted with SPSS 17.0 for windows and significance was assigned at the $p \leq 0.05$ level.

3. Results

3.1 Biochar characterization

The content of P in the raw sludge, B400, and B700 were 39.0, 75.7, and 99.1 mg g^{-1}, respectively. The studied raw sludge and biochar contained higher P than those reported in other literatures [3-5], which was due to the high P-removal ability of the original sludge. The content of K, Ca, Mg, Al, and Fe in raw sludge, B400, and B700 is listed in Table S2. The content of the HMs (Cr, Mn, Ni, As, Mo, Cd, Pb and Zn) in B400 and B700 and the release of these HMs in 0.01 M NaCl solution are shown in Fig. S2.

Two species, i.e. orthophosphates (Ortho-P) and pyrophosphates (Pyro-P), were identified in the NaOH-EDTA extracting solution (Fig. 1a and 1b). The proportion of released Pyro-P was higher than that of Ortho-P according to the peak height of these two species in ^{31}P NMR spectroscopy. Thus, understanding the transformation behavior of Pyro-P from biochar is also important for the biochar-P application. By using current extracting method, it was found that more than 60 % of the P species in biochar was unextractable (Table S3) and remained unidentified. Thus, biochar was subjected to XPS and XRD analysis.

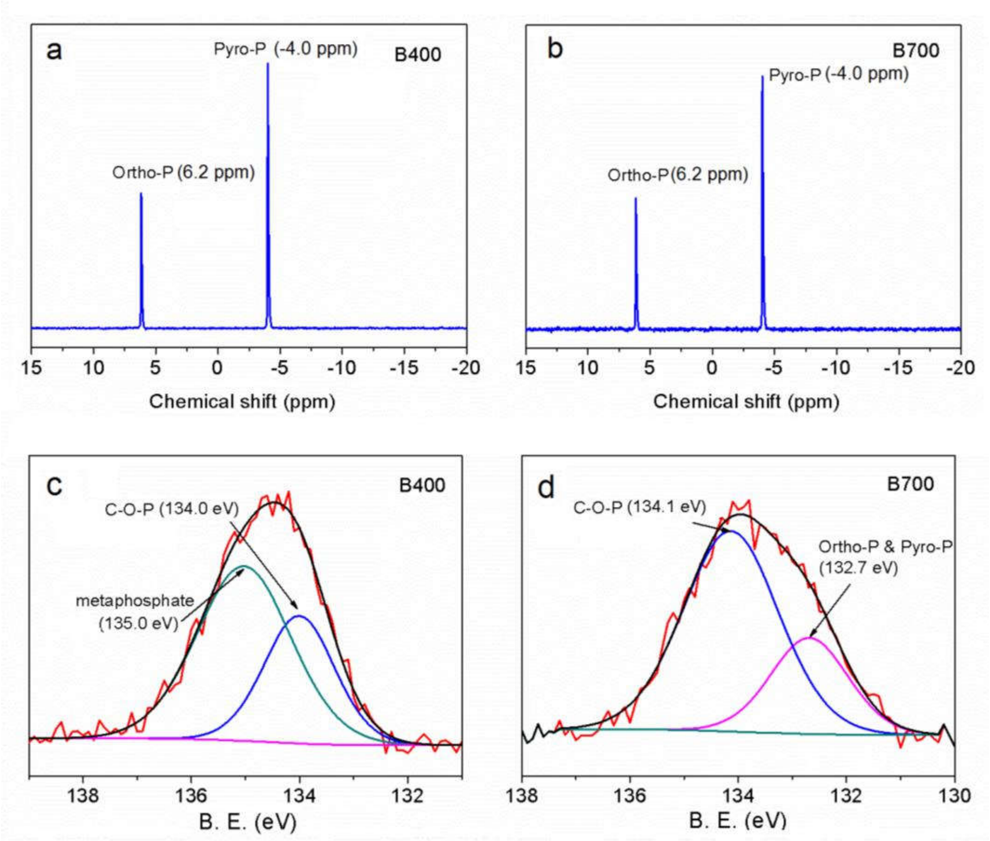

Fig. 1 Identification of P species on biochar. ^{31}P NMR spectra of the liquid samples extracted from B400 (a) and B700 (b) by the solution of NaOH-EDTA; XPS spectra of P 2p of B400 (c) and B700 (d)

Fig. 1c and 1d show the XPS analysis of the chemical states of P in B400 and B700. The P 2p peaks of B400 and B700 were deconvoluted into two components. For B400, the peak of 135.0 eV was assigned to metaphosphate; the peak of 134.0 eV was assigned to C-O-P, which represents the P associated with C in biochar bridged by O [22-25]. For B700, the peaks of 134.1 and 132.7 eV were respectively assigned to C-O-P and Ortho-P/Pyro-P. The percentages of the peak area (PPA, shown in Table S4) can be used to semi-quantitatively analyze the P species. Thus, the higher PPA of C-O-P in B700 (71.9 %) than that of C-O-P in B400 (35.4 %) indicated that the amount of C-O-P in B700 can be higher than that of C-O-P in B400. The chemical states of C on B400 and B700 were also acquired and the results are shown in Figure S3. Compared with the C species in B400, the absence of O-C=O and the higher PPA of the graphite in B700 indicated that the organic carbon compounds in B700 was much more recalcitrant than those in B400, which also suggested that the P species that associate with C on biochar surface (C-O-P) in B700 was more stable than those in B400.

The identification of the P compounds in B400 and B700 was also accomplished by XRD analysis. It was displayed that no peaks could be found in the XRD spectrum of B400 (Fig. S4), suggestive of the poor crystal structure of P compounds in B400. For B700, several peaks arised from its spectrum and these peaks may be attributed to the P compounds, MgP_4O_{11}, $Fe_2P_2O_7$, and $Mg_3(PO_4)_2$. The presence of $Fe_2P_2O_7$ and $Mg_3(PO_4)_2$ in B700 was consistent with the XPS results of B700. It should be noted that the dissolution of metaphosphates was always accompanied with the hydrolysis of these compounds, leading to the major released P form of Pyro-P and Ortho-P [26, 27], this is why only two species can be detected by liquid-state ^{31}P NMR.

3.2 *P. putida* enhanced biochar-P release

The release of biochar-P with the presence of *P. putida* was studied in a time span of 80 days, control experiment was also conducted for comparison. As shown in Fig. 2a, the amount of released TP from B400 and B700 increased with the incubation time. The data of released TP in each experimental/control group were fitted well with zero-order model with the R^2 higher than 0.97 (Fig. 2a and Table S5), which suggested that most of the released P was derived from the dissolution of P-containing compounds [28]. Although the P content in B400 was lower than that in B700, the amount of TP released from B400 was much higher than that released from B700 during the 80 days incubation. For B400, 46.3 and 53.1 % of TP was released from $B400_{control}$ and $B400_{\textit{P. putida}}$, respectively, while for B700, 11.4 and 12.1 % of TP was released from

$B700_{control}$ and $B700_{P. putida}$, respectively. When the biochar was incubated with *P. putida*, more P was released from $B400_{P. putida}$/$B700_{P. putida}$ than that from $B400_{control}$/$B700_{control}$ from the 10th day of incubation onwards (Fig. 2a). The paired sample *t*-test was conducted on the released TP data which were from the 10th to 80th day of the incubation. The result shows that the TP released in experimental group ($B400_{P. putida}$/$B700_{P. putida}$) was significantly higher than that released in control group ($B400_{control}$/$B700_{control}$) ($p<0.05$). The amount of released TP from $B400_{P. putida}$ was 14.7 % higher than that in $B400_{control}$ by the end of the incubation; while the TP released in $B700_{P. putida}$ was only 6.1 % higher than that in $B700_{control}$. The release results of the TP from different biochar were in agreement with the XPS results, that is, the P species in B400 were more labile than those in B700.

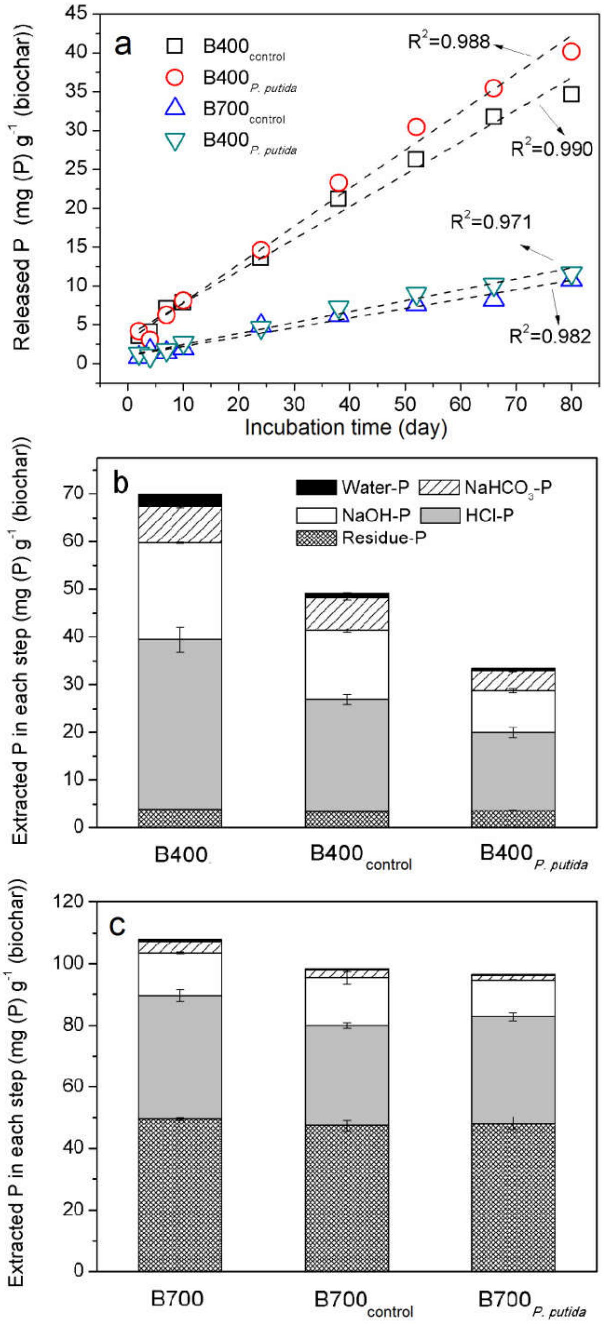

Fig. 2 The amount of TP released from B400/B700 with or without *P. putida* at each time interval (a); The amount of TP released from B400/B400$_{control}$/B400$_{P.\ putida}$ (b) and B700/B700$_{control}$/B700$_{P.\ putida}$ (c) in different steps of sequential extraction

It was found that *P. putida* could enhance the release of biochar-P, especially for the P in

B400. To reveal such enhancing mechanisms by *P. putida*, it will be essential to reveal the types of the P that were affected by *P. putida* and the extent of the effects. Fig. 2b and 2c show the sequential extraction results of the biochar before and after the 80 days incubation. The recovery rate of the sequential extraction was 92.4 % for B400 and 114.9 % for B700. The main types of P in B400 were NaOH-P and HCl-P, which accounted for 29.0 % and 51.1 % of the total P in B400 (Fig. 2b). The sum of Water-P and $NaHCO_3$-P exceeded 14 % of the total P in B400. As Residue-P in B400 only occupied a small portion, it is assumed that most of the P in B400 could be released and utilized [21]. The four portions of the extractable P decreased substantially after 80 days incubation regardless of the presence of *P. putida*. For $B400_{control}$, Water-P, $NaHCO_3$-P, NaOH-P and HCl-P decreased by 63.1 %, 8.6 %, 29.1 %, and 33.6 %, respectively. For $B400_{P. putida}$, Water-P, $NaHCO_3$-P, NaOH-P and HCl-P decreased by 83.5 %, 43.0 %, 56.7 %, and 54.1 %, respectively. The results suggest that *P. putida* could efficiently enhance the release of almost all types of P in B400.

In Fig. 2c, the sum of Water-P and $NaHCO_3$-P was less than 5 % of the total-P in B700. The proportion of NaOH-P and HCl-P were 12.8 % and 36.9 %, respectively. The Residue-P in B700 accounted for 45.9 % of the total P, which was much higher than that in B400. The proportion of P in different P pools in B700 suggests that the P species in B700 were much more stable than those in B400, which is consistent with the XPS results. For $B700_{control}$, HCl-P decreased by 18.1 % after 80 days incubation, while NaOH-P was increased by 11.5 %. For $B700_{P. putida}$, HCl-P decreased by 13.1 % after 80 days incubation, and NaOH-P reduced by 13.6 %. The reduction of HCl-P in $B700_{control}$ and $B700_{P. putida}$ was comparable (i.e. 18.1 % and 13.1 %), suggesting *P. putida* did not have significant effect on HCl-P of B700. For B700, *P. putida* could only enhance the release of a small portion of NaOH-P (Fig. 2c). As the quantity of Water-P and $NaHCO_3$-P in

B700 was small, the changes of P in these two pools will not be discussed hereafter.

It should be noted that the amount of P released from B400/B700$_{\text{control}}$ and B400/B700$_{\textit{P. putida}}$ obtained from sequential extraction experiments was lower than those obtained by using the TP concentrations (Fig. 2a, Table S6). This may be attributed to the heterogeneity of the samples in different experiment. Compared with soil, biochar contains much more P. If large quantity of OH^- is present in the solution, the precipitation of P would be enhanced. Thus, the extraction agent, i.e. 0.1 M NaOH solution, in the sequential extraction would cause the increase of the HCl-P of the sample. Therefore, the decrease of HCl-P in B400/B700$_{\text{control}}$ occurred at pH of 7.0. Theoretically, the amount of HCl-P in B400/B700$_{\text{control}}$ should be the same to that in B400/B700. Similarly, the increase of NaOH-P in B700$_{\text{control}}$ after 80 days incubation was also attributed to the error caused by the extraction method. The P species extracted from B400$_{\textit{P. putida}}$ and B700$_{\textit{P. putida}}$ by NaOH-EDTA solution were also examined by the method of liquid-state ^{31}P NMR spectroscopy (Fig. S5). The peaks belong to organic P were not found in the ^{31}P NMR spectroscopy of B400$_{\textit{P. putida}}$ and B700$_{\textit{P. putida}}$, which indicates that *P. putida* was almost completely removed from biochar before the NaOH-EDTA extraction of biochar, and the P species in *P. putida* did not influence the results of sequential extraction of biochar.

3.3 Effect of *P. putida* on the transformation of P species released from biochar

To understand the transformation of released biochar-P with the presence of *P. putida*, the absorption and release of biochar-P by *P. putida* were studied. The changes of different P species in a time span of 80 days are shown in Fig. 3. As the P species released from biochar were Ortho-P and Pyro-P (Fig. 1a and 1b), the difference between TP and Ortho-P released from B400$_{\text{control}}$/B700$_{\text{control}}$ was considered as Pyro-P. The results show that the amount of Pyro-P released from B400$_{\text{control}}$/B700$_{\text{control}}$ was about 2-fold higher than that of Ortho-P. When biochar was incubated with *P. putida*, the amount of Ortho-P and Pyro-P decreased dramatically. More than 88 and 67 % of the released TP were transformed into Microbe-P for B400$_{P. putida}$ group and B700$_{P. putida}$ group, respectively. Interestingly, similar as Ortho-P, Pyro-P could be also easily utilized by *P. putida*, indicating the excellent microbial availability of P released from biochar. The density of viable bacteria in the suspension of B400$_{P. putida}$ group at the end of each cycle was higher than that in B700$_{P. putida}$ group (Fig. S6). This probably because more P, as energy source, was released from B400$_{P. putida}$ compared to B700$_{P. putida}$.

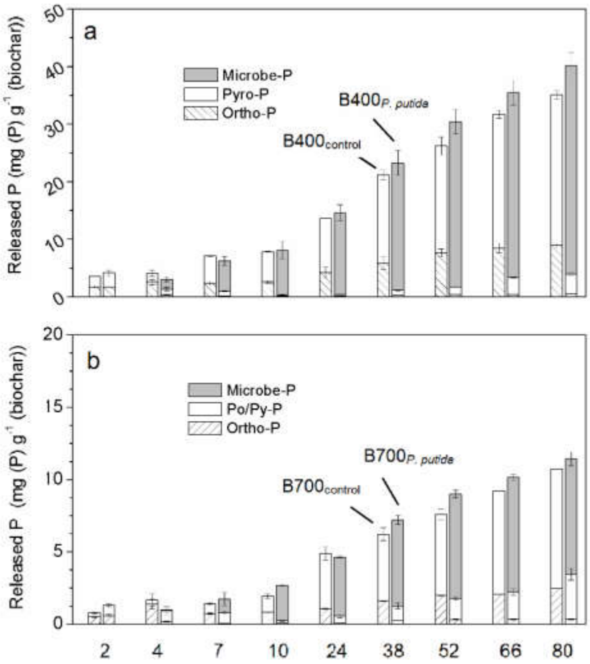

Fig. 3 The amount of released P from B400 (a) and B700 (b) with or without the presence of *P. putida* at each time interval

The soil microorganisms often act as a P pool in soil [29]. Thus, it is crucial to understand the fate of biochar-P stored by *P. putida,* and whether the P absorbed by *P. putida* could be released to the environment, as well as what the main form of the released P is. The abundance of different P species in the suspension before and after 40 days starvation is shown in Fig. 4. The concentration of different P species in the same suspension is shown in Table S7. When the suspension was withdrawn from B400/B700$_{P.\ putida}$ group at the end of the first cycle, almost all the P in the suspension was Microbe-P (Fig. 4). During the first 25 days incubation, nearly no Ortho-P could be detected in the bulk solution (data were not shown). However, from 40th day onwards, high level of Ortho-P was detected in the bulk of Starvation-B400$_{P.\ putida}$. The proportion of Ortho-P increased dramatically from 0.2 % to 53.4 % and TDP increased from 2 % to 76.4 %. For Starvation-B700$_{P.\ putida}$, the proportions of Ortho-P and TDP in the suspension also increased while the increment was not as much as that of Starvation-B400$_{P.\ putida}$. The release of the P from *P. putida* implied that *P. putida* can facilitate the transformation of Pyro-P released from biochar into Ortho-P, which is a form of plant available-P.

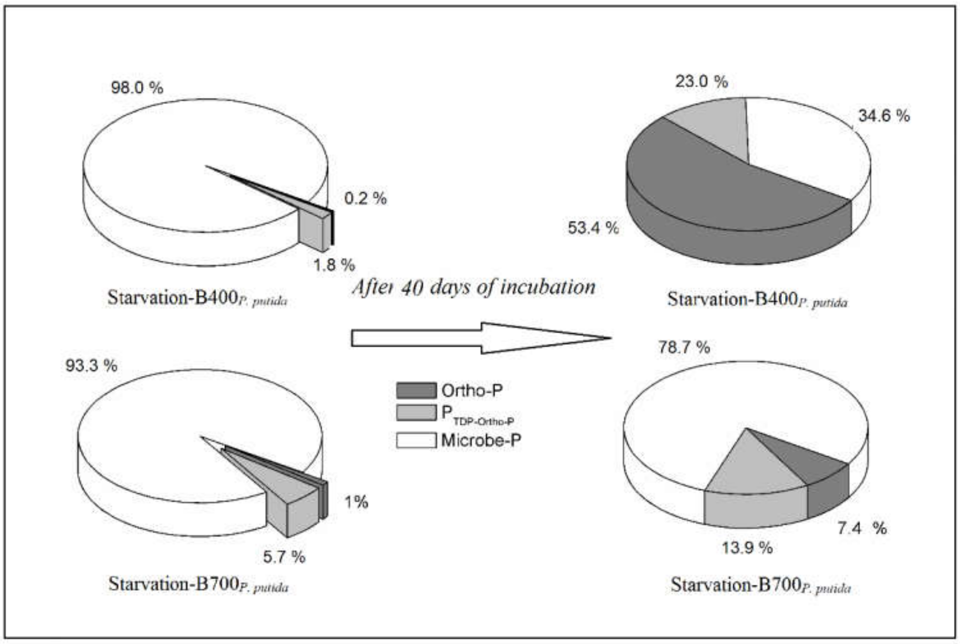

Fig. 4 The abundance of different P species in Starvation-B400$_{P.\ putida}$/Starvation-B700$_{P.\ putida}$ before and after 40 days incubation

4 Discussion

4.1 The stability of P in B400 and B700

Previous studies showed that the stability and release pattern of biochar-P are related to the type of the P-containing compounds, the amount of Fe/Al-containing compounds, and the functional groups of biochar [3, 5, 30]. In this study, the XPS result shows that metaphosphate and the P associated with C bridged by O (C-O-P) were the main species in biochar. Thus, the polymerization degree (chain length) of the metaphosphate [26, 31], the stability of the metaphosphate structure as well as the stability of the organic matters associated with P via the mode of C-O-P in biochar, would also influence the stability and release rates of biochar-P.

When pyrolysis temperature was low (400 ^{o}C), some labile C-containing compounds could remain in the biochar (Fig. S3), these compounds may mix with the P compounds during the formation of metaphosphate, which could facilitate the formation of short chain metaphosphate with poor crystal structure and cannot be detected by XRD analysis (Fig. S4). When the pyrolysis temperature was high (700 ^{o}C), plenty of labile C-containing compounds were removed, then the remaining compounds could evolve into the stable C-containing compounds (see Fig. S3). Without the presence of the labile C-containing compounds during the formation of the metaphosphate, pyrophosphates, and phosphates, the structure of these P species on B700 could be more stable than those in B400. As the short chain metaphosphates with poor crystal structure were more vulnerable than the long chain metaphosphates with stable structure in the solution [26, 31], more P could be released from B400 than B700. The cleavage of the organic matter in the sludge could lead to the formation of C-O-P [32, 33]. At 400 ^{o}C, the main carbon evolution was the decomposition/evaporation of labile organic matter [34, 35]. The mode of C-O-P could mainly occur on labile organic matters during the pyrolysis and only a small portion of P could be associated with the carbon on the newly formed biochar. While at 700 ^{o}C, the cleavage reaction of the organic matter could be more severe than that occurred at 400 ^{o}C [36, 37], and more condensed aromatic structure could form in B700 [36, 38]. Thus, more P could be doped in the stable carbon compounds in B700 via C-O-P bond, leading to less P available for release in B700. Given the high availability of P in B400, it can be concluded that biochar produced at low temperature could be more suitable as a slow release P fertilizer. According to the discussion on the stability of the P species in different biochar, it was found that the release ability of biochar-P mainly depended on the property of the inorganic P compounds, e.g., the polymerization degree and the quality of the crystal structure. The C-containing compounds in biochar had two opposing effects on the release of biochar-P. At low pyrolysis temperature (400 ^{o}C), the C-containing compounds in the biochar could facilitate the release of biochar-P; while at the high pyrolysis temperature (700 ^{o}C), it could inhibit the release of biochar-P.

4.2 The role of *P. putida* on the fate of biochar-P

The effects of *P. putida* on biochar-P primarily reflected in enhancing the release of biochar-P and increasing the proportion of Ortho-P in the environment. Some studies reported that the P solubilizing capacity of PSM was attributed to the acid effect of the organic acids and complex effect of the organic acids secreted by PSM [15, 39]. This study found out that certain amount of organic acids (i.e. acetic acid and butyric acid) presented in the *P. putida* suspension (Fig. S7). As the pH of the suspension was in the range of 7.0–8.3 during the incubation time (Table S8), the acid effect of the organic acids on the release of P could be minor and the complex ability of the organic acids could be the main reason for the enhanced P-release. The organic acids produced by *P. putida* could attack some insoluble P-containing compounds and Fe/Al-containing compounds [3], which resulted in the release of HCl-P and NaOH-P. They could also complex with the Ca^{2+} and Mg^{2+} adsorbed on the oxygen-containing functional groups of biochar, and released the P adsorbed on biochar bridged by Ca^{2+} and Mg^{2+}, and then causing the release of $NaHCO_3$-P (Fig. 5).

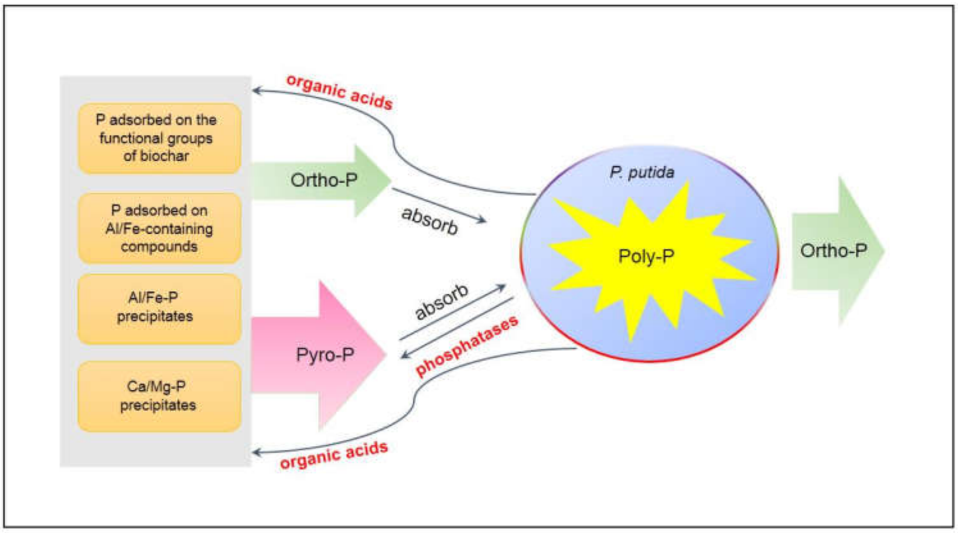

Fig. 5 The role of *P. putida* on the fate of biochar-P

For B400, the crystal structure of the insoluble P-containing compounds in biochar was very poor [3, 5], and the P-containing compounds with poor crystal structure could be easily attacked by the organic acids. Hence, the P species in all the extractable P pools (i.e. Water-P, $NaHCO_3$-P, NaOH-P and HCl-P) in B400 decreased with the presence of the *P. putida*. However, *P. putida* had minor effect on B700-P due to the stable crystal structures of the P-containing compounds and C associated P.

The results also demonstrate that in addition to Ortho-P, Pyro-P released from biochar, could also be utilized by *P. putida* (Fig. 5). As the hydrolysis of many condensed P at the neutral pH and room temperature is negligible [40, 41], the hydrolysis of Pyro-P on biochar was probably catalyzed by some enzymes.. According to the structures of Pyro-P and some organic P in soil (e.g. phytic acid), it is clear that the bonding means of phosphate group on the bulk molecules of these two types of P compounds are similar. It is reasonable that some phosphatases, which are secreted from the microorganisms in soil, may take part in the hydrolysis of Pyro-P and play a predominant role on the hydrolysis of Pyro-P released from biochar. As many soil microorganisms can produce such kind of phosphatases [42, 43], the Pyro-P released from biochar would be utilized easily by soil microorganisms. Most of the previous studies show that the main P species released from the microorganisms in soil under harsh conditions were organic P (molybdate-unreactive P), which were caused by cell lysis or osmotic shock. However, for Starvation-B400$_{P.\ putida}$, the major P species released was Ortho-P. As the poly-P granules were found in *P. putida* (Fig. S8) when *P. putida* was incubated with B400 and B700, the Ortho-P could be actively released from the living cells via the degradation of the intracellular polyphosphate [44] (Fig. 5). Hence, *P. putida* facilitated the transformation of Pyro-P released from biochar into Ortho-P, which would benefit other species (such as plant) in the soil.

5. Conclusions

To apply sludge biochar in soil as a P fertilizer, it is crucial to understand the interaction between biochar-P and soil microorganisms. In this study, two sludge biochars produced at different temperature (i.e., 400 and 700 °C) were incubated with soil bacteria *P. putida*. The results demonstrate that the release of biochar-P was mainly caused by the dissolution of the P-containing compounds in biochar and could be enhanced by *P. putida*. Metaphosphates and P associated with C in biochar are the main P species in biochar. The P-containing compounds with poor crystal structure and low polymerization degree (short chain) were more vulnerable to *P. putida* compared to those with good crystal structure and high polymerization degree, implying that biochar produced at low temperature (e.g. 400 °C) could be used as a slow-release P fertilizer and perfectly involved in soil P cycle. *P. putida* played a regulating role on biochar-P. It could not only enhance the release of biochar-P and utilize all types of the released P (i.e. Ortho-

P and Pyro-P) in biochar, but also release the P back to the environment. This study provides insights on the release behavior of sludge biochar-P under a biotic condition and confirms its suitability for soil application.

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Appendix A. Supplementary data

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Supplementary Material