Waste Management xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Waste Management

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

A comparison between biostimulation and bioaugmentation in a solid treatment of anaerobic sludge: Drug content and microbial evaluation

G. Llorens-Blanch [a,1,2], E. Parladé [b,1,3], M. Martinez-Alonso [b,3], N. Gaju [b,3], G. Caminal [c,2], P. Blánquez [a,*,2]

[a] Departament d'Enginyeria Química Biològica i Ambiental, Escola d'Enginyeria, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain
[b] Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain
[c] Institut de Química Avançada de Catalunya (IQAC) CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain

ARTICLE INFO

Article history:
Received 12 July 2017
Revised 26 October 2017
Accepted 27 October 2017
Available online xxxx

Keywords:
Bioremediation
Trametes versicolor
Biopiles
Pharmaceutical products
Sewage sludge
Microbial shifts

ABSTRACT

Emerging pollutants can reach the environment through the sludge of Wastewater Treatment Plants. In this work, the use of *Trametes versicolor* in biopiles at lab-scale was studied, evaluating its capacity to remove the most hydrophobic Pharmaceuticals and assessing the evolution of the biopiles microbial communities. The total removal of drugs at real concentrations from sewage sludge was assessed for non-inoculated and fungal inoculated biopiles, testing if the re-inoculation of the biopiles after 22 days of treatment would improve the removal yields. It was found that 2 out of the 15 initially detected pharmaceuticals were totally degraded after 22 days, and re-inoculated fungal biopiles achieved higher removal rates than non-re-inoculated fungal biopiles for single compounds and for all the drugs simultaneously: 66.45% and 49.18% re-inoculated and non-re-inoculated biopiles, respectively. Finally, the study of the bacterial and fungal communities revealed that fungal inoculated and non-inoculated biopiles evolved to similar communities adapted to the presence of those drugs.

1. Introduction

The main residue of any Wastewater Treatment Plant (WWTP) is the sludge, which is originated during the solid-liquid separation (Fytili and Zabaniotou, 2008) performed in primary, secondary and tertiary treatments, and its composition and quantity depend on several factors such as the general operational methods and the geographic situation of the plant (Eddy et al., 1991). Furthermore, the wastewater source had an important role not only in the formation of the sludge, but also in its final composition and physicochemical properties. The most common wastewaters treated in WWTPs have an urban, domestic and/or hospital origin (Harrison et al., 2006).

The use of WWTP's sludge in agricultural and forestry activities has become an interesting valorisation method because of its ability to fertilise soils and the low cost of these materials compared to fertilisers. These actions improve the physicochemical properties of the land and can increase the crops yield (Singh and Agrawal, 2008). Nevertheless, the application of untreated sludge into soils can increase the potential risks for human and animal health (Dean and Suesst, 1985), as it can contain different types of pollutants. Consequently, sludge must be treated before its application into soil in order to remove micro-pollutants.

The sludge produced in a WWTP usually has a high concentration of solids – between 0.25 and 15% in weight – which is mainly composed of organic matter. Its treatment and disposal is one of the most complex and expensive problems during wastewater treatment. In general, sewage sludge must be stabilized, thickened and disinfected before its disposal out of the plant. Common techniques to stabilize the sludge are: anaerobic and aerobic digestion, lime stabilization, composting and heat drying; while the general thickening treatments are: centrifugation, filtration and water evaporation (Eddy et al., 1991; Ramalho, 1996). However, it has been proven that these traditional treatments are not capable of removing emerging pollutants (EPs) from the sludge (Clarke and Smith, 2011; Semblante et al., 2015; Stasinakis, 2012).

Expensive tertiary or advanced treatments have been developed in the last years in order to decrease the presence of EPs in wastewater: adsorption into activated carbon, advanced oxidation

Abbreviations: BQL, Below Quantification Limit; DGGE, Denaturing Gradient Gel Electrophoresis; DW, Dry Weight; EP, Emerging Pollutant; ITS, Internal Transcribed Spacer; ND, Non-Detected; PhAC, Pharmaceutical Active Compound; PPCP, Pharmaceutical and Personal Care Products; WHC, Water Holding Capacity; WRT, White-Rot Fungi; WWTP, Wastewater Treatment Plant.

* Corresponding author.
E-mail address: paqui.blanquez@uab.cat (P. Blánquez).

[1] These two authors contributed equally to this work.
[2] These are the authors responsible of treatment.
[3] These are the authors responsible of microbiologic studies.

https://doi.org/10.1016/j.wasman.2017.10.048
0956-053X/

(e.g. ozone and ultra-violet), UV photolysis, ion exchange and membrane filtration (Bolong et al., 2009; Gavrilescu et al., 2014). Nevertheless, these technologies have high implementation, operational and maintenance costs and have not yet been applied to sewage sludge (Heal the Ocean, 2001; USEPA, 2015).

Nowadays, fungal bioremediation has arisen as an economical and sustainable alternative. Fungi are known to degrade a wide variety of compounds and have been in depth studied in the removal of EPs produced by human activities. In this regard, in biopiles systems the sludge being treated is mixed with a bulking material, which improves the aeration, gives structure (Environment Protection Authrority, 2005; Juwarkar et al., 2010; Khan et al., 2004), and it is used as co-substrate by the fungal inoculum (Gadd, 2001; Singh, 2006). Furthermore, these systems require minimum maintenance and inputs (i.e. energy and water), making them cost-effective processes even for long time treatments (Gomez and Sartaj, 2014; Jørgensen et al., 2000; Nano et al., 2003).

The substrate plays an important role in biopiles inoculated with white-rot fungi (WRF), being one of the key factors for a successful mycoremediation application (Leštan et al., 1996). In those cases, a lignocellulosic waste from agriculture, forestry and/or food industry is supplied as substrate, providing the essential lignocellulosic nutrients that the fungus needs to growth, and promoting the production of lignin-modifying enzymes (Rodríguez Couto and Sanromán, 2005). The most studied and used lignocellulosic substrates are: sawdust, wood chips and barks, wheat straw, corn cobs, grape stalks, and olive oil waste (Kassaveti, 2008; Rigas et al., 2007; Stahl and Aust, 1998). The correct selection of a ligninolytic substrate will lead to better pollutant removal by the inoculated WRFs, with low operational time and minor investments.

In previous studies, dry WWTP sludge was treated in biopiles inoculated with *Trametes versicolor* (Rodríguez-Rodríguez et al., 2014). Straw was used as a substrate and microbial analysis demonstrated that *Trametes versicolor* can still be detected, at least, until day 22. From these results, a re-inoculation strategy for the biopiles after 22 days of treatment – in order to enhance the removal of pharmaceutical and personal care products (PPCPs) – was implemented. The aim of the present work was to determine if bioaugmentation with *T. versicolor* could be used to treat thermal dry WWTP sludge in biopiles systems using pine bark as a substrate. Although bark is more difficult to degrade, hydrolyze and colonize than straw, it is a better bulking material compared to straw and allows the scale-up of biopiles. Biopiles systems under non-sterile conditions were constructed using a non-spiked sludge, treating it for 42 days. A re-inoculation was carried out after 22 days of treatment, PPCPs removals were assessed and microbial community analysis were performed.

2. Materials and methods

2.1. Chemicals

All pharmaceutical compounds, methanol and acetonitrile were of high purity grade (>90%) and were purchased from Sigma-Aldrich (Steinheim, Germany), US Pharmacopeia USP (MD, USA), Europea Pharmacopeia EP (Strasbourg, France) and Toronto Research Chemicals TRC (Ontario, Canada). Further information can be consulted in Gros et al. (2012). The individual standard solutions were prepared according to Gros et al. (2012).

2.2. Microorganisms

The strain *T. versicolor* ATCC 42530 was obtained from the American Type Culture Collection, and maintained by subculturing every 30 days on 2% malt extract agar slants (pH 4.5) at 25 °C.

2.3. Sludge and lignocellulosic substrate

20 L of dry sewage sludge were collected from the final stage of the sludge processing system at El Prat de Llobregat WWTP (Spain) in January of 2014. This plant is designed to treat 419,000 m^3 d^{-1} of wastewater for an equivalent population of two million inhabitants. It is a typical activated sludge (AS) plant that uses anaerobic digesters followed by dehydration and thermal drying techniques to treat the produced sludge. The initial water content of the sludge was 16.71 ± 0.03%, and its water holding capacity was 1.19 ± 0.06 $gH_2O{\cdot}gDW^{-1}$. At the arrival to the laboratory the sludge was frozen (−20 °C) until its use.

10 L of commercial decorative pine bark (*Pinus halepensis*) were bought from a local supplier and used as lignocellulosic substrate for the biopiles systems. The initial size of the pine barks ranged from ca. 2.5 cm to 10 cm. Pruning scissors were used in order to make the small pieces of pine bark not >1.5 cm in the tests. The initial water content was 21.27 ± 0.43%, and its water holding capacity was 1.28 ± 0.01 $gH_2O{\cdot}gDW^{-1}$. The substrate was kept at room temperature until its use.

2.4. Experimental procedures

2.4.1. Fungal mycelial suspension

Blended mycelial suspension and pellet suspension were prepared according to Blánquez et al. (2004). Mycelial suspension was made as follows: Erlenmeyer flasks (500 mL) with 150 mL of malt extract medium (2%) were inoculated with 1 cm^2 plugs from agar cultures in Petri dishes and shook (orbital shakers: 135 rpm and r = 25 mm) for 5 days at 25 °C; the resulting fungal mass was homogenized (X10/20, Ystral GmbH) and stored in sterilized saline solution (0.85% NaCl) at 4 °C.

2.4.2. Water holding capacity and moisture content

The initial moisture of the sewage sludge from El Prat de Llobregat WWTP and the pine bark were determined weighing homogeneous volumes of sludge or substrate, and drying them for 24 h at 105 °C in an oven. They were expressed as percentage of moisture.

The water holding capacity (WHC) of the sewage sludge and the pine bark was determined as described by the European Committee for Standardization: metal cylinders with one of its ends covered with paper filter were filled (up to 2/3 parts of their volume) with sludge or substrate and placed in trays; water was added until it fully covered the sludge or substrate, without exceeding it; next, after two hours in contact with water, the cylinders with sludge or substrate were dried by capillarity for 30 min and weighted (wet weight); and finally, the cylinders were dried for 24 h at 105 °C in an oven and weighted (dry weight – DW). The WHC was calculated as the difference between the dry and the wet weights and expressed as grams of water by gram of dry sludge/substrate ($gH_2O{\cdot}gDW^{-1}$) (CEN, 1999).

2.4.3. Solid-phase experiments

Cultures were performed in Schott bottles (250 mL, 95 × 105 mm; GLS 80; Duran, Inc) equipped with 4-port screw caps (GL 18; Duran, Inc). Three ports of the caps were hermetically closed, while one was kept opened, using a 0.45 μm filter as passive air intake. First, 6 g of sterile lignocellulosic substrate (20 min at 120 °C) were placed in each bottle and inoculated with 2 mL of mycelial suspension, setting humidity to 60% of the water holding capacity. After 7 days of static incubation (25 °C), biopiles were prepared adding 14 g of non-sterile dry WWTP sludge in every single pre-grown fungal culture and then homogenized though circular movements, without screwing the bottle; the moisture level was adjusted to 60%. Biopile cultures were incubated at 25 °C in static conditions until sampling. After 22 days of incubation, half

Please cite this article in press as: Llorens-Blanch, G., et al. A comparison between biostimulation and bioaugmentation in a solid treatment of anaerobic sludge: Drug content and microbial evaluation. Waste Management (2017), https://doi.org/10.1016/j.wasman.2017.10.048