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Biodegradation of 2-naphthalensulfonic acid polymers by white-rot fungi: Scale-up into non-sterile packed bed bioreactors

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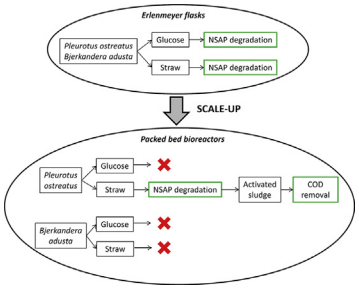
HIGHLIGHTS

- Two white-rot fungi are able to degrade NSA polymers in a petrochemical wastewater.
- A first scale-up of the process into fungal packed-bed bioreactor is proposed.
- A bioreactor worked in continuous mode for three months in non-sterile conditions.
- The use of straw as carbon source allowed fungi to outcompete bacteria.
- The treatment increased the biodegradability of the wastewater from 9% up to 40%.

GRAPHICAL ABSTRACT



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ABSTRACT

This paper presents a first scale up under non-sterile conditions of the biodegradation process of 2-naphthalensulfonic acid polymers (NSAP) contained in a petrochemical wastewater by two white-rot fungi (*Bjerkandera adusta* and *Pleurotus ostreatus*). The biodegradation experiment was conducted first in flasks and then in packed-bed bioreactors filled with inert and biodegradable carriers (straw), the latter acting as both physical support and carbon source. Reactor inoculated with *P. ostreatus* attached on straw worked under non-sterile conditions for three months showing 30 ± 5% NSAP degradation. Respirometric tests showed that the fungal treatment was also able to significantly increase the biodegradable fraction of the wastewater COD, which rose from 9% to 40%. It was observed that the fungal degradation of the straw in the bed releases non-biodegradable by-products. Taking into account this contribution to nbCOD, the combined treatment of fungi and activated sludge could theoretically be able to reduce the original COD by up to 73%.

1. Introduction

Naphthalene sulphonic acid polymers (NSAP) are commonly employed in many industrial sectors covering a wide range of

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activities from textile mills and leather tanning operations to the production of pharmaceuticals, pesticides, cosmetics, polymers, optical brighteners, dispersants, stabilizers, wetting agents and construction materials (Avetta et al., 2012; Germirli Babuna et al., 2009). These compounds are known to have an highly hydrophilic nature (Avetta et al., 2012; Germirli Babuna et al., 2009) and a very low biodegradability (Song and Burns, 2005), resulting in their transport and accumulation in both surface and ground-waters (Avetta et al., 2012; Germirli Babuna et al., 2009; Song and Burns, 2005). Most of NSAP are labeled as toxic chemicals (Germirli Babuna et al., 2009; Shiyun et al., 2002; Song and Burns, 2005), exert genotoxicity activity (Germirli Babuna et al., 2009) and have the ability to remobilize toxic hydrophobic compounds (Germirli Babuna et al., 2009; Song and Burns, 2005).

Conventional biological treatments do not appear effective in removing these compounds (Shiyun et al., 2002); in fact, a study conducted by Song and Burns (2005) demonstrated that four activated sludge (AS) and two single bacterial strain (*Arthrobacter* sp. and *Comamonas* sp.) degraded only the monomers of a mixture of NSAP. The same study demonstrated, on the other hand, that the fungus *Cunninghamella polymorpha* was able to degrade also oligomers from n = 2 to n = 11.

Fungi, in particular white-rot type (WRF) are organisms capable of degrading several recalcitrant compounds such as dyes (Anastasi et al., 2011), pesticides (Xiao et al., 2011), polycyclic aromatic hydrocarbons (Chen et al., 2010), pharmaceuticals (Cruz Morató et al., 2013; Buchicchio et al., 2016) and many others through the non-specific extracellular enzymatic systems composed mainly by Laccases (Lac), Manganese Peroxidases (MnP) and Lignin Peroxidases (LiP) (Harms et al., 2011). Despite the massive work already done by the scientific community, the use of fungi in the field of wastewater treatment still find poor application. Most works are still at the lab scale and/or done under strict limitations for full scale application (e.g. in sterile conditions). The main issue is to design an engineered ecosystem composed mainly by fungi which can allow long-term operations under real conditions, since the typical environmental conditions of wastewater treatment plants (WWTPs) are favorable to bacteria and fungi are easily outcompeted. Indeed, it has been largely demonstrated that the presence of bacteria very often compromises fungal activities (Blánquez et al., 2008; Borchert and Libra, 2001; Hai et al., 2009). In particular, it has been shown that bacteria 1) affect fungus morphology (Hai et al., 2012; Yang et al., 2013b), 2) lead to cessation of fungal growth (Yang et al., 2013a), 3) inhibit enzyme secretion capacity (Yang et al., 2013a), 4) denaturate the secreted fungal enzymes (Yang et al., 2013a) and 5) outperform fungi in the competition for the substrate (Yang et al., 2013b).

Many authors studied some strategies to foster fungal growth and suppress bacterial ones, by changing process parameters or culture conditions. In particular, acid pH seems to play a key role in maintaining fungal biomass active (Gao et al., 2008; Libra et al., 2003). Other strategies include a) the use of nitrogen-limiting conditions, which may, in some cases, foster fungal growth and inhibit bacterial one (Gao et al., 2008; Libra et al., 2003), b) the use of selective carbon sources such as ligno-cellulosic materials (Libra et al., 2003) and c) the use of immobilized or encapsulated mycelium, more resistant to bacterial attacks (Gao et al., 2008; Leidig et al., 1999). Moreover, it could be helpful the use of low substrate concentration, as highlighted by Yang et al. (2009), who found out that in a non-sterile bioreactor treating textile wastewater, the ratio between fungal and bacterial biomasses increased after a decrease in glucose feeding.

The objective of this study is to design a suitable fungal reactor for the degradation of NSAP contained in a petrochemical wastewater working under non-sterile conditions. In particular, to foster fungal growth and enhance biodegradation activity, two WRF selected in a previous study (Gullotto et al., 2015), a *Bjerkandera adusta* and a *Pleurotus ostreatus*, were immobilized on an inert support (open cell polyurethane foam, PUF) and on a lignocellulosic one (straw). Lignocellulosic materials are already been proved to successfully sustain fungal biodegradation; examples are pinewood chips (Ehlers and Rose, 2005), hay, rye, spelt grains, peanut shells (Libra et al., 2003), grape seeds (Lorenzo et al., 2002) and wheat straw (Rodríguez-Rodríguez et al., 2011). The use of these materials could bring some advantages: they are easily available and economical, could be a selective carbon source for fungi and help them to outcompete bacteria (Libra et al., 2003), represent a physical support, enhance the expression of ligninolytic enzymes (Libra et al., 2003) and are source of natural mediators (Camarero et al., 2005; Palli et al., 2015), which are often necessary for degradation of non-phenolic compound (such as NSAP) by laccases (Camarero et al., 2005).

The objective of the present work is to design and present a scaling-up of the NSAP degradation process by white-rot fungi. In this paper first preliminary studies in Erlenmeyer flasks are presented in order to study the degradation abilities of selected WRF (*B. adusta* and *P. ostreatus*) in different conditions (attached on biodegradable and inert support). After these studies, the same processes have been scaled-up into continuous treatments using packed bed bioreactors (PBRs) working under non-sterile conditions.

2. Materials and methods

2.1. Wastewater and chemicals

The wastewater used in this study is a NSAP-containing petrochemical wastewater, collected from a petrochemical factory located in Italy. Once received, raw wastewater was filtered with qualitative filter paper to remove coarse residues of the industrial process and then stored at 4 °C until use. A further 0.45 μm filtration with cellulose acetate filter (Sartorius) was made in order to assess the total suspended solids (TSS) and the soluble COD of the wastewater. During experiments, wastewater pH was lowered to 6 using HCl 0.2 M, in order to make it more suitable for fungal treatment (Gao et al., 2008).

All chemicals were purchased from Sigma-Aldrich.

2.2. Fungus: inoculums preparation and culture conditions

In the present study two WRF, a *Bjerkandera adusta* (MUT 1236) and a *Pleurotus ostreatus* (NCBI KJ020935) were used, both grown and maintained on Malt Extract Agar (MEA) plates (ATCC medium 325) at 4 °C.

For conducted experiments, two types of inoculums were prepared: immobilized mycelium on PUF and on straw.

In order to prepare immobilized mycelium on PUF, ten mycelial plugs made from MEA plates were transferred into 250 mL Erlenmeyer flasks containing 100 mL of Basidiomycetes Rich Medium (BRM) (Bezalel et al., 1997) and grown in the dark at 26 °C in orbital shaker (110 rpm) for seven days. Afterwards, the mycelium was rinsed with sterilized water and then homogenized with a blender (Ultra turrax IKA T18 basic) for 30–40 s at 6000 g/min in 100 mL of modified Czapek Dox (6 g/L $NaNO_3$, 0.52 g/L KCl, 0.52 $MgSO_4 \cdot 7H_2O$,1.52 g/L KH_2PO_4, 10 mg/L $FeSO_4$, 3 mg/L $CuSO_4$, 2 mg/L $ZnSO_4$). Subsequently, eight cubes with 2 cm sides of PUF were added to the flask and colonized by the fungus in 3–4 days in the same conditions (i.e. 26 °C, 110 rpm).

In order to prepare immobilized mycelium on straw it was followed the same procedure, adding 7 g of washed, chopped and