Waste Management xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Effect of bacterial inoculants on phytomining of metals from waste incineration bottom ash

Theresa Rosenkranz [a,*], Petra Kidd [b], Markus Puschenreiter [a]

[a] University of Natural Resources and Life Sciences, Vienna, Department of Forest and Soil Sciences, Institute of Soil Research, Konrad-Lorenz-Straße 24, 3430 Tulln, Austria
[b] Instituto de Investigaciones Agrobiológicas de Galicia (IIAG), Consejo Superior de Investigaciones Científicas (CSIC), Santiago de Compostela 15705, Spain

ARTICLE INFO

Article history:
Received 19 June 2017
Revised 5 December 2017
Accepted 7 December 2017
Available online xxxx

Keywords:
Secondary raw materials
Phytoextraction
Plant growth promoting bacteria
Salix smithiana
Nicotiana tabacum

ABSTRACT

Waste incineration bottom ash is considered a secondary resource for valuable trace elements (TE), which is currently neglected in most European countries. Phytomining could potentially recover valuable TE from such waste materials but is still at an exploratory stage with many challenges. The use of bioaugmentation to improve plant growth and TE accumulation of metal-tolerant high biomass plants growing on waste incineration bottom ash was evaluated. Bacterial strains that were previously isolated from rhizosphere, roots and contaminated soil were selected according to their plant growth promoting characteristics and tolerance to the bottom ash substrate. Those selected bacterial strains were tested for their beneficial effects on *Nicotiana tabacum* and *Salix smithiana* with regards to phytomining. The rhizobacterial strain *Rhodococcus erythropolis* P30 enhanced the shoot dry weight of *N. tabacum* by on average 57% compared to the control plants. Several bacterial inoculants enhanced biomass production and the nutritional status of *S. smithiana*. Moreover, those bacterial strains previously described to enhance biomass production of *N. tabacum* and members of the Salicaceae on TE-contaminated soils, also enhanced biomass production of these species on bottom ash. However, bacterial inoculants could not enhance trace element accumulation in plants.

1. Introduction

Hyperaccumulators and metal-tolerant high biomass plants have been widely used for the clean-up, stabilisation and rehabilitation of contaminated land since the concept of phytoremediation was first introduced by Chaney (1983) and Baker and Brooks (1989). Whereas the process of phytoremediation generally aims to decontaminate polluted soils, phytomining aims to recover a target element from the above-ground biomass of a plant (Bani et al., 2015; van der Ent et al., 2015). Several field demonstration trials of phytomining can be found, all of these cultivated nickel (Ni)-hyperaccumulators, such as *Streptanthus polygaloides* (Nicks and Chambers, 1995), *Berkheya coddii* (Robinson et al., 1997a), *Alyssum bertolonii* (Robinson et al., 1997b) and *Alyssum murale* (Bani et al., 2015) and were implemented on ultramafic soil, which is naturally enriched in Ni. Phytomining represents an alternative use for these soils which are unattractive for conventional agriculture due to their low fertility and productivity (Bani et al., 2007). Moreover, phytomining may target low-grade ores that cannot be exploited profitably using conventional mining methods (van der Ent et al., 2013).

The European Commission has drawn attention to possible raw material shortages and supply insecurities (EC, 2014). In this context, the use of so-called secondary raw materials and the transition to a circular economy is highly encouraged (EC, 2015). Waste incineration in waste-to-energy plants is a technique widely used in Western Europe (Brunner and Rechberger, 2014) and waste incineration bottom ash accounts for the biggest volume of waste incineration residues. Waste incineration bottom ash can be considered a secondary raw material but its use is limited to substituting natural gravel in building and construction works in some European countries (e.g. Germany, Denmark and the Netherlands), and in other countries the material is directly landfilled without any further use (Crillsen et al., 2006). Either way, trace elements (TE) bound to the aggregates of the bottom ash material are lost for further use. Recent studies have considered the potential of waste incineration bottom ash for TE recovery and urban mining (Morf et al., 2013; Allegrini et al., 2014; Fellner et al., 2015). In a previous study the potential of phytomining from waste incineration bottom ash with hyperaccumulating and metal-tolerant high biomass plants was evaluated (Rosenkranz et al., 2017). Hyperaccumulators had severe problems to establish and produce biomass

* Corresponding author.
E-mail address: theresa.rosenkranz@boku.ac.at (T. Rosenkranz).

https://doi.org/10.1016/j.wasman.2017.12.006
0956-053X/

on the bottom ash substrate due to the high ionic strength and high soluble Cu concentrations. Metal-tolerant *Brassica napus* and *B. juncea* were able to grow well without any toxicity symptoms but showed limited TE accumulation. Two metal-accumulating varieties of *Nicotiana tabacum* showed poor germination and growth of surviving plants was limited. Thus, the current study aimed to improve plant health and growth of metal-tolerant high biomass plants on amended waste incineration bottom ash by inoculating with plant growth promoting (PGP) bacterial strains.

PGP-bacteria benefit their host plants by stimulating growth and biomass production, nutrient acquisition and increasing resistance to various stresses, e.g. toxicity from heavy metals or organic pollutants (Mendes et al., 2013). Bacteria can have a beneficial influence on plant nutrition by increasing the availability of essential nutrients, such as nitrogen (N_2-fixing organisms), phosphorus (by solubilisation or mineralisation through the production of organic acids and/or phosphatases) or iron (by releasing Fe(III)-specific chelating agents or siderophores) (Ma et al., 2009; Rajkumar et al., 2010). PGP-bacteria can also enhance biomass production through the production of phytohormones, such as indole-3-acetic acid (IAA) (Duca et al., 2014). Another PGP-trait is the production of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase that cleaves the precursor of ethylene which is produced by plants under various stresses (Glick, 2014). These plant-associated bacteria can not only enhance growth but also the accumulation of certain TE in the above-ground biomass in phytoremediation-based experiments (Ma et al., 2011a; Sessitsch et al., 2013; Kidd et al., 2017). Metal-accumulating willow varieties are commonly used for phytoextraction (Dos Santos Utmazian and Wenzel, 2007; Wieshammer et al., 2007; Puschenreiter et al., 2013) and several authors have investigated the beneficial effects of PGP-bacteria on their metal accumulation (Kuffner et al., 2008, 2010; Becerra-Castro et al., 2012; Álvarez-López et al., 2017). *Nicotiana tabacum* as a high biomass metal-accumulating plant was frequently tested in Zn/Cd-phytoextraction experiments (Loosemore et al., 2004; Fässler et al., 2010; Herzig et al., 2014a) and inoculation with metal-tolerant PGP-rhizobacterial strains enhanced growth and metal yields (Álvarez-López et al., 2016, 2017). Some of these bacterial inoculants have beneficial effects on a wide range of plant hosts (Grandlic, 2008; Ma et al., 2011b; Becerra-Castro et al., 2012; Balseiro-Romero et al., 2016a).

In the current study high biomass metal-accumulating plant species were grown on a waste incineration bottom ash substrate that was previously characterised and amended to support plant growth. Since plant growth on the optimised substrate was still limited the aim of this study was to improve growth on the waste incineration bottom ash and hence phytomining efficiency by inoculating with PGP-rhizobacterial strains.

2. Materials and methods

2.1. Pot experiment and bacterial inoculants

A pot experiment was carried out in the greenhouse facilities of the IIAG-CSIC from April to June 2015. The experiment involved one substrate, two plant species, four treatments with bacterial inoculants and a non-inoculated control per species. Five replicates were established per treatment. The substrate consisted of 70% (w/w) hazardous waste incineration bottom ash (HWI BA), 20% (w/w) material from mechanical-biological-treatment of municipal solid waste and 10% (w/w) biochar from wood chips. This substrate mixture was selected as the optimum mixture to aid plant growth, although it still represented limitations. Detailed preparation and characterisation of the substrate was provided previously (Rosenkranz et al., 2017). The plant species used in the experiment were *Nicotiana tabacum* and *Salix smithiana*. Seeds of *N. tabacum* were obtained from Phytotech Foundation, Switzerland. Herzig et al. (2014a) used conventional selection and *in vitro* breeding methods to select metal-tolerant tobacco variants with enhanced biomass and metal-uptake. In this study the tobacco clone NBCu-10-8 (F_3 generation) was used. Fresh cuttings of *S. smithiana* (*S. caprea* L. × *S. viminalis* L., clone BOKU 03 CZ-001) were obtained from Silva Tarouca Research Center, Průhonice, Czech Republic. This clone was previously identified for high phytoextraction potential on contaminated soils (Dos Santos Utmazian and Wenzel, 2007; Wieshammer et al., 2007; Iqbal et al., 2012; Puschenreiter et al., 2013;). Pots were filled with fresh HWI BA substrate corresponding to 300 g of dry substrate. Tobacco plants were pre-grown on the HWI BA substrate in a growth chamber and transferred to the experimental pots in the greenhouse two weeks after germination. The willow cuttings were placed directly in the experimental pots. Plants were watered regularly to maintain substrate moisture and grown under a natural day/night light cycle in greenhouse conditions. Pots were re-positioned weekly in order to ensure homogenous light conditions.

Bacterial strains were previously isolated from rhizosphere, roots or soil and were selected according to their PGP-characteristics, metal tolerance and previous inoculation trials (Table 1). Rhizobacterial strains P29, P30 and P87 enhanced the growth of *Nicotiana tabacum*, *Festuca pratensis* or *Salix spp*. when growing in metal-contaminated substrates (Becerra-Castro et al., 2012; Álvarez-López et al., 2016). Strain RP92 was previously isolated from the rhizoplane of *Cytisus striatus* growing in a lindane-contaminated soil (Becerra-Castro et al., 2011) and increased the survival and growth of this species in hexachlorocyclohexane (HCH)-contaminated soils (unpublished results). The same strain improved the growth, nutritive status and increased stress tolerance of *Cytisus striatus* and *Lupinus luteus* when growing in diesel-contaminated or nutrient-deficient soils (Balseiro-Romero et al., 2016a, 2016b). Also, in combination with other PGP-bacterial strains it has been shown to improve the dissipation of diesel range organics (Balseiro-Romero et al., 2016b). Both strains P87 and RP92 have been found to improve the growth of *Tagetes patula* when growing in bottom river sediments contaminated with TE (Ba, Zn), polycyclic aromatic hydrocarbons (PAHs) and polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated furans (PCDFs) (Urbaniak et al., 2015). Strains Con8.28, B32.24 and MR28 have been found to stimulate growth of *Populus* cv. Skado growing in agricultural soils with mixed contamination of PAH and TEs (Cd/Zn/Pb) (Cerdeira Pérez, 2016).

Prior to the inoculation experiment, all the selected strains were pre-adapted to the HWI BA substrate in order to improve their survival and growth in these conditions. Pre-adaptation was done *in vitro* by growing the strains in liquid 284 medium amended with increasing amounts of filter-sterilised HWI BA extracts. The 284 medium contains (per litre): 6.06 g Tris–HCl, 4.68 g NaCl, 1.49 g KCl, 1.07 NH_4Cl, 0.43 g Na_2SO_4, 0.2 g $MgCl_2{\cdot}6H_2O$, 0.03 g $CaCl_2{\cdot}2H_2O$, 0.04 g $Na_2HPO_4{\cdot}2H_2O$, 10 ml Fe(III)NH_4 citrate solution (containing 48 mg/100 ml) plus oligoelements (1.5 mg $FeSO_4{\cdot}7H_2O$, 0.3 mg H_3BO_4, 0.19 mg $CoCl_2{\cdot}H_2O$, 0.1 mg $MnCl_2{\cdot}4H_2O$, 0.08 mg $ZnSO_4{\cdot}7H_2O$, 0.02 mg $CuSO_4{\cdot}5H_2O$, 0.036 mg $Na_2MoO_4{\cdot}2H_2O$) adjusted to pH 7. The medium was supplemented with a mixture of different carbon sources: lactate (0.7 g L^{-1}), glucose (0.5 g L^{-1}), gluconate (0.7 g L^{-1}), fructose (0.5 g L^{-1}), and succinate (0.8 g L^{-1}). Extracts were obtained by shaking 250 g of HWI BA substrate with 625 ml sterile deionised water overnight on an orbital shaker followed by centrifugation (6000 rpm) and filter sterilisation (0.22 μm). The adaptation was initiated by growing the bacteria in 1:10 extract: medium and the amount of extract was progressively increased in successive re-cultures until growth was achieved in 1:1 extract: medium. For each extract: medium combination the strains were re-cultivated twice.

Please cite this article in press as: Rosenkranz, T., et al. Effect of bacterial inoculants on phytomining of metals from waste incineration bottom ash. Waste Management (2017), https://doi.org/10.1016/j.wasman.2017.12.006