

# **Lethbridge Landfill Drill Sample Methane Potential Measurements and Molecular Characterization**

Final Project Report

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## Executive Summary

Methane is a major greenhouse gas (GHG) with a global warming potential more than 25 times the carbon dioxide equivalent. Biologically derived methane is commonly released from microbial degradation of organic materials in anaerobic environments such as landfills, the second largest contributor of global anthropogenic methane. In Canada, methane accounts for approximately 13% of GHG emission, 20% of which is derived from landfills. Quantification of landfill methane emission is therefore imperative for the design and implementation of mitigation strategies of landfill GHG emission.

This project involves the biochemical methane potential, laboratory-scale degradation rates and physical and microbial characterization of 45 spent municipal solid waste (MSW) samples collected from two landfills in the City of Lethbridge. The biochemical methane potential and physical waste characterization, reported in [Part 1](#), were performed by Alberta Innovate – Technology Futures, while the molecular characterization of microbial communities, reported in [Part 2](#), was performed by Alberta Livestock Research Branch.

Physical characterization revealed that spent MSW were highly heterogeneous, containing residual organic materials (fibre, fabric, varieties of wood, branches, leaves, garden clippings, paper) and inert materials (plastics, rocks, metal, ceramic and glass). On dry weight basis, the average waste composition was 49% organics, 23% visible inerts, 16% paper and 13% wood.

The average maximum methane yields ( $B_0$ ) were 63.2  $\text{Nm}^3/\text{dry Mg MSW}$  and 73.9  $\text{Nm}^3/\text{dry Mg MSW}$  for the operational and closed landfills, respectively. Regardless of its high organic carbon content, wood had a very low  $B_0$  of 41.3  $\text{Nm}^3/\text{dry Mg wood}$ , contributing an average maximum methane yield per dry ton of MSW of 6.7 and 3.2  $\text{Nm}^3$  in the operational and closed landfills, respectively. The average laboratory-based degradation rate ( $k_{\text{lab}}$ ) for the whole landfill samples were 20.0  $\text{yr}^{-1}$  and 19.6  $\text{yr}^{-1}$  for the operational and closed landfills, respectively. The  $k_{\text{lab}}$  value for wood in the operational landfill was 12.41  $\text{yr}^{-1}$  whereas that in the closed landfill was not determined due to insufficient amount of data. The average  $B_0$  for wood from both landfills combined was not statistically different from that of a non-landfilled softwood reference sample, indicating that wood did not undergo significant degradation in the landfill. However, the  $k_{\text{lab}}$  for wood from the landfill was ~1.5 times that of fresh softwood, which suggests that conditions in the landfill acted as some sort of 'pretreatment' for enhanced degradation of the wood samples. Overall, there was no statistically significant difference between the physical characteristics, degradation rates and maximum methane yields for samples from both landfills.

Molecular identification of microbial communities showed the presence of fungi, highly salt-tolerant bacteria species and anaerobic bacteria. However, methanogenic bacteria and archaea, the major contributors to biological methane, have not been identified. Further analysis may be required to identify the predominant microbial communities contributing to methane production and GHG emission from the landfill sites.

The Alberta Landfill Gas Quantification (ALGQ) Model requires several inputs, some of which may have to be determined experimentally. This study has generated experimental landfill  $B_0$  data relevant to the City of Lethbridge landfill and has improved our understanding of landfill waste characteristics in the region. It has therefore provided Alberta-relevant data to support or fine-tune the province's landfill gas offset protocol. Where a reliable field-based  $k$  is available, the data may represent a basis for relating  $k_{lab}$  to field-applicable rates for the verification or validation of landfill gas emission models.

This report has been produced independently by researchers at Alberta Innovates Technology Futures and the Alberta Livestock research Branch through funding provided, in part, by the Climate Change Emissions Management Corporation (CCEMC). The views expressed in this report are not necessarily the views of the Climate Change Emissions Management Corporation (CCEMC).

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**PART 1: Physical Characterization and Methane  
Potential Measurements of Lethbridge Landfill  
Samples**

# 1. Introduction

## 1.1 Methane as Greenhouse Gas

Methane (CH<sub>4</sub>) is a major greenhouse gas (GHG) - approximately 13% of Canadian GHG emission,<sup>1</sup> - and thus plays an important role in climate change mitigation strategies. Methane is commonly released from degradation of organic material in anaerobic environments such as wetlands, rice paddies, digestive tracts of ruminants and oxygen-deficient organic waste piles, notably landfills.

The deposition of municipal solid waste (MSW) in landfills remains a common practice in Canadian MSW management industry where up to 77% of the MSW is landfilled.<sup>2</sup> In 2012, 25 million tons of non-hazardous waste was sent to Canadian waste disposal facilities and Alberta had the highest per capita waste disposal rate at just over 1 ton per person compared to a national average of 720 kilograms.<sup>3</sup> In landfills, organic waste is entombed, colonized by microbial communities and biodegraded slowly over decades. Depending on the availability of oxygen, the microbial biodegradation can be either aerobic or anaerobic. Anaerobic biodegradation, driven mainly by bacteria and archaea in the absence of oxygen, leads to the production of GHGs, mainly CH<sub>4</sub> and carbon dioxide (CO<sub>2</sub>). The presence of methanogenic communities and the availability of free water are the two major limitations for microbial degradation of waste into GHGs. A certain level of free water or water activity (a measure of the amount of moisture moving in and out of a substance) is required to sustain microbial activity, which is further governed by the ambient temperature. The amount of GHG production by methanogenic communities is further limited by the total amount of energy present within the organic material, referred to as the volatile fraction.

Differences in methane generation may also be a function of the methanogenic and other anaerobic microbial communities present in the waste, which may metabolize the energy content differently. For example, acetoclastic and hydrogenotrophic methanogens,<sup>4,5</sup> sulphur-reducing bacteria<sup>6</sup> and other non-methanogenic microbes<sup>7</sup> require different substrates which are processed through different metabolic pathways. The phylogenetics of microbial communities present in organic material can be characterized through (meta)genomic DNA extraction, DNA amplification and the ever-increasing variety and capability of sequencing analytical tools available. Thus, the physical characteristics of organic waste buried in landfill and the phylogenetic characteristics of the microbial communities therein are complimentary to the rate and extent of waste degradation, and to the maximum potential amount of GHG released.

Methane emission from landfills is of greater concern than CO<sub>2</sub> since the former is widely reported to have more than 25-fold the global warming potential of the latter.<sup>8</sup> Moreover, according to Environment Canada's and Alberta Environment's GHG regulation (adapted from the USEPA<sup>9</sup>), CO<sub>2</sub> produced from organic waste degradation is biogenic in origin and is not classified as anthropogenic GHG emission. On the other hand CH<sub>4</sub> emission from waste degradation in landfills, although biogenic, is considered as anthropogenic GHG emission since it would not have occurred if not for waste deposition in landfills.<sup>10</sup> The huge amounts of solid waste being buried in landfills make MSW

the second largest contributor of anthropogenic methane.<sup>11,12</sup> Quantification of landfill methane emission is therefore imperative for reduction or offset of GHG emissions from landfills.

## 1.2 Estimating Methane Emission from Landfills

Various models and protocols have been developed for the quantification of methane emissions from landfills.<sup>13, 14,26</sup> These predictive models enable estimation of the rate and quantity of methane production via the waste decay rate constant,  $k$  and the maximum methane yield,  $B_0$ . Examples of landfill gas estimation models include the First Order Model of the Netherlands,<sup>15</sup> the California Landfill Methane Inventory Model (CALMIM),<sup>16,17</sup> GasSim by the Environmental Agency of England and Wales,<sup>18,19</sup> the EPER Model used in France and Germany<sup>14</sup>, the Intercontinental Panel on Climate Change (IPCC) Model<sup>20,21,22</sup> and the USEPA's Landfill Gas Emission Model - LandGEM,<sup>23</sup> (eq. 1). LandGEM is the most commonly used landfill gas generation model in North America.

$$Q_n = kB_0 \sum_{i=0}^n \sum_{j=0.0}^{0.9} \frac{M_i}{10} e^{-kt_{i,j}} \quad \text{eq. 1}$$

where  $Q_n$  is the  $\text{CH}_4$  generated ( $\text{m}^3 \text{yr}^{-1}$ ) in a given year of examination  $n$ ;  $k$  the waste degradation rate ( $\text{yr}^{-1}$ );  $B_0$  the maximum  $\text{CH}_4$  generation potential ( $\text{m}^3 \text{Mg}^{-1}$  wet waste);  $M_i$  the waste mass deposited in  $i$  (Mg);  $j$  an 0.1 year time increment used to calculate  $\text{CH}_4$  generation; and  $t$  the time (yr).<sup>23</sup>

Alberta has developed its own model for estimating landfill gas emission. The Alberta Landfill Gas Quantification (ALGQ) Model (eq. 2 and 3) developed by Alberta Environment in collaboration with Alberta Research Council is based entirely on the IPCC First-Order Decay Model.<sup>24,25</sup>

$$k = 0.00003 \times (PCPN + AL) + 0.01 \quad \text{eq. 2}$$

$$B_0 = MCF \times DOC \times DOC_F \times F \times \left(\frac{4}{3}\right) \quad \text{eq. 3}$$

where  $k$  is the waste degradation rate ( $\text{yr}^{-1}$ ),  $PCPN$  the precipitation in the year of calculation ( $\text{mm yr}^{-1}$ ),  $AL$  additional liquids deposited in the landfill in the year of calculation ( $\text{mm yr}^{-1}$ ),  $B_0$  the maximum  $\text{CH}_4$  yield ( $\text{ton CH}_4/\text{wet ton MSW}$ ),  $MCF$  the methane correction factor,  $DOC$  the total degradable organic carbon in MSW (wet weight, obtained as the sum of the weighted degradable organic carbon fractions from each waste components: paper & textile, garden/park, food and wood/straw wastes),  $DOC_F$  the fraction of non-lignin based degradable organic carbon,  $F$  the volume fraction of  $\text{CH}_4$  in generated landfill gas and  $\left(\frac{4}{3}\right)$  the  $\text{CH}_4/\text{Carbon}$  molecular weight ratio.

As indicated in eq. 2 & 3, the model requires several landfill-specific inputs as well as regional assumptions/defaults. Like the IPCC Model, the ALGQ Model specifies a default  $k$  value of 0.02 – 0.04 for landfills in the province (corresponding to temperate, semi-arid regions where average annual temperature and precipitation are less than 20°C and 25 inches, respectively).

As cited by Scharff and co-workers,<sup>26</sup> the IPCC model was not designed for individual landfills, but rather for larger geographic entities in similar climatic zone – usually including several countries, sub-categorized simply as dry or wet. In other words, a default  $k$  value in the IPCC Model is a calculated average of landfill  $k$  values obtained from a relatively small number of randomly chosen landfills within such broad climatic zone. However, intrinsic sub-regional climatic differences may exist within a broad climatic zone. Furthermore, the degradation rate of organic material is dependent on a broad spectrum of factors: waste type or composition (e.g. food waste, yard clippings, garden waste, textile, paper, wood, construction waste, etc.); waste physical properties (e.g. moisture content, density, porosity, permeability, compactability, etc.); moisture/water activity (e.g. wet, dry); presence or absence of oxygen; availability and type of microbial communities (aerobic or anaerobic bacterial/archaeal consortia); availability of nutrients for microbial sustenance; intrinsic climatic conditions of the landfill location (Tropical, Boreal, Temperate, Alpine, semi-Arid, Arid, etc.); landfill topography; waste depth; etc. Since several of these factors are significantly different from one landfill to another, it is not unusual to find, within a broad geographic region, outlier landfills with atypically low or high landfill gas emission rates. The IPCC-based ALGQ Model defaults may therefore not be applicable to all landfills in Alberta, which covers an extensive surface area with up to four distinct climatic sub-regions: subarctic climate in the north, continental climate in the central part of the province, humid-continental in the lower regions of the Rocky Mountains and Cypress Hills, and semi-arid climates in the south.

Although atypical landfill emission rates may cancel each other out when the emission of a broad climatic region is estimated, landfill-specific data remains essential in situations where emissions are tied to landfill-specific benefits or liabilities (e.g. carbon credit allocation or levying of fines, respectively). Applicability of the ALGQ Model on Alberta's landfill carbon credit allocation scheme necessitates validation via comparing the modelled estimates ( $k$  and  $B_0$ ) with actual gas emission data obtained experimentally. The importance of model validation is highlighted in studies which show inherent assumption-driven uncertainties associated with landfill gas estimation models, with huge differences observed between modelled estimates and whole-site experimental data.<sup>26,27,28,29</sup>

Experimental determination of the landfill  $k$  and  $B_0$  is typically done in the field and requires the installation of active gas collection systems for direct whole-site gas volume/velocity measurement,<sup>26,30</sup> or the monitoring of emitted landfill gas via:

- (i) direct non-intrusive techniques via gas sampling/velocity vents, passive sampling techniques and flux chamber/enclosure techniques such as baro-pneumatic<sup>31</sup> and flux chamber methods,<sup>32,33,34</sup> or
- (ii) indirect methods which measure ambient air concentration under defined meteorological conditions and integrate the results with atmospheric dispersion models for rate determination.<sup>35,36</sup>

Although both field methods are typically expensive, the time and cost of installing an active direct gas collection system exceeds the passive non-intrusive techniques by huge margins. A cheaper, time-saving alternative is to evaluate the biodegradability of MSW samples in the laboratory via the

biochemical methane potential (BMP) assay.<sup>37,38,39,40,41,42</sup> In this assay, representative MSW is subjected to physical conditions which promote accelerated degradation, reducing the time of decomposition from >100 years in landfills to 30 to 500 days in the laboratory. The BMP assay can also provide insight into the influence of physical (temperature, moisture, particle size, waste age, etc.), chemical (carbohydrate, lipid and protein content, salinity, alkalinity, acidity, inhibitors, pH, etc.) and biological/microbial characteristics of the MSW on rate and extent of degradation. Several user-friendly first-order rate models have been developed unto which standard BMP assay data can be fitted to obtain key degradation outputs such as the laboratory-based  $k$  ( $k_{lab}$ ) and  $B_0$ .<sup>43,44</sup> The  $B_0$  obtained is directly applicable to landfill scenarios. However, with accelerated decomposition,  $k_{lab}$  is typically amplified 10 to >100-fold over the field-scale equivalent ( $k_{field}$ ), and is therefore not directly applicable to landfill scenarios.

A recent report suggests that, for a given landfill, a relationship can be established between  $k_{lab}$  and  $k_{field}$  through a conversion factor,  $f$ .<sup>45</sup> Determination of the conversion factor warrants, among other prerequisites, an established  $k_{field}$ , which remains a major challenge as it is not available in many landfills, including the two landfills in the City of Lethbridge. In this study, we propose a strategy for the establishment of  $f$  through sub-regional  $k_{field}$  and landfill-specific  $k_{lab}$ .

### 1.3 Project Background

Territorial governments generally specify guidelines and protocols for the mitigation and offset of GHG emissions from sources such as landfills within their jurisdiction. The Alberta Government's ALGQ Model, based on IPCC defaults specified in the Guidelines for National GHG Inventories,<sup>22,24,25</sup> is deemed central to the province's protocol for reduction or offset of landfill CH<sub>4</sub> emissions through programs such as waste prevention, waste diversion, carbon sequestration and carbon credit allocation. These programs, especially, the carbon credit allocation, must be dependent on landfill- or Alberta-specific  $k$  and  $B_0$  of the waste-in-place. Estimation of a reliable Alberta-specific landfill  $k$  value would necessitate determination of several landfill site-specific  $k$  values, the average of which may be used province-wide. This approach will further the scientific impetus on reference data used in the provincial protocol and help fine-tune the regulatory compliance requirements for mitigation and offset of landfill GHG emissions.

The City of Lethbridge, located in the Prairie region of Southern Alberta has been using the ALGQ Model to estimate  $k$  and  $B_0$  on its landfills. The City's operational landfill (Figure 2) has the following parameters required by the model (eq. 2 & 3):

- (i) Waste composition in wet weight basis as reported in the City of Lethbridge Comprehensive Waste Diversion and Waste Prevention Master Plan<sup>46</sup>: paper (27%), garden/park waste (10.8%), food waste (12.1%) and wood and straw waste (7.4%);
- (ii) Default landfill depth of 30m;
- (iii) Methane correction factor of 0.8, reflecting a high methane generation rate in unmanaged landfills of depth  $\geq$  5m or with high water table;

- (iv) Default fraction of CH<sub>4</sub> in landfill gas of 50%;
- (v) Fraction of lignin-free degradable organic carbon in MSW converted to landfill gas (77%);
- (vi) Default fraction of CH<sub>4</sub> migrating from landfill to baseliner (0%);
- (vii) Default fraction of methane oxidized by landfill cover (0%);

Based on these inputs, the calculated annual  $k$  values for the years 1985 through 2013 ranged between 0.016 and 0.027 yr<sup>-1</sup>, the average of which is in agreement with the IPCC default for the region. Experimental data was needed to evaluate the relevance and applicability of information acquired from the ALGQ Model to this landfill site. The City of Lethbridge therefore decided to conduct baro-pneumatic testing and modelling of landfill gas emissions to obtain an experimental site-specific  $k$  value for comparison with the provincial Model value. With the potential recovery of deeply buried organic waste material from the drilling phase of the baro-pneumatic testing project, the City, in collaboration with Alberta Government Agencies contracted the Bio-Processing Group at AITF to physically characterize the recovered waste samples and to determine the laboratory-based degradation rate,  $k_{lab}$  and maximum methane yield,  $B_0$ .

### 1.3.1 Project Objectives

The objective of this study was to:

- (i) Characterize the spent landfill samples with respect to age and depth in landfill matrix, waste composition, moisture content, water activity, metabolizable energy content and diversity of methanogenic microbiota therein;
- (ii) Determine the laboratory-based degradation rate,  $k_{lab}$  and maximum methane yield,  $B_0$  of samples via the BMP assay;
- (iii) Evaluate the influence of the physical and biochemical characteristics of the waste material, and the diversity of microbial communities therein on  $k_{lab}$  and  $B_0$ ;
- (iv) Establish laboratory-based landfill waste degradation data for the Lethbridge area which can potentially be used in the validation of the ALGQ Model estimates or in fine-tuning degradation models relevant to the greenhouse gas mitigation protocols for landfills in Alberta.

### 1.3.2 Site Information

The City of Lethbridge ([Figure 1](#)), the fourth largest city in Alberta, located in the Prairie region of Southern Alberta (Lat 49°42' N, Long 112°49' W, Elevation 929 m) has a fairly cool, semi-arid climate

with annual precipitation averaging 15.0 inches. The city owns a landfill purchased in 2001 for MSW management. The landfill has two sites (Figure 2):

- (i) The closed landfill site: This site was operated from 1975 to 1985;
- (ii) The operational landfill site: This site has been operational since 1985.

By 1985, an estimated 920,000 metric tons of MSW was deposited on the City of Lethbridge landfill sites. This number rose to 2,557,844 metric tons at the close of 2013, the equivalent of 3,410,459 m<sup>3</sup> of waste.

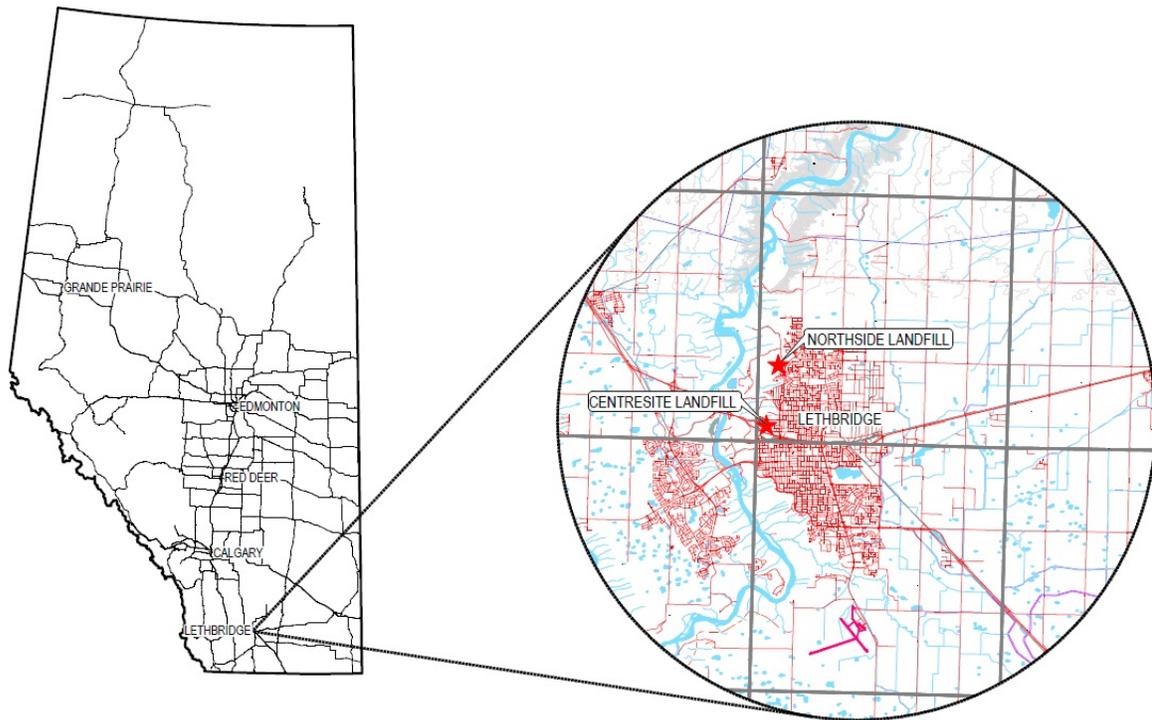


Figure 1. Maps of Alberta and City of Lethbridge showing the closed Northside Landfill (N1/2 7 & SW1/4 18 009-21-W4M) and the operational Centresite Landfill (SW 1/4 6-009-21 W4M) - courtesy of the City of Lethbridge.



### 1.3.3 Sampling

The baro-pneumatic testing project at the City of Lethbridge landfill began in the summer of 2014 with the drilling phase. The City of Lethbridge in collaboration with the Livestock Research Branch used appropriate scientific sampling methods and background information to choose the sites in the landfill for the auger and core drilling of a total of 28 holes: 18 auger drilled in the closed site, and 10 core drilled in the active landfill site. The holes were drilled in order to:

- (i) Install equipment for the estimation of landfill gas generation rates at the operating part of landfill, and
- (ii) Install passive gas vents at the closed part of the landfill to better control gas emission discharges.

This drilling project presented an excellent opportunity to retrieve spent MSW samples (Figure 3 & Figure 4) from various spots on the landfill sites representing different waste ages and depths, for experimental evaluation of residual  $k$  and  $B_0$  as well as the phylogenetics of microbes in the samples.



Figure 3. Auger drilling operation (left) and resultant MSW sample (right) - courtesy of the City of Lethbridge.



Figure 4. Core drilling operation (left) and MSW core drilled sample (right) - courtesy of the City of Lethbridge.

Samples were collected such that materials from each auger and core drilled well would represent the characteristics of that selected sampling location (spot) on the landfill. This approach was intended to ensure that the BMP and characterization results for all analysed samples are representative of the site average.

The auger drilling method yielded a disturbed, loose sample. Each auger drilled sample was sub-sampled into two (one from the top and one from the bottom regions of the drill holes), yielding a total of 18 sub-samples from each of the 1975 and 1985 eras. The core drilling process yielded relatively intact core sample which were readily sub-sampled into three fractions representing 3 depths and/or ages, resulting in a total of 30 samples spanning the 1985 to 2010 era. Each final sub-sample was partitioned into two parts (approximately 1.5kg portions) and quickly placed in airtight, freezer-compatible Ziploc bags; one part was stored at -80 °C for subsequent phylogenetic analysis and the other at -20 °C for BMP tests. The samples for BMP were packed in totes and shipped frozen to AITF where they were stored in a walk-in freezer (-20 °C) until use. All sub-samples for shipment to AITF were prepared in duplicate. Pictures of each sample was taken and archived for reference.

Dating of samples was based primarily on landfill records. Confirmation of these records and estimation of sample age in cases where records were unclear was through legible dated documents in the recovered samples.

## 2. Materials and methods

### 2.1 Equipment and Supplies

Equipment included:

- Two-litre Pyrex glass bottles, with caps encasing a thick rubber septum customized in-house with gas inlet and outlet fittings, were used as reactors for the BMP batch cultures.

- Ritter MGC-1 MilliGas Counters containing silox packing liquid obtained from Ritter GmbH (Bochum, Germany) for biogas volume measurement.
- Microcrystalline cellulose was purchased from Alfa Aesar (Heysham, UK).
- Methanogenic inoculum was obtained from an in-house well-mixed, 80L fed-batch anaerobic digester loaded twice a week with dairy manure and maintained at 8-10% total solids and mesophilic temperature (38°C).
- Cali-5-Bond™ sampling gas bags purchased from Calibrated Instruments Inc. (Hawthorne, NY).
- A CP-4900 Micro-GC gas chromatograph used for gas quality analysis from Agilent Technologies (Santa Clara, CA).
- Etalon-certified compressed gas mixture of nitrogen (79%), carbon dioxide (10%), methane (5%), hydrogen (5%) and oxygen (1%) from Praxair Canada Inc. (Mississauga, ON) was used as standard.
- Nitrogen gas (99.998%) used for the purging of reactors was purchased from Air Liquide Canada Inc. (Edmonton, AB).
- Leco TruSpec CN for the determination of total carbon (TC) was from Leco Instruments ULC (Mississauga, ON).
- KitchenAid® 7 cup food processor, Vita-Mix VitaPrep 3 blender and Cuisinart SG-10 dry grinder were used for substrate homogenization.
- Excella E25 and classic C24 incubator shakers were from New Brunswick Scientific Inc. (Enfield, CT) – now part of Eppendorf Group.
- High temperature-resistant crucibles were from CoorsTek Inc. (Red Deer, AB).
- Precision benchtop weighing balances: Shimadzu UW8200S from Shimadzu Inc. (Columbia, MD); EC30 and Adventurer® from Ohaus Corporation, (Parsippany, NJ); and XS204 Excellence Analytical balance from Mettler Toledo Canada (Mississauga, ON).
- Benchtop lab oven OV-490A-2, maintained at 105°C, was from Blue M Electrical Company (Island, IL) – now LR Environmental Equipment Inc.
- Bench top muffle furnace, Omegalux LMF-3550 was from Omega Engineering Inc. (Stamford, CT).

## 2.2 Methods

### 2.2.1 Sample Preparation and Mass Distribution Analysis

Substrates were allowed to thaw overnight at 4°C and then weighed to record the mass received, spread out on clean plastic film in a fume hood and separated into the following categories:

- i) Paper
- ii) Wood
- iii) Inerts: included glass, rocks, metal, ceramic, plastics and other non-biodegradable, excluding fine sand, silt, clay, soil which were difficult to separate because they stuck tightly to organic material.

- iv) 'Other organics' fraction: included all residual materials after the removal of wood, paper and visible inerts. Fine sand, silt, clay and soil were included in the 'other organics' fraction with degradable organic material such as plant and animal residue, yard clippings/garden waste, fabric, etc. The term 'other organics' was only used for simplicity and strictly in the context hitherto.
- v) The BMP fraction (the paper, fabric and 'other organics' fractions re-combined for BMP testing)

Pictures of sample received and of each category were taken and catalogued. The wet weight of each category and its corresponding fraction of the whole were recorded. The inerts were re-sealed in the original sample Ziploc bags and frozen at -20°C. Similarly, the wood fraction was stored at -20°C in new Ziploc bags. Paper was shredded by hand into approximately 1 inch size and mixed well to ensure homogeneity i.e. prevent similar paper types (e.g. office paper, newsprint, cardboard, construction paper, etc.) from clumping together (Figure 9). Fabric was similarly cut into ~1 inch size using a pair of scissors and mixed well to evenly distribute the different types of fabric. The 'other organics' fraction was mixed by hand. Paper, fabric and 'other organics' constituted the portion of the sample used for the BMP assay. Twenty-five percent of each of shredded paper, fabric and 'other organics' was withdrawn, pooled/mixed and rigorously homogenized in a series of kitchen food processors and blenders for the determination of total solids (TS), volatile solids (VS), and total carbon (TC) content. Samples for TS and VS were analysed immediately as described in section 3.2.3, whereas those for TC were stored at -20°C until analysed. The residual 75% of each of paper, fabric and 'other organics' were stored separately at -20°C until use in the BMP assay. Four wood samples which match the following categories were selected, washed with nanopure water to remove any non-wood residual organic material, air-dried in a fume hood for 4 hours and frozen at -20°C until use in BMP assay. The sample categories included: 6 year old mixture of construction wood and tree/shrub branches from operational landfill (sample ID# MH2-D 4-3-20); 15 year old construction wood from operational landfill (ID# P2S-D 1-3-15); 17 year old tree/shrub branches from operational landfill (ID# 1-1-7) and 35 year old mixture of construction shreds and engineered wood from closed landfill (ID# G12 2-12 to 13m). For comparison with spent wood material from the landfill, shavings and chips (1-2cm) from fresh (non-landfilled) 1" x 4" softwood lumber were also prepared and frozen for BMP assay.

### 2.2.2 Water Activity

The water activity of samples was analyzed by Dr Tim Reuter at the Livestock Research Branch, Alberta Agriculture, Lethbridge using an  $a_w$  Lab Set F instrument (Novasina AG, Lachen, Switzerland) according to the manufacture instructions.

### 2.2.3 Dry Matter Determination: Total Solids (TS) and Volatile Solids (VS)

The total solids (TS) and volatile solids (VS) were measured according to standard methods for the examination of water and waste water.<sup>47</sup> Samples were dried in a forced-air oven at 105°C for 16 to

24 h After TS determination, the VS content was determined by combusting the bone-dry samples in a muffle furnace at 550°C for 2.5 h.

#### 2.2.4 Total Carbon (TC)

Frozen samples (-20°C) were allowed to thaw at room temperature and the wet sample analyzed using Leco TrueSpec CN for Total carbon as specified by the manufacturer.

#### 2.2.5 Biochemical Methane Potential (BMP)

The BMP assay determines the potential methane yield and biodegradability of a feedstock. BMP assay was done according to ASTM D 5210-92(2007)<sup>48</sup> and DIN 38414-S8<sup>49,50</sup> standard methods for biochemical methane potential test. The test was performed at mesophilic temperature (38°C) in 2L glass bottles containing a 1L reaction volume at 5% total solids content. The inoculum was collected and allowed to cool to room temperature overnight before loading into reactors and a substrate to inoculum VS ratio of 0.6 was used (which, from our in-house experience provides sufficient bioactive biomass for anaerobic degradation of spent organic material while ensuring that the inoculum is not overwhelmed by substrate). The total amount of substrate loaded in the reactor was the sum of the weighted fractions of the sample constituents (i.e. paper, fabric and other organics). Nanopure water was used to make up the reaction volume to 1L. All culture inputs (substrate, inoculum and water) were loaded strictly on weight basis. It was assumed that 1g sample is equivalent to 1ml as is the case with water. The reactors were purged for 10 min through the inlet port on the lid to ensure an oxygen-free environment in the culture headspace. Thereafter, each inlet port was quickly sealed and the outlet connected to gas bags through Ritter MilliGas Counter MGC-1 for volume measurement. To monitor inoculum performance, a positive control containing inoculum and microcrystalline cellulose was included in each test batch. According to Standard ASTM D 5210-92 and Standard DIN 38414-S8, the inoculum must convert at least 70% of the reference substrate, cellulose, to biogas during the incubation period. All samples and controls were run in triplicate.

The gas line linking each MilliGas Counter to a sampling gas bag was connected through a 3-way gas valve which could deliver biogas from the reactor to the gas sampling bag or from the gas bag to the GC for gas quality analysis, while maintaining a closed system from the culture bottles through the bag and MilliGas Counter at all times. A pre-set threshold biogas volume of 400mL in the gas sampling bags was programmed to trigger biogas quality analysis followed by bag emptying (venting). The experimental set-up (Figure 5) was PLC-controlled, enabling automation of valve functioning, GC analysis, bag venting, data integration and automatic data logging in real time via Siemens WinCC software.

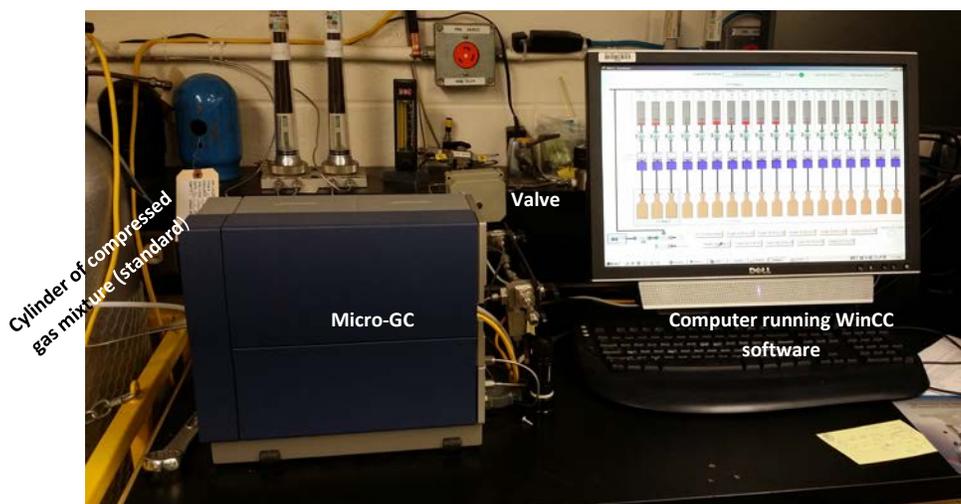
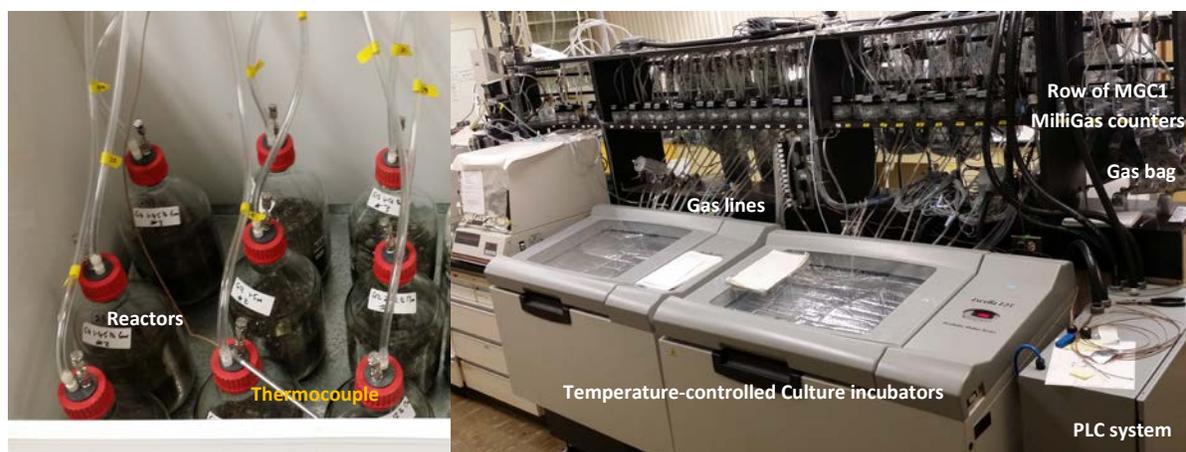


Figure 5. The BMP experimental set-up

The quality of biogas from the culture headspace was measured using CP-4900 Micro-GC which carries two heated-injector equipped analytical channels and a thermal conductivity detector (TCD). One of the channels houses a 10m Molsieve column for the resolution of hydrogen ( $H_2$ ), oxygen ( $O_2$ ), nitrogen ( $N_2$ ) and methane ( $CH_4$ ); while the other carries a 10m PPU column for the resolution of carbon dioxide ( $CO_2$ ) and hydrogen sulfide ( $H_2S$ ). Accumulated gas volumes were normalized to standard conditions (temperature of 273.15 K and pressure of 101.325 kPa). The cumulative gas volumes per unit volatile solids from each substrates (including cellulose control) were corrected by subtracting that of the inoculum controls from the former. The BMP test was stopped when the daily biogas production was below 1% of the total accumulated biogas, in accordance with Standard DIN 38414 - S8. It took 30 days or more for this condition to be met.

### 2.2.6 Specific Methanogenic Activity (SMA)

For each landfill waste sample, specific methanogenic activity (SMA) was monitored by quantifying the amount of methane produced per unit amount of volatile solids in culture and dividing by the

time of incubation under anaerobic conditions. The amount of inoculum was kept constant by maintaining the substrate/inoculum volatile solids ratio at 0.6.

### 2.2.7 Estimation of maximum methane yield, $B_0$ and laboratory-based degradation rate, $k_{lab}$

With data acquired from the BMP assay, the laboratory-based degradation rate,  $k_{lab}$  and maximum methane yield,  $B_0$  respectively, were calculated using a non-linear, first-order model-based fitting tool developed by Jensen *et al.* 2011.<sup>44</sup> For comparison, degradation rates were also estimated via the linearized first-order fit represented in eq.4

$$\ln\left[\frac{B_0 - B}{B_0}\right] = kt \quad \text{eq. 4}$$

where  $B_0$  is the maximum attainable methane yield under the defined experimental conditions, and  $B$ , the methane yield at any given time during the test.

### 2.2.8 Quality Control

Gas flow meters were calibrated in accordance with the manufacturer's specifications before use, the MicroGC was calibrated before each run with Etalon-certified compressed gas mixture standard; precision benchtop weighing balances calibrated annually and digital temperature reading on incubators and ovens checked against thermometers placed in each of the chambers. Leco TruSpec CN for the determination of total carbon (TC) was regularly maintained and calibrated against low, medium and high carbon standards prior to use in each run as specified by the manufacturer. In all cases where experiments were performed in replicates, a confidence limit of mean $\pm$ 5% was set for data acceptance, with any outlier rejected. BMP tests were performed according to the ASTM D 5210-92<sup>48</sup> standard method for biochemical methane potential test.

### 3. Results

#### 3.1 Landfill Sample Characterization

Table A1 in Appendix 1 shows the samples as received and Table A2 in Appendix 1 and Figure 6 – Figure 10 represent the sorted fractions of samples (i.e. ‘other organics’, paper, wood and visible inerts). The reader is reminded that, in addition to degradable organic material, the ‘other organics’ fraction also included fine sand, silt, clay and soil which are non-degradable. The materials were very heterogeneous in nature, with varying amounts of the components. In some cases, wood, paper or visible inerts were completely absent as shown in Table A2 in Appendix 1. Generally, moisture appeared to be retained mainly in the ‘other organics’ fraction and most samples appeared fairly dry and loose, with the extent of degradation far higher for the ‘other organics’ fraction (with the exception of fabric components) than for paper and wood.

The ‘other organics’ fraction, the most readily biodegradable, was evidently spent (Figure 6) in all but a few cases. In the cases where materials did not appear degraded, dark-looking herbaceous leaves, pine-like branches/needle leaves, green-looking material with semblance to garden/yard clippings and cattle manure-like material were visible (Figure 7). The cattle manure-like material had a characteristic ammonia-latent cattle manure odour. The relatively less degraded ‘other organics’ samples generally appeared to retain a high amount of moisture compared to those which were spent, with the exception of a few spent samples that were water saturated. The water saturated, spent samples had a dark humic appearance with a dense, sticky, clay-like clumpy feel. In these samples, the moisture was fairly evenly distributed among all components of the whole (other organics, paper, wood and visible inerts).



Figure 6. Examples of some highly spent ‘other organics’ fraction of some samples. Top from left to right to right: sample ID# MH2-D 4-3-10, 6 years old; sample ID# 3-3-20, 14 years old; sample ID# P2S-D 1-3-12, 19 years old. Bottom from Left to right: sample ID # 2-3-25, 27 years old; sample ID# G4 2-13.5 to 15m, 36 years old sticky clay-like lump; sample ID# G13 3-19m, 40 years old.



Figure 7. Examples of 'other organics' fraction of some samples with limited degradation. Top left: sample ID# MH2-D 4-3-20 – visible greenish material similar in appearance to garden clippings and leaves, 6 years old. Top centre: sample ID# P2S-D 1-3-7 – greenish material similar in appearance to garden clippings, 16 years old. Top right: sample ID# P2S-D 1-3-26m – organic material rich in assorted undegraded fluffy substances and leaves, 28 years old. Bottom left: sample ID# G10alt to G11 1-6.8 to 8m – dry, soil-rich organic material with visible dry leaves, 36 years old. Bottom centre: sample ID# G12 2-12 to 13m – damp, soil rich organic material with visible leaves, 35 years old. Bottom right: sample ID# G12 3-14.5m – visible cattle manure-like material rich in of fat tissue-like lumps, 37 years old.

The paper fraction appeared relatively less degraded than the 'other organics' fraction. In most samples, the paper fraction, especially the newsprint, office paper and product packaging paper appeared fairly dry and well preserved, indicating limited degradation in more than 70% of the samples analysed (Figure 8 and Table A2 in Appendix 1). Compositional variations in the paper fractions were also evident, with some comprised mainly of cardboard paper, construction paper, newsprint, office paper, or various mixtures thereof. Before use in the BMP assay, the paper samples were hand shredded into 2-3 cm size to ensure homogeneity as shown in Figure 9.

The type of wood material (e.g. branches, construction wood and engineered wood) varied significantly among the samples, although construction wood shreds/chips appeared fairly predominant (Figure 10 and Table A2 in Appendix 1). Wood material did not show visible evidence of decomposition, except in a few cases involving mainly branches.

A wide variety of materials constituted the visible inert fraction (Table A2 in Appendix 1) including glass, metal, hard plastic, plastic bags and wraps, synthetic fabric, ceramic and stones/rocks. With the exception of synthetic non-degradable fabric which retained significant amount of moisture, the visible inerts generally retained the least amount of moisture compared to other fractions. As expected, this fraction showed no visual evidence of biodegradation.



Figure 8. Examples of sample paper fractions with varying degrees of degradation. Top left: sample ID# MH2-M 4-2-10-1 – moist, relatively undegraded Tim Hortons cup (foreground) and spent cardboard paper (background), 4 years old. Top centre: sample ID# 2-2-14 – dry, relatively undegraded cardboard, newsprint and office paper, 20 years old. Top right: sample ID# 2-3-25 – moisture-saturated, highly spent cardboard (background) and less degraded stacks of magazine print (foreground), 27 years old. Bottom left: sample ID# G5 2-10.5 – very moist, highly spent cardboard paper, 42 years old. Bottom centre: sample ID# G9alt 1-5m – dry, relatively undegraded newsprint, cardboard and office paper, 33 years old. Bottom right: sample ID# G10 alt to G11 2-12m – very dry, relatively undegraded newsprint, cardboard and office paper, 40 years old.



Figure 9. Example of hand-shredded mixed paper, including cardboard, construction, newsprint and office paper types.



Figure 10. Examples of wood fractions of samples showing the varieties of wood types. Top left: sample ID# 1-1-7 – small tree branches with no signs of degradation, 17 years old. Top centre: sample ID# 2-2-14 –engineered wood showing no signs of degradation, 20 years old. Top right: sample ID# MH2D 4-3-20 – significantly degraded construction wood shreds, 6years old. Bottom left: sample ID# G12 2-12 to 13m – relatively undegraded mixture of construction wood shreds and engineered wood, 35 years old. Bottom centre: sample ID#: P2S-D 1-3-26, compact construction wood chips, with little signs of degradation, 28 years old. Bottom right: G4 2-13.5 to 15m - very moist, highly degraded branches and wood shreds, 36 years old.

## 3.2 Sample Mass Distribution Analysis

### 3.2.1 Wet mass distribution

Results of the wet mass distribution analysis are shown in (Figure 14 & Figure 15). For each of the component fractions a wide range of variation was observed, with the ‘other organics’ fraction representing the widest of these. The other organics fractions constitute about half the average wet weight of the samples analysed, the rest being shared equally amongst the other three categories (Figure 11). Of the 45 samples analysed, 20 had a compositional ‘other organics’ proportion of 50% or more on wet weight basis (Figure 12). There was no visible similar or inverse trend between the ‘other organics’ with sample age or depth in the landfill. Similarly, no such trends was observed between paper, wood and visible inerts with age or depth (Figures A1-A3 in Appendix 1). The sample water activity was in the range 0.69-0.96, with only 5 of the 45 samples having a water activity value > 0.94 (the optimum for growth of most bacteria). Furthermore, there was no clear correlation between  $a_w$  and moisture content. However, the moisture content of the BMP fraction was inversely related to that of the visible inerts (Figure 13). Overall, results from the moisture content, water activity, compositional and wet mass distribution analyses revealed variations with respect to sample age and depth, as well as with the status of the landfill (operational or closed).

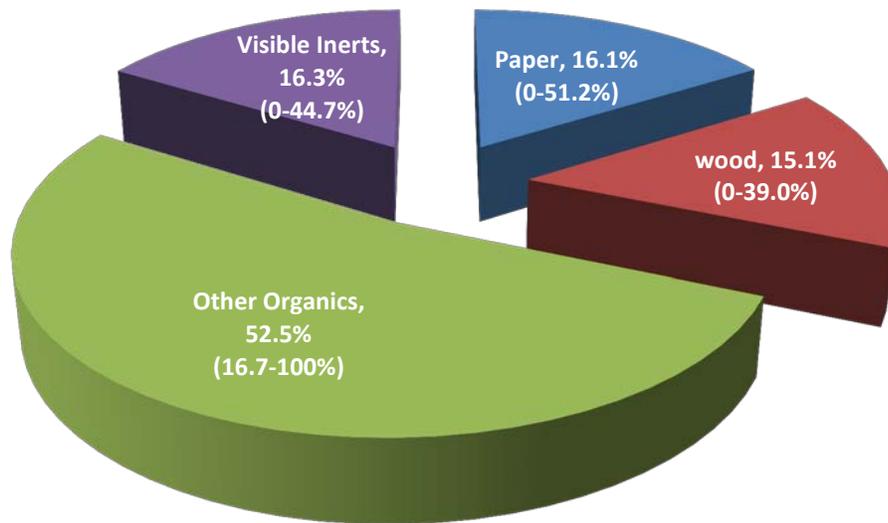


Figure 11. Sample composition (dry mass basis)

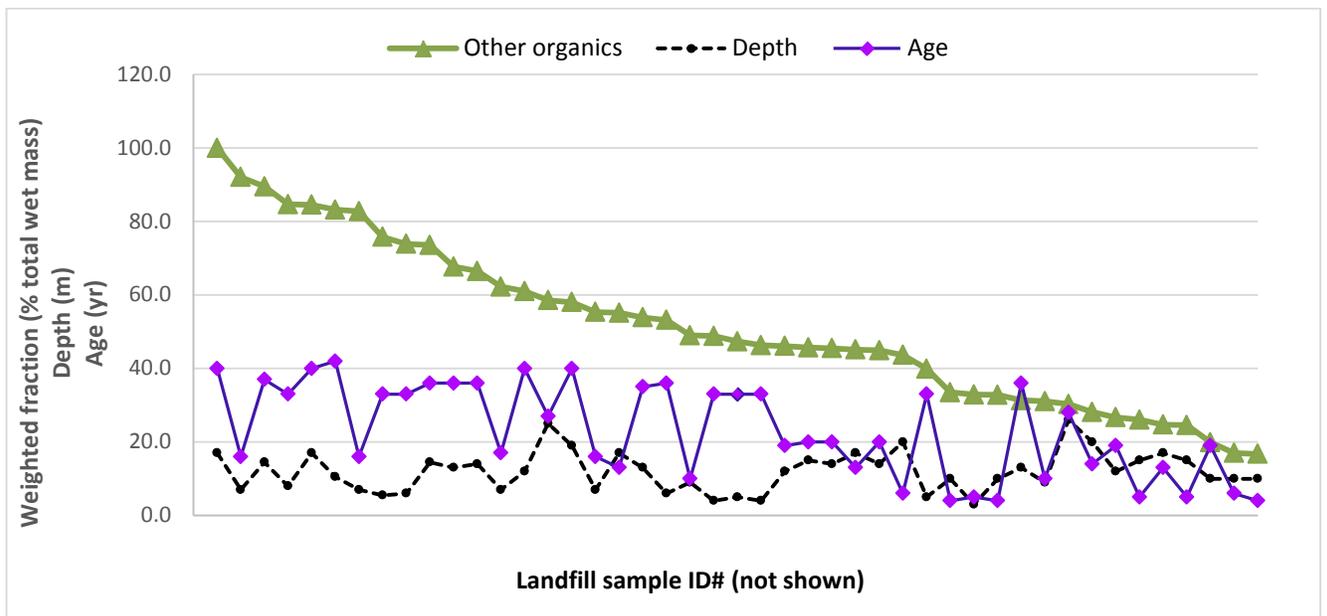


Figure 12. Trends of sample 'other organics' fraction (wet mass basis) and age and depth in the landfill.

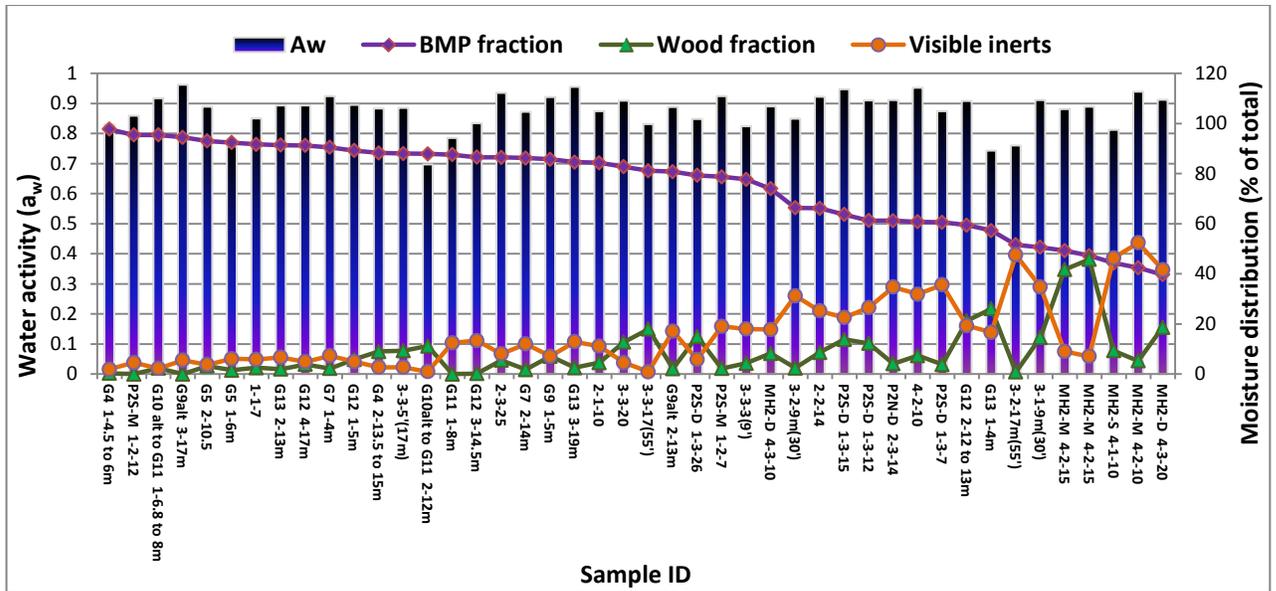


Figure 13. Sample water activity ( $a_w$ ) and moisture content distribution for the BMP, wood and visible inerts fractions. The fraction of moisture contained in each of the waste components was calculated by dividing the total moisture content in each waste component with the total moisture content of the whole MSW sample. Total moisture content in each BMP fraction was calculated from results from TS analysis and the total wet mass of the BMP fraction. The total moisture content of each wood fraction was assumed to be the average obtained from result of TS analysis of 8 randomly selected wood samples and the wet mass of the wood fraction of each MSW sample. The total moisture content of the visible inerts was calculated as the difference between the total moisture content of each sample and those of the BMP and wood fractions.

### 3.2.2 Dry mass distribution

Dry mass distribution results are represented in Figure 14 – Figure 17. Average dry mass proportions appear to be very similar to the corresponding wet mass values for the ‘other organics’ and paper fractions. However, the value increased to 23% (a 30% increase) and dropped to 13% (a 13% drop) for visible inerts and wood, respectively (Figure 14). In decreasing order, the average dry weight proportion of the various components was as follows: ‘other organics’, paper, visible inerts and wood fractions. The paper and ‘other organics’ dry mass content in the BMP fractions were spread through a very wide range: 0-73% and 27-100%, respectively, and averaged 25% and 75% (Figure 15). The relationship between the BMP fraction with age and depth is represented in Figure 16. In more than 84% of the samples analysed (37 of 45), ‘other organics’ exceeded 50% of the BMP fraction (i.e. ‘other organics’ and paper) - Figure 17. The ‘other organics’ fraction showed a weak linear correlation with age but not with depth. Paper and total BMP fraction did not show any clear relationship with sample age and depth (Figure 16 & Figure 17 and Figures A1-A5 in Appendix 1).

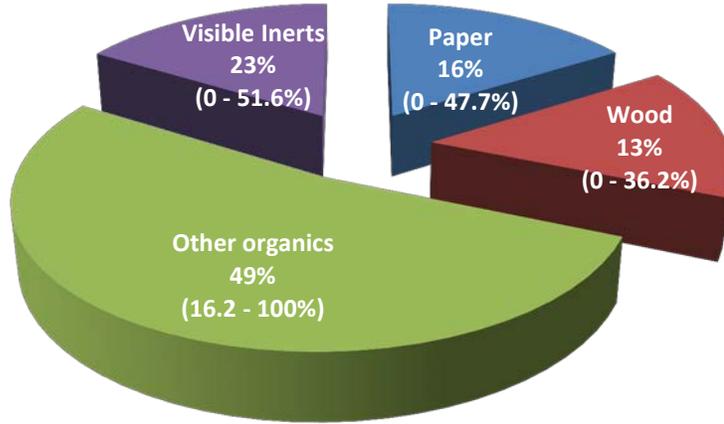


Figure 14. Sample composition, dry mass basis

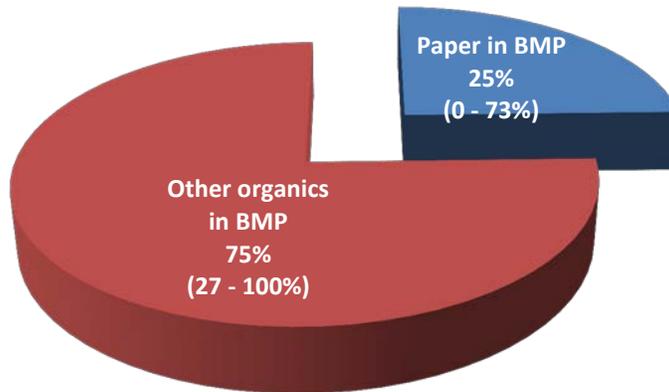


Figure 15. Average dry mass of paper and other organics in fraction used for BMP assay

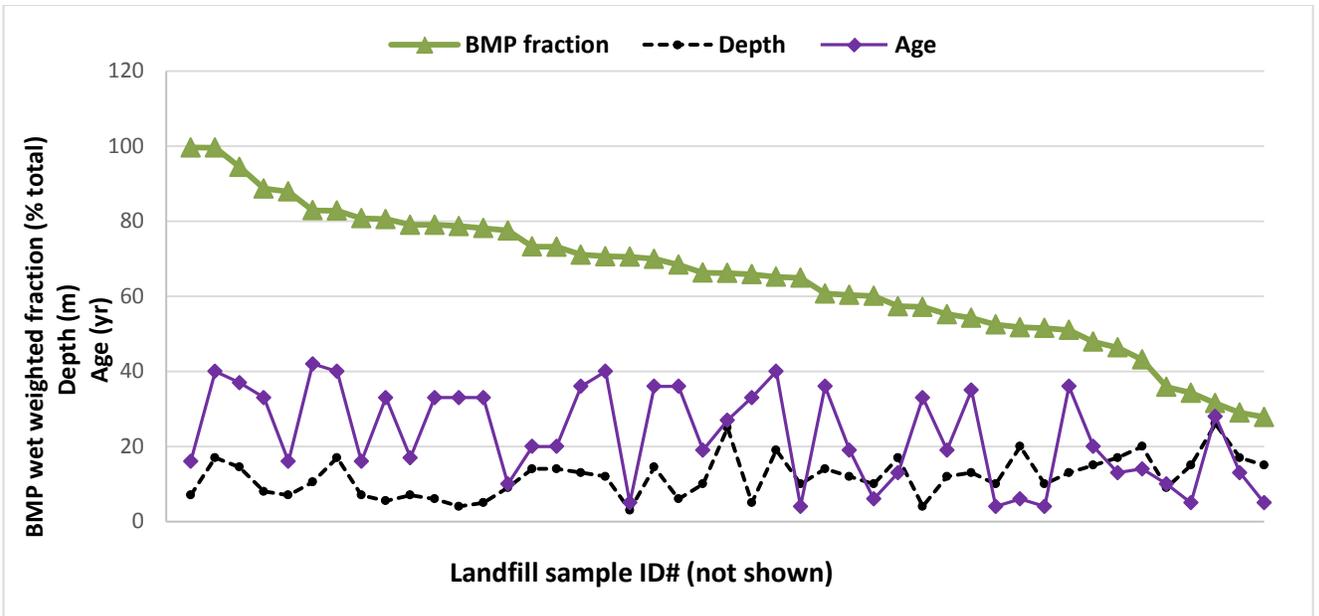


Figure 16. Trends of the BMP fraction (dry weight basis), age and depth for the landfill samples. BMP fraction includes the 'other organics' and paper.

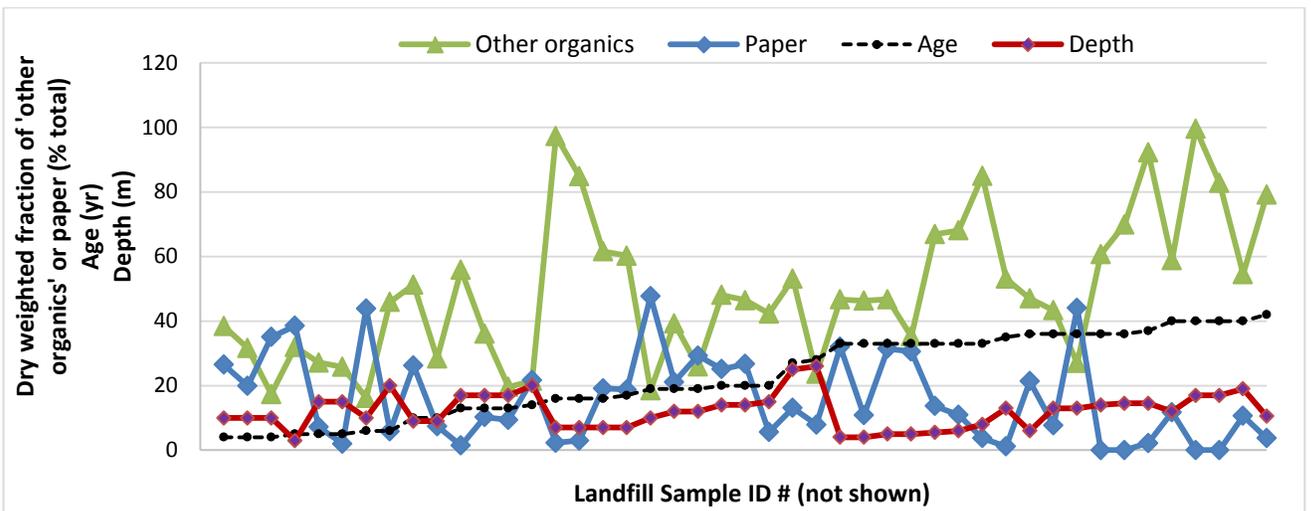


Figure 17. Trends of paper and 'other organics' in BMP fraction (dry mass content), age and depth of the landfill samples.

The physical characteristics of the landfill samples are summarized in Table 1. Samples from the closed landfill were on average 10 years older than those from the operational landfill. For all other physical parameters analyzed, there was no difference between the characteristics of sample from the operational landfill and those from the closed landfill.

Table 1. Summary of physical characteristics of MSW samples obtained from two landfills in the City of Lethbridge. The operational and closed landfills are compared. Values are represented  $\pm$  standard deviation.

Parameter (Average)	Landfill	
	Operational	Closed
Sample age (yr)	13.4 $\pm$ 7.1	36.1 $\pm$ 3.0
Sample depth (m)	13.0 $\pm$ 5.6	10.7 $\pm$ 4.9
Water activity - $a_w$	0.88 $\pm$ 0.05	0.87 $\pm$ 0.07
Moisture (% wet weight)	23.7 $\pm$ 8.8	20.5 $\pm$ 10.2
Dry matter (% wet weight)	76.3 $\pm$ 8.8	80.2 $\pm$ 11.9
Average wood dry matter (% dry weight)	64.8 $\pm$ 1.2	64.5 $\pm$ 2.3
BMP fraction (% dry weight)	58.6 $\pm$ 18.8	73.6 $\pm$ 13.2
'Other organics' in whole sample (% dry weight)	40.3 $\pm$ 20.3	61.2 $\pm$ 19.8
Paper in whole sample (% dry weight)	18.2 $\pm$ 13.4	12.4 $\pm$ 13.3
Wood in whole sample (% dry weight)	16.1 $\pm$ 10.6	7.7 $\pm$ 8.3
Visible inerts in whole sample (% dry weight)	25.4 $\pm$ 15.0	18.6 $\pm$ 11.0
'Other organics' in BMP fraction (% dry weight)	68.6 $\pm$ 20.6	82.6 $\pm$ 18.4
Paper in BMP fraction (% dry weight)	31.4 $\pm$ 20.6	17.4 $\pm$ 18.4
Volatile organic solids (% dry weight of whole MSW sample)	24.2 $\pm$ 1.4	20.6 $\pm$ 1.8
Average volatile solids in wood (% dry weight)	95.3 $\pm$ 0.2	92.6 $\pm$ 0.5

### 3.3 Methane Yield

#### 3.3.1 Methane Yield (B)

In this study, methane yield at any given time (B) was assessed per unit amount of:

1. Volatile organic solids present in the BMP fraction, expressed in NmL CH<sub>4</sub>/gVS. This was to provide information about the portion of the BMP fraction converted to methane.
2. Whole MSW sample, organic and non-organic solids inclusive, expressed in Nm<sup>3</sup> CH<sub>4</sub> per dry megagram MSW (Nm<sup>3</sup> CH<sub>4</sub> /dry Mg MSW). This enables assessment of how much of the whole MSW was converted to methane.

Based on the volatile organic solids in the BMP fraction, the calculated maximum CH<sub>4</sub> yield (B<sub>0</sub>) and the measured CH<sub>4</sub>, biogas and CO<sub>2</sub> from the BMP assay are shown in Figure 18. Three samples did not produce any measurable amount of methane. While most samples with measurable methane production had B<sub>0</sub> values evenly spread in the range 108 – 505 NmL CH<sub>4</sub>/gVS, one sample was a clear outlier: sample ID# G12 3-14.5m from the closed landfill with an exceptionally high B<sub>0</sub> value of 749 NmL CH<sub>4</sub>/gVS. As shown by the polynomial fit in Figure 18 and linear regression in Figure A6 in Appendix 1, correlation (R<sup>2</sup> = 0.4386) was observed between B<sub>0</sub> and the maximum Specific methanogenic activity (SMA), which is presented in greater detail in section 3.3.2 below. The measured CH<sub>4</sub>, CO<sub>2</sub> and total biogas were in the range 0 – 573, 0 – 278 and 0 – 851 NmL CH<sub>4</sub>/gVS respectively.

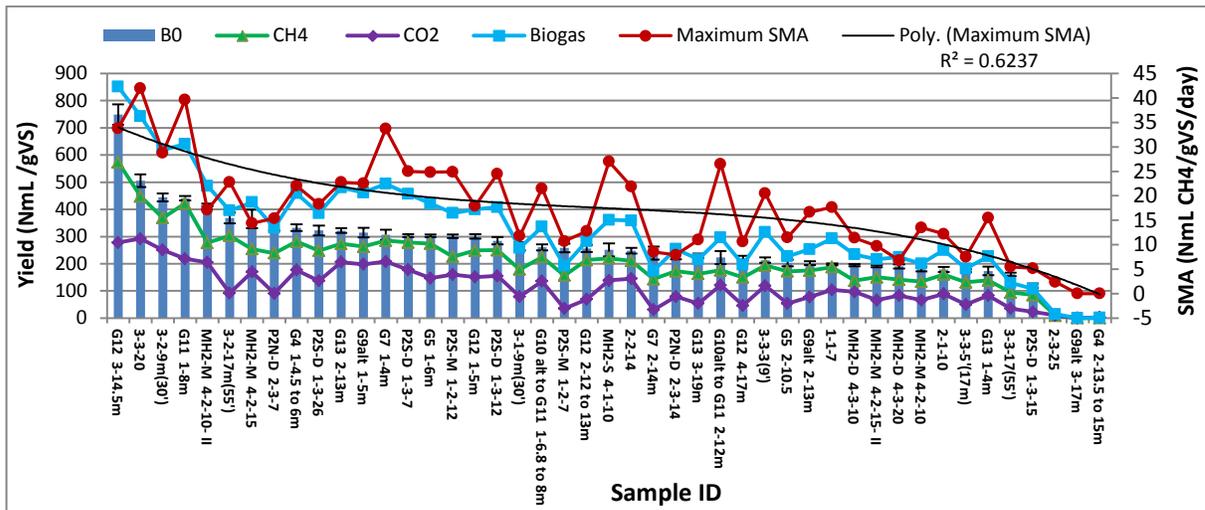


Figure 18. Trends of methanogenic activity, methane, carbon dioxide and biogas yield for the landfill samples.

Maximum methane yield results based on the whole MSW samples are shown in (Figure 19). With the exception of one outlier sample ID# G12 3-14.5m from the closed landfill which had a  $B_0$  value ( $443 \text{ Nm}^3 \text{ CH}_4/\text{dry Mg MSW}$ ) at least 2.5 times that of any other sample from both landfills, samples with measurable methane yield had  $B_0$  values in the range  $14 - 175 \text{ Nm}^3 \text{ CH}_4/\text{dry Mg MSW}$  for the operational landfill and  $13 - 159 \text{ Nm}^3 \text{ CH}_4/\text{dry Mg MSW}$  for the closed landfill. The maximum methane yield from the outlier was noticeably higher than that for cellulose. But for the outlier, the general distribution pattern of  $B_0$  was apparently similar in both landfills. One sample (ID# 2-3-25) from the operational landfill and two (ID# G9alt 3-17m and ID# G4 2-13.5 to 15m) from the closed landfill had  $B_0$  value of  $0 \text{ Nm}^3 \text{ CH}_4/\text{dry Mg MSW}$ . Sample age did not affect the total available carbon, SMA and maximum methane yield. However, sample depth had a negative impact on degradation rates and maximum methane yield.

Maximum methane yields for wood samples were as shown in Table 2. Taking the low average fraction of wood in MSW samples (16.1% and 7.8% for the operational and closed landfills respectively) into consideration, the average maximum methane yield from wood per dry ton of MSW was calculated to be  $6.7 \text{ Nm}^3$  and  $3.2 \text{ Nm}^3$ , respectively.

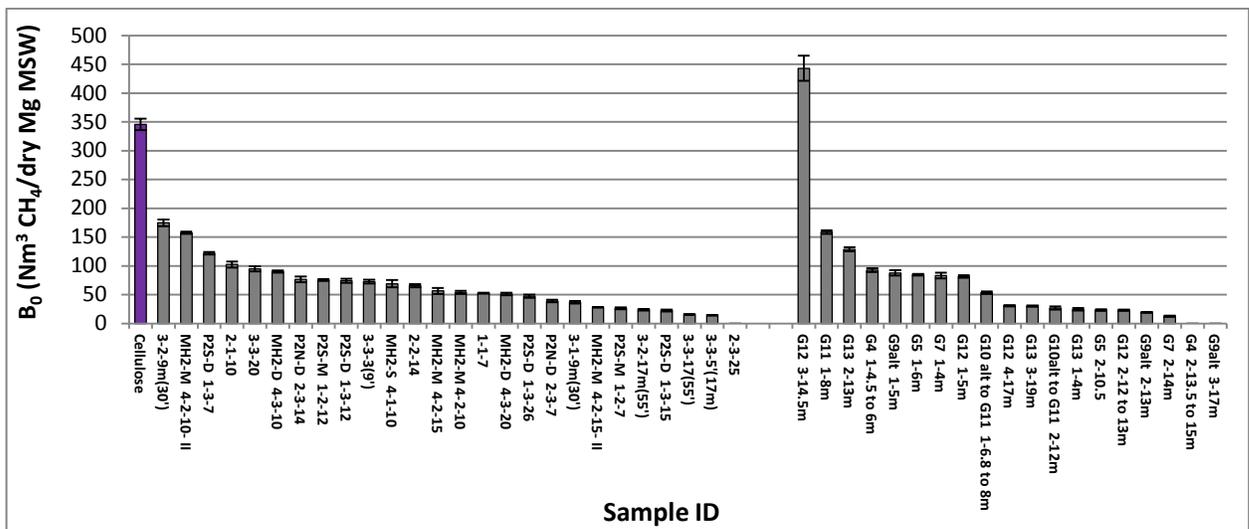


Figure 19. Maximum methane yields for landfill samples. Clusters of bars on the left and right in chart represent the operational and closed landfills, respectively. The purple bar represents the maximum methane yield from cellulose (positive control). Error bars represent 95% confidence limit.

**Table 2. BMP and maximum methane yields for wood samples from the operational and closed landfills in the City of Lethbridge. Values are represented  $\pm$  95% confidence limit.**

Sample ID	Operational Landfill	Closed landfill
Wood B <sub>0</sub> - ID# 1-1-7 (Nm <sup>3</sup> CH <sub>4</sub> /dry Mg wood)	52.9 $\pm$ 0.7 <sup>s</sup>	na
Wood B <sub>0</sub> - ID# P2S-D 1-3-15 (Nm <sup>3</sup> CH <sub>4</sub> /dry Mg wood)	21.9 $\pm$ 1.0 <sup>s*</sup>	na
Wood B <sub>0</sub> - ID# MH2-D 4-3-20 (Nm <sup>3</sup> CH <sub>4</sub> /dry Mg wood)	52.1 $\pm$ 1.3 <sup>s</sup>	na
Wood B <sub>0</sub> - ID# G12 2-12to13m (Nm <sup>3</sup> CH <sub>4</sub> /dry Mg wood)	na	37.4 $\pm$ 1.3 <sup>s*</sup>
Average Wood B <sub>0</sub> (Nm <sup>3</sup> CH <sub>4</sub> /dry Mg wood)	42.3 $\pm$ 1.0	nd
Average Wood B <sub>0</sub> - both landfills (Nm <sup>3</sup> CH <sub>4</sub> /dry Mg wood)	41.3 $\pm$ 1.1	
Fresh soft wood B <sub>0</sub> (Nm <sup>3</sup> CH <sub>4</sub> /dry Mg wood)	42.1 $\pm$ 15	

<sup>s</sup>Values are represented  $\pm$  95% confidence interval.

<sup>\*</sup>Values represented for wood are BMP values measured in the laboratory after 70 days because the methane production curves from wood BMP cultures did not reach the plateau phase to fit in the first-order rate/B<sub>0</sub> estimation model (the 70 day incubation time was not long enough).

nd: not determined due to irregular degradation curve or insufficient amount of data as CH<sub>4</sub> production curves did not plateau to fit on the B<sub>0</sub> estimation model.

na: not applicable

### 3.3.2 Methanogenic Activity (MA)

Specific methanogenic activity (SMA) estimates the activity of methanogenic microbial groups present in anaerobic inoculum (and in the context of this study, on the methanogenic microbiota contained in the landfill samples). It is expected that samples in this study may contain *in-situ* methanogenic microbiota which can have additive effect with seed inoculum on methane production. SMA results obtained in this study are represented in [Figure 20](#). The average SMA and maximum SMA were not influenced by sample age or depth.

Three samples recorded average SMA values of approximately zero ( $\leq 0.4$  NmL CH<sub>4</sub>/gVS/day) and did not yield methane. Two of these samples (ID# G4 2-13.5to15m, 36 years old and ID# G9alt3-17m, 40 years old) were from the closed landfill and the third (ID# 2-3-25, 27 years old) was from the operational landfill. The average SMA for all other samples were in the range 2.3 and 15.4 NmL CH<sub>4</sub>/gVS/day, and a mean of 6.7 NmL CH<sub>4</sub>/gVS/day. Only two samples had average SMA values higher than 10 NmL CH<sub>4</sub>/gVS/day, one (G12 3-14.5m) of which was from the closed landfill and the other (3-3-20) from the operating landfill. For samples with measurable methane production, maximum SMAs were between 2.4 and 42 NmL CH<sub>4</sub>/gVS/day. Of the methane-producing samples, 37% were below 5 NmL CH<sub>4</sub>/gVS/day and 23% higher than 25 NmL CH<sub>4</sub>/gVS/day. It took an average of 7 days for the cultures to attain maximum SMA. Samples with average SMA values less than 5 NmL CH<sub>4</sub>/gVS/day took longer to attain maximum SMA.

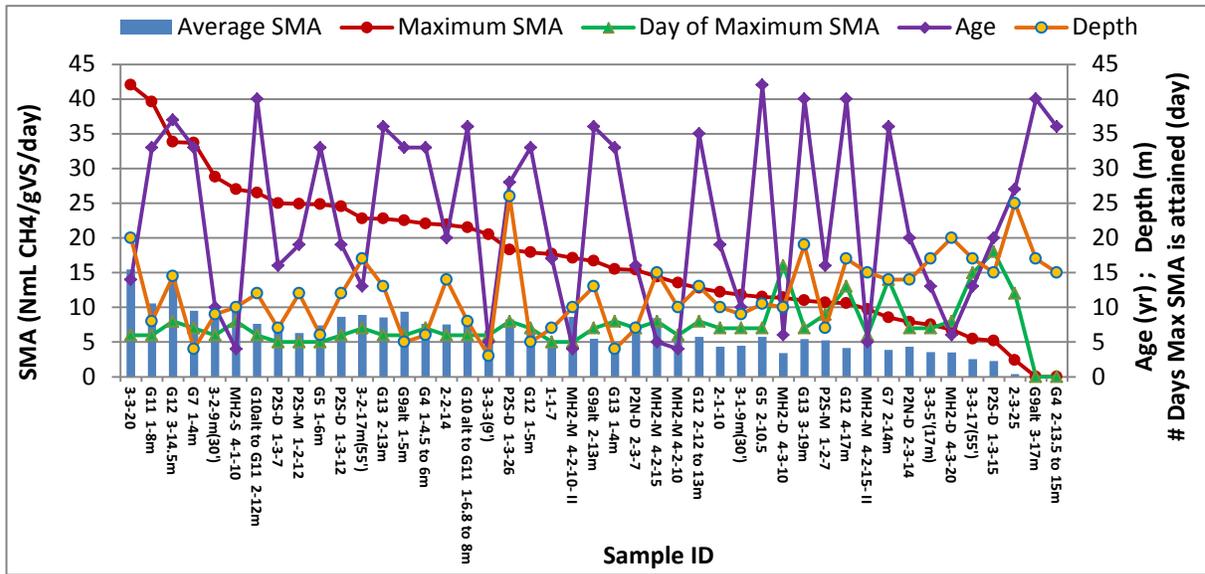


Figure 20. Trends of sample methanogenic activity and age and depth in landfill.

### 3.3.3 Effects of sample age, depth and moisture on maximum methane yield ( $B_0$ )

While the combined data for both landfills did not show correlation between age and depth, data from each landfill revealed the expected, albeit weak correlation between sample age and depth (Figures A7-A11 in Appendix 2). Data from both landfills was collectively analyzed to assess the influence of sample age, depth and in-situ moisture content on  $B_0$  as shown in Figure 21– Figure 29. Neither the sample age nor depth within the landfill matrix had any influence on  $B_0$  (Figure 21 & Figure 22). A weak linear correlation between MSW sample moisture content and  $B_0$  was observed (Figure 23). However, this correlation was not evident when only the volatile fraction of the waste was taken into account (Figure 23). However, when data from each landfill was evaluated separately (Figures A8 & A9 in Appendix 2), it was observed that sample age and depth had a negative impact on maximum methane yield. Water activity did not show any correlation to  $B_0$  either (Figure 24).

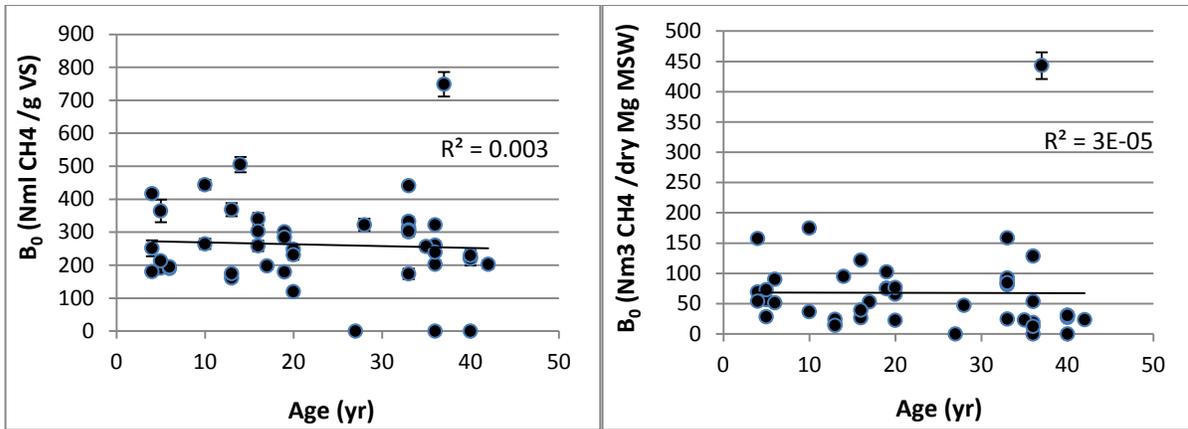


Figure 21. Maximum methane yield ( $B_0$ ) with sample age. Chart on the left:  $B_0$  expressed in  $\text{Nml CH}_4 / \text{g VS}$ . Chart on the right:  $B_0$  expressed in  $\text{Nm}^3 \text{CH}_4 / \text{dry Mg MSW}$ . Linear regression fit is indicated in both charts. Error bars represent 95% confidence limit.

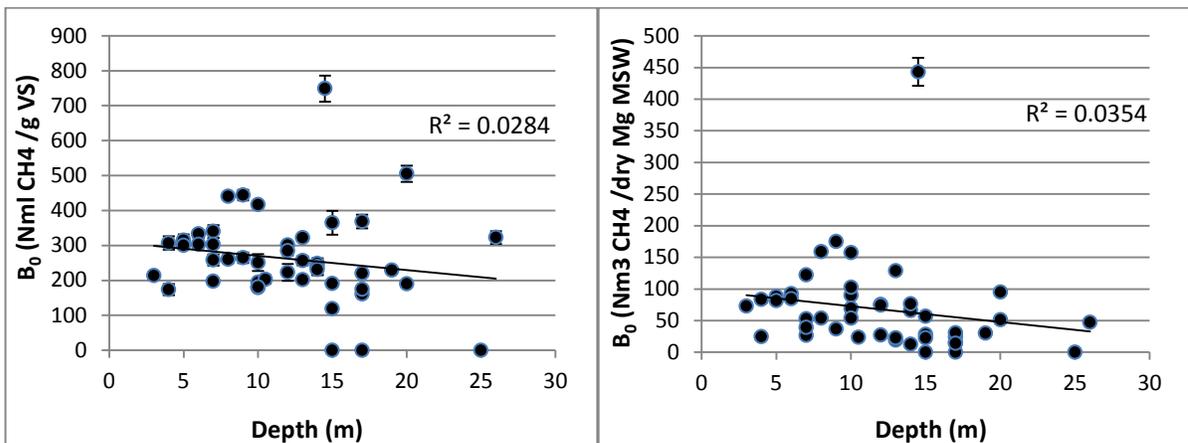


Figure 22. Maximum methane yield ( $B_0$ ) with sample depth. Chart on the left:  $B_0$  expressed in  $\text{Nml CH}_4 / \text{g VS}$ . Chart on the right:  $B_0$  expressed in  $\text{Nm}^3 \text{CH}_4 / \text{dry Mg MSW}$ . Linear regression fit is indicated in both charts. Error bars represent 95% confidence limit.

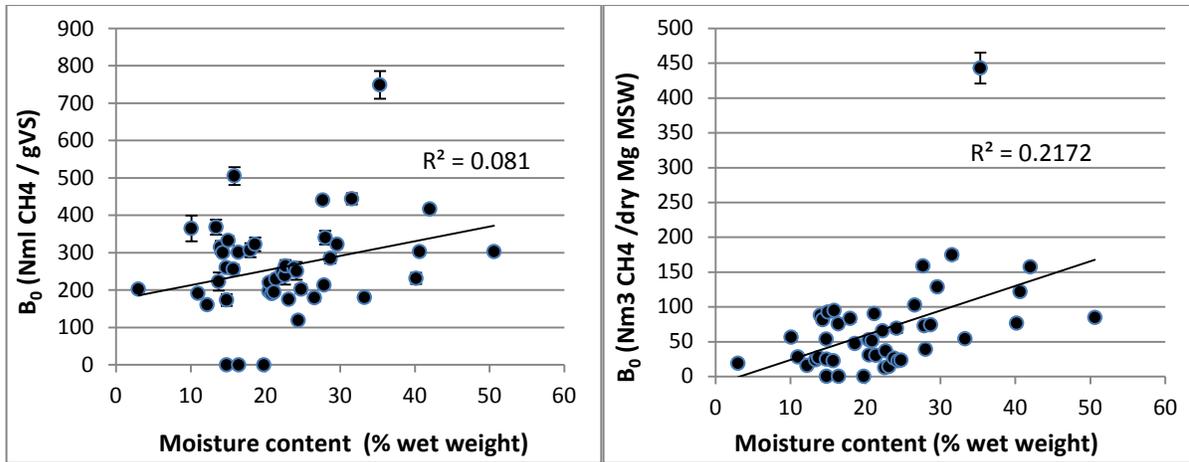


Figure 23. Maximum methane yield ( $B_0$ ) with sample moisture content. Chart on the left:  $B_0$  expressed in Nml  $CH_4$  / g VS. Chart on the right:  $B_0$  expressed in  $Nm^3 CH_4$  / dry Mg MSW. Linear regression fit is indicated in both charts. Error bars represent 95% confidence limit.

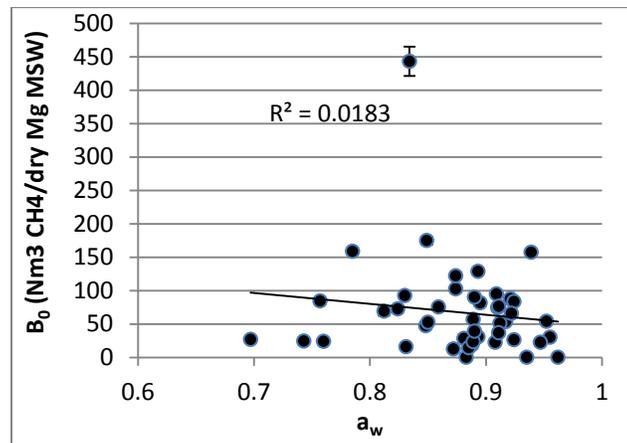


Figure 24. Effect of water activity ( $a_w$ ) on maximum methane yield ( $B_0$ ). Linear regression fit is indicated. Error bars represent 95% confidence limit.

### 3.3.4 Effects of sample components on maximum methane yield ( $B_0$ )

The effect of various components on the maximum methane yield was examined and the results are represented in Figure 25 – Figure 30. On wet and dry weight bases, there was no correlation between any of the waste components and  $B_0$  (Figure 25 - Figure 29). However, when the outlier was not considered, a fairly weak linear correlation between the BMP and paper fractions with  $B_0$  became evident (Figure 25 & Figure 27). Although the data pattern appeared to suggest an inverse linear relationship between wood or visible inerts and  $B_0$ , the correlation was too weak to suggest

any significant influence by these components on the maximum methane yield (Figure 28 & Figure 29).

The volatile organic solids (% dry weight of total MSW sample) and total available organic carbon (amount of carbon in the 'other organics' and paper fractions as a percentage of the whole MSW sample on dry weight basis) show remarkable correlation with  $B_0$ , with  $R^2$  values of 0.6203 and 0.5784, respectively. These correlation coefficients became stronger (0.7022 and 0.7062, respectively) when the outlier was not taken into account (Figure 30).

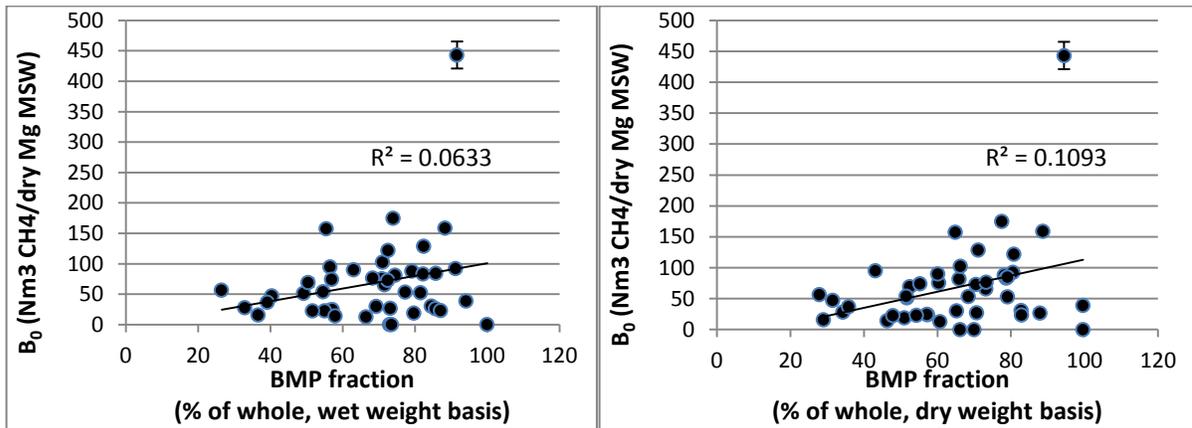


Figure 25. Influence of the BMP compositional fraction on the maximum methane yield ( $B_0$ ). BMP fraction represents the sum of the 'other organics' and paper fractions. The charts on the left and right are presented on wet and dry weight bases, respectively. Linear regression fit is indicated in both charts. Error bars represent 95% confidence limit. Data for the BMP fraction were within  $\pm 5\%$  mean.

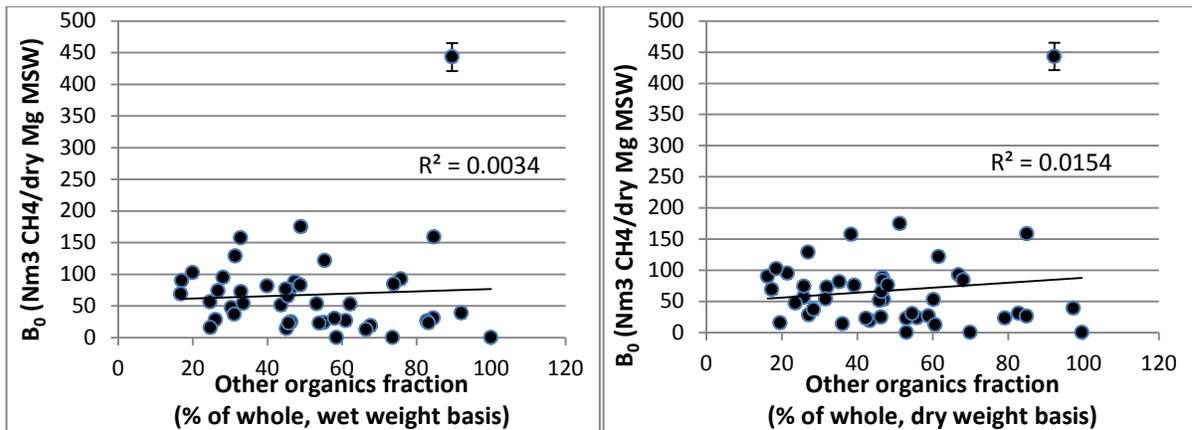


Figure 26. Influence of the 'other organics' waste fraction on the maximum methane yield ( $B_0$ ). The charts on the left and right are presented on wet and dry weight bases, respectively. Linear regression fit is indicated in both charts. Error bars represent 95% confidence limit. Data for the 'other organics' fraction were within  $\pm 5\%$  mean.

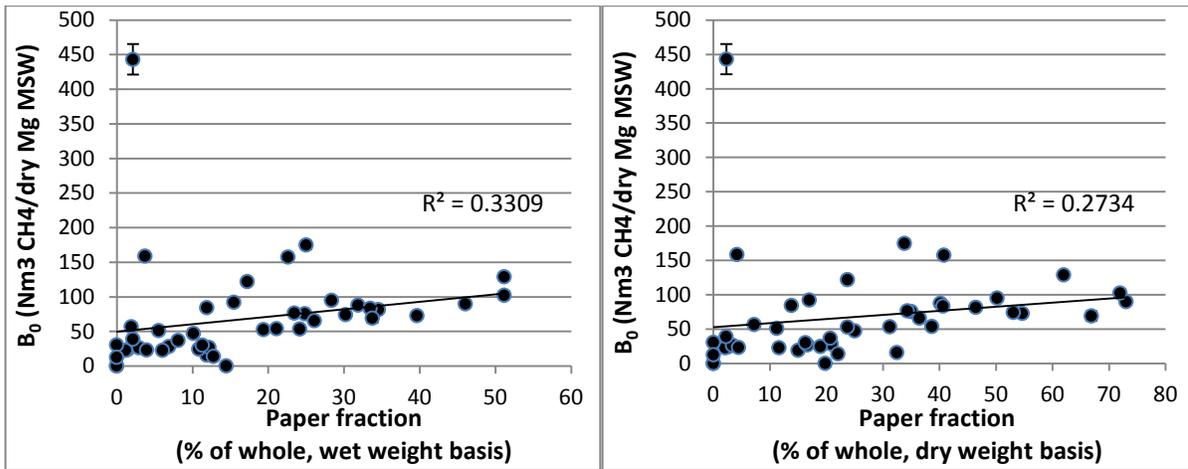


Figure 27. Influence of paper content of waste on the maximum methane yield ( $B_0$ ). The charts on the left and right are presented on wet and dry weight bases, respectively. Linear regression fit is indicated in both charts do not take into account the outlier data point. Error bars represent 95% confidence limit. Data for the paper fraction were within  $\pm 5\%$  mean.

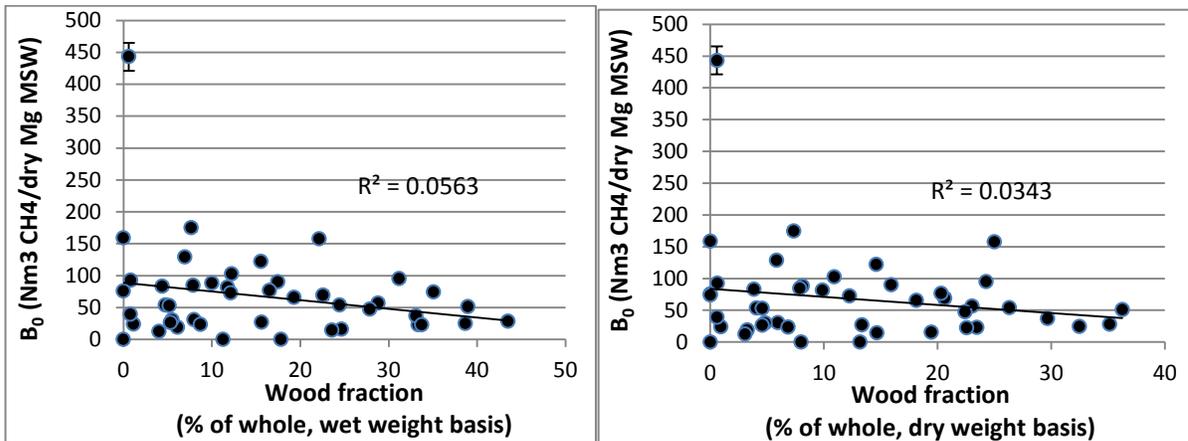


Figure 28. Influence of the wood fraction of waste on the maximum methane yield ( $B_0$ ). The charts on the left and right are presented on wet and dry weight bases, respectively. Linear regression fit is indicated in both charts. Error bars represent 95% confidence limit. Data for the wood fraction were within  $\pm 5\%$  mean.

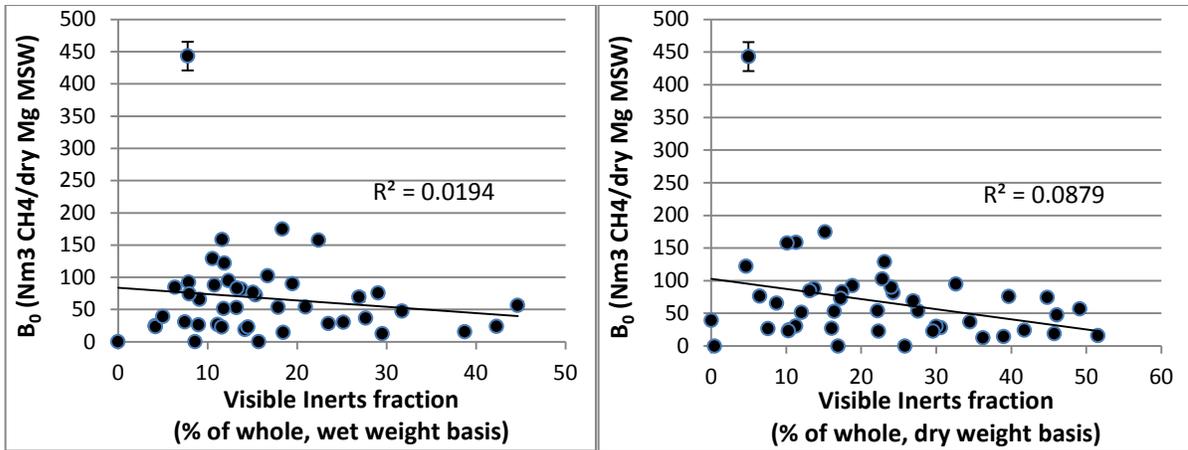


Figure 29. Influence of the visible inerts fraction of waste on the maximum methane yield ( $B_0$ ). The charts on the left and right are presented on wet and dry weight bases, respectively. Linear regression fit is indicated in both charts. Error bars represent 95% confidence limit. Data for visible inerts fraction were within  $\pm 5\%$  mean.

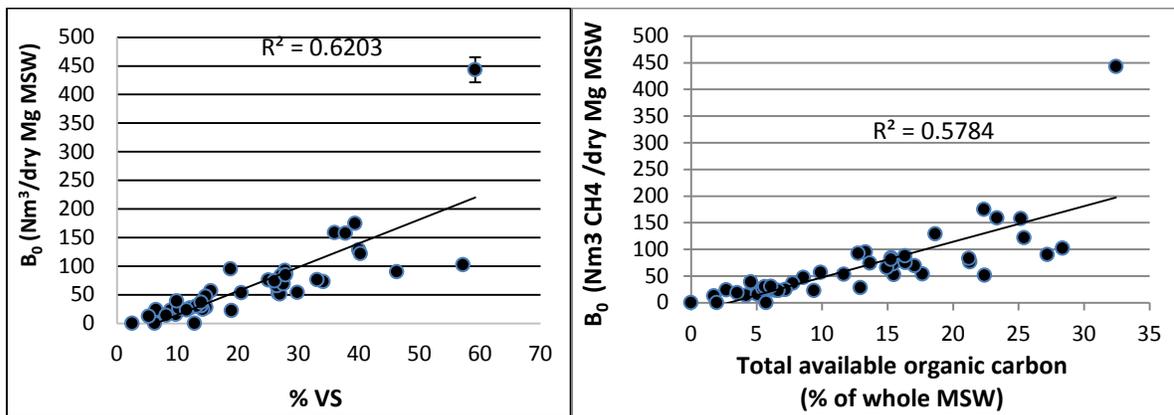


Figure 30. Effect of volatile organic solids and available organic carbon on the maximum methane yield ( $B_0$ ). Linear regression fit is indicated. Error bars represent 95% confidence limit. Data for volatile organic solids and total available organic carbon were within  $\pm 5\%$  mean.



Table 3. Laboratory-based degradation rates for wood samples from the operational and closed landfills in the City of Lethbridge. Values are represented  $\pm$  95% confidence limit.

Sample ID	Operational Landfill		Closed landfill	
	$k_{lab}$ (day <sup>-1</sup> )	$k_{lab}$ (yr <sup>-1</sup> )	$k_{lab}$ (day <sup>-1</sup> )	$k_{lab}$ (yr <sup>-1</sup> )
Wood $k_{lab}$ - ID# 1-1-7	0.026 $\pm$ 0.001	9.49 $\pm$ 0.37	na	na
Wood $k_{lab}$ - ID# P2S-D 1-3-15	nd	nd	na	na
Wood $k_{lab}$ - ID# MH2-D 4-3-20	0.042 $\pm$ 0.003	15.33 $\pm$ 1.10	na	na
Wood $k_{lab}$ - ID# G12 2-12to13m	-	-	nd	nd
Average $k_{lab}$ for Wood samples	0.034 $\pm$ 0.002	12.41 $\pm$ 0.73	nd	nd
Fresh soft wood $k_{lab}$	0.023 $\pm$ 0.001	8.40 $\pm$ 0.37	nd	nd

nd: not determined due to insufficient amount of data.

na: not applicable

Table 4. Degradation rates represented per year  $\pm$  95% confidence limit. 'na' represents not applicable.

Operational Landfill			Closed Landfill		
Sample Location	Sample ID	$K_{lab}$ (yr <sup>-1</sup> )	Sample Location	Sample ID	$K_{lab}$ (yr <sup>-1</sup> )
	1-1-7	31.8 $\pm$ 1.5	G7	2-14m	7.8 $\pm$ 1.1
P2S-M	1-2-7	16.7 $\pm$ 1.9	G7	1-4m	32.9 $\pm$ 4.8
P2S-M	1-2-12	22.7 $\pm$ 1.2	G5	1-6m	25.6 $\pm$ 1.0
P2S-D	1-3-7	28.3 $\pm$ 1.3	G4	1-4.5 to 6m	23.4 $\pm$ 1.9
P2S-D	1-3-12	29.3 $\pm$ 3.1	G5	2-10.5	21.1 $\pm$ 2.7
P2S-D	1-3-15	10.9 $\pm$ 1.3	G4	2-13.5 to 15m	na
P2S-D	1-3-26	17.4 $\pm$ 1.7	G12	3-14.5m	14.0 $\pm$ 1.2
MH2-S	4-1-10	31.4 $\pm$ 7.5	G12	4-17m	15.1 $\pm$ 1.1
P2N-D	2-3-14	12.5 $\pm$ 1.4	G12	1-5m	20.2 $\pm$ 1.1
P2N-D	2-3-7	14.5 $\pm$ 1.3	G12	2-12 to 13m	17.7 $\pm$ 1.7
MH2-M	4-2-10	35.2 $\pm$ 3.9	G11	1-8m	31.5 $\pm$ 1.6
MH2-M	4-2-10	12.8 $\pm$ 0.2	G13	3-19m	16.5 $\pm$ 1.0
MH2-M	4-2-15	12.4 $\pm$ 1.9	G9alt	3-17m	na
MH2-M	4-2-15	19.7 $\pm$ 0.9	G10 alt to G11	1-6.8 to 8m	26.4 $\pm$ 2.4
MH2-D	4-3-20	11.8 $\pm$ 0.9	G13	2-13m	24.7 $\pm$ 1.5
MH2-D	4-3-10	28.8 $\pm$ 1.6	G13	1-4m	24.4 $\pm$ 4.5
	2-1-10	19.2 $\pm$ 1.9	G9alt	2-13m	20.8 $\pm$ 1.3
	2-2-14	25.6 $\pm$ 2.1	G9 alt	1-5m	19.7 $\pm$ 1.9
	2-3-25	na	G10 alt to G11	2-12m	31.5 $\pm$ 6.8
	3-3-20	30.0 $\pm$ 3.2	<b>Average</b>		<b>19.6<math>\pm</math>2.0</b>
	3-3-3(9')	30.5 $\pm$ 3.2			
	3-3-5'(17m)	16.3 $\pm$ 0.9			
	3-3-17(55')	10.4 $\pm$ 0.6			
	3-1-9m(30')	12.5 $\pm$ 1.3			
	3-2-9m(30')	18.9 $\pm$ 1.3			
	3-2-17m(55')	21.0 $\pm$ 2.2			
<b>Average</b>		<b>20.0<math>\pm</math>1.9</b>			

### 3.4.1 Effects of sample age, depth, moisture and water activity on degradation rate ( $k_{lab}$ )

Results from the assessment of the influence of sample age, depth and *in-situ* moisture content on  $k_{lab}$  were as shown in Figure 32 & Figure 33. Pooled data from both landfills showed that waste age had no influence on  $k_{lab}$ , although depth of sample within the landfill matrix demonstrated a weak, inverse, linear correlation ( $R^2 = 0.2661$ ) with  $k_{lab}$  (Figure 32). However, when data from each of the landfills was evaluated separately, age and depth had a negative impact on degradation rate. Moisture content and water activity had no effect on  $k_{lab}$  (Figure 33 and Figures A9 & A10 in Appendix 2).

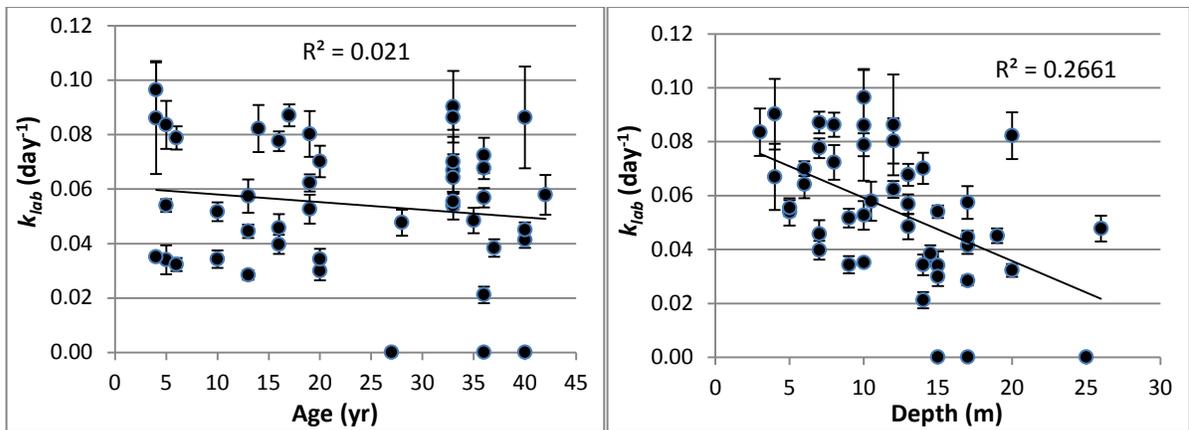


Figure 32. Effects of sample age and depth on  $k_{lab}$  in both landfills. Linear regression fit is indicated in both charts. Error bars represent 95% confidence limit.

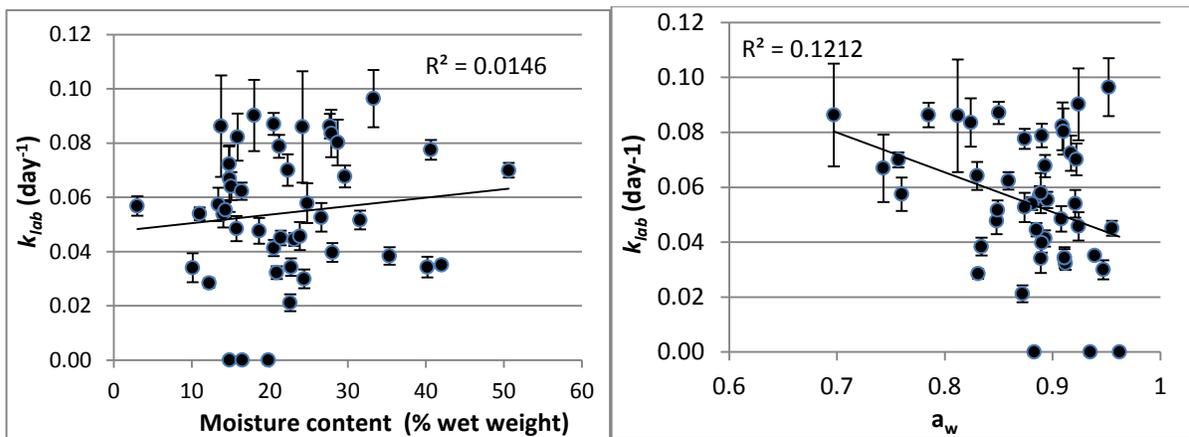


Figure 33. Effects of *in-situ* sample moisture content and water activity on  $k_{lab}$  in both landfills. Linear regression fit is indicated. Error bars represent 95% confidence limit.

### 3.4.2 Effects of sample components on degradation rate ( $k_{lab}$ )

The effects of waste components on a dry weight basis on  $k_{lab}$  were as shown in Figure 34 – Figure 38. The relative abundance of the BMP fraction ('other organics' and paper fractions combined) in the whole waste samples did not have any specific effect on degradation rate (Figure 34). However, a high relative abundance of the individual 'other organics' or paper components in the BMP fraction had an inversely linear or directly linear relationship with  $k_{lab}$ , respectively; each with identical, fairly weak correlation coefficients ( $R^2 = 0.2391$ ) as shown in Figure 35.

While the 'other organics' fraction of the whole waste sample did not affect sample degradation rate, paper showed a weak, direct linear correlation ( $R^2 = 0.2522$ ) with  $k_{lab}$  as shown in Figure 36. There was no evidence to suggest that the relative abundance of wood or visible inerts in the landfill samples had any measurable effect on the degradation rate (Figure 37). The volatile organic solids and total available organic carbon showed a very weak direct correlation ( $R^2 = 0.1517$  and  $R^2 = 0.1665$ , respectively) with  $k_{lab}$  (Figure 38).

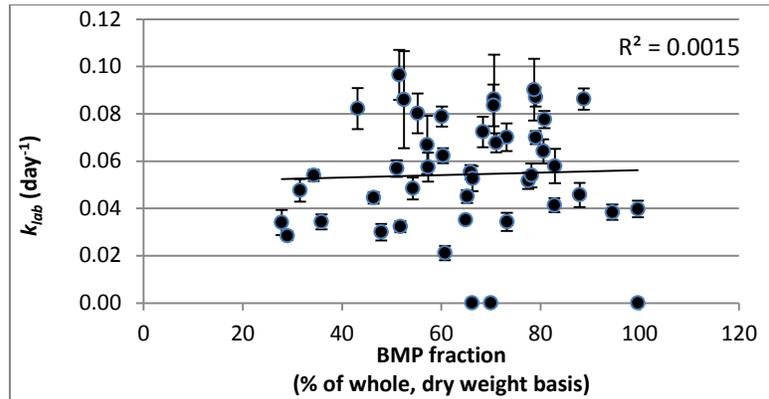


Figure 34. Effect of the BMP compositional fraction on  $k_{lab}$ . BMP fraction represents the sum of the 'other organics' and paper fractions. The charts on the left and right are presented on wet and dry weight bases, respectively. Linear regression fit is indicated in both charts. Error bars represent 95% confidence limit. Data for the BMP fraction were within  $\pm 5\%$  mean.

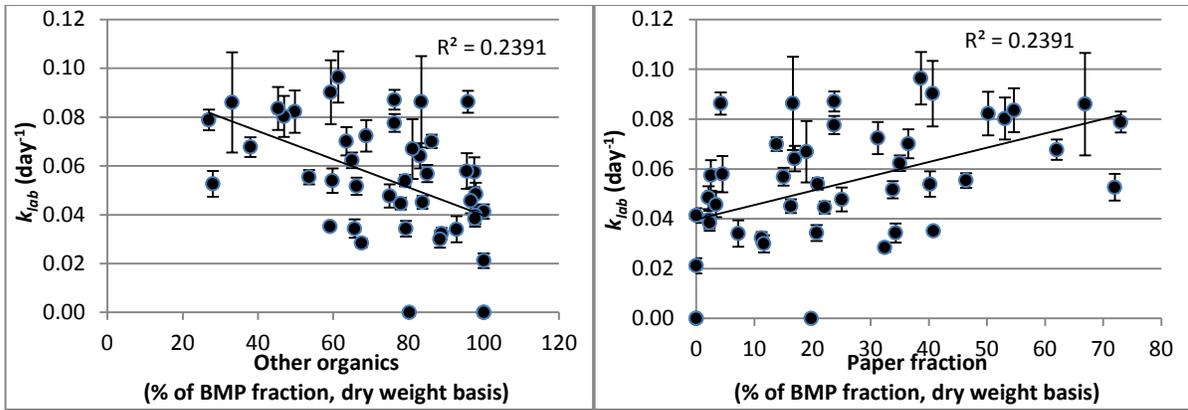


Figure 35. Effects of 'other organics' and paper fractional abundance in BMP fraction on  $k_{lab}$ . The charts on the left and right represent other organics and paper fractions, respectively. Linear regression fit is indicated in both charts. Error bars represent 95% confidence limit. Data for 'other organics' and paper were within  $\pm 5\%$  mean.

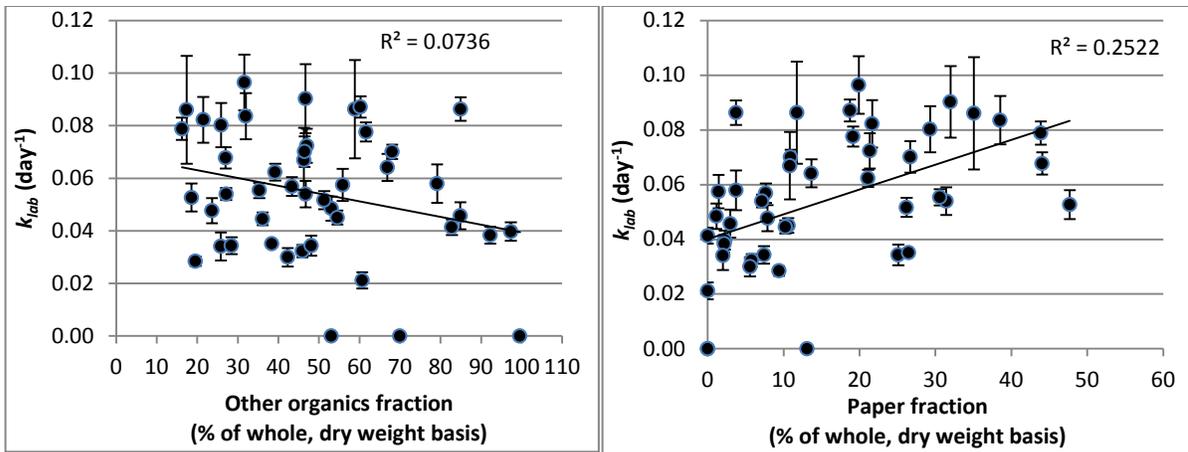


Figure 36. Effects of the relative abundance of 'other organics' and paper fractions on  $k_{lab}$ . The charts on the left and right represent other organics and paper fractions, respectively. Linear regression fit is indicated in both charts. Error bars represent 95% confidence limit. Data for 'other organics' and paper were within  $\pm 5\%$  mean.

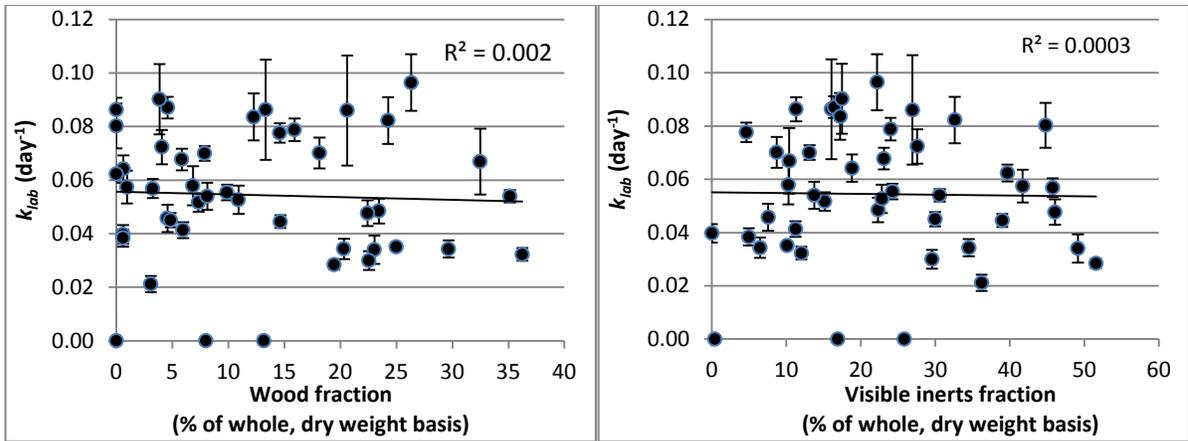


Figure 37. Effect of the wood and visible inerts fractions on  $k_{lab}$ . The charts on the left and right represent other wood and visible inerts fractions, respectively. Linear regression fit is indicated in both charts. Error bars represent 95% confidence limit. Data for wood and visible inerts were within  $\pm 5\%$  mean.

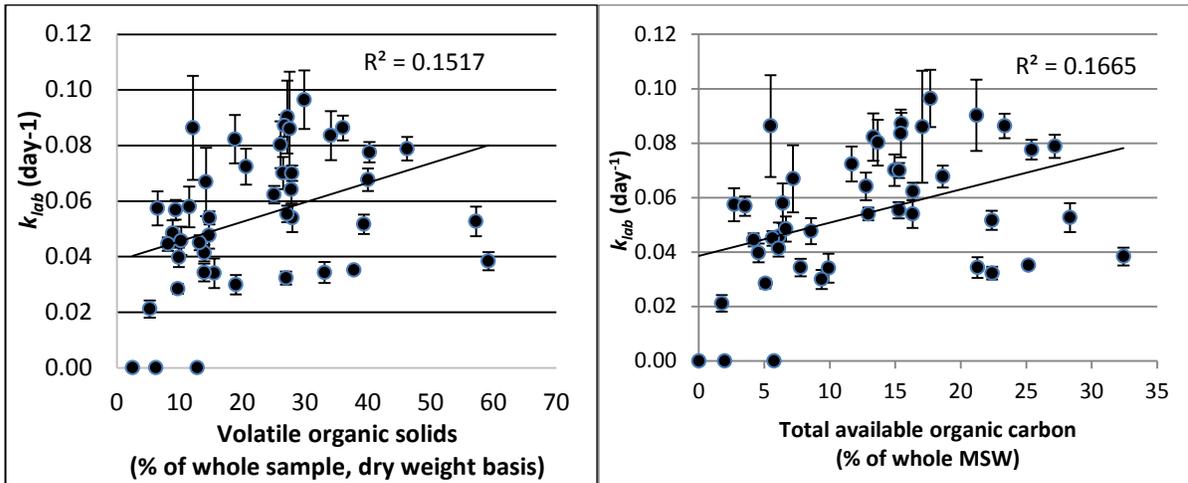


Figure 38. Influence of volatile organic solids and available organic carbon content on  $k_{lab}$ . Linear regression fit is indicated. Error bars represent 95% confidence limit. Data for organic carbon and total carbon were within  $\pm 5\%$  mean.

A summary of the biochemical degradation kinetics parameters for samples from both landfills is shown in Table 5. No significant difference was observed between the characteristics of sample from the operational landfill and those from the closed landfill; particularly, the maximum methane yields and degradation rates were identical.

**Table 5. Summary of biochemical degradation kinetics parameters for MSW samples obtained from two landfills in the City of Lethbridge. The operational and closed landfills are compared.  $B_0$  and  $k_{lab}$  Values are represented  $\pm$  95% confidence limit. All others are represented  $\pm$  standard deviation.**

Parameter (Average)	Landfill	
	Operational	Closed
Total carbon (% dry weight)	25.5 $\pm$ 12.0	15.5 $\pm$ 9.3
SMA (NmL CH <sub>4</sub> /gVS/day)	6.3 $\pm$ 3.1	6.6 $\pm$ 3.4
Maximum SMA (NmL CH <sub>4</sub> /gVS/day)	16.3 $\pm$ 9.1	18.5 $\pm$ 10.7
Time of digestion (day)	33.2 $\pm$ 5.4	32.2 $\pm$ 5.4
Maximum methane yield, $B_0$ (Nm <sup>3</sup> CH <sub>4</sub> /dry Mg MSW)	63.2 $\pm$ 42.4	73.9 $\pm$ 99.7 (53.4 $\pm$ 45.4) <sup>y</sup>
Degradation rate, $k_{lab}$ (day <sup>-1</sup> )	0.055 $\pm$ 0.024	0.054 $\pm$ 0.026
Degradation rate, $k_{lab}$ (yr <sup>-1</sup> )	20.0 $\pm$ 1.9	19.6 $\pm$ 2.0
Average Wood $B_0$ (Nm <sup>3</sup> CH <sub>4</sub> /dry Mg wood)	42.3 $\pm$ 1.0	37.4 $\pm$ 1.3
Average Wood $B_0$ - both landfills (Nm <sup>3</sup> CH <sub>4</sub> /dry Mg wood)	41.3 $\pm$ 1.1	
Average $k_{lab}$ for Wood samples (day <sup>-1</sup> )	0.034 $\pm$ 0.002	nd
Average $k_{lab}$ for Wood samples (yr <sup>-1</sup> )	12.41 $\pm$ 0.73	nd
$k_{lab}$ for fresh soft wood reference sample (day <sup>-1</sup> )	0.023 $\pm$ 0.001	
$k_{lab}$ for fresh soft wood reference sample (yr <sup>-1</sup> )	8.40 $\pm$ 0.37	

<sup>y</sup>Data in parenthesis does not include the exceptionally high outlier (G12 3-14.5m) from the closed landfill.

nd: not determined due to insufficient amount of data.

## 4. Discussion

### 4.1 Landfill Sample Characterization

The primary focus of this study was to determine the physical characteristics, maximum potential methane yield, laboratory-based degradation rates, phylogenetic characteristics of microbes and their interrelations thereof for samples from multiple drill wells from two landfill sites in the City of Lethbridge. Waste characterization data such as composition, moisture content, available organic carbon, age and depth in the landfill matrix are useful for the interpretation of degradation rates and maximum methane yields. Physical characterization showed that spent MSW from both landfills were highly heterogeneous. Components included construction wood, engineered wood, tree branches and leaves, garden clippings, office paper, construction paper, newsprint, cardboard paper, plastic bags and wraps, hard plastic, rocks, metal, glass, fabric and a mixture of assorted residual materials including organic fibre, soil, sand, silt, clay, etc. Cattle manure containing lumps of fat-like tissue material was also identified in some samples, which may seem unusual since animal waste is not normally disposed of in landfills. However, this sample was from the closed landfill where regulations might have permitted deposition of animal carcasses in landfills prior to 1985. The presence of tissue material in the manure-like sample tends to point to (the rumen of) a carcass deposited in the landfill rather than some manure waste.

On dry weight basis, this study revealed the average compositional profile of spent MSW from the City of Lethbridge landfills to be 49% organics (including non-wood organic material, non-synthetic fabric, sand, silt, clay, soil, and other unidentified non-biodegradable material), 23% visible inerts, 16% paper and 13% wood. Wet weight-based values have also been obtained (52.5%, 16.3%, 16.1% and 15%, respectively). The waste heterogeneity in this study is similar to what is reported on MSW from some major North American cities<sup>51</sup> as well as from the City of Lethbridge which included paper (27%), garden/park waste (10.8%), food waste (12.1%), wood and straw waste (7.4%) and inerts (42.8%) on wet weight basis.<sup>46</sup>

With age and depth ranges of 4-42 years and of 3-26m, respectively, sample composition analysis revealed a considerably wide range in relative abundance of the various compositional fractions. Although the relative abundance of each of the compositional fractions of sample spanned a broad range (0-44%, 0-51%, 0-39% and 16.7-100% for visible inerts, paper, wood and 'other organic', respectively), the averages of waste components fractions from this study are significantly different from that reported in reference [46]. Particularly, on wet weight basis, visible inerts and paper are markedly lower 16.3% and 16.1%, respectively. At 15%, wood accounted for more than double the value reported previously for fresh MSW in the City of Lethbridge. This may be due to limited biodegradation of wood in the landfill, and with progressive degradation of the paper, food, garden clippings and yard waste, wood tends to gain an overall increase in relative abundance.

At 52.5%, the 'other organics' fraction is more than double the food and garden waste fractions reported previously. This is likely due to other unidentified non-degradable materials included in this category such as soil, clay, sand, silt, and a variety of small particle-size inerts. Unlike in the

previous report on MSW from the City Lethbridge, food waste was not identified in this study as this waste fraction is usually the first to be completely degraded. It is worth noting however that samples used in reference [46] were collected from fresh MSW before deposition in the landfill, which makes comparison with spent landfill samples difficult.

The combined moisture content of the 'other organics' and paper fractions showed an inverse relationship with that of visible inerts, which indicates that moisture was not necessarily distributed uniformly within samples. Samples with high organic load such as cattle manure, garden clippings, leaves, etc. tend to hold higher amounts of moisture in the 'other organics' fraction, whereas inerts such as non-biodegradable synthetic fabric, sponges, etc. may retain more moisture than metal, rocks, glass, plastic bags/wraps, hard plastic, some paper types as well as some spent organic material with high amounts of soil, sand or silt.

Waste age and depth in the landfill did not affect degradation rate or maximum methane yield. These results may seem unusual as one would expect MSW deposited earlier (and therefore buried deeper in the landfill matrix) to be spent to a greater extent, degrade slower and yield lower amount of methane than that deposited later (younger material). In order to better understand these results, it is essential to carefully examine the existing knowledge on MSW degradation profile in the landfills and to relate this with the Lethbridge landfill peculiarities. Methane production rate curves for MSW suggest that it takes an average of 100 years for the maximum methane yield to be achieved, at which point the degradation rate approaches zero. However, degradation rates peak within the first 3-5 years during which most of the methane is produced, mainly from the readily biodegradable fraction of the waste (such as fats/lipids, starchy carbohydrates and readily solubilizable proteins) and thereafter the rate reduces asymptotically toward zero.<sup>37,45</sup> The residual waste material after 3-5 years in the landfill therefore becomes predominantly composed of recalcitrant lignin-rich organic material and other non-biodegradable constituents. In other words, while a marked difference in biodegradability may be observed between fresh MSW waste and waste which has been on the landfill for a few years, little difference would exist between samples older than 3-5 years on the landfill as their residual material would be predominantly recalcitrant. Differences in biodegradability observed for such materials are likely influenced by other dissimilar characteristics such as source/type (garden clippings, food waste, paper, wood, etc.), composition (heterogeneous vs homogeneous), presence and absence of inhibitors, waste preservation in the landfill influenced by waterproof barriers/compartmentalizing materials which prevent moisture penetration and limit the ability for microbiota to thrive. With all samples in this study having spent a minimum of 4 years on the landfill, their physical characteristics support this hypothesis as some older samples appear better preserved than samples that have spent a shorter time on the landfill. For instance, samples such as ID#MH2-D 4-3-20, ID#P2S-D 1-3-7, ID#P2S-D 1-3-26m, ID#G10alt to G11 1-6.8 to 8m, ID# G12 2-12 to 13m and ID#G12 3-14.5m with respective landfill ages of 6, 16, 28, 35, 36 and 37 years appeared better preserved, with characteristics similar to those of relatively fresh organic wastes such as garden clippings, dry leaves, branches, cattle manure, assorted fluffy organic materials and/or dry paper. On the other hand, samples such as ID#MH2-D 4-3-10, ID#3-3-20, ID#P2S-D 1-3-12, ID#2-3-25, ID#2-13.5 to 15m and ID#G13 3-19m with respective landfill ages of

6, 14, 19, 27, 36 and 40 years were extensively spent, having appearances of dry loose soil, moist lumps of sticky clay-like material and dry loose mature compost-like material.

The wood fraction did not show much signs of degradation and in most cases they appeared very dry. The low biodegradability of wood in landfill environments has been reported, and is thought to be imposed by lignin which forms a structural barrier around holocellulose, the crystallinity of cellulose microfibrils which makes them challenging to solubilize, and the toxic and inhibitory effects from phenolic derivatives from lignin and plant pigments.<sup>37</sup> Engineered woods manufactured by binding or fixing the strands, particles, fibers, veneers or boards of wood together, appeared virtually undegraded. This is mainly due to additives used in their manufacture (chemical treatment, resins and binders), which impair their biodegradation as demonstrated by Wang *et al.*<sup>38</sup> Softwoods are generally more recalcitrant than hardwoods and inhibition of anaerobic degradation has been observed from softwood oriental strand boards and certain hardwood types like eucalyptus. It is therefore not unusual to find relatively undegraded wood material in landfill matrices after several decades.

## 4.2 Methane yields and Degradation rates

The BMP assay relies on methanogenic microbial communities which convert substrate to methane under anaerobic conditions. For a given amount of substrate, the specific methanogenic activity (SMA) provides insight into the amount and viability of methane producing microbiota in the BMP cultures, and permits estimation of the maximum possible methane production rate and substrate biodegradability under specific experimental conditions. In this study, BMP cultures would gain methanogenic microbiota from two sources: (i) the seed inoculum and (ii) the residual *in-situ* methanogenic microbiota from the landfill samples. It is therefore necessary to integrate and contextualize the SMA results with substrate characteristics,  $B_0$  and  $k_{lab}$  observed.

High SMA usually indicates a viable methanogenic microbial community, a readily degradable substrate and, in the case of a substrate with high calorific value (metabolizable energy), high  $B_0$  and vice versa. Limited metabolizable energy inhibits microbial activity as observed in three samples which recorded average SMA values and  $CH_4$  yield of approximately zero. Each of these samples had 'other organics' fractions composed mainly of moist clay or soil-rich material with very limited or no bioavailable organic carbon. The fact that one of these samples had a paper fraction present and still yielded no  $CH_4$  suggests that the paper type was likely rich in inhibitors such as ink and lignin-rich recalcitrant residue. It is not uncommon to find an assortment of materials or chemical species in a landfill matrix which may inhibit anaerobic bacteria and archaea.

The exceptionally high SMA and  $B_0$  from the outlier sample (ID# G12 3-14.5m) was likely due to the presence of fat-rich material in the manure observed as greasy lumps of ligament-like tissue, which would represent a relatively higher Carbon/VS ratio. The presence of tissue in this sample suggests the sample originated from the rumen of a carcass buried in the landfill. The fat explains why the  $B_0$  from this sample exceeded that of cellulose. Nonetheless, it remains a mystery how this 37 year old

sample collected from a depth of 14.5m in a landfill that was closed since 1985 may be that well preserved. The sample was very moist which rules out the possibility of dry preservation within the landfill matrix. Although it is difficult to attribute high or low SMA results to substrate degradability, calorific value or methanogenic microbiota content due to the interplay of other variables such as inhibition, it is clear that relatively undegraded cattle manure entombed deep in the anaerobic core of a landfill, would have a higher calorific value and be readily degradable. Being moisture-rich and yet undegraded is indicative of limited amount of viable methanogenic microbiota or severe *in-situ* microbial inhibition/toxicity. At 5% solids, the BMP test diluted the MSW samples several fold, thereby reducing the inhibitor concentrations and facilitating microbial growth on the substrate. For all other samples on both landfills, the positive of sample moisture content on maximum methane yield is related to a favourable environment for *in-situ* colonization by anaerobic microbiota.

The correlation between sample age and depth with maximum methane yields and degradation rates on each of the landfills can be interpreted in two ways: (i) samples that are deeper in the landfill matrix are older, highly spent and therefore have higher relative abundance of non-degradable components such as soil, sand, silt, clay and other unidentified inerts and recalcitrant organic residuals; (ii) samples that are deeper are exposed to higher concentrations of leachate which confer toxicity and inhibition to methanogenic cultures.

$B_0$  and  $k_{lab}$  were not affected by the 'other organics' fraction due to the heterogeneity of this fraction and the presence of fine non-degradable components and residual recalcitrant organic materials.  $B_0$  and  $k_{lab}$  were found to depend on the fraction of paper in the sample, though with a weak correlation coefficient. For lignocellulosic feedstocks,  $B_0$  and  $k$  are known to be strongly influenced by the degree of lignification of the material such that a high degree of lignification (low carbohydrate-to-lignin ratio) advertently reduces  $B_0$  and  $k$ , and vice versa.<sup>37</sup> Since biochemical analysis for the determination of the cellulose, hemicellulose, lignin, proteins, ash and extractives was not done in this study, it is difficult to relate the effect of the paper fraction on  $B_0$  and  $k_{lab}$  to the degree of lignification of samples. The paper fraction appeared to be relatively similar in compositional profile from one sample to another. Therefore, a high relative abundance of paper in the sample would introduce a high amount of bioavailable carbohydrate into the BMP culture. This assertion is supported by the strong correlations observed between the total available carbon or volatile solids with  $B_0$  and  $k_{lab}$ .

$B_0$  and  $k_{lab}$  measured for wood were very low and consistent with results reported elsewhere for wood. Softwood lumber and four types of engineered softwoods subjected to high-solids anaerobic biodegradation by Wang *et al.* in a laboratory-scale landfill simulator for up to 1347 days produced very low methane yields and degradation rates in the range 0 — 6.3 Nm<sup>3</sup> CH<sub>4</sub>/dry Mg MSW and 1.7 — 21.5 yr<sup>-1</sup>, respectively. Hardwood lumber and engineered hardwood gave much higher yields of 32.5 Nm<sup>3</sup> CH<sub>4</sub>/dry Mg wood and 84.5 Nm<sup>3</sup> CH<sub>4</sub>/dry Mg wood, respectively; with corresponding degradation rates of 2.31yr<sup>-1</sup> and 1 yr<sup>-1</sup>.<sup>38</sup> In the current study however, wood degradation tests were performed at very low solids loading (5%) using wood shreds of sizes several fold smaller than that used in reference [38] - small enough to fit through a 1.5 inch mouth of the culture bottle. Since the wood types (soft or hardwood) were not identified in this study and the substrate consistency

was markedly different from that in reference [38], direct comparison of methane yields and degradation rates is difficult.

Addition of the wood  $B_0$  on each of the whole landfill sample  $B_0$  (which did not include the wood fraction) does not cause a noticeable shift in the landfill sample  $B_0$ . With only one wood sample tested from the closed landfill, it was difficult to compare degradation parameters of wood from the operational and closed landfills. Nonetheless, four varieties of wood samples from both landfills with an average age of 18 years produced a higher  $B_0$  than fresh softwood. While this observation clearly suggests that no significant degradation of wood had taken place on the landfill, several reasons may be responsible for the difference between landfilled and fresh wood degradation: (i) wood type, softwood generally degrades slower than hardwood due to higher lignin content in the former; (ii) wood on the landfill may have undergone significant interaction with ligninolytic or lignocellulolytic organisms which provide some sort of 'biological pre-treatment' on the wood thereby improving on its degradability; (iii) prolonged hydration on the landfill might help soften the wood and open up micro-pore spaces for microbial degradative enzyme attack; (iv) prolonged exposure to anaerobic environment might have 'seeded' the wood substrate with immobilized methanogenic biofilm; etc. Since Alberta's lumber industry is predominantly softwood-based, one would expect most of the wood in Alberta landfills to be softwood.

Overall,  $B_0$  values obtained in this study are comparable to those reported elsewhere for MSW. Barlaz and co-workers reported the  $B_0$  for MSW as  $92 \text{ Nm}^3/\text{dry Mg MSW}$  and for various fresh MSW components, typically in the range  $15.2 - 300 \text{ Nm}^3/\text{dry Mg MSW}$ .<sup>37</sup> Fresh MSW has been reported to be approximately 60% biodegradable, with a methane recovery potential of 90% the theoretical maximum. Unlike the MSW samples used in the study, samples in our study had undergone degradation in the landfill for up to four decades and were likely in contact with physical and chemical inhibitors such as leachate. The extent of degradation and interaction with inhibitors in the landfill would be different from one sample to another, which may severely affect their response to  $B_0$ ,  $k_{\text{lab}}$ . The weak correlation coefficients with sample physical parameters may therefore still convey very informative trends. Overall, with 45 samples spanning more than 4 decades and landfill depth profile of 26m, the average  $B_0$  and  $k_{\text{lab}}$  achieved in this study would be a fairly reliable estimate of the actual landfill data.

### 4.3 Carbon Sequestration in Landfill Waste

A fraction of carbon in waste paper and wood buried in landfills can be sequestered, expressed as carbon storage factor (CSF).  $B_0$  and CSFs of different paper types have been reported in the literature.<sup>38,45</sup> With  $B_0$  and CSFs in the range  $74 - 217 \text{ Nm}^3 \text{ CH}_4/\text{dry Mg paper}$  and  $0.05 - 0.42 \text{ kg Carbon/dry kg paper}$ , respectively, newsprint and glossy paper were shown to have the lowest  $B_0$  and the highest CSF. Office paper had the highest  $B_0$  ( $217 \text{ Nm}^3 \text{ CH}_4/\text{dry Mg}$ ) and the lowest CSF ( $0.05 \text{ kg Carbon/dry kg paper}$ ). Cardboard paper and mixed paper had similar  $B_0$  ( $\sim 140 \text{ Nm}^3 \text{ CH}_4/\text{dry Mg}$ ) and CSFs ( $\sim 0.25 \text{ kg Carbon/dry kg paper}$ ). With the mixed paper samples evaluated in this

study, this suggests that up to a quarter of the carbon content of paper buried in the landfill can be sequestered.

Sequestration of carbon from waste wood in landfills owes to its inherently low biodegradability. A study involving the estimation of CSFs from forest products deposited in U.S. landfills revealed that lumber and engineered wood had a CSF in the range 0.37 – 0.41 gram biogenic carbon stored per gram of wood, summing up to 10 terra grams (Tg) of sequestered biogenic carbon in 2006 alone.<sup>38</sup> With a much lower annual average temperature in Alberta (2.8°C) than in the USA (11.6°C) and the predominance of softwood in the Alberta lumber industry, the biogenic CSF for wood products in landfills in the province is expected to be higher.

#### 4.4 Comparison of the two landfills in the City of Lethbridge

With respect to landfill waste sample characteristics, degradation rates and maximum methane yield, results from this study clearly indicate that there is no significant difference between the currently operational and closed landfills in the City of Lethbridge.

#### 4.5 Relevance of study to existing offset protocols

Validation of the Alberta Landfill Gas Emission Model is essential for carbon credit allocation scheme and for regulatory compliance enforcement on landfill operators in the province. The importance of validation owes to assumption-driven uncertainties associated with landfill gas estimation models, with huge differences observed between modelled estimates and whole-site experimental data.<sup>37,39</sup> Model validation requires comparison of modelled estimates ( $k$  and  $B_0$ ) with actual gas emission data obtained experimentally.

The methane potential assay in this study was laboratory-based, with the peculiarity of accelerating waste degradation from several decades in the landfill to approximately 30 days on the laboratory bench. Laboratory scale degradation rates are therefore several orders of magnitude higher than what is actually applicable to landfill scenarios. For instance, reports suggest  $k$  values between 0.02 – 0.06 yr<sup>-1</sup> for landfills.<sup>22,23,25,45</sup> The degradation rates observed in this study range between 7.8 – 35 yr<sup>-1</sup>. In order to use the data to validate GHG offset protocols, a reliable conversion factor must be established for translating the laboratory based degradation rates into field applicable values. De la Cruz *et al*<sup>45</sup> reported the estimation of waste component-specific landfill degradation rates,  $k_{field}$  using laboratory scale data,  $k_{lab}$  for separated waste components through a conversion factor  $f$  as shown in equation 4 below:

$$k_{field,MSW} = f \times \sum k_{lab,i} \times (weighted\ fraction)_i \quad \text{Eq. 4}$$

where  $i$  is the  $i^{\text{th}}$  waste component and  $f$  is a correction factor.

To relate  $k_{lab}$  to  $k_{field}$  however, the following conditions and assumptions must apply:

- i. It is assumed that the weighted average decay rate for a waste mixture is equal to the bulk MSW decay rate.
- ii. The  $k_{\text{field}}$  must be known for the landfill in question
- iii. Samples used in the determination of laboratory-scale  $k$  should be a reasonably true representative of the MSW composition in the landfill
- iv. For each MSW sample, it is assumed that no interaction occurs between the waste components used in separate BMP assays. In our study, these components represent the 'other organics', paper and fabric and wood fractions.

While most of these conditions may be satisfied, experimentally determined  $k_{\text{field}}$  from the two landfills in the City of Lethbridge remains uncertain. Recently, a report on a baro-pneumatic testing project for the determination of decay rates on the closed landfill in the City of Lethbridge was released.<sup>31</sup> This study revealed the  $k_{\text{field}}$  for the closed landfill to be  $0.022 \text{ yr}^{-1}$ . While this value agrees with the provincial landfill gas emission model default, the data used to draw this conclusion was from two drill wells only, which makes it debatable using it in the determination of the correction factor  $f$ , for conversion of laboratory data to field rates. Our opinion is that baro-pneumatic data from a number of wells which reasonably span the surface area of the landfill would be a solid reference for GHG emission model validation for the Lethbridge region. Consequently, the determination of a correction factor  $f$  based on the  $k_{\text{field}}$  from obtained from the baro-pneumatic testing project was not pursued in this study.

## **PART 2: Molecular Identification of Microbial Communities in Landfill Samples**

## 5. Background

The level of bioactivity and the physiochemical conditions within a landfill reservoir might determine the fate of organic and inorganic compounds. Landfill environments may harbour cryptic microbial communities. In commercial applications, microbial communities may effectively break down organic matter under aerobic or anaerobic conditions.

As part of a comprehensive experiment, drill samples were collected from the Lethbridge landfill (Figure 2 & Figure 4) from various depths (Table 6). Samples from different locations and ages were evaluated for microbial communities. The objective of the present study was to characterize bacterial communities which potentially contribute to greenhouse gas emissions during biological landfill degradation.

## 6. Results and Discussion

### 6.1 Physical appearance of samples

A total of 45 subsamples (44 samples with one duplicated) were received from the operational ( $n=20 + 1$ ) and closed ( $n=24$ ) landfill sites at various depths ranging from 1 to 26 meters (Table 6). The age of the samples was estimated to range from 1973 to 1982 (old site) and at the new site from 1987 to 2011. The total weight of samples was 62.8 kg with an average individual sample weight of  $1134.7 \pm 248.1$  g. From a number of samples the age of the drill site could be verified based on newspapers, journals and documents found with noticeable dates. Considering the composition and size of drill samples, a number of those samples are unlikely to be representative of the entire landfill as a lot/unit.

Compared to other biodegradation processes like anaerobic digestion and/or composting, the biomass decomposition at the landfill site must be considered slow due to the fact that especially highly fibrous materials remained intact or even in unspoiled condition after 30 or more years buried at various depths. However, the composition of samples suggests that readily available nutrients like fat or simple carbohydrates are metabolized initially whereas more complex substrates like hemicellulose remain untouched for decades.

### 6.2 Moisture content and water activity

The sample dry matter (DM) ranged between 49% to 90% with an average of  $78\% \pm 10$  and a corresponding moisture content of  $22\% \pm 10$ . The activity of water ranged from 0.70 to 0.96 with an average of  $0.88 \pm 0.06$  (Table 6). In fact, the monitored values for moisture content and the activity of water ( $a_w$ ) reflect a limiting factor for microbial growth, primarily impacting bacteria and to a minor extent fungi, considering bacteria usually require at least 0.91, and fungi at least 0.7. The measured levels of  $a_w$  may draw the conclusion that both Lethbridge landfills are a very dry environment and/or that, according to personal communication with a local manager, the landfill

contains high amounts of salt which in return are binding water and lowering the  $a_w$ . However, at the measured  $a_w$  levels bacteria are either very limited (salt tolerant organisms) or completely restricted. On the contrary, fungi are less or not restricted by the  $a_w$  levels found at the landfill samples.

### 6.3 Molecular analysis

After freeze-drying and grinding, the initial attempt to extract DNA from landfill samples using a commercially available kit resulted in insufficient and/or detectable amount of nucleic acids. After using advanced extraction methods, we were able to extract small amounts of DNA slightly above the detectable limit (Figure 39a). After extraction bacterial 16S DNA gene fragments were amplified using primer set to highly conserved areas and including less conserved areas as a unique identifier for bacterial taxonomy (Figure 39b).

Individual gene fragments were separated by DGGE (Figure 40) based on G+C content differences in the nucleotide composition of the amplicons generated by PCR. In approximately 50% of positive PCR samples, the results from DGGE analysis are suggesting a variety of bacteria species. Individual DGGE bands were excised from the gel and used as PCR template to amplify specific bands for sequencing (Figure 41). Sequencing successfully identified 24 bacterial 16S DNA fragments (430bp) covering a region with variable nucleotide composition among species.

Altogether, 120 successfully sequenced fragments from 16S genes were unique to bacterial identities (Figure 42). Bacterial taxonomies were rooted against the 16S sequences of the selected reference strain *Bacillus subtilis*, a facultative anaerobic bacterium (Figure 43). From a total of 60 data sets, approx. 30 of the taxonomies were identified as sequences from uncultured bacterial according to the NCBI database.

The majority of microbial genera identified belong to the anaerobic or facultative anaerobic bacteria and were separated into two clusters (blue and green, Figure 43). Among the sequenced bacteria, the majority of identities belonged to genus *Halomonas* highlighted in blue. *Halomonas* are halophilic proteobacteria which can tolerate salt concentration equal to 5 to 25% of NaCl. The frequency of finding numerous salt tolerant identities is supporting the not yet confirmed statement of high salt concentration in the Lethbridge landfill as well as the low activity of water. In addition, the frequency of *Halomonas* bacteria in the samples suggests that the physiochemical landfill conditions are inhospitable for many other bacteria.

The second-most identified sequences belong to uncultured bacteria found in various reservoirs including anaerobic reactors, oil contaminated soil and leachate sediments (Figure 43), green background). As most of the bacteria are uncultured, many assumptions on their metabolism are solely hypothetical.

In addition, preliminary data are suggesting that the Lethbridge landfill accommodates fewer bacteria than fungi. Preliminary DGGE analysis revealed more fungi specific 18S gene fragments across all samples and sample depths compared to bacterial 16S fragments.

**Table 6. Sample ID, location, depth, activity of water, dry matter, moisture, estimated age and status from Landfill samples in 2014**

Sample ID	Location	Depth (m)	A <sub>w</sub>	% Dry Matter	% Moisture	Estimated	active/passive
1	1	7	0.85	79.5	20.5	1998	Older part
2	1	7	0.92	76.2	23.8	1999	
3	1	12	0.86	83.6	16.4	1996	
4	1	7	0.87	59.4	40.6	1999	
5	1	12	0.91	71.3	28.7	1996	
6	1	15	0.95	75.6	24.4	1995	
7	1	26	0.85	81.4	18.6	1987	
8	4	10	0.81	75.8	24.2	2011	
9	2	14	0.91	59.8	40.2	1995	
10	2	7	0.89	72.0	28.0	1999	
11	4	10	0.95	66.7	33.3	2011	2008-2013
12	4	15	0.89	89.9	10.1	2010	
13	4	20	0.91	79.1	20.9	2009	
14	4	10	0.89	78.8	21.2	2009	
44	4	10	0.94	58.0	42.0	2011	Older part
15	2	10	0.87	73.4	26.6	1996	
16	2	14	0.92	77.7	22.3	1995	
17	2	25	0.94	83.6	16.4	1988	
18	3	20	0.91	84.2	15.8	2001	2006-2008
19	3	3	0.82	72.1	27.9	2010	
20	3	5	0.89	76.9	23.1	2002	
21	3	17	0.83	87.8	12.2	2002	
22	3	9	0.91	77.3	22.7	2005	
23	3	9	0.85	68.4	31.6	2005	
24	3	17	0.76	86.6	13.4	2002	
25	G7	14	0.87	77.4	22.6	1979	
26	G7	4	0.92	82.0	18.0	1982	
27	G5	6	0.76	49.4	50.6	1982	
28	G4	6	0.83	85.0	15.0	1982	
29	G5	10	0.89	75.2	24.8	1973	
30	G4	15	0.88	80.2	19.8	1979	
31	G12	15	0.83	64.7	35.3	1978	
32	G12	17	0.89	79.5	20.5	1975	
33	G12	5	0.90	85.7	14.3	1982	
34	G12	12	0.91	84.3	15.7	1980	
35	G11	8	0.79	72.3	27.7	1982	closed 1985
36	G13	19	0.96	78.6	21.4	1975	
37	G9	17	0.96	85.2	14.8	1975	
38	G11	8	0.92	85.2	14.8	1979	
39	G13	13	0.89	70.4	29.6	1979	closed 1985
40	G13	4	0.74	85.2	14.8	1982	
41	G9	13	0.89	NT	NT	1979	
42	G9	5	0.92	86.0	14.0	1982	
43	G11	12	0.70	86.3	13.7	1975	

NT = not analysed

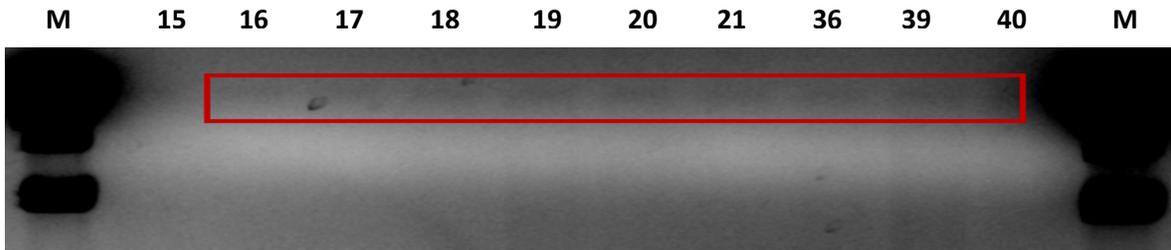


Figure 39a. Extracted DNA from selected landfill samples. Overexposed to make DNA visible with Lamda-HindIII size marker on the outer lanes.

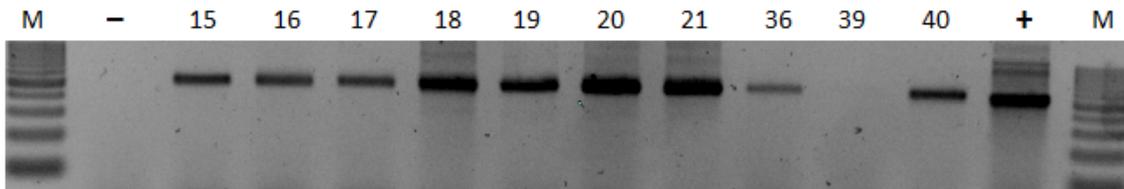


Figure 39b. Amplified 16S GC PCR fragments from selected landfill samples.

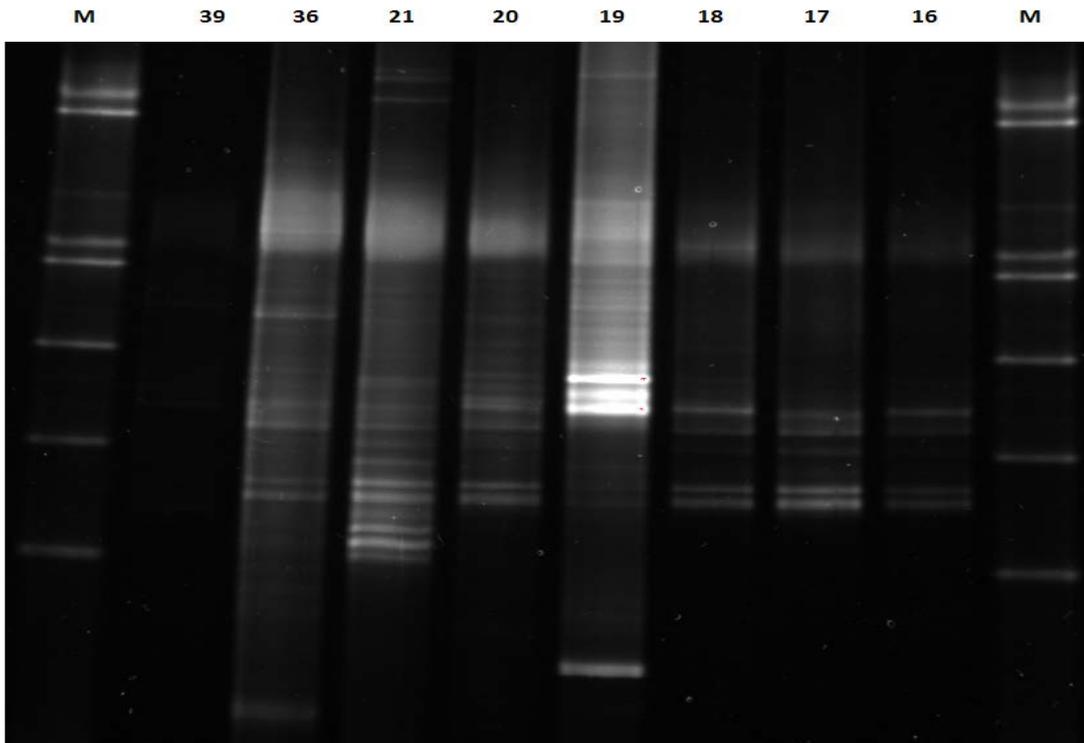


Figure 40. DGGE image of selected 16S PCR products amplified from DNA extracted from landfill samples in Lethbridge.

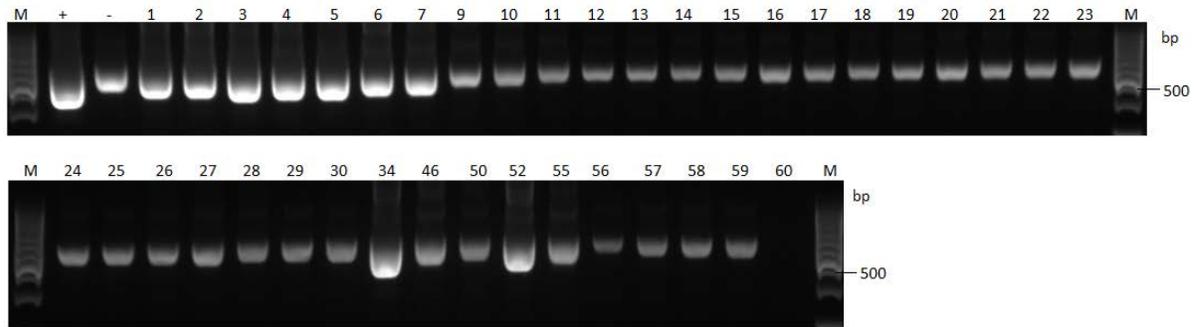


Figure 41. Re-amplified 16S PCR fragments from gel extracted 16S fragments after DGGE separation for sequencing.

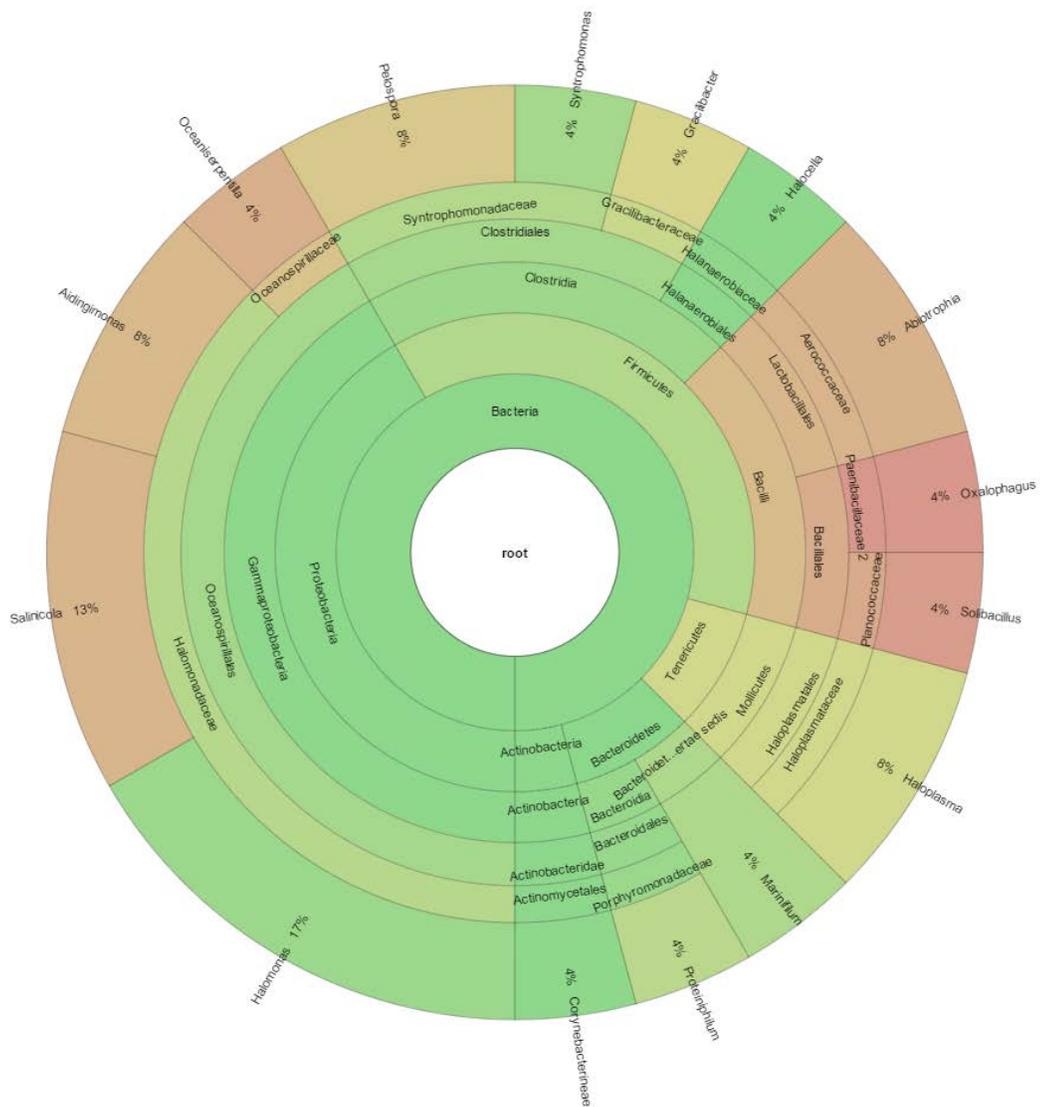


Figure 42. Pie chart of identified bacteria from Lethbridge landfill samples.



## 7. Summary of Molecular Identification of Microbial Communities

The microbial degradation of organic substances especially in anthropogenic landfills may generate climate relevant greenhouse gases (GHG) like methane. A number of factors are essential for microorganism to colonize and degrade MSW in the landfill and produce GHGs. In order to proliferate, microbes have essential needs, with water and availability of energy and essential nutrients (descending a hierarchal order), being of priority.

Samples of different ages and various depths from two Lethbridge landfill sites have been collected to investigate microbial colonization. Physiochemical data indicate that availability of water is limited, which may be attributed in part by high salt contents at both sites. Molecular analyses showed the presence of two major bacterial identities with salt tolerant species on the one hand and anaerobic bacteria previously reported in environments like sludge and sediments on the other.

Our results suggest that bacterial growth is present at a very low level. In addition, based on our analytic methods and capacity, preliminary data showed that the Lethbridge landfill sites accommodate fewer bacteria than fungi. While the landfill conditions are inhospitable for most bacteria, biodegradation and/or GHG production might be preliminary driven by fungi.

Methane-producing bacteria have not been identified by the time this project was concluded. One of the technical challenges was the lack of positive control of methanogens for validation of the primers and probes in the PCR-based assays. The presence and specific species of methane-producing bacteria in MSW samples retrieved from Lethbridge Landfill sites remain to be determined in future.

## 8. Material and methods

### 8.1 Landfill sampling

During the summer 2014 the City of Lethbridge had conducted a landfill drilling project, which allowed samples to be recovered from either auger drilling or core drilling. The drilling project retrieved samples from landfill locations of various age and depth. The City of Lethbridge used auger drilling to retrieve 20 samples from the 1975 to 1985 closed landfill site and core drilling to retrieve 24 samples from their operational (1985 to 2010) landfill site from various depth. The sample dating was primarily relying on landfill records. Between, the 15<sup>th</sup> and 30<sup>th</sup> of July 2014 a total of 45 samples (44 plus a duplicate of one of the samples) were received. Initially the total amount of each individual sample was recorded. Next, a number of pictures were taken for each sample. Sub-samples were oven dried at 60°C for 5 days to determine gravimetrically moisture and dry matter (DM). Organic matter (OM) was measured gravimetrically after combusting oven-dried samples at 600°C for 2 h in a muffle furnace. Activity of water ( $a_w$ ) was measured using Water Activity Meter (Novasina, Lachen, Switzerland) with a 5 grams wet sample.

## 8.2 Sample preparation and DNA extraction

After recording sample weight, samples were individually freeze-dried with subsequent grinding at 26 Hz for 5 min repeated 3 times using a TissueLyser (Qiagen Retsch). The powders of individual samples were stored at 4°C for DNA extraction. Genomic DNA was extracted from the samples according to the following protocol.

### ROCEDURE:

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#### I. Pre-processing of sample:

1. Collect sample:
  - a. Landfill: Weigh approximately 30 mg of freeze dried sample into a sterile 2.0 mL Safe-lock tube
2. For each samples from Step 1, resuspend (mix well) in 600 µL of RESUSPENSION BUFFER [600 mM NaCl, 120 mM Tris-HCl, 60 mM EDTA, 200 mM Guanidine isothiocyanate]
3. Transfer Step 2 into sterile 2 mL Eppendorf safe-lock snap-cap tubes containing 0.4 g of sterile zirconia beads (0.3 g of 0.1 mm and 0.1 g of 0.5 mm).

#### II. Cell lysis:

6. Add 5 µL β-Mercaptoethanol to the thawed mixture and mix well by inverting the tube several times.
7. Add 200 µL of pre-heated (70°C) 10% SDS [final concentration = 1.67%], very gently mix.
8. Homogenize for 3 min at maximum speed on a Qiagen TissueLyser™ (setting = 30).
9. Incubate at 70°C for 15 min, with gentle shaking.
10. Centrifuge at 4°C for 5 min at 16,000× g. Transfer the supernatant (approx. 800 µL) to a fresh 2-mL Eppendorf® tube.
11. Add 800 µL of fresh RESUSPENSION BUFFER, 5 µL β-Mercaptoethanol and 200 µL of 10% SDS to the tube and mix well.
12. Repeat steps 9-11. Do not pool lysate.

#### III. Precipitation of nucleic acids:

13. Add 200 µL of 10 M AMMONIUM ACETATE [final concentration = ~2.0 M] to each lysate tube, mix well, and incubate on ice for 5 min.
14. Centrifuge at 4°C for 10 min at 16,000 × g.
15. Pipet the supernatant to two 1.5-mL Eppendorf tubes (approx. 475 µL each), add one volume of isopropanol and mix well, and incubate on ice for 30 min.
16. Centrifuge at 4 °C for 15 min at 16,000 × g, remove the supernatant, wash the nucleic acids pellet with 750 µL of 70% ETHANOL (spin briefly), and dry the pellet under vacuum for 3 min or by leaving the tube open/inverted for 5-10 min.
17. Dissolve the nucleic acid pellet in 100 µL of TE, pH 7.4, pool together aliquots from duplicate isopropanol precipitation (recall Step 16).

#### IV. Removal of RNA, protein and purification (QIAamp DNA Stool Mini Kit)

18. Add 2  $\mu\text{L}$  of DNase-free RNase (10 mg/mL) **per** 200  $\mu\text{L}$  of sample from Step 18 and incubate at 37°C for 15 min.
19. Add 15  $\mu\text{L}$  of proteinase K (20 mg/mL) **per** 200  $\mu\text{L}$  of sample from Step 18 and 200  $\mu\text{L}$  of Buffer AL **per** 200  $\mu\text{L}$  from Step 18 (from the QIAamp DNA Stool Mini Kit), mix well, and incubate at 70 °C for 10 min.
20. Add 200  $\mu\text{L}$  of ABSOLUTE ETHANOL **per** 200  $\mu\text{L}$  from Step 18 and mix well.
21. Sequentially transfer all aliquots (recall Step 12) of the same sample to a QIAamp column and centrifuge at 16,000  $\times$  g for 1 min. Repeat until all aliquots have been run through the column.
22. Discard the flow through, add 500  $\mu\text{L}$  of Buffer AW1 (Qiagen), and centrifuge for 1 min at room temperature.
23. Discard the flow through, add 500  $\mu\text{L}$  of Buffer AW2 (Qiagen), and centrifuge for 1 min at room temperature.
24. Dry the column by centrifugation at room temperature for 1 min.
25. Add 150  $\mu\text{L}$  of pre-warmed (70 °C) nuclease-free water and incubate at room temperature for at least 2 min into a clean 1.5 microcentrifuge tube.
26. Repeat Elution with 100  $\mu\text{L}$  of pre-warmed (70 °C) nuclease-free water into the 1.5 mL microcentrifuge tube from step 26
  - a. If the pellet in Step 17 was small and/or DNA concentration is anticipated to be low, elute the second elution with 100  $\mu\text{L}$  and/or, elute into a new centrifuge tube.
27. Centrifuge at room temperature for 1 min to elute the DNA.
28. Run 2-4  $\mu\text{L}$  on a 0.8% gel to check the DNA quality. Quantify the DNA purity and concentration using spectrophotometric (A260) and/or fluorescent (PicoGreen) measures. The presence of PCR-inhibitors should also be evaluated by running a sample and corresponding dilution with common PCR primers (eg. 16S).
29. Store the DNA solutions at -20°C.

### 8.3 PCR analysis

PCR amplifications (Qiagen HotStar Plus MasterMix) of the V6 - V8 region (~400bp) bacterial 16S rRNA gene were performed with universal bacterial primers sets 954f-GC and 1369r as previously described (Yu and Morrison 2004). The 16S touchdown PCR temperature cycle consisted of: an initial activation at 95°C for 5 min followed by 12 cycles of denaturation at 94°C for 1 min, annealing from 65°C to 55°C for 30 s decreasing by 1K every cycle and extension at 72°C for 1 min and 25 subsequent cycles of denaturation at 94°C for 1 min, annealing at 60°C for 30 s, extension at 72°C for 1 min, and a final extension of 30 min at 72°C.

Amplicons were analysed by 2% (w/v) agarose gel electrophoresis and visualized by an AlphaImager (Alpha Innotech Corporation).

### 8.4 DGGE analysis

DGGE analysis was performed using a DCode Universal mutation detection system (Bio-Rad) on 7.5% (m/v) polyacrylamide (37.5:1 acrylamide – bisacrylamide) gels prepared with 40% - 70% urea and formamide linear gradient for bacterial amplicons. A total of 20  $\mu\text{L}$  of PCR product was loaded into each

lane of the gel and electrophoresis was performed in 1 × TAE buffer (40 mM Tris base, 20 mM glacial acetic acid, 1 mM EDTA) for 17 h at 60°C, and 70 V for bacterial amplicons. Gels were stained for 30 min in SYBR Gold (Invitrogen). The gels were visualized and photographed using a UV transilluminator (UVP). Dendrogram analysis was performed with UPGMA clustering of Dice coefficient values with 1.0% optimization and 1.5% position tolerance settings.

## 8.5 Sequencing analysis

Individual DGGE bands were excised from the gel and rinsed 3 times in tubes containing 300 µL nuclease-free H<sub>2</sub>O followed by 3 cycles of freeze-thawing at -80°C and 37°C. Gel plugs were crushed with sterile pestles and re-suspended in 30 µL H<sub>2</sub>O and centrifuged at 15,350 ×g for 5 min. Supernatants (5 µL) were used as templates for re-amplification using target primer pairs without GC clamp and conditions as mentioned above. Amplicons were commercially sequenced (Eurofins MWG Operon) using both the forward and reverse target primers. Sequencing data were analyzed by using GeneiousPro R9 software including NCBI Blast nucleotide query.

## 9. Concluding Remarks

The Alberta landfill gas emission model requires several inputs, some of which may have to be determined experimentally for reliability. Reliable experimental data on the maximum methane yield from MSW residuals in both landfills in the City of Lethbridge has been obtained, which has further enhanced our understanding of the waste characteristics within the city's landfills. The degradation rates and maximum methane yields obtained are comparable with rates reported by De la Cruz *et al* and therefore would be useful in establishing a corrections factor for laboratory to field degradation rates. Data generated from this study will therefore be a valuable laboratory-based asset for landfill gas emission model validation. For a comprehensive validation of the ALGQ Model, this study will hopefully be the first of many toward a province-wide model validation initiative.

## 10. Future Work

For province-wide assessment of the ALGQ Model, similar tests are recommended on other landfills. To reduce cost, we recommend that a representative landfill be selected in each specific provincial sub-region with distinct climatic features for baro-pneumatic testing on a reasonably adequate number of wells in order to obtain baseline field degradation rate data for preliminary model validation. BMP tests could then be performed on numerous core MSW samples taken from across the entire surface area and depth profile of the landfill to establish a lab-to-field rate correction factor,  $f$  which can be applied to landfills in the sub-region or throughout the province. Once the major sub-regions are covered,  $k_{\text{field}}$  values for landfills in the vicinities for which field data may be unavailable can still be determined in the laboratory using a common regional  $f$  value. This approach presents the unique advantage of rapid, cost-

effective determination of landfill gas emission rates and provide reliable experimental data for the validation of the provincial landfill gas emission model.

Molecular analyses of landfill core samples revealed the presence of fungal communities, highly salt-tolerant bacteria species and a few anaerobic bacteria previously identified in anaerobic environments such as sludge and sediments. Unfortunately, methanogenic bacteria and archaea communities were not observed in the samples, which leaves several key questions unanswered, especially the biological basis of methane production and GHG emission from the two landfills sites in the City of Lethbridge. This aspect of the work needs to be pursued in order to advance our knowledge on the biological basis of landfill waste degradation and GHG emission.

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## **Scientific Achievements and Communication Plans**

The results from this project will be discussed at two upcoming conferences as follows:

- 8th Canadian Waste Resource Symposium. April 27th – 29th, 2016, Halifax, NS; organized by the Solid Waste Association of North America (SWANA) atlantic chapter, and the Waste Resources Association of Nova Scotia (WRANS).
- SWANA's Northern Lights Chapter annual conference May 11-13, 2016, at the Deerfoot Inn and Casino in Calgary, Alberta.

Additionally, there is a plan to develop a manuscript for publication in the future but the journal has not yet been selected.

In all cases the contents of the presentations and manuscripts will be or have been approved by the CCEMC project sponsor.

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