Enhancing Phosphorus Solubility and Bioavailability from Animal Bones: Effects of Thermochemical and Biological Treatments

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SYNOPSIS

Due to uncertainty about accessibility, amount, and quality of the remaining phosphate rock stocks, a replacement of mineral phosphorus (P) fertilizers by sustainable alternative sources is desirable. Recycling P from animal bone waste by a process called pyrolysis is one of such alternatives to secure the demand. However, plant P availability in bone char is usually low. Factors affecting P solubility and bioavailability during thermal treatments have not been fully understood, moreover biological treatment to enhance P solubility of thermally treated bones has rarely studied. This study aimed to elucidate different factors affecting P solubility and bioavailability (experiment 1), and investigate effects of biological treatment (i.e., phosphate-solubilizing microorganisms; PSM) on P solubility (experiment 2) from thermally treated bones. In experiment 1, X-ray diffraction and FTIR spectroscopy techniques, chemical extraction, soil incubation and pot experiments were conducted to elucidate the effects of bone type, thermal processing method, and production temperature on P fertilizing value of animal bones. In experiment 2, solubilization potential of four different *Penicillium* strains from different bone chars were determined. The findings demonstrated that P solubility from bone char largely depended on bone type, processing method, and production temperature as well as soil type used. High water soluble P was recorded from chicken bone. Pyrolysis resulted in more soluble P than combustion particularly at higher temperature. Overall P fertilizing effect of bone was more pronounced in lower pH soil. Solubilizing potential of different *P.* strains was affected by bone char production temperature, biomass co-pyrolysis with bone, and soil carbon content. *P. bilaiae* was most effective in solubilizing P from bone chars. P solubilization potential and survival rate of the strain was positively influenced by co-pyrolysis and soil carbon content. The current study demonstrated the effects of thermochemical and biological treatments on P solubility and bioavailability from thermally treated animal bone and its potential utilization as alternative P fertilizer.

Keywords: bone ash, bone char, co-pyrolysis, P bioavailability, *Penicillium*

INTRODUCTION

Phosphorous (P) is one of the most important plant nutrients, and mainly derived from rock phosphate (RP), a non-renewable and fatally depleting resource[1]. It is estimated that the world reserves will only last for about 50 to 125 years. High production cost coupled with meager P quality (e.g., heavy metal contamination), are an environmental concern and among the challenges to meet global food demand. As global P demand is expected to double by 2050, future availability of RP is threatened[2]. P fertilizer shortages challenge food security around the world, particularly in Africa where the poorest farmers face the highest fertilizer prices[3].

An alternative and renewable solution to reduce the dependency on RP includes P recycle from organic waste disposal[2]. Animal bone is one of such renewable P sources with huge potential to secure the demand. Recycling P from animal bone waste, that would otherwise generate environmental and health concerns, can secure significant fraction of P fertilizer demand. Bone char, the heating of bone under no or low oxygen conditions (i.e., pyrolysis), has been suggested recently as economically viable and sustainable P fertilizer[4]. However, P solubility in bone char is usually low due to the formation of crystalline, insoluble calcium-P during pyrolysis. Up to date, there have been considerable variations among the reported studies in the P fertilizing effect of bone char, ranging from positive effect[4,5] to non-significant effect[6,7].

The low P solubility from bone char and inconsistency in the past studies calls for more studies to understand factors controlling P solubility and bioavailability in thermally treated bone, which may include physical treatments such as processing method (pyrolysis vs. combustion) and processing temperature, bone type, and soil environments to be applied. Another alternative low-cost biotechnological strategy such as use of phosphorus solubilizing microorganisms (PSMs) could be one way to enhance P solubility from bone char. However, researches on this topic have been lacking, thus underlying mechanism has not been clear.

The overall aim of the present study is, therefore, (i) to determine effects of bone type, thermal processing method, production temperature, and soil pH on P solubility and bioavailability from thermally processed bone and to understand the underlying mechanism; and (ii) to elucidate the potential application of different PSM strains to enhance P solubility and bioavailability from different bone chars both in vitro and in vivo.

MATERIALS AND METHODS

Experiment 1: Effects of bone type, thermal processing method and production temperature on P availability

P solubility was investigated from thermally treated animal bones produced from: (i) 3 bone types (chicken, sheep, and pig); (ii) 2 thermal processing methods (pyrolysis and combustion); and (iii) 4 production temperatures (300°C, 500°C, 700°C, and 900°C). Chemical extraction, incubation, and pot experiments were performed to evaluate P availability. Infrared splitting factor (IFRS) and width of 604 cm^{-1} peak (FW85%) were calculated from FTIR spectra, and X-ray powder diffractometer (XRD) to determine bioapatite crystallinity of thermally treated animal bones.

Experiment 2: Microbial Phosphorous Solubilization of Bone Char: Effects of Pyrolysis Temperature, Biomass Co-pyrolysis, and Soil Carbon Content

P solubilizing potential of 4 different *Penicillium* stains (*P. bilaiae*, *P. glanrum*, *P. expansum* and *P. aculeatum*) was investigated using 4 different bone chars (BC450, Co-BC450, BC850, and Co-BC850) pyrolyzed without and with biomass with 2 different pyrolysis temperatures (450ºC and 850ºC) using a liquid medium. The concentration of low molecular weight organic acids, pH, and solubilized P over time was measured. Additionally, soil incubation and pot experiment were carried out using 2 strains and 2 bone chars selected based on the liquid medium experiment. The survival and P solubilization rate of the strains were investigated under different soil C contents.

RESULT AND DISCUSSION

Experiment 1: P solubility from thermally treated bones

P solubility of thermally treated bones largely depended on bone type, thermal processing method, and production temperature (Fig.1). Chicken bone showed the highest water soluble P content (p < 0.001), irrespective of processing methods and temperatures. In contrast, pig bone exhibited the lowest P despite its high total P content. Pyrolysis resulted in higher water soluble P than combustion, particularly at temperatures >500^0C.

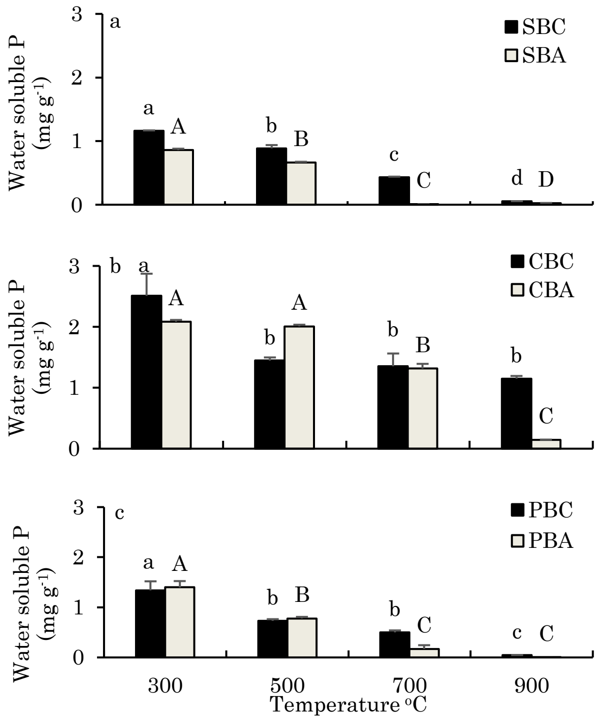

Figure 1. Water soluble P from (a) sheep bone char (SBC) and ash (SBA), (b) chicken bone char (CBC) and ash (CBA), and (c) pig bone char (PBC) and ash (PBA) treated at 300°C, 500°C, 700°C, and 900°C

The XRD and FTIR studies confirmed higher degree of crystallization for those produced from pig bone, combustion than pyrolysis, and higher temperatures (>700ºC) (Fig 2). Significant negative correlations were observed between IRSF and both formic P (p < 0.5) and

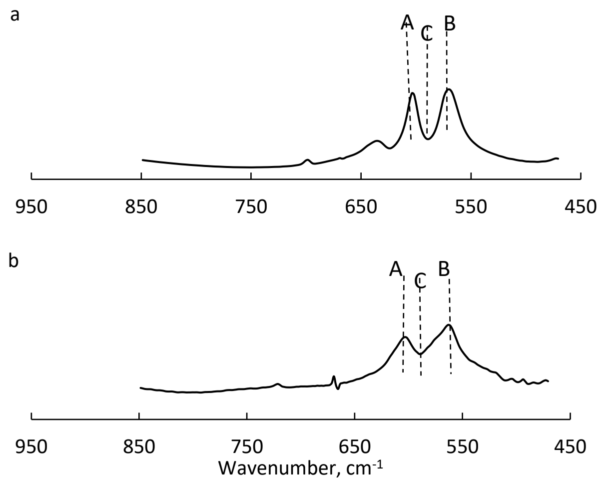

Figure 2. FTIR spectra of (a) pig bone char produced at 900°C and (b) chicken bone char produced at 300°C

water soluble P ($p < 0.001$), and significant positive correlation between FW85% and water soluble P (Table 1; $p < 0.001$). Furthermore, IRSF values of chicken bone were overall lower by 25% than those of pig bone across all processing methods, and FW85% values of chicken bone were overall higher by 12% than those of pig bone, indicating lower degree of bioapatite crystallinity or higher P availability in chicken bone than pig bone. Similarly, the XRD traces also showed one broad peak in chicken bone, whereas pig bone exhibited more than one peaks at lower temperatures. Higher degree of crystallization, therefore, explained lower P availability from those with higher production temperatures and combustion process.

Table 1. Pearson's correlation coefficients between bioapatite crystallization index and different P forms

	Water soluble P	Formic extractable P	Total P
IRSF	–0.79***	–0.52*	0.59*
FW85%	0.71***	0.43ns	–0.74***
Ca:P ratio	–0.49**	–0.27ns	0.38ns

P availability from thermally treated bone chars in soil

Both incubation and pot experiments demonstrated higher available P in lower pH soil (Fig. 3). This can be explained by the dissolution of bioapatite minerals at low pH[5]. The highest Olsen-P was observed at pyrolysis temperature of 700°C (BC700) and combustion temperature of 300°C (BA300).

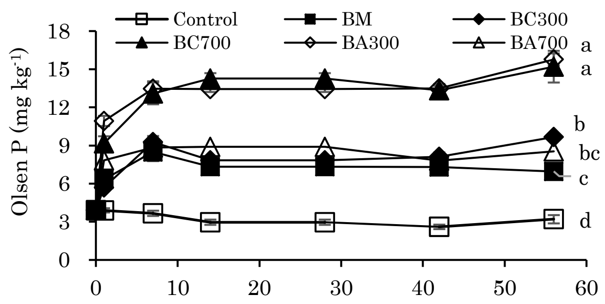

Figure 3. Olsen extractable P of sheep bone char and bone ash amended to low pH soil

Experiment 2: P solubilizing potential of PSM stains from bone char

Solubilized P was significantly influenced by PSM type, processing temperature and biomass addition (Fig. 4; $p <$

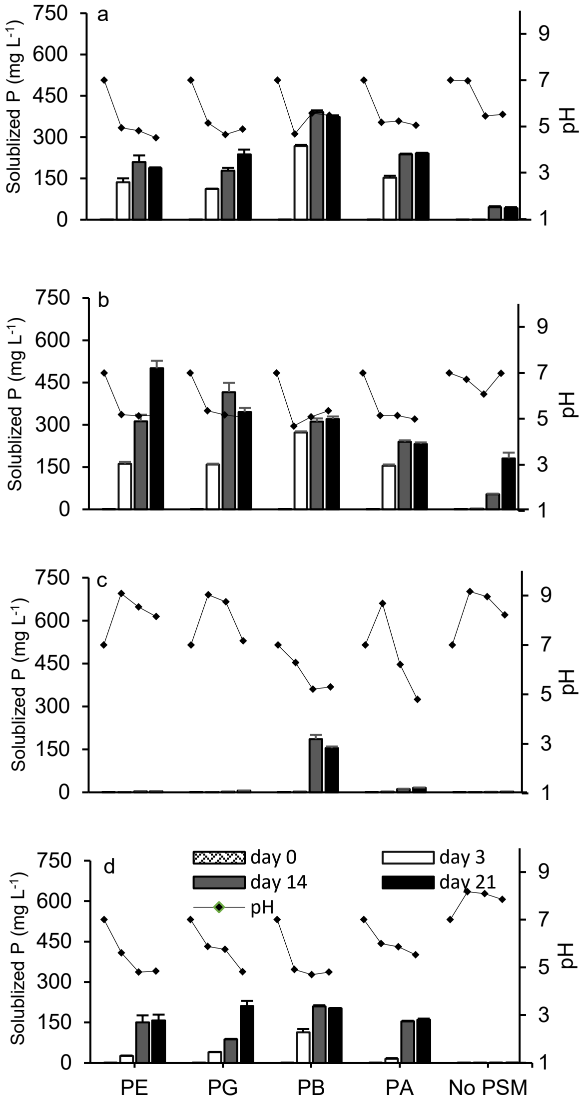

Figure 4. Soluble P and pH in liquid medium from (a) BC450, (b) Co-BC450, (c) BC850, (d) Co-BC850 inoculated with different PSM strains

0.001). Bone chars produced at 450ºC was the most responsive to inoculation of PSM strains than bone char produced at 850ºC. At lower pyrolysis temperature, some labile C-containing compounds could remain in the bone char, which may have resulted in Ca-P with poor crystal structure, hence more P could have been solubilized from BC450.

As for BC850, only *P. bilaiae* was able to increase P solubility, while the others resulted in slight increases compared to non-inoculated control (Fig. 4c). However, co-pyrolysis of bone with biomass was found to enhance P solubilization potential of all the strains particularly at high temperature (Figs. 4b and 4d). The total C in the co-pyrolyzed bone chars was two to five-fold higher. These bone chars may have contained a fraction of labile C that was easily degradable for microorganisms enhancing microbial activity, which may have resulted in high P solubilization.

P. bilaiae showed the highest P solubilization than other strains. This stain produced higher concentration of pyruvic, formic butyric and acetic acids compared to the other strains, which could play a significant role in the acidification of the medium facilitating P solubilization. Effects of PSM and bone char types on solubilized P, and their interaction were highly significant for pH of the culture medium and the concentration of organic acid produced (Table 2; $p < 0.001$). The amount of solubilized P from all the bone chars were negatively correlated with pH and the total amount of organic acid produced.

Table 2. Correlation coefficients between solubilized P and pH or organic anion concentrations in the medium by different bone chars

Bone char type	BC450	Co-BC450	BC850	Co-BC850
Soluble P vs pH	-0.72^{***}	-0.63^{***}	-0.69^{***}	-0.79^{***}
Soluble P vs OA	0.77^{***}	0.56^{***}	0.82^{***}	0.74^{***}

Interaction between soil C and P solubilization

Carbon addition to the soil significantly influenced efficiency of PSM to solubilize P over time. After 35 day of incubation period, *P. bilaiae* showed better P solubilization efficiency with C addition, while *P. expansum* and none-inoculated control treatments decreased very significantly (Fig. 5). Carbon plays important role in promoting growth and survival of microbes, however the different response of the strains shows such effect is strain dependent.

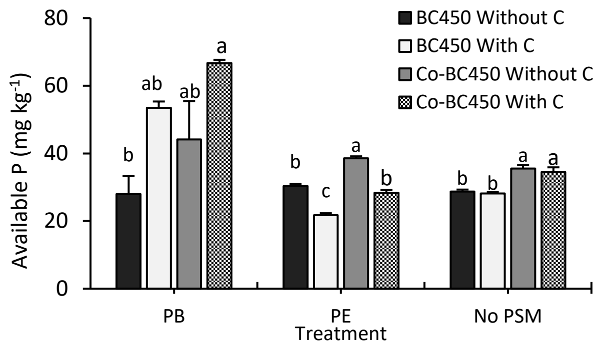

Figure 5. Available P from different bone chars as affected by C addition and PSM inoculation after 35 days of incubation

CONCLUSION

The findings revealed that P solubility from animal bones largely depended on bone type, thermal processing method, and production temperature. XRD traces, IRSF, and FW85% confirmed higher crystalline bioapatite formation with higher production temperatures and during combustion than pyrolysis process.

It was also demonstrated that inoculation of PSM enhanced P solubility from bone char. Bone chars produced at lower temperature were more vulnerable to PSM inoculation. Co-pyrolysis with biomass and C addition to soil enhanced P solubility by PSM.

The current study demonstrated positive effects of thermochemical and biological treatments on P solubility and bioavailability from thermally treated animal bone and its potential utilization as alternative P fertilizer.

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