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Fungal and enzymatic treatment of mature municipal landfill leachate

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ABSTRACT

The aim of our study was to evaluate biotreatability of mature municipal landfill leachate by using white rot fungus and its extracellular enzymes. Leachates were collected in one active and one closed regional municipal landfill. Both chosen landfills were operating for many years and the leachates generated there were polluted by organic and inorganic compounds. The white rot fungus *Dichomitus squalens* was able to grow in the mature leachate from the closed landfill and as it utilizes present organic matter as a source of carbon, the results were showing 60% of DOC and COD removal and decreased toxicity to the bacterium *Aliivibrio fischeri*. On the other hand, growth of the fungus was inhibited in the presence of the leachate from the active landfill. However, when the leachate was introduced to a crude enzyme filtrate containing extracellular ligninolytic enzymes, removal levels of COD and DOC reached 61% and 44%, respectively. Furthermore, the treatment led to detoxification of the leachate to the bacterium *Aliivibrio fischeri* and to reduction of toxicity (42%) to the plant *Sinapis alba*. Fungal and enzymatic treatment seems to be a promising biological approach for treatment of mature landfill leachates and their application should be further investigated.

1. Introduction

Biological treatment is often used due to its reliability, simplicity and high cost-effectiveness and provides many advantages in terms of biodegradable matter and nitrogen compounds removal. However, the efficiency of biological processes is strongly limited in presence of refractory or inhibitory compounds in wastewaters (Renou et al., 2008). One of the wastewaters often treated by biological processes is the landfill leachate. Landfill leachate is generated in every landfill and the leachate composition greatly varies depending mainly on the age of the landfill (Neczaj et al., 2005). In the beginning of the landfill operation, acidogenic phase takes place. This early phase of the landfill lifecycle leads to the release of a large quantity of highly biodegradable volatile fatty acids: they create as much as 95% of the organic content (Armstrong and Rowe, 1999). Such a young leachate can be successfully treated by different biological methods. After the relatively short acidogenic phase (up to 5 years after the waste placement) the efficiency of biological treatment plants slowly decreases due to changing conditions in the body of landfills. Organic matter produced during the acidogenic phase is utilized by the microorganisms presented in the landfill which reached the methanogenic phase. Thus the landfill becomes anaerobic digester by itself. During this phase, high concentrations of refractory humic and fulvic acids, which are the products of microbial degradation, appear in the methanogenic leachates (Batarseh et al., 2010). Concentration of biodegradable organic matter in an old but active landfill can be relatively stable after many years due to the continual waste filling which provides a source of carbon available for microbial growth (Armstrong and Rowe, 1999). However, the efficiency of the aerobic treatment is also affected by a high concentration of inhibitory compounds in the methanogenic leachate, such as ammonium nitrogen, which has a significant effect on slow growing nitrifying organisms. Salinity of leachate also increases with landfill age and causes sludge bulking resulting in high concentration of suspended solids in the effluent of the activated sludge system (Di Iaconi et al., 2006).

Recently, fungal treatment has been intensively studied and white rot fungi have shown a great potential for removal of hazardous and toxic pollutants. White rot fungi produce various extracellular enzymes, including laccase (Lac), lignin peroxidase (LiP) and manganese peroxidase (MnP) which are involved in the degradation of lignin and their natural lignocellulosic substrates (Wesenberg et al., 2003). Ligninolytic enzymes are even capable of degrading various pollutants as phenols, pesticides, polychlorinated biphenyls, chlorinated insecticides, dyes and a range of other compounds (Brijwani et al., 2010). White rot fungi enzymes were most often applied for treatment of textile wastewaters (Chander and Arora, 2007; Nilsson et al., 2006; Rodríguez-Couto, 2012; Wesenberg et al., 2003) due to their excellent ability of decolorization and detoxification of dyes (Erkurt et al., 2007). Ellouze et al. (2009) have reported a successful treatment of a young landfill leachate by different strains of white rot fungi. However, fungal

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treatment of mature leachate generated in old landfills has not been investigated so far. Furthermore, treatment involving fungi and their extracellular enzymes may be beneficial during the whole landfill lifecycle, since it offers easy degradation of non-stabilized organic matter as cellulose, hemicellulose and lignin occurring in the leachate at the beginning of landfill operation, and degradation of stable, refractory organic matter as humic and fulvic acids appearing in the later years of landfill maturing (Zavarzina et al., 2004).

In this context, the aim of our study was to evaluate the potential of using the white rot fungus *Dichomitus squalens* and its extracellular enzymes for treatment and reduction of toxicity of mature landfill leachates from active and closed landfills with different composition and content of organic matter.

2. Material and methods

2.1. Characterizations of landfill leachates

Landfill leachates were sampled in two regional municipal landfills. The sampling sites were chosen to obtain leachates from landfills of different ages and thus different contents of biodegradable organic matter. First landfill selected was approximately 30 years old and it is still active with continuous waste disposal. The leachate was sampled in March 2011 (L1) at the effluent from retention basin. The second chosen landfill operated from 1960, it has been closed since 2001 and it was used for disposal of municipal waste and co-disposal of different wastes from tannery industry. The leachate was sampled in March 2012 (L2) from the open retention basin (Martić, 2012). The sample was immediately transported in high density polyethylene containers to the laboratory. Physico-chemical parameters and toxicity were evaluated immediately; the samples for treatment experiments were frozen at −28 ± 2 °C.

The quality of landfill leachates was evaluated by physico-chemical parameters: pH, BOD_5 (Biochemical Oxygen Demand) (ISO 5815-1, 2003), COD (Chemical Oxygen Demand) (ISO 6060, 1989), DOC (Dissolved Organic Carbon) (ISO 8245, 1999), ammonium nitrogen (ISO 7150-1, 1984), nitrite and nitrate nitrogen, orthophosphates and chlorides (Public Health Association, 2012). Each analysis was performed twice in two parallels and standard deviation (SD) was calculated. The samples were not filtered prior analyses. All measurements were validated by using reference materials.

In addition to physico-chemical parameters, toxicity of the leachates was monitored during the treatment procedures by two toxicity tests using the marine bacterium *Aliivibrio fischeri* and the terrestrial plant *Sinapis alba*. These organisms belong to sensitive species, they are often used for toxicity testing and both tests require only a low amount of the sample. The marine bacterium is tolerant to high concentrations of inorganic ions in landfill leachates and thus the changes in toxicity refer to changes in organic pollutants content while the terrestrial plants present high sensitivity to both organic and inorganic components. The test using freeze-dried luminescent bacterium *Aliivibrio fischeri* (ISO 11348-3, 2007) is based on the measurement of inhibition of bioluminiscence in presence of a toxic sample. The bioluminescence was measured prior and after incubation (30 min) by a LUMIStox luminometer (DR. LANGE, Germany) and the inhibition (%) was calculated. The second toxicity test uses seeds of white mustard *Sinapis alba.* Phytotoxic effects of leachates which result in inhibition (%) of root growth in the first 3 days of the plant germination period were measured (MŽP, 2007). Each tested leachate was diluted by growth medium to obtain test concentrations which caused more than 50% and less than 100% inhibition.

2.2. Fungal and enzymatic treatment

White rot fungus *Dichomitus squalens* was chosen for fungal and enzymatic treatment due to its excellent ability to produce extracellular enzymes laccase (Lac) and manganese peroxidase (MnP) which degrade different pollutants. The fungus *Dichomitus squalens* MZKI B1233 was obtained from the MZKI culture collection (National Institute of Chemistry, Ljubljana, Slovenia). The strain was maintained on 2.5% malt agar plates at 4 °C as described previously (Pavko and Novotný, 2008). The mycelial suspension of *Dichomitus squalens* was prepared by inoculation of four 1 cm diameter plugs from the fungus growing zone on malt agar, in 50 mL of nitrogen limited mineral medium (Tien and Kirk, 1988) in a 250 mL Erlenmeyer flask. This was incubated at 28 °C in an incubator (Heraeus, Function Line IP 20, Germany). After 6–7 days a dense mycelial mass was formed. To prepare the mycelium suspension for inoculation, the suspension was disrupted with an Ultra-Turrax T25 (Janke & Kunkel, IKA Labortechnik, Germany) at 9000 rpm under sterile conditions prior to inoculation.

2.2.1. Growth of D. squalens in medium with landfill leachate

First, growth of fungus in landfill leachates was investigated. The treatments were prepared by inoculating 100 mL of growth medium (Babič and Pavko, 2012) with 50% v/v of landfill leachate without nitrogen and carbon source, but with beech wood sawdust, in 250 mL Erlenmeyer flask with 5% v/v of mycelial suspension. The control medium was prepared and inoculated in the same way. It contained 50% v/v of deionized water with pH 4.5. For a positive control the optimized growth medium with beech wood sawdust was used. The Erlenmeyer flasks with fungus were incubated on a RVI-403 rotary shaker (Tehtnica, Slovenia) under constant temperature (28 °C ± 1 °C) and agitation of 150 rpm for 8 days. Aliquots of the liquid culture were collected for determination of extracellular laccase (Lac) and manganese peroxidase (MnP) enzymes activities; DOC, COD and N-NH_4^+ removal as well as decrease of toxicity to *Aliivibrio fischeri* and *Sinapis alba* were monitored. For the determination of biomass (with beech wood sawdust) dry weight, duplicate flasks were harvested at indicated times and filtered through Whatman No. 1 filter paper that had previously been dried at 105 °C to a constant weight. The biomass (with beech wood sawdust) retained on the filter paper was dried at 105 °C to a constant weight, and the biomass (with beech wood sawdust) weight and concentration of TOC (%) in biomass were determined.

2.2.2. Ligninolytic enzyme production in shaken cultures

For production of ligninolytic enzymes of white rot fungus *Dichomitus squalens*, the shaken cultures were prepared by inoculating 200 mL of the optimized growth mineral media containing beech wood sawdust (Babič and Pavko, 2012) in a 500 mL Erlenmeyer flask with 5% v/v of the mycelial suspension. The fungus was incubated on a RVI-403 rotary shaker under constant temperature (28 °C ± 1 °C) and agitation of 150 rpm. *Dichomitus squalens* biomass was separated after 7 days of cultivation from the extracellular medium with ligninolytic enzymes through Whatman filter No. 1. Crude enzyme filtrate was used for the evaluation of toxicity and biodegradability of landfill leachates.

2.2.3. Toxicity of the landfill leachates to ligninolytic enzymes

The experiments for determination of toxicity of landfill leachates to Lac enzymes were prepared in 250 mL Erlenmeyer flasks with 100 mL of medium with 10% v/v of the crude enzyme filtrate and 90% v/v of properly diluted landfill leachate. Dilutions were: 10, 30, 50, 70 and 90% v/v. Deionized water with pH 4.5 was used for dilution. Aliquots of the liquid culture were collected for determination of activity of Lac enzymes.