RESILIENT INFRASTRUCTURE
June 1–4, 2016
RESILIENT INFRASTRUCTURE LONDON 2016

ANALYSIS OF REACTION KINETIC PARAMETERS IN ENZYME CATALYSED AEROBIC WASTE DEGRADATION

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ABSTRACT

The study discussed here is an attempt at comparing three of the most important kinetic parameters in an enzyme catalysed aerobic system; overall reaction kinetic constant, hydrolysis rate constant and oxygen assimilation constant. The kinetic parameters are compared with each other as well as between uncatalyzed and enzyme catalyzed reactions. Theatrical reaction kinetic models were developed for the analysis. Batch experiments were conducted to characterize solid waste behaviour and the results used to calibrate the developed models. Lignin content, cellulose/hemicellulose content, total organic content, oxygen depletion and CO_2 production was used as responses in the experiments. Manganese peroxidase from white-rot-fungi is used as the enzyme for catalysing treatments. The catalyzed reactions showed higher reaction rates than the un-catalyzed reactions. Further analysis revealed that catalyzed reactions have higher hydrolysis rates compared to the overall rates of metabolism. The O_2 assimilation analysis revealed that catalyzed reactions require 1.66 times more O_2 than un-catalyzed reactions.

Keywords: Aerobic degradation, Reaction Kinetics, Hydrolysis, Enzymes, Oxygen Assimilation

1. INTRODUCTION

The biodegradation rate in aerobic landfills is significantly reduced in latter stages of operation, due to the decrease in availability of readily degradable organic matter. This is mainly caused by the presence of organic compounds known as lignins. Different enhancement techniques can be used in the latter stages to overcome this barrier. Enhancing aerobic degradation of waste can be achieved using the following methods: control of landfill bioreactor temperature, leachate augmentation and bioventing (Ishigaki et al., 2003). Leachate augmentation techniques include addition of sludge, addition of supplemental nutrients, and augmentation of leachate with enzymes. Among these techniques, the addition of sludge is the most common and oldest practice (Jayasinghe et al., 2011).

Lignin is a long chain aromatic hydrocarbon found in organic substances, and is difficult to biodegrade. Lignocellulose, which consists of cellulose, hemicellulose, and lignin, is the major organic component of municipal solid waste (MSW). Lignin is less inhibitory to substrate decomposition in an aerobic environment than in an anaerobic one, 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). The biodegradation of lignin is limited to several types of white-rot fungus (WTF), brown-rot fungus (BRF), and some bacteria that can be found in nature (Bugg et al., 2011; Hofrichter, 2002). The WRF *Nematoloma frowardii* is a type of basidiomycete that can degrade lignin. This fungus produces several types of enzymes that can break down the lignocellulose structure.

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 include an array of oxidases and peroxidases. Examples of commercially available lignin degraders 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). 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 dissolve phenolic compounds, hence it has a lower potential to degrade lignin than MnP.

Although it has been validated that enzyme augmentation can enhance waste biodegradation by degrading lignin, the exact biological behaviour has not been quantified (Hettiaratchi et al., 2014). The biological activity inside a landfill bioreactor can be described with reaction kinetics. The parameters in reaction kinetics, or reaction rate constants, are a key aspect in landfill design. Development of a comprehensive reaction kinetic model and model calibration can be a complex process. However, there are several key parameters in the reaction process that need to be determined for all MSW bioreactors waste cells which are hydrolysis rate; overall reaction rate; and oxygen assimilation/consumption rate. The addition of ligninolytic enzymes essentially increases the hydrolysis of the organic material. Also, for aerobic reactions, the oxygen assimilation/ consumption rate becomes important, since this can determine the rates of aeration.

In this study a theoretical reaction kinetics model was first developed for aerobic waste degradation. The model was then calibrated using batch experiments. The objective of the study was to determine the reaction kinetic parameter values of an aerobic waste degradation process and compare the values of catalyzed and uncatalyzed reactions.

2. MATERIALS AND METHODS

2.1 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). In order to obtain samples representative of the entire landfill cell, ASTM standard procedure D4687 was followed during the sample collection.
Once collected, the waste samples were mixed thoroughly. The samples were then shredded and sieved to an average particle size of 1 mm prior to use in batch experiments. The characteristics of shredded and sieved waste samples were determined experimentally according to the standard test methods (APHA, 2005) and are presented in Table 1. 10 Samples were analyzed for each test.

Table 1: Characteristics of the waste material

Parameter	Value
Moisture Content (% of TS)	24.00 +/- SD 0.04
Dry Solids (% of TS)	76.00 *
Volatile Solids (% of DS)	22.21 +/- SD 4.00
Lignin Content (% of VS)	14.00 +/- SD 4.00
Field Capacity (%)	44.00 +/- SD 6.40
Cellulose and Hemicellulose to Lignin ratio, (C+H)/L ratio	1.63 +/- SD 0.39
Ash %	77.79 *

* no standard deviation since these are calculated values

MnP from *Nematoloma frowardii*, purchased from Sigma-Aldrich Co. (Product #: 41563), was used for the experiments.

Table 2: Elemental analysis of waste

	Dry Solid					Ash		
	C %	**H %**	**N %**	**O%**	**Ash %**	**C %**	**H %**	**N %**
CHN Analysis data	10.37	1.41	0.52			1.33	0.12	0.04
Volatile solids (VS)	9.04	1.28	0.48	11.41	77.79			
# of moles per 100 g	0.75	1.28	0.03	0.71				
Chemical ratio of VS	21.97	37.39	1.00	20.80				

Waste samples were further tested for elemental analysis using a CHN analyzer (Perkin Elmer Model 2400 series II). The results are shown in Table 2, and each data has an error of ±17%. Using this elemental analysis, an approximate molecular formula for the Volatile Solids (VS) was estimated as $C_{22}H_{37}O_{21}N$.

2.2 Experimental Method

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 m L 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 maintained at room temperature ($22 \pm 1\ ^0C$), which is representative of the average temperature in a bioreactor landfill (Hunte, 2010). The glass bottle reactors were opened every two days to ensure there was no gas build up and increase in pressure within the reactors. Opening the bottles also ensured O_2 levels were maintained over time, which can impact the microbial population in the bottles. The headspace gas was collected and measured using a syringe and Micro GC. Headspace CO_2 was used as the response of the system. 1 L of headspace volume was used in the CO_2 concentration calculations. The experiment was conducted until a stable CO_2 production as achieved. This experiment was able to achieve a stable CO_2 Production in 60 days.

Every 14 days the bottles were mixed, and 1 g solid samples collected and analyzed. The lignin content, cellulose, hemicellulose content, and TOC were measured as responses during the solid sample analysis. In order to use substrate utilization as an indication of the success of experiments, the lignin contents of the waste before and after experiments were determined according to the ASTM-D1106 standard test method with minor modifications. First, the waste samples were grounded to pass a 0.5 mm sieve and dried at 105 ^0C for 2 hrs. 1 g of prepared sample was added to an extraction thimble, and the test samples were extracted with 1:2 ethanol: benzene solution for 8 hrs in a soxlet extraction apparatus. The sample was then transferred into a beaker; 400 mL of water was added and digested in a hot water bath at 100 ^oC for 3 hrs. The digested sample was washed with 100 mL hot water and then dried in the air. The dried sample was transferred to a glass bottle and 15 mL of 72 % H_2SO_4 was added slowly while stirring. Once the sample was well mixed with acid, it was kept in a water bath at 20 ^oC for 2 hrs. The acid digested sample was diluted by adding 560 mL of distilled water and boiled for 4 hrs. After allowing the insoluble materials to settle, the sample was washed again with 500 mL hot water and dried at 105 ^oC for 2 hrs. The weight of the sample was measured and then combusted at 550 ^oC for 2 hrs in a muffle furnace (Isotemp, Fisher Scientific). The weight of the ignited sample was measured. The weight loss on ignition represents lignin.

3. REACTION KINETIC MODEL

In aerobic degradation, two distinct stages can be identified which are; hydrolysis stage and oxidation stage. The initial hydrolysis stage can be modeled based on first order reaction kinetics as;

$$[1] \quad -\frac{dC_s}{dt} = K_h C_s$$

where, K_h is hydrolysis rate of waste (s^{-1}) and C_s is the substrate or the non-dissolved organics concentration.

Dissolved organic carbon concentration is impacted by hydrolysis, microbial growth, and microbial death. Dissolved organic carbon concentration can then be expressed as follows (Slezak et al., 2012);

[2] $$\frac{dC_h}{dt} = K_h C_s - \mu_X \frac{C_h}{K_s + C_h} C_X + K_D C_X$$

where, K_D is the microbial death rate constant in dissolved organics (day^{-1}), K_s is the half saturation constant for substrate during aeration (kg m^{-3}), μ_x is microbial biomass maximum specific growth rate constant (day^{-1}). C_h and C_X are the concentrations of the hydrolysis and intermediate complex. Intermediate complex represents the biomass concentration.

The enzymatic reaction kinetics is based on the Michaelis and Menten (1913) model. The rate of formation and dissociation of the intermediates are assumed to be zero using pseudo-steady-state hypothesis. Neufeld and Hernandez-Garcia (2010) showed that if enzymes have only one binding site then the reaction rate equation can be written as given in Eq. 3.

[3] $$\frac{dC_s}{dt} = -K_3 \frac{C_s}{K_M + C_s} E$$

where, K_1, K_2, K_3 are reaction rate constants for substrate to intermediate complex forward reaction, substrate to intermediate complex backward reaction, and intermediate complex to product, respectively. K_M is the Michaelis constant $K_M = (K_2 + K_3)/K_1$. The concept discussed here is widely known as Michaelis–Menten kinetics. Also $K_3 E = V_{max}$ is the maximum rate of reaction (velocity of reaction) (g_{TOC}/g_{DS}.day). C_s and E are concentrations for the substrate and enzyme, respectively.

The modified hydrolysis rate equation for an enzyme catalyzed aerobic system is as follows;

[4] $$\frac{dC_h}{dt} = V_{max} \frac{C_s}{K_M + C_s} - \mu_X \frac{C_h}{K_s + C_h} C_X + K_D C_X$$

However, C_h is much lower than K_s for landfill bioreactors resulting in a first order approximation (Neufeld and Hernandez-Garcia, 2010). Hence, the above equation is approximated to;

[5] $$\frac{dC_h}{dt} = V_{max} \frac{C_s}{K_M + C_s} - K_G C_X + K_D C_X$$

where, K_G is the first order growth rate of microorganisms (day^{-1}),

Also, for modelling purposes, it is assumed that the dead micro-organisms cause the production of CO_2 in the decomposition process (El-Fadel et al., 1996). CO_2 production can be estimated as follows (Slezak et al., 2012);

[6] $$\frac{dC_{CO2}}{dt} = (1 - Y_a) K_G C_X$$

where, Y_a is the yield coefficient representing the amount of dissolved organic carbon used for microbial growth (Slezak et al., 2012).

Based on first order general reaction kinetics, a direct relationship between the refuse mass and the production of CO_2 can be developed. A similar equation to Scholl-Canyon model (USEPA, 2005) used for anaerobic CH_4 generation is presented in Eq. 7 for aerobic CO_2 production.

[7] $$\gamma = mM_{C0}(1 - e^{-K_R t})$$

where, t is the time elapsed (days), and K_R is the first-order rate constant (day^{-1}), Y is the cumulative CO_2 generated from the beginning of life to time t (m^3); M_{C0} is the CO_2 generation potential (m^3 kg^{-1}), and m is the remaining mass of refuse (kg).

The rate of CO_2 production is obtained by differentiating Eq. 7 with respect to time and is as follows;

[8] $$\alpha_c = kM_{C0}me^{-kt}$$

where, α_c is the CO_2 production rate at time t (m^3 days^{-1}).

Unlike the anaerobic process the depletion or the assimilation of O_2 is also a factor in the aerobic process (Lin et al., 2008). Similarly, O_2 production can be approximated to (Slezak et al., 2012);

[9] $$\frac{dC_{O2}}{dt} = \frac{\mu_o}{\mu_X} K_G C_X$$

where, μ_o is the O_2 maximum specific assimilation rate constant (day^{-1}).

4. ANALYSIS AND RESULTS

The model discussed in the previous section is analyzed for the three kinetic parameters: hydrolysis, overall reaction, and O_2 assimilation.

The hydrolysis stage is analyzed separately for catalyzed and uncatalyzed systems. The hydrolysis stage reaction is in Eq. 1 for un-catalyzed reactions and in Eq. 3 for catalyzed reactions.

Eq. 1 can be written in the form;

[10] $$-\int_{C_{s0}}^{C_s} \frac{1}{C_s} dC_s = \int_{t_0}^{t} K_h dt$$

Integration results in;

[11] $$ln\left(\frac{C_{s0}}{C_s}\right) = K_h(t - t_0)$$

By finding the slope in $ln\left(\frac{C_{s0}}{C_s}\right)$ vs $(t - t_0)$, the value of K_h is determined as 0.0058 (day^{-1}). Date from control experiments or the enzyme dose 0 experiments are used for calculating this value.

Similarly for the catalyzed reaction, Eq. 3 is rewritten in the form;

[12] $$-\int_{C_{s0}}^{C_s} \left(\frac{K_M}{C_s} + 1\right) dC_s = \int_{t_0}^{t} V_{max} dt$$

Integration results in;

[13] $$\frac{(C_{s0} - C_s)}{(t - t_0)} = V_{max} - K_M \frac{ln\left(\frac{C_{s0}}{C_s}\right)}{(t - t_0)}$$

This equation is in the form of y = mx +c, where, the slope of the equation (m) is $-K_M$ and the intercept (c) is V_{max}. The analysis is conducted for 3 different enzyme doses. The results obtained for the 3 different enzyme doses for the enzyme-catalyzed reaction are presented in Table 3. Enzye dose 0.04 mg/g of DS did not show any time dependent relationship and the results could not be analyzed. The highest reaction kinetic constant among the catalyzed systems was observed in the enzyme dose of 0.08 mg/g of DS.

Table 3: Reaction kinetic parameters

Enzyme Dose (E) (mg/g of DS)	**0.08**	**0.12**	**0.16**
V_{max}	0.0056	0.0078	0.0075
K_m	0.33	0.54	0.44
$K_{cat} = K_3$	0.07	0.07	0.05
K_{cat}/K_h	12.07	11.21	8.08

The reaction kinetic constant for catalyzed hydrolysis, K_{cat}, can be compared with the un-catalyzed hydrolysis K_h. The catalyzed reaction rate for an enzyme dose of 0.08 mg/g of DS is 12 times faster than the uncatalyzed reaction rate. Certain enzyme based reaction rates could be million times faster than that of comparable un-catalyzed reactions (Jayasinghe, 2013). However, a comparison of the kinetic constant with those reported in literature was not possible due to the unavailability of similar results.

The CO_2 generation potential, M_{co}, is estimated from the theoretical stoichiometric chemical formula developed during the CHN analysis and is estimated as 0.76 m^3/kg of waste.

Eq. 8 is used for the kinetic model fitting and is modified as;

[14] $$ln\left(1-\frac{\gamma}{mM_{co}}\right) = -kt$$

This equation is in the form of y = mx +c where the slope of the equation is $-k$. Plots are generated for the catalyzed and un-catalyzed reactions, shown in Figure 1 and Figure 2, respectfully. The data used for the plots are from the conducted batch experiments.

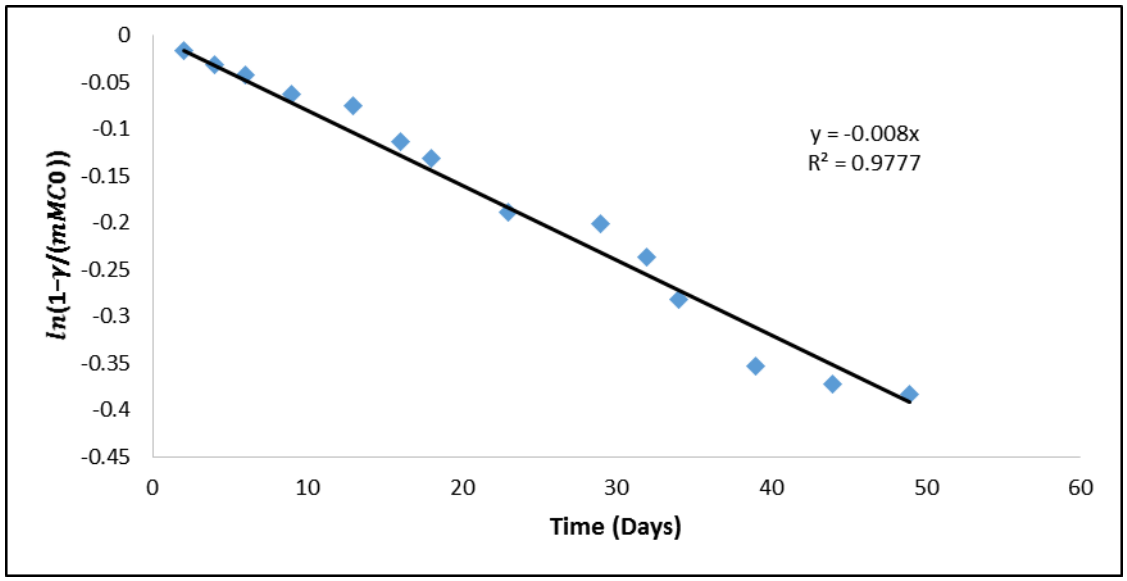

Figure 1: Kinetic model fitting for CO_2 production in uncatalyzed reaction

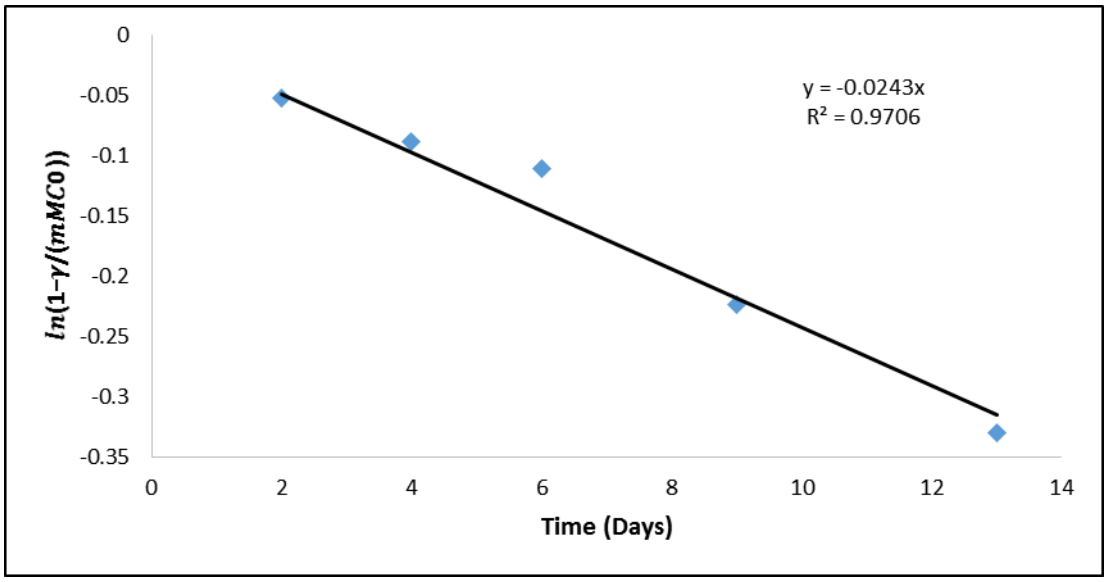

Figure 2: Kinetic model fitting for CO_2 production in catalyzed reaction

The uncatalyzed reaction shown in Figure 1 was used to measure an overall reaction rate constant of 0.008 day^{-1}, which is comparable to that of the hydrolysis rate 0.0058 day^{-1} of the un-catalyzed reaction. The catalyzed overall reaction rate constant is 0.024 day^{-1}. This value is lower than the catalyzed hydrolysis rate of 0.07 day^{-1}. In other words, in catalyzed reactions, the overall reaction rate constant was 2.92 times lower than the rate of hydrolysis. This observation indicates that although the enzymes are capable of achieving rapid hydrolysis, the overall increase in reaction rate is much lower. This effect can be attributed to many other limiting conditions that affect microbial growth, such as lack of trace nutrients, heterogeneity, and limited O_2 supply (Barlaz, 1996).

Evaluation of O_2 assimilation or consumption is an important aspect of aerobic landfill bioreactors in understanding the aeration flow rates required. Excess aeration results in increased moisture evaporation and in turn reduces the moisture content in the waste matrix. The loss of moisture impacts the rate of degradation. Another advantage of determining the O_2 assimilation rate is that it can be used to accurately determine other parameters such as Radius of Influence (ROI) (Hinchee and Leeson, 1962)

The modified O_2 assimilation as a first order model is presented as;

[15] $$\frac{dC_{O2}}{dt} = \frac{\mu_o}{\mu_X} K_G Y_{O2} C_{O2}$$

where, Y_{O2} is the yield coefficient representing substrate mass and O_2 consumption.

The equation is modified as;

[16] $$\frac{dC_{O2}}{dt} = K_O C_{O2}$$

where, $K_O \left[= \frac{\mu_o}{\mu_X} K_G Y_{O2}\right]$ is the rate of O_2 assimilation.

Eq. 17 can be written in the form;

[17] $$\int_{C_{O2,0}}^{C_{O2}} \frac{1}{C_{O2}} dC_s = \int_{t_0}^{t} K_O dt$$

Integration of Eq. 17 results in;

[18] $$ln(C_{O2}) - \ln(C_{O2,0}) = K_O(t - t_0)$$

This equation is in the form of y = mx +c where the slope of the equation (m) is the K_O. The two slopes for catalysed and uncatalyzed systems were analyzed using the batch experimental data. Catalyzed system was analyzed at an enzyme dose of 0.08 mg/g of DS.

K_O for the un-catalyzed reaction is 0.074 day^{-1} and K_O for the catalyzed reaction is calculated as 0.123 day^{-1}. This states that 1.66 times more O_2 is required for the catalyzed reaction than the uncatalyzed reaction.

5. CONCLUSIONS

The reaction kinetic analysis proved that a MnP catalyzed reaction could achieve as much as 12 times higher reaction rate constants compared to an un-catalyzed reaction, under aerobic conditions for waste degradation. In addition, the analysis showed that the enzymatic enhancement of the waste increases the hydrolysis at a faster rate than the overall reaction kinetics. This suggests that although MnP is capable of achieving rapid hydrolysis, the microbial growth could be limited by other factors resulting in lower rates of overall degradation. The O_2 assimilation analysis revealed that catalyzed reactions require 1.66 times more O_2 than un-catalyzed reactions.

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