



# Enhanced performance of the aerobic landfill reactor by augmentation of manganese peroxidase



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## HIGHLIGHTS

- Waste treated with MnP reaches stable conditions and increases biodegradability.
- MnP enhancement of waste resulted in increasing nutrient availability for microbes.
- Biogas production could be increased over three times after enzymatic enhancement.
- Faster hydrolysis rates in MnP enhanced lignocellulose waste material.

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## ABSTRACT

The aim of the work discussed in this article was to determine the ability of an MnP augmented aerobic waste cell to reach stable conditions rapidly in terms of gas production, nutrient content and cellulose and hemicellulose to lignin ratio (C + H/L). Two types of experiments were conducted; small batch and laboratory scale lysimeter experiments. Results from batch experiments showed that enzyme added treatments have the capability to reach a stable C + H/L and lower gas production rates, faster than the treatments without enzyme addition. Enzyme enhancement of the lysimeter increased the rate of biodegradability of the waste; gas production increased more than two times and there was clear evidence of increase in nutrients (nitrogen, dissolved carbon, biological oxygen demand) in the lysimeter leachate.

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## 1. Introduction

Landfill bioreactors are commonly operated under anaerobic conditions. The anaerobic operation enhances landfill gas (LFG) production, which can be used for energy recovery. Aerobic waste degradation, on the other hand, shows reduced waste stabilization periods and reduced greenhouse gas (GHG) production rates compared to anaerobic operation (El Fadel et al., 2012; Hashisho and El Fadel, 2014). Also, anaerobic waste cells are not feasible in all scenarios. For example, aerobic waste cells are more suited for remote locations and small scale landfills. Recent trends in hybrid anaerobic/aerobic landfills have prompted new research in aerobic landfilling technologies (Rich et al., 2008). Several experimental studies have demonstrated the viability of operating waste cells as aerobic bioreactors to accelerate waste decomposition to a level where the cell could be mined (Read et al., 2001; Ko et al., 2013).

One of the barriers to implementing aerobic landfill technology, is the presence of compounds that biodegrade slowly (Mussatto et al., 2008). In addition, the biodegradation rate is significantly reduced in latter stages of aerobic waste cells, due to the decrease in availability of readily degradable organic matter. Hence, enhancement of biodegradation is very important for the final phase of the waste cell operation. The enhancement of aerobic degradation of waste can be achieved in several ways: control of waste cell temperature, leachate augmentation, and bioventing (Ishigaki et al., 2003). This enhancement could be achieved through leachate augmentation including addition of sludge, addition of supplemental nutrients, and augmentation of leachate with potential enzymes. Among these techniques, the addition of sludge is the most common and oldest practice (Jayasinghe et al., 2011).

Very low rates of degradation due to the presence of lignin could be the main factor that limits the waste stabilization in aerobic waste cells. Lignin is a long chain aromatic hydrocarbon found in organic substances, and is difficult to naturally biodegrade. Lignocellulose, which consists of cellulose, hemicellulose and lignin, is

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the major organic component of MSW (Lin et al., 2010). Lignin is less inhibitory to substrate decomposition in an aerobic environment than in an anaerobic one. This is due to the physical association of lignin with cellulose. Also, lignin is relatively degradable in aerobic environments but refractory in anaerobic ones (Komilis and Ham, 2003). White Rot Fungus (WRF) is a type of basidiomycete that can degrade lignin, and hence is also known as wood-decay-fungi. Brown rot Fungi (BRF) completes a similar function to WRF but to a lesser extent. The biodegradation of lignin is limited to several types of WRF and BRF that can be found in nature (Brown and Chang, 2014). These fungi produce several types of enzymes that can biodegrade lignocellulose. Other lignin degrading methods, such as photochemical strategies also has been studied recently (Nguyen et al., 2014), however, this paper only focuses on waste lignin degradation using fungi.

Hettiaratchi et al. (2014) have demonstrated that enzyme addition can increase the lignin degradation of landfilled waste under both anaerobic and aerobic conditions. The lignin degrading enzymes in general include an array of oxidases and peroxidases. Laccase, manganese peroxidase (MnP), lignin peroxidase (LiP) and versatile peroxidase (VP) are assumed to be the first in the line of proteins expressed during the fungal catabolism of lignin (Janusz et al., 2013). Examples of commercially available lignin degraders are LiP, MnP, soybean peroxidase (SbP), horseradish peroxidase (HRP), and laccases. Of these peroxidases, LiP and MnP are described as true lignin degraders due to the high potential redox value (Jayasinghe et al., 2011). Also, Hettiaratchi et al. (2014) demonstrated that MnP is a better enhancement agent than LiP. Although LiP is a true lignin degrader, it does not hydrolyse phenolic compounds, hence it has a lower potential to degrade lignin than MnP. A similar trend was observed by Yazici et al. (2012) and reported that faster and complete removal of phenol, chlorophenol, dichlorophenols, and trichlorophenol were achieved in the aerobic landfill, while aerobic treatment was less effective on tetrachlorophenol and pentachlorophenol, compared to anaerobic environments.

This study is aimed at understanding the biochemical behaviour of MnP augmented waste in terms of lignin, cellulose, and hemicellulose, and total organic content (TOC). The study also demonstrates the behaviour of a larger-scale lysimeter experiment that simulates a bioreactor aerobic landfill, in order to study the expected field behaviour.

## 2. Materials and methods

### 2.1. Characteristics of waste material

Samples of partly degraded MSW were collected from a 30-year old landfill cell located at the City of Calgary Shepard landfill, Calgary, Canada. The sampled cell had an average depth of approximately 12 m, area of one hectare, and a cover thickness of 1 m. Samples were taken from 3 boreholes at different depths (every 1 m up to a total depth of 8 m). ASTM (2014) Standard Procedure D4687 was followed during the sample collection. This standard ensures the collected samples are representative of the entire landfill cell.

Once collected, the waste samples were mixed thoroughly. The samples were then shredded and sieved (Canadian standard sieve series – maximum sieve size 14 mm) to an average particle size of 4.2 mm prior to use in lysimeter experiments. The material required for batch experiments were further sieved to 2 mm (Canadian standard sieve series – No 8–2.38 mm).

The characteristics of shredded and sieved waste samples were determined experimentally according to the standard test methods. The moisture content of the waste sample was 24% of TS

and the field capacity was measured as 44%. The volatile solids content was 22.21% of dry solids. Standard test methods for moisture content, dry solids and volatile solids were used as discussed by Jayasinghe (2013). Funnel experiments were conducted to determine the FC of partly degraded solid waste material (Jayasinghe, 2013).

Substrate utilization can be used as an indication of the success of experiments. In order to measure substrate utilization, the cellulose, hemicellulose and lignin contents were determined according to the ASTM-D1106 standard test method with minor modifications, as described by Liferi (2010). Lignin content was measured as 14% of volatile solids.

A total of 10 samples were analyzed for characterization experiments.

Unlike in anaerobic conditions, it is not necessary to activate peroxidase enzymes using an oxidising agent under aerobic conditions as explained by Hettiaratchi et al. (2014). MnP from *Nematoloma frowardii* purchased from Sigma-Aldrich Co. (Product #: 41563) was used for the experiments.

### 2.2. Batch experiments

The laboratory experiments were conducted in batch reactors of 1 L glass bottles with plastic caps and a septum. 25 g of dry waste, 15 mL of distilled water, and a variable dose of MnP (0, 0.04, 0.08, 0.12, or 0.16 mg/g DS in sample) were added to each bottle. The experimental bottles were kept at room temperature ( $22 \pm 1$  °C), which is representative of the average temperature in a bioreactor landfill (Hettiarachchi et al., 2013). Each treatment had duplicate samples. The glass bottle reactors were opened every two days to ensure that there was no gas build up and increase in pressure within the reactors. Opening the bottles also ensured that O<sub>2</sub> levels did not decrease over time. The O<sub>2</sub> level can impact the microbial population in the bottles. The headspace gas was collected and measured using a syringe and Micro GC. Headspace CO<sub>2</sub> was used as the response of the system. 1 L of headspace volume was used in the CO<sub>2</sub> concentration calculations.

The bottles were mixed every 6–8 days and 1 g of solid samples were collected from each bottle for solid sample analysis. The lignin, cellulose and hemicellulose contents and TOC were measured as responses.

### 2.3. Lysimeter experiments

A laboratory cylindrical plastic lysimeter bioreactor unit with an internal diameter of 35 cm and waste filled volume of 30 L was filled with 90 kg of wet waste and the waste was kept at field capacity. The lysimeter was designed such that leachate could be collected at the bottom and re-circulated to the top of the waste matrix using a leachate distribution system at the top. A leachate collection tank was connected to lysimeter to collect and store the leachate before re-circulation. Lysimeter was aerated using a vertical piping system. A representative diagram of the lysimeter is illustrated in Fig. 1. Once a stable gas production rate was reached, i.e., rate of gas production stopped increasing, at day 28, the lysimeter leachate was supplemented with a 50 mg of MnP. The experiment was conducted for 65 days.

The leachate recirculation rate and the aeration rate were controlled based on predetermined values. The aeration flow rate was maintained at 1 LPM (Bartholameuz and Hettiaratchi, 2016). The moisture inside the lysimeter was kept at field capacity by leachate recirculation. Every 2 days, headspace gas concentrations were measured and leachate samples were collected. Total Nitrogen (TN), pH, Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), and Dissolved Organic Carbon (DOC) were measured in the leachate. TN was measured according to the APHA

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