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Enhancing the phosphorus bioavailability of thermally converted sewage sludge by phosphate-solubilising fungi

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ABSTRACT

Biochars and ashes from sewage sludge have a high phosphorus (P) content, but plant P availability is typically rather low. Phosphate-solubilising microorganisms (PSM) have been shown to have the ability to solubilise P from different compounds. The aim of this study was to explore the P-solubilisation potential of different PSM on various biochars and ashes, and the effect of the addition of different carbon (C) and nitrogen (N) sources on their P-solubilisation activity. The most promising combination of PSM, thermal residue and nutrients was then tested for its effect on plant growth and P uptake in a pot trial. Six PSM strains (four *Penicillium bilaiae (Pb), one Penicillium aculeatum (Pa)* and one *Aspergillus niger (An)*) were tested on two sewage sludge ashes and one biochar. *Pb.4* and *An* showed the highest P-solubilisation rates on fluid-bed incineration (FB-I) ash. *Pb.4* solubilised higher amounts of P when it was supplied with fructose in combination with NH_4-N, while *An* performed equally well with fructose, maltose, mannose and xylose in combination with NH_4-N. Increasing the concentration of the C source generally also increased the P solubilisation. However, when FB-I ash was inoculated with *Pb.4* plus xylose/NH_4-N and applied to spring wheat in a pot trial with γ-irradiated soil, the inoculation did not significantly affect plant shoot biomass or P uptake. The results indicate that the amount and temporal availability of P solubilised by the fungal strain from the ash did not match plant requirements, suggesting that further work is required that focuses on further increasing solubilisation efficiency.

1. Introduction

Ashes and biochars from the thermal treatment of sewage sludge are residual waste products with comparatively high phosphorus (P) contents and could therefore represent an interesting and more sustainable alternative to mineral P fertilisers (Bierman et al., 1995; Herzel et al., 2016) derived from non-renewable rock phosphate. A barrier to the application of ash and biochar from sewage sludge as a P fertiliser is the often reported low immediate P availability for plants (Mackay et al., 2017; Schiemenz and Eichler-Löbermann, 2010), which has been found to be related to the structural order of the amorphous P forms in the ash (Nanzer et al., 2014a, 2014b). Several industrial processes, such as acid leaching of P from the sewage sludge ash (Cohen, 2009) or thermochemical treatment with either $CaCl_2$ or $MgCl_2$ (Nanzer et al., 2014a) have been described, but still represent a complex and expensive process (Cieślik and Konieczka, 2017) feasible only at industrial scale. Phosphate-solubilising microorganisms (PSM) are among the beneficial microorganisms that are considered to be potential biofertilisers, meaning that they are able to increase plant nutrient uptake when applied to a soil/plant system (Vessey, 2003) and can represent a low-cost alternative to industrial processes. The efficiency of the beneficial microorganisms is dependent on a successful inoculation, especially in soil that represents a competitive and harsh environment. Biochars from different origins have been reported to be beneficial for soil microorganisms (Lehmann et al., 2011), and their use as an inoculant carrier for beneficial microorganisms such as plant growth promoting bacteria (Hale et al., 2015, 2014; Sahu and Brahmaprakash, 2016; Sun et al., 2016; Zayed, 2016) has also been suggested. In a previous study, the concept of inoculating P-rich biochars and ashes with PSM to enhance their P availability was introduced, thereby simultaneously providing a P source to solubilise and an inoculation carrier (Raymond et al., 2018). The P-solubilising fungus *Penicillium bilaiae* could grow on and solubilise P from different types of biochars and ashes derived from sewage sludge. However, the proportion of total P actually solubilised was still relatively small and needs to be enhanced in order to achieve levels of available P sufficient to sustain plant P nutrition.

One of the main mechanisms associated with microbial P solubilisation is the acidification of the local environment and the production

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and excretion of organic anions. Different PSM synthesize various organic anions types, which is strain specific, but also strongly influenced by the nutritional substrate and the P form to solubilise. Accordingly, the capacity of soil microbes to dissolve relatively insoluble P forms has been described as being heavily dependent on the nature and availability of substrates found in soil (Nahas, 2007). Several studies show the beneficial effect of specific carbon (C) sources and their concentrations (Nahas, 2007; Scervino et al., 2011), however the final amount of P solubilised has been described as also being dependent on the type and amount of nitrogen (N) source used (Barroso et al., 2006; Cerezine et al., 1988; Scervino et al., 2011). Therefore, a non-optimal nutritive environment for PSM growth and acidification activity may be one of the main reasons why the beneficial effect often disappears when they are applied to soil (Herrmann and Lesueur, 2013). Providing optimal nutrition for PSM growth and P solubilisation will therefore contribute alleviating the different bottlenecks to the performance of PSM in the natural soil/plant environment. However, biochars and ashes provide no or only small amounts of C and N for fungal growth, therefore supplementation with easily available nutrients is necessary to enhance P solubilisation from these materials.

When inoculated to soil, the low competitiveness of the PSM with the indigenous soil microbial communities has also been reported to be a barrier to their success (Bashan et al., 2014; Richardson, 2001; Richardson et al., 2011). If PSM are not able to grow and/or perform P-solubilisation activity *in vivo* in soil, other strategies can be explored, such as performing P solubilisation *in vitro* and then applying the soluble P fertiliser to the soil (Vassilev et al., 2014). In that way, the plant is provided with a pool of soluble P at early growth stages, and the degradation of the microbial P pool or the re-activation of the PSM by the renewed substrate availability through root exudation may add some further possibilities to increase P availability from the sewage sludge thermal residues.

The overall aim of this study was to identify a suitable combination of ash/biochar material, PSM strain and C and N sources to maximise P availability from the material both *in vitro* and *in vivo* when applied to a soil-plant system. The combination aimed at improving the performance of inoculated PSM by providing a protective environment and ensuring proximity of the P source to be solubilised. Such a combined application of ash/biochar, a PSM, and a substrate may be an easily applicable procedure, even for small-scale or on-farm development, while avoiding a complex and expensive industrial process. The specific objectives were therefore i) to investigate the P-solubilisation activity of different fungal strains inoculated on sewage sludge ashes and biochar converted in different thermal processes, ii) to test the temporal dynamics of P solubilisation to optimise the incubation time, iii) to determine the C and N sources (and their dosage) that maximise P solubilisation, and finally iv) to test the effect of the most effective combination of fungal strain/biochar-ash material/nutrient sources on plant biomass production and P uptake in a spring wheat pot trial.

It was hypothesised that: (1) Available P released is strongly dependent on the ash/biochar type and P-solubilising fungal strain used, (2) a particular combination of C and N sources is needed for optimal P-solubilisation performance, (3) soil application of the ash/fungus/CN combination that increases plant-available P most effectively *in vitro* results in increased plant growth and P uptake compared to the application of non-inoculated ash, and (4) *in vitro* P solubilisation before application of the ash has a greater effect on plant response than the simultaneous inoculation of ash, fungal spores and nutrients to soil.

2. Materials and methods

2.1. Sewage sludge ashes and biochar

Two ashes and one biochar from the thermal treatment of sewage sludge were selected for this study. The different materials were prepared from sludge originating from the Bjergmarken Renseanlæg wastewater treatment facility (Roskilde, Denmark) as part of the Cross-Platform Sludge Experiment (CPSE) campaign (Thomsen et al., 2017). The thermal conversion processes to produce the materials were slow pyrolysis (SP) (~2 h, 600 °C), low temperature circulating fluidised bed gasification (LT-CFBG, 750 °C) and fluid-bed incineration (FB-I, 850 °C). Chemical and physical properties of the resulting products are described in detail in Raymond et al. (2018) and Thomsen et al. (2017) and are summarised in Table 1. Briefly, the materials were crushed, sieved (< 1 mm) and sterilised by autoclaving for 20 min at 121 °C. pH was measured by suspending 1 g of material in 25 mL milliQ water and electrical conductivity (EC) was obtained by shaking and filtrating 1 g material in 20 mL milliQ water. Total C and total N were determined by Thomsen et al. (2017), while total P was measured in a 25 mg material digested in 2.5 mL 70% nitric acid and 1 mL 30% hydrogen peroxide and mixed with 500 µL hydrofluoric acid before being measured by ICP-OES (Agilent 5100, Agilent Technologies, Manchester, UK). Water extractable P (WEP) was measured by shaking 0.5 g material suspended in 30 mL milliQ water for 16 h. After filtration of the suspension, orthophosphate P concentration was determine by the molybdate blue-ascorbic acid assay as described by Murphy and Riley (1962) and Watanabe and Olsen (1965).

Table 1
Chemical and physical properties of the two ashes and the biochar produced from sewage sludge.

Properties	LT-CFBG ash	FB-I ash	SP biochar
pH	10.70	9.18	11.58
EC (µS cm^{-1} (25 °C))	322 ± 22.2	946 ± 32.0	420 ± 6.7
Total C (mg g^{-1})[1]	72	5	226
Total N (mg g^{-1})[1]	5	–	22
Total P (mg g^{-1})	101.5	95.2	75.9
WEP (mg g^{-1})	0.004	0.008	0.002
Water holding capacity (%)	32 ± 3.5	29 ± 0.8	24 ± 1.1
BET[2] surface area (m^2 g^{-1})	25 ± 0.33	5 ± 0.06	25 ± 0.35

LT-CFBG: low temperature circulating fluidized bed gasification ash; FB-I: fluid-bed incineration ash; SP: slow pyrolysis biochar.
[1] From Thomsen et al. (2017) and Raymond et al. (2018).
[2] Brunauer Emmett Teller method.

2.2. Phosphate-solubilising strains and inoculum preparation

The ability of six fungal strains to solubilise P from sewage sludge ashes and biochar was tested. Five *Penicillium* fungal strains were selected according to their P solubilisation ability (tested by Novozymes, Denmark, based on a large commercial strain collection): *Penicillium bilaiae* ATCC 20851 (*Pb.1*), *Penicillium bilaiae* ATCC 18309 (*Pb.2*), *Penicillium bilaiae* ATCC 22348 (*Pb.3*), *Penicillium bilaiae* DBS-5 (*Pb.4*), *Penicillium aculeatum* ATCC 10409 (*Pa*). *Aspergillus niger* ATCC 9142 (*An*) was also included because of its strong P solubilisation ability on rock phosphate, as described by Schneider et al. (2010). From frozen spore stocks (−80 °C), fungal strains were grown for about two weeks on potato dextrose agar (PDA) plates before being sub-cultured for a further two weeks on a fresh PDA plate.

The second-generation spores were collected by washing the plate with sterile MilliQ water. The spore suspension was then filtered through sterile glass wool (Miracloth, EMD Millipore Corporation, Billerica, MA 01821 USA) and centrifuged at 4000 rpm and 4 °C for 10 min. Spore concentration in the suspension was adjusted with MilliQ water by determining the spore concentration with a hemacytometer (Improved Neubauer, Brand, Germany).

2.3. Fungal strain and ash/biochar selection

A volume of 300 µL of ash/biochar supplemented with glucose/ammonium sulphate or sucrose/ammonium sulphate (10 mg C g^{-1}