EVALUATION OF SOURCE SEPARATED ORGANICS AFTER INOCULATION WITH EFFECTIVE MICROORGANISMS AND THE EFFECT ON COMPOST QUALITY.

by

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Dedication page

This thesis is dedicated to my grandmother, mom, dad, and brother without whose support it would have been impossible to achieve.

Thank you is not enough.

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ABSTRACT

A large volume of source-separated organics (SSO) are generated around the world and there are limited management strategies to reduce their effect on the environment. The volume of compost can be reduced through applications to agricultural soils after achieving a cured and stabilized product, however this is often a lengthy process. Composting time can be reduced by using specialized organisms called effective microorganisms (EM). The current project examines the effect of two commercially available EM products on different compost materials. This is achieved in two studies: study one aims at examining the role of EM toward reducing the time of composting for five different compost materials. The second study examines role of EM toward reducing the time composting for fresh green waste and barley straw. Results from both the studies showed that EM products were only marginally successful in reducing the time of compost.

LIST OF ABBREVIATIONS AND SYMBOLS USED

C:N	Carbon Nitrogen ratio
CCME	Canadian council of ministers of environment
CO_2	Carbon dioxide
d	day
dm	dry matter
EC	Electrical conductivity
EM	Effective microorganisms
g	gram
MSW	Municipal solid waste
MT	Metric tonnes
NH_4^+	Ammonium ion
SSO	Source-separated organics
TMECC	Test Methods for the Examination of Composting and Compost
VAM	Vesicular-arbuscular mycorrhiza
VS	Volatile solids

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CHAPTER 1 INTRODUCTION

Large amounts of municipal solid waste (MSW) are generated around the world due to rapid urbanization and industrialization. According to Statistics Canada, 25.8 million MT of waste was produced in Canada in 2008, of which non-residential sources accounted for 67% and the remaining 23% came from residential waste. In the same year, Nova Scotia produced 321,352 MT of municipal solid waste which consisted of 58% non-residential and 42% of residential wastes (Statistics Canada, 2010). Non-residential waste includes materials disposed of by industries, commercial centers (shopping centers, restaurants, offices) and institutions (schools, hospitals, government facilities). This category also includes waste produced from construction, renovation and demolition activities and residential waste.

Solid waste-resource management facilities in Nova Scotia receive a wide variety of material and they vary significantly in a composition which can directly influence the management process and economic benefits associated with their operations. A study conducted by HMJ Consulting Limited (2008) showed the price of compost from these facilities can range from \$2.50 MT^{-1} to over \$50.00 MT^{-1} due to the different levels of maturity and quality of final products. Nova Scotia Environment (NSE) has recommended that these facilities should increase the quality of their product by accelerating the rate of decomposition through improved operating conditions (HMJ Consulting Limited, 2008). One possible means to achieve this is through the addition of products containing specialized composting microorganisms called effective microorganisms (EM). This present study aims to examine the effect of two such EM products to accelerate the decomposition process of SSO to achieve a mature product.

CHAPTER 2 LITERATURE REVIEW

The following section outlines the theoretical and methodological contributions to all the topics listed below.

2.1 Municipal Solid Waste

MSW is defined as the waste produced by private households and collected by local municipal authorities (Strange, 2002). A more precise classification of MSW is based on the existing nature or structure of the municipality of the country (Al. Ansari, 2012**).** Generally, MSW is the unwanted waste material from households and offices disposed of by human activity, but this does not include construction, hazardous and industrial wastes (Adefemi and Awokunmi, 2009**)**.

2.1.1 Problems generated from MSW

As the world population is rising, by 2025, it is expected that two-thirds of the world's population will be living in cities. As a result of this rapid urbanization and increased wealth, concomitant increases in waste production is also expected. For example in China and Columbia, there is a huge rise in meat consumption and cars (Johnstone and Labonne, 2004). Along with this, disorganized and unplanned growth can lead to environmental problems like water and air pollution and solid waste production (UNEP, 2001). In more developed countries, such as in Canada, landfill diversion rates of 67–70% from municipal solid waste facilities have already been reported (Wang and Nie, 2001).

MSW also causes other problems including contaminated water bodies due to illegal dumping which releases inorganic and organic pollutants. If left unmanaged, MSW disposal can be a serious threat to the environment posing problems including the

spread of disease, human health deterioration, surface water pollution, unpleasant odour production and pest infestations (Turan et al., 2009). In Quebec, Canada, there are incidences reported of people living near municipal solid waste sites becoming afflicted with cancer (Goldberg et al., 1999). Many developed countries which produce large amounts of MSW have raised an alarm about the economic feasibility and environmental tolerability of the present waste disposal methods (Daskalopoulos et al., 1998). In addition to this, anaerobic degradation of organic wastes in landfills can generate greenhouse gases like methane which exacerbates global warming.

2.1.2 Managing MSW

Of the methods used for disposing of MSW, incineration, landfilling, and composting of organics are the most common**.** Incineration is a process where solid waste materials are combusted to ash to reduce the volume of waste being landfilled (Li et al., 2003). Although this method is quick, it has some limitations including concentrating heavy metals in the bottom ash, fly ash, and grate sifting (Zhang et al., 2010). Incinerators can also release toxic gases which can cause health concerns for human beings. Volatile organic compounds, such as dioxins and furans present in the air emitted from some incinerators can persist in the environment for decades without degrading and may accumulate in the soil, plants, and water.

A landfill is the oldest form of waste disposal and can be defined as a place where waste materials are buried. This is usually done to avoid contact between waste materials and surrounding areas. Most landfills are not managed properly, municipal wastes dumped in open areas can cause environmental deterioration (Madi and Ozerkan, 2012). Most landfills also produce leachate that may contain toxic and carcinogenic organic

compounds (Brown and Donelley, 1988) like benzene, toluene, xylene, methylene chloride, 1,1,2-trichlorocthylene, 1,1-dichloroethane and trans-l,l-dichloroethylene which takes years to degrade (Sabel and Clark, 1984). These chemicals can also contaminate the groundwater and pose a severe threat to the ecosystem and human health if the leachate reaches the drinking water (Baun et al., 1999).

The third method of managing MSW is composting, which is often considered as the most suitable method for managing and recycling the organic portion of MSW, which is also referred to as SSO compost. The composted product can be applied to soils to improve their quality and fertility (Larney and Hao, 2007). Composting of organics for use as a soil conditioner is a more cost-effective and sustainable alternative for SSO management.

2.2 Composting

Composting is defined as the biological breakdown of organic matter under controlled conditions forming a nutrient-rich product called humus (Narayana, 2008). It is managed as an aerobic process in which the microorganisms break down complex organic particles into simpler products to produce a final product which can be used as a stable soil conditioner (Gajdos, 1992).

2.2.1 Composting Process

Composting typically occurs in three stages: the mesophilic and thermophilic stages, and a curing or maturing stage over a period of several months. The duration of the composting stages depends on the type of feedstock used, the amount of agitation and aeration, and operating conditions such as moisture content. In the beginning of the process, at ambient temperature, the microbial populations usually feed on the labile

carbon sources like lipids, monosaccharides and starch which are easily broken down to Carbon dioxide (CO_2) and energy. A portion of this energy is used by the microorganisms for their growth and reproduction and the rest is liberated as heat increasing the temperature of the compost pile gradually. Substances such as organic acids are also produced which in turn can decrease the pH of the compost pile. As the temperature gradually increases, the compost pile changes from the mesophilic to the thermophilic stage and the mesophilic bacteria are slowly replaced by thermophilic bacteria. During the thermophilic stage, the proteins are degraded by microorganisms liberating ammonium (NH_4^+), thus increasing the pH. Increased metabolic activity and growth of microorganisms can elevate the temperature to 65 ºC or higher at which point most pathogenic organisms are destroyed making the product safer for handling and agricultural use. The next phase is the most important and longest of all stages, curing phase. After the degradation of all simple carbon sources, the resistant carbon compounds like lignin, cellulose and hemicellulose are broken down and transformed partially into stable humic-like products (Crawford, 1980). The temperature subsequently declines to 25–30 ºC during which the compost is cured. The cation exchange capacity and humus content gradually increase at which point the final product is typically stabilized and free of harmful pathogens (de Araújo et al., 2010).

2.2.2 Microbiology of Composting

The composting process is carried out by a succession of microorganisms that play a very important role in the process of decomposition. The composting process involves three main groups of microorganism: bacteria, actinomycetes and fungi. The total microbial population usually changes during composting with many microbial

species varying significantly during different stages of composting (Atkinson et al., 1996). The decomposition process is dependent on several factors such as temperature, moisture content, C:N ratio, metabolic generation of heat, oxygen access and micronutrients. Monitoring of microbial activity, using temperature as a proxy, leads to effective management of the composting process (Ishii et al., 2000). In the initial phase of composting there is a rapid increase in temperature involving a rapid conversion of feedstock carbon into microbial biomass. At this stage, mesophilic bacteria are dominant and as the temperature rises to around 40 °C, thermophilic bacteria and fungi take over. A variety of microorganisms which are thermo-tolerant, including aerobic thermophilic and mesophilic organisms including certain bacteria, yeasts, molds, actinomycetes and fungi are reported to show activity in the temperature range of 20–60 °C (Fergus, 1964). When the temperature exceeds 60 °C, an overall decrease in the microbial activity results (McKinley and Vestal, 1985). In the second phase, the readily available substrates decline, decreasing the overall microbial population and thus decreasing the microbial respiration. During these temperature transitions, another succession of microorganisms takes place changing the microbial composition back to mesophilic species (Beffa et al., 1996). The state of microbial diversity, predominantly bacterial, in the curing phase decreases but the metabolic diversity and taxonomy increases (Finstein and Morris, 1975). The mesophilic and thermotolerant fungi and actinomycetes which mostly degrade polymers namely cellulose, lignin and hemi-cellulose tend to increase during the curing phase (Finstein and Morris, 1975).

2.2.2.1 *Bacteria*

Due to their small size, 0.5–3.0 μm, bacteria have a very large surface to volume ratio and hence they can transfer the soluble substrates into their cells very rapidly. Bacteria, therefore, are more dominant than larger microbes like fungi (Haug, 1993). The thermophilic phase is dominated by the bacterial species such as *Bacillus subtilis, B. licheniforms and B. circulans*. Along with these, the genus *Thermus* can be also found in higher temperatures ranging from 65–82 °C (Beffa et al., 1996).

2.2.2.2 *Actinomycetes*

Actinomycetes are the bacterial species which produce multicellular filaments resembling fungi. They are found both in thermophilic and maturing phase and sometimes are visible on the surface of the compost. Actinomycetes degrade lignin and cellulose and can withstand high temperatures and pH compared to fungi. They grow slowly compared to most bacteria and fungi and compete ineffectively when the availability of nutrients is high. Thermophilic actinomycetes tend to be more tolerant compared to fungi at higher temperatures and reproduce quickly at their optimum temperatures of 50–55 °C (Fergus, 1964). Their population, however, decreases above 60 °C and their significance in the decomposition process also decrease during such adverse conditions and they tend to survive as spores (Cross, 1968). The thermophilic actinomycetes that have been isolated from compost include Streptomyces, Micromonospora, Thermoactinomycetes and Nocardia (Strom, 1985).

2.2.2.3 *Fungi*

Fungal growth is mainly affected by temperature and pH, and available carbon and nitrogen. Fungi are predominantly mesophiles that grow between 5–37 °C. The

cellulolytic and thermo-tolerant characteristics of *Aspergillus fumigatus* make it the most dominant fungal species (Beffa et al., 1996) in thermophilic. Thermophilic fungi has the highest growth at a temperature of 50 °C or higher and the lowest growth rate at a temperature of 20 °C or lower (Cooney and Emerson (1965). The thermophilic fungi are either killed or are present in the form of spores above 60 °C.

2.2.3 Factors Affecting Composting

Although composting is a natural process, good management can help increase the efficiency and obtain a stable end product. This can be achieved by controlling several parameters such as pH, moisture content, temperature, bulk density, porosity, particle size, nutrient content, C:N ratio and oxygen supply which all help to achieve optimum conditions required for the growth of microbes and the breakdown of organic matter (Agnew and Leonard, 2003; Das and Keener, 1997; de Bertoldi et al., 1983; Haug, 1993; Miller, 1992; Richard et al., 2002). All these parameters help to enhance the process of organic matter decomposition and are described in more detail below (Bernal et al., 2009).

2.2.3.1 *pH and EC*

The optimum pH in a compost pile should be between 5.5 and 8.0 (Miller, 1992). Biodegradation of substrates releases NH_4^+ which acts as an alkali and increases the pH of the compost to more than 8. High pH is an indication of the presence of NH_4^+ and, therefore, immaturity. A high pH coupled with a high temperature can lead to nitrogen (N) loss by ammonia volatilization (de Bertoldi et al, 1983). A decrease in pH at the beginning of the composting process can be attributed to the activity of acid-forming bacteria to degrade the carbonaceous material into organic acids, lactic and acetic acid.

However as these acids are neutralized, the pH will increase (Ko et al., 2008). A drop in pH can also be prevented by the addition of lime to accelerate the composting process (Finstein and Morris, 1975). Compost is considered mature when the pH is neutral (Ko et al., 2008). Hence, it can be considered as a potential measure of compost maturity.

Brewer and Sullivan (2003) and Avnimelech et al. (1996) found that a near-neutral pH coincided with compost stability in yard waste and MSW. pH changes during the course of composting depend on the source or feedstock used in composting (Avnimelech et al., 1996).

Electrical conductivity (EC) is the measure of the amount of salts present in compost and high levels of this can be phytotoxic to plants and when applied can reduce seed germination, nutrient uptake, water availability and can cause root damage (TMECC, 2001). As the compost matures EC may decrease due to organic acid decomposition and leaching of salts (Avnimelech et al., 1996). Along with pH, EC is often used as a suitable indicator to measure compost maturity. The acceptable range of EC for compost is usually considered to be 1–10 mmhos cm^{-1} (USDA, 2015).

2.2.3.2 *Particle Size and Surface area*

Since microbial decomposition occurs on the surface of the organic material, particle size and particle distribution affects microbial activity and thus the composting process. If the particle size is large, the surface area to mass ratio will be smaller and vice versa. The composting process is more efficient if the particle size is small as they can be easily degraded by the microorganisms. However, if the particle size is too small, then it becomes too compacted for the microbes to degrade the organics (Bernal et al., 2009).

2.2.3.3 *Porosity and Aeration*

Porosity is the amount of pore space preset between the particles. Porosity greatly influences the distribution of air which in turn impacts the composting process. Composting piles are expected to have a pore space of 35–50% (CWMI, 1996). If the porosity exceeds 50%, there will be a significant drop in temperature as the heat loss (through pores) exceeds the heat generated. If the pore space is less, anoxic conditions can develop and create odor problems. Porosity also affects the amount of carbon stabilized within the pores and thus the stability of the compost is affected. Porosity also affects the water holding capacity of the compost which will affect plant growth, as well as an oxygen movement without which the compost pile becomes anaerobic (CWMI, 1996). According to Miller (1992), compost piles should have an optimum oxygen concentration between 15 and 20%. An oxygen concentration below 5% is insufficient for microbial activity (Morse, 2001). Turning the compost pile provides proper aeration by delivering oxygen necessary for adequate biological activity, regulates the temperature and also eliminates additional moisture. On the other hand, too much turning may not give sufficient time for the compost pile to heat up. Optimum porosity is required for oxygen movement and microbial activity without which the decomposition process may be slowed. Optimum aeration helps build up microflora, which play an important role in degrading the compost.

2.2.3.4 *Moisture*

Water is crucial for the survival of microbes and ensuring an acceptable compost moisture content sustains good microbial activity (Richard et al., 2002). The exchange of chemicals and nutrient transfer occurs through water as a carrier. The optimum moisture

content for a compost system should range from 50–60% depending on the material to be composted (Gajalakshmi and Abbasi, 2008). An increase in the compost moisture content beyond a certain level can impede the free pore space, inhibiting the movement of oxygen which leads to anaerobic conditions (Das and Keener, 1997).

2.2.3.5 *Temperature*

Temperature plays a critical role in composting as it determines the elimination of pathogens before the compost is applied to soil (Barrena et al., 2006). The optimum temperature range required for composting is 40–65 ºC (de Bertoldi et al., 1983) and pathogenic microorganisms are killed above 55 ºC (Miller, 1992). The decomposition process is active between 5 and 60 ºC (Miller, 1992) and the activity of microbes tends to decline above 63 ºC, as the optimum temperature required by thermophiles is exceeded. It is very important to regulate the temperature which can be achieved by maintaining the shape and size of the composting mass and by turning the compost pile at regular intervals.

2.2.3.6 *Organic Matter Content*

Compost feedstocks contain various types of organic compounds such as proteins, carbohydrates, lipids and lignin. During biodegradation, approximately one-half of this material is converted to CO_2 and released into the atmosphere while the remaining material is converted into more stable humic compounds (Wichuk and McCartney, 2010). The biodegradation of organic matter is impacted by numerous factors which fall into two categories: (1) those that regulate the concentration and degradability of the compound to be broken down or the one which affects the population of microbes and

activity; and (2) those that directly regulate the kinetics of the reaction itself, such as moisture content, population size, and temperature (Hamoda et al., 1998).

2.2.3.7 *C:N ratio*

Carbon is considered as the source of energy for microorganisms and is also the major component of microbial cells (Pare et al., 1998; Tiquia et al., 2002). The second element which is of utmost importance for microbial growth is nitrogen which is the major component of enzymes, nucleic acids, amino acids and co-enzymes. Microorganisms in the compost pile can proliferate in the presence of adequate carbon and nitrogen (Kalbasi et al., 2005). A balance between carbon and nitrogen is necessary for healthy microbial activity. The optimal ratio of carbon and nitrogen for the recipe of the compost pile is considered to be 30:1 (USDA, 2015). The C:N ratio is the weight of the total carbon to the weight of the total N (Rynk, 1992; Kalbasi et al., 2005). The various kinds of carbon amendments are wood shavings, straw, wood chips, sawdust, and corn stalks which possess high C:N ratios (Morse, 2001). A balanced C:N ratio also reduces the odor problems produced during composting. Compost feedstocks are often tested for their C:N ratio before processing. As the decomposition of organic material proceeds the C:N ratio decreases first, then eventually stabilizes due to the release of CO_2 which results in a loss of organic carbon (Bio-Logic, 2001). A high C:N ratio results in a slower composting process and requires a longer time, whereas compost piles with low C:N ratios produce more ammonia and are carbon deprived. Sullivan and Miller (2001) proposed that the C:N ratio of compost as an amendment to be from 10:1–15:1 (Wichuk and McCartney, 2010).

2.3 Composting as a Means to Manage the Organic Fraction of MSW

Managing MSW organics is a critical global issue due to the increasing amounts generated and loss of resources associated with disposal (Chen et al., 2010). Large volumes of organic material create problems in urban environments and organics in MSW can be managed effectively with techniques such as composting (Gautam et al., 2010). Composting is considered as a suitable alternative for waste management through the diversion of organic waste materials from landfills and for creating a new product, which can meet agricultural demands for soil amendments (Eriksen et al., 1999; Wolkowski, 2003; Kanat et al., 2006). When the fresh organic material is directly added to the soil, it negatively affects crop development (Kononova et al., 1961) as its decomposition by soil fauna can release intermediate metabolites that are incompatible with plant growth (Zucconi et al., 1981 a, b). Other disadvantages of adding fresh organic matter to soil include the competition between microbes and plant roots for nitrogen, higher C:N ratio and ammonia production in the soil (Golueke, 1977). Composting is therefore considered a suitable way of creating a stable humic material from MSW organics and restoring the soil fertility. Although it is an old technology, composting produces a stable product which can improve the quality of soil (Araujo et al., 2001; Kaushik and Garg, 2003; Arau´jo and Monteiro, 2005; Larney and Hao, 2007) which can improve the soil structure, water holding capacity, soil fertility and buffering capacity (Reeves, 1997). Additional advantages of composting SSO include enhancement of plant growth (Bulluck and Ristaino, 2002), replacement of chemical fertilizers, storing of carbon in soil, and increasing the microbial biomass of soil (Bulluck and Ristaino, 2002; Arau´jo and Monteiro, 2006). Many countries have been applying SSO compost because

of its environmental benefits, including recycling nutrients and reducing costs of agricultural production (Pokhrel and Viraraghavan, 2005). Roca-Perez et al. (2009) demonstrated that the use of SSO compost on two Spanish soils increased soil fertility and organic matter content which in turn enhanced plant growth. Selivanovskaya and Latypova (2006) reported that applications of large quantities of SSO compost stimulated microbial biomass growth in forest soils of Russia. The increase in microbial biomass of soil is due to the presence of the organic carbon in compost (de Araújo et al., 2010).

There are, however, some negative effects of using MSW sourced compost rather than SSO. Some toxic heavy metals including mercury, selenium, cadmium and lead can be found in MSW compost and the application of this compost to soils may impact food safety (Gillet, 1992). Heavy metals that may be found in composts mostly arise from contaminants associated with non-source-separated municipal solid waste collection programs which include contaminants such as batteries, foils, and paint chips (Hamdi et al., 2003). Heavy metals present in MSW compost may negatively affect microorganisms, water quality, plant growth and animal/human health (Woodbury, 1992). The heavy metals content of compost may increase during the composting process due to weight loss by organic matter decomposition and the release of water (Yangsheng et al., 2007). Heavy metals are a major concern especially when their concentration increases due to repeated applications of compost to the soil. Richard (1992) showed that composting does not degrade heavy metal content and can be concentrated in compost. Serious consequences on plant and soil microbes can occur as a result of excess heavy metals in soils (Smith, 2009). Hence, SSO waste is recommended in order to produce high quality and safer compost (Deportes et al., 1995).

2.4 Maturity of Compost

The end product of composting MSW organics is considered beneficial if it meets safety guidelines for human health and the environment, and it can also enhance soil health (Wichuk and McCartney**,** 2010). Compost maturity is defined as a property that, when applied to plants, does not cause adverse effects including phytotoxic effects. In general, mature compost can be defined as a material which is ready for agricultural use or any particular purpose (Wichuk and McCartney, 2010). Compost maturity differs from compost stability in that, stability relates to the relative activity of microorganisms due to composting conditions while maturity applies to the absence of remaining readily compostable organics. Hence, maturity is related to the state of the material which can be more easily quantified and measured while stability depends on the temporal activity of the microbes at any point in time and involves some judgement. A mature compost has a very slow rate of biological activity under any conditions, contains no easily decomposable molecules, is usually dark in colour, and has an earthy smell with fine texture (CCME, 2005).

It is very important that the final product of composting be mature. Immature compost causes several detrimental effects including reducing the available soil nitrogen, which in turn causes nitrogen deficiency in crops (Jiménez and Garcia, 1989). In addition, due to the speedy degradation of immature compost, an anaerobic environment is often created around the plant roots, depleting the supply of oxygen. Furthermore, acidic compost also increases the solubility of heavy metals and inhibits the germination of plant seeds by producing phytotoxic substances (Rosen et al., 1997) like ethylene oxide, organic acids and ammonia (Sellami et al., 2008). High levels of microbial activity

in an immature product can also lead to self-heating which may cause fires due to entrapment of combustible gases in large piles (Brinton, 2000). Compost maturity, therefore, is chosen to be one of the primary parameters for determining the compost grade in Canada so as to protect the process of bioconversion of waste.

2.4.1 Measurement of Compost Maturity

Although there are no globally accepted test methods available to evaluate the maturity of compost (Wichuk and McCartney, 2010), some effective techniques are recognized to examine maturity. The most commonly used method is physical and sensory methods for testing compost maturity which include monitoring pile temperature, colour, and odour. Biological methods such as respiration (CO_2 evolution), phytotoxicity, and enzyme activity are often used to test the final compost products while chemical measurements of C:N ratio, organic matter, humification parameters, cation exchange capacity, EC, pH, ammonia and nitrate, spectroscopy and dissolved organic carbon can indicate changes in the material (Wichuk and McCartney, 2010).

Carbon dioxide is produced due to the activity of microorganisms and it is estimated to decrease, with the decline in activity of microbes as the compost material stabilizes. Hence, CO_2 evolution can be considered as a good indicator of maturity. CO_2 evolution testing is less expensive, accurate and simpler and many samples can be analyzed in less time (Wichuk and McCartney, 2010).

According to the Canadian Council of Ministers of Environment (CCME, 2005) guidelines, compost has to undergo a curing phase of 21 days and satisfy one of the following three conditions:

1. Possess a respiration rate ≤ 400 mg of $O_2\, kg^{-1}$ of OM h^{-1}.

2. Possess a CO_2 evolution rate ≤ 4 mg of CO_2-C g^{-1} of OM day^{-1}.
3. Experience a rise in compost temperature < 8 ºC above the ambient temperature.

The composting process can take from several months to years to attain maturity, but, this time, period may be reduced by an alternative technique that utilizes EM.

2.5 Effective Microorganisms

Higa (1991) from the University of Ryukyus, Okinawa, Japan, introduced the concept of EM and defined them as a group of mutually compatible species of microorganisms used to accelerate the decomposition process. They include lactobacilli (*Streptococcus lactis, Lactobacillus plantarum and L. casei*), yeasts (*Saccharomyces* species), photosynthetic bacteria (*Rhodobacter sphac*rodes and *Rhodopseudomonas plastris*) and actinomycetes (*Streptomyces* species) (Xi et al., 2002). The initial solution of EM prepared by Dr. Higa consisted of 80 different species and 10 genera of microbes isolated from Okinawa and other places of Japan. Eventually, the technology was redefined and only four of the above-mentioned species were included. This mixture of microorganisms is later blended in a sugar-based medium and the entire solution is maintained at low pH of approximately 3.0. The mixture does not contain any genetically modified microorganisms. EM products are manufactured in over 40 countries from species isolated from various countries. The technology is considered safe and environmentally-friendly (Sangakkara, 2002). Daly and Steward (1999) showed that a one mL quantity of EM contains at least 10^5 viable species of *Propionibacterium freudenreichil, Aspergillus oryzae, Candida utilis, Streptomyces albus, Mucor hiemalis, Streptococcus lactis* and *Saccharomyces cerevisiae* and an unspecified number of Rhodopseudomonas species, *Streptomyces griseus* and Lactobacillus species. A 40–60%

increase in worldwide agricultural production was observed from the year 1930–1998 as a result of extensive utilization of chemical fertilizers (Roberts, 2009). However, this has caused soil degradation in some areas and, financial instability for many small-scale farmers who comprise a major part of food growers in developing nations, as they cannot afford the increasing costs of these fertility products (Tittonell et al., 2005). In some cases, food has been contaminated with pesticides and these can also harm the environment to a great extent (Javaid and Bajwa, 2011). Recently, attempts have been made to replace the use of chemical applications with biological ones. The following sections outline the descriptions for various microorganisms present in EM solution.

2.5.1 Photosynthetic bacteria

These bacteria produce bioactive substances (amino acids, nucleic acids and sugars), that are secreted from roots and synthesize food materials by utilizing energy from the infrared spectrum (700–1200 nm) of solar radiation ranging while plants cannot do this. The plants tend to absorb these metabolites and act as substrates enhancing the biodiversity of microorganisms. Photosynthetic microorganisms help the proliferation of other microorganisms by producing nitrogenous compounds which are utilized by vesicular-arbuscular mycorrhiza (VAM). VAM absorb soluble phosphates from the soil which in turn supplies phosphorus to plants. VAM is in a symbiotic relationship with Azotobacter and enhances nitrogen fixation in legumes.

2.5.2 Lactic Acid Bacteria

Lactic acid bacteria are considered strong sterilizers; they suppress the growth of harmful microorganisms and speed up the microbial decomposition of organic matter. This bacteria accelerates the degradation of lignin and cellulose and ferments them,

which otherwise are hard to breakdown. They also suppress the growth of harmful fungi like *Fusarium*, a common plant pathogen.

2.5.3 Yeasts

Yeasts synthesize antimicrobial substances, essential for plant growth, from the amino acids and sugars produced by photosynthetic bacteria, plant roots and organic matter. Yeasts also produce bioactive substances like hormones and enzymes which promote cell and root division. Their secretions are useful for the growth of other EM.

2.5.4 Actinomycetes

Actinomycetes are the intermediate microorganisms between fungi and bacteria. They are able to synthesize antimicrobial compounds from amino acids produced by photosynthetic bacteria and organic matter and inhibit the growth of harmful fungi and bacteria. They mutually co-exist with photosynthetic bacteria and improve the quality of soil by enhancing the microbial activity (Condor et al., 2006).

2.6 Why Use EM?

Although the composting process is carried out by naturally occurring microbes, it may result in poor quality compost if the conditions are not adequately managed. The natural composting processes often take a significant amount of time to decompose organics from MSW due to the highly nitrogenous content of the feedstocks and often times the amount of area available to deal with large amount of organic waste is limited (Awasthi et al., 2014). Inoculation of compost enhances the composting process and quality of the final product (Mirdamadian et al., 2011). In this century, EM has been diversely utilized in agricultural and environmental management. Higa (1993, 2001, 2003) proposed that EM produces antioxidants such as inositol, saponin, ubiquinone,

low-molecular polysaccharides, chelating agents and polyphenols. These substances inhibit the harmful microbial population, enhance the multiplication of beneficial microorganisms and decontaminate harmful substances simultaneously. However, a single microorganism cannot produce all the necessary enzymes for complete degradation of SSO but use of microbial groups such as EM which act synergistically for rapid biodegradation of organic residues can help produce all the necessary enzymes.

There have been reports that research carried out on EM in Japan and the USA have shown a decrease in the dioxin content in soils (Sangakkara, 2002). The application of EM to decrease the composting time is not a rare practice. It is reported that a cellulose decomposing fungus, *Trichoderma harzianum* has reduced the composting time from three months to one month (Misra et al., 2003). Ghaffari et al. (2011) showed that the cellulase activity and decomposition of organic matter were increased by microbial inoculation. Cellulosic compounds such as vegetables, fruits and kitchen refuse are difficult to degrade and require a considerable amount of time (Nair and Okamitus, 2010). Inoculation increases the microbial number, expresses the beneficial microbial communities, improves the microbiological quality, produces desired enzymes and also enhances the degradation of organic material (Ohtaki et al., 1998). Application of EM increases the biodiversity of soil microorganisms and enhances the quality and yield of crops (Higa and Parr, 1994). Parameters such as temperature and organic matter content will change throughout the composting process affecting the microbial activity and rates of decomposition (He et al., 2011). Hence, inoculation may play an important role in promoting overall composting efficiency. Microorganisms decompose a broad range of chemical compounds from SSO and thus accelerate the decomposition process (Lei,

1999). Thermophilic bacteria degrade proteins and prevent the pH from dropping at the beginning of the process, allowing the proliferation of other thermophiles (Nakasaki al., 1994). Bacterial inoculum enhances the degradation of keratin and the formation of biofilms in poultry manure composting (Ichida et al., 2001). Photosynthetic bacteria produce nucleic acids, sugars, amino acids, bioactive substances and polysaccharides which enhance plant growth. These microorganisms are more active when adequate amounts of carbon and nitrogen are available, so it is recommended to apply some organic matter along with EM application (Yamada and Xu, 2000). Daly and Stewart (1999) showed that the yield of sweet potatoes, peas and onions increased 23, 31 and 29%, respectively after EM- treated compost was added. Even though pine bark co-composting with EM did not alter the process nor the quality of compost, adding this finished compost improved seedling growth in cabbage (Mupondi et al., 2006). Javaid and Mahmood (2010) showed that EM use in compost treatments produced had better plant growth and yield than conventional farming.

In contrast, the application of EM produced negative results on crop yield from studies by Daiss et al. (2008) and Javaid and Shah (2010). Lindani and Bvenura (2012) reported that the application of EM reduced the tomato yield as a result of immobilization of nutrients. Javaid (2009) showed that efficiency of these microorganisms is dependent on the soil fertility and other management factors. Although EM increased fruited plants of tomato, but the yield was not significantly higher as EM was inefficient to control tomato blight that affected the crop during a late growing season (Ncube et al., 2012). These researchers showed that inoculation did not accelerate the decomposition process during composting, as both the inoculated microbes and native microbes evolved at the

same time. Vargus-Garcia et al. (2006) showed that the effect of inoculum on composting processes depends on the chemical composition of raw materials and the microorganisms applied (Vargus-Garcia et al., 2006). In yet another study, the effect of EM application on the microbial decomposition process and the growth of banana plants was negligible (Formmwitz et al., 2007).

As with the benefits of EM, there is abundant literature that concludes EM is a poor amendment to speed up the composting process (Faure and Deschamps, 1991) and very sparse information is available about processes like carbon and nitrogen turnover and changes in fungal and bacterial population during the composting process (Formmwitz et al., 2007). There is a lot of unreliable information available on EM which is mostly business oriented (Condor et al., 2006). Even though some research has been done related to EM and plant growth, very little information is available on EM accelerating the composting process. Hence, it is essential to study the efficacy of EM when applied to compost

2.7 Research Objectives

The aim of the current study was to evaluate the effects of applying EM to the SSO portion of MSW from municipalities across Nova Scotia. The specific objectives were as follows:

1. To examine the potential of EM to improve the chemical and physical properties of maturing SSO compost.
2. To examine the effect of EM on the entire microbial decomposition process beginning with raw SSO compost.

2.8 Hypothesis

Based on the previous studies, it is hypothesized that the application of EM to SSO compost enhances the microbial decomposition process and produces a mature product in a shorter time period.

CHAPTER 3 MATERIALS AND METHODS

This thesis project is divided into two studies. The objective of study one was to examine the effectiveness of the commercially available EM in promoting microbial decomposition in maturing compost samples obtained from various municipal SSO composting facilities across Nova Scotia. In the second study, the effect of EM to promote microbial decomposition in a fresh SSO compost of barley straw and fresh green waste from the cafeteria kitchen of Dalhousie Agricultural Campus was investigated. In both studies, EM is regularly referred to as inoculants.

3.1 Study One: Evaluation of Inoculants to Enhance the Microbial Decomposition in Curing SSO

This study was designed to examine the ability of EM-inoculated composts (deemed to be immature by the facility managers), to accelerate the remaining decomposition process. The goal of this study was to evaluate the efficiency of the inoculants on SSO compost from various municipal sites around Nova Scotia.

3.1.1 Compost Sample Collection

Source-separated organics compost materials used in this study were collected in July and August 2012 from five composting facilities across Nova Scotia: the Cumberland Central Composting Facility (Cumberland), the Lunenburg Regional Community Recycling Facility (Lunenburg), the Pictou County Solid Waste Management Composting Facility (Pictou), the composting facility owned by New Era Technologies in Halifax (New Era), and the compost plant owned by Northridge Farms in Kings County (Northridge). SSO compost samples collected included curing samples deemed to be immature by site facility managers, except for New Era which provided only a mature

compost sample. The amount of sample collected from each of these five facilities is given in Table 3.1

Table 3.1 Mass of composts sample collected from facilities across Nova Scotia.

Facility Name	Amount Collected (kg)
Cumberland	33.7
Lunenburg	17.2
New Era	25
Northridge	17.4
Pictou	31

Compost samples were randomly collected with a shovel from various compost piles at different locations and depths and made into a composite sample. These samples were stored in plastic bins and subsequently screened using a 19 mm sieve to get rid of foreign materials, such as plastics, nails, screws and other metal objects. Sieved samples were then stored at 4º C for 11 months until the experiments were conducted. The criteria used by these facilities to classify immature compost from mature is given in Table 3.2.

Table 3.2 Characteristics of compost facilities across Nova Scotia.

Compost facility	Compos-ting technology	Annual capacity (Tonnes)	Feedstock received	Mature compost on-site criteria used	Immature compost on-site criteria used	Age of compost (months)
Cumber-land	Windrow	5,000	Residential yard trimmings and food waste	Cured for minimum of 21 days, respiration rate is ≤ 400 mg of O_2 kg^{-1} of volatile solids/hr, CO_2 evolution rate is ≤ 4mg of CO_2-C/g OM/day, Temp rise of compost above ambient temperature is < 8 °C	Not fulfilling the maturity requirements.	6
Lunen-burg	Aerated, turned wide bed	10,000	Residential yard trimmings and food waste	Temperature 15 °C	Temperature >15 °C	6
Pictou	Aerated, turned wide bed	5,000	Residential yard trimmings, food waste and commercial wastes from restaurant	No steam from pile after turning it, temperature around 20 °C.	Steam from pile after turning and temperature between 50–60 °C	12
New Era	Aerated container	25,000	Residential yard trimmings	Follow CCME guidelines (category A) and NSDE for compost maturity guidelines	Follow CCME (cat A) & NSDE compost maturity guidelines	
North-ridge	Bunker	12,000	Residential yard trimmings, food & commercial wastes	Smells like black earth.	Compost has stinky odour.	15

3.1.2 EM Products

The EM products chosen were TeraGanix Bokashi (EMRO, Tucson, Arizona), commonly referred to as Bokashi and ProBio Balance Plus (Emerald Earth, Santa Fe, NM), referred to as Pro-bio and were selected on the basis that they both contain several species of beneficial microorganisms. The former product contains *Bacillus subtilis, B. Cereus, B. megaterium, Azotobacter vinelandi, Lactobacillus acidophilus, Rhizobium japonicum and A. oryzae* along with alfalfa meal and seaweed extract as base ingredients and the latter product contains purified water, probiotic lactic acid cultures, organic sugarcane molasses, mineral powder, sea salt and rice bran liquid extract. These EM products are promoted to help prevent odor problems (Teraganix, 2015) and promote the growth of naturally occurring beneficial bacteria to achieve a healthy microbial balance when these microorganisms are introduced into the compost. The active compost treatment was a one-year-old compost made from horse manure and bedding that was made available through the Innovative Waste Management Research Program, Faculty of Agriculture, Dalhousie University. It was expected that the active compost would also increase the microbial population in the compost samples evaluated through inoculation of pre-established microorganisms. The molasses product chosen was the commercially available Plantation Blackstrap Molasses.

3.1.3 Experimental Design

Initially, the maturity of compost samples was tested using a modified respirometry method based on the Test Methods for the Examination of Composting and Compost (TMECC) (USDA and CCREF, 2002). In the modified method, sodium hydroxide traps were not used but instead CO_2 gas evolved from the compost samples

was measured by collecting gas samples from the head space of the Mason jars. The respirometry method followed involved, a collection of 25 g of as-is compost sample for each of the five facilities and two empty jars as a control, totaling 27 experimental units. The moisture content of the compost samples was adjusted to 60% on a gravimetric basis. The samples were pre-incubated at room temperature of (approximately 25 °C) for 24 hours to allow the microflora of all the compost samples to adapt to the mesophilic environment. After the period of pre-incubation, the samples were sealed in the 1000 mL Mason jars with a lid containing a rubber septum attached to it. All the jars were incubated at 37 °C for five days. The jars were placed in the incubator in a completely randomized design with five replications. A CO_2 gas sample was removed with a 20 mL syringe from the headspace air every day at the same time. After the sampling time was over, the headspace of all the jars was purged with ambient air and the bottles were resealed. This process was repeated over five days. This maturity testing procedure was repeated again on all the samples at the end of the study.

To test the efficiency of EM, a completely randomized design study was set up with one main factor, inoculum, and four levels with a control and four replicates. All the compost samples from the five different municipal sites were inoculated with four different inoculants (day 0): two commercially available EM inoculants (Bokashi, and Pro-bio), active compost, and molasses with the as-is un-inoculated compost acting as a control. Samples were incubated for 105 days at 37 °C (Fig. 3.1) to evaluate the decomposition through measurements of carbon evolved as CO_2. As the goal was to evaluate the efficiency of the EM (Bokashi and Pro-bio) within the sites, and not compare the effect of inoculants from site to site, only one factor of inoculants is involved.

A stock EM solution of all the inoculants, Bokashi, Pro-bio, active compost and molasses, was prepared by mixing 5 g (Bokashi or active compost) and 5 mL (Pro-bio or molasses) in 100 mL water to prepare a 5 % stock solution. This stock solution was later used to inoculate the compost samples. A 225 g compost sample was brought at 60% gravimetric moisture content on a wet basis by adding water of which 20% was 5% stock EM solution (Jusoh et al., 2013). The final amount of EM solution added for Cumberland, Lunenburg, New Era, Northridge and Pictou was 19.09, 14.49, 23.65, 23.09 and 16.72 mL, respectively. This quantity of solution was the same for all the inoculants (Bokashi, Pro-bio, active compost and molasses) but differed slightly among facilities. These inoculated samples were transferred to 1000 mL Mason jars for incubation. Active compost and molasses treatments were also added as inoculants in order to compare against both the EM treatments and the control. The choice of incubation temperature was based on reported optimum temperatures for the growth of bacteria at 37 °C (Gomez et al., 2006). Gas samples were taken using a 20 mL syringe inserted into each jar through septa on the lids of each experimental unit for the first 10 days and subsequently at five-day intervals. These gas samples were used to determine CO_2 evolved from each sample by GC analysis (Varian, 3800) (Fig. 3.2). After sampling the headspace, the lid of the sampled jar was opened to allow for air exchange with the additional use of a hair dryer (with no heat) for 30 seconds to create air movement. Later the lids were closed again until the next sampling time. Initially, the jars were sealed for 24 hours prior to sampling during the first 10 days (June 26th to July 4th, 2013) of the study and subsequently, from July 10th to October 15th, 2013 the jars were sealed only for one hour prior to sampling of the headspace. It was observed that keeping the jars closed

continuously for the entire 24 hours period lead to high CO_2 evolution rate. Hence, a small experiment was conducted whereby the lids of treatment jars were sealed for one hour, two hours, three hours and four hours intervals and gas samples were taken to determine the rate of CO_2 being evolved. It was found that closing lids for one hour yielded an optimum evolution rate of CO_2. The procedure for gas sampling from the treatment jars was changed following day 11 (July 10^{th}), the lids of all jars were sealed for one hour only just prior to collecting gas samples from the head space. Over the five-day intervals, the jars were covered with parafilm wax to allow for sufficient gas exchange but to reduce moisture loss. The parafilm would still allow for gas exchange in the jars (Bemis Company, 2010). The moisture content was maintained at 60% throughout the experiment by adding water at the time of sampling if the weight changed from the initially measured weight. At the end of the experiment, all the compost samples were analyzed for moisture content, pH and EC, organic matter content (loss on ignition), total carbon and nitrogen content.

Figure 3.1 Mason jars containing compost samples inoculated and incubated at 37 °C in a walk-in incubator, Faculty of Agriculture, Dalhousie University.

Figure 3.2 Gas chromatograph used to analyse gas samples obtained from headspace to determine CO_2 evolved from compost samples.

3.2 Study Two: Evaluation of Microbial Inoculants Added at Different Composting Stages in a Food Waste-Based Compost

3.2.1 Compost Pile Description

A one cubic meter compost pile was built in the Bio-Environmental Engineering Centre, in Bible Hill, NS on October 10, 2013. The feedstocks used were food waste from the student cafeteria (Faculty of Agriculture, Dalhousie University) as a nitrogen source, and barley straw as a carbon source. The amount of green waste and barley straw added were 68.34 kg and 94.70 kg (wet weight), respectively, to produce a compost mixture which had a C:N ratio of 29:1 (Faucette et al., 2000). The moisture content of as-is green waste was 80% and barley straw was 15%. The feedstocks were individually weighed in a plastic pail and then transferred to an in-vessel compost mixer for uniform mixing (Fig. 3.3). The in-vessel composter was maintained at two revolutions per minute and a built-in exhaust fan was used to draw air into the system. The feedstock was mixed for one hour and subsequently placed in a wire gauge cage, measuring approximately 1 m^3, and covered with foil insulation to reduce the loss of heat. Barley straw was also placed on top of the pile to prevent heat loss. Temperature probes were placed at three locations in the pile: bottom, center and top of the pile. Temperatures were monitored and logged every minute using a Campbell Scientific CRX100 data logger. The moisture content of the pile was maintained at approximately 50% to 60% by performing the squeeze test wherein the compost sample was squeezed by hand to estimate the percentage of moisture content. A sample which showed little drops of water dripping through the squeezed hand was considered to have the moisture of approximately 50% to 60%.

3.2.2 Sampling for EM Inoculation

During each compost sample collection period, the material from the compost pile was dumped on the ground and turned and mixed using a shovel and then transferred back into the wire cage. Water was added to the pile to maintain the moisture content based on the squeeze test as above. A total of 3.6 kg of material was collected from the compost pile during each collection date. The unscreened compost samples were transferred to 16 one-litre Mason jars with 225 g (wet basis) of compost in each container. The sample jars were then inoculated with three different inoculants (Bokashi, Pro-bio, active compost), and the un-inoculated sample acted as a control. The procedure for treating compost samples with inoculants is described in section 3.1.2. All the jars were incubated at 37 °C for 50 days. Gas was sampled from the headspace using a 20 mL syringe daily for the first ten days and then once every five days. Mason jars were sealed for one hour prior to the sampling to allow gas accumulation. After sampling the headspace, the lid of the sampled jar was opened to allow for air exchange which was assisted using a hair dryer (with no heat) for 30 seconds. A parafilm wax was placed on the jar openings to permit air exchange but to prevent moisture losses over the experimental period. Moisture content was maintained at 60% throughout the experiment by adding water during each sampling period based on gravimetric changes in the jars.

3.2.3 Experimental Design

A one-factor study was carried out with three EM treatments, Bokashi, Pro-bio and active compost, against a control in a completely randomized design with four replications in order to determine the effect of compost inoculum (i.e. EM treatments) on composting of cafeteria food waste mixed with barley straw. In this study, the molasses

treatment was not included in order to reduce the number of treatments. Compost samples were taken at three different composting stages (2, 50, 100 days from establishment of the compost pile), based on temperature ranges from the literature, from the main compost pile for inoculation and incubation: mesophilic (10–40 °C), thermophilic (>40 °C) and a second mesophilic or curing phase (10–40 °C). Therefore, the compost samples were collected from three different stages to examine how the EM inoculation interacted with material from each of the three different stages of composting. The first compost sample was collected one day after the compost pile was built to determine the effect of EM inoculation on the microbial decomposition process during the initial stages of a composting process with fresh material. All the raw materials were still fresh and the temperature was observed to have begun increasing. The second set of samples were collected from the same compost pile during the thermophilic stage when the temperature was between 60–70 °C. This period was mostly inhabited by thermophilic bacteria and actinomycetes and also heat-tolerant fungi. The high temperatures were due to heat generated by the intense metabolic activity and breakdown of proteins, fats, cellulose and hemicellulose by the microorganisms. The third set of samples was collected again from the same compost pile during the curing stage when the temperature was below 25 °C, as the mesophilic actinomycetes and bacteria are thought to be slow degraders of lignin and other resistant compounds.

Compost samples were tested for CO_2 evolution, pH, EC, and C:N ratios at each of the three stages before and after inoculation. Moreover, a maturity test was also conducted on samples collected from each of the three composting stages, but prior to inoculation or incubation, following modified procedures outlined in the TMECC (USDA

and CCREF, 2002). Briefly, 25 g of the as-is sample was weighed from each of the three composting stages tested in the study. The moisture content was adjusted to 60% gravimetrically. The samples were pre-incubated at room temperature of around 25 °C for 24 hours to allow the microflora of all the compost samples to adapt to the mesophilic environment. After the period of pre-incubation, the samples were transferred to 1000 mL Mason jars containing a seal with a rubber septum attached to it. All the jars were incubated at 37 °C for five days. The jars were placed in the incubator in a completely randomized design with five replications. A gas sample was collected with a 20 mL syringe from the headspace every day at the same time and transferred to vacuum vials for CO_2 analysis. Prior to each gas sampling, Mason jars were sealed for one hour. This process was repeated over five days. This maturity testing procedure was repeated again on compost samples collected at the end of the inoculation and incubation study for each composting stage.

Figure 3.3 SSO waste from student cafeteria mixing in the in-vessel composter.

3.3 Physical and Chemical Analysis of Compost for Study One and Two

Chemical and physical characteristics like pH, EC, organic matter, total carbon and total nitrogen (with three replicates) were measured initially prior to inoculating the compost samples, and at the end of the experiment (105^{th} day) after the inoculation and incubation periods. This was performed to examine the changes before and after inoculation on all the compost samples. The data from all the physical and chemical measurements was analyzed statistically based on four replications.

Physical and chemical analyses of the compost samples were conducted on samples from both studies 1 and 2. A measure of pH and EC was taken in an aqueous extract of compost according to procedures described in the TMECC (2001) manual (04.11 Electrometric pH Determinations for Compost). The aqueous extract was prepared

by mixing a 1:5 (w/v) ratio of compost and water and this mixture was shaken for 20 min and filtered. pH and EC were measured on the extract using an Accumet XL50 dual channel pH/Ion/Conductivity meter.

Table 3.3 Bulk densities of compost before inoculation on immature compost by host facilities.

	Bulk density (g cm^{-3})
Cumberland	0.8
Lunenburg	0.52
New Era	0.5
North Ridge	0.12
Pictou	0.59

To determine the moisture content present in the compost samples, the gravimetric water content of the samples was determined on samples dried in an oven at 60 ºC for 24 hours or until a constant weight was achieved.

The bulk density (Table 3.3) was measured by taking a representative compost sample after it was screened and transferred into an empty 20-liter pail. One-third of the material was added and the container was tapped five times and then filled to the top of the container without tapping. The weight of filled container was recorded. Bulk density was calculated by dividing the mass of the filled container less the mass of the empty container by the container volume (Dougherty, 1999).

To measure porosity (Table 3.4), a two-litre graduated cylinder was half-filled with compost and tapped firmly on the ground several times to settle the sample and the volume was recorded. The sample was removed and saved. The graduated cylinder was filled with 70 mL of water. Gradually, the previously saved compost was added, stirred

and allowed to stand for five minutes so that the bubbles escaped. The final volume of water and compost mixture was recorded (Trautmann and Krasny, 1997). Porosity of the sample is calculated using the following formulas:

Volume of solids (mL) = volume of compost and water mix – 70 mL water

Volume of pore space (mL) = volume of packed sample – volume of solids (mL)

$$Porosity = \frac{Volume\ of\ Pore\ Space}{Volume\ of\ packed\ sample}\ x\ 100$$

For determination of the C:N ratio, compost samples were first dried at 60 °C in an oven until a constant weight was achieved and these dried samples were then ground using a Retsch MM300 ball mill (Retsch Hann, Germany). Total carbon and nitrogen of the compost samples were analysed by taking a subsample for analysis using a LECO CN-2000 (Leco Corporation, St. Joseph, Michigan, U.S.A) by following the manufacturer's analytical method for compost samples. The LECO instrument was calibrated using blanks and Ethylene diamine tetra acetic acid (EDTA) samples. A 0.5 g certified alfalfa sample was also used throughout the LECO runs as a quality assurance measure of combustion and validity of the total carbon and nitrogen values.

The reduction in organic matter shows the stabilization of compost material and was estimated by measuring the volatile solids (VS) (Sullivan and Miller 2001). As the total carbon decreases, the stability of compost increases. So, organic matter content can be measured to assess changes in volatile components from the compost. Organic matter content was determined by drying the compost sample at 80 °C using a hot air oven for 12 hours and then weighing 10 g of this dried sample, which had been sieved using a 10.1 mm sieve.

Table 3.4. Porosity of compost before inoculation on immature compost by host facilities

	Porosity (%)
Cumberland	48
Lunenburg	6
New Era	66
Northridge	10
Pictou	6

The samples were then kept in a programmable muffle furnace (Fisher Scientific). The furnace temperature was slowly increased to 550 °C and the samples were combusted at 550 °C for 2 hours. After that, the temperature of the furnace was slowly decreased to approximately 200 °C. The ashed samples were removed from the furnace and transferred to desiccators to cool to the ambient temperature. The content of OM was then calculated based on the differences in weight of the initial dried sample and the final ashed sample.

3.4 Statistical analysis

Carbon dioxide data for both study one and two were analyzed as a repeated measures ANOVA using the most appropriate covariance structure (Littell et al., 1998). Based on the Akaike's information, the various covariances like Autoregressive (1), Compound Symmetry, Huynh-Feldt and Unstructured were run and the lowest AICC value was chosen. The repeated measures analysis was done separately for the first ten days of the study where the jars were sealed for 24 hours and then separately for the data of the remaining time periods sampled. If data did not meet the assumptions of normality

an appropriate transformation was applied and the means were back-transformed and presented in the results. The analyses were completed using the Mixed Procedure of SAS v.9.3 (SAS Institute Inc., Cary, NC, USA), and further multiple means comparison was completed for significant ($P < 0.05$) effects by comparing the least squares means (LSD) of the corresponding treatment combinations. A one-way analysis of variance was used to test the significance for pH, EC, organic matter content, and C:N ratio with Minitab 17 (Minitab Inc, State College, PA). The letter groupings were generated using LSD at a 5% level of significance.

For both of the studies, some of the data points for CO_2 evolution were not included for repeated measures analysis to protect from type I experimental error.

CHAPTER 4 RESULTS AND DISCUSSION

The following chapter is divided into two parts and the results from the effectiveness of the commercially available EM in promoting microbial decomposition during the curing phase (Study One) are presented in the first part which is followed by the results from second study which evaluated the effect of EM on microbial decomposition of SSO compost made from barley straw and fresh green waste (Study Two).

4.1 Study One: Evaluation of Inoculants to Enhance the Microbial Decomposition in Curing SSO

4.1.1 Physical Parameters of Compost Samples Before Inoculation

The initial values refers to different parameters of compost samples before inoculating the compost samples and final values refers to the after inoculation at the end of the 105 days of composting period (Table 4.1).

Compost samples collected from different facilities were analysed for pH before inoculation and the statistical analyses showed no significant differences between different treatments with inoculants (p -value = 0.90, Table 4.2). Similarly, statistical analyses performed on EC values of different compost samples from SSO composting facilities also identified no significant differences (p - value = 0.35. Table 4.3).

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Table 4.1 Initial and final values for chemical parameters measured in uninoculated (initial) and inoculated (final compost samples at end of incubation period) composts from municipal facilities.

Facility	pH		EC (dS m^{-1})		Organic matter (%)		Total carbon (%)		Total nitrogen (%)	
EM	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
Cumberland										
Control	7.99	7.50	1.98	8.29	39.48	35.91	-	-	1.98	2.47
Bokashi		7.85		8.24		38.28		-		2.27
Pro-bio		7.75		9.86		35.77		-		2.30
Active compost		7.91		7.47		35.34		-		2.25
Molasses		7.40		8.43		38.62		-		2.16
Lunnenburg										
Control	8.00	7.38	2.15	12.92	-	-	23.16	19.27	2.15	2.30
Bokashi		7.29		12.18		-		18.56		2.18
Pro-bio		7.27		13.36		-		18.87		1.80
Active compost		7.48		13.66		-		19.74		2.28
Molasses		-		11.99		-		20.06		2.39
New Era										
Control	8.17	7.48	2.60	10.07	56.06	36.79	22.86	16.98	2.81	2.01
Bokashi		7.69		9.19		47.08		20.54		2.49
Pro-bio		7.35		10.53		42.36		17.34		2.09

Facility	pH		EC (dS m^{-1})		Organic matter (%)		Total carbon (%)		Total nitrogen (%)	
EM	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
Active compost		7.41		11.06		45.68		19.88		2.45
Molasses		7.54		11.57		50.53		19.23		2.17
North Ridge										
Control	7.62	7.70	2.25	9.62	61.72	47.77	24.04	21.23	2.25	2.51
Bokashi		7.40		12.49		49.25		18.06		2.20
Pro-bio		7.78		4.01		47.03		18.34		2.14
Active compost		7.84		5.90		43.55		20.25		2.46
Molasses		7.83		9.90		50.17		16.86		1.90
Pictou										
Control	7.81	7.40	2.17	4.84	-	-	21.20	19.82	2.17	2.51
Bokashi		7.71		6.433325		-		19.76		2.48
Pro-bio		7.65		1.45709		-		19.61		2.46
Active compost		7.56		1.865956		-		19.84		2.39
Molasses		7.75		1.55		-		18.48		2.06

Table 4.2 Initial mean (n=3) pH values of compost samples from various composting facilities.

Facility	pH
Cumberland	7.99^{A}
Lunenburg	8.00^{A}
New Era	8.17^{A}
Northridge	7.62^{A}
Pictou	7.81^{A}

Means sharing same letters are not significantly different from each other at p = 0.05).

Table 4.3 Initial mean (n=3) EC values (dS m^{-1}) of compost samples from various SSO composting facilities.

Facility	EC (dS m^{-1})
Cumberland	1.98^{A}
Lunenburg	2.15^{A}
New Era	2.60^{A}
Northridge	2.25^{A}
Pictou	2.17^{A}

Means sharing same letters are not significantly different from each other at p = 0.05)

However, compost samples obtained from the different composting facilities had significant differences in C:N ratio (p - value = 0.00, Table 4.4). Among the five facilities, samples from Northridge had the highest C:N ratio followed by Lunenburg and Pictou whereas the New Era compost samples had the lowest C:N ratios.

The analysis of pH of compost materials from the facilities showed that the compost samples are slightly alkaline in nature suggesting they have been through a thermophilic stage when they were originally collected (Ko et al., 2008).

Table 4.4 Initial mean (n=3) C:N ratio values of compost samples from various SSO composting facilities.

Facility	C:N ratio
Cumberland	9.7^{C}
Lunenburg	11.3^{B}
New Era	8.2^{D}
Northridge	12.1^{A}
Pictou	10.5^{BC}

Means sharing same letters are not significantly different from each other at p = 0.05

However, EC and C:N ratio suggested that the compost samples were in a later phase of decomposition, curing phase, as evident by their values which fall in optimum ranges of 1–10 dS m^{-1} for EC and 10:1–15:1 for C:N ratio (Wichuk and McCartney, 2010). It is possible that the low C:N ratios were also suggestive of mixtures that had low carbon or high nitrogen.

4.1.2 Effect of Inoculating Composts

Table 4.5 Analysis of variance of chemical indicators for compost samples (at the end of 105 days) treated with different inoculants.

Parameter	Cumberland	Lunenburg	New Era	Northridge	Pictou
pH	0.003*	0.09	0.31	0.68	0.02*
EC	0.32	0.75	0.19	0.47	0.13
OM content	0.4	0.49	0.005*	0.46	0.09
C:N ratio	0.46	0.67	0.85	0.66	0.25

*significant at p = 0.05

After inoculating the compost samples with different treatments, only the compost samples from Cumberland and Pictou facilities showed significant differences (p – value

= 0.003 and 0.02 respectively) for pH, while New Era compost samples had significant differences (p – value = 0.005) in organic matter content after inoculating the samples with EM (at the end of 105 days) (Table 4.5).

4.1.2.1 *Effect of Inoculation on Compost pH*

Compost samples treated with different inoculants differed significantly in their pH values for both Cumberland and Pictou facilities. Cumberland samples treated with Bokashi, Pro-bio and active compost had the highest pH and those treated with molasses and control had the lowest pH. The starting pH of these samples, before adding inoculants was 8, which was reduced to 7.8, 7.7 and 7.9 respectively. These pH values were not statistically different from each other (Table 4.6). Compost material from the Pictou facility had significantly higher pH in the Bokashi, Pro-bio and Molasses treatments than the control but there was no statistical significance between the treatments (Table 4.6). The initial pH of the Pictou samples was 7.8.

The initial pH for Cumberland, Lunenburg, New Era, Northridge and Pictou was high. At the beginning of the composting process, the expectation was that a relatively high amount of carbon would be available for microbial consumption leading to the production of organic acids thereby decreasing the pH (Benito et al., 2003; Bertoldi). As the composting process moves further, microbial decomposition typically leads to a process of ammonification and also mineralization of organic nitrogen producing NH_4^+ (Huang et al., 2004, Petric et al., 2009 and Benito et al., 2003). This has the effect of raising the pH of compost materials during decomposition. Tuomela et al. (2009) also reported that microorganisms degrade protein to release NH_4^+ thus increasing the pH.

Table 4.6 Mean pH (n=4) values of Cumberland and Pictou compost samples inoculated with different inoculants).

Inoculants	Cumberland	Pictou
Bokashi	7.85^{A}	7.71^{AB}
Pro-bio	7.75^{AB}	7.65^{AB}
Active compost	7.91^{A}	7.56^{BC}
Control	7.50^{BC}	7.40^{C}
Molasses	7.40^{C}	7.75^{A}

Means sharing the same letters in a column are not significantly different from each other at p = 0.05.

4.1.2.2 *Effect of Inoculation on Compost EC*

The EC of inoculated compost samples was measured at the end of the study on 105 days. The addition of various microbial inoculants did not have a statistically significant effect on EC between different compost samples. However, by the end, EC increased two to six times compared to the initial samples in all treatments. The increase in EC may have been due to the release of NH_4^+ salts which is caused during organic matter decomposition (Abid and Sayadi, 2006). Under outdoor composting conditions, changes in EC may not be clear, as precipitation can leach these salts but in our study accumulation was measured since it was conducted in jars.

4.1.2.3 *Effect of Inoculation on Compost OM content*

The final OM content decreased in compost samples from Cumberland, New Era and Northridge facilities. However, a statistically significant difference ($p < 0.05$) was only observed for the New Era samples (Table 4.5). A reduction in the organic matter content can be attributed to microbial activity in the compost material which actively feed on organic molecules thus reducing the total carbon present (Benito et al., 2005).

Compost samples from New Era had an organic matter content of 56.06% before inoculation of the samples which was reduced significantly by Pro-bio and active compost. Table 4.7 shows that the New Era compost samples with Bokashi had a similar organic matter content as that of the control but Pro-bio greatly reduced the organic matter content in the compost samples. According to Garcia et al. (1992), this signifies that the organic matter is being mineralized by the microbial population as they utilize these compounds as a carbon source to produce energy. Ingelmo et al. (2012) also reported that the decrease, in the form of CO_2-C by microbial decomposition and mineralization, can be significant under conditions of high microbial activity in low stability organic materials. The lack of a significant reduction in organic matter in the Bokashi treated samples compared to Pro-bio might be due to two possible reasons, firstly, Pro-bio contains more organic products such as probiotic lactic acid cultures, organic sugarcane molasses, and rice bran liquid extract compared to Bokashi which might have provided an active medium for microbes to act upon. Secondly, there might not be adequate amounts of carbon material available for microorganisms to carry out decomposition process leading to very low microbial activity. Unfortunately, samples from Lunenburg and Pictou facilities were lost before inoculation due to analytical error and thus the initial organic matter content was undetermined

4.1.2.4 *Effect of Inoculation on Compost Total Carbon and Nitrogen*

There were small reductions in total carbon in compost samples from Lunenburg, New Era, Northridge and Pictou facilities signifying minor microbial feeding on the carbon material. On the contrary, total nitrogen increased only for Cumberland, Lunenburg, and Pictou with the maturity process and decreased for New Era. During the

composting process, all the New Era samples lost moisture very rapidly and dried up which resulted in disruption of the samples. This process might have led to an increase in ammonia loss and a corresponding decrease in nitrogen content. At the end of the composting period, the C:N was lower than 12:1 which was not significantly different from the initial C:N ratios.

Table 4.7 Mean (n=4) organic matter (%) content (after 105 days of composting) of New Era compost samples inoculated with different inoculants.

Inoculants	Organic matter (%)
Bokashi	47.08^{AB}
Pro-bio	42.36^{C}
Active compost	45.68^{BC}
Control	36.79^{B}
Molasses	50.53^{A}

Means sharing the same letters in a column are not significantly different from each other at p = 0.05.

4.2 Maturity test

A maturity analysis conducted on all the original samples obtained from the five SSO composting facilities (deemed to be immature) produced CO_2 evolution rates lower than the CCME threshold of 4 mg CO_2-C g^{-1} of OM day^{-1} (Table 4.8) (CCME, 2005). These very low values were likely a function of modified method used to collect CO_2 samples (Linda 2001, Benito et al. 2005) instead of the traditional alkaline trap method. A previous study showed that respiration rate measured from compost piles using alkaline trap method for one hour was almost double the respiration rate measured from head space method (1.5 vs. 0.7 mg C-CO_2 g^{-1} dw d^{-1} on day one of horse manure

compost). Similar results were also observed by Brewer and Sullivan (2001). This might be due to the reason that only a small amount of sample is used to determine the amount of CO_2 evolved in alkaline trap method compared to a larger sample in CO_2 gas sampling. This high sample size limits the oxygen available per gram of sample limiting CO_2 evolution rates as observed with CO_2 headspace samples. Benito et al. (2005) also found that increasing the sample size from 31 g to 125 g reduced the amount of CO_2 evolved by 40%. Another potential reason could be long storage period of compost samples at 4 °C after collection as the incubation study was not initiated until 11 months. Despite refrigeration, biological activity is known to continue and it is possible that the test materials decomposed sufficiently to impact the quality of the composts. Also the CO_2 values were expressed in CO_2-C g OM^{-1} day^{-1} instead of the CO_2-C g C^{-1} day^{-1} which might have also contributed to the low values.

Table 4.8 Comparison of mean (n=5) CO_2 -C evolution rates from Cumberland, Lunenburg, New Era, Northridge and Pictou facilities before inoculation and at the conclusion of test at 105 days with various inoculants.

Inoculants	Cumberland		Lunenburg		New Era		Northridge		Pictou	
	Before	After	Before	After	Before	After	Before	After	Before	After
	mg CO_2-C g^{-1} OM day^{-1}									
Bokashi	0.06	0.22	0.24		0.15	0.3	0.31	0.41	0.08	0.25
Pro-bio	0.06	0.16	0.24	0.29	0.15	0.25	0.31	0.55	0.08	0.47
Active compost	0.06	0.22	0.24	0.24	0.15	0.26	0.31	0.27	0.08	0.43
Control	0.06	0.25	0.24	0.37	0.15	0.34	0.31	0.33	0.08	0.28
Molasses	0.06	0.35	0.24	0.25	0.15	0.19	0.31	0.28	0.08	0.34

Even though the maturity tests results showed the compost samples to be already mature, it was determined important to evaluate whether residual microbial activity remained in the samples. Irrespective of the low CO_2 values in the maturity test, the compost samples from the five facilities were inoculated with the different EM treatments and monitored for CO_2 evolution for 105 days.

A subsequent maturity test was also conducted after the study period (105 days) on all the inoculated samples and the values were slightly higher but well below the CCME guidelines for maturity. The differences are possibly the result of natural variability between sample batches tested and experimental error.

4.3 Carbon dioxide Evolution from EM Inoculated SSO Composts

This study was divided into two phases. During the first phase, the CO_2 evolved was sampled after a 24 hours accumulation period in the headspace of the sealed container for 10 consecutive days. During the second phase of the study (11^{th} day of incubation onwards), CO_2 evolution was sampled at five-day intervals (with a one hour accumulation period) from the headspace of the sealed container. Even though the second phase of study began from 11^{th} day but the repeated measure analysis was performed from days 30−105 instead of days 11−105. These time points were reduced in order to avoid the type I experimental error in repeated measure analysis. The following data is expressed in CO_2-C g^{-1} C day^{-1} instead of CO_2-C g^{-1} OM day^{-1} which is the traditional unit for expressing the respiration rate in composting.

4.3.1 Cumberland SSO Compost

In the first phase, CO_2 evolved from different compost samples treated with different inoculants decreased significantly over time ($F_{36, 129} = 1.62$, p - value = 0.02).

Bokashi had higher effect on CO_2 evolved compared to other inoculants in the majority of the sampling dates (Fig. 4.1). Molasses and active compost had high rates of CO_2 evolution on the second day but decreased thereafter and remained low. In the first phase, the CO_2 values were below 3 mg CO_2-C g^{-1} C day^{-1}. The highest value observed was for Bokashi treated samples on 2nd day, 2.43 ± 0.01 mg CO_2-C g^{-1} C day^{-1}. During the second phase of the study (Fig. 4.2), no statistically significant differences between inoculants were observed. But carbon mineralization rates peaked for the Bokashi sample at 17.04 ± 0.19 mg CO_2-C g^{-1} C day^{-1} and the control at 17.34 ± 0.19 mg CO_2-C g^{-1} C day^{-1} on the 75th day.

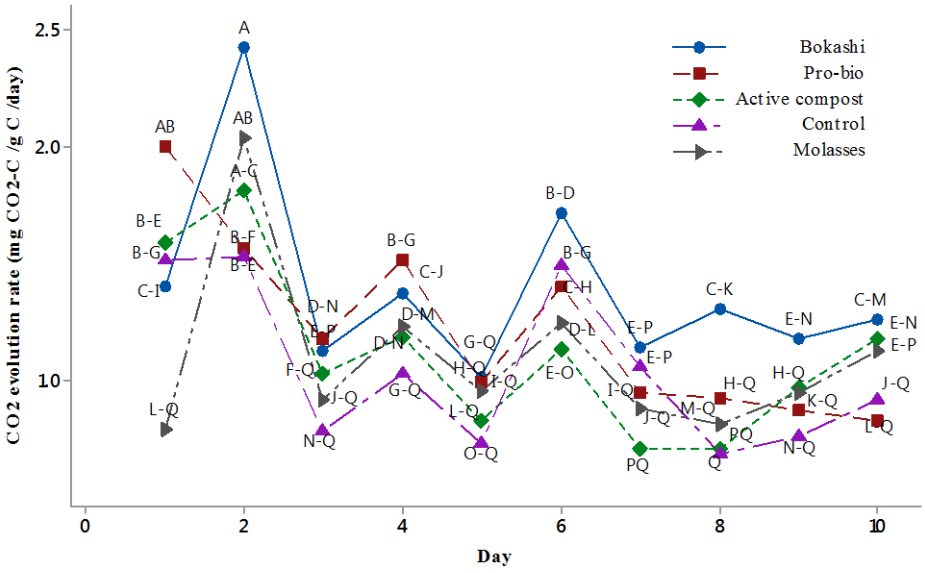

Figure 4.1 CO_2-C evolved from Cumberland compost treated with different inoculants over the first ten days period. (Means sharing the same letters are not significantly different from each other at p=0.05)

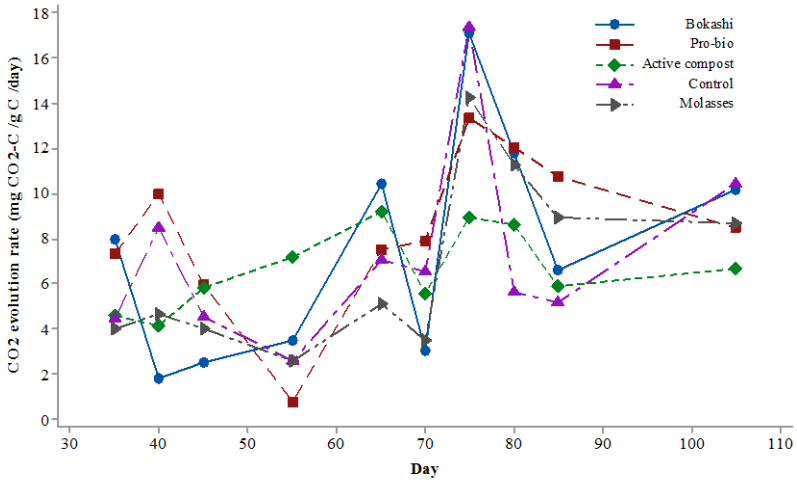

Figure 4.2 CO_2-C evolved from Cumberland SSO compost treated with different inoculants from Days 30 to 105.

4.3.2 Lunenburg SSO Compost

There was no significant effect of inoculants on first 10 days ($F_{36, 127} = 0.9$, p - value = 0.62) and from days 30 to 105 ($F_{36, 134} = 1.25$, p - value = 0.18) on CO_2 evolved for compost samples from the Lunenburg facility (Fig. 4.3). During first ten days, the highest CO_2-C evolution rate was observed on the second day for Pro-bio and control which are 3.15 ± 0.01 CO_2-C g^{-1} of C day^{-1} and 3.35 ± 0.01 CO_2-C g^{-1} C day^{-1} respectively and later it peaked around the 75th day at 14.93 ± 0.11 mg CO_2-C g^{-1} C day^{-1} for Bokashi and 14.12 ± 0.11 mg CO_2-C g^{-1} C day^{-1} for active compost (Fig. 4.4).

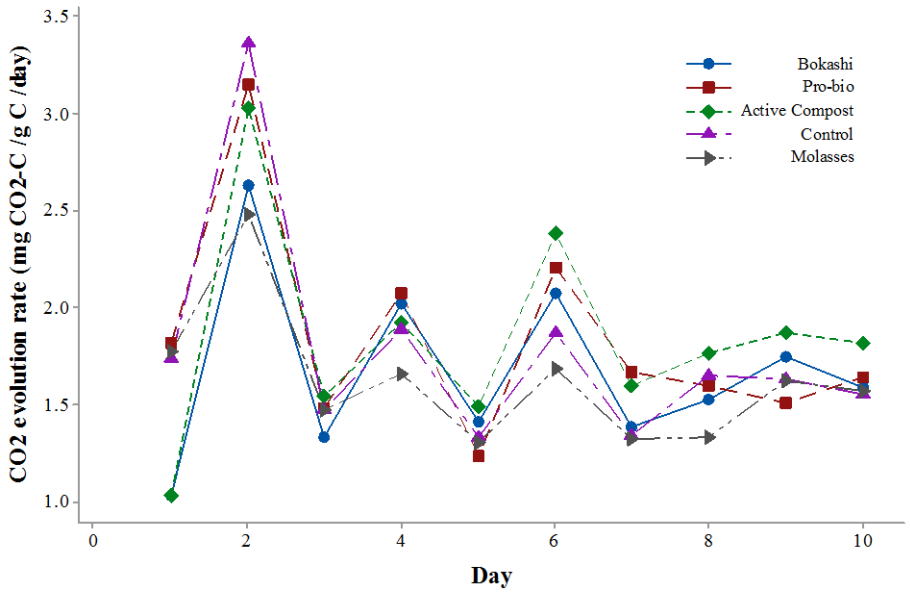

Figure 4.3 CO_2-C evolution rate from Lunenburg compost treated with different inoculants over the initial ten days of the study.

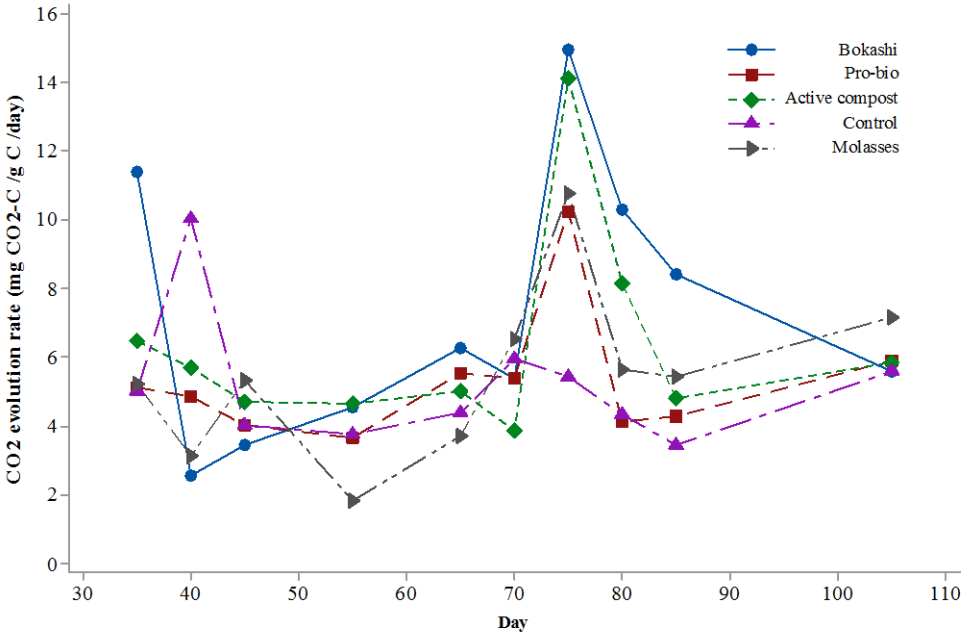

Figure 4.4 CO_2-C evolution rate from Lunenburg SSO compost treated with different inoculants from days 30 to 105.

4.3.3 New Era SSO Compost

The addition of inoculants failed to show any significant effect on CO_2 evolved ($F_{36, 128} = 1.26$, p - value = 0.17) during the first 10 day period. But from days 40 to 105, inoculants had a significant effect ($F_{24, 90} = 5.92$, p - value = 0.01) on CO_2 evolved (Fig. 4.5, Fig 4.6). During the experiment all the compost samples from New Era started caking up. However, the specific reason for this was not understood. Therefore, on 40th day, samples were re-inoculated at the same rate and were also mixed by adding water to bring the samples back up to a 60% moisture content. All this was done on the 40th day and the peak in the CO_2-C evolution rate observed on day 40 was 23.14 mg ± 0.05 and 23.43 mg ± 0.05 CO_2-C g^{-1} C day^{-1} for molasses and active compost treatments, respectively (Fig. 4.6).

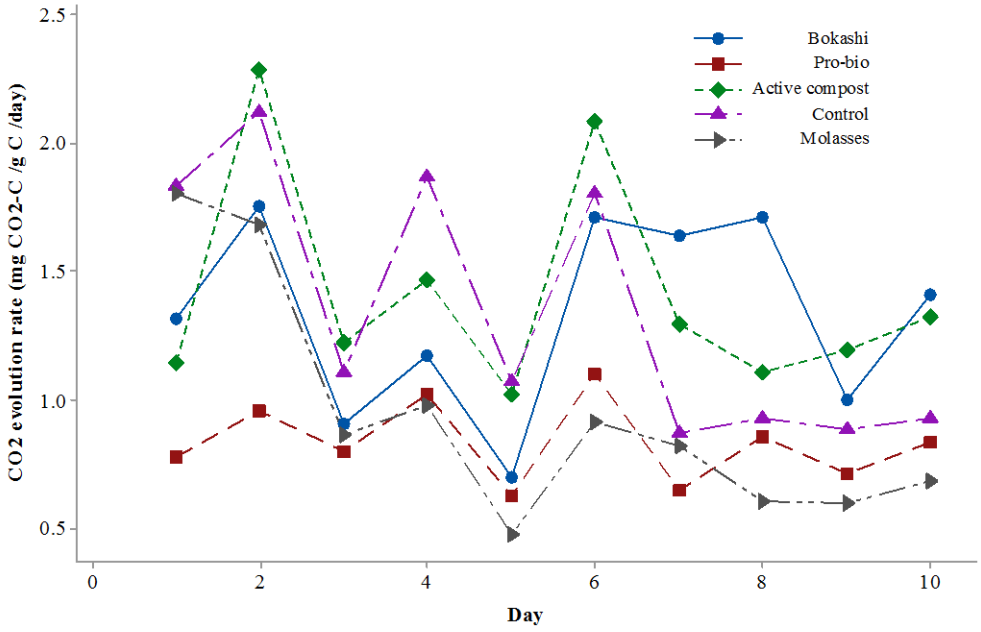

Figure 4.5 CO_2-C evolution rate from New Era compost treated with different inoculants over the initial ten days of the study.

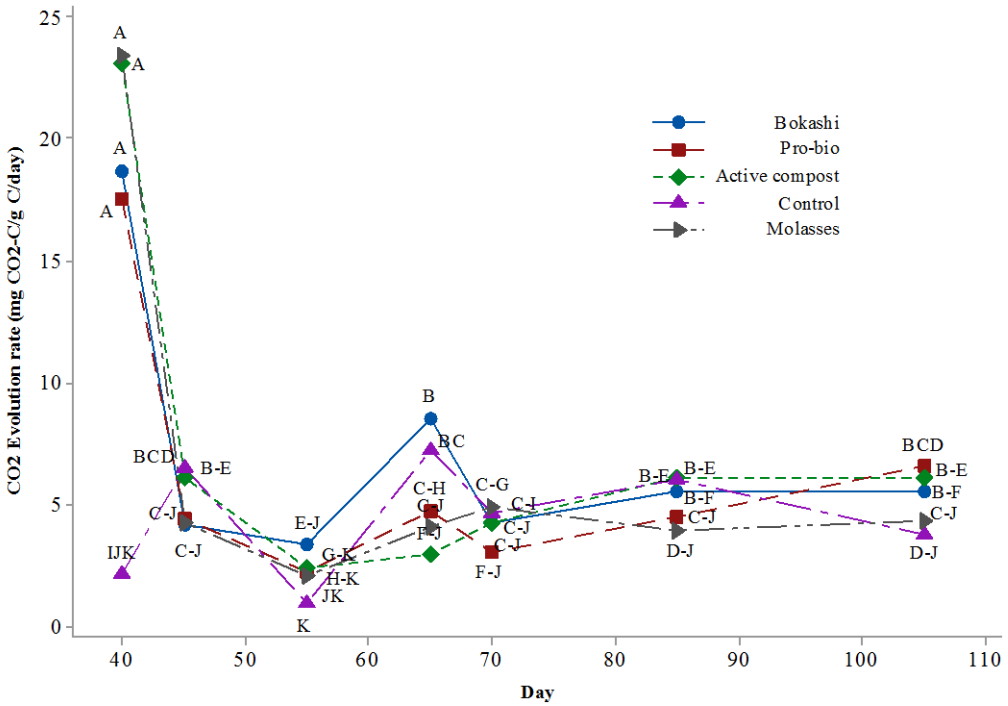

Figure 4.6 CO_2-C evolution rate from New Era SSO compost treated with different inoculants from Days 30 to 105. (Means sharing the same letters are not significantly different from each other at P=0.05)

4.3.4 Northridge SSO Compost

There were no significant effects of inoculants on CO_2 evolved during the first 10 days ($F_{36, 123} = 1.14$, p - value = 0.29) nor from days 30 to 105 ($F_{36, 127} = 1.35$, p - value = 0.11) in compost samples from the Northridge facility (Fig. 4.7, Fig 4.8). The highest rate of CO_2-C evolution was observed for Bokashi on second day at 3.66 ± 0.01 mg CO_2-C g^{-1} C day^{-1} followed by the control around the 35th day with a rate of 25.98 ± 0.18 mg CO_2-C g^{-1} C day^{-1} (Fig. 4.8). After this day, carbon mineralization rates decreased rapidly with a slight elevation of CO_2 evolved on 75th day.

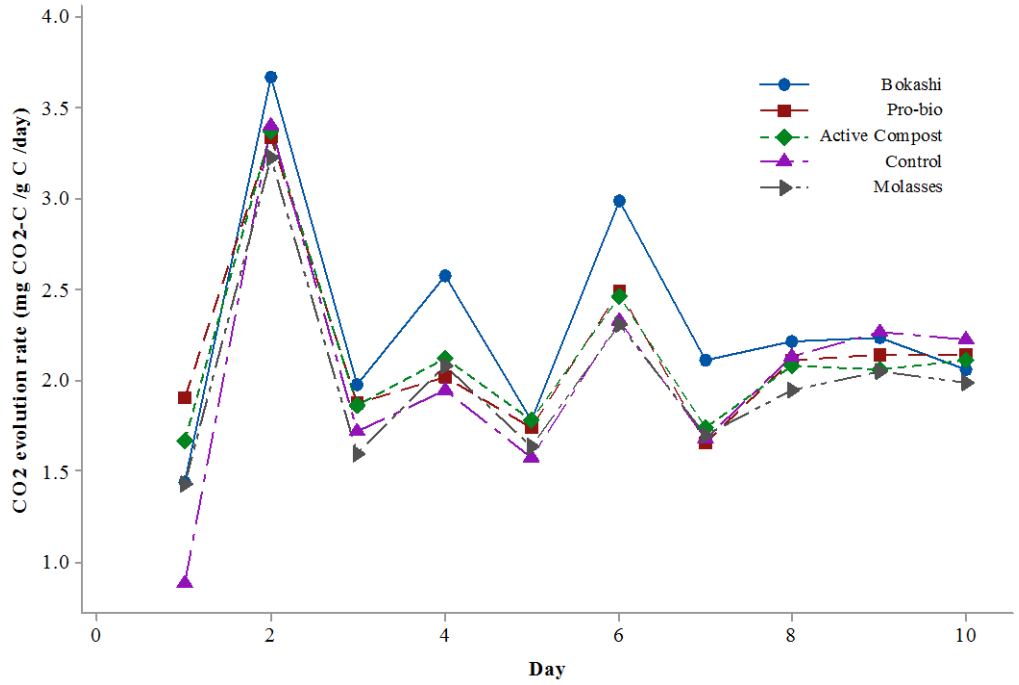

Figure 4.7 CO_2-C evolution rate from Northridge compost material treated with different inoculants over the initial ten days of the study.

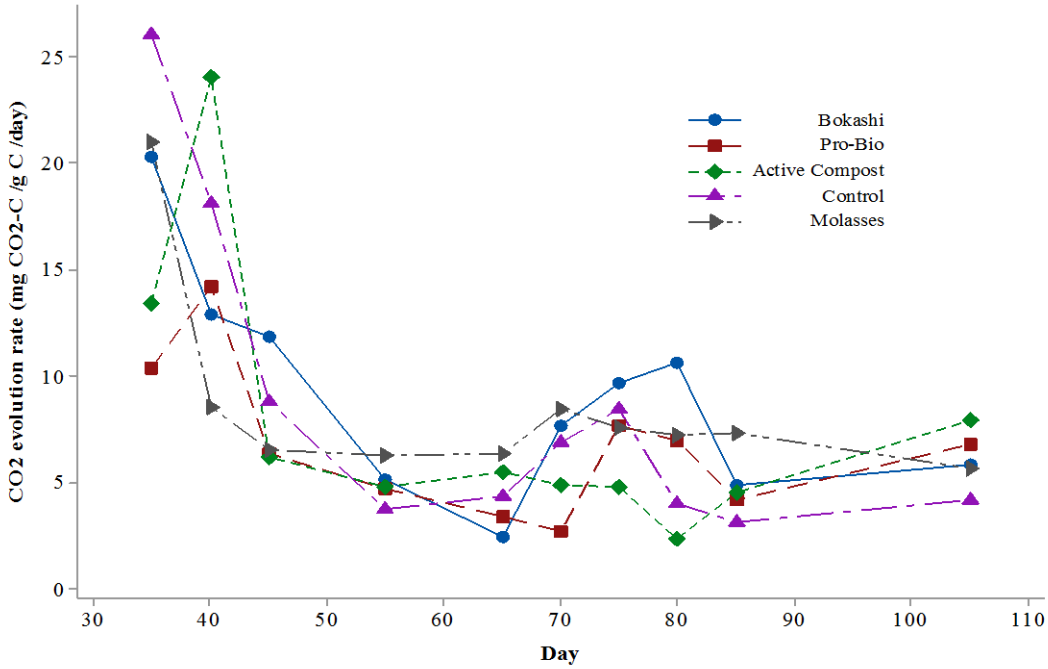

Figure 4.8 CO_2-C evolution rate from Northridge SSO compost treated with different inoculants from days 30 to 105.

4.3.5 Pictou SSO Compost

The CO_2-C evolution rates for Pictou were higher compared to other facilities over the first 10 days. However, as observed in other facilities CO_2 evolved did not differ significantly ($F_{36,129} = 1.12$, p – value = 0.3). Among the various compost samples treated with inoculants, the control and active compost showed the highest evolution rates at 7.54 ± 0.01 and 7.41 ± 0.01 mg CO_2-C g^{-1} C day^{-1} respectively (Fig. 4.9). Over the remaining study period, CO_2 evolved decreased with a slight elevation of the rate observed on the 75th day (Fig. 4.10).

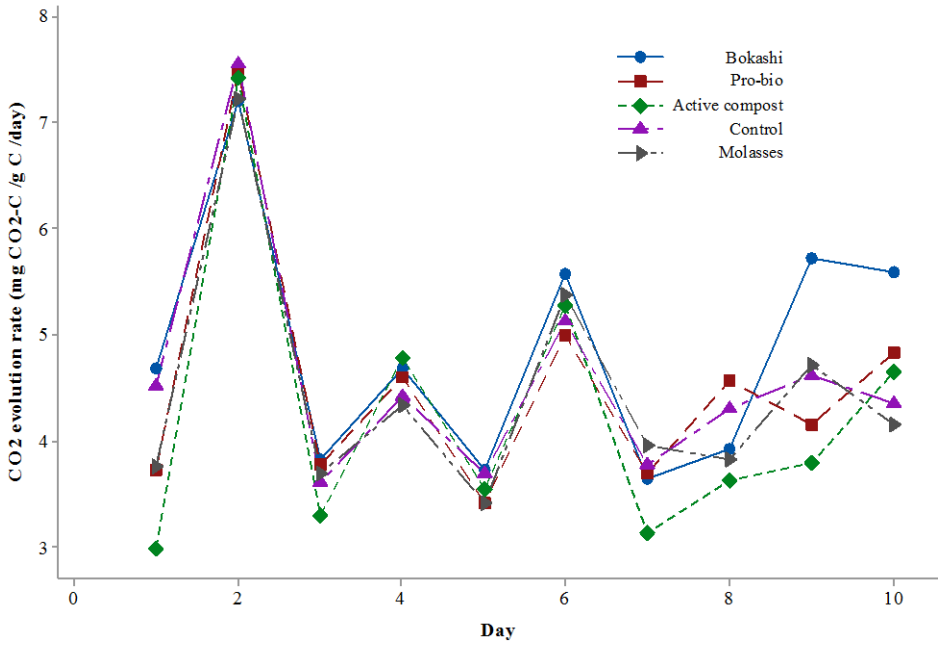

Figure 4.9 CO_2-C evolution rate from Pictou compost material treated with different inoculants over the initial ten days of the study.

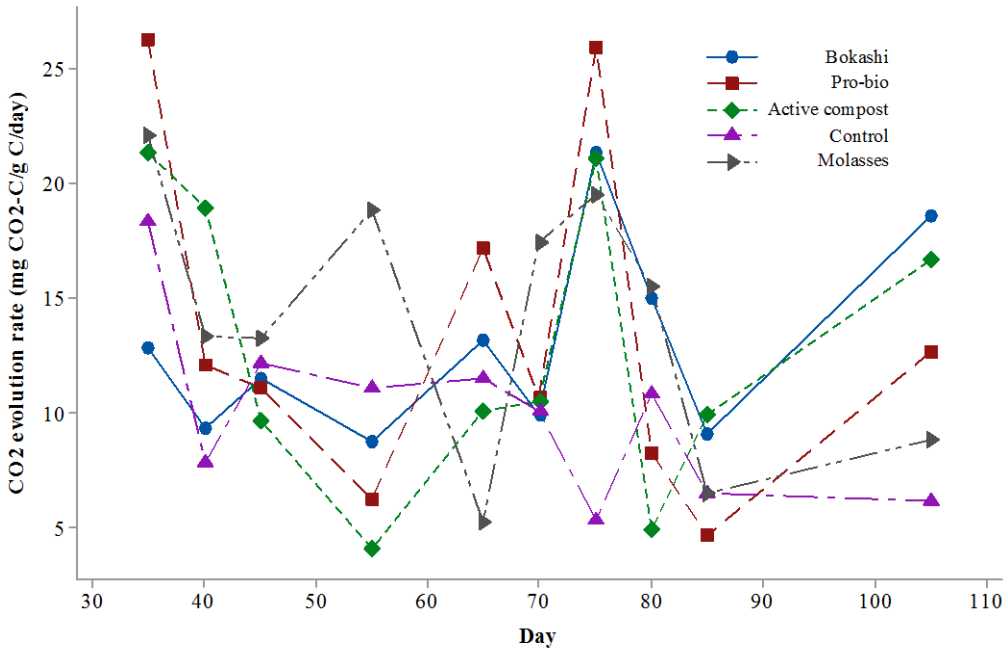

Figure 4.10 CO_2-C evolution rate from Pictou SSO compost treated with different inoculants from days 30 to 105.

In all the facilities, both the EM inoculants Bokashi and Pro-bio, had different effects on CO_2 evolution rates due to the fact that each inoculum contained different species composition and population of microorganisms (Ohtaki et al., 1998). In all the facilities, except for New Era, an elevation in CO_2 evolution rates was observed on the 75^{th} day. The reason for the peak at this time is not clear and is probably unlikely, but may have been due to an equipment malfunction. Additionally, Mason jar lids were sealed for 24 hours intervals between sampling during the first 10 days of the experiment which might have led to reduced oxic conditions or created anaerobic conditions decreasing the microbial activity which were in turn reflected in the low CO_2 values (Chen et al., 2012). Respiration values in the later part of the experimental period, days 11 to 105, were higher compared to the first 10 days, as sealing the lids for a shorter

period might have provided optimum aerobic conditions leading to higher microbial degradation. To maintain compost samples at 60% moisture content, an appropriate amount of water was added to compensate for the loss of moisture and bring back the moisture level to 60%. Nevertheless, loss of organic matter in the form of carbon was not taken into consideration from the weight loss measurements and it is possible that too much water was added throughout the study period.

The use of a gas headspace technique can be quite sensitive, but there is only limited work that has been done in the past comparing it to the alkaline trap approach and the majority of the studies report CO_2 evolution rates using alkaline (NaOH) trap method (Sadaka et al., 2006; Benito et al., 2005; Brewer and Sullivan 2001; Linda 2001). The major difference between the two methods is that, in the alkaline trap method CO_2 evolved is continuously trapped by the alkaline solution present, however in the head space sampling when the jars are sealed for 24 hours, the gradual buildup of CO_2 can decrease oxygen availability to the microbial community reducing metabolic activity and organic matter decomposition. The amount of gas sampled from the head space can also be affected by the volume of the samples. Compost samples having high volume occupies more space in the jars thereby influencing the amount of space available for CO_2 accumulation. Compost materials attain the optimum respiration rates depending upon the environmental conditions and the management practices (Matteson and Sullivan, 2006). Conducting studies of this type requires very tight control over the environmental conditions which is possibly a strong influencing factor in the results observed over this study.

There are some disadvantages associated with the respirometry technique employed in this study. A small amount of sample is often used to represent a very large compost pile collected from the facility (Chica et al., 2003). Some studies have shown that microbial respiration rates are not accurate as the microbial community is sensitive to variations in temperature, moisture, nitrogen and oxygen availability (Hermann and Shann, 1993).

4.4 Cumulative CO_2 Evolution

The cumulative carbon evolved by samples from all the five facilities were obtained by averaging the replications from each sampling time point and adding these values to the next day averages. These cumulative graphs are presented to represent the overall pattern of CO_2-C evolved over the entire time period from day one to day 105.

4.4.1 Cumberland SSO Compost

During the first 10 days after inoculation, the microbial inoculants, Bokashi and Pro-bio, had the highest microbial activity followed by molasses and active compost (Fig. 4.11). In all treatments, during the first 10 days period, the increases in CO_2-C evolution rates appeared to follow a linear response which is not typical of a highly volatile organic material. After day 45, and until day 60, the control treated had higher CO_2 evolution rates than the microbial inoculants (Fig. 4.12). The reason for this might be due to the small amount of EM applied which was less likely to dominate the naturally occurring microorganisms in the compost samples (Formowitz et al., 2007).

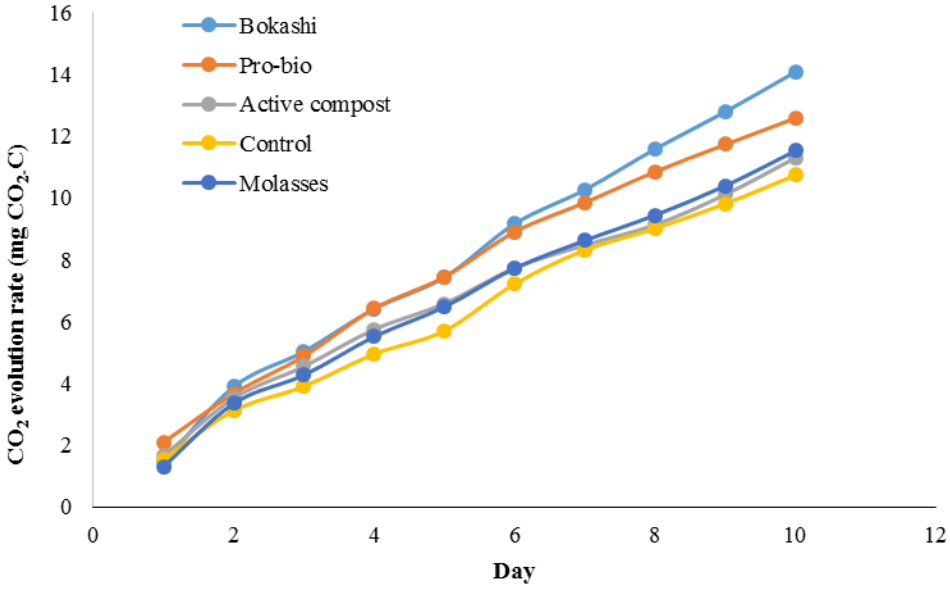

Figure 4.11 Cumulative CO_2-C evolved from Cumberland SSO compost over the initial ten days of the study.

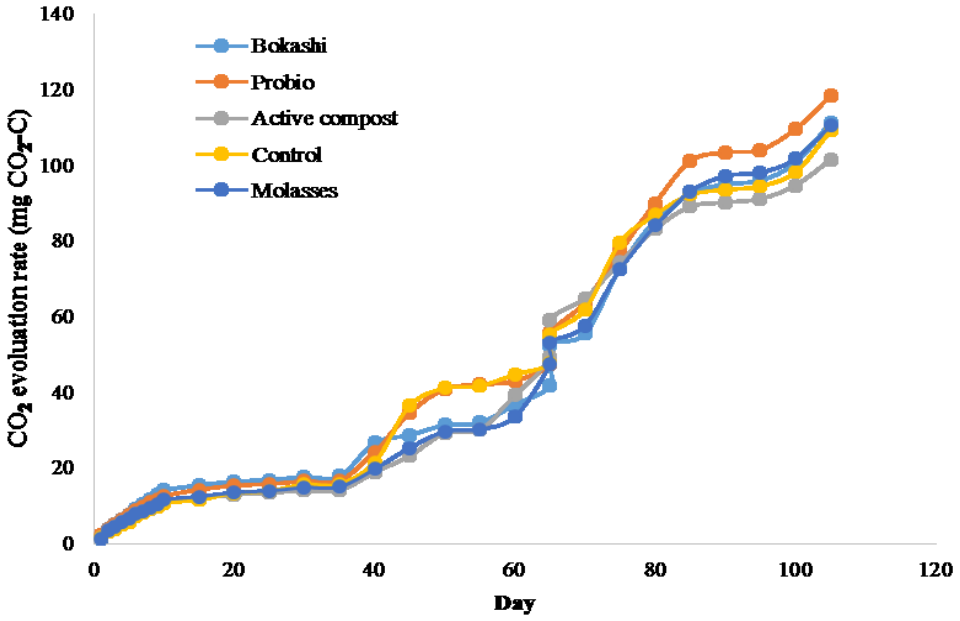

Figure 4.12 Cumulative CO_2-C evolved from Cumberland SSO compost over a period of 105 days.

4.4.2 Lunenburg SSO Compost

The first 10 day period did not show any distinct activity of CO_2 production for all the inoculants (Fig. 4.13). The rate of carbon mineralization in the control treatment and the inoculants were not appreciably different until day 80 (Fig. 4.14). After day 80, the Bokashi showed the highest evolution of CO_2. For the first 10 days, the compost samples all produced a near-linear increase, similar to material from Cumberland and within the same magnitude of CO_2-C evolved. Slightly greater separation in treatments occurred, with some apparent suppression in the inoculant treatments relative to the control. The compost control had microbial activity similar to Pro-bio but lower than the rate in the Bokashi and active compost treatments throughout the study period.

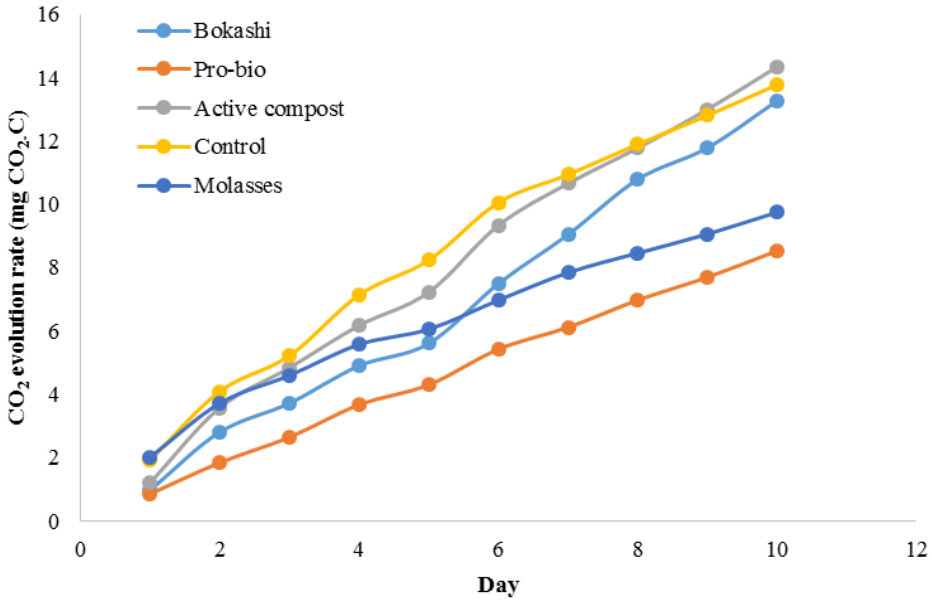

Figure 4.13 Cumulative CO_2-C evolved from Lunenburg SSO compost over the initial 10 days of the study.

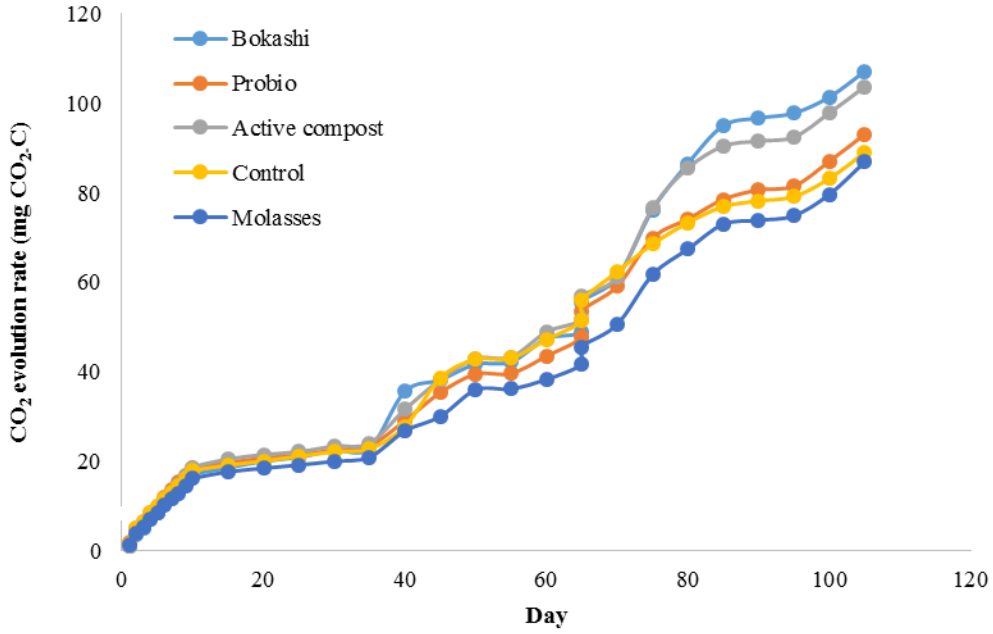

Figure 4.14 Cumulative CO_2-C evolved from Lunenburg SSO compost over a period of 105 days.

4.4.3 New Era SSO Compost

The Pro-bio, compost control and active compost treatments had slightly higher rates of activity during the first 10 days of the study (Fig. 4.15). From day one to 40, the control and active compost treatments had higher CO_2 evolution rates (Fig. 4.16). On day 40, a sudden increase in the CO_2 evolution rates resulted from removal and re-moistening of the samples. Due to extreme drying of the samples; they were inoculated again at the same rate and were also mixed by adding water to bring the samples to 60% moisture content. A rapid increase in the CO_2-C evolution rates was observed in the inoculant treatments relative to the control after that point.

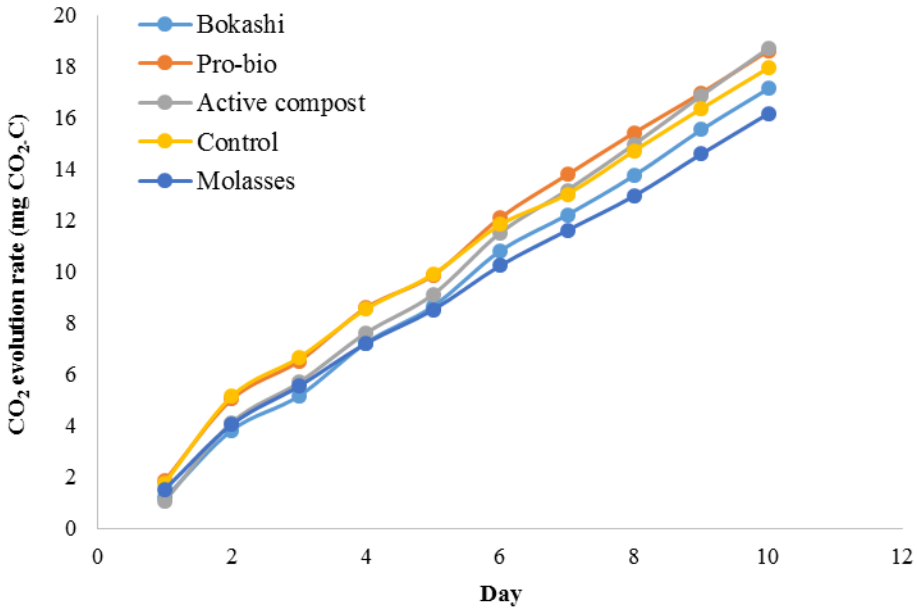

Figure 4.15 Cumulative CO_2-C evolved from New Era SSO compost over the initial ten days of the study.

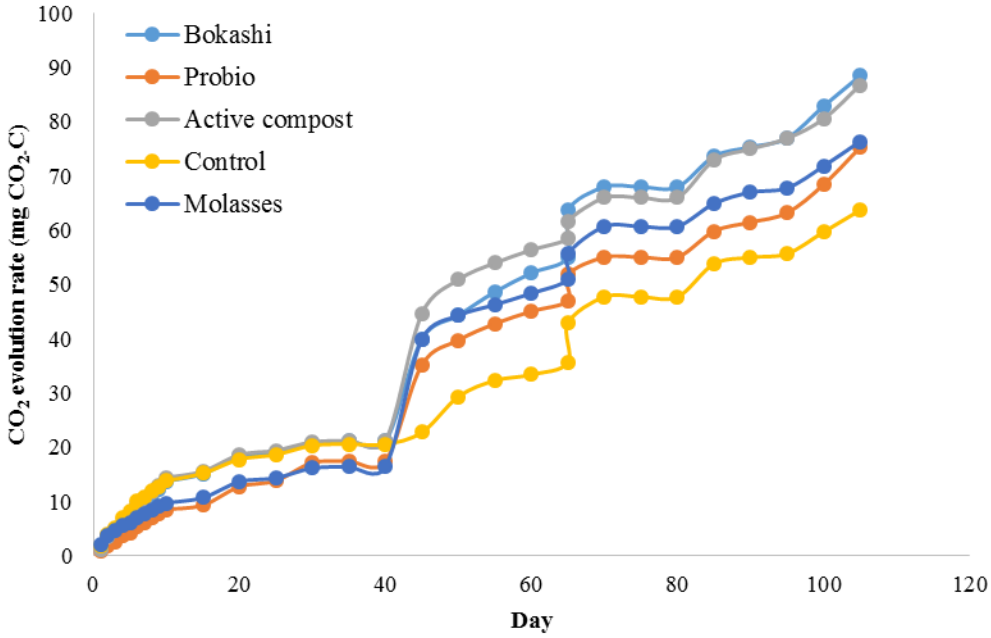

Figure 4.16 Cumulative CO_2-C evolved from New Era SSO compost over a period of 105 days.

4.4.4 Northridge SSO Compost

Over the initial ten days period, the Bokashi treatment was slightly more active than the other inoculant treatments (Fig. 4.17) but all the treatments followed a linear response through most of the study. The Bokashi treatment had the highest CO_2 evolution rates, except from day 40 to 70, where the control showed the highest value of CO_2 evolved (Fig. 4.18). Overall, there was no clear separation between the inoculant treatments with compost samples from this site.

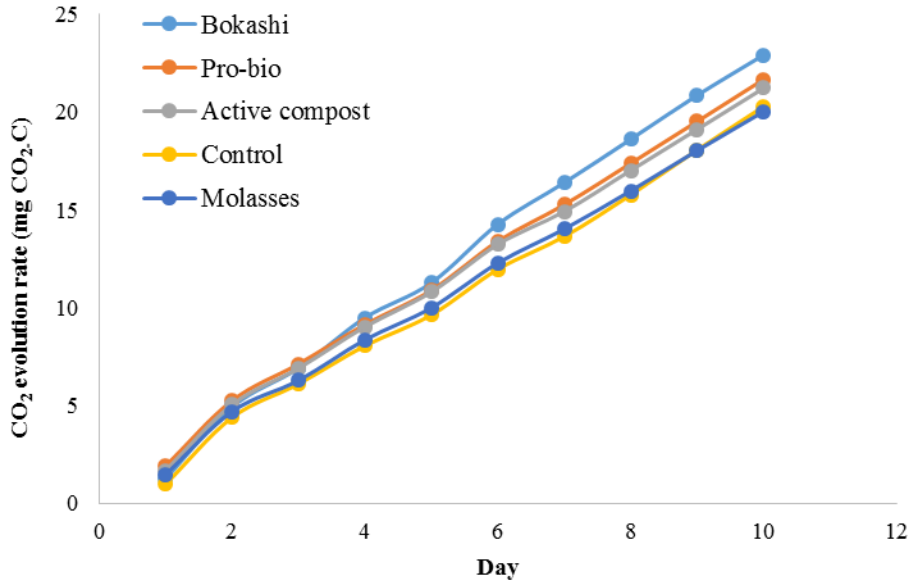

Figure 4.17 Cumulative CO_2-C evolved from Northridge compost over the initial ten days of the study.

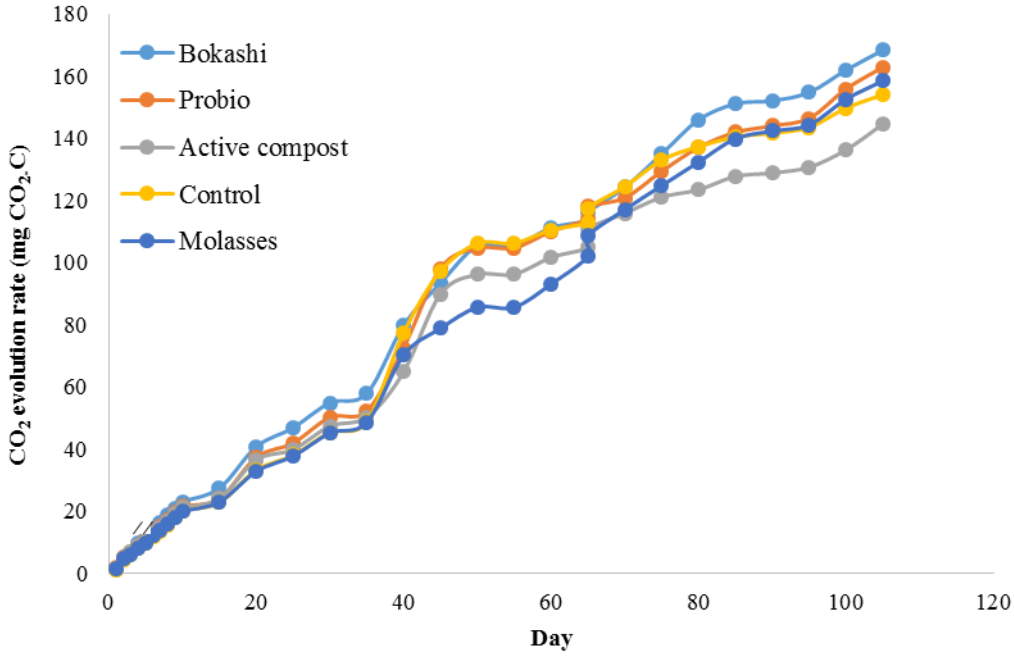

Figure 4.18 Cumulative CO_2-C evolved from Northridge SSO compost over a period of 105 days.

4.4.5 Pictou SSO Compost

There were only minor differences in the rates of evolution of CO_2 over the initial ten days (Fig. 3. 19) but all responses were linear. Bokashi and Pro-bio had the highest effect of inoculation for CO_2 evolution rates (Fig. 3.20). The CO_2 evolution rate from Bokashi treated samples were highest up to the 40^{th} day and from which point Pro-Bio had higher evolution rates followed by the molasses treatment. The magnitude of CO_2 evolution rates for material from the Pictou SSO facility was almost twice those observed in inoculated compost samples from other facilities.

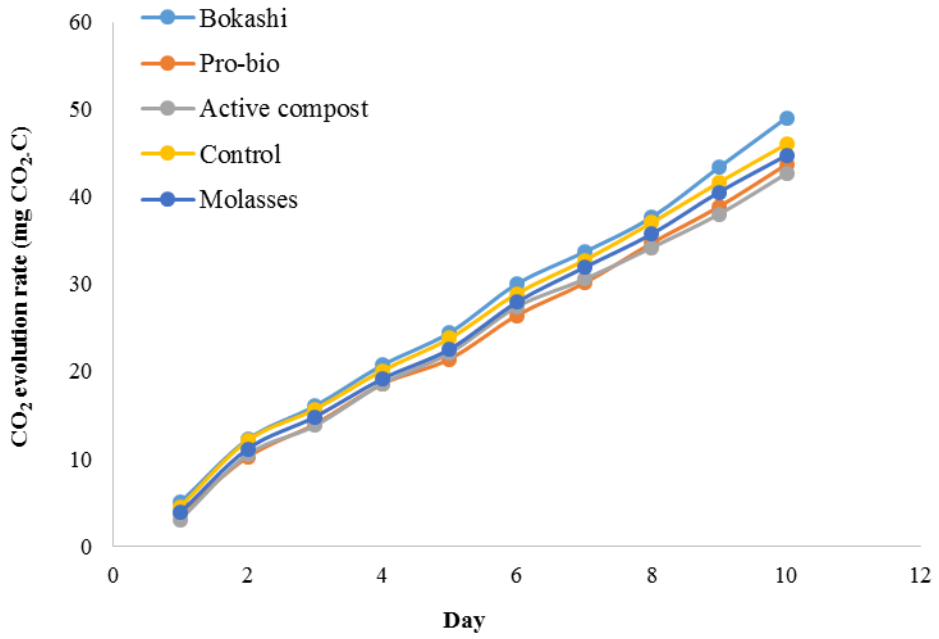

Figure 4.19 Cumulative CO_2-C evolved from Pictou compost material over the initial ten days of the study.

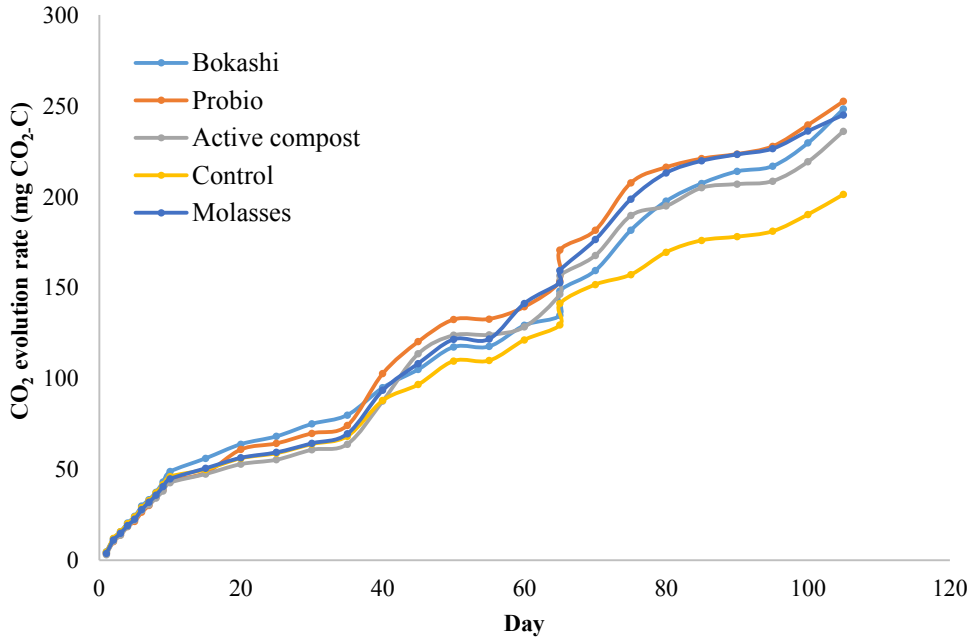

Figure 4.20 Cumulative CO_2-C evolved from Pictou SSO compost material over a period of 105 days.

4.5 Study Two: Evaluation of Microbial Inoculants Added at Different Composting Stages in a Food Waste-Based Compost

4.5.1 Compost Temperature Profile

Temperature data was collected at hourly intervals at three different depths throughout the entire study in a food waste compost pile. The following Fig. 4.21 shows the temperature data as an average daily temperature obtained by averaging the hourly temperatures recorded throughout the day. The focus of the discussion will be on the temperature at the center of the compost pile as it is the most stable and unaltered by the external factors. Initially, a rapid increase in temperature was observed within the first few days of making the compost pile which supports other studies showing high initial microbial activity when the raw feedstock is mixed together (Tiquia et al., 1997; Sivakumar et al., 2008). High initial temperatures and subsequent rapid decline achieved in this study are due to a static compost pile which after some initial compaction and high microbial activity might have significantly depleted the oxygen levels in the pile. After achieving peak temperatures within 24 to 48 hours, a linear decrease occurred over the following eight to ten days. The reason for the sudden decrease in temperatures is a combination of consumption of carbon sources by microorganisms and a dwindling supply of oxygen leading to a suppression of microbial activity. At temperatures above 65 °C inhibition of microbes takes place (Petric et al., 2009) but only certain species of fungi, thermophilic bacteria, and actinomycetes are only inactive (Gray et al., 1971). Temperatures increased rapidly after each turning event, 10 days and 35 days after pile preparation, peaking at approximately the same temperature (73 °C) each time but declining more rapidly thereafter each time.

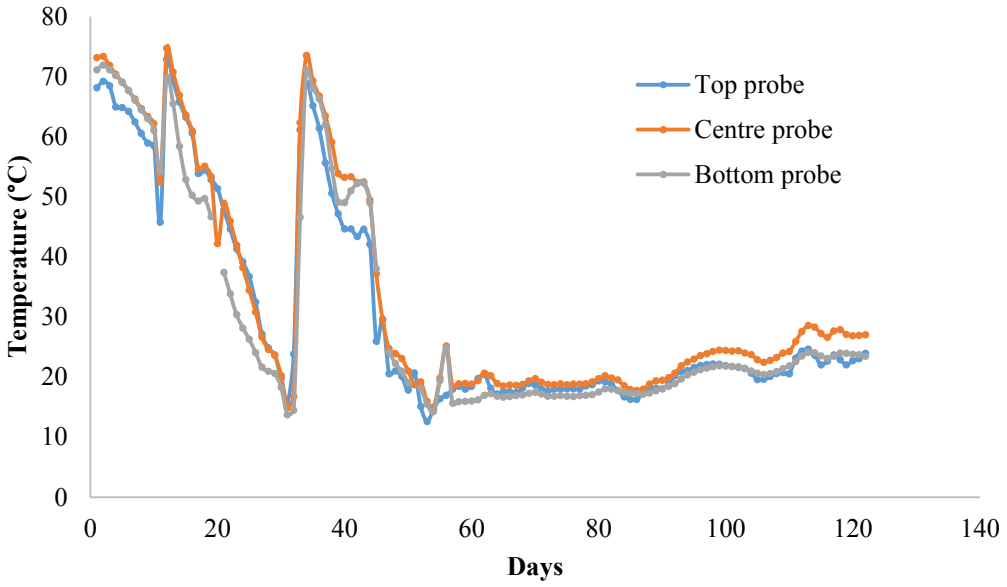

Figure 4.21 Average daily temperature profile recorded at three depths of the SSO compost pile.

After thermophilic conditions were achieved the temperature appeared to be maintained at 55 °C or greater for 3 to 15 days. By day 60, the temperature in the core of the compost pile had stabilized at higher than ambient but mesophilic temperatures. The pile was built during the fall season as temperatures began to drop below zero (appendix H) and, therefore, the temperature was stabilized by day 60 but remained above ambient conditions.

4.5.2 Compost pH

The initial pH of compost samples taken at each of the three stages prior to inoculation was neutral (Table 4.9). After inoculation, the final pH decreased slightly by the end of each 50th day of composting stage and by the end of the entire study remained slightly acidic (Table 4.9). The decrease in the pH values over the 50-day test period might be due to the release of organic acids from the breakdown of organic matter by

microbial activity. The inoculants had a significant effect on pH for stage one and three (Table 4.10). By the end of the study, the final pH for all the three stages remained in between the optimum pH range of 6 to 8. In stages 1 and 3 of the composting process, Bokashi, active compost and control treatments had the highest pH followed by Pro-bio whereas for the stage 2, the addition of inoculum did not cause any significant differences in pH (Table 4. 11). Wu et al. (2000) also found that the final pH values in the range of 5.8 to 8.4 for food waste, animal manure and wood chip using forced aerated windrow composting method.

4.5.3 Electrical conductivity

The initial EC before inoculation was low but increased after inoculation for all the treatments (Table 4.9). This is probably due to the decomposition of organic matter which releases mineral salts, such as aluminium ions (Abid and Sayadi, 2006). The addition of inoculants had a significant effect on the EC for all the three stages studied (Table 4.10). In stage 1, the EC was highest for the control compost sample and all the inoculated samples had lowest EC, however, these three did not differ statistically. On the other hand, Pro-bio had the lowest EC during stage 2 of the composting process and Bokashi and control treatments had the highest (Table 4.12). In stage 3, samples treated with active compost had the lowest EC measurement but was significantly different from Bokashi and Pro-bio but the latter two were not different from each other. EC measured during these three stages showed that there was a gradual increase in the conductivity values. The incubation was set up as a closed environment which precluded any leaching from the compost samples, which under outdoor conditions would have resulted in the solution being removed and possible loss of electrolytes. Electric conductivity values

obtained in the present study are on par with the final EC values (0.38 to 0.48 S m^{-1}) for food waste compost reported by Wu et al. (2000).

Table 4.9 Initial (before inoculation) and final (after completing each stage) values of chemical parameters measured at each stage of the composting study.

Composting stage	pH		EC (dSm^{-1})		Organic matter (%)		Total carbon (%)		Total nitrogen (%)	
	Initial	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
Stage 1										
Control	7.23	7.10	2.34	1.75	87.27	82.27	38.35	34.15	1.31	2.28
Bokashi		7.36		1.47		85.09		34.79		2.55
Pro-bio		7.03		1.40		84.13		35.84		3.09
Active compost		7.17		1.43		79.49		36.29		2.55
Stage2										
Control	7.05	6.75	3.43	4.52		—	41.12	36.41	1.17	2.25
Bokashi		6.71		4.73		—		32.47		2.24
Pro-bio		6.79		3.71		—		32.18		2.36
Active compost		6.74		3.93		—		35.26		2.19
Stage3										
Control	6.95	6.08	2.56	4.99	85.26	79.95	40.62	35.06	2.55	2.31
Bokashi		6.14		5.49		76.17		34.13		2.39

Composting stage	pH		EC (dS m^{-1})		Organic matter (%)		Total carbon (%)		Total nitrogen (%)	
	Initial	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
Pro-bio		5.87		6.20		77.10		35.32		2.37
Active compost		6.18		4.23		52.66		34.35		2.20

Table 4.10 Analysis of variance for chemical analysis indicators from samples taken at stages 1, 2 and 3 of the composting process after inoculation with EM compounds.

	Stage 1	Stage 2	Stage 3
pH	0.05*	0.75	0.02*
EC	0.03*	0.01*	0.02*
OM content	0.92	0.03*	0.82
C:N ratio	0.07	0.33	0.33

p-values are significant at p = 0.05

Table 4.11 Mean (n=4) pH of the compost samples treated with four inoculants studied at stage 1, stage 2 and stage 3 measured at the end of each stage.

Inoculants	Stage 1	Stage 2	Stage 3
Control	7.20^{A}	6.71^{A}	6.08^{A}
Bokashi	7.36^{A}	6.79^{A}	6.14^{A}
Pro-bio	7.03^{B}	6.74^{A}	5.87^{B}
Active compost	7.17^{AB}	6.74^{A}	6.18^{A}

Means sharing the same letters in a column are not significantly different from each other at p = 0.05.

Table 4.12 Mean (n=4) EC of the compost samples treated with four inoculants studied at stage 1, stage 2 and stage 3 measured at the end of each stage.

Inoculants	Stage 1	Stage 2	Stage 3
	dS cm^{-1}		
Control	1.75^{A}	4.52^{AB}	4.99^{AB}
Bokashi	1.47^{B}	4.73^{A}	5.49^{A}
Pro-bio	1.40^{B}	3.71^{C}	6.20^{A}
Active compost	1.43^{B}	3.93^{BC}	4.23^{B}

Means sharing the same letters in a column are not significantly different from each other at p = 0.05.

4.5.4 Organic Matter

Measurement of organic matter content showed there was a gradual decline throughout the composting process. The inoculants had significant effects on stage 2 organic matter content which might be due to the fact that the microbial activity was very high during this period leading to high microbial degradation and thermal breakdown of carbon bonds. These values are on par with the organic matter content values of Bustamante et al. (2008) and are attributed due to organic matter degradation. According to Bustamante et al. (2008), lowest mineralization of organic matter was observed in the maturation phase.

4.5.5 Total Carbon and Nitrogen

There were considerable differences between the total carbon values before inoculation in stage 1 and after inoculation in stage 3 (Table 4.9). As expected, the total carbon decreased by the end of each of the three stages. The high temperatures achieved throughout each turning of the compost suggested a large amount of microbial metabolism taking place which suggests that the substrate was highly degradable.

The final nitrogen content increased for stages one and two but was not significantly different at stage three. The increase is due to an overall reduction in the size of the compost pile and transition from organic nitrogen to a mineralized form which is subsequently re-absorbed into microbial tissue. The transition and accumulation of nitrogen in the composting system are often reported when a compost becomes more stabilized (Tiquia and Tam, 2000). No significant differences in the C:N ratio were observed between different treatments throughout the experiment (Table 4.10). A trend of higher C:N ratios in the control was observed for stages 1 and 2. The final C:N ratio

across all treatments was below 16.1, an optimal range for a curing compost. The same values (14;1 to 16:1) were also obtained for a food waste compost studied by Wu et al. (2000)

4.6 Comparison of CO_2 Evolution for a Food Waste Compost Inoculated during the Three Composting Stages

The following sections outline the results for CO_2 evolved for stage 1, stage 2 and stage 3 after inoculating the compost in each stage and also the cumulative accumulation of CO_2 of all the three stages separately.

4.6.1 Stage 1

Treating compost samples with different inoculants only had a marginally significant influence on respiration rates compared to the uninoculated control in this stage ($F_{33, 132}$ =1.51, p-value = 0.055, Fig. 4.22). CO_2 activity was high during the first nine days, exceeding 9 mg CO_2-C g^{-1} C day^{-1} but declined to 1 mg CO_2-C g^{-1} C day^{-1} by day 10. The high CO_2 values observed at the beginning were due to the availability of high amounts of carbon and nitrogenous material leading to high microbial activity (Barren et al., 2007). With the progress of the composting process, the availability of carbon material and oxygen decreases consequently reducing the activity of microbes (Barren et al., 2007). It was noted that a small amount of water had pooled at the bottom of some containers, likely due to water which was added at the start of the incubation draining over time, which may have created a slightly anaerobic environment. Temperature variations were observed in the incubator due to a malfunctioning temperature controller, leading to temperatures being reduced from 37 to 19 °C over a two–week period during the initial stage. In this instance, the final rates of respiration

measured may have been affected by other factors and not directly related to any treatment effects.

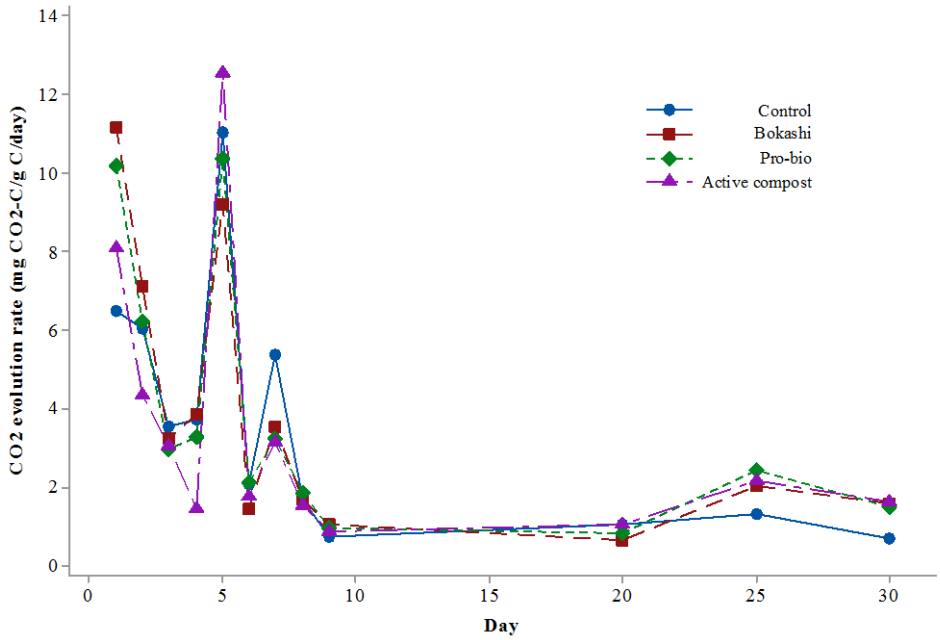

Figure 4.22 CO_2-C evolution rate from stage 1 inoculated food-waste based compost material in a controlled environment study.

4.6.2 Stage 2

The inoculants had a significant effect on CO_2-C evolution rates ($F_{32,122} = 4.67$, p - value = 0.0001) during the incubation period of stage 2. Samples from stage 2 treated with different inoculants responded differently than the control. Overall, the control seemed to have maintained higher respiration rates of CO_2 than the inoculants (Fig. 4.23). Bokashi, Pro-Bio and active compost treatments had slightly lower respiration rates. The food-waste used in this experiment was not shredded which might have led to slower organic matter degradation due to lower surface area and reduced access to available carbon. Therefore, increasing surface area through physical breakdown of particles could

have enhanced organic matter degradation and thus the amount of CO_2 evolved (Tremier et al., 2005).

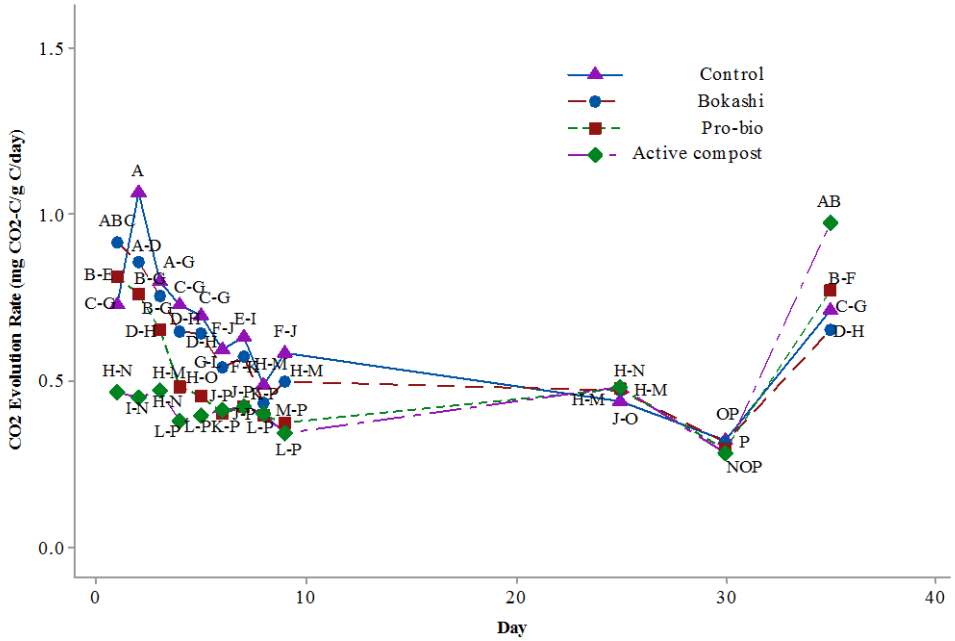

Figure 4.23 CO_2-C evolution rate from stage 2 compost from the pile treated with different inoculants. (Means sharing the same letters are not significantly different from each other at p = 0.05).

4.6.3 Stage 3

There were no significant differences in CO_2-C evolution rates ($F_{33, 119} = 1.09$, p - value = 0.35) between treatments over the incubation period of inoculated compost material in stage 3 (Fig. 4.24).

The final CO_2 evolution rates in stage 3 for the compost samples treated with different inoculants were in the range of 01.5 mg CO_2-C g^{-1} C day^{-1} which is in the range of CO_2 evolved from a mature compost of 0–3 mg CO_2-C g^{-1} C day^{-1} obtained by Brewer and Sullivan (2001) using NaOH trap method. However, Sadaka et al. (2006) observed a

CO_2 evolution rates as high as 120 mg CO_2-C g^{-1} C day^{-1} during the curing stage of composting, also using an alkali trap method.

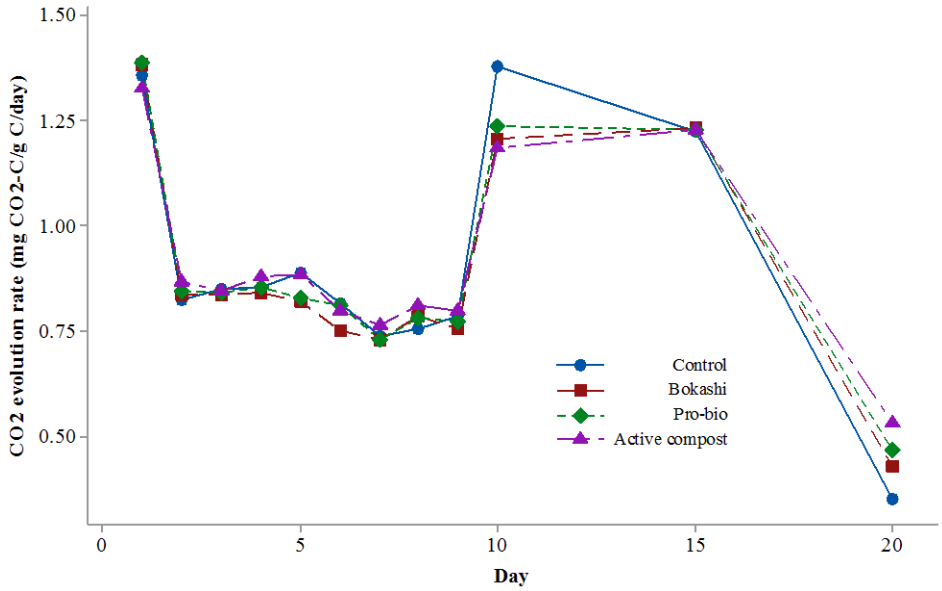

Figure 4.24 CO_2-C evolution rate from stage 3 compost material from the pile treated with different inoculants.

The low CO_2 emission rates during the mesophilic and thermophilic stages of the current study might be due to lack of proper aeration and large pieces of food waste as the compost was not shredded before utilizing in the process. Guo et al. (2012) studied composting of pig feces and corn stalks and found that increasing aeration rate from 0.24 $L \cdot kg^{-1}$ $dm \cdot min^{-1}$ to 0.72 L kg^{-1} $dm \cdot min^{-1}$ significantly increased CO_2 evolved from approximately 7-10 g kg^{-1} DM d^{-1} to 17.5-24.5 g kg^{-1} DM d^{-1}. The authors of this study also attributed the low CO_2 evolution rates due to low aeration rates and larger pieces of waste material. Another possible reason might be the addition of water without taking into account the weight loss of compost which might have created oversaturated conditions leading to an anaerobic environment thus reducing the microbial degradation

process. Results from the temperature profile of the compost pile showed that the temperature was as high as 70 °C during the first two weeks of the composting process. But the samples used in our study were inoculated and incubated at 37 °C. Similar to the compost pile, the temperature of the samples might have increased significantly adversely affecting the added EM populations thus leading to low respiration rates. Most compost microflora might not have adapted to high composting process temperatures and are very susceptible to sudden changes in temperature (Iannotti et al., 1993) (for example 70 °C to 37 °C as observed in the present study).

Chen et al. (2013) inoculated vegetable and fruit compost with matured compost and observed the CO_2 emission rates as high as 14.6 g kg^{-1} VS h^{-1} (189 mg g^{-1}C day^{-1}) and 7.5 g kg^{-1} VS h^{-1} (97.2 mg g^{-1}C day^{-1}) after 12 and 34 hours, respectively, starting the composting process but soon decreased to approximately 1 g kg^{-1} VS h^{-1} (12.96 mg g^{-1}C day^{-1}) on the fifth day of composting. A study conducted by Linda (2001) on yard waste composting showed the CO_2 rates in the range of 12 mg CO_2-C g C^{-1} day^{-1} during the first five days of composting when alkaline trap method was used for collecting CO_2. But in the same study CO_2 samples collected from head space had an evolution rate of 8 mg CO_2-C g C^{-1} day^{-1} on day one and decreasing rapidly to 1 mg CO_2-C g C^{-1} day^{-1}around fifth day of composting. These values are on par with those observed in this present study in stage one of composting process. Formowitz et al. (2007) reported negative impact of EM on the degradation process and growth of the banana plant and did not measure an increase in the microbial population.

Overall, from stages 1 through 3 the current experiment showed that there was only a marginally significant influence on all the three stages of the compost pile.

Golueka et al (1954) did not find a significant effect on adding inoculants on the composting process even though the inoculants used were rich in bacterial microflora. They concluded that the inoculum would have an effect if the indigenous microorganisms were inadequate to act on the compostable material. The compost prepared in this study might have had sufficient native flora to mask the effect caused by added inoculants or the concentration of EM added failed to dominate the innate microbial communities. Hence, it might be more beneficial if the amount of microbial inoculants added to the samples was increased. Nakasaki et al (1992), found similar results when testing for the effect of inoculants to accelerate the microbial decomposition process in composting. They did discover a change in the pattern of CO_2 evolved but failed to find a significant difference in the final conversion of carbon.

4.7 Maturity Assessment

Table 4.13 Comparison of CO_2-C evolved before and after inoculation from stage 1, stage 2 and stage 3.

Inoculants	Stage 1		Stage 2		Stage 3	
	mg CO_2-C g OM^{-1} day^{-1}					
	Before	After	Before	After	Before	After
Bokashi	6.54	0.20	0.41	0.002	5.46	0.03
Pro-bio	11.67	0.51	0.70	0.004	5.31	0.01
Active compost	10.29	0.45	0.57	0.007	5.11	0.03
Control	8.2	0.30	0.01	0.002	4.21	0.02

The respiration rates before and after inoculation were compiles to establish the relative maturity of various samples. The initial CO_2 data refers data was from the compost samples before inoculation with EM while the final data referred to samples

after inoculation with EM. The maturity tests indicated that the average daily release of CO_2-C at the end of all the three stages were well below 4 mg CO_2-C g^{-1} of OM day^{-1} which follows the CCME guidelines (CCME, 2005) of mature compost (Table 4.13). Maturity test results of material from stage 2, prior to inoculation, were very low compared to the other two stages. The reason for these results is unclear and are likely due to an error in the experimental conditions.

4.8 Cumulative CO_2 Evolution

The cumulative C evolved by samples from all the five facilities were obtained by averaging the replications from each sampling time point and adding these values to the next day averages. These cumulative graphs are presented to represent the overall pattern of CO_2-C evolved over the entire time period from day one to day 150.

The Bokashi treatment had the greater cumulative evolution of CO_2 during stage 1 of the composting process (Fig. 4.25). All the treatments of the incubated stage 1 compost samples followed a non-linear response function which is reflective of high resource consumption in a limited resource environment. The inoculants appeared to evolve slightly larger quantities of carbon than the control compost. The compost samples recovered from the thermophilic stage of composting, stage 2, all produced low cumulative CO_2 numbers with higher nonlinear responses in the control treatment than the inoculants or active compost (Fig. 4.26). In fact, the active compost treatment in stage 2 had an extremely low CO_2 evolution rate which peaked suddenly on day 30. It is possible that greater decomposition of organic matter in the inoculated treatments during stage 1 reduced the available carbon pool for decomposition at later stages. Whereas, in the control treatment, organic matter was not decomposed in the stage 1 and sufficient

amount of labile carbon was still available for decomposition to continue a higher rate of respiration in material taken during the thermophilic phase of the composting process.

During the incubation with material from stage 3 of the composting process, the CO_2 evolved was not much different between the control and the two EM inoculants while the active compost treatment mineralized less CO_2 (Fig. 4.27). A largely nonlinear response was also observed over the incubation of stage 3 material but the magnitude of CO_2 -C evolved was low compared to the stage 1 material. Some experimental management issues that occurred over the course of the study might have affected some of the results. During the incubation of stage 1, compost samples water drained to the bottom of the incubation containers and pooled potentially causing some limited anaerobic conditions. A second issue arose as a result of a malfunction in the incubator during this incubation period leading to lower incubation temperatures for approximately ten days.

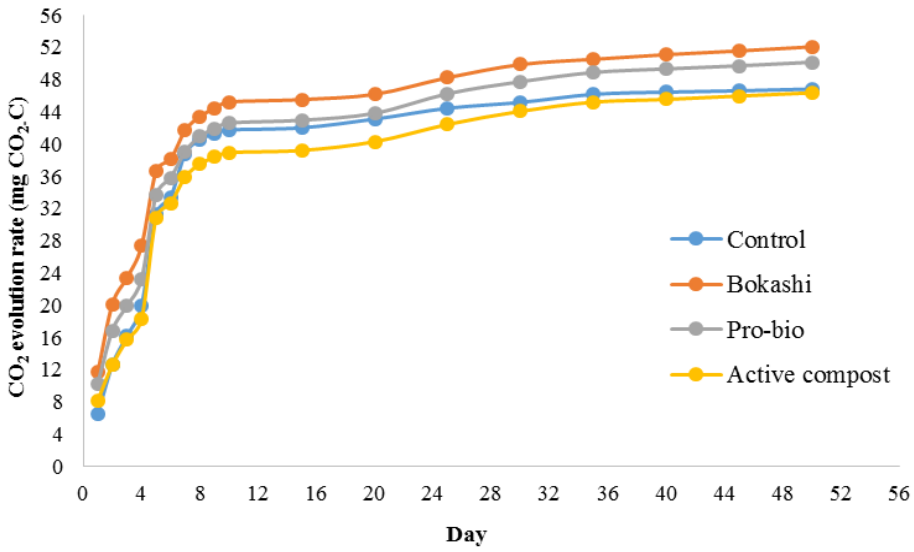

Figure 4.25 Cumulative CO_2-C evolved of inoculated compost material from stage 1 in a controlled environment incubation.

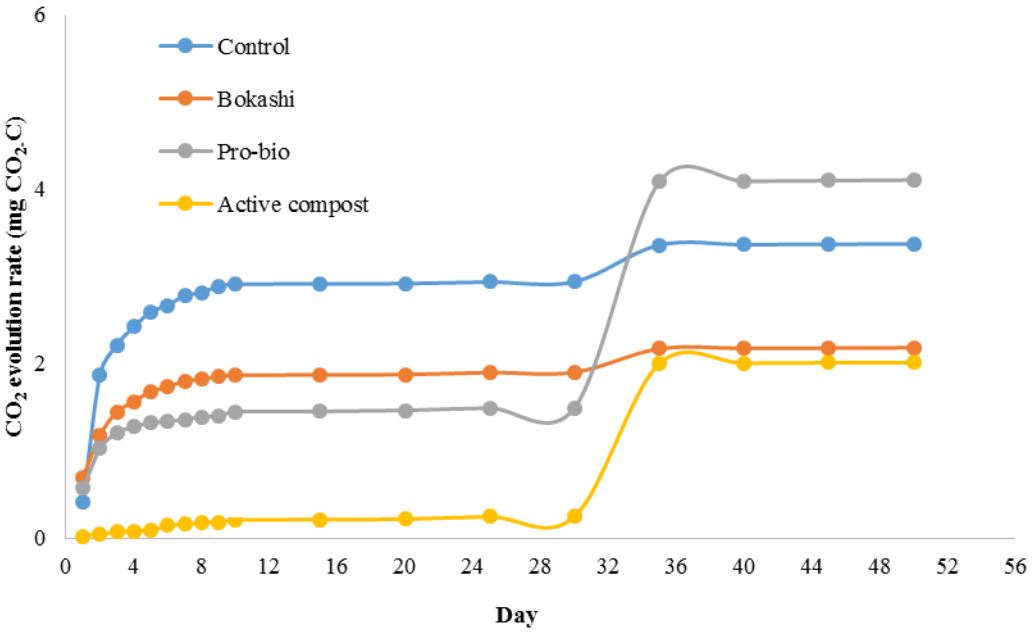

Figure 4.26 Cumulative CO2-C evolved of inoculated compost material from stage 2 in a controlled environment incubation.

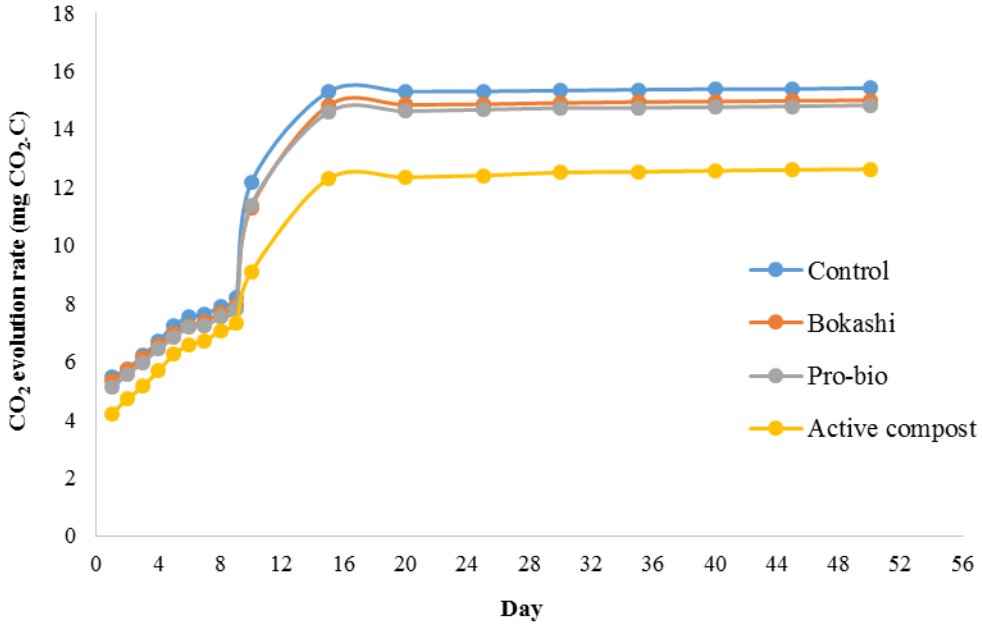

Figure 4.27 Cumulative CO_2-C evolved of inoculated compost material from stage 3 in a Controlled environment incubation.

CHAPTER 5 CONCLUSION

Application of commercially available inoculants to enhance the microbial decomposition process in curing the SSO samples obtained from all the five facilities was investigated in study one. Overall, the use of inoculants did not produce any significant effect on the CO_2 evolved as the samples appeared to already be stable and mature. A maturity test performed before conducting study one, on the compost samples obtained from the various facilities showed very low respiration values implying the compost is already matured by the time of collection. Hence, the inoculants might have failed to show their true potential influencing the microbial respiration rate. Applying EM products on a raw compost pile might yield significantly different results and hence a second study was carried out to test this. The addition of microbial inoculants had a marginal influence on the composting process in stage one and a significant influence in stage two, but the respiration rates remained relatively similar in stage three of the SSO raw compost study. Stage three of the composting process is the curing phase where the available carbon is utilized by the microorganisms in the stage one and two and there might be no adequate available carbon for EM to act. This is consistent with the results from study one where the addition of EM to compost samples (in curing phase) collected from different compost facilities across Nova Scotia. The range for CO_2 value for immature compost is usually between 10–20 mg CO_2-C g^{-1} C day^{-1}. But the values for the current studies were below this range which in study one might be due to the material already being mature due to prolonged storage time before testing and in study two a combination of environmental controls and the method of measuring respiration which might have contributed to the lack of treatment differences. A more precise control of the

environmental factors could have yielded more fruitful results. Hence the future studies should consider using freshly obtained samples and avoid storing the samples too long before doing respiration analysis. The incubation temperature might have been too high and may need to be reduced to 32 °C or lower, to reduce the risk of sample drying. At the same time, compost samples in the Mason jars may need to be mixed frequently to promote aeration which might increase the microbial decomposition and produce a better measurement of CO_2 evolution rates. Collecting larger samples to represent the compost from entire facility might change the respiration rates significantly. The SSO compost consists a large population of different kinds of microorganisms and the small quantity of EM suspension added in the current study might have failed to express their effect in the presence of native populations. A higher concentration of EM solutions can also significantly different results than the current study. Current pricing of EM solutions available in the market is around 17$ per liter and there are different varieties available commercially. It is still unclear how much EM material would be required in order to optimize the efficacy of compost degradation and as such an estimate of real costs is not possible at this time.

The chemical characteristics were analyzed at the beginning and end of both the experiments. For study one, Cumberland and Pictou facility showed a significant difference for the pH samples treated with an inoculant. There was no difference between the initial and final pH for all the five facilities as it was slightly alkaline in both the cases. Similarly, EC did not show any significant difference among the facilities. The inoculants did not have a significant effect on total carbon and nitrogen in any of the facilities. The total carbon content decreased for all the facilities. There was no

significant difference between the initial and final value of nitrogen for all the facilities. The maturity test results show that the CO_2 evolved fulfilled the CCME guidelines (CCME, 2005) as it was less than 4 mg CO_2-C g^{-1} of OM day^{-1}. For study two, pH showed significant differences among treatments for stage 1 and 3. The final pH slightly decreased for all the stages. The EC showed significant differences among treatments for all the stages and the final EC was quite high for all the stages. The total carbon at the end of the composting period decreased as all the easily available carbon material was consumed by the microorganisms. No significant difference was observed for nitrogen for all the stages after the inoculation. The chemical characteristics do not give a clear picture even though there are significant differences among various treatments.

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Appendix A. Carbon dioxide evolution (mg CO_2-C g^{-1} of C day^{-1}) rate from Cumberland compost material treated with different inoculants.

Inoculants					
Days	**Bokashi**	**Pro-bio**	**Active compost**	**Control**	**Molasses**
1	1.402^{D-K}	2.51^{A}	1.59^{C-F}	1.51^{C-G}	0.79^{O-W}
2	2.43^{AB}	1.56^{C-G}	1.81^{B-D}	1.53^{C-G}	2.03^{A-C}
3	1.12^{F-T}	1.17^{F-R}	1.19^{I-V}	0.78^{Q-VW}	0.91^{L-V}
4	1.37^{D-L}	1.51^{C-H}	1.19^{F-Q}	$1.03^{H\&J-V}$	1.23^{E-P}
5	1.02^{I-V}	0.99^{K-V}	$0.83^{N\&RW}$	0.73^{S-V}	0.95^{J-V}
6	1.71^{C-E}	1.40^{D-J}	1.13^{F-S}	$1.49^{C-F\&I}$	1.24^{E-O}
7	1.15^{F-T}	0.94^{K-V}	0.71^{UV}	$1.05^{GH\&JU}$	0.88^{M-V}
8	1.30^{D-M}	0.92^{K-V}	0.71^{T-V}	0.69^{V}	0.81^{P-W}
9	1.18^{F-R}	0.87^{M-V}	0.97^{J-V}	0.76^{R-V}	0.94^{J-V}
10	1.266^{E-N}	0.83^{N-V}	1.17^{F-R}	0.91^{L-V}	1.13^{F-T}

Means followed by the same letter within the same column are not significantly different at p = 0.05

Appendix B. Carbon dioxide evolution (mg CO_2-C g^{-1} of C day^{-1}) rate from Lunenburg compost material treated with different inoculants.

Days	
1	1.45^{DE}
2	2.91^{A}
3	1.46^{DE}
4	1.91^{BC}
5	1.35^{E}
6	2.03^{B}
7	1.45^{DE}
8	1.57^{DE}
9	1.67^{CD}
10	1.63^{CD}

Means followed by the same letter within the same column are not significantly different at p = 0.05

Appendix C. Carbon dioxide evolution (mg CO_2-C g^{-1} of C day^{-1}) rate from New Era compost material treated with different inoculants.

Day	
1	1.34 ^{BC}
2	1.72 ^{A}
3	0.97 ^{EF}
4	1.28 $^{B-D}$
5	0.76 ^{F}
6	1.49 ^{AB}
7	1.02 $^{C-E}$
8	1.01 ^{DE}
9	0.86 ^{EF}
10	1.01^{DE}

Means followed by the same letter within the same column are not significantly different at p = 0.05.

Appendix D. Carbon dioxide evolution (mg CO_2-C g^{-1} of C day^{-1}) rate from North Ridge compost material treated with different inoculants.

Day	
1	1.44^{E}
2	3.39^{A}
3	1.79^{D}
4	2.13^{C}
5	1.70^{D}
6	2.50^{B}
7	1.77^{D}
8	2.09^{C}
9	2.14^{C}
10	2.09^{C}

Means followed by the same letter are not significantly different at p = 0.05.

Appendix E. Carbon dioxide evolution (mg CO_2-C g^{-1} of C day^{-1}) rate from Pictou compost material treated with different inoculants.

Day	
1	3.9D^{E}
2	7.37^{A}
3	3.63^{E}
4	4.55^{C}
5	3.55^{E}
6	5.26^{B}

Day	
7	3.63^{E}
8	4.04^{D}
9	4.57^{C}
10	4.7^{C}

Means followed by the same letter are not significantly different at p = 0.05.

Appendix F. Carbon dioxide evolution (mg CO_2-C g^{-1} of C day^{-1}) rate from Cumberland compost material treated with different inoculants.

Day	
35	5.55^{CD}
40	5.39^{CD}
45	4.46^{DE}
55	2.98^{E}
65	7.76^{BC}
70	5.13^{CE}
75	14.0^{A}
80	9.69^{B}
85	7.33^{BC}
105	8.83^{B}

Means followed by the same letter are not significantly different at p = 0.05.

Appendix G. Carbon dioxide evolution (mg CO_2-C g^{-1} of C day^{-1}) rate from Lunenburg compost material treated with different inoculants.

Day	
1	6.46^{B}
2	4.95^{BCD}
3	4.27^{CD}
4	3.60^{D}
5	4.93^{BCD}
6	5.39^{BCD}
7	10.81^{A}
8	6.29^{B}
9	5.14^{BCD}
10	$6.02B^{C}$

Means followed by the same letter are not significantly different at p = 0.05.

Appendix H. Carbon dioxide evolution (mg CO2-C g-1 of C day-1) rate from New Era compost material treated with different inoculants.

Day	
40	18.7^{A}
45	4.13^{C-J}
55	3.32^{E-J}
65	8.52^{B}
70	4.26^{C-J}
85	5.52^{B-F}
105	5.54^{B-F}
40	17.55^{A}
45	4.35^{C-J}
55	2.17^{H-K}
65	4.73^{C-H}
70	2.98^{F-J}
85	4.47^{C-J}
105	6.55^{B-D}
40	23.14^{A}
45	6.11^{B-E}
55	2.39^{G-K}
65	2.94^{F-J}
70	4.23^{C-J}
85	6.07^{B-E}
105	6.11^{B-E}
40	2.11^{I-K}
45	6.46^{B-D}
55	0.96^{K}
65	7.23^{BC}
70	4.65^{C-I}
85	5.99^{B-E}
105	3.78^{D-J}
40	23.43^{A}
45	4.19^{C-J}
55	2.08^{JK}
65	4.05^{C-J}
70	4.88^{C-G}

Day	
85	3.92^{D-J}
105	4.34^{C-J}

Means followed by the same letter are not significantly different at p = 0.05.

Appendix I. Carbon dioxide evolution (mg CO_2-C g^{-1} of C day^{-1}) rate from North Ridge compost material treated with different inoculants.

Day	
1	19.20^{A}
2	16.69^{A}
3	7.77^{B}
4	4.85^{CD}
5	4.23^{D}
6	5.85^{BCD}
7	7.49^{BC}
8	5.84^{BCD}
9	4.61^{CD}
10	5.98^{BCD}

Means followed by the same letter are not significantly different at p = 0.05.

Appendix J. Carbon dioxide evolution (mg CO_2-C g^{-1} of C day^{-1}) rate from Pictou compost material treated with different inoculants.

Day	
1	19.93^{A}
2	12.00^{C}
3	11.51^{CD}
4	9.18^{CD}
5	11.07^{CD}
6	11.57^{CD}
7	17.72^{AB}
8	10.48^{CD}
9	7.19^{D}
10	12.13^{BC}

Means followed by the same letter are not significantly different at p = 0.05.

Appendix K. Carbon dioxide evolution (mg CO_2-C g^{-1} of C day^{-1}) rate from Stage 1 compost material treated with different inoculants.

Day	
1	8.89^{B}
2	5.87^{C}
3	3.20^{D}
4	2.98^{D}
5	10.74^{A}
6	1.85^{EF}
7	3.78^{D}
8	1.68^{EF}
9	0.9^{G}
20	0.88^{G}
25	1.96^{E}
30	1.31^{FG}

Means followed by the same letter are not significantly different at p = 0.05.

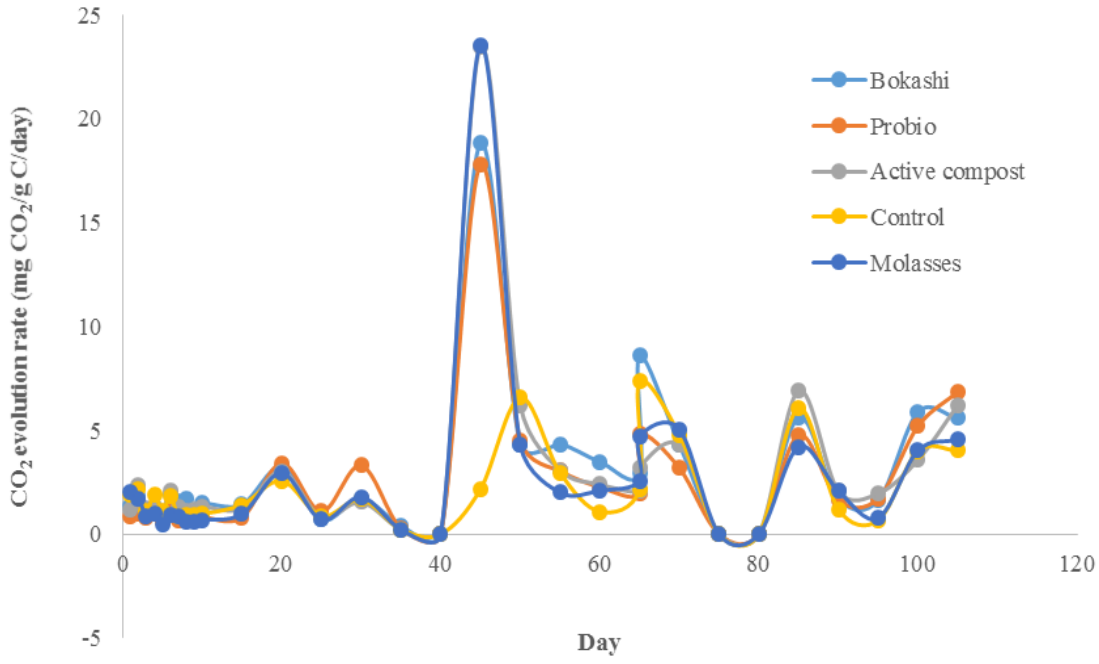
Appendix L. The carbon dioxide evolution (mg CO_2-C g^{-1} of C day^{-1}) rate from Stage 2 compost material treated with different inoculants.

	Inoculants			
Days	Control	Bokashi	Pro-bio	Active compost
1	0.73^{DH}	0.91^{AC}	0.81^{B-E}	0.47^{J-O}
2	1.06^{A}	0.85^{BD}	0.75^{CG}	0.45^{K-Q}
3	0.79^{B-F}	0.75^{CG}	0.65^{F-I}	0.47^{J-N}
4	0.72^{DH}	0.64^{E-I}	0.48^{J-M}	0.38^{M-T}
5	0.69^{D-I}	0.64^{F-I}	0.46^{J-P}	0.39^{M-S}
6	0.59^{G-J}	0.55^{I-L}	0.4^{M-R}	0.41^{L-S}
7	0.63^{F-I}	0.57^{HK}	0.42^{L-R}	0.42^{L-R}
8	0.32^{R-T}	0.32^{QT}	0.39^{MT}	0.33^{R-T}
9	0.32^{R-T}	0.31^{R-T}	0.29^{ST}	0.28^{T}
25	0.71^{D-I}	0.65^{E-I}		0.97^{B}
30	0.31^{R-T}	0.32^{QT}	0.34^{N-T}	0.32^{R-T}
35	0.33^{QT}	0.32^{RT}	0.34^{O-T}	0.33^{P-T}

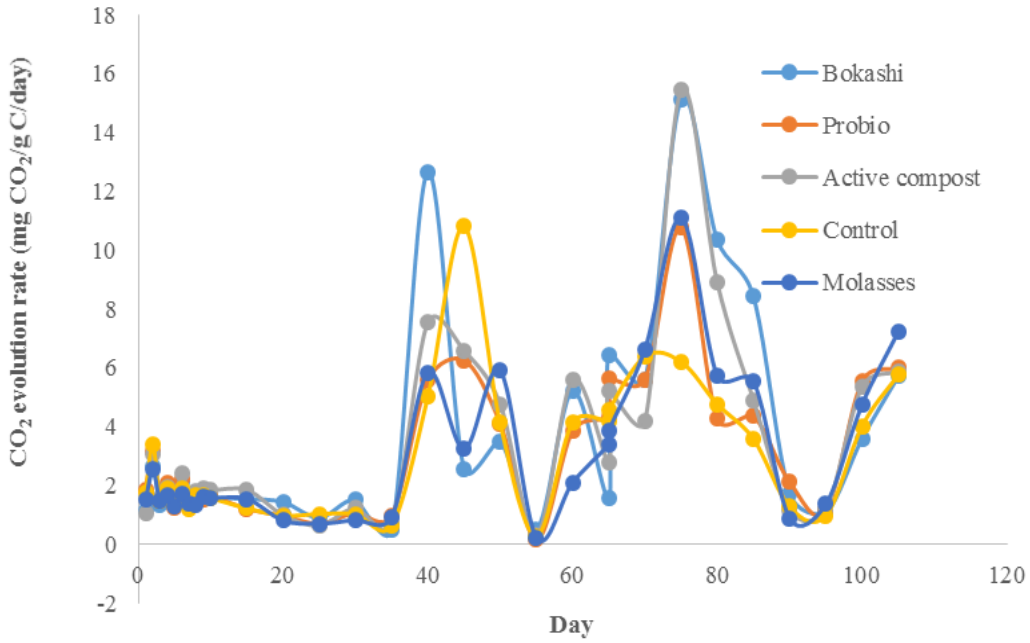
Means followed by the same letter are not significantly different at p = 0.05.

Appendix M. Carbon dioxide evolution (mg CO_2-C g^{-1} of C day^{-1}) rate from Stage 3 compost material treated with different inoculants.

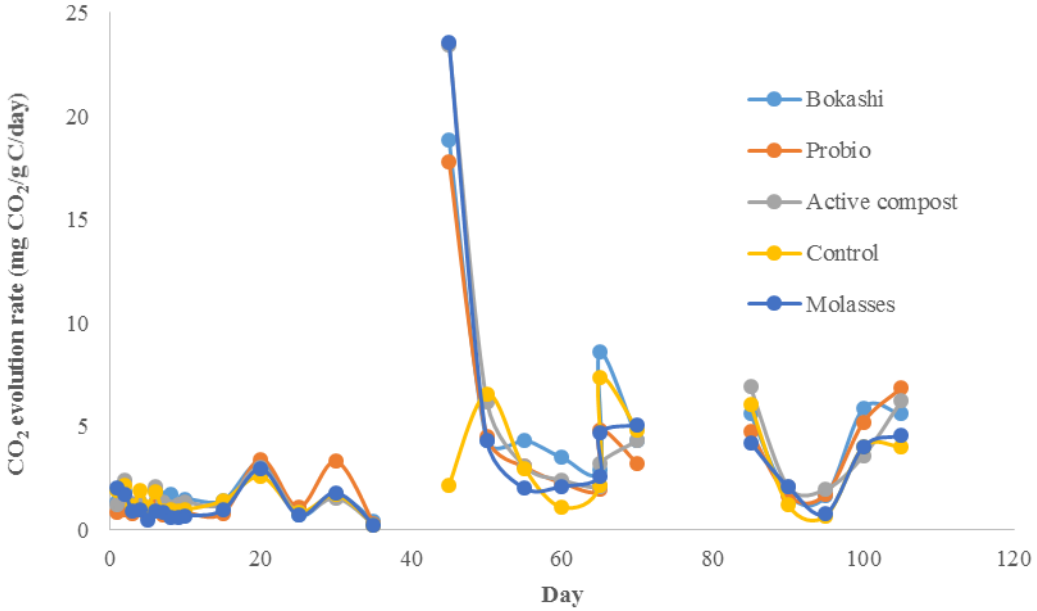
	Day
1	1.36^{A}
2	0.84^{CD}
3	0.84^{CD}
4	0.85^{C}
5	0.85^{C}
6	0.79^{DE}
7	0.73^{E}
8	0.78^{E}
9	0.77^{E}
10	1.25^{B}
15	1.22^{B}
20	0.44^{F}



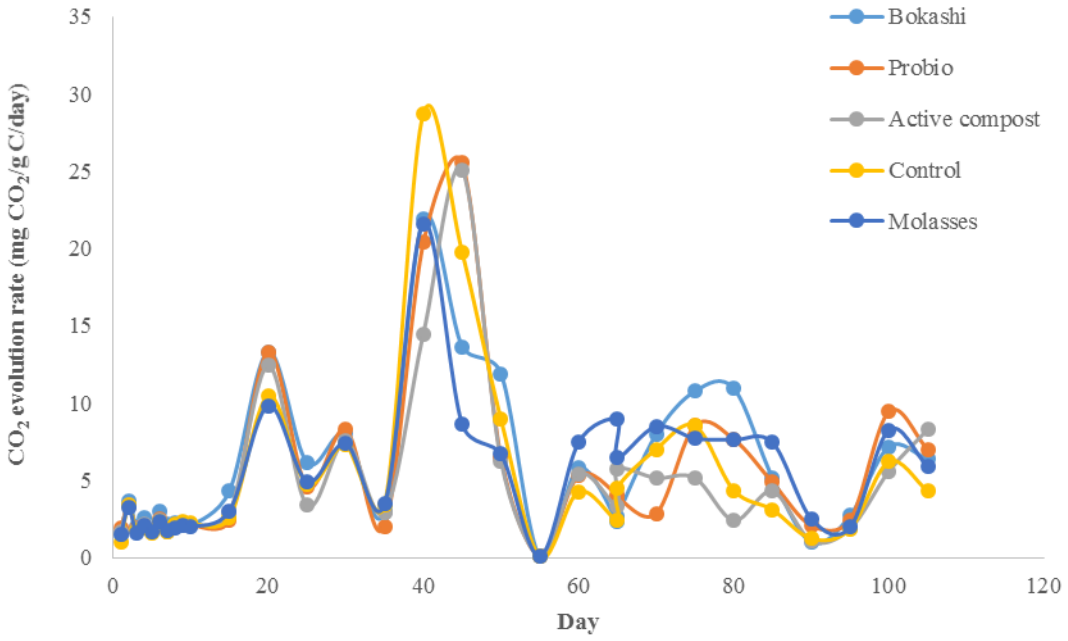
Appendix N. CO_2-C evolved from Cumberland SSO compost treated with different inoculants



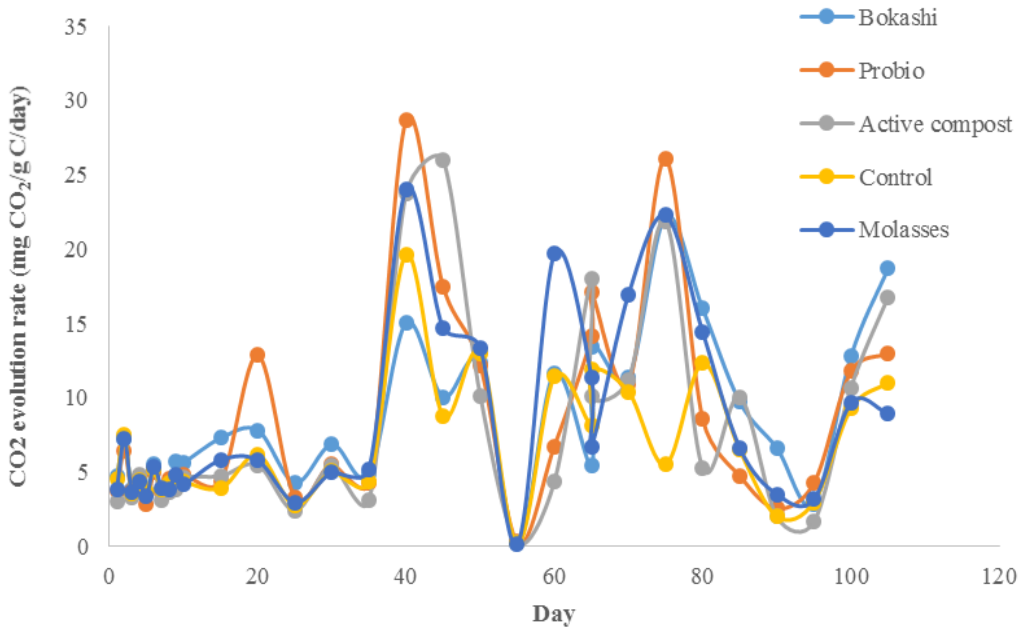
Appendix O. CO_2-C evolved from Lunenburg SSO compost treated with different inoculants



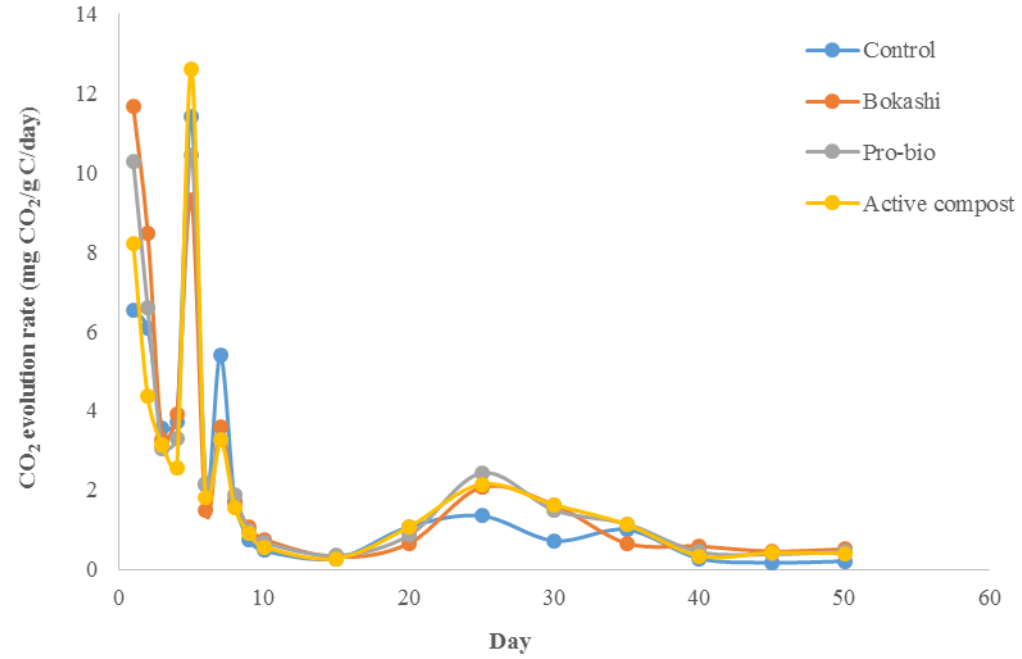
Appendix P. CO_2-C evolved from New Era SSO compost treated with different inoculants



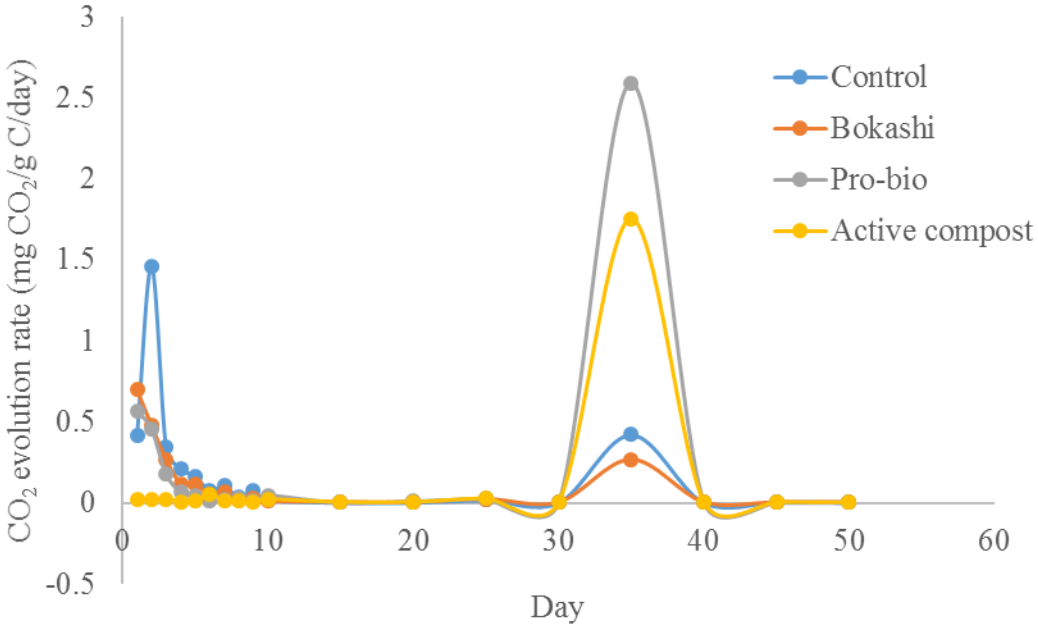
Appendix Q. CO_2-C evolved per day from Northridge SSO compost treated with different inoculants



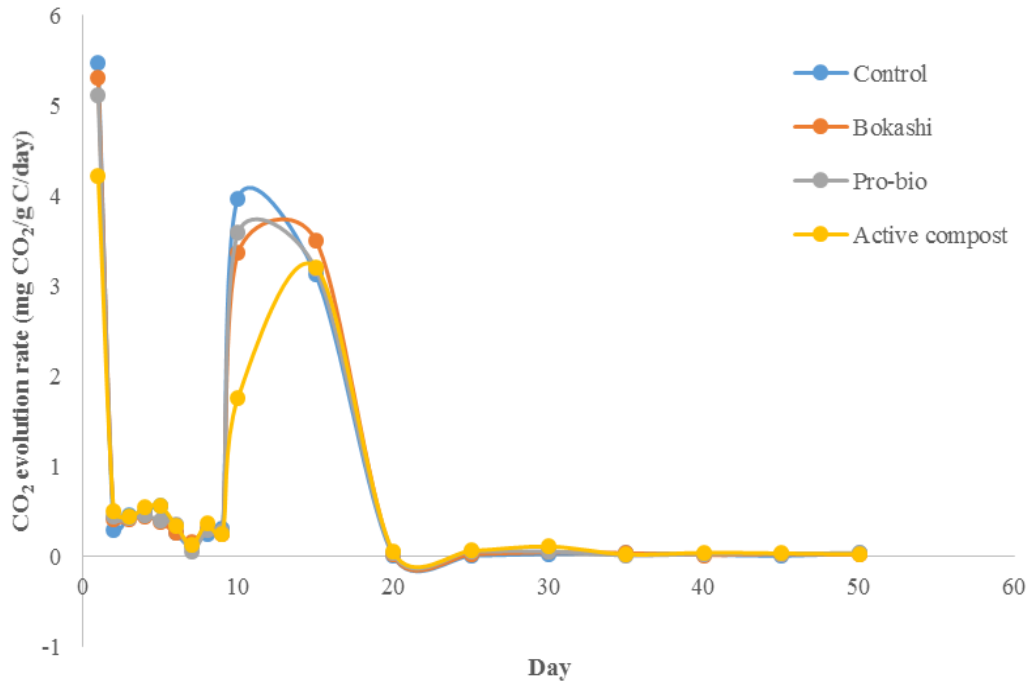
Appendix R. CO_2-C evolved from Pictou SSO compost treated with different inoculants



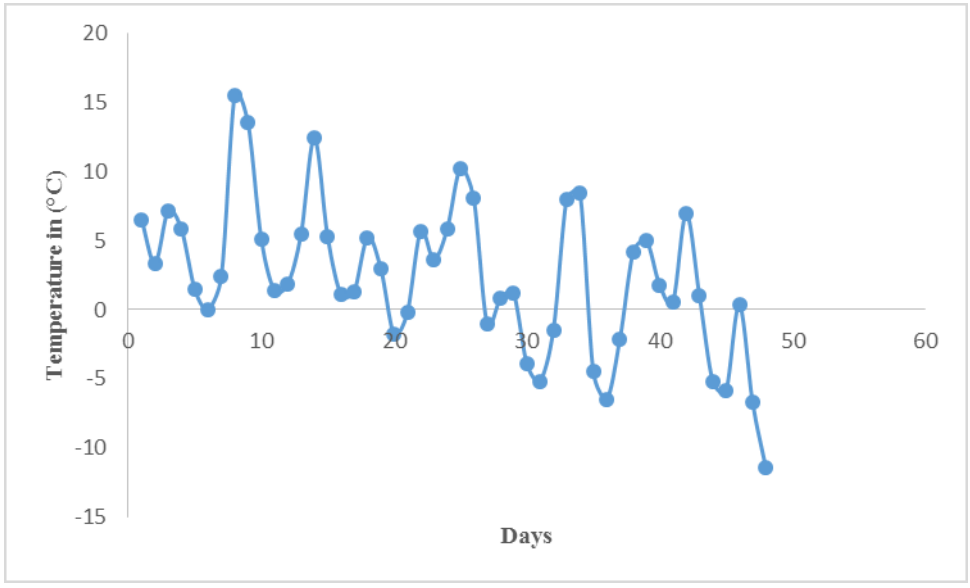
Appendix S. CO_2-C evolved for stage 1 SSO compost treated with different inoculants



Appendix T. CO_2-C evolved from stage 2 SSO compost treated with different inoculants



Appendix U. CO_2-C evolved from stage 3 SSO compost treated with different inoculants



Appendix V. Ambient temperature profile of the compost pile in Study two.