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Effect of cellulose as co-substrate on old landfill leachate treatment using white-rot fungi

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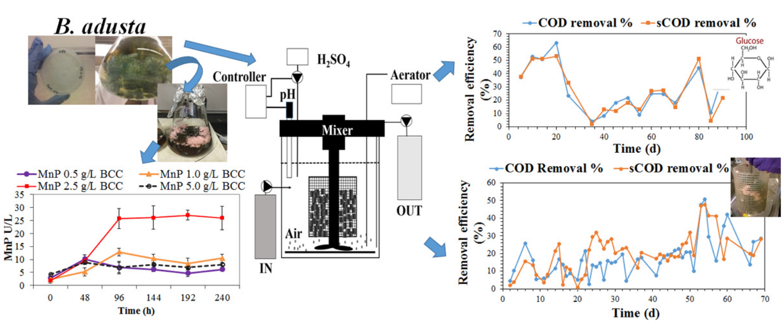
HIGHLIGHTS

- Old LFL treatment with *B. adusta* was investigated through batch and continuous tests.
- *B. adusta* produced MnP with cellulose, glucose, and malt as co-substrates.
- Irregular co-substrate dosages significantly increased COD and sCOD removal.
- COD removals of 63% and 51% were achieved with glucose and cellulose, respectively.
- sCOD removals of 53% and 51% were achieved with glucose and cellulose, respectively.

GRAPHICAL ABSTRACT



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ABSTRACT

Conventional wastewater treatment technologies are ineffective for remediation of old LandFill Leachate (LFL), and innovative approaches to achieve satisfactory removal of this recalcitrant fraction are needed. This study focused on old LFL treatment with a selected fungal strain, *Bjerkandera adusta* MUT 2295, through batch and continuous tests, using packed-bed bioreactors under non-sterile conditions. To optimize the process performance, diverse types of co-substrates were used, including milled cellulose from beverage cups waste material. Extracellular enzyme production was assayed, in batch tests, as a function of a) cellulose concentration, b) leachate initial Chemical Oxygen Demand (COD) and Soluble COD (sCOD), and c) co-substrate type. Bioreactors were dosed with an initial start-up of glucose (Rg) or cellulose (Rc). An additional glucose dosage was provided in both reactors, leading to significant performance increases. The highest COD and sCOD removals were i) 63% and 53% in Rg and ii) 54% and 51% in Rc.

1. Introduction

Landfill Leachate (LFL) is defined as the liquid produced by rainwater percolation in landfill waste layers. Since sanitary landfilling is one of the most common methods for disposing Municipal Solid

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Waste (MSW) (Nghiem et al., 2016), the generation of LFL cannot be prevented (Ghosh et al., 2014a). LFL contains recalcitrant organic compounds that standard biological processes are unable to efficiently degrade (Vedrenne et al., 2012; Zhao et al., 2013a; Tigini et al., 2014). Therefore, the search for innovative and sustainable technologies to reduce the impact of untreated leachate is a serious environmental concern (Jones et al., 2006; Ghosh et al., 2014b). Although the composition of LFL varies widely, depending on diverse factors including age and degree of stabilization of waste, several common features can be observed (Umar et al., 2010; Razarinah et al., 2015), such as the presence of high ammonia concentrations, high organic loads, and the presence of inorganic compounds, including heavy metals and salts (Kamaruddin et al., 2014).

With landfill aging, the ratio between Biological and Chemical Oxygen Demand (BOD_5/COD) decreases due to the hydrolysis of the biodegradable organic fraction of LFL, while the non-biodegradable fraction of COD remains unchanged. In particular, three stages of LFL have been classified according to landfill age (Peyravi et al., 2016). Young leachate (<5 years) presents higher concentrations of biodegradable organic loading, with a BOD_5/COD ratio >0.3 composed mainly (about 70%) of Volatile Fatty Acids (VFA). Intermediate leachate, from 5 to 10 years, presents a BOD_5/COD ratio between 0.3, and 0.1 and its composition includes 5–30% VFAs, as well as humic and fulvic acids (Renou et al., 2008). In contrast to young LFL, old leachate (>10 years) presents a low BOD_5/COD ratio (<0.1) and high concentrations of refractory humic and fulvic acids as a consequence of microbial activity (Batarseh et al., 2010; Kalčíková et al., 2014; Ghosh and Thakur, 2016). However, the cut-off between intermediate and old leachate is not strictly defined (Peyravi et al., 2016), and often the same treatments are applied to both intermediate and old LFL (Bohdziewicz and Kwarciak, 2008).

Although biological treatments can effectively remove non-stabilized organic matter and toxic compounds in young LFL, the efficiency decreases with the age of the leachate and, generally, further physical-chemical treatment are required before discharging old LFL in receiving waters. Therefore, the achievement of sustainable technologies for old LFL treatment is still a challenge (Peyravi et al., 2016).

The movement of LFL into the surrounding soil, ground water, or surface water, may lead to severe pollution (Razarinah et al., 2015; Kumari et al., 2016) and thus regulations concerning LFL discharge into receiving waters are becoming more and more stringent (Renou et al., 2008; Peyravi et al., 2016). Indeed, the traditional hauling of LFL to wastewater treatment facilities can interfere with UV disinfection (Zhao et al., 2013b) and LFL composition can inhibit the biological treatment resulting in increased concentrations of effluents (Neczaj and Kacprzak, 2007).

For all these reasons, LFL has been regarded with particular interest as a highly polluted wastewater whose treatment is generally complex and expensive (Kamaruddin et al., 2014). The increasing attention on LFL treatment is clearly visible in the growing number of articles related to this topic. Over 110 articles concerning LFL treatment were published in the scientific literature between 1970 and the end of 20^{th} century, and >600 have been published since the beginning of the 21^{st} century (source ISI Web of Science).

Innovative biological treatments, such as the use of white-rot fungi, have been widely investigated, resulting effective remediation of several problematic wastewaters (Lopez et al., 2002), including pharmaceutical wastewater (Marco-Urrea et al., 2009), olive mill wastewater (Kissi et al., 2001), bleaching wastewater from pulp paper industries (Fang and Huang, 2002), textile wastewater (Rodriguez-Couto, 2013), and petrochemical wastewater (Palli et al., 2016). Effective remediation of soils contaminated with polycyclic aromatic hydrocarbons has also been achieved using white-rot fungi (Di Gregorio et al., 2016).

The use of white-rot fungi has been recently applied in combination with more common approaches, including other biological treatment methods (Gullotto et al., 2015), as well as physical and chemical methods (Castellana and Loffredo, 2014; Loffredo et al., 2016). Recalcitrants of LFL have been in part identified as natural macromolecules including lignins, tannins, humic materials, folic acids, carbohydrates (Gourdon et al., 1989) and, partially, as organic pollutants such as preservatives used in personal care products (PCPs), such as methylparaben (MP), ethylparaben (EP), propylparaben (PP), and butylparaben (BP), hormones, pharmaceuticals, halogenated hydrocarbons, and pesticides (Peyravi et al., 2016). When considering the refractory fraction of LFL, the use of white-rot fungi, with their ligninolytic systems, could play an important role in its treatment (Ellouze et al., 2008). Effective fungal treatments are often associated with the production of extracellular ligninolytic enzymes, such as manganese-dependent peroxidases (MnP), lignin-peroxidases (LiP), and laccases (LaC) (Wesenberg et al., 2003; Ellouze et al., 2008), all of which are expressed by white-rot fungi (Razarinah et al., 2015).

Studies of fungal treatment of LFL in the scientific literature have focused, mainly, on remediation of young LFL. For example, a COD reduction of up to 90% with 50% diluted leachate has been associated with laccase activity up to 4000 U/L (Ellouze et al., 2008, 2009). In contrast, inhibition of fungal enzymatic activity has been reported using 90% old LFL (Kalčíková et al., 2014), although normal enzymatic activity was restored when the concentration of old LFL was reduced. Tigini et al. (2013) reported the association of decolourisation with ligninolytic enzymatic activity, through batch experiments on LFL using autochthonous and allochthonous fungal strains. The authors also quantified the fungal load and ecotoxicological features of LFL (Tigini et al., 2014).

Although promising, the majority of the results achieved with fungal treatment on LFL have been attained in batch experiments. Only a limited number of experiments have been carried out in continuous bioreactors (Ghosh et al., 2014a; Saetang and Babel, 2009), and no full-scale applications have been reported.

In this paper the treatment efficiency of a selected white-rot fungus, *Bjerkandera adusta* MUT 2295, on old LFL (from a landfill site in Winnipeg, Canada) has been investigated, under non-sterile conditions, through batch and continuous experiments. In particular, batch tests were performed to evaluate the enzymatic activity of the fungus using glucose, malt extract or milled cellulose as co-substrate under different experimental conditions including a) different cellulose concentrations and b) leachate dilutions. Continuous experiments were carried out using bench-scale packed-bed trickling bioreactors in which *Bjerkandera adusta* was inoculated as immobilized on polyurethane foam carriers.

2. Material and methods

2.1. Chemicals, fungal strain, and substrates

All chemicals used in this study were of analytical grade and purchased from VWR Canada. The fungal strain used in this study, *Bjerkandera adusta* MUT 2295, was obtained from *Mycotheca Universitatis Taurinensis* (MUT). The strain, previously used to treat textile, tannery and pharmaceutical wastewaters (Anastasi et al., 2010; Spina et al., 2012), was selected during previous experiments (Bardi et al., 2016) on account of its capability of decolourizing a sample of leachate (Italy) up to 40%. The color removal was associated with MnP production up to 40 U/L.

The strain was preserved on Malt Agar plates (MEA, glucose 20 g/L, malt extract 20 g/L, yeast extract 20 g/L and peptone 2 g/