**STABILIZATION OF PAPER WASTE IN BIOREACTOR LANDFILLS USING
SOYBEAN PEROXIDASE ENZYME**

by

Ram Kumar Sah, B. Eng., Kathmandu, 1990

A thesis

Presented to Ryerson University

in partial fulfillment of the
requirements for the degree of
Master of Applied Science
in the Program of Civil Engineering

Toronto, Ontario, Canada, 2006

© Ram Kumar Sah

UMI Number: EC53612

INFORMATION TO USERS

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleed-through, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

UMI Microform EC53612
Copyright 2009 by ProQuest LLC
All rights reserved. This microform edition is protected against
unauthorized copying under Title 17, United States Code.

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

Author's Declaration

I hereby declare that I am the sole author of this thesis.

I authorize Ryerson University to lend this thesis to other institutions or individuals for the purpose of scholarly research.

I further authorize Ryerson University to reproduce this thesis by photocopying or by other means, in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

Borrower's Page

Ryerson University requires the signatures of all persons using or photocopying this thesis.

Please sign below, and give address and date.

Name

Address

Signature

Date

Stabilization of Paper Waste in Bioreactor Landfills using Soybean Peroxidase Enzyme

By

Ram Kumar Sah

Master of Applied Science, 2006

Department of Civil Engineering

Ryerson University

Abstract

One obvious way to reduce the amount of paper waste being discarded, and to conserve the forest resources, is to recycle more of the waste paper. But most common waste management technique for solid waste is the use of landfills. This study was aimed at decomposing the waste in aerobic condition before the anaerobic condition using soybean peroxidase enzyme (SBP). One bioreactor cell without enzyme was compared with two bioreactor cells, which contained different concentration of soybean peroxidase enzyme with hydrogen peroxide (H_2O_2). The rate of reaction was analyzed by performing COD, BOD_5 , NH_3 , TS, and pH tests as well as monitoring the settlement.

The findings showed that the bioreactors with enzyme had faster decomposition rate than the control one. Final COD concentration in control one was 62.6% higher than the bioreactor having higher concentration of enzyme. Similarly, final BOD_5 concentration in control one was 70.7% higher than the bioreactor having higher concentration of enzyme. Furthermore, the results were compared with the previous study, the bioreactors having municipal solid waste (MSW) with biosolids. The results showed that the strength of leachate quality was much better in bioreactors having paper waste with enzyme than the bioreactor having MSW with biosolids. The COD reduction rate in case of enzyme was about more than double than the bioreactor having biosolids. Similarly, BOD_5 reduction rate in case of enzyme was 1.6 times higher than the biosolids and TS reduction rate was about 1.4 times higher than the bioreactor having biosolids.

Acknowledgements

I would like to thank Dr. Mostafa Warith, P. Eng., for his continuous guidance and generous support in the completion of this thesis. I would also like to thank Dr. Grace Luk for providing environmental laboratory for the experimental work and for valuable comments and input as examination committee member. I am also thankful to Dr. Khaled Sennah for valuable comments and input as examination committee member. I would also like to thank Dr. Patrick Hettiratchi for his continuous support in the completion of the research work.

I would like to thank Hui Jin for providing data for the research work. I am also thankful to Mr. Robin Luong, Mr. Dan Peneff, Mr. Nidal Jaalouk, and Mr. Domenic Valle for their help in experiment work. I would also like to thank Dr. Bryce for supplying the soybean peroxidase enzyme for the experimental work.

Finally, I would like to thank my wife for her encouragement and support in the completion of this study.

Dedication

For my parents

Table of Contents

	Page
Author's Declaration	ii
Borrower's Page	iii
Abstract	iv
Acknowledgement	v
Dedication	vi
List of Tables	xii
List of Figures	xiii
List of Acronyms	xv
1. Introduction	1
1.1 Sources of Waste	1
1.2 Scope of the Study	1
1.3 Waste Management	2
1.4 Objective of the Study	3
1.5 Organization of Report	4
2. Literature Review	5
2.1 Basic Concept of Enzyme	5
2.1.1 Definition	5
2.1.2 Historical Overview	5
2.2 Enzyme Kinetics	6
2.3 Rate of Reaction	9
2.3.1 Enzyme Concentration	9
2.3.2 Substrate Concentration	10
2.3.3 Time	10
2.3.4 Effect of pH	11
2.3.5 Effect of Temperature	12

	Page
2.4 Peroxidases	13
2.4.1 Introduction of Peroxidases	13
2.4.2 Soybean Peroxidases	14
2.4.3 Horseradish Peroxidases	15
2.4.4 Other Peroxidase	16
2.4.5 Inactivation of Peroxidase Enzymes	18
2.4.6 Introduction of Hydrogen Peroxide	19
2.4.7 Polymerization of the Enzyme	19
2.5 Application of Enzyme	19
2.5.1 Application to Waste Water Treatment	19
2.5.2 Application to Solid Waste Treatment	20
2.5.3 Application to Sludge Treatment	22
2.6 Advantages and Disadvantages of Enzyme Treatment	22
2.6.1 Advantages of Enzyme Treatment	22
2.6.2 Disadvantages of Enzyme Treatment	23
2.7 Selection of Enzyme	23
2.7.1 Soybean versus Horseradish Peroxidases	24
2.7.2 Economics	25
2.7.3 Selection of Soybean peroxidase	27
2.7.4 Extraction Method of SBP Enzyme	28
2.8 Solid Waste Management	29
2.8.1 Open Dump	29
2.8.2 Engineered Landfill	30
2.8.3 Bioreactor Landfill	31
2.8.4 Types of Bioreactor Landfills	32
2.9 Aerobic Decomposition of Waste in Bioreactor Landfill	34
2.9.1 Aerobic Decomposition	34
2.9.2 Benefits of Air Injection	35

	Page
2.10 Anaerobic Decomposition of Waste in Bioreactor Landfill	36
2.10.1 Anaerobic Decomposition	36
2.10.2 Factors for Anaerobic Decomposition	36
2.11 Landfill Leachate	38
2.11.1 Leachate Recirculation	38
2.12 Generation of Landfill Gases	38
2.12.1 Initial Adjustment Phase	38
2.12.2 Transition Phase	39
2.12.3 Acid Formation Phase	39
2.12.4 Methane Fermentation Phase	40
2.12.5 Maturation Phase	40
3. Materials and Methods	41
3.1 Bioreactor Landfill System	41
3.2 Components and Configuration of Bioreactor Landfill	42
3.2.1 Bioreactors	44
3.2.2 Leachate Collection Tanks	44
3.2.3 Leachate Recirculation Reservoirs and Pumps	44
3.2.4 Leachate Distribution Apparatus	45
3.2.5 Air Distribution Pipes	45
3.2.6 Air Compressor	45
3.2.7 Temperature Sensor and Thermometer	45
3.2.8 Gas Discharge System	46
3.3 Leachate Recirculation System	46
3.4 Air Injection and Exhaust System	46
3.5 Maintenance of Bioreactor System	46
3.6 Preparation of Waste	50
3.6.1 Waste Composition	50
3.6.2 Collection and Shredding of Paper Waste	50

	Page
3.6.3 Loading and Waste Compaction into Bioreactors	51
3.7 Source and Dose of Enzyme	51
3.8 Rate of Airflow	52
3.9 Leachate Recirculation Rate	53
3.10 Buffering Amount	53
3.11 Analytical Methods	53
3.11.1 Physical Analysis of Paper Waste	53
3.11.2 Chemical Analysis of Leachate	54
3.11.3 Statistical Analysis	56
4.0 Results and Discussions	57
4.1 Variations in Chemical Oxygen Demand (COD)	57
4.2 Variations in Biochemical Oxygen Demand (BOD ₅)	59
4.3 Variation in BOD ₅ / COD Ratio	60
4.4 Variations in Ammonia Nitrogen (NH ₃ -N)	61
4.5 Variations in Total Solids (TS)	62
4.6 Variations in pH	63
4.7 Variations in Waste Settlement Rate	64
4.8 Changes in Mass, Density, Field Capacity and Settlement Rates in the Bioreactors	65
5.0 Comparison of Results	67
5.1 Generation and Management of MSW	67
5.2 MSW Composition	67
5.3 Loading and Operation of Bioreactor Landfill	68
5.4 Comparison of Relative COD Variations	69
5.5 Comparison of Relative BOD ₅ Variations	71
5.6 Comparison of Relative NH ₃ -N Variations	73
5.7 Comparison of Relative TS Variations	75
5.8 Comparison of Variations in Settlement Rates	77

	Page
5.9 Enzymatic Treatment	78
5.10 Economic Feasibility of Soybean Peroxidase	80
6.0 Conclusions and Recommendations	81
6.1 Conclusions	81
6.2 Recommendations	82
REFERENCES	83
APPENDIX A- Experimental Data	91
APPENDIX B- Experimental Photographs	119

List of Tables

	Page
Table 2-1 Calculation of Soybean Peroxidase Extractable from Soybean Hulls	26
Table 2-2 Estimated value of Soybean Peroxidase per Bushel of Soybean Peroxidase	27
Table 3-1 Laboratory Bioreactor Landfills	42
Table 4-1 COD Concentrations and Reduction Rate in Bioreactors	58
Table 4-2 BOD ₅ Concentrations and Reduction Rate in Bioreactors	59
Table 4-3 BOD ₅ /COD in Bioreactors	60
Table 4-4 TS Concentrations and Reduction Rate in Bioreactors	63
Table 4-5 Changes in Mass, Density, Field Capacity, and Settlement Rates in Cells	66
Table 5-1 Laboratory Bioreactor Matrix	67
Table 5-2 Composition of MSW	68
Table 5-3 Relative COD Concentrations and Reduction rate in Bioreactors	71
Table 5-4 Relative BOD ₅ Concentrations and Reduction rate in Bioreactors	73
Table 5-5 Relative TS Concentrations and Reduction rate in Bioreactors	77

List of Figures

	Page
Figure 1-1 Management of Paper Waste in Canada	2
Figure 2-1 Enzymatic Reaction	8
Figure 2-2 Zero order reaction rate independent of substrate	9
Figure 2-3 Effect of Substrate Concentration	10
Figure 2-4 Effect of pH on Reaction Rate	12
Figure 2-5 Effect of Temperature on Reaction Rate	13
Figure 2-6 Seed of Soybean Plant	15
Figure 3-1 Components and Configuration of Bioreactor Landfill	43
Figure 3-2 Leachate Recirculation Mode	47
Figure 3-3 Air Injection and Exhaust Mode	48
Figure 3-4 Air injection through air compressor into all three bioreactors	49
Figure 3-5 Waste Composition	50
Figure 4-1 COD variations in the Bioreactors	57
Figure 4-2 BOD ₅ variations in the Bioreactors	59
Figure 4-3 NH ₃ -N variations in the Bioreactors	61
Figure 4-4 TS variations in the Bioreactors	62
Figure 4-5 pH variations of leachate in the Bioreactors	64
Figure 4-6 Settlement rate of waste mass in the Bioreactors	65
Figure 5-1(a) Relative COD variations in Bioreactor having MSW with Biosolids	70
Figure 5-1(b) Relative COD variations in Bioreactor having Paper waste with Enzyme	70

	Page
Figure 5-2(a) Relative BOD ₅ variations in Bioreactor having MSW with Biosolids	72
Figure 5-2(b) Relative BOD ₅ variations in Bioreactor having Paper waste with Enzyme	72
Figure 5-3(a) Relative NH ₃ –N variations in Bioreactor having MSW with Biosolids	74
Figure 5-3(b) Relative NH ₃ –N variations in Bioreactor having Paper waste with Enzyme	74
Figure 5-4(a) Relative TS variations in Bioreactor having MSW with Biosolids	76
Figure 5-4(b) Relative TS variations in Bioreactor having Paper waste with Enzyme	76
Figure 5-5(a) Settlement rate variations in Bioreactor having MSW with Biosolids	77
Figure 5-5(b) Settlement rate variations in Bioreactor having Paper waste with Enzyme	78

List of Acronyms

BOD ₅	5-Day Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
ES	Enzyme –Substrate Complex
HCL	Hydrochloric Acid
HRP	Horseradish Peroxidase
H ₂ O ₂	Hydrogen Peroxide
LFG	Landfill Gas
MOE	Ministry of Environment
MSW	Municipal Solid Waste
NaOH	Sodium Hydroxide
NH ₃ -N	Ammonia Nitrogen
SBP	Soybean Peroxidase
TS	Total Solids
TDS	Total Dissolved Solids
TSS	Total Suspended Solids

1.0 Introduction

1.1 Sources of Waste

People have been using paper for hundreds of years, although its use was limited by cost and availability. With the introduction of mechanized paper making machines in the 18th Century, paper became a cheap and readily available material. Paper today is made from wood pulp derived from wood chips, either from short-fibred hardwoods or long-fibred softwoods (Cleanaway 2000).

Paper is currently used for a wide variety of purposes including writing, packaging, printing and photocopying. The paper categories include newspaper, school and office paper, packaging paper, magazines, cardboards, flyers and posters, etc. Several major wastepaper categories are of primary concern for recycling. Corrugated boxes, newspapers, high grade office papers and mixed papers are the standard for separation. In order to keep paper recycling economically attractive to prospective investors, it is essential that each of these are well sorted (Murphy 1990).

Wastepaper is one of the constituents of the Municipal Solid Waste (MSW) diverted to the landfill site. MSW consists of food and plant wastes, paper, plastics, wood, leather and various fabrics (Themelis and Kim 2002).

1.2 Scope of the Study

Paper and paper products account for more than one third of the materials discarded into Canada's municipal waste stream. Today, it is widely recognized that the volume of paper products we discard must be dramatically reduced.

One obvious way to reduce the amount of paper waste being discarded, and to conserve our forest resources, is to recycle more of our waste paper. It is estimated that less than one quarter of the 6 million tonnes of paper and paperboard used annually in Canada is recycled (Environment Canada 1992). About 60 percent of paper waste goes to the waste stream, 20 percent is recycled and remaining 20 percent is unrecoverable as shown in Figure 1-1.

Figure 1-1 Management of Paper Waste in Canada

Waste management is everyone's responsibility. We all have a role to play in encouraging the recycling of waste paper but majority of paper waste goes to the waste stream.

1.3 Waste Management

The increase in solid waste generation poses numerous questions concerning the adequacy of conventional waste management systems and their environmental effects. Landfill disposal is the most commonly waste management method worldwide. Landfills have served as ultimate waste receptors for municipal refuse, industrial residues and recycle discards (Warith 2003).

Landfills are mainly categorized as open dumps, engineered landfills and bioreactor landfills. Open dumps are sites where all types of waste are stacked on top of each other and are in contact with the environment. There is little or no site preparation. The capacity of the landfill is unknown and poorly sited (Tchobanoglous et al. 1977). In open dump, there is no leachate and gas management. The leachate accumulating from the dump may seep into the ground and contaminate the groundwater.

Engineered landfill is the most predominant MSW disposal option. The wastes are delivered to the landfill, spread out, compacted and covered at the end of the day with a

thin layer of soil until a planned depth is reached. Then the waste is covered with an impermeable cap. Landfills are constructed with liner system that contains leachate and minimizes its impact on sub-surface environment. Leachate is collected via series of pipes and drainage layer either for treatment or for recirculation into the landfill.

Bioreactor landfill is an emerging technology for solid waste management. The basic concept of bioreactor landfill is to use specific design and operation practices to accelerate the decomposition of waste by promoting optimum moisture content and enzymes to degrade the waste. The method not only enhances the degradation processes, but also stabilizes the landfill as quickly as possible (Warith 2003). The bioreactor landfill attempts to control, monitor, and optimize the waste stabilization process rather than contain the wastes as prescribed by most regulations (Reinhart et al. 2002). This landfill technology is gaining popularity both in pilot and full scale in various landfills in North America, particularly in areas where landfill closure is costly and where landfill space is crucial. It provides accelerated waste stabilization, a means for recovery of capacity or air space and a means to enhance landfill gas (LFG) generation rates (McCreanor and Reinhart 1996).

1.4 Objectives of the Study

The obvious way to reduce the amount of paper waste being discarded is to recycle more of our waste paper. But most of paper waste is diverted to the municipal waste stream. This study aims at examining the effect of decomposing the solid waste with moisture contents and enzymes to degrade under aerobic conditions followed by anaerobic conditions. Air addition into the waste mass accelerates the decomposition of the easily biodegradable organic materials as well as creates optimum environment for the downstream anaerobic degradation in bioreactor landfills. If waste is decomposed into anaerobic condition directly, the pH values will decrease sharply, which will seriously affect the degradation of the waste.

The objectives of this study are as follows.

1. Examine the performance of aerobic and anaerobic bioreactor landfill for decomposing the waste.

2. Examine the strength of leachate by measuring the leachate parameters.
3. Monitor the effect of enzymes in stabilization of paper waste.
4. Compare the performance of different concentration of enzymes with the control one.
5. Compare the effect of enzyme with biosolids in decomposition of waste.

1.5 Organization of Thesis

This thesis is organized into six Chapters. Chapter 1 outlines the introduction and objectives of the research. The literature review is discussed in Chapter 2. Material and methods are provided in Chapter 3. Experimental results and discussions are presented in Chapter 4. The comparisons of results are presented in Chapter 5. Conclusions of the research and recommendations are presented in Chapter 6.

2.0 Literature Review

2.1 Basic Concept of Enzyme

2.1.1 Definition

Enzymes are macromolecules, mostly of protein nature, that occur in all living organisms. They have a number of functions in the living cell, but ultimately, they help to convert food into energy and new material for the growth and repair of the organism in which they function (Mathewson 1998). Acting as natural catalysts, they enable the biochemical reactions necessary for life to take place by increasing the reaction rates. Enzymatic reactions require neither high temperatures nor high-pressure conditions, and are therefore less energy-intensive than processes dependent on chemical catalysts. Enzymes have a high specificity. In general, an enzyme catalyzes only one reaction type and operates on only one type of substrate.

Enzymes bind temporarily to one or more of the reactants of the reaction they catalyze. In doing so, they lower the amount of activation energy needed and thus speed up the reaction. It catalyzes the decomposition of hydrogen peroxide into water and oxygen.

One molecule of catalase can break 40 million molecules of hydrogen peroxide each second.

2.1.2 Historical Overview

Although enzymes have been among us since time immemorial, recently their potential was realized in the food, textile, leather, and paper industries, in the wastewater-treatment sector, and as a washing-detergent additive.

The enzyme complex of malt is isolated for the first time in 1833. Chymosine is exerted from calf stomachs for the first time in 1874. Glucose is converted into ethanol and carbon dioxide using cell-free yeast extract, proving that the conversion is due to the action of enzymes and not to that of living yeast cells themselves.

A method for the industrial production of the enzyme amylase (starch-degrading enzyme), which occurs in the fungus *Aspergillus oryzae*, is developed in 1900. The effectiveness of washing detergents is enhanced by the addition of the proteolytic (protein-degrading) enzyme trypsin, which is obtained from animal pancreas in 1913. The product is marketed under the "Burnus" trademark until well into the 1960s. For the first time in 1917, an amylase from a bacterium is used to replace the acids, bases, and oxidants that have previously been used in the desizing of textiles. Mass production of the enzyme begins only after the end of the Second World War.

The first washing detergent using a bacterial protease (proteolytic enzyme) in place of pancreatic trypsin is developed in 1950. The enzyme amyloglucosidase is first used to fully degrade starch into glucose (sugar syrup), which rapidly leads to the use of acid becoming virtually redundant in the production of glucose. Bacterial chymosin is available for the first time in 1971 as a substitute for the calf-stomach enzyme for the production of cheese.

Enzymes are used for the first time to enhance the efficiency of chlorine in paper-bleaching processes, enabling the consumption of chlorine to be cut drastically. Amylase for the starch industry is produced in genetically modified microorganisms. Chymosin is first produced in genetically modified yeast cells in 1988. Chymosin from genetically modified microorganisms is given a marketing licence in Germany in 1997. Many of the enzymes used in the food sector are produced by genetic modification. Additionally, in washing detergents enzymes are in use those are modified in terms of their molecular structure for special applications. Similar developments are predicted for the food industry.

2.2 Enzyme Kinetics

The kinetics of enzyme reactions is similar to those for non-enzymatic reactions except those enzymes have a unique way of accelerating the reaction. Enzymes are protein found in the plants, milk and white blood cells. They act as catalysts by bringing the reactants together so as to promote the chemical reaction. First, there is rapid combination of enzyme (E) with the reactant molecule or substrate (S) to form a reactive intermediate, in the case, the enzyme-substrate complex (ES). This complex then breaks down into product

(P) and releases the enzyme, unchanged, to combine with another substrate molecule. This is represented in the following general equation for a one-substrate enzyme-catalyzed reaction (Mathewson 1998).

As indicated by the arrows, the formation of the enzyme-substrate complex and the breakdown of ES to product can both be reversible reactions. The coefficients K_{+1} , K_{-1} , K_{+2} , and K_{-2} represent the rate constants for each reaction and depend on the specific conditions. The formation of the ES complex is usually rapid, whereas the breakdown to products is a slower, rate-limiting reaction that normally proceeds in an energetically preferred direction (i.e., K_{+2} is normally much greater than K_{-2}). However, under certain conditions, an enzyme-catalyzed reaction can proceed in either direction. For example, some enzyme-catalyzed reactions that proceed in a forward direction in aqueous media can be made to proceed in the reverse direction in a non-aqueous media.

The rate v , at which product is formed is proportional to the concentration of the ES complex, according to the equation:

$$v = K_{+2} (ES) \quad [3]$$

Equations 2 and 3 provide a basis for calculation of reaction rates, substrate-binding energy, and many other important characteristics of enzymatically catalyzed reactions.

One important concept in enzyme kinetics is reaction order, defined as zero-order, first-order, second-order, or third-order based on the rate of reaction under defined conditions where only the concentration of reactants varies and the enzyme concentration is held constant. In a zero-order reaction, the rate of reaction is independent of reactant (substrate) concentration. In first, second, and third-order reactions, the rate is dependent on one, two, or three reactants, respectively. The substrate concentration must be kept very high relative to the enzyme concentration so that the reaction is zero-order with respect to substrate.

Under such conditions (at constant pH, temperature, etc.), the reaction is independent of substrate concentration and depends on enzyme concentration only (Mathewson 1998).

The enzymic reactions are shown in Figure 2-1. An active enzyme and substrate combine to form an enzyme-substrate complex. The complex goes through a transition state and leads to a molecule with a covalent bond between the enzyme and the substrate.

Source: <http://ull.chemistry.uakron.edu/genobc/>

Figure 2-1 Enzymatic Reaction

After decomposition, the substrate is converted to stable products and a complex of enzyme and product is produced. Finally, the enzyme and product complex breaks off into original enzyme and product. The enzyme is ready to combine with the new substrate and the process is continued until complete decomposition of the substrate. This catalytic cycle allows for small concentration of enzyme to impact large concentration of substrate (Campbell 1993).

2.3 Rate of Reaction

2.3.1 Enzyme Concentration

In order to study the effect of increasing the enzyme concentration upon the reaction rate, the substrate must be present in an excess amount; i.e., the reaction must be independent of the substrate concentration. Any change in the amount of product formed over a specified period of time will be dependent upon the level of enzyme present. Graphically this can be represented as in Figure 2-2.

Figure 2-2 “Zero order” reaction rate is independent of substrate

The amount of enzyme present in a reaction is measured by the activity it catalyzes. The relationship between activity and concentration is affected by many factors such as temperature, pH, etc. An enzyme assay must be designed so that the observed activity is proportional to the amount of enzyme present in order that the enzyme concentration is the only limiting factor. It is satisfied only when the reaction is zero order.

2.3.2 Substrate Concentration

It has been shown experimentally that if the amount of the enzyme is kept constant and the substrate concentration is gradually increased, the reaction velocity will increase until it reaches a maximum. After this point, increase in substrate concentration will not increase the velocity. This is represented graphically in Figure 2-3.

Figure 2-3 Effect of Substrate concentration

It is theorized that when this maximum velocity had been reached, the entire available enzyme has been converted to ES, the enzyme substrate complex. This point on the graph is designated V_{max} .

2.3.3 Time

In the initial period of time, the amount of substrate, which has been transformed, is directly proportional to the length of time for which the reaction has been proceeding (Wynn 1973). After this initial period, the rate of reaction starts to decrease and the amount of reaction is no longer directly proportional to the time. Provided the substrate is present in excess, the explanation of this phenomenon is the progressive loss of enzyme activity after a period of time. This may be due to the effect of heat on the tertiary structure of the

enzyme to the formation of some product or side-product of the reaction, which inhibits the enzyme. In the initial investigation of any enzyme reaction, it is important to establish the period over which the reaction varies linearly with time. This period can be as short as a minute while some enzymes have a linear variation over a period of days. For meaningful results, the initial rate of reaction should be measured.

2.3.4 Effect of pH

At a neutral pH (7.0), most proteins have both positive and negative charges available along the amino acid chain. The opposite charges attract each other, while like charges repel each other. Although the force is not very strong, these repulsion/attraction forces play a significant role in maintaining the overall three-dimensional (tertiary) structure of the protein, which is so important to its functionality.

The ability of the amino acids at the active site of an enzyme to interact with substrate depends on their electrostatic state, that is, whether they are properly charged or uncharged, as well as their spatial orientation. If the pH is not correct, the charge on one or all of the required amino acids is such that the substrate can neither bind nor react to produce product. Most enzymes have a rather narrow range in which they can catalyze a reaction efficiently. The majority works in the pH range of 6.0-8.0, although there are exceptions to this. For example, pepsin, the enzyme in our stomach that helps to digest protein, works best at a pH of about 2.0, which is very acidic. Lipxygenase enzymes from soybeans work best around pH 9.0. In general, commercial enzymes prefer a pH around neutral (Mathewson 1998).

The most favorable pH value, the point where the enzyme is most active is known as the optimum pH. This is graphically illustrated in Figure 2-4. Extremely high or low pH values generally result in complete loss of activity for most enzymes. The pH is also a factor in the stability of enzymes. As with activity, for each enzyme, there is also a region of pH optimal stability.

The optimum pH value will vary greatly from one enzyme to another. The optimum pH for Soybean Peroxidase (SBP) treatment of 2, 4-Dichlorophehenol (DCP) is approximately 8.2 (Kennedy et al. 2002). The optimum pH for Horseradish Peroxidase (HRP) treatment of 2, 4-Dichlorophehenol (DCP) is 6.5.

Figure 2-4 Effect of pH on reaction rate

2.3.5 Effect of Temperature

The effect of temperature is similar to the effect of pH in that some heat can help an enzyme but too much can permanently inactivate it. Putting more heat into the system helps to overcome the energy barrier. The warmer the solution, the faster the molecules move. Thus, the reactants collide more often and with greater energy.

In general, chemical reactions, including enzymatic reactions, obey what is known as the Q_{10} rule (Mathewson 1998). A ten degree Centigrade rise in temperature will increase the activity of most enzymes by 50 to 100%. Variations in reaction temperature as small as 1 or 2 degrees may introduce changes of 10 to 20% in the results. In the case of enzymatic reactions, this is complicated by the fact that many enzymes are adversely affected by high temperatures. As shown in Figure 2-5, the reaction rate increases with temperature to a maximum level, then abruptly declines with further increase of temperature. Because most

animal enzymes rapidly become denatured at temperatures above 40°C, most enzyme determinations are carried out somewhat below that temperature.

Figure 2-5 Effect of Temperature on Reaction Rate

Over a period of time, enzymes will be deactivated at even moderate temperatures. Storage of enzymes at 5°C or below is generally the most suitable. Some enzymes lose their activity when frozen.

2.4 Peroxidases

2.4.1 Introduction of Peroxidases

Peroxidases are oxidoreductases produced by a number of microorganisms and plants (Karam and Nicel 1997). They catalyze a variety of reactions but they all require the presence of peroxides such as hydrogen peroxide (H_2O_2) to activate them. Hydrogen peroxide first oxidizes the enzyme, which in turn oxidizes the substrate. Peroxidases that have been used for the laboratory-scale treatment of aqueous aromatic contaminants include soybean peroxidase, horseradish peroxidase, lignin peroxidase and a number of other peroxidases from different sources.

Peroxidase is a type of heme protein, which is a class of oxidative enzymes found in plants, milk and white blood cells (Goetz 1996). It can reduce environmental

contamination caused by manufacturing processes, and it's gaining an industrial following. Peroxidase can be used for many industrial and commercial processes, such as (Producers' Natural Processing Inc, N.D.):

- Wastewater treatment
- Soil remediation
- Medical diagnostics
- Pulp and paper bleaching (substitute for chlorine bleach)
- On-site waste destruction
- Bread dough conditioner

The baking industry in Europe has started using soybean peroxidase instead of potassium bromate in bread making and dough conditioning. A U.S. company is planning to use it for labeling antibodies in medical and research diagnostics. Bioremediation companies are considering peroxidase to treat industrial wastewater. There is on- going research for its future use in manufacturing computer chips, adhesives, car parts, varnishes and linings of drums and cans. Peroxidase someday may also be used for bioremediation of water or soil. Peroxidase replaces a lot of really harsh chemicals and eliminates harsh reaction conditions. It's a cleaner technology, and it's cost effective (Goetz 1996).

2.4.2 Soybean Peroxidases

Soybean Peroxidase (SBP) is an enzyme found in the husk or seed of the soybean plant (Driscoll et al. 2000; Alemany 2000) as shown in Figure 2-6.

It has properties that make it particularly suited for practical applications. Therefore, it is essential to know its enzymatic composition and properties. SBP is an anionic peroxidase that has a pH of 4.1 and molecular weight of 37,000 Daltons or atomic mass unit. Soybean peroxidase, like all peroxidases, is activated in the presence of hydrogen peroxide, which then catalyzes the oxidation of many organic and inorganic substances (Driscoll et al. 2000).

Figure 2-6 Seeds of Soybean Plant

The characteristics of the polymer formed when using SBP as an enzyme is still unknown. This uncertainty may pose potential problems when decomposing solid waste. Furthermore, if these insoluble toxic polymers seep into the groundwater and are not properly treated, they may be harmful to the public's health.

The SBP treatment process is still in the experimental stage so researchers are continually studying and optimising treatment efficiency while studying other characteristics of the process (Kennedy et al. 2002).

2.4.3 Horseradish Peroxidases

Horseradish peroxidase (HRP) is undoubtedly one of the most studied enzymes in the relatively new area of enzymatic waste treatment. Once activated by H_2O_2 , HRP can catalyze the oxidation of a wide variety of toxic aromatic compounds including phenols, biphenols, anilines, benzidines and related heteroaromatic compounds (Karam and Nicel 1997). The reaction products are polymerized through a non-enzymatic process, which leads to the formation of water-insoluble precipitates that can be easily removed from water or wastewater by sedimentation or filtration. HRP is particularly suitable for wastewater treatment because it retains its activity over a broad range of pH and

temperature. The mechanism of HRP action is relatively well understood and has been mathematically modeled.

Most applications have focused on the treatment of phenolic contaminants. The use of HRP for the treatment of contaminants including anilines, hydroxyquinoline and arylamine carcinogens such as benzidines and naphthylamines has also been demonstrated in the laboratory. In addition, HRP has the ability to co-operate certain difficult to remove contaminants, including non-substrates of HRP, along with the more easily removable compounds. It also includes the formation of mixed polymers that behave similarly to the polymeric products of easily removable compounds. This phenomenon has an important practical implication for wastewater, which usually contain many different pollutants. An extension of this principle to hazardous wastes was demonstrated when it was observed that polychlorinated biphenyls could be removed from solution through co-precipitation with phenols. However, this particular application of HRP does not appear to have been pursued in any subsequent research.

Considerable efforts have aimed at optimizing the HRP catalyzed removal of phenols from aqueous solutions. Improvements in the useful life of the enzyme, and thereby a reduction in treatment cost, have been accomplished through selection of an appropriate reactor configuration, enzyme immobilization, the use of additives such as sodium borate, gelatin and polyethylene glycol to protect the enzyme from entrapment in the precipitating polymers, and the addition of adsorbents such as talc, which protect the enzyme from inhibition by oxidation products.

2.4.4 Other Peroxidase

Lignin peroxidase (LiP), also known as ligninase or diarylopropane oxygenase was first reported in 1983. It is part of the extracellular enzyme system of the white-rot fungus *phanerochaete chrysosporium*. LiP was shown to mineralize a variety of recalcitrant aromatic compounds and to oxidize a number of polycyclic aromatic and phenolic compounds. LiP's role in lignin depolymerization has also been confirmed (Karam and Nicel 1997). Its mechanism is very similar to that of HRP.

Lip's stability, which influences greatly the economic and technical feasibility of the enzyme's application in waste treatment, was studied by Aitken and Irvine (1989). The authors reported that Lip was readily inactivated at low pH. It was found that optimized conditions for phenolics removal included high enzyme concentration at pH above 4.0 and controlled addition of H_2O_2 . Cornwell et al. (1990) reported an immobilization of Lip on porous ceramic supports did not adversely affect Lip's stability and showed a good potential for degradation of environmentally persistent aromatics.

Chloroperoxidase from the fungus *Caldariomyces fumago* has been reported to oxidize several phenolic compounds (Karam and Nicel 1997). In addition, it has been shown to catalyze certain oxygen transfer reactions such as the oxidation of ethanol to acetaldehyde or the oxidation of chloride ions. This latter reaction might lead to the formation of a different range of products (which may be more toxic) when chloride ions are added to reaction mixtures containing chloroperoxidase.

Manganese peroxidase, produced by *Phanerochaete chrysosporium* has also been observed to catalyze the oxidation of several monoaromatic phenols, and aromatic dyes, but these reactions depend on the presence of both divalent manganese and certain types of buffers. In fact, manganese peroxidase catalyzes the oxidation of Mn(II) to Mn(III) in the presence of Mn(III) stabilizing ligands. The resulting Mn(III) complexes can then carry out the oxidation of organic substrates. However, the enzyme's requirement for high concentrations of Mn(III) makes its feasibility for wastewater treatment applications doubtful.

The use of a microbial peroxidase from *Coprinus macrorhizus* as an alternative to HRP for the removal of aromatic compounds from wastewater was recently investigated. The use of hemoglobin as an HRP substitute was suggested by Chapsal et al. (1986) and was deemed feasible from a cost and efficiency point of view.

2.4.5 Inactivation of Peroxidase Enzymes

There are several mechanisms by which peroxidase enzymes become inactivated. As a result of inactivation of the enzyme, larger quantities of the enzyme are required, which dramatically increase the cost of treatment. Several mechanisms for inactivation are thermal, hydrogen peroxide or polymerization of the enzyme.

There are various conditions for which enzymatic activity is decreased. The ideal temperature range varies depending on the thermal stability of each peroxidase. At temperatures above this range inactivation may occur rapidly.

The number of turnovers the enzyme exhibited was higher when the reaction occurred at lower temperatures. They hypothesize that the high temperature is not inactivating the enzyme, but that at lower temperatures the reaction proceeds at a slower rate. This results in a lower concentration of substrate radicals thus reducing the probability of free radicals binding to the active site and rendering the enzyme inactive (Nicell et al. 1992).

The pH of the reactor can also have a dramatic effect on the activity of the enzyme. An ideal range for each peroxidase is where the most efficient removal occurs but at pH levels outside this range, the enzyme may exhibit little or no activity.

Enzyme inactivation may result from the presence of excess hydrogen peroxide. If excess H_2O_2 is available in the solution, then Compound II may react with the excess of H_2O_2 to form Compound III, an inactive verdohaemoprotein rather than returning to the original form of the enzyme.

Mechanism based inactivation occurs when peroxidase enzyme becomes inactivated as a result of becoming entrapped in the insoluble polymer product of the binding of the phenoxy radical to the active site as a result of the reaction. Thus there is no active site for the substrate to enter and be consumed. Several studies compared the peroxidase activity in the solution than in the supernatant after centrifuging. The activity was found to be higher in solution than in the supernatant alone. This indicated that during initial entrapment of

the enzyme it still retains some enzymatic activity, but as polymerization continues the active site becomes increasingly difficult to access and inactivation occurs (Driscoll et al. 2000).

2.4.6 Introduction of Hydrogen Peroxide

Hydrogen peroxide is required to catalyze the reaction or oxidation of aromatic compounds. An insufficient dose results in H_2O_2 being the limiting reagent for the reaction, while excessive dosing will encourage inactivation of the enzyme. The peroxide reacts with Compound II and forms Compound III, an inactive verdohaemoprotein rather than returning to the original form of the enzyme. Although Compound III is inactive, it may spontaneously decompose back to the original. But this spontaneous return to the enzyme is very slow and the overall catalytic ability of the enzyme is hampered. Hence amount of H_2O_2 is reduced and the required quantity and cost of the enzyme is increased for the removal of the same contaminant as would be removed if the optimum amount of H_2O_2 is used (Driscoll et al. 2000).

2.4.7 Polymerization of the Enzyme

When peroxidase enzymes become entrapped in the insoluble polymer product, the inactivation occurs through polymerization. In order to operate the peroxidases at their full potentials, it is necessary to have an operating and storage temperature within the range of the enzyme's thermal stability and an optimum amount of Hydrogen Peroxide to activate the enzyme to limit inactivation due to polymerization.

2.5 Application of Enzyme

2.5.1 Application to Waste Water Treatment

During the past two decades, considerable research has been conducted to investigate the new possibilities offered by enzymes in waste treatment. The rate of introduction of xenobiotics and recalcitrant organic pollutants into the environment is on the rise, and it is becoming increasingly difficult to achieve the required degree of removal of these pollutants using conventional chemical and biological processes. Therefore, there is a need

for the development of alternative treatment methods that are faster, cheaper, more reliable, and simpler to implement than current processes. There is a growing recognition that enzymes can be used to target specific pollutants for treatment. The recent biotechnological advances have allowed the production of cheaper and more readily available enzymes through better isolation and purification procedures (Karam and Nicel 1997).

Aromatic compounds, including phenols and aromatic amines, constitute one of the major classes of pollutants and are heavily regulated in many countries. They are found in the waste water of a wide variety of industries including coal conversion, petroleum refining, resins and plastics, wood preservation, metal coating, dyes and other chemicals, textiles, mining and dressing, and pulp paper. Most aromatic compounds are toxic and must be removed from wastewater before they are discharged into the environment. Enzymatic treatment has been proposed by many researchers as a potential alternative to conventional methods. Firstly, enzymes are highly selective and can effectively treat even dilute wastes. Secondly, they are less likely to be inhibited by substances, which may be toxic to living organisms and their cost could eventually be less than that of other methods if commercially available enzymes are produced in bulk quantities. Moreover, enzymes operate over a broad aromatic concentration range and require low retention times with respect to other treatment methods.

2.5.2 Application to Solid Waste Treatment

For the past decade, there has been an increasing interest in the enzymatic hydrolysis of cellulose. This interest stems from the advantages that such a process would offer, namely, the conversion of lignocellulosic and cellulosic wastes to a useful energy source through the production of sugars, ethanol, biogas or other energetic need products. However, until now, the industrial production of sugars from cellulose has been plagued with a number of constraints pertaining to enzyme production and cost and substrate preparation, among others.

The hydrolysis process in a landfill anaerobic degradation system is promoted by enzymes produced by fermentative bacteria. Cellulose is a main component in the degradable fraction of MSW. One possibility of controlling and enhancing the hydrolysis of cellulose is to manipulate the natural enzyme activities. The industrial manufacture of these enzymes has now reached a level of efficiency where large scale application to waste treatment could be economic (Lagerkvist and Chen 1993).

Hakulinen (1991) used cellulose enzyme on MSW under semi aerobic conditions (Composting) and yielded approximately 30% enhancement by simulating the growth of different fungi.

Lagerkvist and Chen (1993) used laboratory-scale simulators to investigate the effect of adding industrial cellulolytic enzymes into MSW during both acidogenic and methanogenic states. Because these two degradation steps in combination i.e. the Two Step Anaerobic Degradation (TSAAD) is the primary landfill process for which a possible application for commercial enzymes are identified. Their results suggested that it is viable to intensify both acidogenic and methanogenic by enzyme addition (Yuen 2001).

There have been numerous reports on the possible ways of improving the enzymatic hydrolysis of the cellulose contained in the organic fraction of municipal solid waste (MSW) in order to produce fermentable sugars and eventually ethanol or butanol (Karam and Nicel 1997). Rivers and Emert (1988) studied the effects of a number of parameters, such as substrate composition, cellulose crystallinity and particle size, on the yields of enzymatic hydrolysis for two major MSW components, newspaper and corrugated cardboard. Their primary aim was to try to determine the modifications of MSW that would lead to increased conversions to ethanol. The enzyme system consisted of endoglucanase, cellobiohydrolase and cellobiase. The results seemed to indicate that cellulose crystallinity and particle size were not major factors in determining the accessibility and the susceptibility of lignocellulosics to cellulolytic enzymes. It was also reported that the best pretreatment for newspaper was wet attrition while that for corrugated cardboard was dry attrition.

2.5.3 Application to Sludge Treatment

There has been some work done concerning the use of enzymes to improve sludge dewatering. Thomas et al. (1991) used an enzyme product containing carbohydrase, lipase and proteinase activities to improve the dewater ability of sewage sludge and to increase the quantity of water released during pressing in order to achieve a true reduction in volume. The effect of enzyme addition was to cause the cleavage of the water-binding macromolecules. However, whereas an enzyme dose of $2.5\text{--}5\text{ mg dm}^{-3}$ gave good results, an increased dose led to the creation of more hydrophilic end groups, which negatively affected the dewatering process butanol (Karam and Nicel 1997).

Attempts to dewater phosphate slimes were reported by Anazia and Misra (1989). Phosphatic slimes contain considerable amounts of swelling clay-like material and their fine particle size, along with their slow settling behavior, makes them particularly difficult to dewater. Anazia and Misra (1989) proposed the use of peroxidase to improve the dewatering of these slimes. They reported that peroxidase significantly promoted the growth of algae and mold with the beneficial effect of enhanced aggregation of the particles and very high viscosity and gel strength. Such high viscosity and gel strength would help in post-sedimentation handling.

Hakulinen (1988) reported on the use of cellulase and the bacterial enzyme lysozyme or muramidase, for sludge dewatering while cellulase was used with penicillin and gave rather poor results, lysozyme was allegedly able to alter floc matrices and to cause a dramatic increase in dewatering rates.

2.6 Advantages and Disadvantages of Enzyme Treatment

2.6.1 Advantages of Enzyme Treatment

There are many advantages to using enzymes over conventional treatment methods. Enzymatic treatment of contaminated water provides remediation of a wider range of compounds than conventional treatment. As well as a wide range of operating conditions

are possible with remediation occurring at extreme temperature, pH, salinity levels, and high or low concentrations.

2.6.2 Disadvantages of Enzyme Treatment

There are some disadvantages to enzyme treatment. The high cost of the enzyme required to treat the contaminant, and the uncertainty of the toxicity of the end product polymer (Driscoll et al. 2000). The use of enzymes may not be feasible when there is a variety of pollutants present and their reactants vary in toxicity.

2.7 Selection of Enzyme

The choice of selection of the most suitable enzymes is a function of the specificity of the enzyme, its need for cofactors (such as H_2O_2) and its capacity to retain a substantial amount of its activity for a reasonably long period under normal operating conditions. The specificity of the enzyme is important especially in cases where removal of specific chemicals from dilute mixtures is mandated. An enzyme with too broad a specificity might have reduced efficiency. Enzymes that require cheap cofactors (such as O_2 for polyphenol oxidases as compared to H_2O_2 for HRP) or that do not require cofactors at all are obviously preferred. Finally, the improvement of the catalytic life of the enzymes (i.e. their ability to retain their activity for extended periods of time) may be improved by immobilization of the enzymes on solid supports. Indeed, in most of cases, immobilized enzymes have been found to be more effective than free enzymes. The advantages of immobilization include improved stability, possibility of continuous processing and enzyme reuse (Karam and Nicell 1997).

There is great potential for enzymes in a large number of waste treatment areas. Particularly notable is the possibility of using enzymes to biconvert wastes from municipal solid waste. This bioconversion has the double advantage of reducing the amount of valueless material that has to be landfilled or disposed of in other ways, while at the same time creating valuable products such as fuel (e.g. ethanol), food, feed or other products.

2.7.1 Soybean versus Horseradish Peroxidases

In the past, many studies have been performed on horseradish peroxidase, which have shown that it can oxidize a large number of aromatic compounds, up to 99% removal over a very short contact time. More recently studies have examined the use of SBP as an alternative to HRP due to its superior stability (Driscoll et al. 2000).

SBP is much more stable than HRP when in the presence of high levels of H_2O_2 (Nicell and Wright 1997) and at pH levels below 3, which seems to be the result of SBP having a stronger hold on its heme (McEldoon et al. 1995). SBP retains over 50% of its activity at a pH of 3 while horseradish becomes inactivated in this acidic environment. Additional experiments examined the oxidation of veratryl alcohol, which occurs at an optimum pH of 2.4, while SBP is quite efficient at the oxidation. The structural differences and strength of heme bond indicate that SBP has a great advantage over HRP in acidic conditions. The pH range for HRP has shown excellent contaminant removal between a pH of 6 to 9 and reduced removal between 4 to 6 and 9 to 10. If the pH is below 2 and above 11, no catalytic ability or contaminant removal occurred (Nicell et al. 1992). It is possible that these pH levels lead to the loss of the prosthetic group containing the enzyme.

SBP has been found to be more thermally stable compared to HRP allowing reactors to operate at higher temperatures without experiencing thermal inactivation. The temperature range for HRP to remove contaminants effectively is 5° to 35°C . If the temperature is increased above 45°C , inactivation of horseradish peroxidase occurs (Bewtra et al. 1995). SBP essentially exhibits no activation at temperatures below 70°C by the 12th hour. Its catalytic activity is optimal at pH 6.4. SBP activity is very stable at 25°C at neutral and alkaline pH. Additionally, SBP remains thermally stable at high temperatures, even when high temperatures are combined with acidic conditions (i.e. 60°C in pH of 2.4). SBP has a higher resistance to inactivation than HRP, when they react with hydrogen peroxide (Wright and Nicell 1999).

In comparison with HRP, the activity of SBP is less sensitive to pH, and is significantly more stable at elevated temperatures. SBP is less susceptible to permanent inactivation by

hydrogen peroxide. However, SBP is catalytically slower than HRP and a larger molar quantity of phenolic substrate. Both SBP and HRP are susceptible to increased inactivation in the presence of phosphate or orthophosphite ions under acidic conditions (Wright and Nicell 1999).

The advantage of HRP is that it appears to oxidate phenols at an order of magnitude faster than SBP. This may result in longer retention times, and larger facilities for the treatment of phenols by SBP. While this poses an additional initial cost in the long term, this may be overcome if crude SBP is used (Nicell and Wright 1997).

Overall, the advantage of SBP as a result of its stability for temperature, pH, and high hydrogen peroxide concentrations may lie in its ability to remediate sites for alternative peroxidases or treatment procedures. This may lead to the development of remediation tool with commercial and environmental (Driscoll et al. 2000).

2.7.2 Economics

Despite the promising results, peroxidases have shown in the laboratory. The cost of these enzymes for use in larger scale projects has been prohibitive. Much of the expense is a result of the high cost of the enzyme itself. However, an alternative is to buy enzymes from a chemical supplier, which is to puree the original form of the enzyme, such as horseradishes. Even though this method will save some capital, it is still more expensive to use enzyme treatment than the conventional treatment methods due to the high cost of raw horseradish (Driscoll et al. 2000).

The advantage of SBP is that the enzyme is found in the seed coat, which is a byproduct of the soybean industry and can be utilized without reducing the value of the soybean's oil and meat. Since there is currently no market for this byproduct, it is difficult to estimate an associated cost, but could be assumed to be very inexpensive. Recent research indicates the use of crude soybean hulls to be extremely efficient (Flock et al. 1999).

Soybean seed coats or hulls have a protein content of 0.5% and are added back to soymeal following oil extraction to increase the fiber content of the meal. This provides a value to soybean hulls equal on a per weight basis to soymeal. SBP represents 3% of the total protein content of soybean seed hulls. HRP has been used widely in a broad range of diagnostic kits, coupled with glucose oxidase as a color indicator. SBP is more heat stable than HRP and thus provides a functional improvement for commercial applications. The analysis of the available data is used to determine the relative value of soybean peroxidases per bushel of soybean. Unfortunately, the cost of commercially extracted SBP has not been established yet, but it has been provisionally estimated it at \$250.00/bushel for pharmaceutical grade peroxidase (Penner 2003). The calculation of the amount of soybean peroxidase extractable from soybean hulls is shown in Table 2-1.

Table 2-1 Calculation of Soybean Peroxidase Extractable from Soybean Hulls

Soybean Peroxidase Input	Units
Cost of soybean grain	\$ 8.50/bu
Soybean hull yield	1.15 Lbs/bu
Peroxidase concentration	3% (%of total protein)
Extractable total protein in seed hulls (0.5%)	5 g/kg of hulls
Extractable total protein in seed hulls	2.61 g/bu
Peroxidase extracted from hulls/bu	78.24 mg/bu
Cost peroxidase extraction	\$250.00/bu

Source: Penner, 2003 Market Opportunities analysis for Canadian Soybean

The commercial catalogue price is used for horseradish peroxidase to estimate the potential market price and market opportunity for soybean peroxidase. The estimated value of SBP is shown in Table 2-2.

Table 2-2 Estimated value of Soybean Peroxidase per Bushel of Soybean Peroxidase

Value	Units
Commercial price for horseradish Peroxidase	\$ 41.30/2000 units
Activity	250.00 units/mg
Price/mg	\$ 5.16/mg
Value of Soybean Peroxidase	\$403.94/bu
Operating margin for Soybean Peroxidase	\$153.94/bu

Source: Penner, 2003 Market Opportunities analysis for Canadian Soybean

A reduction in cost could also be achieved by increasing the peroxidase activity in the seed coat, increasing the enzyme catalytic lifetime, or the number of turnovers. The raw soybean seed coat may contain zero to five- percent peroxidase by dry weight, although genetically engineered soybean levels of the peroxidase enzyme (Goetz 1996). Increasing the catalytic lifetime can be accomplished by minimizing the adverse conditions, which result in the inactivation of the enzyme. Optimum conditions for the enzyme can be determined through research (Trombly 1995).

2.7.3 Selection of Soybean Peroxidase

Soybean peroxidase has ability to catalyze the polymerization and precipitation of phenolic compounds from water. It can act on a broad range of compounds and retains its catalytic ability over wide ranges of temperature and pH (Wright and Nicell 1999). It remains thermally stable at high temperatures, even when high temperatures are combined with acidic conditions (i.e. 60⁰C in pH of 2.4). Its catalytic activity is optimal at pH 6.4, with more than 90% of its maximum activity observed between pH 5.7 and 7.0, and more than 10% between pH 3 and 9. SBP activity is very stable at 25⁰C at neutral and alkaline pH. It is most thermally stable at approximately pH 6 (Wright and Nicell 1999).

In comparison with HRP, the activity of SBP is more sensitive to pH and is more stable at elevated temperatures. It has a higher resistance to inactivation than HRP, when they react with hydrogen peroxide (Wright and Nicell 1999). Particularly, the characteristics of SBP

are very favorable. For these reasons, this enzyme will be used to decompose organic solid wastes. However, there are some concerns when it comes to the inactivation of peroxidase, the unknown toxicity of the end-product polymer and the excessive cost to buy it.

2.7.4 Extraction Method of SBP Enzyme

2.7.4.1 Enzyme Purification

The SBP enzyme is an abundant protein within the seed coat, which represents the dominant peroxidase isozyme, and accumulates during the maturation of the tissue. The seed coats are obtained by soaking whole seeds in distilled water for approximately 5 minutes or until the coats become wrinkled (Gillikin and Graham 1991). The coats are then removed from the seed and homogenized with a Tekmar Tissuemizer in extraction buffer 25 mM KH_2PO_4 having pH 7.5 at a ratio of 10 mL extraction per gram tissue. All purification steps are performed at 4⁰ C. The homogenate is filtered through two layers of Miracloth and the filtrate centrifuged (12,000g, 10 min). The supernatant fluid is applied to a DEAE sepharose fast flow column (2.5 ×20 cm) previously equilibrated with a liner salt gradient from 0 to 300 mM KCl in extraction buffer. Fractions containing peroxidase activity are made 1×in Con A-Sepharose binding buffer by addition of one-fourth volume of a 5× stock solution (250 mM Tris-HCl, 2.5 M NaCl, 25 mM MnCl_2 , and 25 mM CaCl_2). This is then applied to a Con A-Sepharose column (2.5 ×20 cm) previously equilibrated with 1 ×binding buffer. Peroxidase activity retained on the column is eluted with a linear gradient from 0 to 1 M methyl α -D-mannopyranoside in 2.5 M NaCl, 50 mM Tris-HCl (pH 6.8), and detected as described above. The activity is concentrated to 5 mL using an Omegacell, 10,000 NMWL ultrafiltration systems, and loaded directly onto a Sephadex G-75 column (2.5 ×120 cm) equilibrated in water. Fractions containing peroxidase activity are pooled and lyophilized.

2.7.4.2 Determination of Seed Coat Peroxidase Isoelectric Point

The Isoelectric point of the seed coat isozyme is determined by analytical flatbed isoelectric focusing polyacrylamide gels containing ampholines in the pH range of 3.5 to

9.5. The gel is loaded with a seed coat extract in one lane and is mock loaded with H₂O in an adjustment lane. The gel is subjected to electrophoresis for 1.5h at 0.125 W/cm² at 10 °C (Lagrimini and Rothstein 1987). The lanes are then separated and the one containing the seed extract is stained with 4-chloro-1-naphthol solution to determine the distance the peroxidase activity migrated relative to the anode. The mock loaded lane is sliced into 0.25 cm pieces. Gel slices are then added to 2 mL of H₂O and incubated at room temperature overnight with shaking. The pH of the solution containing each gel slice is determined and plotted as a function of distance from the anode. By comparing the distance from the anode that the activity migrated to the pH of the gel at that position, the peroxidase isoelectric point of enzyme is ascertained.

2.7.4.3 Determination of Peroxidase Isozyme Tissue-Specific Activity

The determination of isozyme activity levels in specific tissues is accomplished by densitometric tracing of peroxidase activity-stained gels. The cotyledons and seed coats are homogenized with a Tekmar Tissuemizer in distilled H₂O at a ratio of 10 mL per g of tissue. Samples are then centrifuged (12,000g, 10 min). Samples are electrophoresed on analytical IEF gels. Gels are then placed in 4-choloro-1 naphthol solution and allowed to develop for 60s followed by a 5 min wash in distilled H₂O. Densitometric tracings of the activity stained gels are performed at 582 nm using a shimadzu CS-930 dual wavelength TLC scanner. Units of activity are arbitrary values calculated by measuring peak areas of peroxidase activity in the IEF gel. The specific activity of seed coat peroxidase is defined as units/mg of soluble protein applied to IEF gels.

2.8 Solid Waste Management

2.8.1 Open Dump

In case of open dump, the waste is unloaded in piles, make very uneconomical use of the available space, allow free access to waste pickers, animals and flies and often produce unpleasant and hazardous smoke from slow-burning fires (Tchobanoglous et al. 1977). Since there is no leachate and gas management, the leachate accumulating from the dump may seep into the ground and contaminate the groundwater, which leads to increased costs to treat the water. Furthermore, lack of gas management causes risk of fire and explosion.

Open dumps have the lowest initial capital investment and operating cost. Such costs can include site acquisition and some activities carried out by municipal officials. Additionally, many open dumps start off as controlled dumps and degrade due to lack of management and other resources.

Due to the low initial costs of open dumps, and lack of expertise and equipment, these sites are common in developing countries. They pose significant risks to human health and the environment, especially as MSW becomes more complex in industrializing countries. In addition, the cost of remediating these sites can easily exceed their total lifetime capital and operating costs. Contaminated groundwater may never be returned to usable condition and other environmental impacts may take many decades to ameliorate. Open dumps attract numerous birds that feed on the wastes, which can make them more serious disease vectors than flies (Tchobanoglous et al. 1977).

The practice of open dumping is a dilemma for the poorer and smaller cities and towns of developing countries. Certainly, this method is not sound practice. The reality of inadequate technical and managerial resources in many places, however, means that solid waste managers must attempt to ameliorate open dumping practices and gradually upgrade the sites.

2.8.2 Engineered Landfill

An Engineered landfill site is an engineered waste disposal facility where garbage is deposited in the ground compacted into a cell and covered with earth fill materials. Environmental controls are incorporated into the engineering design of the facility to protect both the human and natural environments. In Ontario, the Ministry of the Environment (MOE) regulates the development and operation of such facilities (Warith 2003).

The inclusion of environmental barriers such as liners and caps frequently excludes moisture that is essential to waste biodegradation (Francis 2003). Consequently, waste is

contained in a “dry tomb” and remains intact for long period of time ranging from 30 to 200 years, possibly in excess of the life of the landfill barriers and covers. The liner failure could happen in conventional dry landfill sometime in future, which can cause serious groundwater contamination (Warith 2003).

With a better understanding of landfill decomposition processes and behavior, there has been a strong trend in recent years to shift the philosophy of landfill design from the permanent storage concept to bioreactor approach (Krol et al. 1994; Pacey et al. 1999).

2.8.3 Bioreactor Landfill

Bioreactor landfill uses specific design and operation practices to accelerate the decomposition of food waste, green-waste, paper and other organic wastes in a landfill by promoting optimum moisture content and sufficient nutrients for the microorganisms to degrade the waste. The techniques used to enhance biological degradation are shredding the waste, leachate recirculation and addition of nutrients and sludge. The other techniques include controlling features, such as temperature and moisture content are proved in accelerating MSW biodegradation (Warith 2002).

The bioreactor landfill allows active landfill management based on an understanding of the biological, chemical and physical processes involved. It utilizes a landfill as a bioreactor to treat waste rather than merely as burial ground. It offers the potential to avoid the long-term environmental risks associated with the dry cell approach (Yuen 2001).

Waste stabilization means the environmental performance parameters such as landfill gas composition, generation rate and leachate concentration remains at a steady level. Biological processes reduce the fraction of solid waste. Leachate recirculation can be used to enhance solid waste biodegradation. Landfill’s leachate may contain high concentration of organic and inorganic materials including toxic compounds and heavy metals (Carven et al. 1999). The recirculation of leachate accelerates the rate at which the waste is broken down and decreases the time required to stabilize the waste (Warith 2003).

2.8.4 Types of Bioreactor Landfills

The bioreactor landfills can be categorized broadly as aerobic or anaerobic. However, there are also ongoing studies of Hybrid or semiaerobic bioreactors, which combine elements of both aerobic and anaerobic systems.

2.8.4.1 Aerobic Bioreactor

The aerobic bioreactor accelerates waste degradation in presence of air, which contains oxygen. Air is injected into the waste mass using vertical or horizontal wells to promote aerobic bacteria to accelerate waste decomposition. Prior to air injection, liquid is pumped under pressure into the waste mass through injection wells in order to wet the waste mass to moisture content between 50% and 70% by weight. Once optimal moisture conditions have been reached, air injection commences (Campman and Yates 2002).

The degradation of waste occurs under conditions similar to compost operation (Repa 2003). The aerobic process continues until most of the easily and moderately degradable compounds have been degraded and the compost temperature gradually decreases during the final phase of "curing" or maturation of the remaining organic matter.

The byproducts of aerobic degradation are carbon dioxide and water. The aerobic process does not generate methane. The aerobic process is much accelerated and typically requires less than two years for full biodegradation (Warith 2003).

2.8.4.2 Anaerobic Bioreactor

The anaerobic bioreactor accelerates waste degradation in absence of oxygen. Methanogenetic bacteria are promoted to accelerate the waste degradation without air. The byproducts of anaerobic degradation are methane and carbon dioxide. The moisture content required to optimize anaerobic degradation is about field capacity or 35-40% moisture. The moisture content in typical landfills is around 10-20% water (Warith 2003). The average landfilled MSW has a moisture content of 25% wet weight (Emcon Associates 1980). Moisture is added in a form of leachate. Sewage sludge can also be added as a source of moisture. This leads to an increase in the amount of landfill gas

production that is collected by the gas collection system in the landfill. The maximum methane production in landfills occurred at moisture content of 60 to 80% (Farquhar and Rovers 1973). This suggests that most landfills are well below the optimum moisture content for methane production.

Anaerobic bioreactor landfills initially should be carefully monitored. If the waste is wetted too rapidly, a buildup of volatile organic acids might lower the leachate pH, inhibiting the methane-producing bacteria population and reducing the rate of biodegradation. Leachate parameters (such as pH, volatile organic acids, and alkalinity) and LFG parameters (such as methane content) are direct indicators of an established methane-producing bacteria population. Optimal conditions for methane-producing bacteria are a pH of greater than 6.5. A high volatile organic acids-to-alkalinity ratio (>0.25) indicates that the leachate might have a low buffering capacity and conditions could soon inhibit methane generation (Francis 2003).

The gas content of anaerobic bioreactors is similar to that of conventional landfills, with methane and carbon dioxide each making up approximately 50% of the total LFG volume. When the methane content of the LFG exceeds approximately 40%, the methane-producing bacteria population can be considered established. A decrease in the methane gas content below 40% is a possible indication that the waste is becoming too wet or dry. Once the methane-producing bacteria population has become established, the rate of leachate recirculation may be increased (Campman and Yates 2002).

2.8.4.3 Hybrid (Aerobic-Anaerobic) Bioreactor

In a hybrid bioreactor, the waste is first degraded under aerobic conditions followed by anaerobic conditions. The objective is to cause a rapid biodegradation of food and other easily degradable waste in the aerobic stage resulting in the earlier methanogenesis. In this system, the uppermost lift of waste is aerated while the lifts below receive liquids (Warith 2003). Horizontal wells are used to transport landfill gases, liquids and air.

2.9 Aerobic Decomposition of Waste in Bioreactor Landfill

2.9.1 Aerobic Decomposition

When organic materials decompose in the presence of oxygen, the process is called aerobic. The aerobic process is most common in nature. The concept of aerobic bioconversion by injecting air in a landfill was examined in a relatively large scale in California (Merz and Stone 1962). However, results were inconclusive due to the fact that the rate of airflow was too low and, therefore, both aerobic and anaerobic reactions were encountered (Themelis and Kim 2002). Haug (1993) discussed the injection of air in enclosed static piles in Maryland. The raw material was sludge from a wastewater treatment plant, admixed with wood chops. The average aeration rates were of the order of 2,000 ft³/h per ton of dry solids treated and the mean temperature was about 70°C over the 24-day campaign (Themelis and Kim 2002).

Aerobic biodegradation of MSW is carried out in a section of the Columbia County landfill near Augusta, Georgia (Hudgins and Harper 1998). In aerobic decomposition, living organisms, which use oxygen, feed upon the organic matter. They use the nitrogen, phosphorus, some of the carbon, and other required nutrients. Much of the carbon serves as a source of energy for the organisms and is burned up and respired as carbon dioxide (CO₂) and water, instead of methane, with stabilized humus remaining.

The aerobic degradation of MSW is mostly covered in the composting literature. Rich (1963) suggested the following stoichiometric equation for aerobic composting:

Where: $r = 0.5[b - nx - 3(d - nz)]$; $s = a - nw$

The terms $C_aH_bO_cN_d$ and $C_wH_xO_yN_z$ represent the compositions of feed substrate and final product, respectively.

Aerobic decomposition demonstrates that many of the organic compounds found in MSW can be stabilized in significantly short time frames as compared with anaerobic conditions

by the introduction of air and moisture in the proper proportions (Stessel and Murphy 1992; Hudgins and Harper 1999; Read et al., 2001).

2.9.2 Benefits of Air Injection

2.9.2.1 Accelerating MSW Decomposition

Aerobic decomposition occurs much faster rate and to a greater extent than anaerobic conditions in a given time period (Stessel and Murphy 1992). The aerobic biodegradation of MSW carried out in a section of the Columbia County landfill near Augusta, Georgia (Hudgins and Harper 1998) has shown a 50% increase in biodegradation rate in comparison to the anaerobic section of the landfill (Themelis and Kim 2002).

2.9.2.2 Leachate Volume Reduction

After injecting air through the waste mass, it is heated by the aerobically degraded materials, and picks up moisture as well as dries the remaining materials. So the volume of leachate is reduced. The aerobic decomposition in a section of the Columbia County landfill near Augusta also showed 86% reduction in leachate flow (Themelis and Kim 2002).

2.9.2.3 Waste Settlement

The volatile carbon that is amenable to degradation decomposes very fast and weakens the waste mass structurally that promote the settlement. The aerobic degradation showed enhanced degradation compared to the traditional anaerobic decomposition (Stessel and Murphy 1992).

2.9.2.4 Removing Anaerobic Odors

There is no accompanying bad smell when there is adequate oxygen present. Stessel and Murphy (1992) reported that aerobic lysimeters smelled clean and earthy, while anaerobic were somewhat offensive. Because wide range of compounds is included in the anaerobic odors such as hydrogen sulfide, carbon monoxide, methane, hydrogen, nitrogen, volatile fatty acids and ammonia, etc.

2.10 Anaerobic Decomposition of Waste in Bioreactor Landfill

2.10.1 Anaerobic Decomposition

Anaerobic decomposition is the breakdown of organic contaminants by microorganisms when oxygen is not present. Some anaerobic bacteria use nitrate, sulfate, iron, manganese, and carbon dioxide as their electron acceptors, and break down organic chemicals into smaller compounds, often producing carbon dioxide and methane as the final products. Ammonia, hydrogen sulfide and mercaptans (sulfonated hydrocarbons) are also generated (Themelis and Kim 2002).

This general mechanism of anaerobic biodegradation is an example of anaerobic respiration. Alternatively, some anaerobic microorganisms can break down organic contaminants by fermentation. Fermentation is where the organic chemical acts as an electron acceptor.

The other gases include the ammonia, carbon monoxide, hydrogen sulfide, nitrogen, hydrogen and oxygen.

2.10.2 Factors for Anaerobic Decomposition

2.10.2.1 Moisture Content

Moisture is the primary limiting factor in waste degradation (McBean et al. 1995; Reinhart et al. 1996). Therefore, adequate moisture is an essential requirement in a functional bioreactor. The maximum moisture content of the waste, prior to the production of leachate is referred to as field capacity. The addition of moisture to attain and exceed field capacity conditions in the waste results in enhanced biodegradation rates. The specific moisture content of the waste at field capacity varies with the waste compaction, density and porosity of the waste. Various studies have determined field capacity moisture content ranges between 29 to 44% of the waste volume (Yuen et al. 2001).

In the absence of adequate moisture content throughout the reactive landfill, the rate of decomposition of waste is very slow or can cease altogether. Old landfills have been opened after several decades and little decomposition of the entombed material has been observed at locations where there was no ingress of moisture. On the other hand, if a large amount of precipitation water is allowed to permeate through the landfill, the leachate solutions formed within it must be collected and treated continuously. So they do not contaminate the groundwater and adjacent bodies of water (Themelis and Kim 2002).

2.10.2.2 Landfill pH

The pH of the refuse and leachate significantly influences the chemical and biological processes. The optimum pH for anaerobic reaction is 6.7 to 7.5 (Warith 2003). Within the optimum range, methanogens grow at high rate leading to maximum methane production. The rate of methane production is seriously limited when the pH level is lower than 6 or higher than 8 (Barlaz et al. 1987). During the initial stages of anaerobic decomposition, organic acids forms and result in an acidic pH and eventually stops methane production (Warith and Sharma 1998). Therefore, addition of buffering materials during bioreactor landfill operation is a critical strategy to maintain appropriate pH as well as balance relations between the various bacterial groups.

2.10.2.3 Landfill Temperature

The microbiological degradation rate increases along with temperature increase. Rees (1980) observed that the optimum temperature for methane production from domestic refuse in a conventional anaerobic digester is about 40°C. Hartz et al. (1982) found that 41°C was the optimum for the generation of methane on a short term basis and generation would cease from 48 to 55°C. Mata-Alvares and Martina-Verdure (1986) reported the optimum temperature is 34°C to 38°C. The rate of methane generation can be increased up to 100 times when the temperature raises from 20 to 40°C (Christensen and Kjeldsen 1989).

2.11 Landfill Leachate

Leachate is a liquid that has percolated through solid waste and has extracted, dissolved and suspended materials that may include potentially harmful materials (Warith 2003). The quality of leachate depends on the type of solid waste, physical, chemical and biological activities that occur in the solid waste. The quantity of leachate seeping from the landfill is proportional to the build up of leachate within the landfill. An effective method for the treatment of the leachate is to collect and recirculate leachate through the landfill.

2.11.1 Leachate Recirculation

Leachate recirculation is one of the emerging techniques that can be used to manage leachate from landfill. One of the main purposes of leachate recirculation is to optimize the water content in order to accelerate waste degradation (Barina 2005). It increases the moisture content and movement inside the waste matrices. Not only moisture content but moisture movement also affects the waste stabilization.

During the early stages of landfill operations, the leachate contains significant amounts of BOD, COD, TDS and heavy metals. When leachate is recirculated, biological, physical and chemical reactions will occur and provide treatment of BOD (Warith 2003). Leachate recirculation can provide a means for the distribution of nutrients and enzymes; a medium for pH buffering; dilution of inhibitory compounds; distribution of methanogens and liquid storage (Conestoga-Rovers and Associates 2004). Beneficial effects of the leachate recirculation include the accelerated waste stabilization process, enhanced gas production, greater volume reduction and leachate strength.

2.12 Generation of Landfill Gases (LFG)

The generation of the principal landfill gases can be divided into five phases.

2.12.1 Initial Adjustment Phase

The organic biodegradable components in MSW undergo microbiological decomposition with presence of oxygen soon after the waste is placed in the landfill. The oxygen is consumed by the aerobic microorganisms. Digested wastewater treatment plant sludge

disposed of in many MSW landfills and recycled leachate is the sources of organisms (Tchobanoglous et al. 1993).

2.12.2 Transition Phase

In this phase, oxygen is depleted and anaerobic conditions begin to develop. As landfill becomes anaerobic, nitrate and sulfate, which can serve as electron acceptors, are reduced to nitrogen gas and hydrogen sulfide. The onset of anaerobic conditions can be monitored by measuring the oxidation/reduction potential of the waste. The production of methane occurs when the oxidation/reduction potential values are in the range from 150 to 300 millivolts (Rovers et al. 1995). In this phase, the pH of the leachate starts to drop due to presence of organic acids and effect of the elevated concentration of CO₂.

2.12.3 Acid Formation Phase

In this phase, the microbial activities initiated in second phase accelerate with the production of significant amount of organic acid and lesser amount of hydrogen gas. There are two major steps that occur in this phase. The first step involves the enzyme-mediated transformation of the higher molecular mass compounds (e.g., lipids, polysaccharides, proteins, and nucleic acids) into compounds that can be used by microorganisms as a source of energy and cell carbon. Acidogenesis is the second step involved in the microbial conversion of the compounds into lower molecular mass compounds such as acetic acid (CH₃COOH). Carbon dioxide (CO₂) and smaller amount of hydrogen gas (H₂) are generated in this phase. The microorganisms involved in this conversion are known as nonmethanogenic or acidogens (Rovers et al., 1995). The pH of leachate drops to 5 or lower because of presence of organic acids and concentrations of CO₂ within the landfill. The biochemical oxygen demand (BOD₅), chemical oxygen demand (COD), and the conductivity of the leachate increase due to dissolution of the organic acids (Tchobanoglous et al. 1993). Because of low pH, inorganic constituents, particularly heavy metal will be solubilized and many nutrients will be lost in this phase.

2.12.4 Methane Fermentation Phase

In this phase, a group of microorganisms convert the acetic acid and hydrogen gas to CH_4 and CO_2 . The microorganisms responsible for this conversion are called methanogenic. The rate of methane production increases rapidly to some maximum value. Methane gas constitutes approximately 50-60% (by volume) of gas composition (Barlaz et al. 1990; Warith and Sharma 1998). The pH value will rise to more neutral values in the range of 6.8 to 8.0 and consequently heavy metals are removed by precipitation. The BOD_5 and COD will be reduced.

2.12.5 Maturation Phase

This phase starts after biodegradable organic material has been converted to CH_4 and CO_2 . The rate of landfill gas is reduced in this phase because most of the available nutrients have been removed with the leachate during the previous phases and the remaining landfill substrates are slowly biodegradable. The principal landfill gases evolved in this phase are CH_4 and CO_2 . The small amount of nitrogen and oxygen may also be found in the landfill gas. The leachate will contain humic and fulvic acids, which are difficult to process further biologically.

3.0 Materials and Methods

3.1 Bioreactor Landfill System

Three bioreactor landfills were operated in the laboratory for the experimental works. Each bioreactor had a volume capacity of 220L and contained approximately 150L synthetic MSW. The paper and paper products, which are constituent of MSW, were used in this experiment. The paper categories include newspaper, school and office paper, packaging paper, magazines, cardboards, flyers and posters, etc. The paper waste compositions were shredded and then mixed up thoroughly to get uniformity before loading into the bioreactors. The bioreactors were loaded up with the waste and compacted to the densities reflecting typical landfill conditions. Tap water was added into the bioreactor landfills, and the produced leachate was pumped back until the waste matrices reached field capacity with respect to their moisture contents.

All three bioreactor landfills were operated for 180 days in the laboratory. The bioreactors were operated in aerobic and anaerobic stages sequentially. Each bioreactor was being supplied 0.20 L S^{-1} of air during the 53-day aerobic stage.

Leachate recirculation and buffering technologies were applied to all bioreactors. Sodium hydroxide (NaOH) solution was used as buffering solution in case of acidic. The amount was decided according to the pH of the generated leachate from respective bioreactor in order to keep the optimum pH range of 6.4 to 7.2.

The all three closed-loop leachate recycling bioreactors were named as Cell 1 (Control one), Cell 2 (Enzyme concentration 1) and Cell 3 (Enzyme concentration 2) as shown in Table 3-1.

Table 3-1 Laboratory Bioreactor Landfills

ID #	Matrix	SBP Enzyme Concentration (Units)	Operation
Cell 1	Paper waste without Enzyme	No Enzyme (Control One)	Leachate Recirculation and buffering; aerobic and anaerobic stages
Cell 2	Paper waste with Enzyme Concentration 1	5 million	Leachate Recirculation and buffering; aerobic and anaerobic stages
Cell 3	Paper waste with Enzyme Concentration 2	10 million	Leachate Recirculation and buffering; aerobic and anaerobic stages

Physical, chemical and statistical analyses of the waste matrices were performed. The physical analyses included the moisture content, volume, density, settlement, and internal temperature of waste matrices. The leachate sample was collected to measure the parameters such as BOD₅, COD, NH₃-N, TS, TSS and pH. All data obtained were analyzed and plotted the trend lines with Microsoft Excel. The performance benefits of air injection were ascertained and characterized through these analyses.

3.2 Components and Configuration of Bioreactor Landfill

The components and configuration of the bioreactor systems are shown in Figure 3-1. The detailed description of design and construction of the systems are as follows.

Figure 3-1 Components and Configuration of Bioreactor Landfill

3.2.1 Bioreactors

Three semi-transparent polyethylene cylindrical containers were used as the bioreactors. Each container had diameter of 0.55 m, height of 0.92m, and total capacity of 220 L. A lid with the suitable size was used to cover each bioreactor. Each bioreactor was installed in the laboratory. After that, the paper waste with tap water was loaded in each bioreactor and the commissioning was finished. The seam between the body of the bioreactor and the lid was sealed by silicone sealant in order to keep air tight and water tight. There is a $\frac{3}{4}$ " port on the bottom and lid of each bioreactor respectively. Two fittings were installed in the ports. One was installed at the bottom for discharging leachate. The lid one was the pathway for the exhaust gas. A stainless steel sink screen is placed onto each bottom fitting in order to prevent clogging or waste loss. About 5cm thick gravel lining layer was maintained at the bottom of each bioreactor. The paper waste was directly loaded onto the gravel liner.

3.2.2 Leachate Collection Tanks

Each bioreactor was connected to one leachate collection tank with a $\frac{3}{4}$ " x 1 $\frac{1}{4}$ " PVC adaptor and suitable vinyl tubing. Each tank had a capacity of 20 L. The purpose of tank is to collect the leachate discharged from the bioreactor to prevent flooding inside the bioreactor. The generated acidic leachate during the anaerobic could be discharged very quickly, and never accumulated inside the paper waste. There was a $\frac{1}{2}$ " PVC adjustable ball valve on the sidewall of each tank for sampling and discharging leachate to the leachate recirculation reservoir.

3.2.3 Leachate Recirculation Reservoirs and Pumps

Three transparent polyethylene tanks were used as leachate recirculation reservoirs. The capacity of each reservoir was 25 L. The reservoir served as the leachate transfer station, where pH and volume of the recirculated leachate could be measured. The acidic and alkaline leachate was also buffered in the reservoir to keep the enzyme active. The submersible pumps (Laguna Statuary Pump 3#) were used for pumping the leachate and makeup water back into the bioreactors.

3.2.4 Leachate Distribution Apparatus

The leachate distribution apparatus was installed into each bioreactor to distribute the recirculated leachate evenly on the top of the waste matrix, just like raining. The apparatus included a circle with a cross inside, and it is made by connecting a ¾" PVC cross, three ¾" PVC tees, and ¾" vinyl tubing. Sixteen holes with 3mm diameter were punched on the vinyl tubing. The apparatus was connected to the leachate recirculation pipe outside the bioreactor. In order to pass the bioreactor wall a ¾" PVC adaptor was used for the pipe. An adjustable PVC ball valve was installed on the recirculation pipe. Every time, the valve was closed after leachate recirculation to air tight the bioreactor.

3.2.5 Air Distribution Pipes

Three air distribution PVC pipes were installed in each bioreactor to distribute the injected air evenly into the waste matrices. One third of each pipe near the bottom of the bioreactor was perforated. The air pipes were covered by plastic insect screen to prevent the waste getting inside the pipes and clog the pipes. All three pipes were connected together by PVC elbows and tubing, and become one entity, which also served as a support for the water distribution apparatus. The injected air reached the entity through one air pipe that passed the bioreactor wall by one PVC adaptor. An adjustable PVC ball valve was installed in the air pipe to adjust the airflow rate and shut down the air during the anaerobic stage in the bioreactor.

3.2.6 Air Compressor

Air compressor (HUSKY, 2.75HP, 8 Gallons, 4.0 SCFM @ 40psi) was used to supply air into all three bioreactors. There was a standby air compressor (DeVILBISS ® Tradesman Model DAC-7118, DeVILBISS, Canada) in the laboratory.

3.2.7 Temperature Sensor and Thermometer

Three temperature sensors were installed at different depths (top, middle and bottom) in each bioreactor. The wires were connected to the sensor with the thermometer (EXTECH

421307 thermometer) outside the bioreactor, which could record the internal temperature of the waste matrix at different depths.

3.2.8 Gas Discharge System

The exhaust gas pipe on the lid of each bioreactor was connected together by tubing and tees. The gases such as carbon dioxide, methane, hydrogen sulfide, hydrogen, nitrogen, and oxygen in bioreactors were discharged to outdoors through $\frac{3}{4}$ " vinyl tubing.

3.3 Leachate Recirculation System

Leachate recirculation system consisted of leachate collection tank, valves, leachate recirculation reservoir, submersible pump, tubing, and leachate distribution apparatus. It was operated in a closed-loop mode. The pathway of the leachate is presented in Figure 3-2. The movement of leachate direction is shown by arrows in the figure.

3.4 Air Injection and Exhaust System

The air injection and exhaust system include air compressor, air supply pipe, gas flow meter, valve, air distribution pipes, gas exhaust port, and gas exhaust pipe. The movement direction of the injected air is presented by arrows in Figure 3-3. The air injection through air compressor into the all three bioreactors and the gas exhaust pipes are shown in Figure 3-4.

3.5 Maintenance of Bioreactor System

The joints and seams were checked often during the study to make sure they were airtight and watertight. The spare parts such as elbows and tubing; leachate recirculation tanks, reservoirs and pumps; and stand-by air compressor were in stock.

1	Gas Exhaust Port	11	Leachate Recirculation Reservoir
2	Lid of Bioreactor	12	Leachate Sampling Port
3	Leachate distribution Apparatus	13	Graduation
4	Body of Bioreactor	14	Leachate Recirculation Pump
8	Screen	15	Leachate Recirculation Pipe
9	Gravel	19	Adjustable Valve
10	Leachate Collection Tank		

Figure 3-2 Leachate Recirculation Mode

1	Gas Exhaust Port	10	Leachate Collection Tank
2	Lid of Bioreactor	16	Air Compressor
4	Body of Bioreactor	17	Air Supply Pipe
7	Waste Matrix	18	Gas Flow Meter
8	Air Distribution Pipes	19	Adjustable Valve
9	Screen		
	Gravel		

Figure 3-3 Air Injection and Exhaust Mode

Figure 3-4 Air injection through air compressor into all three bioreactors

3.6 Preparation of Waste

3.6.1 Waste Composition

The paper waste composed of all kinds of paper such as newspaper; school and office paper; all forms of packaging paper; computer and printing paper; magazines; and cardboards, etc as shown in Figure 3-5.

Figure 3-5 Waste Composition

3.6.2 Collection and Shredding of Paper Waste

All kinds of paper waste were collected from the offices at Ryerson University. These papers were shredded. Shredding may help to increase the homogeneity of the waste by size reduction and mixing and improves the water content distribution in the waste. It also increases the specific surface area of the waste for biodegradation and removes the moisture barriers caused by impermeable materials (Yuen 2001). However, some investigations have suggested that shredding of waste may induce a negative effect on degradation (Christensen et al. 1992) by promoting excessive initial hydrolysis and acid

formation that inhibit the onset of a methanogenic environment. Shredding may prove to be beneficial only if the over-estimated acid production is to be controlled by pH buffering.

3.6.3 Loading and Waste Compaction into Bioreactors

After shredding all the paper waste, they were thoroughly mixed up and about 25 kg of waste was loaded into each bioreactor and it was compacted in different layers. Some studies showed that compaction could affect anaerobic decomposition (Dewalle et al. 1978). If a waste is relatively dry, increasing the compaction or dry density may significantly speed up the degradation processes.

The tap water was added into each bioreactor during compaction of the waste. The higher moisture content (by volume) available in more compacted solids helps to enhance the distribution of nutrients and contact between substrates and bacteria. It also affects the waste degradation.

The leachate was recirculated daily in the first three days and the volume of the leachate was measured until the waste in each bioreactor was brought to field capacity. The field capacity was used to characterize the moisture content of the waste matrix. The field capacity is the moisture content at which the maximum amount of water is held (through capillary forces) against gravity. The addition of more moisture will result in continuous leachate drainage (Reinhart and Townsend 1998). If the amount of water in the leachate collection tank was approximately equal to the amount of leachate recirculated the previous day, the paper waste inside the bioreactors was considered to reach the field capacity. The field capacity is a function of the waste composition, density and porosity, particle sizes, waste overburden, waste age (Yuen et al. 2001; Reinhart and Townsend 1998).

3.7 Source and Dose of Enzyme

The SBP enzyme was received from the Bio-Research Products Inc., North Liberty, Iowa, U.S.A. About 15 million units of SBP were used in this research work. The concentration of enzyme was 6025 U/ml.

The activity of SBP is measured in units. Each unit of enzyme activity has the ability to degrade 1 micromole of phenol per minute for a specific set of conditions at pH 7.4 and temperature 25°C (Driscoll et al. 2000). The enzyme dose added to the bioreactor determines the present removal from solution, although at some point there is little increase in removal with an increase in enzyme dose. On the other hand, the number of turnovers or reactions catalyzed per enzyme increases and inactivation decreases when the concentration is kept low, probably as a result of the low concentration of free radicals plant. In this study, about 5 million units of enzyme was added to cell 2 and 10 million units was added in cell 3.

3.8 Rate of Airflow

All three bioreactors, two having different concentration of enzymes and control one, were operated in two stages. The first 53 days was the aerobic stage. In aerobic stage, air was injected into all bioreactors to accelerate the decomposition of the waste. Haug (1993) has provided the general chemical composition – $C_{266}H_{434}O_{210}N$ for the mixed paper. Following general chemical composition, it is assumed that organics are totally degraded. Equation [4] can be expressed as:

The stoichiometric oxygen demand can be determined as 1.23 g O_2 g⁻¹ of waste (dry). Anderson (1990) presented that a suitable aeration rate is considered by many operators to be between 0.6 to 1.8 m³ air d⁻¹ kg⁻¹ volatile solids during the thermophilic stage with progressive decrease during the cooling down and maturation stages for composting. About 25 kg of dry weight of paper waste was used in the experimental work. Each bioreactor was being supplied 0.2 L S⁻¹ of air. Since all three bioreactors were operated at the same time, 0.6 L S⁻¹ of air was supplied by the air compressor.

3.9 Leachate Recirculation Rate

Leachate recirculation was carried out in both aerobic and anaerobic stage. The optimum leachate recirculation rate is different for aerobic and anaerobic degradation. Stessel and Murphy (1992) concluded that waste moisture levels had to be maintained at 75% for the optimum aerobic degradation. In order to keep the field capacity in the bioreactors, leachate was recirculated daily at the beginning of the study. Later the frequency was reduced to three times a week.

There are many studies conducted on the effects of different leachate recirculation rates on anaerobic degradation. Chugh et al. (1998) utilized three daily recirculation rates – 2%, 10%, and 30% of initial volume of waste matrices. They concluded that the rate and extent of waste decomposition improved with the increase in moisture flow. Warith (2002) used 15% of the total volume of solid waste in each cell and recirculated three times a week. Following the previous studies, daily recirculation rate was determined as 15 % of the total waste matrix volume in each bioreactor for the anaerobic stage.

3.10 Buffering the Leachate

Every time, buffering solution was added into the leachate to maintain the pH in the range of 6.4 to 7.2 to keep the enzyme active. The amount of buffering solution was determined according to the pH value of the leachate. If the leachate was acidic, sodium hydroxide solution (NaOH) was used as buffer solution and hydrochloric acid (HCL) was used in case of alkaline.

3.11 Analytical Methods

3.11.1 Physical Analysis of Paper Waste

In order to characterize the nature of the waste samples and their decomposition as a whole, physical analysis of paper waste was performed. In this study, moisture content, volume, density, settlement, and internal temperature were measured.

3.11.1.1 Moisture Content and Field Capacity

The initial field capacity was derived from the initial moisture content of waste and the amount of tap water added into the waste matrices for saturating the waste. The moisture content was measured again at the end of the study to find out the final field capacity for comparison.

3.11.1.2 Settlement of Waste

After loading the waste into landfill, thickness of the waste decreases with time. The analysis of the waste settlement is very important, because it helps in making projections of the remaining life and the design of the landfills' components. It also gives the picture of the post closure development of the landfills. The rate of landfill settlement depends primarily on the waste composition, operational practices and factors affecting biodegradation of the landfill waste, particularly moisture content (El-Fadel 1998). In this study, paper waste was used in bioreactor landfills. Since the bioreactors were semi-transparent polyethylene, the thickness of waste layer was easily measured from outside of the bioreactors. The settlement rates were presented in percentage. The volume and density of the waste were also calculated from the measurement of the waste matrix thickness and mass of the waste.

3.11.1.3 Internal Temperature

Temperature sensors and an EXTECH 421307 thermometer were used for monitoring the internal temperature of the waste to ensure that the heat inside the bioreactor had not reached to a dangerous extent. The mercury thermometers were used to record the ambient air temperature.

3.11.2 Chemical Analysis of Leachate

Every time about 125 to 150 ml of leachate sample was collected throughout the study. In first 84 days, samples were taken three times a week. Later frequency was reduced to two times a week until 137 days, after that once a week. The leachate parameters BOD₅, COD, TS, TSS, NH₃-N and pH were measured.

3.11.2.1 pH of Leachate

In this study, the pH meter was used to measure the pH of leachate. If pH was not in a range of 6.4 to 7.2, the leachate was buffered to keep the enzyme active. Commercial standard solutions including pH 4.0, 7.0 and 11.0 were used for calibration the pH meter.

3.11.2.2 Biochemical Oxygen Demand (BOD₅)

In this study, the standard method 5210B (APHA et al. 1998) was followed for the measurement of BOD₅. The samples were incubated in a Fisher Scientific Isotemp Incubator (Model 637D, Fisher Scientific Ltd.), and dissolved oxygen was measured by Orion 3 Star Benchtop DO Meter (Thermo Electron Corporation). After measuring the COD value of the sample, the BOD₅ value can be predicted, and then three dilution factors were selected for each sample according to Tchobanoglous (2003). Polyseed was used as seed source; 5 ml prepared polyseed solution was added to each 300 ml BOD bottle.

3.11.2.3 Chemical Oxygen Demand (COD)

The standard method 5220D (APHA et al. 1998) was followed to analyze the chemical oxygen demand (COD) of leachate. The digester (Bioscience) was used to digest the samples, blank and one or more standards with reagents. A spectrophotometer Lambda 20 (Perkin Elmer) operating at wavelength 600nm was used for the measurement. The sample was analyzed in duplicate.

3.11.2.4 Total Solids (TS) and Total Suspended Solids (TSS)

The measurement of Total Solids (TS) followed standard method 2540B (APHA et al. 1998). In this study, 20ml sample was selected for the analysis. The measurement of Total Suspended Solids (TSS) followed standard method 2540D (APHA et al. 1998). 1.5 μ m glass fiber filter was used for the TSS test. 20ml sample size was selected in the beginning of study. The sample size was increased to 30 and 40 ml as the strength of leachate decreased with time. The apparatuses used for these two parameters included porcelain dishes, aluminum weighing dishes, Fisher Scientific Isotemp Oven (Model 630G, Fisher Scientific Ltd.), OHAUS ® Precision Standard balance (GENEQ Inc.), desiccators, filtration apparatus and vacuum pump.

3.11.2.5 Ammonia Nitrogen (NH₃-N)

In this study, the standard method 4500-NH₃ F (APHA et al. 1998) was followed for NH₃-N measurement. A spectrophotometer Lambda 20 (Perkin Elmer) operating at wavelength of 640nm was used for the measurement. Each sample was analyzed in duplicate.

3.11.3 Statistical Analysis

The data obtained such as settlement, pH, BOD₅, COD, TS, TSS and NH₃-N were calculated, analyzed and plotted against time with Microsoft® Excel™ 2003.

4.0 Results and Discussions

All three bioreactors were operated for 180 days. The first 53 days was the aerobic stage and later the bioreactors were operated in anaerobic stage. Physical analyses of waste matrices and chemical analyses of leachate in the bioreactors are discussed in this section. The experimental data were analyzed and compared in aerobic and anaerobic stages. The leachate parameters such as BOD₅, COD, NH₃-N, TS, TSS, and pH in bioreactors with SBP enzyme and without enzymes are compared in this section.

4.1 Variations in Chemical Oxygen Demand (COD)

The chemical oxygen demand (COD) variations in the bioreactors are presented in the Figure 4-1 and the data are presented in Appendix A (Table A-1). The COD concentrations and the reduction rates are summarized in Table 4-1. The initial COD concentrations in all three bioreactors were approximately same. The COD in Cell 1, Cell 2 and Cell 3 were 3,667 mg/l, 3,792 mg/l and 3,750 mg/l respectively. The peak COD in Cell 1 (6,583 mg/l) was about 12% higher than Cell 2 (5,792 mg/l) and 17.1% higher than Cell 3 (5,458 mg/l).

Figure 4-1 COD variations in the Bioreactors

Table 4-1 COD Concentrations and Reduction Rate in Bioreactors

COD	Units	Cell 1	Cell 2	Cell 3
Initial COD	mg/l	3,667	3,792	3,750
Maximum COD	mg/l	6,583	5,792	5,458
COD at end of Aerobic stage	mg/l	983	767	567
COD Reduction rate in Aerobic stage	%	85.1	86.8	89.6
Final COD at end of study	mg/l	863	487	323
COD reduction rate at end of study	%	86.9	91.6	94.1

Note: The aerobic stage ends on day 53. The COD reduction rate in aerobic stage was calculated based on the first peak concentration and concentration at the end of aerobic stage. The COD reduction rate at the end of study was calculated based on the first peak concentration and the final concentration in the study.

In first 53 days, all bioreactors were operated in aerobic stage. The COD concentration decreased sharply in the aerobic stage. At the end of aerobic stage, the COD in Cell 1 (983 mg/l) was 22% higher than Cell 2 (767mg/l) and 42.3% higher than Cell 3 (567 mg/l).

After 53 days, all bioreactors were operated in anaerobic stage. The final COD at the end of the study in Cell 1 (863 mg/l) was 43.5% higher than the Cell 2 (487 mg/l) and 62.6% higher than the Cell 3 (323 mg/l). These observations showed that the leachate strength decreased with time and the leachate quality in Cell 2 and Cell 3 was better than the Cell 1. The operation protocols in all three bioreactors were same except different concentrations of enzymes in Cell 2 and Cell 3. The overall reduction rates in Cell 1, Cell 2 and Cell 3 were 86.9%, 91.6% and 94.1% respectively. Thus it is concluded that the addition of enzymes in Cell 2 and Cell 3 improved the quality of leachate comparing with Cell 1 (Control one).

4.2 Variations in Biochemical Oxygen Demand (BOD₅)

The Biochemical oxygen demand (BOD₅) variations in the bioreactors are presented in the Figure 4-2 and the data are presented in Appendix A (Table A-2). The BOD₅ concentrations and the reduction rates are summarized in Table 4-2.

Figure 4-2 BOD₅ variations in the Bioreactors

Table 4-2 BOD₅ Concentrations and Reduction Rate in Bioreactors

BOD ₅	Units	Cell 1	Cell 2	Cell 3
Initial BOD ₅	mg/l	1,926	1,824	1,893
Maximum BOD ₅	mg/l	3,372	2,916	2,568
BOD ₅ at end of Aerobic stage	mg/l	507	348	243
BOD ₅ Reduction rate in Aerobic stage	%	85.0	88.1	90.5
Final BOD ₅ at end of study	mg/l	431	209	126
BOD ₅ reduction rate at end of study	%	87.2	92.8	95.1

Note: The aerobic stage ends on day 53. The BOD₅ reduction rate in aerobic stage was calculated based on the first peak concentration and concentration at the end of aerobic stage. The BOD₅ reduction rate at the end of study was calculated based on the first peak concentration and the final concentration in the study.

The initial BOD₅ concentrations in bioreactors Cell 1, Cell 2 and Cell 3 were 1,926 mg/l, 1,824 mg/l and 1,893 mg/l respectively. The peak BOD₅ in Cell 1 (3,372 mg/l) was about 13.5% higher than Cell 2 (2,916 mg/l) and 23.8% higher than Cell 3 (2,568 mg/l).

In aerobic stage, the BOD₅ concentration decreased sharply same as COD. At the end of aerobic stage, the BOD₅ in Cell 1 (507 mg/l) was 31.4% higher than Cell 2 (348 mg/l) and 50.1% higher than Cell 3 (243 mg/l).

The final BOD₅ at the end of the study in Cell 1 (431 mg/l) was 51.5% higher than the Cell 2 (209 mg/l) and 70.7% higher than the Cell 3 (126 mg/l). The overall reduction rates in Cell 1, Cell 2 and Cell 3 were 87.2%, 92.8% and 95% respectively. The BOD₅ data showed that the addition of enzymes in Cell 2 and Cell 3 improved the quality of leachate comparing with Cell 1.

4.3 Variations in BOD₅/COD Ratio

The BOD₅/COD ratio in bioreactors is presented in Table 4-3 and the data are presented in Appendix A (Table A-3). It showed the decreasing trend in all bioreactors. Initially, the BOD₅/COD ratio in Cell 1 was 0.53 and at the end of aerobic stage, it was decreased to 0.52. Similarly, at the end of aerobic stage, the ratio in Cell 2 and Cell 3 was decreased from 0.48 to 0.45 and 0.50 to 0.43 respectively. The final BOD₅/COD ratio at the end of study in Cell 1, Cell 2 and Cell 3 was 0.50, 0.43 and 0.39 respectively. The decrease in BOD₅/COD ratio showed the decrease in percentage of degradable compounds in the leachate. The ratio of 0.4-0.8 implies a highly degradable leachate (Warith 2002). Initially, the leachate from all three bioreactors belonged to this kind of leachate.

Table 4-3 BOD₅/COD in Bioreactors

BOD ₅ /COD	Cell 1	Cell 2	Cell 3
Initial BOD ₅ /COD ratio	0.53	0.48	0.50
BOD ₅ /COD ratio at the end of aerobic stage	0.52	0.45	0.43
Final BOD ₅ /COD ratio at the end of study	0.50	0.43	0.39

4.4 Variations in Ammonia Nitrogen (NH₃-N)

The ammonia nitrogen (NH₃-N) variations in the bioreactors are presented in the Figure 4-3 and the data are presented in Appendix A (Table A-4). In this study, the mixed paper was used as a substrate. The concentration of nitrogen in paper waste is very less. The initial NH₃-N concentrations in bioreactors Cell 1, Cell 2 and Cell 3 were 0.08 mg/l, 0.04 mg/l and 0.04 mg/l respectively.

Figure 4-3 NH₃-N variations in the Bioreactors

In aerobic stage, the NH₃-N concentration was increased, because CO₂ and H₂O together with ammonia gas were released after the decomposition of waste. At the end of aerobic stage, the NH₃-N in Cell 1 (0.27 mg/l) was 25.9% higher than Cell 2 (0.20 mg/l) and 44.4% higher than Cell 3 (0.15 mg/l).

At the end of study, the concentration of NH₃-N in Cell 1 was increased to 0.31 mg/l i.e. 32.3% higher than Cell 2 (0.21 mg/l) and 48.4% higher than Cell 3 (0.16 mg/l).

4.5 Variations in Total Solids (TS)

The total solids (TS) variations in the bioreactors are presented in the Figure 4-4 and the data are presented in Appendix A (Table A-5). The TS concentrations and the reduction rates are summarized in Table 4-4. The initial TS concentrations in bioreactors Cell 1, Cell 2 and Cell 3 were 4,200 mg/l, 3,900 mg/l and 3,800 mg/l respectively. The peak TS in Cell 1 (6,225 mg/l) was about 10.8% higher than Cell 2 (5,550 mg/l) and 19.7% higher than Cell 3 (5,000 mg/l).

Figure 4-4 TS variations in the Bioreactors

Table 4-4 TS Concentrations and Reduction Rate in Bioreactors

Total Solids	Units	Cell 1	Cell 2	Cell 3
Initial TS	mg/l	4,200	3,900	3,800
Maximum TS	mg/l	6,225	5,550	5,000
TS at end of Aerobic stage	mg/l	2,200	1,600	1,400
TS Reduction rate in Aerobic stage	%	64.7	71.2	72.0
Final TS at end of study	mg/l	2,450	1,900	1,600
TS reduction rate at end of study	%	60.6	65.8	68.0

Note: The TS reduction rate in aerobic stage was calculated based on the first peak concentration and concentration at the end of aerobic stage. The TS reduction rate at the end of study was calculated based on the first peak concentration and the final concentration in the study.

At the end of aerobic stage, the total solids in Cell 1 (2,200 mg/l) were 27.3% higher than Cell 2 (1,600 mg/l) and 36.4% higher than Cell 3 (1,400 mg/l). The final total solids at the end of the study in Cell 1 (2,450 mg/l) were 22.4% higher than the Cell 2 (1,900 mg/l) and 34.7% higher than the Cell 3 (1,600 mg/l). The overall reduction rates in Cell 1, Cell 2 and Cell 3 were 60.6%, 65.8% and 68% respectively.

4.6 Variations in pH

The pH variations of leachate in the bioreactors are presented in Figure 4-5 and the data are presented in Appendix A (Table A-6). The pH was low in the beginning of the study. The pH values in the Cell 1 increased from 5.6 to 6.7. Similarly, the pH values in Cell 2 and Cell 3 increased from 6.0 to 6.9 and 5.5 to 6.9 respectively. Since the pH was low, the leachate in Cell 1 was buffered in first 25 days in order to keep the enzyme active. The sodium hydroxide (NaOH) was used in buffering the leachate. The leachate in Cell 2 and Cell 3 was buffered first 23 days and 18 days respectively. At the end of aerobic stage, the pH in Cell 1 started to drop from 6.7 to 6.5 and later increased to 6.7. The pH values after aerobic stage in case of Cell 2 and Cell 3 started to drop to 6.6 and 6.65 respectively. The pH of leachate at the end of study in Cell 2 and Cell 3 was 6.9.

Figure 4-5 pH variations of leachate in the Bioreactors

4.7 Variations in Waste Settlement Rate

The settlement rates of waste mass in the bioreactors are presented in Figure 4-6 and the data are presented in Appendix A (Table A-7). The settlement rate is the thickness reduction of the waste mass divided by the initial thickness of the waste mass inside the bioreactors. The settlement rate in the bioreactors increased with time. The settlement in the bioreactor was faster in aerobic stage than the anaerobic stage. The settlement rates in Cell 1, Cell 2 and Cell 3 at the end of aerobic stage were 6%, 9% and 11% respectively. At the end of study, the settlement rates in Cell 1, Cell 2 and Cell 3 were 12%, 15% and 17% respectively.

Figure 4-6 Settlement rate of waste mass in the Bioreactors

4.8 Changes in Mass, Density, Field Capacity and Settlement Rates in the Bioreactors

The organic materials can be converted to water, carbon dioxide and ammonia in the aerobic environment. In anaerobic condition, the organic wastes, such as cellulose and hemicellulose, protein and fats can be converted to gases such as methane, carbon dioxide, ammonia, hydrogen sulfide and hydrogen. The generation of gases and volatile products can cause the loss of the waste weight in the bioreactors.

The decomposition of paper waste in the bioreactors experienced the reduction in weight of waste after 180 days as shown in Table 4-5. The initial wet weight of waste was about 108.4 kg in all bioreactors. The wet weight reduction in Cell 1, Cell 2 and Cell 3 was 7.8 kg, 14.8 kg and 18.2 kg respectively. Similarly, the dry weight reduction in Cell 1, Cell 2 and Cell 3 was 2.3 kg, 3.7 kg and 4.3 kg respectively.

Both the waste volume and waste weight decreased with time in all the bioreactors. The densities in all bioreactors experienced increase due to greater reduction in waste volume proportional to the waste weight. The changes in the field capacities were very small. The

settlement rates in Cell 1, Cell 2 and Cell 3 with time are presented in Figure 4-6 and data are presented in Appendix A (Table A-7). The overall settlement in Cell 1, Cell 2 and Cell 3 was 12%, 15% and 17% respectively.

Table 4-5 Changes in Mass, Density, Field Capacity, and Settlement Rates in Cells

Items	Units	Cell 1	Cell 2	Cell 3
Initial Wet Waste Weight	kg	108.4	108.4	108.4
Final Wet Waste Weight	kg	100.6	93.6	90.2
Wet Weight Reduction	kg	-7.8	-14.8	-18.2
Wet Weight Reduction Rate	%	-7.2	-13.7	-16.8
Initial Dry Waste Weight	kg	25	25	25
Final Dry Waste Weight	kg	22.7	21.3	20.7
Dry Weight Reduction	kg	-2.3	-3.7	-4.3
Dry Weight Reduction Rate	%	-9.2	-14.8	-17.2
Initial Density of Waste	kg/m ³	651.5	651.5	651.5
Final Density of Waste	kg/m ³	682.7	656.3	654.3
Initial Field Capacity	%	76.9	76.9	76.9
Final Field Capacity	%	77.4	77.2	77.1
Final Settlement Rate	%	12	15	17

Note: Field capacity is the weight of water inside the waste divided by the total wet weight of the waste in the bioreactor. Settlement rate is the thickness reduction of the waste matrix divided by the initial thickness of the waste matrix inside bioreactor.

5.0 Comparison of Results

The results of this study are compared with the previous study. In previous study, the synthetic MSW with biosolids was loaded in the bioreactor. The biosolids were the mixture of primary sludge and secondary activated sludge that have undergone anaerobic digestions process. The identification of laboratory bioreactor landfills in this study and the previous study is presented in Table 5-1.

Table 5-1 Laboratory Bioreactor Landfills

Matrix	Operation	Remarks
Paper waste with Enzyme	Leachate Recirculation and buffering; aerobic and anaerobic stages	Soybean peroxidase
MSW with Biosolids	Leachate Recirculation and buffering; aerobic and anaerobic stages	

5.1 Generation and Management of MSW

The MSW includes the residential, commercial, and non- hazardous industrial waste but excludes the combustion ash, hazardous waste, sludge, and industrial process waste. However, many of these wastes are often deposited in the same landfill that receives MSW (Hilger and Barlaz 2002). Therefore, many researchers use refuse instead of MSW.

The increase in generation of MSW has promoted the development of integrated management of MSW that is accomplished by recycling, composting, incineration, or landfilling of wastes. Among these methods, sanitary landfill is the predominant municipal solid waste disposal alternative because it is less expensive comparing with combustion, and there is a limit to the types of wastes that can be recycled or composted (Hilger and Barlaz 2002).

5.2 MSW Composition

Synthetic MSW used in previous study were waste foods, waste vegetables, plastics, waste paper, textiles, and biosolids. The amount and percentages of compositions are presented in the Table 5-2.

Table 5-2 Composition of MSW

Composition	Weight (Kg)	Percentage (%)
Foods	25.90	35
Vegetables	19.24	26
Paper	7.40	10
Plastics	4.44	6
Textiles	2.22	3
Biosolids	14.80	20
Total Wet Weight	74.00	100

5.3 Loading and Operation of Bioreactor Landfill

The synthetic MSW was loaded into the bioreactor. Then the tap waster was added into bioreactor. After that biosolids taken from Toronto Ashbridges Bay Treatment Plant was placed into the bioreactor. In the beginning, the leachate was recirculated daily and the volume of the leachate was measured until the MSW in bioreactor was brought to field capacity.

First 18 days, the bioreactor was operated in aerobic stage followed by anaerobic stage. The variations in decomposition of MSW were compared with paper waste using enzyme. The parameters COD, BOD₅, NH₃-N, TS and settlement rate are compared in this study.

In order to compare the results, COD, BOD₅, NH₃-N and TS concentrations were converted to the relative COD, BOD₅, NH₃-N and TS respectively, where C₀ was the initial concentration and C was the concentration at that time. Similarly, the elapsed time was converted to the relative time, where T₀ was the particular time and T was the total time period until the end of study.

5.4 Comparison of Relative COD Variations

The relative COD variations in the bioreactor having MSW with biosolids are presented in the Figure 5-1(a) and the data are presented in Appendix A (Table A-10). Similarly, the relative COD variations in bioreactor having paper waste with enzyme are presented in 5-1(b) and the data are presented in Appendix A (Table A-11). In order to compare the results, the COD concentration was converted to the relative COD, where C_0 was the initial COD concentration and C was the COD concentration at that time.

The relative peak COD in bioreactor having MSW with biosolids was lower than the bioreactor having paper waste with enzyme as shown in Figure 5-1(a) and 5-1(b). The relative peak COD for MSW with biosolids was 1.22, whereas that for paper waste with enzyme was 1.46 as shown in Table 5-3. The COD reduction rate at the end of aerobic stage in case of MSW with biosolids was 45.2 %, whereas COD reduction rate in case of paper waste with enzyme was 89.6%. The final COD reduction rate at the end of study in case of biosolids was 41.9% and that in case of enzyme was 94.1%. These observations led to the conclusion that the leachate quality in case enzyme was better than the biosolids.

Figure 5-1(a) Relative COD variations in Bioreactor having MSW with Biosolids

Figure 5-1(b) Relative COD variations in Bioreactor having Paper waste with Enzyme

Table 5-3 Relative COD Concentrations and Reduction rate in Bioreactors

Relative COD	MSW with Biosolids	Paper Waste with Enzyme
Initial Relative COD	1.00	1.00
Maximum Relative COD	1.22	1.46
Relative COD at end of Aerobic stage	0.67	0.15
COD Reduction rate in Aerobic stage (%)	45.1	89.7
Final Relative COD at end of study	0.71	0.09
COD reduction rate at end of study (%)	41.8	93.8

Note: The COD reduction rate in aerobic stage was calculated based on the first peak concentration and concentration at the end of aerobic stage. The COD reduction rate at the end of study was calculated based on the first peak concentration and the final concentration in the study.

5.5 Comparison of Relative BOD₅ Variations

The relative BOD₅ variations in bioreactor having MSW with biosolids are presented in Figure 5-2(a) and the data are presented in Appendix A (Table A-12). The relative BOD₅ variations in bioreactor having paper waste with enzyme are presented in 5-2(b) and the data are presented in Appendix A (Table A-13). The BOD₅ concentration was converted to the relative BOD₅, where C_0 was the initial BOD₅ concentration and C was the BOD₅ concentration at that time.

The BOD₅ variations followed the similar trend as COD variations. The relative peak BOD₅ in bioreactor having MSW with biosolids was lower than the bioreactor having paper waste with enzyme as shown in Figure 5-2(a) and 5-2(b). The relative peak BOD₅ for MSW with biosolids was 1.30, whereas that for paper waste with enzyme was 1.36 as shown in Table 5-4. The BOD₅ reduction rate at the end of aerobic stage in case of MSW with biosolids was 44.1 %, whereas BOD₅ reduction rate in case of paper waste with enzyme was 90.5%. The final BOD₅ reduction rate at the end of study in case of biosolids was 58.4% and that in case of enzyme was 95.1%. The BOD₅ data showed that the strength of leachate in case of enzyme reduced faster than the biosolids.

Figure 5-2(a) Relative BOD₅ variations in Bioreactor having MSW with Biosolids

Figure 5-2(b) Relative BOD₅ variations in Bioreactor having Paper waste with Enzyme

Table 5-4 Relative BOD₅ Concentrations and Reduction rate in Bioreactors

Relative BOD ₅	MSW with Biosolids	Paper Waste with Enzyme
Initial Relative BOD ₅	1.00	1.00
Maximum Relative BOD ₅	1.30	1.36
Relative BOD ₅ at end of Aerobic stage	0.73	0.13
BOD ₅ Reduction rate in Aerobic stage (%)	43.8	90.4
Final Relative BOD ₅ at end of study	0.54	0.07
BOD ₅ Reduction rate at end of study (%)	58.5	94.9

Note: The BOD₅ reduction rate in aerobic stage was calculated based on the first peak concentration and concentration at the end of aerobic stage. The BOD₅ reduction rate at the end of study was calculated based on the first peak concentration and the final concentration in the study.

5.6 Comparison of Relative NH₃-N Variations

The relative NH₃-N variations in bioreactor having MSW with biosolids are presented in Figure 5-3(a) and the data are presented in Appendix A (Table A-14). The relative NH₃-N variations in bioreactor having paper waste with enzyme are presented in Figure 5-3(b) and the data are presented in Appendix A (Table A-15). The NH₃-N concentration was converted to the relative NH₃-N, where C₀ was the initial NH₃-N concentration and C was the NH₃-N concentration at that time.

In both cases, the relative the NH₃-N concentration was increased, because CO₂ and H₂O together with ammonia gas were released after the decomposition of waste.

Figure 5-3(a) Relative $\text{NH}_3\text{-N}$ variations in Bioreactor having MSW with Biosolids

Figure 5-3(b) Relative $\text{NH}_3\text{-N}$ variations in Bioreactor having Paper waste with Enzyme

5.7 Comparison of Relative TS Variations

The relative TS variations in the bioreactor having MSW with biosolids are presented in the Figure 5-4(a) and the data are presented in Appendix A (Table A-16). The relative TS variations in the bioreactor having paper waste with biosolids are presented in the Figure 5-4(b) and the data are presented in Appendix A (Table A-17). The TS concentration was converted to the relative TS, where C_0 was the initial TS concentration and C was the TS concentration at that time.

The relative peak TS in bioreactor having MSW with biosolids was higher than the bioreactor having paper waste with enzyme as shown in Figure 5-4(a) and 5-4(b). The relative peak TS for MSW with biosolids was 2.77, whereas that for paper waste with enzyme was 1.32 as shown in Table 5-5. The TS reduction rate at the end of aerobic stage in case of MSW with biosolids was 61.8 %, whereas TS reduction rate in case of paper waste with enzyme was 72.0%. The final TS reduction rate at the end of study in case of biosolids was 48.0% and that in case of enzyme was 68.0%. Thus, similar to the COD and BOD₅, the TS reduction rate was faster in case of enzyme than the biosolids.

Figure 5-4(a) Relative TS variations in Bioreactor having MSW with Biosolids

Figure 5-4(b) Relative TS variations in Bioreactor having Paper waste with Enzyme

Table 5-5 Relative TS Concentrations and Reduction rate in Bioreactors

Relative TS	MSW with Biosolids	Paper Waste with Enzyme
Initial Relative TS	1.00	1.00
Maximum Relative TS	2.77	1.32
Relative TS at end of Aerobic stage	1.06	0.37
TS Reduction rate in Aerobic stage (%)	61.7	72.0
Final Relative TS at end of study	1.44	0.42
TS reduction rate at end of study (%)	48.0	68.2

Note: The TS reduction rate in aerobic stage was calculated based on the first peak concentration and concentration at the end of aerobic stage. The TS reduction rate at the end of study was calculated based on the first peak concentration and the final concentration in the study.

5.8 Comparison of Variations in Settlement Rates

The settlement rate variations in the bioreactor having MSW with biosolids are presented in Figure 5-5(a) and the data are presented in Appendix A (Table A-9). The settlement rate variations in the bioreactor having paper waste with enzyme are presented in Figure 5-5(b) and the data are presented in Appendix A (Table A-7).

Figure 5-5(a) Settlement rate variations in Bioreactor having MSW with Biosolids

Figure 5-5(b) Settlement rate variations in Bioreactor having Paper waste with Enzyme

The MSW settled faster than the paper waste. The settlement rate at the end of aerobic stage in case of MSW with biosolids was 20 %, whereas settlement rate in case of paper waste with enzyme was 11%. The final settlement rate at the end of study in case of biosolids was 24% and that in case of enzyme was 17%.

5.9 Enzymatic Treatment

Enzymatic treatment involves chemical processes based on the action of biological catalyst. The catalytic abilities of enzymes are located in the active site. The active site is where the enzymatic treatment of the substrate occurs. The first part of the active site is the binding site, where enzyme and substrate are physically bonded to one another. The second part of the active site is the catalytic site where the enzyme reacts with the substrate. The enzyme is activated in presence of hydrogen peroxide and catalyzes the oxidation of the substrate.

The catalytic oxidation of compounds in particular is a three-step process (Driscoll et al. 2000). The first step is the oxidation of the ferric enzyme (heme[Fe(III)]_(PX)) to water and

Compound I. Compound I is a protein, which contains an oxyferryl (Fe(IV)=O) center and an organic cation radical. In the next step, Compound I reacts with a molecule of substrate (S) to oxidize the substrate molecule to a substrate radical (S^*) and Compound II is formed. Compound II then reacts with another substrate to become reduced to the original ferric enzyme (heme[Fe(III)](PX)) and substrate molecule is oxidized to a substrate radicals. The catalytic cycle is given by equations [7] to [9] (Banci 1997).

The substrate radicals can form insoluble polymers, as a result of their low solubility, which settle out of the solution. The polymers may be removed via filtration or sediment collection. The toxicity of the end product is reduced in case of enzyme.

The potential advantages of enzymatic treatment as compared with conventional treatment are numerous: remediation of waste, which is toxic to microorganisms; operation over a wide range of pH, temperature and salinity; absence of shock loading effects; absence of delays associated with the acclimatization of biomass; and no generation of biomass (Nicell et al. 1992).

The results in this study showed that the enzymatic treatment was faster than the conventional treatment. In fact, enzymes require low retention times with respect to other treatment process. The enzymes can accelerate and effectively treat the waste (Karam and Nicell 1997). Since the enzymes speed up the chemical reactions and decompose the organic waste faster than the non-enzymatic treatment, the peak COD concentration in case of enzymes was lower than the control one as shown in Figure 4-1. Similarly, the peak BOD_5 and TS concentrations in bioreactors having enzymes were lower than the control one as shown in Figure 4-2 and Figure 4-4 respectively.

In this study, the temperature in the bioreactors was in the range of 22⁰ C to 26⁰ C. The low temperature was experienced at top of bioreactor and high temperature at the bottom of the bioreactor. Nicel et al. (1993) found that the number of turnovers the enzyme exhibited was higher when the reaction occurred at lower temperatures. The SBP enzymes are found to be more thermally stable, allowing reactors to operate at higher temperatures without experiencing thermal inactivation. The SBP essentially exhibits no inactivation at temperatures below 70⁰ C by the 12th hour. Additionally, SBP remains thermally stable at high temperatures, even when high temperatures are combined with acidic conditions (Driscoll et al. 2000).

5.10 Economic Feasibility of Soybean Peroxidase

The SBP enzyme is found in the seed coat, which is a by-product of the soybean industry and can be utilized without reducing the value of the soybean's oil and meat. Since there is currently no market for this by-product, it is difficult to estimate an associated cost, but could be assumed to be very inexpensive as it is currently considered to be refuse. The recent research has indicated that use of crude soybean hulls is extremely efficient (Flock et al. 1999). The reduction in cost could also be achieved by increasing the peroxidase activity in the seed coat and increasing the enzyme catalytic lifetime. The raw soybean seed coat may contain zero to five percent peroxidase by dry weight, although genetically engineered soybean plants have been shown to contain much higher levels of the peroxidase enzyme. Increasing the catalytic lifetime can be accomplished by minimizing the adverse conditions, which result in the inactivation of the enzyme.

6.0 Conclusions and Recommendations

6.1 Conclusions

One way to reduce the amount of paper waste being discarded is to recycle more of our waste paper. But it is estimated that only about 20 to 25 percent of paper and paperboard used is recycled. About 60 to 80 percent of paper waste goes to the waste stream.

This study was aimed at decomposing the paper waste in bioreactor landfills in aerobic condition before the anaerobic condition using soybean enzymes. All together three bioreactor cells had been set up in the laboratory for experimental works. One bioreactor cell (Cell 1) without enzymes was compared with two bioreactors (Cell 2 and Cell 3), which contained different concentration of soybean peroxidase (SBP) enzymes with hydrogen peroxide (H_2O_2). The bioreactor Cell 2 had 5 million units of SBP enzymes and the bioreactor cell 3 had 10 million units of SBP enzymes.

The pH of the leachate at the beginning was in the range from 5.5 to 5.9. Therefore, the pH had been maintained with a range of 6.4 to 7.2 by using buffer solutions to keep the SBP enzyme active. The aerobic-anaerobic bioreactor landfills enhanced the degradation to transform and stabilize the paper waste. The effect of air injection exhibited that the aerobic decomposition of waste was much faster than anaerobic decomposition.

The strength of leachate reduced faster in aerobic stage than anaerobic stage. After about 180 days of waste treatment, the final COD concentration in Cell 1 was 863 mg/l i.e. 43.5% higher than the Cell 2 and 62.6% higher than Cell 3. Similarly, final BOD_5 concentration in Control cell (Cell 1) was 431 mg/l i.e. 51.5% higher than the Cell 2 and 70.7% higher than Cell 3. The final total solids (TS) concentration in Cell 1 was 2,450 mg/l i.e. 22.4% higher than Cell 2 and 34.7% higher than Cell 3. The settlement rates at end of the study were 12%, 15% and 17% in Cell 1, Cell 2 and Cell 3 respectively.

Hence, it can be concluded that the bioreactors with enzyme had faster decomposition of waste as compared to the bioreactor without enzyme. The bioreactor having higher

concentration of enzyme decomposed faster than the bioreactor having lower concentration.

The result of this study was compared with the previous study. The relative COD, BOD₅, NH₃-N and TS variations in the bioreactors were compared in this study. The relative COD variations showed that the final COD reduction rate at the end of study in case of biosolids was 41.9% and that in case of enzyme was 94.1%. Similarly, the final BOD₅ reduction rate at the end of study in case of biosolids was 58.4% whereas in case of enzyme was 95.1%. And the final TS reduction rate at the end of study in case of biosolids was 48.0% and that in case of enzyme was 68.0%.

The comparison of the results of the bioreactors having MSW with biosolids and paper waste with enzyme showed that the decomposition of solid waste in case of enzyme was faster than the biosolids. The comparison of the result also showed that the leachate quality in case of enzyme was better than the biosolids. The percentage of COD, BOD₅ and TS reduction in case of enzyme was higher than the biosolids. The COD reduction rate in case of enzyme was about more than double than the bioreactor having biosolids. Similarly, BOD₅ reduction rate in case of enzyme was 1.6 times higher than the biosolids and TS reduction rate was about 1.4 times higher than the bioreactor having biosolids. Since the degradation rate in case of enzyme is faster, it can be concluded that the enzymes are better in degradation of the organic waste and the use of enzymes should be promoted.

6.2 Recommendations

This research has been carried out in laboratory bioreactor. The aerobic stage is followed by the anaerobic stage. The air supply to the bioreactor landfills might be costly in the field bioreactors. So, from the economic point of view, the requirement of aerobic stage requires further investigation in case of field bioreactor.

Furthermore, it has been concluded that the decomposition of paper waste in bioreactor using SBP enzyme is faster than control one. However, cost benefit analysis is required for the treatment of waste with enzyme in field bioreactor.

REFERENCES

Aitken, M.D. and Irvine, R. L., 1989. Stability testing of ligninase and Mn-peroxidase from *Phanerochaete chrysosporium*. *Biotechnology and Bioengineering*. 34, 1251-60.

AL-Kassim L., Taylor K. E., Bewtra J. K. and Biswas, N., 1995. Optimization and preliminary cost analysis of soybean peroxidase-catalyzed removal of phenols from synthetic and industrial wastewater. 45th Canadian Chemical Engineering Conference, Quebec.

Aleman, K., 2000. Master's Dissertation submitted to the University of Ottawa.

American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF), 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th Edition. (ed.) Clesceri, L. S., Greenberg, A. E., Eaton, A. D., Washington, DC.

Anderson, J. G., 1990. Treatment of wastes by composting. In *microbiology of landfill sites* (E. Senior, ed.). Boca Raton, FL, U.S.A.: CRC Press, Inc., p 59-80.

Anazia, I. and Misra, M., 1989. Enzymatic dewatering of Florida phosphate slimes. *Minerals and Metallurgical processes*. 6(20), 93-5.

Banci, L., 1997. Structural Properties of Peroxidases, *Journal of Biotechnology*. 53, 253.

Barina, G., 2005. Leachate recirculation at large scale: Investigation on design and waste degradation. International Workshop: Hydro-Physico Mechanics Landfills. LIRIGM Grenoble 1 University, France. 21-22 March 2005.

Barlaz, M. A., Mike, M. W., Ham, R. K., 1987. Gas Production Parameters in sanitary landfill simulators. *Waste management and Research*. 5, 27-36.

Barlaz, M. A., Ham, R. K., Shaefer, D. M., 1990. Methane production from municipal refuse: a review of enhancement techniques and microbial dynamics, *Critical Reviews in Environmental Control*, CRC Press. 19 (6), 557-584.

Bewtra, J. K., Biswas, N., Henderson, W. D., and Nicell, J. A., 1995. Waste Water Quality Resources *Journal Canada*. 30, 115-125.

Campbell, Neil A., 1993. *Biology*. 3rd edition. The Benjamin/Cummings Publishing Company, Inc. pp. 100-110.

Campman, C. and Yates, A., 2002. A Summary of the current Bioreactor Landfill Situation. Walter B. Satterthwaite Associates Inc.

http://www.forester.net/mw_0209_bioreactor.html

Carven, W., Vogel, K., 1999. Field Investigation of Landfill Leachate Collection System. Research Project by Florida Center for Solid and Hazardous Waste Management. pp. 1-9.

Chaplin M. and Bucke C., 1990. *Enzyme Technology*, Cambridge University Press. <http://www.Isbu.ac.uk/biology/enztech>.

Chapsal, J. M., Bourbigot, M. M. and Thomas, D., 1986. Oxidation of aromatic compounds by haemoglobin. *Water Research*. 20, 709-13.

Christensen, T. H., Kjeldsen, P., 1989. Basic biochemical processes in landfills. In *Sanitary landfilling: Process, Technology and Environmental Impact*. Academic Press Ltd., Toronto. pp. 29-49.

Christensen, T. H., Kjeldsen, P., and Stegmann, R., 1992. Effect of Landfill Management Procedures on Landfill Stabilization and Leachate and Gas Quality, *Landfilling of Waste: Leachate*: Elsevier Applied Science.

Chugh, S., Pullammanappallil, P., Rudolph, V., 1998. Effect of recirculated leachate volume on MSW degradation. *Waste Management and Research*. 16 (6), 564-573.

Cleanaway, 2000. Recycling Paper and Cardboard. 'There's no time to Waste' video
Elephant Productions. http://www.wastewise.wa.gov.au/pages/recycling_paper.asp

Conestoga-Rovers and Associates, 2004. Design and Operation report for the optimization
of the green lane landfill site. Literature Review and Application- Landfill Bioreactor
Technology.

Cornwell, R.L., Tinland-Butez, M. F., Tardone, P.J., Cabasso, I and Hammel, K. E., 1990.
Lignin degradation and lignin peroxidase production in cultures of *Phanerochaete*
chrysosporium immobilized on porous ceramic supports. *Enzyme and microbial*
Technology. 12, 916-20.

Dewalle, F. B., Chian, E. S. K., and Hammerberg, E., 1978. Gas Production from Solid
Waste in Landfill. *Journal of the Environmental Engineering Division, ASCE*, 104 (EE3),
415.

Driscoll, N., Warith, M., and Kennedy, K., 2000. The Potential of Soybean Peroxidase for
remediation.

El-Fadel, M., 1998. Leachate Recirculation Effects on Settlement and Biodegradation
Rates in MSW Landfills. *Environmental Technology*. 20, 121-133.

Emcon Associates, 1980. Methane Generation and Recovery from Landfills. Ann Arbor
Science.

Environment Canada, 1992. Recycling Paper and Cardboard. Office of Waste
Management, Conservation and Protection, <http://www.ns.ec.gc.ca/udo/wastepap.html>

Farquhar, G. J. and Rovers, F. A., 1973. Gas Production during Refuse Decomposition.
Water, Air and Soil Pollution. 2 (9), 483-495.

Flock, C., Bassi, A. and Gijzen, M., 1999. Removal of aqueous phenol and 2-chlorophenol
with purified soybean peroxidase and raw soybean hulls. *Journal of Chemical Technology*
and Biotechnology. 74, 303-309.

Francis, A. O., 2003. Bioreactor Landfill, Shanghai University.
<http://www.kunlad.net/BIOREACTORLANDFILLHOMEWORK.doc>

Gillikin, J. W. and Graham, J. S., 1991. Purification and Developmental Analysis of the Major Anionic Peroxidase from the Seed Coat of Glycine Max. *Plant Physiology* 96(1), 214-220.

Goetz, Rebecca, 1996. New test kit adds 'green' value to soybeans. Retrieved on November 19, 2004. <http://www.purdue.edu/UNS/html4ever/9605.Vierling.html>

Hakulinen, R., 1988. The use of enzymes for wastewater treatment in the pulp and paper industry- a new possibility. *Water Science and Technology*. 20(1), 251-62.

Hakulinen, R., 1991. Personal communication, Alko Ltd. Biotechnology Division, SF-05200 Rajamaki, Finland.

Hartz, K. E., Klink, R. E., and Ham, R. K., 1982. Temperature effects: methane generation from landfill samples. *Journal of Environmental Engineering Division, Proceedings of the American Society of Civil Engineers*. 108, 629-638.

Haug, R. T., 1993. *The Practical Handbook of Composting Engineering*, Chapter 2, 10, Lewis Pub.

Hilger, H. H., Barlaz, M. A., 2002. Anaerobic decomposition of refuse in landfills and methane oxidation in landfill cover soils. In *Manual of Environmental Microbiology*, Hurst, C. J. (ed), ASTM Press, 2002, 696-718.

Hudgins, M. and Harper, S., 1998. Successful Demonstration of Two Aerobic Landfills- Leading towards a More Sustainable Solid Waste Management Approach, Environmental Control Systems, Inc., Aiken, SC.

Hudgins, M., Harper, S., 1999. Operational characteristics of two aerobic landfill systems. 7th Int. Waste Management and Landfill Symposium in Sardinia, Italy.

Karam, J. and Nicel, J. A., 1997. Potential Applications of Enzymes in Waste Treatment. *Journal of Chemical Technology and Biotechnology*. 69, 141-53.

Kennedy, K., Alemany, K. and Warith, M., 2002. Optimisation of Soybean Peroxidase Treatment of 2, 4-dichlorophenol. *Water SA*. 28, 149-58.

Krol, A., Rudolph, V., and Swarbrick, G., 1994. Landfill: A Containment Facility or a Process Operation. Paper presented at the 2nd National Hazard and Solid Waste Convention, Melbourne.

Lagerkvist, A., and Chen, H., 1993. Control of Two Step Anaerobic Degradation of Municipal Solid Waste (MSW) by Enzyme addition. *Water Science and Technology*. 27(2), 47.

Lagrimini, L. M. and Rothstein, S. 1987. Tissue specificity of tobacco peroxidase isozymes and their induction by wounding and tobacco mosaic virus infection. *Plant Physiology*. 84, 438-442.

Mata-Alvares, J., Martina-Verdure, A., 1986. Laboratory simulation of municipal solid waste fermentation with leachate recycles. *J. Chem. Tech. Biotechnology*. 36, 547-556.

Mathewson, P. R., 1998. *Enzymes*, Eagan Press Handbook Series.

McBean, E., Rovers, F. and Farquhar, G., 1995. *Solid Waste Landfill Engineering and Design*. Prentice- Hall Publishing Co., New Jersey.

McCreanor, P. T., and Reinhart, D. R., 1996. Hydrodynamic Modeling of Leachate Recirculation Landfills. *Water Science and Technology*. 34 (7-8), 463-470.

McEldoon, J. P., Pokora, A. R., and Dordick, J. S., 1995. Lignin Peroxidase-type Activity of Soybean Peroxidase. *Enzyme and Microbial Technology*. 17, 359-365.

Merz, R. C. and Stone, R., January 1962. Sanitary landfill Behavior in an Aerobic Environment, *Public Works*. 68-70.

- Murphy, R. J., 1990. Waste Reduction/ Recycle of Plastic and Paper Waste. University of South Florida, Tampa, Florida. http://www.floridacenter.org/publications/murphy_90-8.PDF
- Nicell, J. A., Bewtra, J.K., Taylor, K.E., Biswas, N., and St. Pierre, C., 1992. Water Science and Technology. 25(3), 157.
- Nicell, J. A., Bewtra, J. K., Biswas, N., and Taylor, E., 1993. Water Resources. 27, 1623.
- Nicell, J. A., and Wright, H., 1997. A Model of Peroxidase Activity with Inhibition by Hydrogen Peroxide. Enzyme and Microbial Technology. 21, 302.
- Nicell, J. A., Saadi, K. W., and Buchanan, I. D., 1995. Phenol polymerization and precipitation by peroxidase and an additives. Bioresource Technology. 54, 5-16.
- Nicell, J. A., AL-Kassim L., Bewtra J. K. and Taylor K. E., 1993. Wastewater treatment by enzyme catalyzed polymerization and precipitation. Biodeterioration Abstr. 7, 1-8.
- Pacey, J., Augstein, D., Morck, R., Reinhart, D., Yazdani, R., 1999. The Bioreactive Landfill. MSW Management. http://forester.net/msw_9909_bioreactive_landfill.html
- Penner, G., June 2003. Market Opportunities Analysis for Canadian Soybeans. http://www.soy2020.ca/pdfs/Soy2020_June_2003_Section_2_Annual_Report_Alternatives_Per.pdf
- Read, A. D., Hudgins, M., Harper, S., Phillips, P., Morris, J., 2001. The successful demonstration of aerobic landfilling. The Potential for a More Sustainable Solid Waste Management Approach, RCR. 32, 115–146.
- Reinhart, D. R., Al-Yousfi, A., 1996. The Impact of Leachate Recirculation on Municipal Solid Waste Landfill Operating Characteristics. Waste Management and Research 14, 337-346.

Reinhart, D. R., Townsend, T. G., 1998. Landfill bioreactor design and operation. CRC Press LLC.

Reinhart, D. R., McCreanor, P. T., Townsend, T., 2002. The Bioreactor Landfill: Its status and future. Waste Management and Research 20, 172-186.

Rees, J. F., 1980. Optimisation of methane production and refuse decomposition in landfills by temperature control. J. Chem. Tech. Biotechnology. 30, 458-465.

Repa, E. W., 2003. Bioreactor Landfills: A Viable Technology. National Solid Wastes Management Association (NSWMA) Research Bulletin 03-02. Washington, DC.
<http://wastec.isproductions.net/webmodules/webarticles/articlefiles/463-Bioreactor%20Landfills.pdf>

Rich, L. G., 1963. Unit Processes of Sanitary Engineering. John Wiley and Sons, Inc., New York.

Rivers, D. B. and Emert, G. H., 1988. Factors affecting the enzymatic hydrolysis of municipal solid waste components. Biotechnology and Bioengineering. 31, 278-81.

Rovers, F., Grahame, J., 1995. Solid Waste Landfill Engineering and Design. Prentice Hall Inc., New Jersey.

Stessel, R. I., Murphy, R. J., 1992. A Lysimeter Study of the Aerobic Landfill Concept. Waste Management and Research. 10, 485-503.

Tchobanoglous, G., Theisen, H., and Eliassen, R., 1977. Solid Wastes: Engineering Principles and Management Issues. New York: McGraw-Hill, Inc.

Tchobanoglous, G., Theisen, H., and Vigil, S., 1993. Integrated Solid Waste Management: Engineering Principles and Management Issues. New Jersey: McGraw-Hill, Inc.

Tchobanoglous, G., 2003. Wastewater engineering: Treatment and reuse 4th ed./ revised by George Tchobanoglous, Franklin L. Burton, H. David Stensel. Metcalf & Eddy Inc.

Themelis, J. N. and Kim, Y. H., 2002. Material and Energy Balances in a Large-Scale Aerobic Bioconversion Cell, 20, 234-242.

Thomas, O. R. T. and White, G. F., 1991. Immobilization of the surfactant-degradading bacterium *Pseudomonas C12B* in polyacrylamide gel. III. Biodegradation specificity for raw surfactants and industrial wastes. *Enzyme and Microbial Technology*, 13, 338-43.

Trombly, J., 1995. *Environmental Science and Technology*, 29, 12, 560A.

U.S. Environmental Protection Agency. 1979. U.S. Environmental Protection Agency catalogs. EPA, Washington, D.C.

Warith, M. A., Sharma, R. 1998. Technical review of methods to enhance biological degradation in sanitary landfills. *Water Quality Resource Journal Canada*. 33(3), 417-437.

Warith, M. A., 2002. Bioreactor landfills: Experimental and Field Results. *Waste Management*. 22(1) 7-17.

Warith, M. A., 2003. Solid waste management: New trends in landfill design. *Emirates Journal of Engineering Research*. 8(1), 61-70.

Wright, H. and Nicell J. A., 1999. Characterization of soybean peroxidase for the treatment of aqueous phenols. *Bioresource Technology* 70, 69-79.

Wynn, C. H., 1973. *The Structure and Function of Enzymes*, Edward Arnold (Publishers) Limited.

Yuen, S. T. S., 2001. Bioreactor Landfills: Do they work? Paper presented at Geoenvironment 2001: 2nd ANZ Conference on Environmental Geotechnics, Newcastle, Australia.

Yuen, S. T. S., Wang, Q. J., Styles, J. R., McMahon, T. A., 2001. Water balance comparison between a dry and wet landfill- a full scale experiment. *Journal of Hydrology*. 251, 29-48.

APPENDIX A – Experimental Data

Table A-1 COD Concentrations (mg/l)

Date	Day	Cell 1	Cell 2	Cell 3
08/07/2005	1	3,667	3,792	3,750
10/07/2005	3	5,042	4,208	3,875
12/07/2005	5	5,417	4,625	4,083
14/07/2005	7	5,850	5,000	4,500
15/07/2005	8	6,583	5,042	4,542
17/07/2005	10	6,458	5,250	4,917
20/07/2005	13	6,333	5,792	5,458
22/07/2005	15	6,167	5,625	5,292
25/07/2005	18	6,125	5,625	4,875
30/07/2005	23	5,938	5,000	4,313
01/08/2005	25	5,625	4,875	4,250
04/08/2005	28	5,313	4,750	4,125
06/08/2005	30	5,133	4,533	4,067
09/08/2005	33	4,875	4,208	3,958
11/08/2005	35	4,750	4,000	3,750
13/08/2005	37	4,292	3,667	3,583
16/08/2005	40	3,983	3,317	2,900
18/08/2005	42	3,267	2,433	2,150
20/08/2005	44	2,950	2,267	2,083
21/08/2005	45	2,567	2,000	1,667
24/08/2005	48	2,083	1,667	1,517
25/08/2005	49	1,983	1,417	1,233
26/08/2005	50	1,550	1,250	1,017
28/08/2005	52	1,333	983	800
29/08/2005	53	983	767	567
02/09/2005	57	1,183	850	717
06/09/2005	61	1,227	867	700
08/09/2005	63	1,200	800	667
12/09/2005	67	1,200	883	700

Table A-1 COD Concentrations (mg/l) Cont^d

Date	Day	Cell 1	Cell 2	Cell 3
15/09/2005	70	1,233	833	683
18/09/2005	73	1,150	817	667
20/09/2005	75	1,000	700	567
22/09/2005	77	1,033	683	583
25/09/2005	80	1,100	700	567
27/09/2005	82	1,150	683	550
29/09/2005	84	1,150	700	567
03/10/2005	88	1,100	683	550
07/10/2005	92	933	633	517
11/10/2005	96	950	617	467
13/10/2005	98	983	567	450
17/10/2005	102	950	600	467
20/10/2005	105	900	538	425
24/10/2005	109	838	513	375
27/10/2005	112	825	500	363
31/10/2005	116	838	613	463
03/11/2005	119	825	588	363
07/11/2005	123	813	550	338
10/11/2005	126	863	463	313
14/11/2005	130	850	450	350
18/11/2005	134	825	450	325
21/11/2005	137	800	450	338
28/11/2005	144	797	497	327
05/12/2005	151	817	500	342
12/12/2005	158	821	508	350
20/12/2005	166	773	473	313
28/12/2005	174	792	504	329
03/01/2006	180	863	487	323

Table A-2 BOD₅ Concentrations (mg/l)

Date	Day	Cell 1	Cell 2	Cell 3
08/07/2005	1	1,926	1,824	1,893
10/07/2005	3	2,196	1,863	1,998
12/07/2005	5	2,388	2,088	2,046
14/07/2005	7	2,712	2,352	2,088
15/07/2005	8	3,264	2,832	2,238
17/07/2005	10	3,354	2,892	2,418
20/07/2005	13	3,372	2,916	2,568
22/07/2005	15	3,186	2,652	2,190
25/07/2005	18	2,952	2,466	2,103
30/07/2005	23	2,766	2,100	2,007
01/08/2005	25	2,484	1,890	1,758
04/08/2005	28	2,091	1,680	1,572
06/08/2005	30	1,827	1,650	1,560
09/08/2005	33	1,806	1,578	1,476
11/08/2005	35	1,785	1,584	1,446
13/08/2005	37	1,656	1,434	1,302
16/08/2005	40	1,491	1,278	1,206
18/08/2005	42	1,350	1,191	1,101
20/08/2005	44	1,320	1,143	1,011
21/08/2005	45	1,257	1,119	948
24/08/2005	48	1,329	1,056	898
25/08/2005	49	1,215	834	688
26/08/2005	50	920	664	502
28/08/2005	52	690	544	406
29/08/2005	53	507	348	243
02/09/2005	57	567	367	291
06/09/2005	61	603	391	302
08/09/2005	63	623	406	316
12/09/2005	67	637	432	310

Table A-2 BOD₅ Concentrations (mg/l) Cont^d

Date	Day	Cell 1	Cell 2	Cell 3
15/09/2005	70	647	453	330
18/09/2005	73	599	398	297
20/09/2005	75	579	350	264
22/09/2005	77	573	330	252
25/09/2005	80	611	330	275
27/09/2005	82	617	330	243
29/09/2005	84	603	350	252
03/10/2005	88	570	347	283
07/10/2005	92	585	345	300
11/10/2005	96	570	360	309
13/10/2005	98	510	315	276
17/10/2005	102	555	300	281
20/10/2005	105	546	332	286
24/10/2005	109	530	327	247
27/10/2005	112	525	315	245
31/10/2005	116	495	357	287
03/11/2005	119	468	309	234
07/11/2005	123	476	329	222
10/11/2005	126	488	309	211
14/11/2005	130	462	311	210
18/11/2005	134	450	308	216
21/11/2005	137	453	306	221
28/11/2005	144	423	306	201
05/12/2005	151	412	301	207
12/12/2005	158	403	273	180
20/12/2005	166	398	266	172
28/12/2005	174	403	244	145
03/01/2006	180	431	209	126

Table A-3 BOD₅/COD

Date	Day	Cell 1	Cell 2	Cell 3
08/07/2005	1	0.53	0.48	0.50
10/07/2005	3	0.44	0.44	0.52
12/07/2005	5	0.44	0.45	0.50
14/07/2005	7	0.46	0.47	0.46
15/07/2005	8	0.50	0.56	0.49
17/07/2005	10	0.52	0.55	0.49
20/07/2005	13	0.53	0.50	0.47
22/07/2005	15	0.52	0.47	0.41
25/07/2005	18	0.48	0.44	0.43
30/07/2005	23	0.47	0.42	0.47
01/08/2005	25	0.44	0.39	0.41
04/08/2005	28	0.39	0.35	0.38
06/08/2005	30	0.36	0.36	0.38
09/08/2005	33	0.37	0.37	0.37
11/08/2005	35	0.38	0.40	0.39
13/08/2005	37	0.39	0.39	0.36
16/08/2005	40	0.37	0.39	0.42
18/08/2005	42	0.41	0.49	0.51
20/08/2005	44	0.45	0.50	0.49
21/08/2005	45	0.49	0.56	0.57
24/08/2005	48	0.64	0.63	0.59
25/08/2005	49	0.61	0.59	0.56
26/08/2005	50	0.59	0.53	0.49
28/08/2005	52	0.52	0.55	0.51
29/08/2005	53	0.52	0.45	0.43
02/09/2005	57	0.48	0.43	0.41
06/09/2005	61	0.49	0.45	0.43
08/09/2005	63	0.52	0.51	0.47
12/09/2005	67	0.53	0.49	0.44

Table A-3 BOD₅/COD Cont^d

Date	Day	Cell 1	Cell 2	Cell 3
15/09/2005	70	0.52	0.54	0.48
18/09/2005	73	0.52	0.49	0.45
20/09/2005	75	0.58	0.50	0.47
22/09/2005	77	0.55	0.48	0.43
25/09/2005	80	0.56	0.47	0.49
27/09/2005	82	0.54	0.48	0.44
29/09/2005	84	0.52	0.50	0.44
03/10/2005	88	0.52	0.51	0.51
07/10/2005	92	0.63	0.54	0.58
11/10/2005	96	0.60	0.58	0.66
13/10/2005	98	0.52	0.56	0.61
17/10/2005	102	0.58	0.50	0.60
20/10/2005	105	0.61	0.62	0.67
24/10/2005	109	0.63	0.64	0.66
27/10/2005	112	0.64	0.63	0.68
31/10/2005	116	0.59	0.58	0.62
03/11/2005	119	0.57	0.53	0.65
07/11/2005	123	0.59	0.60	0.66
10/11/2005	126	0.57	0.67	0.68
14/11/2005	130	0.54	0.69	0.60
18/11/2005	134	0.55	0.68	0.66
21/11/2005	137	0.57	0.68	0.65
28/11/2005	144	0.53	0.62	0.62
05/12/2005	151	0.50	0.60	0.61
12/12/2005	158	0.49	0.54	0.51
20/12/2005	166	0.51	0.56	0.55
28/12/2005	174	0.51	0.48	0.44
03/01/2006	180	0.50	0.43	0.39

Table A-4 NH₃-N Concentrations (mg/l)

Date	Day	Cell 1	Cell 2	Cell 3
08/07/2005	1	0.08	0.04	0.04
10/07/2005	3	0.09	0.06	0.05
12/07/2005	5	0.11	0.08	0.06
14/07/2005	7	0.13	0.09	0.07
15/07/2005	8	0.15	0.13	0.09
17/07/2005	10	0.23	0.18	0.12
20/07/2005	13	0.26	0.19	0.14
22/07/2005	15	0.25	0.20	0.15
25/07/2005	18	0.26	0.18	0.14
30/07/2005	23	0.28	0.18	0.13
01/08/2005	25	0.27	0.17	0.13
04/08/2005	28	0.29	0.19	0.13
06/08/2005	30	0.29	0.21	0.15
09/08/2005	33	0.26	0.20	0.15
11/08/2005	35	0.27	0.20	0.15
13/08/2005	37	0.28	0.23	0.20
16/08/2005	40	0.28	0.25	0.20
18/08/2005	42	0.28	0.24	0.21
20/08/2005	44	0.29	0.25	0.18
21/08/2005	45	0.29	0.23	0.17
24/08/2005	48	0.28	0.24	0.17
25/08/2005	49	0.26	0.23	0.15
26/08/2005	50	0.28	0.22	0.16
28/08/2005	52	0.26	0.19	0.16
29/08/2005	53	0.27	0.20	0.15
02/09/2005	57	0.28	0.24	0.16
06/09/2005	61	0.27	0.21	0.16
08/09/2005	63	0.27	0.20	0.16
12/09/2005	67	0.28	0.21	0.15

Table A-4 NH₃-N Concentrations (mg/l) Cont^d

Date	Day	Cell 1	Cell 2	Cell 3
15/09/2005	70	0.29	0.21	0.15
18/09/2005	73	0.28	0.20	0.15
20/09/2005	75	0.27	0.19	0.14
22/09/2005	77	0.28	0.21	0.15
25/09/2005	80	0.28	0.19	0.16
27/09/2005	82	0.26	0.21	0.18
29/09/2005	84	0.27	0.21	0.17
03/10/2005	88	0.26	0.18	0.17
07/10/2005	92	0.24	0.18	0.16
11/10/2005	96	0.25	0.19	0.17
13/10/2005	98	0.26	0.21	0.16
17/10/2005	102	0.27	0.21	0.15
20/10/2005	105	0.26	0.18	0.16
24/10/2005	109	0.26	0.20	0.17
27/10/2005	112	0.25	0.19	0.16
31/10/2005	116	0.26	0.21	0.15
03/11/2005	119	0.25	0.19	0.14
07/11/2005	123	0.26	0.21	0.16
10/11/2005	126	0.28	0.19	0.15
14/11/2005	130	0.26	0.21	0.15
18/11/2005	134	0.27	0.20	0.17
21/11/2005	137	0.26	0.21	0.17
28/11/2005	144	0.28	0.22	0.16
05/12/2005	151	0.29	0.23	0.16
12/12/2005	158	0.28	0.22	0.17
20/12/2005	166	0.30	0.21	0.16
28/12/2005	174	0.31	0.22	0.15
03/01/2006	180	0.31	0.21	0.16

Table A-5 TS Concentrations (mg/l)

Date	Day	Cell 1	Cell 2	Cell 3
08/07/2005	1	4,200	3,900	3,800
10/07/2005	3	4,850	4,000	3,950
12/07/2005	5	5,400	4,350	4,300
14/07/2005	7	5,625	4,200	4,100
15/07/2005	8	5,850	4,400	4,150
17/07/2005	10	6,075	4,750	4,250
20/07/2005	13	6,000	5,200	4,500
22/07/2005	15	6,225	5,550	4,950
25/07/2005	18	6,000	5,150	4,800
30/07/2005	23	5,925	4,950	5,000
01/08/2005	25	5,775	4,800	4,500
04/08/2005	28	5,475	4,850	4,350
06/08/2005	30	5,100	4,500	3,900
09/08/2005	33	5,025	4,350	3,550
11/08/2005	35	4,725	3,350	3,150
13/08/2005	37	4,275	3,300	2,900
16/08/2005	40	3,600	3,100	2,600
18/08/2005	42	3,525	2,900	2,500
20/08/2005	44	3,300	2,850	2,350
21/08/2005	45	3,150	2,650	2,050
24/08/2005	48	3,075	2,300	1,900
25/08/2005	49	3,225	2,500	2,000
26/08/2005	50	2,900	2,450	2,050
28/08/2005	52	2,700	2,000	1,750
29/08/2005	53	2,200	1,600	1,400
02/09/2005	57	2,300	1,650	1,450
06/09/2005	61	2,450	1,700	1,500
08/09/2005	63	2,400	1,650	1,450
12/09/2005	67	2,550	1,700	1,400

Table A-5 TS Concentrations (mg/l) Cont^d

Date	Day	Cell 1	Cell 2	Cell 3
15/09/2005	70	2,400	1,550	1,400
18/09/2005	73	2,550	1,650	1,450
20/09/2005	75	2,500	1,600	1,400
22/09/2005	77	2,350	1,650	1,450
25/09/2005	80	2,400	1,750	1,450
27/09/2005	82	2,250	1,800	1,550
29/09/2005	84	2,200	1,800	1,450
03/10/2005	88	2,250	1,850	1,550
07/10/2005	92	2,350	2,000	1,750
11/10/2005	96	2,400	2,050	1,700
13/10/2005	98	2,450	1,950	1,650
17/10/2005	102	2,500	2,050	1,800
20/10/2005	105	2,400	1,900	1,750
24/10/2005	109	2,450	2,000	1,700
27/10/2005	112	2,350	1,950	1,600
31/10/2005	116	2,450	2,100	1,650
03/11/2005	119	2,400	2,150	1,750
07/11/2005	123	2,350	2,100	1,750
10/11/2005	126	2,300	2,050	1,600
14/11/2005	130	2,400	2,000	1,750
18/11/2005	134	2,300	1,900	1,550
21/11/2005	137	2,450	2,000	1,650
28/11/2005	144	2,300	1,850	1,600
05/12/2005	151	2,350	1,750	1,500
12/12/2005	158	2,300	1,900	1,550
20/12/2005	166	2,250	1,850	1,600
28/12/2005	174	2,450	1,950	1,550
03/01/2006	180	2,300	1,900	1,600

Table A-6 pH of Leachate

Date	Day	Cell 1	Cell 2	Cell 3
08/07/2005	1	5.65	5.99	5.56
10/07/2005	3	5.91	6.18	6.13
12/07/2005	5	5.99	6.20	6.21
14/07/2005	7	5.99	6.09	6.15
15/07/2005	8	6.18	6.30	6.31
17/07/2005	10	6.26	6.30	6.37
20/07/2005	13	6.32	6.35	6.44
22/07/2005	15	6.30	6.38	6.44
25/07/2005	18	6.36	6.40	6.46
30/07/2005	23	6.44	6.46	6.60
01/08/2005	25	6.50	6.51	6.59
04/08/2005	28	6.61	6.63	6.64
06/08/2005	30	6.65	6.67	6.73
09/08/2005	33	6.67	6.69	6.79
11/08/2005	35	6.78	6.86	6.89
13/08/2005	37	6.81	6.83	6.90
16/08/2005	40	6.85	6.93	6.91
18/08/2005	42	6.71	6.81	6.86
20/08/2005	44	6.68	6.80	6.89
21/08/2005	45	6.71	6.81	6.81
24/08/2005	48	6.78	6.88	6.88
25/08/2005	49	6.78	6.91	6.95
26/08/2005	50	6.75	6.88	6.94
28/08/2005	52	6.69	6.85	6.92
29/08/2005	53	6.67	6.89	6.90
02/09/2005	57	6.61	6.79	6.93
06/09/2005	61	6.52	6.62	6.71
08/09/2005	63	6.60	6.65	6.74
12/09/2005	67	6.58	6.64	6.65

Table A-6 pH of Leachate Cont^d

Date	Day	Cell 1	Cell 2	Cell 3
15/09/2005	70	6.60	6.62	6.71
18/09/2005	73	6.61	6.67	6.69
20/09/2005	75	6.68	6.70	6.63
22/09/2005	77	6.73	6.79	6.82
25/09/2005	80	6.74	6.82	6.83
27/09/2005	82	6.72	6.84	6.85
29/09/2005	84	6.72	6.86	6.86
03/10/2005	88	6.73	6.87	6.89
07/10/2005	92	6.74	6.85	6.88
11/10/2005	96	6.75	6.85	6.83
13/10/2005	98	6.76	6.84	6.86
17/10/2005	102	6.78	6.83	6.81
20/10/2005	105	6.78	6.82	6.81
24/10/2005	109	6.69	6.77	6.76
27/10/2005	112	6.67	6.79	6.81
31/10/2005	116	6.66	6.78	6.79
03/11/2005	119	6.64	6.75	6.79
07/11/2005	123	6.66	6.75	6.84
10/11/2005	126	6.64	6.75	6.88
14/11/2005	130	6.68	6.79	6.85
18/11/2005	134	6.72	6.78	6.88
21/11/2005	137	6.65	6.82	6.86
28/11/2005	144	6.67	6.79	6.83
05/12/2005	151	6.69	6.78	6.87
12/12/2005	158	6.71	6.84	6.89
20/12/2005	166	6.74	6.86	6.90
28/12/2005	174	6.69	6.87	6.92
03/01/2006	180	6.69	6.88	6.93

Table A-7 Settlement of Waste Matrix

Date	Day	Cell 1		Cell 2		Cell 3	
		Matrix Height (m)	Settlement Rate (%)	Matrix Height (m)	Settlement Rate (%)	Matrix Height (m)	Settlement Rate (%)
08/07/2005	1	0.70	0	0.70	0	0.70	0
10/07/2005	3	0.70	0	0.70	1	0.69	1
12/07/2005	5	0.69	1	0.68	3	0.67	4
15/07/2005	8	0.69	2	0.67	4	0.66	6
17/07/2005	10	0.69	2	0.67	4	0.66	6
20/07/2005	13	0.68	3	0.67	5	0.65	7
22/07/2005	15	0.68	3	0.67	5	0.65	7
25/07/2005	18	0.68	4	0.66	6	0.65	8
30/07/2005	23	0.68	4	0.66	6	0.65	8
01/08/2005	25	0.68	4	0.66	6	0.65	8
04/08/2005	28	0.68	4	0.66	6	0.65	8
06/08/2005	30	0.68	4	0.66	6	0.65	8
09/08/2005	33	0.67	4	0.65	7	0.64	9
11/08/2005	35	0.67	4	0.65	8	0.64	9
16/08/2005	40	0.67	4	0.65	8	0.64	9
18/08/2005	42	0.67	5	0.65	8	0.64	9
20/08/2005	44	0.67	5	0.64	9	0.64	9
21/08/2005	45	0.67	5	0.64	9	0.63	10
24/08/2005	48	0.66	6	0.64	9	0.63	10
27/08/2005	51	0.66	6	0.64	9	0.63	11
29/08/2005	53	0.66	6	0.64	9	0.63	11
31/08/2005	55	0.66	6	0.64	9	0.62	11
06/09/2005	61	0.66	6	0.64	9	0.62	11
08/09/2005	63	0.66	6	0.64	9	0.62	11
12/09/2005	67	0.66	6	0.64	9	0.62	12

Table A-7 Settlement of Waste Matrix Cont^d

Date	Day	Cell 1		Cell 2		Cell 3	
		Matrix Height (m)	Settlement Rate (%)	Matrix Height (m)	Settlement Rate (%)	Matrix Height (m)	Settlement Rate (%)
20/09/2005	75	0.65	7	0.63	10	0.62	12
22/09/2005	77	0.65	8	0.63	11	0.61	13
27/09/2005	82	0.64	9	0.62	11	0.61	13
29/09/2005	84	0.64	9	0.62	12	0.61	13
03/10/2005	88	0.64	9	0.62	12	0.61	14
07/10/2005	92	0.64	9	0.62	12	0.61	14
11/10/2005	96	0.64	9	0.62	12	0.61	14
13/10/2005	98	0.64	9	0.61	13	0.61	14
17/10/2005	102	0.64	9	0.61	13	0.60	14
20/10/2005	105	0.64	9	0.61	13	0.60	14
24/10/2005	109	0.64	9	0.61	13	0.60	14
27/10/2005	112	0.63	10	0.61	13	0.60	14
31/10/2005	116	0.63	10	0.61	13	0.60	14
03/11/2005	119	0.63	10	0.61	14	0.60	15
07/11/2005	123	0.63	10	0.61	14	0.60	15
10/11/2005	126	0.63	11	0.61	14	0.60	15
14/11/2005	130	0.63	11	0.60	14	0.59	16
18/11/2005	134	0.63	11	0.60	14	0.59	16
21/11/2005	137	0.62	11	0.60	14	0.59	16
28/11/2005	144	0.62	11	0.60	14	0.59	16
05/12/2005	151	0.62	11	0.60	14	0.59	16
12/12/2005	158	0.62	11	0.60	15	0.58	17
20/12/2005	166	0.62	12	0.60	15	0.58	17
28/12/2005	174	0.62	12	0.60	15	0.58	17
03/01/2006	180	0.62	12	0.60	15	0.58	17

Table A-8 Previous Data (MSW with Biosolids)

Date	Day	COD (mg/l)	BOD ₅ (mg/l)	NH ₃ -N(mg/l)	TS (mg/l)
06/09/2004	1	30,000	14,160	342	12,500
07/09/2004	2	33,560	17,760	-	29,700
08/09/2004	3	36,494	18,450	351	-
09/09/2004	4	-	-	-	34,600
11/09/2004	6	-	-	-	21,200
12/09/2004	7	29,301	15,557	269	19,700
15/09/2004	10	23,872	9,128	385	-
16/09/2004	11	22,555	11,950	335	13,900
19/09/2004	14	21,000	8,751	535	13,000
21/09/2004	16	19,088	8,333	572	13,600
23/09/2004	18	20,001	10,312	454	13,200
26/09/2004	21	20,022	6,909	646	13,500
28/09/2004	23	25,289	13,539	665	15,100
30/09/2004	25	26,323	13,149	704	18,000
03/10/2004	28	24,743	11,250	786	15,300
05/10/2004	30	21,874	9,458	774	14,000
07/10/2004	32	23,907	9,025	612	15,000
11/10/2004	36	21,966	9,573	783	16,500
14/10/2004	39	22,782	9,025	653	20,000
18/10/2004	43	23,770	10,833	959	19,800
21/10/2004	46	25,882	8,957	697	19,800
25/10/2004	50	24,843	10,750	724	20,400
28/10/2004	53	21,974	12,535	873	21,300
04/11/2004	60	23,253	11,750	796	21,100
11/11/2004	67	21,778	10,999	713	19,100
18/11/2004	74	20,876	7,000	621	17,200
25/11/2004	81	21,223	7,042	725	16,200
02/12/2004	88	20,600	6,428	730	19,500
09/12/2004	95	21,250	7,999	830	18,300
16/12/2004	102	21,215	7,678	1,021	18,000

Table A-9 Settlement Rate (MSW with Biosolids)

Date	Day	Settlement Rate (%)
06/09/2004	1	0
07/09/2004	2	4
08/09/2004	3	5
09/09/2004	4	8
10/09/2004	5	13
11/09/2004	6	14
13/09/2004	8	17
14/09/2004	9	19
15/09/2004	10	19
16/09/2004	11	19
18/09/2004	13	19
19/09/2004	14	19
21/09/2004	16	19
23/09/2004	18	20
25/09/2004	20	20
27/09/2004	22	20
02/10/2004	27	20
05/10/2004	30	20
07/10/2004	32	22
11/10/2004	36	22
17/10/2004	42	22
20/10/2004	45	22
24/10/2004	49	22
01/11/2004	57	22
09/11/2004	65	22
14/11/2004	70	22
21/11/2004	77	22
24/11/2004	80	22
07/12/2004	93	22
12/12/2004	98	24
18/12/2004	104	24

Table A-10 Relative COD (MSW with Biosolids)

Day	Relative Time (T_0/T)	COD (mg/l)	Relative COD (C/C_0)
1	0.01	30,000	1.00
2	0.02	33,560	1.12
3	0.03	36,494	1.22
7	0.07	29,301	0.98
10	0.10	23,872	0.80
11	0.11	22,555	0.75
14	0.14	21,000	0.70
16	0.16	19,088	0.64
18	0.18	20,001	0.67
21	0.21	20,022	0.67
23	0.23	25,289	0.84
25	0.25	26,323	0.88
28	0.27	24,743	0.82
30	0.29	21,874	0.73
32	0.31	23,907	0.80
36	0.35	21,966	0.73
39	0.38	22,782	0.76
43	0.42	23,770	0.79
46	0.45	25,882	0.86
50	0.49	24,843	0.83
53	0.52	21,974	0.73
60	0.59	23,253	0.78
67	0.66	21,778	0.73
74	0.73	20,876	0.70
81	0.79	21,223	0.71
88	0.86	20,600	0.69
95	0.93	21,250	0.71
102	1.00	21,215	0.71

Table A-11 Relative COD (Paper Waste with Enzyme)

Day	Relative Time (T_0/T)	COD (mg/l)	Relative COD (C/C_0)
1	0.01	3,750	1.00
3	0.02	3,875	1.03
5	0.03	4,083	1.09
7	0.04	4,500	1.20
8	0.04	4,542	1.21
10	0.06	4,917	1.31
13	0.07	5,458	1.46
15	0.08	5,292	1.41
18	0.10	4,875	1.30
23	0.13	4,313	1.15
25	0.14	4,250	1.13
28	0.16	4,125	1.10
30	0.17	4,067	1.08
33	0.18	3,958	1.06
35	0.19	3,750	1.00
37	0.21	3,583	0.96
40	0.22	2,900	0.77
42	0.23	2,150	0.57
44	0.24	2,083	0.56
45	0.25	1,667	0.44
48	0.27	1,517	0.40
49	0.27	1,233	0.33
50	0.28	1,017	0.27
52	0.29	800	0.21
53	0.29	567	0.15
57	0.32	717	0.19
61	0.34	700	0.19
63	0.35	667	0.18
67	0.37	700	0.19

Table A-11 Relative COD (Paper Waste with Enzyme) Cont^d

Day	Relative Time (T_0/T)	COD (mg/l)	Relative COD (C/C_0)
70	0.39	683	0.18
73	0.41	667	0.18
75	0.42	567	0.15
77	0.43	583	0.16
80	0.44	567	0.15
82	0.46	550	0.15
84	0.47	567	0.15
88	0.49	550	0.15
92	0.51	517	0.14
96	0.53	467	0.12
98	0.54	450	0.12
102	0.57	467	0.12
105	0.58	425	0.11
109	0.61	375	0.10
112	0.62	363	0.10
116	0.64	463	0.12
119	0.66	363	0.10
123	0.68	338	0.09
126	0.70	313	0.08
130	0.72	350	0.09
134	0.74	325	0.09
137	0.76	338	0.09
144	0.80	327	0.09
151	0.84	342	0.09
158	0.88	350	0.09
166	0.92	313	0.08
174	0.97	329	0.09
180	1.00	323	0.09

Table A-12 Relative BOD₅ (MSW with Biosolids)

Day	Relative Time (T₀/T)	BOD₅ (mg/l)	Relative BOD₅ (C/C₀)
1	0.01	14,160	1.00
2	0.02	17,760	1.25
3	0.03	18,450	1.30
7	0.07	15,557	1.10
10	0.10	9,128	0.64
11	0.11	11,950	0.84
14	0.14	8,751	0.62
16	0.16	8,333	0.59
18	0.18	10,312	0.73
21	0.21	6,909	0.49
23	0.23	13,539	0.96
25	0.25	13,149	0.93
28	0.27	11,250	0.79
30	0.29	9,458	0.67
32	0.31	9,025	0.64
36	0.35	9,573	0.68
39	0.38	9,025	0.64
43	0.42	10,833	0.77
46	0.45	8,957	0.63
50	0.49	10,750	0.76
53	0.52	12,535	0.89
60	0.59	11,750	0.83
67	0.66	10,999	0.78
74	0.73	7,000	0.49
81	0.79	7,042	0.50
88	0.86	6,428	0.45
95	0.93	7,999	0.56
102	1.00	7,678	0.54

Table A-13 Relative BOD₅ (Paper Waste with Enzyme)

Day	Relative Time (T_0/T)	BOD ₅ (mg/l)	Relative BOD ₅ (C/C_0)
1	0.01	1,893	1.00
3	0.02	1,998	1.06
5	0.03	2,046	1.08
7	0.04	2,088	1.10
8	0.04	2,238	1.18
10	0.06	2,418	1.28
13	0.07	2,568	1.36
15	0.08	2,190	1.16
18	0.10	2,103	1.11
23	0.13	2,007	1.06
25	0.14	1,758	0.93
28	0.16	1,572	0.83
30	0.17	1,560	0.82
33	0.18	1,476	0.78
35	0.19	1,446	0.76
37	0.21	1,302	0.69
40	0.22	1,206	0.64
42	0.23	1,101	0.58
44	0.24	1,011	0.53
45	0.25	948	0.50
48	0.27	898	0.47
49	0.27	688	0.36
50	0.28	502	0.27
52	0.29	406	0.21
53	0.29	243	0.13
57	0.32	291	0.15
61	0.34	302	0.16
63	0.35	316	0.17
67	0.37	310	0.16

Table A-13 Relative BOD₅ (Paper Waste with Enzyme) Cont^d

Day	Relative Time (T ₀ /T)	BOD ₅ (mg/l)	Relative BOD ₅ (C/C ₀)
70	0.39	330	0.17
73	0.41	297	0.16
75	0.42	264	0.14
77	0.43	252	0.13
80	0.44	275	0.15
82	0.46	243	0.13
84	0.47	252	0.13
88	0.49	283	0.15
92	0.51	300	0.16
96	0.53	309	0.16
98	0.54	276	0.15
102	0.57	281	0.15
105	0.58	286	0.15
109	0.61	247	0.13
112	0.62	245	0.13
116	0.64	287	0.15
119	0.66	234	0.12
123	0.68	222	0.12
126	0.70	211	0.11
130	0.72	210	0.11
134	0.74	216	0.11
137	0.76	221	0.12
144	0.80	201	0.11
151	0.84	207	0.11
158	0.88	180	0.10
166	0.92	172	0.09
174	0.97	145	0.08
180	1.00	126	0.07

Table A-14 Relative NH₃-N (MSW with Biosolids)

Day	Relative Time (T ₀ /T)	NH ₃ -N (mg/l)	Relative NH ₃ -N (C/C ₀)
1	0.01	342	1.00
3	0.03	351	1.03
7	0.07	269	0.79
10	0.10	385	1.13
11	0.11	335	0.98
14	0.14	535	1.56
16	0.16	572	1.67
18	0.18	454	1.33
21	0.21	646	1.89
23	0.23	665	1.94
25	0.25	704	2.06
28	0.27	786	2.30
30	0.29	774	2.26
32	0.31	612	1.79
36	0.35	783	2.29
39	0.38	653	1.91
43	0.42	959	2.80
46	0.45	697	2.04
50	0.49	724	2.12
53	0.52	873	2.55
60	0.59	796	2.33
67	0.66	713	2.08
74	0.73	621	1.82
81	0.79	725	2.12
88	0.86	730	2.13
95	0.93	830	2.43
102	1.00	1,021	2.99

Table A-15 Relative NH₃-N (Paper Waste with Enzyme)

Day	Relative Time (T ₀ /T)	NH ₃ -N (mg/l)	Relative NH ₃ -N (C/C ₀)
1	0.01	0.04	1.00
3	0.02	0.05	1.40
5	0.03	0.06	1.65
7	0.04	0.07	2.00
8	0.04	0.09	2.43
10	0.06	0.12	3.18
13	0.07	0.14	3.73
15	0.08	0.15	4.13
18	0.10	0.14	3.85
23	0.13	0.13	3.53
25	0.14	0.13	3.58
28	0.16	0.13	3.45
30	0.17	0.15	3.99
33	0.18	0.15	4.19
35	0.19	0.15	4.16
37	0.21	0.20	5.39
40	0.22	0.20	5.45
42	0.23	0.21	5.74
44	0.24	0.18	5.08
45	0.25	0.17	4.77
48	0.27	0.17	4.77
49	0.27	0.15	4.22
50	0.28	0.16	4.46
52	0.29	0.16	4.34
53	0.29	0.15	4.12
57	0.32	0.16	4.39
61	0.34	0.16	4.30
63	0.35	0.16	4.27
67	0.37	0.15	4.09

Table A-15 Relative NH₃-N (Paper Waste with Enzyme) Cont^d

Day	Relative Time (T ₀ /T)	NH ₃ -N (mg/l)	Relative NH ₃ -N (C/C ₀)
70	0.39	0.15	4.12
73	0.41	0.15	4.23
75	0.42	0.14	3.90
77	0.43	0.15	4.10
80	0.44	0.16	4.27
82	0.46	0.18	4.83
84	0.47	0.17	4.67
88	0.49	0.17	4.60
92	0.51	0.16	4.50
96	0.53	0.17	4.67
98	0.54	0.16	4.34
102	0.57	0.15	4.13
105	0.58	0.16	4.27
109	0.61	0.17	4.54
112	0.62	0.16	4.34
116	0.64	0.15	4.17
119	0.66	0.14	3.90
123	0.68	0.16	4.40
126	0.70	0.15	4.07
130	0.72	0.15	4.20
134	0.74	0.17	4.54
137	0.76	0.17	4.67
144	0.80	0.16	4.34
151	0.84	0.16	4.40
158	0.88	0.17	4.77
166	0.92	0.16	4.47
174	0.97	0.15	4.17
180	1.00	0.16	4.30

Table A-16 Relative TS (MSW with Biosolids)

Day	Relative Time (T_0/T)	TS (mg/l)	Relative TS (C/C_0)
1	0.01	12,500	1.00
2	0.02	29,700	2.38
4	0.03	34,600	2.77
6	0.07	21,200	1.70
7	0.10	19,700	1.58
11	0.11	13,900	1.11
14	0.14	13,000	1.04
16	0.16	13,600	1.09
18	0.18	13,200	1.06
21	0.21	13,500	1.08
23	0.23	15,100	1.21
25	0.25	18,000	1.44
28	0.27	15,300	1.22
30	0.29	14,000	1.12
32	0.31	15,000	1.20
36	0.35	16,500	1.32
39	0.38	20,000	1.60
43	0.42	19,800	1.58
46	0.45	19,800	1.58
50	0.49	20,400	1.63
53	0.52	21,300	1.70
60	0.59	21,100	1.69
67	0.66	19,100	1.53
74	0.73	17,200	1.38
81	0.79	16,200	1.30
88	0.86	19,500	1.56
95	0.93	18,300	1.46
102	1.00	18,000	1.44

Table A-17 Relative TS (Paper Waste with Enzyme)

Day	Relative Time (T_0/T)	TS (mg/l)	Relative TS (C/C_0)
1	0.01	3,800	1.00
3	0.02	3,950	1.04
5	0.03	4,050	1.07
7	0.04	4,100	1.08
8	0.04	4,150	1.09
10	0.06	4,250	1.12
13	0.07	4,500	1.18
15	0.08	5,000	1.32
18	0.10	4,800	1.26
23	0.13	4,800	1.26
25	0.14	4,500	1.18
28	0.16	4,350	1.14
30	0.17	3,900	1.03
33	0.18	3,550	0.93
35	0.19	3,150	0.83
37	0.21	2,900	0.76
40	0.22	2,600	0.68
42	0.23	2,500	0.66
44	0.24	2,350	0.62
45	0.25	2,050	0.54
48	0.27	1,900	0.50
49	0.27	2,000	0.53
50	0.28	2,050	0.54
52	0.29	1,750	0.46
53	0.29	1,400	0.37
57	0.32	1,450	0.38
61	0.34	1,500	0.39
63	0.35	1,450	0.38
67	0.37	1,400	0.37

Table A-17 Relative TS (Paper Waste with Enzyme) Cont^d

Day	Relative Time (T₀/T)	TS (mg/l)	Relative TS (C/C₀)
70	0.39	1,400	0.37
73	0.41	1,450	0.38
75	0.42	1,400	0.37
77	0.43	1,450	0.38
80	0.44	1,450	0.38
82	0.46	1,550	0.41
84	0.47	1,450	0.38
88	0.49	1,550	0.41
92	0.51	1,750	0.46
96	0.53	1,700	0.45
98	0.54	1,650	0.43
102	0.57	1,800	0.47
105	0.58	1,750	0.46
109	0.61	1,700	0.45
112	0.62	1,600	0.42
116	0.64	1,650	0.43
119	0.66	1,750	0.46
123	0.68	1,750	0.46
126	0.70	1,600	0.42
130	0.72	1,750	0.46
134	0.74	1,550	0.41
137	0.76	1,650	0.43
144	0.80	1,600	0.42
151	0.84	1,500	0.39
158	0.88	1,550	0.41
166	0.92	1,600	0.42
174	0.97	1,550	0.41
180	1.00	1,600	0.42

APPENDIX- B Experimental Photographs

B-1 Air Injection into the Bioreactors

B- 2 Leachate Recirculation (Aerobic Stage)

B- 3 Leachate Recirculation (Anaerobic Stage)

B-4 BOD₅ Measurement