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Effect of enzyme additions on methane production and lignin degradation of landfilled sample of municipal solid waste

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ABSTRACT

Operation of waste cells as landfill bioreactors with leachate recirculation is known to accelerate waste degradation and landfill gas generation. However, waste degradation rates in landfill bioreactors decrease with time, with the accumulation of difficult to degrade materials, such as lignin-rich waste. Although, potential exists to modify the leachate quality to promote further degradation of such waste, very little information is available in literature. The objective of this study was to determine the viability of augmenting leachate with enzymes to increase the rate of degradation of lignin-rich waste materials. Among the enzymes evaluated MnP enzyme showed the best performance in terms of methane yield and substrate (lignin) utilization. Methane production of 200 mL CH_4/g VS was observed for the MnP amended reactor as compared to 5.7 mL CH_4/g VS for the control reactor. The lignin reduction in the MnP amended reactor and control reactor was 68.4% and 6.2%, respectively.

1. Introduction

Recent research has shown the possibility of operating waste cells as landfill bioreactors as opposed to "dry tomb" landfills, the preferred option in most jurisdictions. In dry tomb landfills, the waste is kept dry to minimize leachate production, whereas, in landfill bioreactors, the waste is kept moist to promote microbial activity. Landfill bioreactors could be operated either in anaerobic or aerobic mode or in hybrid mode (sequential or simultaneous anaerobic/aerobic modes). Sequential operation has the advantage of energy recovery as well as resource/space recovery, if the stabilized waste is mined at a later stage (Hettiaratchi et al., 2010). One of the primary aims of landfill bioreactors is to achieve a high rate of waste degradation, thereby enhancing waste stabilization and methane generation (Kumar et al., in press; Mehta et al., 2002).

The methane production in anaerobic bioreactors is not constant, but decreases over time (Pohland et al., 2003), and the rate depends on the rate of substrate hydrolysis (Kjeldsen et al., 2002). Modification of leachate quality before recirculation is a promising technique to increase the methane production rate at the later stages of bioreactor operation. Most of the past research on leachate modification has focused on nutrient enhancement and pH balance (Barlaz et al., 1990). Barlaz et al. (1990) noted that methane production from fresh waste could be considerably enhanced by nutrient addition and pH neutralization. Few past studies have investigated enzyme enhancement of leachate before recirculation (Lagerkvist and Chen, 1993; Cirne et al., 2008). These studies focused on the use of cellulase enzyme to enhance the degradation of cellulose-rich waste. In biosolids management field, researchers have studied the enzymatic hydrolysis of biosolids. The pre-mixing of mixed biosolids with enzymes prior to anaerobic digestion was shown to improve the biosolids degradation and enhance methane production (Delgenes et al., 2003; Wawrzynczyk et al., 2008).

Lignocellulose, consisting mainly of the polymers, cellulose, hemi-cellulose, and lignin, is a major component of municipal solid waste (MSW) (Sanchez, 2009), and is not easily biodegradable. Many microorganisms are capable of degrading and utilizing cellulose and hemi-cellulose as carbon and energy sources while lignin is highly resistant to degradation (Higuchi, 2006). Therefore, at later stages of bioreactor operation, most of the undigested MSW could be lignin-rich waste materials. Lignin is a three-dimensional polymer connected by several acid resistant C–C linkages. Lignin could be only partly degraded to monomeric compounds by hydrolysis and is mostly degraded by oxidative attack on the C–C bonds (Martinez et al., 2005; Higuchi, 2006). The white rot fungi have the unique ability of degrading lignin by oxidation (Higuchi, 2006; Sanchez, 2009). A special category of commercially available enzymes made from white rot fungi are peroxidases. Peroxidases could potentially catalyze the lignin degradation process.

There is considerable interest in using peroxidases in contaminated site remediation (Husain et al., 2009) and sludge dewatering (Neyens and Baeyens, 2003). Recent studies have shown that the

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use of peroxidases in waste management processes could be effective in breaking down many organic pollutants. However, enhancing degradation of lignin-rich waste materials by the addition of peroxidase enzymes has not been studied. This paper presents results from the first stage of a multi-stage project to determine the feasibility of augmenting leachate with different peroxidase enzymes to increase the rate of waste degradation during later stages of anaerobic landfill bioreactor operation. In this stage of the research program, laboratory batch experiments were conducted to determine the effectiveness of using peroxidise enzymes to enhance methane production; determine the factors affecting the enzyme supported degradation process, and identify the enzyme type most suitable for enhancing methane production. The subsequent stages of the multi-stage project will include laboratory flow-through lysimeter column studies and full-scale leachate recirculation experiments at a field landfill bioreactor known as the Calgary Biocell (Hettiaratchi et al., 2010).

2. Methods

2.1. Waste samples

Batch experiments were conducted using partly degraded MSW samples collected from a 30-year old landfill cell located at the city of Calgary landfill site. The average depth of the sampled cell is about 12 m. The cover thickness is 1 m. Samples were taken from 3 boreholes, at different depths up to a total depth of 10 m. In order to obtain samples representative of the entire landfill cell, ASTM standard D4687 procedure was followed during sample collection. Once collected, the samples were shredded to an average particle size of about 2 mm. The mass of wet waste used for each batch experiment was 2 g (with 0.37 g of volatile solids (VS)).

The characteristics of waste samples were determined according to the standard test methods (APHA, 2005), and are shown in Table 1. In order to use substrate utilization as an indicator of the success of experiments, the lignin contents of the waste before and after experiments were determined according to the methodology described by Lifrieri (2010). The initial and final pH values were measured and found no significant difference.

2.2. Types of enzymes

Examples of commercially available peroxidases are lignin peroxidase (LiP), manganese peroxidase (MnP), soybean peroxidase (SbP), horseradish peroxidase (HRP), and laccases. Of these peroxidases, LiP and MnP are described as true lignin degraders because of their high potential redox value (Martinez et al., 2005). For the present study, three types of peroxidase enzymes, LiP, MnP, and SbP, were selected to evaluate their ability to further degrade partly degraded MSW. The enzymes were purchased from Sigma Aldrich Canada Ltd. These peroxidases needed to be activated by mixing with hydrogen peroxide (H_2O_2).

Table 1
Waste characteristics.

Parameters	Value
Moisture content (%)	18.0
Total solids (%)	82.0
Volatile solids (%)	18.6
Lignin content (% of TS)	81.9
Cellulose and hemicellulose to lignin ratio, (C + H)/L ratio	0.2
Soluble chemical oxygen demand, SCOD (mg/L)	390.3

2.3. Batch experiments

The laboratory experiments were conducted in small batch reactors consisting of 125 mL glass bottles closed by plastic caps with a septum. Two grams of waste and the corresponding amount of moisture, enzymes, and H_2O_2 were added to the bottles. One batch reactor was kept as a control, with no enzymes/H_2O_2. The glass bottles were sealed to prevent air entry and purged with pure nitrogen (N_2) gas for approximately 20 min to create anaerobic conditions inside the bottles. All of the bottles were kept in an incubator operating at a temperature of 35 °C. Bottles were shaken manually every day during the 40 day monitoring period. Methane production was measured daily by collecting 1.5 mL sample of head-space gas using a pressure lock glass syringe which was analyzed using a VARIAN 4900 Micro gas chromatograph (GC). The bottles were purged with N_2 gas on a weekly basis to replace the collected gas to maintain the inside pressure. The gas sampling continued until methane production reached a steady state.

The yield of CH_4 was calculated using Eq. (1) proposed by Soto et al. (1993). In Eq. (1), the net production of N_2 was considered negligible in relation to CH_4 production.

$$v_{CH_4} = \frac{v x^0_{N_2} x_{CH_4}}{(1 - x_{CH_4} x_{CO_2})} \quad (1)$$

where,

v_{CH_4} = volume of CH_4 (mL);
v = volume of gas phase (mL);
$x^0_{N_2}$ = initial composition of N_2 gas;
x_{CH_4}, x_{CO_2} = mole fractions of CH_4 and CO_2 at time t.

2.4. Experimental protocol

The experimental variables were; enzyme type, enzyme dose, and the ratio of enzyme:H_2O_2. Three enzyme types were tested in combination with H_2O_2 at five levels of enzyme dose and enzyme:H_2O_2 ratio. The five levels of enzyme dose were 0.1, 0.2, 0.3, 0.4, and 0.5 mg per 2 g of waste (or 0.37 g VS). For each level of enzyme dose, five different ratios of enzyme:H_2O_2 *i.e.*, 0.0023, 0.0027, 0.0034, 0.0046, 0.0068 g/g, were tested.

The amount of waste and moisture content of waste, mixing frequency, and environmental conditions were kept constant for all the bottles throughout the experiment. Each experiment was conducted in triplicate and the average response was used for data analysis. The standard deviation for the results obtained from each replicate sample was less than 4%.

3. Results and discussion

The cumulative methane yield and the substrate utilization were used as indicators of the effect of enzyme amendment on waste degradation. As described earlier, methane yield was calculated from headspace gas compositions and substrate utilization was determined from the percentage reduction in lignin mass.

3.1. Cumulative methane yield

3.1.1. Time dependent methane production with enzyme enhancement

The cumulative methane yield over time for MnP enzyme at different enzyme:H_2O_2 ratios with an enzyme dose of 0.3 mg is shown in Fig. 1. A significant increase in methane yield was observed in enzyme amended batch reactors compared to the control reactor.

A similar trend of increasing cumulative methane production over time was observed in each of the three enzymes amended reactors tested (see Figs. 2 and 3).