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Reaction mechanisms and rate constants of waste degradation in landfill bioreactor systems with enzymatic-enhancement

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HIGHLIGHTS

- Peroxidases enhanced the waste degradation of lignin-rich waste.
- The enzyme-catalyzed reaction mechanism was identified.
- Rate constants for enzyme-catalyzed an un-catalyzed reactions were estimated.

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ABSTRACT

Augmenting leachate before recirculation with peroxidase enzymes is a novel method to increase the available carbon, and therefore the food supply to microorganisms at the declining phase of the anaerobic landfill bioreactor operation. In order to optimize the enzyme-catalyzed leachate recirculation process, it is necessary to identify the reaction mechanisms and determine rate constants. This paper presents a kinetic model developed to ascertain the reaction mechanisms and determine the rate constants for enzyme catalyzed anaerobic waste degradation. The maximum rate of reaction (V_{max}) for MnP enzyme-catalyzed reactors was 0.076 g_{TOC}/g_{DS}.day. The catalytic turnover number (k_{cat}) of the MnP enzyme-catalyzed was 506.7 per day while the rate constant (k) of the un-catalyzed reaction was 0.012 per day.

1. Introduction

The rate of gas production during anaerobic waste degradation decreases over time (Pohland et al., 2003). One of the main reasons for this observation could be the decrease in available carbon for the growth of microorganism. At later stages of anaerobic bioreactor operation, the majority of the organic fraction is composed of compounds, such as lignocellulosic materials, rich in lignin, which are somewhat resistant to anaerobic microbial degradation (Higuchi, 2004).

Modification of leachate by adding supplemental materials, such as nutrients, buffers, and enzymes, before recirculation is a promising technique for increasing the gas production rate during the gas declining phase of anaerobic bioreactor operation (Barlaz et al., 1990; Lagerkvist and Chen, 1993; Cirne et al., 2008; Jayasinghe et al., 2013). The leachate acts as a carrier medium of supplemental materials. Most of the past research efforts have focused on enhancing gas production from fresh and cellulose-rich waste materials. Very little is known about enhancing gas production from lignin-rich waste material degradation and the use of lignin-degrading enzymes, such as peroxidases, to modify leachate before recirculation (Cirne et al., 2008; Jayasinghe et al., 2011). Recently, Jayasinghe et al. (2011, 2013) reported results of experimental studies conducted to identify the most suitable enzyme for lignin degradation under landfill bioreactor conditions. However, information is not available in literature on the reaction mechanisms of enzymatic-catalyzed lignin-rich waste degradation and associated rate constants. Such information is necessary for process optimization, and the design and operation of full-scale landfill bioreactor systems.

The objective of this study was to identify the mechanisms and rate constants of the process of enzyme catalysis when different peroxidase enzymes are used to treat leachate before recirculation at later stages of anaerobic landfill bioreactor operation. This paper presents the development and evaluation of a kinetic model that could be used to represent the process mechanisms and estimate the rate constants.

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2. Theoretical considerations

2.1. Kinetics of enzyme-catalyzed reactions

The overall rate of reaction depends on the catalytic activity of the enzyme in the waste hydrolysis reaction. It is assumed that enzyme-catalyzed reaction follows the mechanism given in Eq. (1); the Michaelis–Menten mono-substrate, double-intermediate enzyme-catalyzed model (Taylor, 2002; Leskovac, 2003):

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} ES' \xrightarrow{k_4} E + P \tag{1}$$

In this equation, one molecule of an enzyme (E) combines reversibly with a single molecule of substrate (S) to form an enzyme–substrate complex (ES), which is transformed into another different enzyme–substrate complex (ES') before the release of free E and final product (P).

The rate expressions, which describe the rate of change of concentrations of the intermediates, ES and ES', and product P, are written as:

$$\frac{d[ES]}{dt} = k_1[E][S] - (k_2 + k_3)[ES] \tag{2}$$

$$\frac{d[ES']}{dt} = k_3[ES] - k_4[ES'] \tag{3}$$

$$\frac{d[P]}{dt} = k_4[ES'] \tag{4}$$

With the assumption of 'the net rates of formation and dissociation of both the intermediates are equal to zero', the reaction velocity (v') can be simplified to,

$$v' = \frac{d[P]}{dt} = -\frac{d[S]}{dt} = \frac{k_1 k_3 [E][S]}{k_2 + k_3} \tag{5}$$

Assuming that the enzyme is conserved during the process (i.e., by ignoring any short-term de-activation), Eq. (5) can be further simplified as,

$$v' = \frac{k_1 k_3 k_4 [S][E_o]}{(k_2 k_4 + k_3 k_4) + (k_1 k_4 + k_1 k_3)[S]} \tag{6}$$

where, $[E_o]$ is the initial enzyme concentration (0.00015 g_E/g_{DS}).

Eq. (6) can be written in the form of the Michaelis–Menten equation (i.e., Eq. (7)) by defining the relationships in Eqs. (8)–(10):

$$v' = -\frac{d[S]}{dt} = \frac{V_{max}[S]}{K_M + [S]} \tag{7}$$

$$V_{max} = \left(\frac{k_3 k_4}{k_3 + k_4}\right)[E_o] = k_{cat}[E_o] \tag{8}$$

$$K_M = \left(\frac{k_4(k_2 + k_3)}{k_1(k_4 + k_3)}\right) \tag{9}$$

$$k_{cat} = \left(\frac{k_3 k_4}{k_3 + k_4}\right) = \frac{V_{max}}{[E_o]} \tag{10}$$

where, V_{max} is the maximum rate of reaction or velocity (g_{TOC}/g_{DS} day), K_M is the Michaelis constant (g_{TOC}/g_{DS}), and k_{cat} is the catalytic rate constant (day^{-1}). Eq. (7) can be integrated and simplified as:

$$\left(\frac{[S_o] - [S_t]}{t_t - t_o}\right) = -K_M \frac{\ln\left(\frac{[S_o]}{[S_t]}\right)}{(t_t - t_o)} + V_{max} \tag{11}$$

where, $[S_o]$ and $[S_t]$ denote the initial concentration of substrate at time t_o and the concentration of substrate at time t_t, respectively.

According to Eq. (11), a plot of $\left(\frac{[S_o]-[S_t]}{t_t-t_o}\right)$ versus $\frac{\ln\left(\frac{[S_o]}{[S_t]}\right)}{(t_t-t_o)}$ should yield a straight line with the slope equal to K_M and the intercept equal to V_{max}.

2.2. Kinetics of un-catalyzed reaction

The conversion of substrate S to product P, when it is not catalyzed by enzymes, can be represented as:

$$S \xrightarrow{k} P \tag{12}$$

where, k is the un-catalyzed rate constant (in day^{-1}), which is independent of concentration, but dependent on the temperature.

Assuming that the waste degradation follows first-order reaction kinetics, the velocity of the un-catalyzed reaction, v'', can be written as:

$$v'' = \frac{d[P]}{dt} = -\frac{d[S]}{dt} = k[S] \tag{13}$$

Integration and simplification of Eq. (13) yields:

$$\left(\ln\frac{[S_t]}{[S_o]}\right) = -k(t_t - t_o) \tag{14}$$

where, $[S_o]$ and $[S_t]$ are the initial concentration of substrate at time t_o and the concentration of substrate at time t_t, respectively.

The slope of the straight line of $\left(\ln\frac{[S_t]}{[S_o]}\right)$ versus $(t_t - t_o)$ plot will be equal to the rate constant, k.

3. Methods

A set of laboratory batch experiments were conducted to obtain the model inputs and to identify the process mechanisms of enzyme-catalyzed and un-catalyzed reactions. Time-dependent substrate utilization was measured in terms of dissolved organic carbon (DOC) in leachate and total organic carbon (TOC) in solid waste. The TOC values were used as inputs to estimate the model parameters in Eqs. (11) and (14), and both the TOC and DOC values were used to identify the process mechanisms.

The laboratory batch experiments were conducted in 125 mL glass bottle reactors filled with 2 g of partly degraded waste (collected from a 30 year old landfill cell) and pre-determined quantities of water, enzyme, and hydrogen peroxide. Hydrogen peroxide (H_2O_2) was used as the enzyme activator. Manganese peroxidase (MnP) was used as the preferred enzyme, as it was reported to perform better than other peroxidases, such as lignin peroxidase and soybean peroxidase (Jayasinghe et al., 2011).

One batch reactor was kept as the control reactor, in which enzyme and H_2O_2 were not added. Once the samples were mixed, the bottles were purged with nitrogen gas and sealed to ensure anaerobic conditions. Further details of the experimental methodology are provided in Jayasinghe et al. (2011).

The TOC values in the solid waste samples were measured using a modified Mebius procedure, as described by Yeomans and Bremner (1988). The DOC values in leachate samples were measured using Teledyne Tekmar Apollo 9000 combustion DOC analyzer. The extraction of leachate from solid waste for DOC analysis followed the method outlined in Chefetz et al. (1998).

Since these were batch experiments, one batch reactor had to be opened every sampling day to collect waste samples for testing. The opened batch reactor was discarded once the sample was obtained.