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Short Communication

Waste degradation and gas production with enzymatic enhancement in anaerobic and aerobic landfill bioreactors

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HIGHLIGHTS

- Peroxidases enhanced CH_4 yield from lignin rich waste under anaerobic conditions.
- Peroxidases enhanced CO_2 yield from lignin rich waste under aerobic conditions.
- Effect of H_2O_2 on aerobic enhancements was insignificant.

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ABSTRACT

The presence of lignin is the limiting factor at later stages of biodegradation of municipal solid waste under aerobic or anaerobic conditions. Supplying enzymes into the system could facilitate lignin degradation, thereby aiding anaerobic and aerobic waste degradation processes. A comprehensive set of laboratory experiments were conducted under both anaerobic and aerobic conditions to evaluate the feasibility of using enzymes in accelerating lignin-rich waste degradation. After 30 days of anaerobic operation, MnP and LiP enzyme treated reactors produced 36 and 23 times higher cumulative methane (CH_4), respectively, compared to that of the control reactor devoid of enzyme treatments. The carbon dioxide (CO_2) yield of MnP enhanced aerobic reactor showed more than two-fold increase.

1. Introduction

A biocell involves the operation of a waste cell under sequential anaerobic and aerobic bioreactor modes followed by cell mining to recover space and potentially recyclable/reusable material (Hettiaratchi, 2007). It is a further refinement of the landfill bioreactor concept. Although, the biocell concept is an attractive alternative for municipal solid waste (MSW) management, methane (CH_4) production and waste stabilization at later stages could be limited by the presence of lignin in the organic fraction of MSW.

Although lignin is usually resistant to natural biodegradation, white rot fungi (WRF) is known for its ability to degrade lignin by oxidation (Higuchi, 2004; Sanchez, 2009). Peroxidases are enzymes made from WRF that have the unique ability of assisting lignin degradation. Peroxidases catalyze the initial depolymerization of lignin by generating highly reactive free radicals such as oxyferryl (Fe^{+4}-O) cation radical (Barr and Aust, 1994). The free radicals are capable of breaking down lignin into smaller molecules that are easier to hydrolyze than complex lignin molecules. Furthermore, the degradation of lignin allows microorganisms easy access to un-degraded cellulose and hemicellulose in a lignocellulosic substrate, such as woody residues in MSW. Lignin depolymerization thus increases the rate of hydrolysis (i.e., the rate limiting step in waste degradation) and increases the available carbon for microbial reactions. Therefore, one of the options available for stimulating waste degradation in both anaerobic and aerobic stages of a biocell operation is the manipulation of enzymatic activity by supplying additional enzymes into the system (Lagerkvist and Chen, 1993; Jayasinghe et al., 2011, 2013).

The objective of the present study was to evaluate the possibility of enhancing waste degradation in a biocell by the addition of lignin-degrading peroxidase enzymes. It is postulated that, during the anaerobic mode of biocell operation, lignin degradation would enhance biogas production, and therefore, increases the possibility of extracting the gas for energy recovery over a long time period. During the aerobic mode of biocell operation, lignin degradation would enhance waste stabilization rates, and therefore, allow the

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cell operator to mine the biocell for resource and space recovery within a short period of time. This paper presents results from a comprehensive set of laboratory experiments and graphical and statistical analysis of the results to compare the effectiveness of enzymatic enhancement of waste degradation under anaerobic and aerobic conditions.

2. Materials and methods

The samples of partly degraded MSW used in the laboratory experiments were collected from a 30-year old landfill cell located at the City of Calgary's Shepard Landfill site. The moisture content (MC) and field capacity (FC) of waste samples were determined using standard test methods (APHA, 2005). The MC and the FC of the waste samples used in the anaerobic experiments were 18% and 48%, respectively, and those in the waste samples used in the aerobic experiments were 21% and 45%, respectively.

The enzymes, lignin peroxidase (LiP) and manganese peroxidase (MnP from *Phanerochaete chrysosporium*), were used in this study, as they are considered true lignin degraders due to their high potential redox value (Sanchez, 2009). The enzymes were purchased from Sigma Aldrich Canada Ltd. Hydrogen peroxide (H_2O_2) was used to activate the enzymes.

A two-factor, three-level factorial experimental design was used for both anaerobic and aerobic experiments. The experimental factors were enzyme dose and H_2O_2 dose. The two enzyme types were tested individually at three-levels of enzyme doses and three levels of H_2O_2 doses, resulting in 18 treatments. Each treatment had 3 replications. The three levels of enzyme doses were 0 (control), 0.1 and 0.15 mg/g_{DS} and the H_2O_2 doses were 0, 0.01 and 0.02 mL/g_{DS}. The different treatment combinations were labeled with levels of −1, 0, and 1. For example, experiments conducted with an enzyme dose of 0.1 mg/g_{DS} and H_2O_2 dose of 0.02 mL/g_{DS} was labeled as 0,1.

2.1. Anaerobic batch experiments

The anaerobic batch experiments were performed using 125 mL glass bottle reactors. The quantity of dry waste used in the experiments was 2 g. Pre-determined amounts of water, enzymes and H_2O_2 were added to the reactors. Sufficient amount of water was added to the sample to reach a MC of 160% of the FC of the waste. The amount of water added to the sample brought the final MC of the waste to about 60% (w/w) that is within the optimum moisture content for maximum waste degradation (Khanal, 2008).

One batch reactor was maintained as the control, with no enzyme or H_2O_2 addition. The reactors were sealed to prevent air entry and purged with pure nitrogen gas to create anaerobic conditions. The CH_4 concentration within the reactor was measured daily, over the monitoring period of 30 days, by collecting samples of headspace gas and analyzing using a VARIAN 4900 Micro gas chromatograph (Micro GC). The volume of CH_4 produced was calculated as described by Jayasinghe et al. (2011).

2.2. Aerobic batch experiments

The aerobic experiments were conducted in one-liter bottle reactors. The larger volume was necessary to help maintain aerobic conditions throughout the experiment. In addition, the reactors were opened to atmosphere weekly to ensure there is adequate oxygen in the reactors. The quantity of dry waste used was 6 g. Known quantities of water, enzymes, and H_2O_2 were added to the reactors. In contrast to anaerobic experiments, the MC was kept at 80% of the FC, to ensure aerobic conditions could be maintained at all times (Huag, 1993). The carbon dioxide (CO_2) concentration was measured daily, over the 30-day time period, using the Micro GC.

3. Results and discussion

3.1. Cumulative gas production: anaerobic experiments

The experimental response, CH_4 yield, was used as the primary indicator of the effectiveness of enzyme addition on the lignin-rich waste degradation under anaerobic conditions. The cumulative CH_4 yields for LiP and MnP enzyme treatments, as a function of time, are shown in Fig. 1.

Significant increases in CH_4 yields were observed in all enzyme treated batch reactors compared to the control (−1 −1) and the inactivated-enzyme (i.e., no addition of H_2O_2) reactors (0 −1 and 1 −1; note that these are not shown in Fig. 1, as they overlap with the control yields). Furthermore, in reactors treated with only H_2O_2 (i.e., the treatment combinations of −1 0 and −1 1; but only −1 0 is shown in Fig. 1 as they overlap with each other), CH_4 yields were higher than that of the control reactor. In these cases, the increases in CH_4 yields were not as high as in activated-enzyme (i.e., with the addition of H_2O_2) reactors. Although H_2O_2 is a strong oxidant, our results show that H_2O_2 alone is not sufficient in degrading lignin-rich solid waste.

In the case of MnP treatment, the highest CH_4 yield was observed with the 1,1 treatment (i.e., enzyme dose of 0.15 mg/g_{DS} and H_2O_2 dose of 0.02 mL/g_{DS}). The maximum CH_4 yield for this combination was of 39.4 mL CH_4/g_{DS}. However, in the case of LiP treatment, at early stages, 0,0 treatment (i.e., enzyme dose of 0.1 mg/g_{DS} and H_2O_2 dose of 0.01 mL/g_{DS}) exhibited the highest CH_4 yield, but 1,0 treatment (i.e., enzyme dose of 0.15 mg/g_{DS} and H_2O_2 dose of 0.01 mL/g_{DS}) provided the highest CH_4 yield after the 30-day time period. The maximum CH_4 yield for 1,0 treatment was 25.8 mL CH_4/g_{DS}. The cumulative CH_4 yield of the control reactor was much lower (i.e., 1.1 mL CH_4/g_{DS}) than the CH_4 yields of enzyme treated reactors.

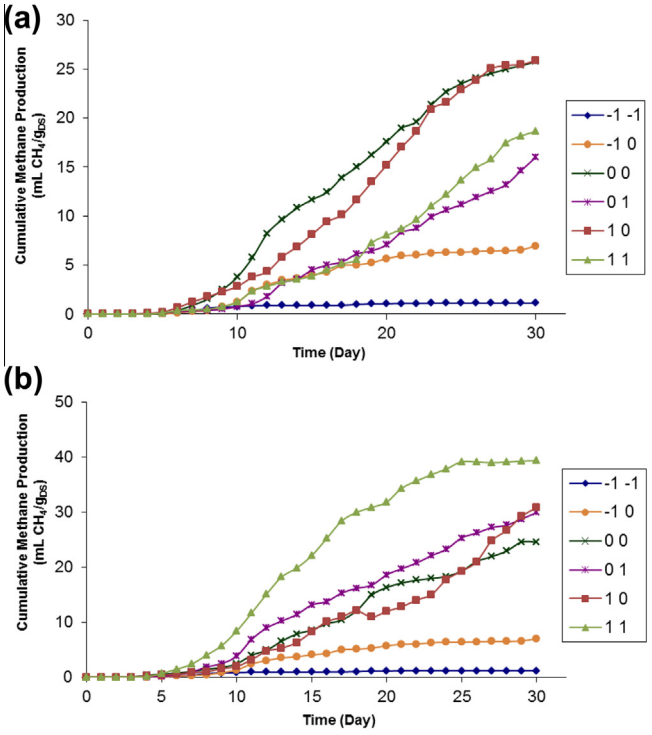

Fig. 1. Cumulative CH_4 yield under anaerobic conditions (a) LiP enhancement (b) MnP enhancement.