CHAPTER 1

Literature Review
**Introduction**

In the past years, nations have begun to understand the relationship between human activities and global climate change. One of the most important issues in this area is that the combustion of fossil fuels releases greenhouse gases into the atmosphere, which, have been implicated in increasing global temperatures. This is due to increased carbon dioxide and other gases released into the atmosphere, and which trap energy on the planet. In turn, increased temperatures result in melting polar ice caps, changes in weather patterns and increased sea levels. However, burning fossil fuels is not the only way in which greenhouse gases finish in the atmosphere. Degradation of waste in landfills produces gases that include methane, which is about 21 times more potent than carbon dioxide (IPCC, 2001). The gases absorb radiation from the sun and trap it in the atmosphere. By doing so heat is not liberated out, resulting in increased temperature and therefore, melting of ice caps.

**Landfill gases in environment**

Global climate change caused by anthropogenic emissions of radiatively active gases may present a serious threat to the Earth’s environment (Bender and Conrad, 1997). One such emission source is the sanitary landfill, especially, the ones accepting biodegradable municipal solid waste (BMSW). The anaerobic decomposition of land filled BMSW generates large amounts of gas composed of approximately 50% methane ($\text{CH}_4$) by volume, 40% carbon dioxide ($\text{CO}_2$) the rest being diols ($\text{NO}_2$ and $\text{SO}_2$) and other trace gases (Stein and Hettiaratchi, 1999). Landfills are estimated to account for approximately 20% of annual anthropogenic $\text{CH}_4$ emissions of the global emissions (Nozhevnikova, *et al.*, 1993). At the majority of these well sites, the combined rates of $\text{CH}_4$ emissions is less than 300 m$^3$/day (Stein and Hettiaratchi, 1999).

In recent years, there has been an explosive increase in population, which results in increased generation of waste (municipal solid waste, MSW). In
order to eliminate this MSW, landfills have been established. With improvement in technology, recycling was suggested to reduce amounts of waste and space for landfills. However, not all wastes can be recycled. Wastes generated, causes greenhouse gases like methane (\( \text{CH}_4 \)) and nitrate (\( \text{NO}_x \)). Based on the recent population trends and the resultant expected increase in MSW, there is a need for control of landfill environments to rapidly degrade this waste (Lindner, 2001). In addition to MSW, landfills are also used as disposal sites for certain industrial wastes and agricultural residues (Barlaz, 1997). Due to the diversity of contents, landfills offer a unique environment that promotes a complete series of biological and chemical reactions (Barlaz, 1997).

**Motivation to change from landfills**

The problem associated with the accumulation of solid waste in landfills is becoming an area of great concern due to the number of environmental problems produced by landfills. A primary concern for environmental engineers is redesigning landfills to optimize waste degradation so as to handle the MSW produced. The predominant method of municipal solid waste disposal is burying it within a landfill. Reuse, recycling and composting reduces some of the waste that would be bound for a landfill, but landfills continue to grow. As solid waste accumulates in landfill, hazardous materials are often emitted from these landfills in the form of gases or leachates, polluting air and water. Landfills have to be re-designed and incorporate new techniques in order to increase solid waste degradation and reduce landfill emissions.
Landfill design

The simplest way to handle solid waste disposal at the lowest cost is in landfills. In this procedure, solid wastes, both organic and inorganic, are deposited in low-lying and hence low value land (Ronald and Richard, 1992). Exposed waste causes various estheric and public health problems, attracts insects and rodents and poses fire hazard. The “sanitary landfill” fig. 1.1 is an improvement over this in which each day’s waste deposited is covered with a layer of soil (U.S. Department of Health, Education and Welfare. 1970). After completion of the landfill, the site becomes usable for recreation and eventually for construction. This simple and inexpensive disposal technique, however, has several disadvantages. The limited number of suitable disposal sites available in urban areas is rapidly becoming filled, necessitating longer hauling of the solid waste to more distant sites. The organic content of the landfill undergoes slow, anaerobic decomposition over a period of thirty to fifty years. During this period, the landfill slowly subsides, and methane (CH\textsubscript{4}) is produced. Premature construction of the landfill site may result in structural damage to the buildings and explosion hazard due to CH\textsubscript{4} seeping into
basements and cellars. CH₄ seepage may also damage planting on the disposal site.

**Selection of soil cover**

Recently, a number of researchers have recognized the potential for designing soil covers and biofilters that maximize CH₄ oxidation (Bogner et al., 1997; Dammann et al., 1999; Humer and Lechner, 1999; Straker et al., 1999). However, design guidelines prescribing the optimal compositions and thickness of such covers have yet to be developed. The rate at which CH₄ is biologically oxidized depends on environmental conditions such as temperature, moisture content and an adequate supply of O₂ (Whalen et al., 1990; Kightley et al., 1995; Czepiel et al., 1996). Soil moisture content depends on the soil type and climatic variables such as precipitation, temperature, solar flux, average wind speed and the type of vegetative cover (Stein, et al., 2002). Hydrologic models such as BROOK90 (Feder, 1995) can be used to predict soil moisture content as a function of these climatic variables and the soil’s physical properties (Czepiel et al., 1996). However, selecting the soil type that optimizes moisture content cannot be the sole design criterion, as the CH₄ oxidative potential of a soil cover also depends on the depth of O₂ penetration. This depends on the soil’s relative air-filled porosity (Stein et al., 2002). The oxidative potential of soil determines the effects of these parameters; a simple one dimensional reactive transport model can be developed. This model will aid in the design of CH₄ oxidative soil cover systems by reducing the number of laboratory experiments required to select the optimal soil type and thickness for a given environment.

**Methane production in solid waste**

Sewage sludge is generally digested under anaerobic conditions. Anaerobic digestion is also commonly used to treat materials with a high content of insoluble organic matter, such as cellulose and to degrade concentrated industrial wastewater such as those from food processing industry. The
Degradative and fermentative reactions in the anaerobic secondary treatment process can be divided into two stages:

- acid forming
- methane forming

Complex organic compounds (polysaccharides, fats and proteins)

HYDROLYSIS

Hydrolysis by extra cellular bacterial enzymes

Monomeric compounds (sugars, fatty acids and amino acids)

Higher organic acids

ACETOGENESIS

Acetic acid

Acetic acid + hydrogen + carbon dioxide

METHANOGENESIS

Methane

Figure 1.2 Degradation of organic matter to methane production

Soon after waste materials have been deposited in landfills, they are subjected to anoxic conditions. This is the result of low $O_2$ diffusion into the waste in relation to its consumption by microorganisms utilizing the organic matter of the waste as an energy and carbon source. The rates of these processes depend on the amount of organic matter, the composition of the waste material and the extent to which the newly deposited material temporarily is covered and/or compacted. Thus, easily degradable organic matter leads to a faster microbial growth and $O_2$ consumption than more resistant materials (Table 4.3).
Gas emissions and environmental effects

Methane emission by soils results from antagonistic but correlated microbial activities. CH$_4$ is produced in the anaerobic zones of submerged soils by methanogens and is oxidized into CO$_2$ by methanotrophs in the aerobic zones of wetland soils and in upland soils. CH$_4$ transfer from soil to the atmosphere occurs mostly through the parenchyma of aquatic plants, but also by diffusion and as bubbles escaping from wetland soils (figure 3.3).

Methane (CH$_4$) is the principal gas produced (about 50%) from the anaerobic decomposition of the organic matter in waste treatment. CH$_4$ is colourless, odourless, combustible hydrocarbon of high fuel value. Normally, large quantities are not encountered in wastewater treatment because even small amounts of oxygen tend to be toxic to the organisms responsible for the production of methane. In treatment plants where methane is produced, notices should be posted about the plant warning of explosion hazards, and plant employees should be instructed in measures to be maintained while working in and about the structure where gas may be present.

\[ \text{H} \]
\[ \text{H} \quad \text{C} \quad \text{H} \]
\[ \text{H} \]

Figure 1.3 Methane structure

Although methane and carbon dioxide are produced in about equal amounts (50 and 45% respectively), methane is of greater concern as a greenhouse gas. The 100-year global warming potential of methane (its infrared absorption potential in the atmosphere) can increase with time because of the emissions. Despite a short residence time in the atmosphere (about 10 years), the CH$_4$ ability to absorb infrared radiation makes it 20 to 30 times more efficient than CO$_2$ as a greenhouse gas (Blake and Rowland, 1988). Carbon dioxide released from the landfills is neutral and amount is negligible (IPCC, 2001). Globally, atmospheric methane concentrations are approximately 1.8ppm by volume, which represents a doubling in the last 100 years (Hube-Humer, 2004). Although many modern landfills operate a gas
extraction methane is a radiation absorbing gas, whose atmospheric concentration has increased significantly during the past few hundred years, contributing to about 15% to the potential global warming (OTA, 1991).

Methane is chemically reactive and is therefore, involved in changes in the chemical composition of the atmosphere (Cicerone and Oremland 1988). In particular it reacts with hydroxyl radicals in the troposphere, reducing its oxidative power and ability to eliminate pollutants such as chloro-fluoro carbons (CFC) and leading to the production of other greenhouse gases (ozone, CO, CO₂) (Borjesson and Svensson, 1993). In the stratosphere, such reactions produce water vapour, which is involved in the destruction of the stratospheric ozone layer, the natural barrier against detrimental solar radiations (Borjesson and Svensson, 1993).

It is estimated to contribute relatively about 19% the increase in radiation absorbing force since pre-industrial times (Intergovernmental Panel on Climate Change [IPCC], 1996; Stein, et al., 2002). One emission source is sanitary landfills, specifically, the ones accepting biodegradable municipal solid waste (BMSW). The anaerobic decomposition of land filled BMSW generates large amounts of gas composed of approximately 50 – 60 % CH₄ (by volume), 40 – 50 % CO₂, and other trace gases such as nitrogen (N₂) and volatile organic hydrocarbons (VOC) (Czepiel, et al., 1996; Kightley, et al., 1995; Stein et al., 2002).

Systems, which collect the gas for flaring for energy use, still lose some gas, which escapes into the atmosphere. In addition, methane is still emitted from older landfills where gas collection systems are not installed, especially in poorer countries. Therefore, highly engineered and low cost technology waste disposal sites could benefit from new methods to reduce methane emissions.
Effect of CH₄ as a greenhouse gas

It has been suggested by Prim et al. (1992) that one of the major cause of the slowdown is the increasing magnitude of CH₄ oxidation by methanotrophic bacteria is in the aerobic zones of soils. Hence Tamai et al. (2003) note, “this biological sink plays an important role in modulating global warming”. It can be appreciated that carbon dioxide (CO₂) induced global warming, would produce two biological-mediated negative feedback to counter the increase in temperature: a warming-induced increase in CH₄ uptake from the atmosphere by essentially all soils. An increase in soil CH₄ uptake from the atmosphere that is produced by increase in plant litter C/N ratio (Tamai et al. 2003). Recent concern about global warming has intensified interest in the role of terrestrial systems in controlling atmospheric CH₄ levels. Terrestrial systems can function as net sources of sinks for atmospheric CH₄. Methane flux measured at the soil/atmosphere interface is the net effect of two processes; CH₄ oxidation and methanogenesis (Knowles, 1993).

Methane in agriculture

The rice plants do not produce methane but their vascular system acts as transport channels of methane dissolved in water (Cicerone and Shetter, 1981; Nouchi et al., 1990). Animal dung can carry microbes that produce large amounts of methane. Apart from methanogens being present in cow dung and farmyard manure, a consortium of microorganisms of the soil, which are capable of producing methane (Stanier et al., 1987), therein degrades the organic matter present. In India (Banik et al., 1995), cow dung and farmyard manure have been used as organic manure for centuries. The methanogens are still active as some are encapsulated deep in the manure (Nayer and Conrad, 1990). With monsoon rains or irrigation water, there is a possibility that the soils are enriched with methanogens in cow dung (Hobson, 1982). While soils have not been considered as significant sinks for methane until recently, methane consumption has been reported in agricultural soils, forest soils, tundra and bogs (Topp and Hanson, 1991).
Methane in other environments

CH$_4$ is a common constituent on the deep subsurface. As much as 20% of the world’s natural gas resources are estimated to have been generated by microbes (Rice, 1993). Subsurface coal deposits, oil wells, natural gas storage in carbonated shelves, coal swamps, coastal plains, deep sea up swellings and hydrocarbon deposits are a major sources of ‘biogenic’ CH$_4$ (Kotelnikova, 2002). Microbial CH$_4$ oxidation is a biogeochemical process that limits the release of CH$_4$ from anaerobic environments (Hanson and Hanson, 1996).

The evolution of CH$_4$ from anaerobic digesters and other bioconversion processes occurs simultaneously with the evolution of CO$_2$. The ratios of CH$_4$ to CO$_2$ depend on the chemical composition of the substrate and the environmental conditions under which the bioconversion is carried out. Biotechnological processes can, and must be, adjusted to maximize the proportions of CH$_4$ in the evolved gases. The CH$_4$ must be trapped and separated from other gases to be a useful energy resource that can supplement and/or replace natural gas as a fuel. This makes it expensive therefore other separation techniques are looked into.

Methane breakdown

CH$_4$ consumption occurs in most soils and exhibits a broad range of values. Highest consumption rates or potentials are observed in soils where methanogenesis is or has been effective and where CH$_4$ concentration is or has been much higher than in the atmosphere (rice fields, swamps, landfills etc.). Aerobic soils consume atmospheric CH$_4$ but their activities are very low and the microorganisms involved are largely unknown (Le Mer and Roger, 2001). CH$_4$ emissions by cultivated or natural wetlands are expressed in mg CH$_4$ m$^{-2}$ h$^{-1}$. CH$_4$ oxidation by aerobic upland soils is rarely higher than 0.1 mg CH$_4$ m$^{-2}$ h$^{-1}$. Forest soils are the most active, followed by grasslands and cultivated soils. Factors that favour CH$_4$ emission from cultivated wetlands are mostly submerged and organic matter addition (Le Mer and Roger, 2001).
Recently, biological oxidation of CH$_4$ has attracted much attention from the research community due to a renewed interest in biofiltration as an inexpensive waste gas treatment mechanism and the potential benefits of oxidation of CH$_4$ by indigenous bacterial populations. The degradable organic matter gets catabolized broken down into a stabilized organic residue, water and carbon dioxide, this contributing to landfill gas. Aerobic fermentation of waste will take place if sufficient moisture is present. With complete absence of oxygen, true anaerobic microorganisms, including methanogens, become established. Organic acids and hydrogen in the waste are then metabolized forming methane and carbon dioxide. If methane migrates to areas of the landfill, which are operated under aerobic conditions, it may be oxidized to CO$_2$ by methanotrophs. Biofiltration is also seen as an attractive treatment technique in light of recent criticisms brought against flaring, which has been identified as a source of gaseous emissions capable of causing human and environmental problems, such as dioxins (Strosher, 1996).

It is assumed to be on an increase at 0.7 – 0.8% per year (Khalil and Rasmussen, 1993; Steels et al., 1989; Watanabe and Matsura, 1992;). Methane, produced through methanogenesis by bacteria, is an important potential fuel source (Pfeffer, 1976; National Academy of Science, 1977; National Research Council, 1979). CH$_4$ can be used in the generation of mechanical, electrical and heat energy. It can be used as a fuel source for homes and industry by transfusion through natural gas pipelines and converted by microbial action or chemical means to methanol, which can be used as fuel in internal combustion engines (Ronald and Richard, 1992).

**Oxidation of methane looking at different variables in soil**

Some reports exist, including the study of temperature (Crill, 1991; Crill et al., 1994; Dunfield et al., 1993; King and Adamsen, 1992; Nesbit and Breitenbeck, 1992; Whalen et al., 1990;); moisture (Whalen et al., 1990; Nesbit and Breitenbeck, 1992; Adamsen and King, 1993; Crill et al., 1994); pH (Dunfield et al., 1993; Yavitt et al., 1993; Hutsh et al., 1994); NH$_4^+$ availability (Steudler et al., 1989; Mosier et al., 1991; Nesbit and Breitenbeck, 1992; Hutsh et al., 1993,1994; Bender and Conrad, 1994; Crill et al., 1994; Schnell
and King, 1994) and particle fraction of soil (Bender and Conrad, 1994). Often, however, field-fresh soils show only a low CH$_4$ oxidizing activity, but have a marked potential to increase their activity when exposed to elevated CH$_4$ concentrations under suitable conditions (Megraw and Knowles, 1987; Nesbit and Breitenbeck, 1992; Bender and Conrad, 1992). The response of the soil microbial community to increased CH$_4$ is of particular interest on paddy soil, swamps landfill soils and other environments that are, at least periodically exposed to high CH$_4$ concentrations (Bender et al., 1995). CH$_4$ oxidizing activity, with a decrease in soil oxygen and an increase in microbial biomass, has also been demonstrated on soils around leaks in natural gas pipes (Adams and Ellis, 1969) and in landfill covers (Kightley, et al. 1995).

**Waste degradation in landfills**

Waste is collected from various areas (industrial and domestic) and deposited in landfills. Here it undergoes different phases of degradation. These include aerobic degradation until all the oxygen (O$_2$) is depleted. The buried waste is a source of carbon (nutrients) to anaerobic and aerobic bacteria including some parasites, this requires collaboration of a variety of organisms that interact and coexist with one another to degrade this waste and transform it into non-toxic by-products (Lindner, 2002). While degradation occurs, volatile fatty acids are formed which in return are not environmentally friendly as the flow into waterbed and contaminate water source.

**Mechanism of waste degradation**

Microbiological processes in landfill

Although new methods of municipal waste utilization are being explored, landfill is still a common disposal method and is likely to remain so in many countries for the foreseeable future for several reasons. It is relatively cheap; the technology and environmental control measures are reasonably well understood; leachates may be collected and treated by anaerobic digestion under controlled conditions to produce high-value chemicals and any methane generated may provide a potential valuable source of energy (Grainger, 1984).
Waste degradation undergoes aerobic degradation produces acidogenic phase. In this phase, organic matter is aerobically oxidized using a cocktail of bacteria available in the environment. Methanogenesis starts when the available oxygen is finished and the reaction goes anaerobic.

This transformation requires successive actions of four populations of microorganisms that degrade complex molecules in simpler compounds:

- Hydrolysis of biological polymers into monomers (simple sugars, glucides, fatty acids, amino acids) by an hydrolytic micro flora that can be either aerobic, or facultative, or strictly anaerobic;
- Acidogenesis from monomeric compounds and intermediary compounds formed during fermentation (production of volatile fatty acids, organic acids, alcohols, H₂ and CO₂) by a fermentative microflora that can; be either facultative or strictly anaerobic;
- Acitogenesis from the previous metabolites by a syntrophic or homoacetogenic microflora;
- Methanogenesis from the simple compounds that can be used by methanogens (in particular H₂ + CO₂ and acetate) which constitutes the last step of the methanogenic fermentation.

Examples of reactions occurring in an anaerobic digestion tank and their products.

1. Hydrogen use by methanogens
   a) \( 4 \text{H}_2 + \text{HCO}_3^- + \text{H}^+ \leftrightarrow \text{CH}_4 + 3\text{H}_2\text{O} \)

2. Some reactions of the hydrogen-producing acetogenic bacteria
   i) Fermentative bacteria without hydrogen using methanogens
   b) Organic acids + water \( \leftrightarrow \) acetate\(^-\) + H\(^+\) + H\(_2\)
   The organic acids include lactate, butyrate propionate and methanol. In some cases succinate acetate and formate. Lactate produces bicarbonate; the amount of hydrogen produced varies with the organic acid used
   ii) Fermentative bacteria with hydrogen using methanogens
   c) Organic acids + H\(_2\)O + H\(_2\)CO\(_3\) \( \leftrightarrow \) CH\(_4\) + H\(_2\)O + H\(^+\)
\( \Delta G \) varies with substrate, for example, glucose has \(-148.1\) while pyruvate has \(-87.0\) (kJ). Ethanol has \( \Delta G \) of \(+9.6\) and propionite has \(+76.1\ \Delta G\) (kJ). The lower the \( \Delta G \) value the more the reaction is favoured. This means that propionite will not be digested in the presence of ethanol, likewise in the presence of pyruvate and glucose. The reaction will favour glucose till it is depleted. Followed by pyruvate and intermediate organic acids, (table 1.1).

**Table 1.1: Methanogenic reactions**

<table>
<thead>
<tr>
<th>Reactants</th>
<th>Products</th>
<th>( \Delta G^0 ) kJ/mol CH(_4)</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 4H_2+HCO^-+H^+ )</td>
<td>( CH_4 + 3H_2O )</td>
<td>-135</td>
<td>H</td>
</tr>
<tr>
<td>( 4HCO^-2+H^++H_2O )</td>
<td>( CH_4 + 3CHO^-3 )</td>
<td>-145</td>
<td>F</td>
</tr>
<tr>
<td>( 2CH_3CH_2OH+HCO^-_{a} )</td>
<td>( 2CH_3COO^-+H^++CH_4+H_2O )</td>
<td>-116</td>
<td>A</td>
</tr>
<tr>
<td>( CH_3COO^- + H_2O )</td>
<td>( CH_4 + HCO^-3 )</td>
<td>-31</td>
<td>Ac</td>
</tr>
<tr>
<td>( 4CH_3OH )</td>
<td>( 3CH_4+HCO^-3+H_2O+H^+ )</td>
<td>-105</td>
<td>M</td>
</tr>
<tr>
<td>( 4(CH_3)_{3}NH^+ + 9H_2O )</td>
<td>( 9CH_4+3HCO^-3+4NH^-4+3H^+ )</td>
<td>-76</td>
<td>MA</td>
</tr>
<tr>
<td>( 2(CH_3)_2S + 3H_2O )</td>
<td>( 3CH_4+HCO^-3+2H_2S+H^+ )</td>
<td>-49</td>
<td>MeS</td>
</tr>
<tr>
<td>( CH_3OH + H_2 )</td>
<td>( CH_4 + H_2O )</td>
<td>-113</td>
<td>H/M</td>
</tr>
</tbody>
</table>

F, Formate: A, Alcohols; Ac, Acetate; H, Hydrogen; MA, Methylamines; M, Methanol

\( a \) Substrate legend: \( b \) assumed all species are mesophiles and freshwater: \( c \) Dueteche Sammlung von microorganism. Data from Thauer et al. (1977)

After hydrolysis and acidogenesis where acids are produced methanogenesis occurs. This stage releases methane from the (acids) chemicals formed. During the first two phases, there is high energy released, and this pushes the reaction to form \( H^+ \) and carbon dioxide (CO\(_2\)). Methanogens covert these into acetate and methane. Depending on the strain of methanogens present, the amount of methane produced varies.
Methanotrophy
This is a process of oxidizing methane that is being produced in anaerobic phase to a lesser potent gas CO₂. Although this is still a greenhouse gas, it is less radio active compared to methane and it can be used in greenhouse for plants photosynthesis. Three options are available for exploiting soil methanotrophy at a variety of specific field sites (Matthews, 2000).

Methane-oxidizing bacteria:
Part of the CH₄ diffusing into landfill cover soils may be oxidized by methanotrophic bacteria that use the following reactions to gain energy and carbon for their growth (Hanson and Hanson. 1996):

\[
\begin{align*}
\text{CH}_4 & \rightarrow \text{CH}_3\text{OH} \rightarrow \text{HCHO} \rightarrow \text{HCOOH} \rightarrow \text{CO}_2 \\
\end{align*}
\]

Energy is yielded in all steps, except in the first step. The importance of intermediate formaldehyde (HCHO) stage is that, the bacteria form synthesis of new cell material that can be used. HCHO can be transformed and stored as polymers. Polymers can also be excreted, sometimes in amounts so huge that the use of PLFA (phospholipid fatty aid) analysis it has been shown that CH₄ oxidation in landfill covers could be linked to the two main types of methanotrophic bacteria, but not in an easy-interpreted pattern (Borjesson et al. 1998).

Besides CH₄ (55 – 60 vol.%) and CO₂ (40 – 45 vol.%) landfill gas also contain numerous trace compounds (up to 5 vol.%) (Brosseau and Heitz, 1994). Microbial oxidation of CH₄ in aerobic soils plays a significant role in reducing the emission of CH₄ to the atmosphere. In landfill top covers CH₄ and O₂ counter-gradients may appear due to emission of CH₄ from the waste and diffusion of O₂ from ambient air. Oxidation of CH₄ by methanotrophic bacteria in landfill top cover soil has been shown to reduce the amount of CH₄ by emitted to the atmosphere. Under CH₄ oxidation conditions the methanotropic bacteria is known to co-metabolize a variety of aliphatic compounds including some halogenated hydrocarbons (Oldenhuis et al. 1989).
Alternatives to control CH$_4$

Recent landfill studies have shown that methanotrophic CH$_4$ oxidation in aerated soils can be a major natural control on net CH$_4$ emissions, with rates as high as 166 g m$^{-2}$ d$^{-1}$ (Nozchevnikova et al., 1993), indicating high capacities for CH$_4$ oxidation in landfill soils. Oxidation of CH$_4$ by methanotropic bacteria provides an important sink for CH$_4$ that would otherwise escape from water and soil environments to the atmosphere.

Biological CH$_4$ oxidation has been found to restrict the fluxes of CH$_4$ produced in lakes and rice paddies by up to almost 100% (Rudd and Hamilton, 1975), while the corresponding values for landfills have been estimated to range from 10 to 70%. Oxidation of CH$_4$ in soil requires the availability of O$_2$, the presence of methanotrophic bacteria and suitable soil conditions that allow the bacteria to be active (King, 1993). The effects of the various soil variables that potentially affect the microbial CH$_4$ oxidation activity are still not well-characterized (Bender, 1995).

It is believed that CH$_4$ emission from landfills could be reduced considerably by the use of improved management practices (Crutzen, 1991). Gas extraction has been proposed as a means of achieving such a reduction (Richards, 1989). Emissions can also be regulated through biological CH$_4$ oxidation (Whalen et al., 1990; Kightley et al., 1995; Czepiel et al., 1996; Borjsson and Svensson, 1997). CH$_4$ oxidation in landfill cover soils has been estimated to reduce CH$_4$ emissions by 35% (Reeburgh, 1996) (4). Obligate methanotrophic bacteria are probably the most important CH$_4$ oxidizing organisms in CH$_4$-rich environments owing to their stronger ability to convert large amounts of CH$_4$ and their shorter generation times (Conrad, 1995).

Among environmental problems with landfills, the greenhouse effect is likely to be the most serious due to the CH$_4$ content of the landfill gas (LFG), which contributes to the increased CH$_4$ concentration in the atmosphere. Besides the contribution to the climate change, CH$_4$ also brings about risks for fires and explosions (Borjensson et al., 1995). Other components in landfill gas can cause odour problems (Young and Parker, 1983). The easiest measure to prevent these negative effects of LFG is the installation of active gas
recovery systems (Borjensson et al., 1995). This phenomenon could potentially have a strong mitigation effect on CH$_4$ emissions from sources such as heavy oil well sites and landfills, and the optimization of this process may serve as an inexpensive strategy for reducing emissions of this potent greenhouse gas.

Methods of controlling waste

Methods for managing municipal solid waste (MSW) vary widely, ranging from open dumps, burning to sanitary landfills. Two main alternatives exist for managing CH$_4$ emission sites (Aitchison, 1993). One option is to undertake landfill gas recovery with associated gas use, and is generally regarded as being the superior choice. The alternative option is that of encouraging CH$_4$ oxidation in the soil covering the landfill (Boeckx, et al., 1996). This is a much cheaper and more effective option for reducing emissions in smaller and older landfills with gas extracting, which becomes inefficient at low CH$_4$ contents. The top soil of a landfill is a dynamic mixing zone for air and landfill gas. O$_2$ and N$_2$ concentrations decrease with depth, while CH$_4$ and CO$_2$ increase with depth (Boeckx et al., 1996). CH$_4$ emissions through the cover soil are greatly reduced by the relative abundance of methanotrophic bacteria within the aerated cover soil (Jones and Nedwell, 1993). Whalen et al. (1990) and Kightley et al. (1995) reported high CH$_4$ oxidation rates in landfill cover soils. These authors found oxidation rates of 45 and 166 mg CH$_4$ m$^{-1}$d$^{-1}$ respectively. Landfill emissions can range over six orders of magnitude (Bogner and Scott, 1995).

Importance of soil cap

Consumption of atmospheric CH$_4$ by soil is one of the important sinks in the global cycle of CH$_4$, which is a green house gas of great importance to earth’s climate (Primm, 1994).

- Optimization of the CH$_4$ oxidation process through the selection, design and maintenance of soil covers.
- Manipulation of existing soil covers to increase their CH$_4$ oxidation potential
The use of CH$_4$ oxidizing biofilters, or simply channeling and distributing CH$_4$ gas through an existing layer of topsoil. Before this technique can be applied, however, a thorough understanding of how environmental variables and soil properties limit a soil’s CH$_4$ oxidation potential is needed (George and Werner, 2001).

Previous studies on non-landfill soils suggest that high capacities may be observed in soils with historically elevated CH$_4$ fluxes (Bogner and Spokas, 1997). At landfill sites, “negative” CH$_4$ emissions (inward fluxes) have also been measured by several investigators using static enclosure (closed chamber) methods (Bogner et al., 1995; Borjesson and Svensson, 1996; Boeckx, et al., 1996). In such cases, methanotrophs in the cover soil are oxidizing all of the CH$_4$ transported upward from landfill sources and additionally are oxidizing CH$_4$ from the atmosphere, resulting in the soil functioning as a sink for atmospheric CH$_4$ as originally documented in tundra soils (Whalen and Reeburgh, 1990). Bender and Conrad (1994) demonstrated direct relationships between the CH$_4$-oxidizing activity determined from soil incubations and the number of methanotrophs (Bogner, et al., 1997). Furthermore, methanotrophs are able to encyst themselves for protection from heat and desiccation, enabling them to survive for extended periods in natural soils under virtually all conditions (Mancinelli, 1995).

CH$_4$ oxidising activity, with a decrease in soil oxygen (O$_2$) and in increase with microbial biomass, has also been observed in soils around leaks in natural gas pipes (Adams and Ellis, 1969) and in landfill covers (MacKay, 1985; Whalen et al., 1990; Stein et al., 2002). Microbial oxidation of CH$_4$ is carried out by methanotrophic bacteria, which are present in small numbers in most soils. Long-term exposure of soils to high levels of CH$_4$ encourages the growth of populations of methanotrophs with a high capacity of CH$_4$ oxidation.

**Gas collection**

Methane migration through landfill caps is the fourth largest source of anthropogenic CH$_4$ emissions worldwide (Stern and Kaufmann, 1996) and it is
the largest source in the United States (US Department of Energy, 1997). These emissions alter the global CH$_4$ budget, and since CH$_4$ is a potent greenhouse gas, they contribute to global, climate change. Microbial CH$_4$ consumption in the aerobic portions of a landfill cap reduces CH$_4$ emissions to the atmosphere. Laboratory and field studies indicate that CH$_4$ oxidizers typically consume 10 – 20% of the CH$_4$ passing through a landfill cover, although under laboratory conditions, up to 60% CH$_4$ oxidation has been reported (Kightley et al., 1995; Hilger, Cranford and Barlaz, 2000). Bogner et al. (1995) have shown that under certain conditions, landfill covers are even a sink for atmospheric CH$_4$. Many bacteria, including CH$_4$ oxidizers, produce exopolymeric substances (EPS) that can serve as a source of anchorage to the soil particles. ESP has been linked to both nutrient imbalance and O$_2$ deficiency (Wrangstadh et al., 1986). It has been proved that biofilm has pores/channels that allow diffusion of water and nutrients to the cell (Hilger et al., 2000). It was thought that accumulation of ESP could alter metabolism of bacteria embedded in a biofilm (Hilger et al., 2000). Composed largely of polysaccharides (Costerton et al., 1981), a viscous film can offer greater resistance to substrate diffusing into the base film (Christensen et al., 1990; Mozes et al., 1992) and the is evidence that diffusivity decreases with increasing film age (Matson and Characklis, 1976; Hilger et al., 2000).

In some countries the landfill gas is collected and combusted to reduce emissions. However, regulations on the gaseous emission from landfill sites, particularly the hazardous components, are deficient.

A negative CH$_4$ flux, that is, consumption of CH$_4$ by soil, occurs when the magnitude if the CH$_4$ uptake process is larger than methanogenesis.

$$\text{CH}_4(\text{emi}) = \text{CH}_4(\text{prod}) - \text{CH}_4(\text{recov}) - \text{CH}_4(\text{oxid})$$

This is common observed in the arable soils, when conditions are predominately-aerobic (Bronson and Mosier, 1993; Hansen et al., 1993). On the other hand, a positive CH$_4$ flux indicates net CH$_4$ production, and is observed when the magnitude of the methanogenic process is larger than CH$_4$ uptake. This is the case in rice paddies and wetlands (flooded or water saturated areas) which are predominately anaerobic (Chan and Parkin, 2000).
Other effects of landfills and dormant landfills

Older landfills can threaten local water quality, although, the number of operating (open) landfills is decreasing the number of closed (dormant) landfills is increasing, posing a continued risk of leachate contamination into underlying aquifers. Numerous studies have examined natural attenuation of landfill CH$_4$ through aerobic oxidation in landfill cover soils (Williams and Ward. 1999), but few have examined attenuation of the landfill CH$_4$ that contaminates groundwater. Moreover, few studies have had the opportunity to investigate the basic process of anaerobic CH$_4$ oxidation in an aquifer setting (Grossman, et al., 2002).

Household waste

Methods of managing household and industrial waste differ substantially among different countries. Landfill is still the predominant method, especially in developing countries where both hazardous and non-hazardous waste may be co-disposed. More than 85% of municipal solid waste is still land filled (IPCC, 1996). Landfilling of waste is inescapable today, with potential threats to public health and the environment if not properly managed. Some fractions can neither be recycled for burned. Therefore, attenuating (satisfying) mechanisms of mobilizing or transporting co-disposed waste is needed to direct appropriate landfill operation and management strategies. By focusing on changes on leachate and gas characteristics during co-disposed loadings of organic and inorganic hazardous wastes, the potential treatment capacity of landfills with leachate containment and circulation (Pohland, 1999). Ashes from incineration of wastes are usually buried, similarly to ashes from coal burning. Ashes contain minerals including heavy metals, but considerable amounts of organic mater (1-2% organic carbon; Pavasars. 1990), which might make the ashes difficult to stabilize using conventional landfill techniques (Bendz et al. 1999).

At present, municipal solid waste treatment, units belong to one of the following classes; incinerator plants or municipal solid waste landfills. Incinerators produce toxic ashes and large volumes of smoke that has dioxins and causes acid rain, whereas landfills are responsible for biogas emissions and pollution by leachate. In some small rural areas, landfills were left in the
open and waste set alight generating toxic fumes and odours. However, recently, waste deposits have been controlled, a watertight landfill, periodically covered with soil layers to minimise pollution is used. This generated gas produced by bacteria filter through, contributes to global warming (Popov and Power, 1999). Rising environmental concern helped promote severe constrains on waste treatment world wide. It is of paramount importance that gas and leachate produced in landfills be collected to minimise contamination in table water. The leachate often carries with it heavy metals, nitrates and some acids. The collection of these by-products can be achieved by pipelines or building a reservoir for leachate collection. The gas can either be used for energy while purification of leachate can be achieved by filtration or water treatment procedures. (Trebovet et al., 1999). Jaffrin et al. (2003) worked on harvesting the biogas and using it for generating heat in plant greenhouses and using the carbon dioxide for carbon supplementation in plant growth (Fig. 1.4).

![Figure 1.4: Harvesting biogas from landfill to be used in greenhouse (Jaffrin et al., 2003)](image)

**Estimation of landfill emission lifespan**

Landfilling is still the economical method of solid waste disposal and are source of strong contaminants. To prevent or control the emission potential and pollutant release in the environment, stringent technical requirements for waste and landfills are being looked into. Currently, the link between
theoretical knowledge and practical experience regarding the long-term behaviour of these systems is rather poor. Characterization of the chemical, physical and biological properties of the deposited solid waste and the understanding of the processes taking place on a long term are essential for finding a solution to the practical problem within the scope of the landfill management. Techniques that could be generally used to estimate time on waste behaviour, taking into account waste composition and thus emission potential during the lifespan of landfills.

The microbial methane oxidation in biofilter systems and cover layers of municipal solid waste represents an alternative way to the biological treatment of the landfill gas (Ustohalova, et al., 2005).

**Modelling an integral part of long-term landfill management**

Development of a numerical model describing the effect of solid phase composition and pore structure of the landfill on the conversion phenomena, gas transportation taking into account the simultaneously occurring settlement process. Due to a strong interrelation between the biological and chemical effects on a landfill, a highly coupled set of differential equations has to be used and solved (Wolfe, 2002). The body that is to be investigated consists of organic, inorganic moisturized phases and a gas phase. All interactions between the constituents like mass transfers, interaction of forces and energy supplied/used has to be considered. The municipal waste is a porous medium with a complicated structure. As the majority of natural porous, it contains an interconnected three-dimensional network of capillary channels of non-uniform sizes and shapes. Fluid flow and gas diffusion in porous media takes place within extremely complicated microscopic boundaries. However, the internal geometry of porous solid materials remains completely unknown. In recent years, the theory of porous media has provided a convenient and flexible base for the study of coupled processes, including phase transitions and gas and liquid transport (Ustohalova et al., 2005). The porous medium is assumed to consist of a solid phase $\Phi^S$ (solid), an organic phase $\Phi^O$, a liquid
phase $\Phi^L$ and a gaseous phase $\Phi^G$. It was assumed that, the porous solid always models a control space and the pores are statistically homogeneously distributed. A porous medium occupying the control space of the porous solid $B_S$ with the boundary $\partial B_S$ consists of constituents;

$$\Phi^\alpha (\alpha = \text{S,O,L,G})$$

with real volumes $v^\alpha$. Where the index $\alpha$ denotes $\kappa$ (individual constituents) (deBoer, 2000). Peer et al. (1993), said estimation of methane produced from the landfills was difficult due to lack of accurate refuse and landfill data, which leads to assumptions being used. The variables (amount and type of refuse; how much of this [type] refuse produce methane) are difficult to monitor and control in landfills. Moreover, the methanogenesis is not known when it starts. The emitted methane is the surplus of the un-oxidized in the cover material. This is based on the laboratory studies to see the overall production.

**Municipal waste incineration and health risks**

In some first world countries, incineration was getting popular with the hope of replacing the landfills in relation to solid waste treatment. There were problems encountered with this technology. Incineration emissions are complex and depend on the type of waste, the design of incinerator and combustion conditions. This posed a health risk problem when considering the toxic fumes released to the atmosphere. The most common emitted pollutants were acid gases, which caused acid rain, metals and various organic compounds and the dioxins (Domingo, 2002). The studies done by Glorennec et al. (2005) showed that the most concern was inhalation of sulphur dioxide at low atmospheric dispersion. However, it is impossible to assess the risk associated with all the pollutants contained in municipal waste incineration. Only a few pollutants can be monitored and there is limited data to compare the risk associated with them. The U.S. National Research Council (NRC, 2000) concluded that most relevant emissions are those of particular matter e.g. lead, mercury and dioxins. The toxicity depends on the value absorbed and current data availability. Waste incineration as air pollutant, was considered to be miniscule compared to traffic source (0.5 versus 20 – 30 $\mu$g/m$^3$) observed in France (Minister of ecology and
Development durability, 2002). Inhalation exposure was assumed to be equal to ambient air around homes (Glorennec et al., 2005). While this assumption depends heavily upon the compound, the house ventilation characteristics and the season, the outdoor/indoor concentration ranges from 0.25 to 1 µg/m³ (Monn et al., 1987).

**Health hazards of landfill gas**

Gases from landfill sites may not only be potential health effects on those people working at the site, but also on those living in the local area (Http://www.doh.gov.uk/landfillrep.pdf). Landfill gases also contain toxic volatile organic compounds (VOC) which have numerous health implications. Environmentally, it reduces detrimental gassed to the environment such as sulphur (acid rain), nitrogen (smog formation) and particles (visibility and breathing problems). Finally can improve in town planning establish collaboration of and common interest and awareness.

(Table 1.2)
**Table 1.2:** some volatile organic compounds emitted from landfills

<table>
<thead>
<tr>
<th>Compound</th>
<th>NOAEL*</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>32mg/m³</td>
<td>Reduced foetal weight</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Retarded,</td>
</tr>
<tr>
<td>Tetrachloroethylene</td>
<td>2000 mg/m³</td>
<td>Embryolethality</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Foetotoxicity</td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td>0.2 mg/ kg</td>
<td>Cardiac defects</td>
</tr>
<tr>
<td>Vinyl chloride</td>
<td>130 mg/m³</td>
<td>Retarded</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male testicular effects</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduced male fertility</td>
</tr>
<tr>
<td>1,3 butadiene</td>
<td>88 mg/m³</td>
<td>Reduced foetal weight</td>
</tr>
<tr>
<td>Carbon disulphide</td>
<td>-----</td>
<td>Uncertain malformation</td>
</tr>
<tr>
<td>Chloroform</td>
<td>147 mg/m³</td>
<td>Reduced foetal weight</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Retarded ossification</td>
</tr>
<tr>
<td>1,2 Dichloroethylene</td>
<td>0.025 mg/ kg</td>
<td>Cardiac defects</td>
</tr>
<tr>
<td>Ethylebenzene</td>
<td>430 mg/m³</td>
<td>Embryolethal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>foetotoxicity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Teratogenic</td>
</tr>
<tr>
<td>Formaldey</td>
<td>12 mg/m³</td>
<td>Reduced foetal weight</td>
</tr>
<tr>
<td>Methyl chloride</td>
<td>525 mg/m³</td>
<td>Cardiac defects</td>
</tr>
</tbody>
</table>


NOAEL* NO-Observable-Adverse-Effect-Level

Unpleasant odours may also result in maladies such as headaches, distributed sleep, shallow breathing, anger or depression and general decrease in well-being. Other health effects are explosion threats, air pollution and seepage of leachate into table water.

**Reduction of health risks by landfill gas**

The gas is removed in various ways by erecting pipelines to collect gas for energy. Flaring has been proven to cause secondary pollution in the atmosphere (acid rain and dioxins). Health risk on animals and vegetation, this is occurs as accumulation of gas.
Environmental effect
Because of the high methane concentration and its high UV, holding landfill gas is associated with increased global warming. Other detrimental effects are smog formation in high concentration of landfill gas (http://www.createionday8.co.uk/greenhouse.asp).

Benefits of gas collection
It eliminates toxic gases from entering the atmosphere and affecting the health of local population. It can be economical viable if used as energy source. Money can be brought in the area to build infra structure of the gas and sell the energy (renewable) source, job creation and low cost operation. The electricity production from this low cost raw material (waste) could supply 7,000,000 kilowatt per hour for a year of gas production (www.epa.gov/lmop/about.htm).

Co-disposal for gas production.
The co-disposal of sewage and municipal refuse enhanced gas production (Chan, Chi and Wong, 1999). They observed that the higher the refuse ration in mixture the more gas production was. 75 : 25% ratio of refuse to sewage increased gas production as compared to waste alone. For other ratios gas production was low, in anaerobic digestion of organic waste for fuel production, it is generally difficult to achieve a daily gas production rate higher than 1 l/kg of waste (Ruskin, 19982). After 36 days of digestion, the treatment with refuse alone was still in its acid formation phase as indicated by the slightly acidic leachate collected (pH6.1) (Chan et al., 1999). High levels of CO₂ and low levels of CH₄ from refuse alone throughout the whole experiment indicate that longer than 36 days was needed, these are the best indicators reflecting the digestion process has not reached active methanogenesis (Pohland and Harper, 1987). The low putrecible content of Hong Kong waste (35%) as compared to UK (75%). Westlake (1995) tends to minimize initial gas generation.
Mixing wastes in a proper ratio can enhance the digestion process and shorten time to reach anaerobiosis, as indicated that batch reactors with high gas production start to decline after 20 days. In landfill conditions, a site may be active for decades. In temperate countries, it may take 12-18 months to reach the methanogenic phase and about 50% of gas evolved within 10 years (Parker, 1983). Leachate circulation can cause a 12-fold increase in gas production and reduce the duration to reach methanogenic phase (Reinhart and Al-Yousfi, 1996). Sewage as compared to sludge has a lower potential gas production due to chemical energy is sludge been depleted in aeration tank. Low moisture content in refuse is also a limiting factor causing slow release of gas. Wong (1987) reported that at least 85% moisture is required for better gas production from municipal and farm wastes. Optimum pH for gas production is at pH 7 and at pH 5.5 gas; production ceases (Farquhar and Rovers, 1973).

Co-disposal for better landfill management
Co-disposal enhances decomposition rate of landfill waste, which in turn maximizes energy recovery. And also reduces costly long-term aftercare for gas migration and cover maintenance. The recent development of landfill technology favours containment concept with proper top and bottom liners to isolate waste (Johnson, 1985; Grantham, 1988; Westlake, 1995). These modern landfills become huge dry bioreactors and the waste may take longer to release gas. Chan et al. (1999) concluded that, it is feasible to co-dispose waste (municipal, sewage) in landfills if mixed accordingly it is advantageous as well as this increases gas (methane) production which can be used as energy source and shortens acidic stage. However, heavy polluted waste cannot be co-disposed in landfills because their adverse effects on leachate quality.

Other sources of methane
Stock pilling of industrial waste and subsequent disposal without end use is common in India (Majumdar et al., 2006). Sometimes the solid waste is sold
to farmers as soil conditioner due to high organic content. The usage of agricultural solid wastes by recycling can improve soil physical conditions and fertility (Mishra et al., 1989; Bhaardwaj, 1995 and Bansal and Kapoor, 2000). Xavier and Nand (1990) found that adding cow dung improved efficiency of methane production in landfills. A 0.419m³ gas was yielded at 8% cow-dung slurry addition.

**Cleaner production: a win-win situation**
The United Nation environment Programme (UNEP) has defined a cleaner production.
“The continuous application of an integrated environment strategy to processes, products and services to increase efficiency and reduce risk to humans and environment”. The idea is that industrial processes can often be improved in ways that not only reduces the amount of waste, and therefore pollution, but also saves or makes money for the company (Australian academy of science, 1994). Not so long ago, polluting companies did not bear the cost of waste disposal, the environment did. In many countries these days, companies discharging pollutants into the environment must purchase a license to do so or pay the municipality to clean their waste.

Sometimes, cleaner production techniques do not always produce a financial windfall for companies. In some cases, cost savings brought about by improved production efficiency may be outweighed, in the short term at least, by the cost of introducing new technologies. The purpose of this study is to investigate CH₄ emission from the landfill cover caps and oxidation of CH₄ by bacteria from the soil with the aim of reducing CH₄ accumulation in atmosphere.

The aim of this study is to evaluate the soils that can be used as cover material in landfills. Soils were analyzed to observe the physical quality as a parameter of cover material. These soils were compared against each other and monitored which is best that oxidises methane as a major gas emitted in landfills.
CHAPTER 2

Pilot Study; Simulation of landfill and setting up parameters of a bioreactor
Design and operation of bench scale simulated landfill bioreactor. Production of landfill gas by waste and oxidation of this gas by soil.
Abstract

Landfill simulations have been studied to monitor the volume of methane production and to a greater extent oxidation of the emitted gases. Various wastes, such as paper, plastics and putrescibles were mixed in a given proportion in the anaerobic compartment of a laboratory-scale bioreactor simulating a landfill. The soils contained in the aerobic compartment included garden soil rich in organic manure, road-side soil which was sandy and used mostly for paving and uncultivated land soil containing few organics. The soils were analysed and found that they have little variation when comparing their physical properties. Within the waste reactor, the methane produced was approximately 6%, which was not as high as the documented volumes of 50%. There were three identical waste reactors used, which had maximum methane composition as follows; 5.7, 6.0 and 6.1 respectively. However, it was observed that oxidation of methane occurred in the soils. More oxidation occurred in garden soil, which was related to available organic matter and less in road-side soil. It was speculated that this latter result was due to permeability of the soil and the inability of the soil to hold water. The bacteria isolated averaged 6 log cfu/ml in garden and uncultivated soils whereas the sand averaged 5 log cfu/ml.
Introduction

Landfills are a means of collecting and disposal of wastes. They have been used for many years, but are now posing a problem as they emit methane, which is a greenhouse gas. There are many ways that are used to eliminate the emitted methane. For the purpose of this study, soil as landfill cover is studied and evaluated to measure its oxidising capability. Natural occurring bacteria found in soil carry out methane oxidation. The soils studied are collected from three different areas for different properties and chemical composition. It is assumed that different soils will have different bacterial composition hence different oxidative capacity. Although the bacteria are naturally occurring, it is believed that the bacteria should be encouraged to grow to their maximum numbers to achieve reasonable oxidation of methane.

Soil has been used as a cover material to control waste being blown away. This controlled the spread of diseases and unsightly waste. However, the gas was not constrained and atmospheric contamination occurred from these emitted gases. Engineered capping was designed to control leachate sipping into table water and controlling of gases into the atmosphere. With all the technology, there are smaller problems that were not visualised. That is, the engineered caps prevented air circulation, which enabled methanotrophs to oxidise the gases. In first world countries, there is strong emphasis on alternate capping materials, which will prevent oxidation to some extent but allow collection of gas to be used for energy purpose. Where soil is used, structure and restoration layer become more complex and the thickness increased to as much as 3 metres resulting in installation costs increasing (IPCC, 2001).

Buried wastes are typically high in organic content, and the decomposition of the organic materials yield gaseous products that migrate towards the atmosphere. Landfill gas consists mainly of methane (CH₄) and Carbon dioxide (CO₂) with trace concentration of nitrates. Carbon dioxide formed and released from landfills is a mainly greenhouse neutral and the amount is negligible, while landfills are the largest
anthropogenic source of atmospheric methane in many developed countries (Huber-Humer, 2004). It is estimated that 37% of methane comes from the landfills while 30% is estimated in Europe (US EPA, 2003: EEA, 2001). Globally, atmospheric methane concentrations are approximately 1.8 ppm by volume. Furthermore, the concentration continues to rise, although the rate of increase is slowing. Methane is also emitted from older and smaller landfill sites, where the installation of a gas collection system is considered costly, and from poorly controlled of abandoned sites in countries where there is little waste management infrastructure. Therefore, highly engineered and low technology waste disposal sites could benefit from new improved methods to reduce emissions.

Recent research suggests that one inexpensive way to reduce methane and nitrates emissions is to exploit the natural process of microbial methane oxidation through improved landfill cover design and maintenance. This process is usually mediated by a group of bacteria called the methanotrophs; this phenomenon has been well documented in emergent wetlands, rice production systems and landfill cover soils (Adamsen and King, 1993; Borgener et al., 1997; Borjensson and Svensson, 1997; Chanton and Liptay, 2000; Hilger et al., 2000 and Hummer and Lechner, 2001). Where there is a natural interface between anaerobic and aerobic systems, methane forming and methane oxidising microbial populations are typically plentiful.

Physical phenomena that affect the integrity of compacted soil barriers in shallow land burial facilities and remediation sites are related to normal cycle of temperature and precipitation. Cycling occurs over three distinct time scales; diurnal (hourly changes), precipitation evapotranspiration (few days to months), and annual (seasonal). The diurnal cycle determines temperature gradients through the soil surface and influences root water uptake and gradient of soil matrix potential. Wetting and drying cycles associated with precipitation and evapotranspiration directly change soil water content and matrix potential. The annual climatic cycle provides the greatest range of temperature and moisture gradients. Long-term climatic cycles have much smaller amplitude and lower frequency and therefore are less important,
although low frequency extreme weather events can damage caps (Smith, Luxmore and Suter. 1994).

The methane oxidisers convert methane to water, carbon dioxide and microbial biomass. Many other organic compounds in landfill gas, for example, aromatic and halogenated hydrocarbons, have also been shown to be degraded in such aerobic environments, (Scheutz and Kjeldsen, 2002).

**Figure 2.1:** Some the pathways used by microbes in methane generation

Three-stage scheme for the complete anaerobic degradation of organic matter showing the general pathways and the three major metabolic groups of bacteria. 1, Fermentative bacteria; 2, hydrogen-producing acetogenic bacteria; 3, methanogenic bacteria. Acetate and sometimes other acids may be produced from H₂ and CO₂ by fourth group of bacteria. (McInerney and Bryant, 1981)
In this study, the main aim was to simulate an MSW landfill to produce methane and evaluate different soils to oxidise methane. The soil that reduces emissions can be used as cover soil material.
Materials and methods

**Figure 2.2:** Bioreactors set-up (anaerobic reactor, aerobic reactor, gas sampling and effluent collection.

Key:
1 – Gas bomb (gas sampling)
2 – Anaerobic reactor (waste)
3 – Aerobic reactor (soil)
4 – Water jacket on aerobic reactor
5 – Tube to collecting jar (leachate)

Waste reactor (anaerobic digestor), inside volume \[\pi r^2 h\]

diameter = 7cm; height = 47cm, therefore radius is 3.5cm.
Total volume = 3.14 x (3.5)² x 47 = 1808.77cm³
The bottom of the reactor is conical to collect leachate; the water jacket cover was 0.7cm. The top (lead) of the reactor had three outlets (figure 2.3); these were used as leachate recycling (B). Manometre connection (A) and gas sampling tube (D). The top has a 2.5cm flap to screw tight the lead which has rubber gaskets for airtight connection. Care should be taken when tightening the screws as the perspex material would break. When the connection was finished, the present air would be used by aerobic bacteria in waste, and when depleted then anaerobic bacteria would start digestion. This allowed for all hydrolysis steps to occur. The sample was taken from the leachate reservoir by using a needle through a rubber bung and taking 10ml sample by a syringe. After six-week run, a 10g waste was taken for further microbiological analysis. This was mixed in nitrate mineral salts (NMS) broth media for dilutions (-10 serial dilutions) and spread plated on solid OCM media below.

Waste composition was as in table 2.1 below; total mass was 450g and compacted in anaerobic reactor. The three reactors were interlinked with water pipe from the water-bath for temperature control. The reactors were built on these specifications;

Waste reactors are bigger than the soil reactors to accommodate more waste whereas the soil reactors were packed with mass of 150g of soil. The leachate was recycled daily and 10 ml of it withdrawn for pH measurement using (Merck) sticks.
Figure 2.3: System in operation (reactors filled with waste and soil)
<table>
<thead>
<tr>
<th>Components</th>
<th>% by wet weight</th>
<th>Sub-components</th>
<th>% by wet weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper</td>
<td>13.30</td>
<td>Cardboard</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Newspaper/directories</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Magazines</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Office paper</td>
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<tr>
<td>Glass</td>
<td>6.62</td>
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<td></td>
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<td>Textiles</td>
<td>5.09</td>
<td>Brushed nylon</td>
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<td></td>
<td></td>
<td>Toweling</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other</td>
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<tr>
<td>Plastics</td>
<td>7.64</td>
<td>Shopping bags</td>
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<td></td>
<td>Bottles (Ace) PVC</td>
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<td></td>
<td></td>
<td>Bottles (Coca-cola)</td>
<td>3.2 [Perspex]</td>
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<td>Metals</td>
<td>6.6</td>
<td>Aluminium cans</td>
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<td>Off-cuts</td>
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<td>Putrescibles</td>
<td>24.05</td>
<td>Meat</td>
<td>1.5</td>
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<td></td>
<td></td>
<td>Bones</td>
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<td></td>
<td></td>
<td>Bread</td>
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<td></td>
<td></td>
<td>Grass</td>
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<td></td>
<td>Mixed garden waste</td>
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<td>Fruit/Vegetables</td>
<td>13.7</td>
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<td></td>
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<td>Rice</td>
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<td>Noodles</td>
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</tr>
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<td></td>
<td></td>
<td>Oats porridge</td>
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</tr>
<tr>
<td>Other</td>
<td>11.91</td>
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<td></td>
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<td>Tyres</td>
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<td></td>
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<td>Soap</td>
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</tr>
<tr>
<td>Ash</td>
<td>25.04</td>
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</tr>
</tbody>
</table>

Table 2.1: Composition for refuse
### Anaerobic media from OCM file for Methanogens

#### Basal medium

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Mass/litre (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract</td>
<td>2 (g/l)</td>
</tr>
<tr>
<td>Fe(NH₄)₂</td>
<td>2 (mg/l)</td>
</tr>
<tr>
<td>NiCl₂</td>
<td>4 (mg/l)</td>
</tr>
<tr>
<td>CoCl₂</td>
<td>4 (mg/l)</td>
</tr>
<tr>
<td>(NH₄)₂HPO₄</td>
<td>60 (mg/l)</td>
</tr>
<tr>
<td>Na₂S</td>
<td>60 (mg/l)</td>
</tr>
<tr>
<td>Na₂HPO₃</td>
<td>4000 (mg/l)</td>
</tr>
<tr>
<td>15 g agar</td>
<td></td>
</tr>
</tbody>
</table>

#### Alternative medium

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Mass/litre (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>256</td>
</tr>
<tr>
<td>Potassium Di-hydrogen phosphate</td>
<td>1.7</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>0.8</td>
</tr>
<tr>
<td>Boric acid</td>
<td>10.3</td>
</tr>
<tr>
<td>Sodium nitrate</td>
<td>0.3</td>
</tr>
<tr>
<td>Iron (III) chloride</td>
<td>0.1</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>15</td>
</tr>
<tr>
<td>Manganese sulphate</td>
<td>0.06</td>
</tr>
<tr>
<td>Trace elements</td>
<td>10 (ml/l)</td>
</tr>
<tr>
<td>Sulphur (NaS₂O₃)</td>
<td>0.05</td>
</tr>
<tr>
<td>Sodium sulphate</td>
<td>0.3</td>
</tr>
<tr>
<td>15g agar for plates</td>
<td></td>
</tr>
</tbody>
</table>
Trace elements

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Volume (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrochloric acid (25%)</td>
<td>10 (ml/l)</td>
</tr>
<tr>
<td>Iron (II) chloride</td>
<td>1.5 (g/l)</td>
</tr>
<tr>
<td>Zinc chloride</td>
<td>70 (mg/l)</td>
</tr>
<tr>
<td>Manganese chloride</td>
<td>100 (mg/l)</td>
</tr>
<tr>
<td>Boric acid</td>
<td>6.0 (mg/l)</td>
</tr>
<tr>
<td>Cobalt chloride</td>
<td>190 (mg/l)</td>
</tr>
<tr>
<td>Copper chloride</td>
<td>2 (mg/l)</td>
</tr>
<tr>
<td>Nickel chloride</td>
<td>24 (mg/l)</td>
</tr>
<tr>
<td>Sodium molybdate</td>
<td>36 (mg/l)</td>
</tr>
<tr>
<td>Distilled water</td>
<td>990 (ml)</td>
</tr>
</tbody>
</table>

Procedure;
Dry chemicals were systematically dissolved in 800ml distilled water and made up to a mark with the remaining water. The solution was bubbled with nitrogen in a chamber for two hours then autoclaved at 121°C for 15 minutes. Trace elements solution was bubbled with the same gas and 10ml was filter sterilized and added to the medium after cooling to about 40°C.

Soil reactor (aerobic reactor), inside volume $\pi r^2 h$
Diametre = 7cm; active height is 30cm and radius = 3.5cm.
The working volume is 1154.54cm$^3$, which could take 1500g of soil. The soil was compressed in the reactor to a height of 28cm.

There is another 20cm above the water-jacket (active height) which allowed air circulation. In this 20cm, there were two outlets (5cm from the soil and 13cm apart. This was for gas circulation (figure 2.5). The soil was weighed and put in aerobic reactor that was connected to the anaerobic by tubing for gas transportation and sampling.
Media for culturing aerobic bacteria

Nitrate Mineral Salts (NMS) Media for Methanotrophs

**Solution 1.** 10x NMS

Dissolve in approximately 700ml of distilled water (in this order) and dilute to 1 litre.

- KNO₃ 10.0g
- MgSO₄ 10.0g
- CaCl₂ 2.0g

**Solution 2.** Iron EDTA. Make up to 100ml with distilled water

FeEDTA 3.8g

**Solution 3.** Sodium molybdate, made up to a litre with distilled water

Na₂MoO₄ 0.26g

**Solution 4.** Trace elements; dissolve the following in the specified order in distilled water and dilute to 5 litres, store in the dark.

- CuSO₄ 1.000g
- FeSO₄ 2.500g
- ZnSO₄ 2.000g
- H₃BO₃ 0.075g
- CoCl 0.250g
- EDTA di-sodium salt 1.250g
- MnCl₂ 0.100g
- NiCl 0.050g

**Solution 5.** Phosphate buffer, dissolved in specified order in 800ml distilled water. Adjust pH to 6.8 and dilute to a litre.

- Na₂HPO₄ 71.6g
- KH₂PO₄ 26.0g

**Preparation of NMS Medium**

10 ml of solution 1 was diluted to a litre, 1ml of solution 3 and solution 4 were added. 0.1ml of solution 2 and 15g agar were added then autoclaved at 121°C for 15 minutes. 10ml of solution 5 was autoclaved separately for every litre of NMS. When the media was cool to hold, ascetically solution 5 was added. If this is done too early the phosphate will precipitate out.
**Microbiological analysis:**

Approximately 1 gram of each different soil from different reactors was taken using a cork borer for microbial analysis. The soil was mixed with sterilised saline with 20 grams of glass beads to dislodge the bacteria from the soil. The mixture was shaken vigorously for 1 minute and let to stand for heavy particle to settle first. The sample was taken from the supernatant and diluted in MNS media for methanotrophs culturing. The media used for methanotrophs was NMS as mentioned above. For methanogens, 10 ml was withdrawn from leachate, a 9ml sample was taken for pH measurement, this was done to minimise aeration of the sample while 1ml was taken for microbiological analysis. The dilution was done in NMS broth media but for growth solid OCM was used as mentioned above. The anaerobic bacteria (methanogens) were incubated at 30°C in an anaerobic hood (Labotec), while the methanotrophs were incubated in a gas jar incubated at 25°C with a 50 : 50 gas concentration (methane : carbon dioxide) from Afrox gases. After a six-week run, a sample of waste (1g) is taken, mixed with NMS broth, diluted and cultured anaerobically. This helps in comparison of the leachate culture and actual waste culture.

**Gas measurement and analysis:**

![Connections from reactors for gas transportation](image)

**Figure 2.4:** Connections from reactors for gas transportation
Key:
A – Outlet to manometre (gas displacement unit, figure 3.4)
B – Leachate inlet tube
C – Aerobic reactor
D – Gas outlet
E – Gas displacement unit
F – Gas bomb

![Diagram of gas displacement unit](image)

**Figure 2.5:** Gas displacement unit

A gas displacement unit was continuously set in instead of a manometer. The gas produced pushes the liquid and we measure the displaced liquid as in figure 2.4. The unit developed bubbles if let to stand for a long period therefore, need to be zeroed by pumping the liquid back to eliminate gas formed. Using the formula given below we calculated the gas production;

**Calculation of gas produced**

The distance water moved = height \( (h) \)

Volume of a cylinder = \( \pi r^2 h \)

\[
\begin{align*}
  r &= 4\text{cm} \quad h = 6\text{cm} \\
  \text{Therefore;} \quad \text{volume} &= \pi \times 4^2 \times 6 \\
  &= 3.14 \times 16 \times 6 \\
  &= 301.44 \text{ cm}^3
\end{align*}
\]

From this we can calculate gas flow rate = ml/hr
Because the measurement was done daily, then we use 24 hour as our baseline.

Flow rate = 301.44ml / 24hr

= 12.56 ml/hr

From the apparatus shown in figure 2.2, the gas bomb was continuously set in to ensure a homogenous flow of gas. The gas was taken when the maximum displacement was measured. To direct the gas, the taps of the gas bomb were closed to send the gas to the gas displacement unit. When the maximum gas displacement was achieved per day, the taps would be opened and the gas would flow in the gas bomb and taps closed, the bomb would be disconnected. Taking out the gas bomb, the tubes are clipped closed to minimize air coming in, then the gas bomb taken out. A new bomb would be set in to continue with sampling. Before inserting the gas bomb, it is connected to a pump to suck out the air. When it is inserted, it will suck in the biogas produced, but because of continuous flow, the gas will homogenise. The sample is taken for analysis (gas chromatography).

DNA extraction was done by PCR primer kit and the marker was met 47 and met 54.

**DNA isolation**

A pure colony was picked up using a loop and mixed with 40ml sterile distilled water. 20 ml of chloroform was added to the mixture, boiled at 100°C for 20 minutes. This was spun down for 5 minutes and 2µl of supernatant was taken for PCR. For PCR 25µl of master mix was added to the mixture with 1µl of forward and reverse primers each and 20.5µl of deionised water incubated in a PCR machine. When finished, this was run on gel electrophoresis (Lindsay, 2006).
Results

Graph 2.1 Total bacterial counts from anaerobic (a) waste reactors [methanogens] and (b) aerobic soil reactors [methanotrophs].
**Graph 2.2** (i) Methane production from waste and (ii) methane oxidation by soil bacteria.
Figure 2.7 Chromatogram of gas analysis

Figure 2.8 Molecular identification of the isolated cultures.
Figure 2.9 Fungal growths on the surface of soil at start of experiment.
Discussion

The pH remained constant with time; it was controlled using a 1molar sodium hydroxide solution if it dropped below 6.5, as the optimum pH for the waste reactors (methanogens) is 7.0. From the experiment, it was observed that within the first and second week the bacteria were still increasing in numbers, refer to graph 2.1 (a), this is when the organic matter was rotting and therefore, in the acidogenesis stage and it is crucial to control the pH, as this will sour the reactor. That is, the reactors will produce too much acid killing the methanogens. This however, will produce other gases like hydrogen or carbon monoxide and organic acids. From the anaerobic counts graph, it was confirmed that, the counts are increasing, showing that the pH is properly controlled. The temperature was controlled from one water bath as the reactors were inter-linked. This has not affected the gas production in that reactor were still producing some amount of gas as the concentration is increasing in all the rest of the reactors. As the reactors stabilized, the numbers of bacteria dropped and settled on 6.5 log cfu/ml in R1, 5.9 log cfu/ml in R2 and while R3 was at 7.8 log cfu/ml.

This however, seemed to be a different scenario in aerobic reactors. The soils have naturally occuring bacteria, as the initial bacterial analysis is the highest at 7.6 log cfu/ml in garden and uncultivated soil while sand soil was at 4.8 log cfu/ml. The bacterial numbers, over time were seen to decline. It can be explained as thus; the number of bacteria declined as the gas was produced. This limited air and other oxidative reactions to occur resulting in the decrease in numbers. On the other hand, the numbers of methanotrophs increased with supply of methane in the biogas production, hence the numbers settled at a high log value that is 4.8 log cfu/ml in sand soil and log 5.6 in garden and uncultivated soils (Graph 2.1b). Although the counts started high, there was some fungal growth on the surface of the soil at the beging of the experiment. This was observed in all the soils, but more on uncultivated soil, as it was reddish and therefore gave a contrasting colour with white fungal. It looked like some spider web on the garden soil and the sand was a thick solid at the surface. This disappeared with time although
some traces were still seen. Some of the isolated cultures from the waste and soils (figure 2.7). [A] were cultures isolated from waste while [B] were cultures isolated from soils.

**Methane**

In comparison, the gases (methane production) show the same trend in that they are increasing, peaking in week 4 and 5 and a slight drop in week six, which probably could stabilise if the experiment ran for a longer period. The soils show oxidation of methane in all different soils, the most effective soil being the garden soil. This is attributed to high organic matter in the soil. However, roadside soil has oxidation effect when looking at its bacterial numbers (methanotrophs). The only disadvantage of this soil is; it is too porous. This, emit more gas faster than it held it back for the bacteria to attach and finally metabolise the gas resulting in oxidation. It is seen that the bacteria uncultivated soil emitted more methane that garden soil and roadside soil. Although the bacterial numbers are high, comparable with garden soil, it can only be that the counted are not methane oxidising (methanotrophs), but others that can withstand minimal nutrient media. The soil was collected 30 cm in depth with no organic matter and no debri from dead plant. This then tells us that the bacteria is naturally found in soil. During the pilot study, the soil grew some fungi on the surface but later disappeared when more methane was produced or during extended exposure to landfill gas.

From the study it can be concluded that the soil as a cover material works to keep the water in place and to oxidise gases (methane) produced to a lesser potent gas carbon dioxide and water. The technical aspect of using soil as a cover is to keep the soil together i.e. more clay to hold together so that it is not blown away. In addition, lessen sand particles to avoid landslide when there are too much rains and the soil is water logged.

From the study there was not enough gas production relative to the literature therefore, we opted to increase the putrecibles for the study purpose. To get more degradable organic matter and increase inoculation by adding sewage.
This will limit the acedogenesis stage, as the acid will be by-passsed in high number of anaerobic cultures carried with sewage. The general feeling is, we are getting the methane but want high concentrations and faster. We observed the oxidation in soils and isolated bacteria that are presumptive methanotrophs. The next stage is to inoculate the soil with the isolated cultures and monitor the oxidation rate.

**DNA extraction**

From figure 3.10 the samples did not run. Picture 1 were waste isolates and picture 2 were soil isolates. Only the marker was seen to have run in picture 2. No sample ran in both purification. This can be concluded that the DNA extraction was not properly extracted for PCR or these were not methanogens/methanotrophs although there was methane production and oxidation.

**Comparison with other work**

Gebert *et al.* (2003) stated that every biochemical reaction is influenced by temperature. Methane oxidation as a chemical reaction is too dependent on temperature. Knowledge of temperature kinetics is particularly relevant as the reaction is maximum at ambient temperature. In this study however, the effect of temperature was not looked into. The study was conducted at $35^\circ C$ for the wastes while the soils that is, oxidation reactors were ran at $30^\circ C$ which accommodated the maximum enzyme activity rate. This study was aimed at looking at different bacterial activity on methane oxidation in different cover soils. Molecular identification of isolated was not achieved (figure 2.9). Over a period of three weeks, the numbers of methane oxidising bacteria stabilised between log 5 and 6 cfu/ml, which was reasonable compared to Gebert study (2003) where their stability was at log 11 cfu/g soil over a year, which is higher than that found in other methane-oxidizing habitat. This indicates the high activity of dense methanotroph population that has developed under conditions of high methane production.
Although slimy cultures were isolated from soils, there was no evidence that they grew in soils. Formation of expolymeric substances as reported by Lechner et al. (2001) and Hilger et al. (2000) for methanotrophic habitat and subsequently clogging the filter (soil) was not observed. However, when cleaning the soil reactor, the soil was caked which could be a result of compaction or the slime formers. This then could result in delay of steady flow of gas for methane removal. It is assumed therefore, that the low effluent of methane was either methanotrophic or slime formation.

**The soil**  
Soil as a cover material should be permeable and hold together, that is, not get blown away by wind. Top soil methane oxidation was increased with time this was probably due to increased methane oxidisers and the substrate (methane) increase. As much as temperature plays a role in methane oxidation, gas diffusion also plays an important role. For oxidation to occur there must be gaseous exchange. The compaction of soil should allow gas to travel either methane upward and oxygen inwards and the bacteria are enzymes that control the rate of reaction. For low concentration of methane, type I methanotrophs are selected. These are high affinity, but when the concentration increases, the low affinity methanotrophs (type II) are selected. These vary in depth in the soil cover (Hanson and Hanson, 1996). Type I methanotrophs are mainly closer to the surface and have membrane bound enzyme that oxidises methane, whereas the type II methanotrophs are deeper in the cover soil and have cytoplamic enzyme.

Finally, there is methane oxidation occurring in the soils chosen in this study. However, the production of methane was very low to conclude on the capability of the isolated bacteria. The soils had naturally occurring bacteria that declined in numbers as the methane started being pumped through. This could be the methanotrophs were isolated and other bacteria suppressed hence the decline in numbers.
Conclusion

For further work, the landfill took about 10 years to start producing methane in large volumes (50%) the low concentration could be to late phase change. Therefore, next is to increase organic matter and decrease metal, bottle and ash. This will enable us to compare other variables such as addition of organics (compost) in soil in relation to amount of methane oxidised. More work on molecular purification will have to be done.
CHAPTER 3

Microbial populations in waste and soils
Isolation, purification and identification of methanogens and methanotrophs from waste and landfill cover soil.
Abstract

The main greenhouse gases are carbon dioxide and methane. Each of these gases has different impact in the atmosphere, that is, each has a different global warming potential. The goal of this study is to isolate methane oxidising bacteria (methanotrophs) from soil landfill. It was found that methane was generated from the waste reactors (simulated landfill) by methanogens, although not in good quality or concentration in comparison to the real landfill. About 8% methane is produced and approximately 95% was oxidised using different soils. Soils used were garden soil, sandy soil and soil from uncultivated soil.

Bacteria occur naturally in the environment, they are either detrimental or beneficial to humans. In this study biodegradation of waste by bacteria was studied to observe which bacteria are involved in methane production. The process starts by aerobic bacteria using available air and later the anaerobic bacteria finishing the process. Thereby, further decomposing waste. In so doing methane is produced which is oxidised to lesser toxic gas. This involves bacteria in soil using diffusing air between soil particles.

The oxidation was more in garden soil due to soil fertility. Addition of compost increased oxidation of methane. Although addition of isolated cultures to soil increased bacterial counts but did not have much effect on methane oxidation. Soil bacterial counts from soils were 6 log, 4 log and 6 log cfu/ml respectively. The final counts were 9 log, 7 log and 8 log cfu/ml respectively. The number of bacteria from waste averages 5 log cfu/ml on waste only, 6 log cfu/ml waste and sewage and 7 log cfu/ml on waste, sewage and cowdung.

There was no link between microbial numbers and methane production, even after adding more inoculum to increase volumes produced. In soil, oxidation occurred, but sandy soil emitted more due to its permeability.

From this study, more production of methane should be achieved to monitor microbial (methanotrophs) population. This will give more evaluation of methanotrophs in soil as to whether they are type I or type II. Likewise, in the landfill simulation more knowledge of methanogens would be achieved if better molecular techniques were employed.
The entire community would be tested for ability to degrade other more toxic compounds. With a thorough understanding of a landfill community and its bioremediative qualities, it may be possible to redesign landfills to reduce methane emissions to a bare minimum and organic compounds to a reasonable measures.
Introduction

Methane bacteria have an extremely limited substrate spectrum (Zehnder and Svensson, 1986). Their main substrates are acetate or carbon dioxide and hydrogen. “Various bacteria intervene in this anaerobic process in a highly organised and coordinated manner, converting the organic components of the waste to methane,” says Professor Colleran. Molecules with more than two carbon except isopropanol (Widdel, 1986) are neither converted to methane or nor can either electrons be used to reduce carbon dioxide to methane in methane bacteria. In case of only carbon dioxide and protons are present as terminal acceptors, other bacteria first have to convert complex organic carbon compounds to acetate or hydrogen and carbon dioxide before methane bacteria can finally produce methane.

Acetogenic dehydrogenase has been assessed in various ecosystems. Kaspar and Wuhrmann (1978) showed that about 60% of the flow of hydrogen in domestic sewage sludge was influenced by increasing the partial pressure of hydrogen.

Ecological aspects

Methanogens are entirely dependent on metabolism of other compounds by aerobes for providing their growth substrates. The breakdown of organic matter in anaerobic ecosystems, proceed sequentially from the complex to the simple compounds. Thus, biopolymers such as cellulose undergo initial attack to yield biopolymers which are eventually degraded to the level of methanogenic substrates.

Anaerobic methane oxidation

Concentration profile of methane in anoxic marine waters and sediment frequently has “concave upward” appearances (Barnes and Golberg, 1976; Reeburg, 1977; Martens and Berner, 1977; Bernard, 1977). Kinetic models of such profile data indicate that there is less methane in these regions than
would be predicted by simple diffusion. In all the methanogenic bacteria tested, the terminal step of methanogenesis involves the reductive demethylation of methylocenzyme M ($\text{CH}_3\text{S—CoM}$) to coenzyme M and methane. McBride and Wolfe (1971) discovered Taylor and Wolfe (1974) established coenzyme M derivatives and their structures.

**Methanogenic waste treatment**

Although anaerobic systems for wastewater treatment have been used since the beginning of the twentieth century (Emscherbrunnen in Germany), they were long considered to be inefficient and too slow to serve the needs of a quickly expanding wastewater volume, especially in industrialized and densely populated areas. Aerobic techniques such as trickling filters and oxidation ponds with more or less intense mixing devices are installed for wastewater treatment in small communities. Larger treatment plants today depend nearly exclusively on the activated sludge process, in which a mixed and so far only marginally identified population of bacteria and protozoa degrades the organic material in intensely aerated basins. The biomass produced (activated sludge) is recycled to a varying proportion within the system to enhance its operational efficiency. The excess activated sludge is often stabilized in an anaerobic fermentation step. The digester gas obtained in this process is mostly flared off; in recent years, it has been applied to cover some part of the enormous energy needs invested in stirring of aeration. The activated sludge can vary sufficiently to qualitative and quantitative changes in waste water composition, although management problems such as bulking are still an ill-understood phenomenon which is an empirical rather than an analytical basis (La Riviere, 1977; Pipes, 1978). The function of the protozoa is mainly the maintanace of a stable and well-settling bacteria community, and the reduction of pathogens (Curds, 1982).

**Mechanisms and microflora involved**

Unlike production of methanol that is carried out by pure cultures of yeasts, a mixed microbial community carries out the production of $\text{CH}_4$. Numerous
different bacterial species participate in the acid forming stage. Some microbial populations are involved in the breakdown of various organic materials, including complex polymers and simple fatty acids, hydrogen, carbon dioxide and alcohols (Ronal and Richard, 1992). In the methane forming stage, strict anaerobes of the genera methanobacterium, methanobacillus, methanococcus and methanosarcina convert acetate, hydrogen and carbon dioxide produced during fermentation to methane. Methane is produced in many natural anaerobic habitats where organic matter is degraded by microorganisms and carbon dioxide is the only available electron acceptor (in absence of oxygen, sulphate and nitrate. Alessandro Volta was the first to discover (1777) that combustible air (CH₄) was generated in sediment rich in organic matter at the bottom of marshes and lakes. All methanogens are archea bacteria that derive their energy by reduction of compounds e.g. carbon dioxide, acetate or methanol.

**Methanogenesis in anaerobic treatment of food-processing waste**

Disposal of waste inorganic matter may involve either treatment on site or discharge via sewage system for treatment by the local authority. Because of the cost of the latter approach, there is increasing interest in treatment on site and, especially in anaerobic treatment. The two principal advantages of these are that the expensive and energy-intensive process of aeration is eliminated and that a useful fuel is obtained. Biogas contains approximately 70% methane and 30% carbon dioxide and may be used in conventional burners with little difficulty.

In spite of its basic attraction, the use of anaerobic digestion for methanogenesis has not become widespread, principally because the conversion of organic matter to methane is often slow, and the process has a reputation of poor reliability.

Methanogenesis is carried out by a group of bacteria known collectively as methanogens. Methanogens are found in abundance in diverse natural anaerobic habitats, such as the rumen and intestinal tract of animals, fresh and marine sediments, water logged soil, compost and landfills. Between 500
and 800 million tons of biologically generated CH$_4$ are released into atmosphere per year (Alexander and Nikaido, 1995). This is approximately 2% of CO$_2$ fixed annually by photosynthesis. A cow produces about 200 liters of CH$_4$ per day (Alexander and Nikaido, 1995) which are released by belching.

Methanogenic bacteria utilize these fermentation products for the production of CH$_4$. Methanogenesis is commonly carried out by microbial populations involved in, synergistic or mutualistic relationships. The organisms responsible for the conversion of organic matter to CH$_4$ represent a balanced microbial community. This mixture presents some problems for bioengineers, who design fermenters for industrial applications that optimize conditions for defined microbial populations. Effective designs for the conversion of organic matter to CH$_4$ require relatively compact fermenters for the ready trapping of gaseous CH$_4$ (Switzenbaum, 1983).

As population growth and MSW generation rates increases in many areas there is an urgent need to develop methods to increase the rate of microbial decomposition of MSW while minimizing the release of CH$_4$, as potent greenhouse gas (Uz, Rascche, Townsend, Orgram and Lindner, 2003). Most landfills are composed primarily of anaerobic zones, where microbial decomposition of MSW follows well-known decomposition processes, including microbial fermentations, sulphate reduction, acetogenesis and methanogenesis (Barlaz. 1997). The last step in the conversion of the complex polymers in MSW to CH$_4$ requires the activity of CH$_4$-producing micro-organisms (methanogens), which use a limited range of growth substrates including acetate, H$_2$/CO$_2$, formate, methanol and methylated amines and dimethyl sulphide (Keltjens and Vogels, 1993; Barlaz.1997). Despite estimates of global net emissions of CH$_4$ from landfills ranging from 9 to 70 Tg yr$^{-1}$ (Nozhevnikova et al. 1993; Boeckx et al. 1996; Czepiel et al. 1996; Bogner et al. 1997; Vosvanathan et al. 1999). Methanotrophs have been reported to oxidize a significant amount of CH$_4$ to CO$_2$, a far less potent greenhouse gas (Whalen et al. 1990; Boeckx et al. 1996; Czepiel et al. 1996; Bogner et al. 1997). Methanogens and methanotrophs has also been linked
in their abilities to sequentially transform chlorinated solvents, such as perchloroethylene (PCE) and trichloroethylene (TCE), known to be present in landfills, yielding CO₂ as the final product (Wilson and Wilson, 1985; Little et al. 1988). Thus, understanding the composition, metabolic potential and interrelationships of these individual groups of microorganisms in landfills will facilitate the development of optimized landfill management strategies.

Landfills require microorganisms to breakdown their solid waste. Most landfills do not allow access to the atmosphere, which inhibits the growth of aerobic microorganisms.

**Microbiological processes in landfill**

Although new methods of municipal waste utilization are being explored, landfills are still a common disposal method and are likely to remain so in many countries for the foreseeable future for several reasons: It is relatively cheap; the technology and environmental control measures are reasonably well understood; leachates may be collected and treated by anaerobic digestion under controlled conditions to produce high-value chemicals and any methane generated may provide a potential valuable source of energy (Grainger, 1984).

**Classifying Archaea bacteria**

The archaea bacteria include three distinct kinds of bacteria, all found in extreme environments: the methanogens, extreme halophiles and extreme thermophiles. The methanogens live only in oxygen free environment and generate methane by the reduction of carbon dioxide.

**Methanogens**

Methanogens are autotrophic archebacteria that use anaerobic respiration for ATP synthesis. They use CO₂ taken from their growth media as the carbon substrate for growth. This CO₂ is the ultimate oxidizing agent of an electron transport chain which, maintains a trans-membrane electrochemical ion gradient which powers ATP production (USEPA, 1994).
**Effect of methanogens**

Methanogens affect the growth of some of the hydrogen producing species of microorganisms in the rumen’s stomach (Martins, 1992; Bogajl, 1994). The growth of *Fibrobacter succinogenes* for example is not affected by presence of methanogens as the hydrogenase activity in *Fibriobacter* is not sensitive to prevailing hydrogen tension. These microorganisms do not therefore, live in syntrophic relations with methanogens but methanogens do use the hydrogen they produce which is available to them as a result of interspecies hydrogen transfer (Martins, 1992). On the other hand, *Ruminococcus* species do have their metabolism process altered by the presence of methanogens and they do live in syntrophic relations with the methane producing species which, use the hydrogen that they produce. One species, *R. lbus*, produces ethanol, hydrogen, carbon dioxide and acetate but in proportions that vary with the abundance of methanogens in culture. For example, in presence of vigorously growing methanogens the products of fermentation are biased towards acetate and away from ethanol. This change happens because methanogens remove the hydrogen that *R. albus* produces and the decreased hydrogen partial pressure results causes *R. albus* to alter its the way NAD is regenerated so as to reduce ethanol production and increase acetate production, *R. flavifaciens* shows the similar change in fermentations products brought about by co-cultures.

The figure below (fig. 3.1) shows hydridogenase sensitivity, that is, it is inhibited by hydrogen. The enzyme is reduced its functionality when dehydrogenase that regenerates oxidized ferredoxins used as an oxidizing agent in pyruvate conversion in not removed by co-cultured methanogens (Bogajl, 1994. picture from the same journal). The decrease in some organic acids (intermediates) and increase in acetate production by *R. flavifaciens* when grown in co-culture with methanogens has profound consequences for feed utilization on the host ruminant. The decrease in succinate production by *Ruminococcus* growing in the presence of methanogens means that species like *Selenomoas* and *Veillonella* have less substrate available for making propionate.
Methanogenesis

The microbiological degradation of organic materials is due mainly to anaerobic bacteria and their dynamic interactions. The optimal degradation and energy gain for the anaerobic bacteria in a landfill environment, where CO₂ is the dominant compound for respiratory activity, takes place with methanogens as the most essential group of bacteria. Therefore, anything that hampers the activity of these bacteria will put constraints of the waste mineralisation (Ejlertsson et al. 2003). The biotic and abiotic transformation processes of different wastes give rise to pools of organic and inorganic compounds in the gaseous and liquid phases. Such compounds may be emitted to the atmosphere of surface and groundwater basins in the drainage area of the landfill. The formation, appearance, and disappearance of a specific pollutant in a landfill depends on several factors, including the chemical and physical properties of the compound itself, e.g. its chemical reactivity, water solubility, absorption capacity, volatility and biodegradability.

The complete mineralization of organic matter in anaerobic environments where sulphate and nitrate concentrations are low, occurs through
methanogenic fermentation, which produces CH₄ and CO₂ according to the reaction

\[ C_6 H_{12} O_6 \rightarrow 3CO_2 + 3 CH_4 \]

This transformation requires successive actions of four populations of microorganisms that degrades complex molecules in simpler compounds:

- Hydrolysis of biological polymers onto monomers (glucides, fatty acids, amino acids) by an hydrolytic microflora that can be either aerobic, or facultatively, or strictly anaerobic;
- Acidogenesis from monomeric compounds and intermediary compounds formed during fermentation (production of volatile fatty acids, organic acids, alcohols, H₂ and CO₂) by a fermentative microflora that can be either facultatively or strictly anaerobic;
- Actogenesis from the previous metabolites by a syntrophic or homoacetogenic microflora;
- Methanogenesis from the simple compounds that can be used by methanogens (in particular H₂ + CO₂ and acetate) which constitutes the last step of the methanogenic fermentation.

Anaerobic organisms generally show high degree of metabolic specialization. The success of anaerobic digestion therefore, depends on cooperative interaction between microorganisms with different metabolic capabilities.

**Examples of reactions occurring in an anaerobic digestion tank and their products.**

1. Hydrogen use by methanogens
   a) \( 4 H_2 + HCO_3^- + H^+ \leftrightarrow CH_4 + 3 H_2O \)

2. Some reactions of the hydrogen-producing acetogenic bacteria
   a) organic acids + water \( \leftrightarrow \) acetate^- + H^+ + H₂

   The organic acids include lactate, butyrate propionate and methanol. In some cases succinate acetate and formate. Lactater produces
bicarbonate, the amount of hydrogen produced varies with the organic acid used

Figure 3.2 Methane production (Ronald and Richard 1992)

Methanogenesis, which requires strict anaerobiosis and low reduction potentials, involves aspecialized, strictly anaerobic microflora that can develop in synergy or in syntrophy with other anaerobic bacteria. Methanogens belong to the domain Archaea (Hanson, 1998). Methanogens have a limited trophic spectra comprised of a small number of simple substrates: H₂ + CO₂, acetate, formate, methylated compounds (methanol, methylamines, dimethylesulphur), and primary and secondary alcohols. This allows to distinguish five trophic groups of methanogens (table 3.1). Carbon monoxide (CO) can be used by methanogens but is not an important substrate. The two major pathways of CH₄ production in most environments where organic matter decomposition is significant are acetotrophy and CO₂ reduction by H₂ (Le Mer and Roger 2001).
Table 3.1 Characteristics of trophic and morphological groups of methanotrophs.

<table>
<thead>
<tr>
<th>Trophic groups and substrate</th>
<th>Cocci</th>
<th>Rods</th>
<th>Rods with sheath</th>
<th>Sarcinae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogenotrophs ( \text{H}_2 + \text{CO}_2 )</td>
<td>most</td>
<td>most</td>
<td>None</td>
<td>Few</td>
</tr>
<tr>
<td>Formatotrophs, formotrophs</td>
<td>All are</td>
<td>All are</td>
<td>All are</td>
<td>All are</td>
</tr>
<tr>
<td>Hydrogenotrophs formate</td>
<td>several</td>
<td>several</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Acetotrophs acetate</td>
<td>2 species</td>
<td>none</td>
<td>1 genus</td>
<td>All</td>
</tr>
<tr>
<td>Methylotrophs methylated compounds</td>
<td>4 genera</td>
<td>none</td>
<td>None</td>
<td>All</td>
</tr>
<tr>
<td>Alcohologtrophs (no strict forms) alcohols I, II</td>
<td>none</td>
<td>few</td>
<td>None</td>
<td>Few</td>
</tr>
</tbody>
</table>

Methanogens are responsible for \( \text{CH}_4 \) production in a wide variety of anaerobic habitats. They convert relatively narrow organic substrates to \( \text{CH}_4 \) (table 3.2). Therefore in habitats with complex organic substrates, they must interact with other anaerobes that catalyze complex substrates into simpler methanogenic ones (Hanson, 1998).
**Table 3.2** ∆G produced by methanogenic reactions

<table>
<thead>
<tr>
<th>Reactants</th>
<th>Products</th>
<th>∆G° (kJ/mol CH₄)</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>4H₂+HCO⁻⁺H⁺</td>
<td>CH₄ + 3H₂O</td>
<td>-135</td>
<td>H</td>
</tr>
<tr>
<td>4HCO₂⁺H⁺+H₂O</td>
<td>CH₄ + 3CHO⁻</td>
<td>-145</td>
<td>F</td>
</tr>
<tr>
<td>2CH₃CH₂OH+HCO⁻₃</td>
<td>2CH₃COO⁻⁺H⁺+CH₄+H₂O</td>
<td>-116</td>
<td>A</td>
</tr>
<tr>
<td>CH₃COO⁻ + H₂O</td>
<td>CH₄ + HCO⁻</td>
<td>-31</td>
<td>Ac</td>
</tr>
<tr>
<td>4CH₃OH</td>
<td>3CH₄+HCO⁻₃+H₂O+H⁺</td>
<td>-105</td>
<td>M</td>
</tr>
<tr>
<td>4(CH₃)₃-NH⁺ + 9H₂O</td>
<td>9CH₄+3HCO⁻₃+4NH⁺⁴+3H⁺</td>
<td>-76</td>
<td>MA</td>
</tr>
<tr>
<td>2(CH₃)₂-S + 3H₂O</td>
<td>3CH₄+HCO⁻₃+2H₂S+H⁺</td>
<td>-49</td>
<td>MeS</td>
</tr>
<tr>
<td>CH₃OH + H₂</td>
<td>CH₄ + H₂O</td>
<td>-113</td>
<td>H/M</td>
</tr>
</tbody>
</table>

F, Formate: A, Alcohols; Ac, Acetate; H, Hydrogen; MA, Methylamines; M, Methanol

*Substrate legend: a assumed all species are mesophiles and freshwater: c Duetches Sammlung von mikroorganism

Despite the low number of methanogenic species that can use acetate as carbon and energy source (about 14% corresponding to the genera Methanosarcina and Methanosaeta), acetotrophy is generally considered to be responsible for about two-thirds of the CH₄ produced. This reaction produces little energy in normalized conditions (∆G°) which results in a low growth rate of acetotrophic methanogens (Le Mer and Roger, 2001). About 77% of methanogenic species are hydrotrophic; about 60% also utilize formate. Formate, like H₂, is involved in inter-species transfers during the oxidation of the reduced compounds produced by the anaerobic decomposition of organic matter (Dong and Stams, 1995). The energy produced by these reactions is high, which results in a rapid growth of hydrogenotrophic and formatrophic methanogens (Le Mer and Roger 2001). The larger range of CH₄ production in rice soils, as compared to uncultivated soils, can at least partly be attributed to an usually higher content on easily mineralisable carbon.
**Methanotrophy**

A group of aerobic bacteria known as methanotrophs consumes CH$_4$ and uses it as a carbon and energy source. Methanotrophs are a unique group of bacteria that utilize methane as their sole source of carbon and energy, and are all obligate aerobic, gram-negative bacteria. They are becoming a novel agent of bioremediation (Matthews, 2000). They can also break down complex, possibly carcinogenic compounds into smaller less harmful products. Significant amounts of CH$_4$ produced in landfills is consumed by methanotrophs. Aside from consuming CH$_4$, several methanotrophs species have been already been discovered to biodegrade multi-carbon organic compounds that are potentially harmful to humans including halogenated alkanes, halogenated aromatic compounds, long-chain alkanes, alkenes aromatic and cyclic hydrocarbons, phenols long chain and alicyclic alcohols and multi-ring compounds (Hirsak and Begonja, 1998). This ability has been described as “fortuitous metabolism”, but methane monooxygenase (MMO), the enzyme the reaction that changes CH$_4$ to CO$_2$, is responsible for degrading harmful compounds (Hirsak and Begonja, 2000). However, successful control of microbial activity in landfills can contain the problem using landfill sites for biogas production (Marchant, 1981). Research into bacteria found in landfills may also reveal methanotroph or other bacteria species that may be useful in optimizing solid waste degradation (Lindner, 2002). An improved understanding of the microbial processes involved is a necessary preliminary to successful control of hazards and maximization of benefits because landfill microbiology is still in its infancy (Grainger, 1984).

Methane oxidizing bacteria (methanotrophs) are obligate aerobes, gram negative bacteria that utilize CH$_4$ as their sole source of carbon and energy. Some can also use methanol but none grow on multi-carbon compounds. These bacteria have been isolated from a wide range of environments including soil sediments, fresh water, salty water and more extreme environments such as hot springs (Murrel and Radajewski, 2000). Methanotrophs play a major role in CH$_4$ cycling in the natural environment thereby mitigating the effects of the greenhouse gas. One important discovery obtained from studies of CH$_4$ oxidation kinetics is that there are two
distinct populations of methanotrophs (Hanson and Hanson, 1998). The methanotrophs we have in culture only exhibit low affinity oxidation kinetics but there is also a distinct and as yet uncultured population of methanotrophs, which can oxidize CH$_4$ at lower concentrations (high affinity).

The growth of type II appears to be favoured in environments that contain relatively high levels of CH$_4$, low levels of dissolved oxygen, and limiting concentrations of combined nitrogen and copper. Type I methanotrophs, appear to be dominant in environments in which CH$_4$ is a limiting factor, and combined nitrogen and copper levels are relatively high. These bacteria serve as biofilters for the oxidation of CH$_4$ produced in anaerobic environments, and when oxygen is present in soils, atmospheric CH$_4$ is oxidized. Their activities in nature are greatly influenced by agricultural practices and other human activities. Recent evidence indicates that naturally occurring, uncultured methanotrophs represent new genera (Hanson and Hanson, 1998). Methanotrophs that, are capable of oxidizing CH$_4$ at atmospheric levels exhibit CH$_4$ oxidation kinetics different from those of methanotrophs available in pure cultures (Hanson and Hanson, 1998).

Methane-oxidizing bacteria: Part of the CH$_4$ diffusing into landfill cover soils may be oxidized by methanotrophic bacteria which, use the following reactions to gain energy and carbon for their growth (Hanson and Hanson, 1996):

CH$_4$ $\rightarrow$ CH$_3$OH $\rightarrow$ HCHO $\rightarrow$ HCOOH $\rightarrow$ CO$_2$

Energy is yielded in all steps, except in the first step. The importance of intermediate formaldehyde (HCHO) stage is that the bacteria for synthesis of new cell material can use it. HCHO can be transformed and stored as polymers. Polymers can also be excreted, sometimes in amounts so huge the use of PLFA (phospholipid fatty aid) analysis it has been shown that CH$_4$ oxidation in landfill covers could be linked to the two main types of methanotrophic bacteria, but not in an easy-interpreted pattern (Borjesson et al. 1998).
Two forms of CH$_4$ oxidation are recognized in soils (Bender and Conrad, 1992, 1993). Methanotrophs use CH$_4$ as only a carbon and energy source. O$_2$ availability is the main factor limiting their activity. The first form, known as “high affinity oxidation”, occurs at CH$_4$ concentrations close to that of the atmosphere (<12ppm). This form is apparently ubiquitous in soils that have not been exposed to high NH$_4^+$ concentrations (Le Mer and Roger, 2001). The second form of oxidation, known as “low affinity oxidation” occurs at CH$_4$ concentrations higher than 40ppm. It is performed by bacteria called methanotrophs and is considered as methanotrophic activity *sensu stricto* (Le Mer and Roger, 2001).

Cultivable methanotrophs responsible for “low affinity oxidation” occurs in all soils with a pH higher than 4.4. CH$_4$ oxidation in methanogenic environments (rice fields, peat soils, landfills etc.) is a low affinity. CH$_4$ concentration in the water of the first centimeters of a rice field soil may reach 110 ppm (Conrad and Rothfuss, 1991) and that in the air of drained rice soil is often higher than the 11 – 45 ppm threshold established for a low affinity activity (Bender and Conrad, 1992).

Values are distributed within two large groups. Those corresponding to methanotrophy of high affinity, measured in upland soils, range from 0 to 1.7 Kg CH$_4$.ha$^{-1}$.da$^{-1}$. higher values were obtained in forest soils, lower values were obtained in cultivated soils (table 3.3) (Le Mer and Roger. 2001). Among upland soils, forest soils are probably the most efficient CH$_4$ sink. Their higher methanotrophic activity may be attributed to a stimulation by a significant methanogenic activity if litters
Table 3.3 Methanotrophy in various soil types (g CH$_4$.ha$^{-1}$.d$^{-1}$)

<table>
<thead>
<tr>
<th>Environment</th>
<th>No. of data</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivated soils</td>
<td>13</td>
<td>0.00</td>
<td>866</td>
<td>5.5</td>
</tr>
<tr>
<td>Grassland soils</td>
<td>7</td>
<td>1.75</td>
<td>485</td>
<td>6.5</td>
</tr>
<tr>
<td>Non-cultivated upland soils</td>
<td>6</td>
<td>0.10</td>
<td>228</td>
<td>8.3</td>
</tr>
<tr>
<td>Forest soils</td>
<td>17</td>
<td>0.16</td>
<td>1659</td>
<td>9.9</td>
</tr>
<tr>
<td>Wetland soils</td>
<td>9</td>
<td>0.00</td>
<td>7.10$^5$</td>
<td>172</td>
</tr>
<tr>
<td>Upper soil layer in covered landfills</td>
<td>3</td>
<td>7.10$^4$</td>
<td>1.7.10$^6$</td>
<td>4.5.10$^5$</td>
</tr>
</tbody>
</table>

In the wetlands, methanotrophs develop link to the oxidized soil layer, in the aerobic rhizosphere of plants possessing an aerenchyma, and inside the roots and the submerged part of the leaf sheaths of the plants (Bosse and Frenzel, 1997). Methanotrophs use CH$_4$ as only a carbon and energy source. Oxygen availability is the main factor limiting their activity. However, a partial CH$_4$ oxidation was reported in marine anoxic sediments (Alperin and Reeburh, 1985).

Methanotrophic bacteria seem to oxidize CH$_4$ most efficiently when they occur in consortia among other bacteria, when they may constitute 90% of the microbial population (Burjesson, et al., 1998). In a CH$_4$ oxidizing consortium isolated from a humisol, the uptake of excess CH$_4$, nitrite and hydroxylamine by accompanying organisms was of great importance for methanotrophic activity (Megraw and Knowles, 1989).

**Relations between methanogens and methanotrophs**

Most of the research work indicates that methanogen and methanotrophs maintain their populations under unfavourable conditions; that is, during drainage and drying-up for anaerobic methanogens and during submersion for aerobic methanotrophs (Le Mer and Roger, 2001). A study where both populations are simultaneously counted in a range of soils, confirmed that
methanogens and methanotrophs were present simultaneously in soils and showed that their densities were positively correlated. The densities of culturable methanotrophs and potential methanotrophic activities were higher than the densities of culturable methanogens and potential methanogenic activities (Escoffier, Le Mer, and Roger, 1998).

**Methane emission**

Methane emission by wetland soils results from CH$_4$ production in anoxic zones, CH$_4$ consumption by methanotrophs in oxidized zones (rhizosphere, lower part of culms, soil-water interface and submersion water), and transfer to the atmosphere, mostly through plant aerenchyma and, at a lower level, through diffusion and ebullition (Figure 3.3).
Methane oxidation in the rhizosphere is quantitatively the most important and varies according to the development stage of the rice plant (Danier van der Gon and Neue, 1996). Methanotrophs are also associated with roots and rhizomes of aquatic plants and their activity is correlated with the oxidizing activity of the rhizosphere (Escoffier et al., 1997). In oxic soils, maximum methanotrophy is usually observed in the lower soil layer (Bender and Conrad, 1992). Singh et al. (2003) observed that methane efflux enhanced by 16% in rice straw during heading stage. The fermented manure (cowdung) decreased the fluxes by 64%, indicating that when the paddy was amended with low C/N ration organic manures (fermented cowdung) the methane emission was mitigated between 58 and 64% at heading stage. On the other hand, rice straw can enhance methane flux because of high organic carbon, which serve as substrate for methanogenesis (Minoda and Kimura, 1996).

The traditional field method for measurement of landfill $\text{CH}_4$ emission is the use of static chambers. They have been placed on the landfill surface with an
open part attached to the surface and the accumulated CH₄ concentration on the closed volume has been measured. The method is simple, but laborious if the total emission rate of a landfill is required. This is due mainly to spatial heterogeneity of the landfill cover. However, the method is suitable only for comparisons between different parts of a site, or for following dynamic changes as governed by climate and other factors. Most of the CH₄ escapes from a few weak parts of the landfill cover, which are mostly difficult to identify and measure. Furthermore, recent investigations have indicated that these “hot spots” move over time (Borjesson et al. 2000). The reasons may be that the intensity of the CH₄ production moves between different parts of a site, depending on the composition of the waster and for how long the degradation has proceeded and on changes in the landfill cover material due to moisture differences. This affects the gas diffusion characteristics and thus gas transport and probably also CH₄ oxidation by bacteria in the surface soil (FRTR). Some work has been done by Hilger (1999), assuming that methane oxidizers are embedded in a thin layer of biofilm coating the soil. This however, showed that the thicker the biofilm the more methane is oxidized. This therefore, can be concluded that extra-polysaccharide (EPS) accumulation may regulate methane oxidation rates in landfill covers (Hilger, Liehr and Barlaz, 1999).

**Methane oxidation**

Microbial methane oxidation is a biogeochemical process that limits the release of methane from anaerobic environments (Hanson and Hanson, 1996). An oxidation of methane or higher, saturated hydrocarbons by sulphate-reducing bacteria or other anaerobes was the subject of controversial observations and discussions (Alperin, 1984; Ivanov, 1983). The thermodynamically, and oxidation of these hydrocarbons would be possible, although in the case of an assumed methane oxidation, the free energy change would be low

\[ \text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O} \quad \Delta G^0 = -16.6 \text{ kJ} \]
Environmental factors that affect methane emission

Factors that affect CH\textsubscript{4} emission by soils are those that affect; gas diffusion in relation with the oxy-reduction level and CH\textsubscript{4} transfer, in particular the water content, the nature of the clays and the type of vegetation;

a. Microbial activities in general: temperature, pH, substrate availability, physicochemical properties of soils, Eh, etc.;

b. Methanogenesis and in particular the competition with denitrification and sulphate-reduction;

c. Methane-mono-oxygenase activity: content on H\textsubscript{2}, CH\textsubscript{4}, NH\textsuperscript{+4}, NO\textsubscript{3}, NO and Cu

Competition and predatory may probably affect methanogenic and methanotrophic populations.

![Diagram](image.png)

**Figure 3.4** Methanotrophs in work fields and natural gas effluents (Daniel et.al., 1996)

Physicochemical properties of soils

Little data is available on correlations between soil physicochemical properties and CH\textsubscript{4} emission. The research done deals with potential methanogenic and methanotrophic activities in rice-field soils, for which major physiochemical properties were studied. Most studies showed negative correlations between (a) methanogenic potential and (b) soil conductivity, chlorine content, clay content and C/N ratio (van der Gon and Neue, 1995). Significant correlations
may be obtained when grouping soils according to their level of CH₄ production in the absence of organic manure.

Methanogenic potential and (b) soil conductivity, chlorine content, clay content and C/N ratio (van der Gon and Neue, 1995). Significant correlations may be obtained when grouping soils according to their level of CH₄ production in the absence of organic manure.

**Oxygen availability**
This is the major factor, limiting methanotrophy. Methanotrophs are prominently found (ubiquitous) in soils, where their densities are strongly affected by the oxidation status of the soil. The importance of O₂ availability was also evidenced where methanotrophy was significant in peat, where gas diffusion is easy, whereas methanotrophy was negligible in compacted clay soils. In submerged soils and freshwater ecosystems, high availability of oxygen, which allows benthic photosynthetic activity, increases the thickness of the oxidized soil layer and thus CH₄ oxidation.

**Organic matter**
The intensity of reduction processes in submerged soils depends upon content and nature of organic matter (OM), the ability of the microflora to decompose this OM, and the availability and nature of electron acceptors. In peat soils, the nature of the OM determined both CH₄ production and consumption, both activities being correlated (Le Mer and Roger, 2001).

Methanogenesis can be viewed as the end of a complex series of trophic interactions in which several groups of bacteria work together to oxidize organic carbon, leading to the production of methane (Chauhan et al., 2004). Hydrogen-utilizing fatty acid-oxidizing bacteria, frequently referred to as syntrophs are secondary fermentors that work in concert with methanogens to oxidize fermentation products such as propionate and butyrate that cannot be utilized directly by methanogens (Bachoon and Jones, 1992; Conrad, 2003),
and therefore, play an important role in decomposition of organic matter under methanogenic conditions. Syntrophs require low hydrogen concentration to ferment these substrates to carbon dioxide, and hydrogenotrophic methanogens are responsible for maintaining low hydrogen concentrations.

The aim of this study was to grow both methanogens and methanotrophs from waste and soil respectively, isolate them and try to identify them using molecular techniques.
Materials and methods

Isolation of methanogenic bacteria

The growth media of methanogenic bacteria have been summarised by Balch et al. (1979) and May and Smith (1981). Mesophilic and thermophilic species are known; with few exceptions a pH value close to neutral is required. Although some species have specific organic requirements for growth. Nearly all methanogens utilise hydrogen and carbon dioxide for growth. In addition to these, formate, acetate, methanol and methylamine can be used as sole carbon source and energy source (Weimer and Zeikus, 1978; Balch et al. 1979; Mah, 1980; Huser et al. 1982). The media used has been mentioned in chapter 2, for both anaerobic and aerobic cultures.

Soil sample (1g) was taken from soil reactor mixed in a flask of 9 ml saline. Ten g of glass beads were added, and shaken vigorously for a minute to remove biofilm. Series of dilution were prepared and spread plated. Media used was NMS solidified with bacteriological agar, the plates were incubated in a 50 : 50 % CH₄ : CO₂ at 25⁰C in an air tight jar.

In soils, compost was added at 15g compost to total soil, mixed and moistened with water. Lastly, cultures isolated from previous isolations were rinsed off the agar and the soil was hydrated with this water at 30%.

The same applies with the leachate from waste bioreactor, that is, 1ml leachate was diluted in 9ml liquid NMS media. 1ml of diluted sample was spread plated and incubated in an anaerobic hood at 35⁰C.

There were different treatments done to waste to increase microbial cultures and therefore, increase methane production. The first treatment on waste was to add 300ml sewage in the waste reactor. The duplicate run, cow-dung was added to the waste at 10% of waste mass. That is 45g of cow-dung, was added in waste. In soils 10% of compost was added, and secondly, the soil was hydrated using isolated cultures.
Characterisation of anaerobic (methanogenic) and aerobic (methanotrophic) bacteria.

Molecular tools were used to identify the bacteria isolated. The primers were bought from Inqaba with the primers Met 87F; Met 86F and Met 1340R used from Huang et al. (2003) and Murell et al. (1998). Others were Archeal primers 27F and 1492R Wise et al. (2001). The isolates were treated in chloroform and boiled for 20 minutes. The supernatant spun down, master-mix added as in chapter 2 method.

Gas samples were collected as previously (pilot study and gas production/oxidation [chapter 2]) and sent for analysis.

Statistical analysis was used to evaluate the viability of bacteria for methane production and oxidation in soils (see chapter 4).
RESULTS

Graphical presentation of anaerobic (methanogenic) microbial counts

**Graph 3.1:** Methanogenic counts in original waste

**Graph 3.2:** Methanogenic counts in waste and sewage added
Graph 3.3: Methanogenic counts in waste, sewage and cow-dung added

Graphical presentation of aerobic (methanotrophic) bacteria isolated from soils

Graph 3.4: Methanotrophic (aerobic) counts in soil
Aerobic count from soil and compost

Graph 3.5: Methanotrophic counts in soil and compost

Aerobic counts from soil, compost and purified cultures

Graph 3.6: Metranotrophic counts in soil, compost and isolated cultures
Figure 3.5: Isolated cultures from waste (a) and soil (b). Some of the stained colonies (c and d) waste and soil respectively. Soil sample from the reactor. At the start of experiment the soil grew fungi (e and f) which disappears as more landfill gas was generated.
Wet mounts of bacteria from leachate (a) and soil (b)

Leachate in week 1 (c) week 2 (d) and week 6 (e). Comparison of leachate produced in subsequent times.

**Figure 3.6** Original volume of waste before degradation
(a) change in colour of waste during degradation

(b) drop in volume after degradation

**Figure 3.7** Change in colour and volume during waste degradation

Scanning electro micrographs of isolated bacteria from soil and leachate

(a) Methanogens from leachate

(b) Methanogens from leachate

**Figure 3.8** Methanogens from leachate
Figure 3.8 Mucus (ESP) produced by soil bacteria

DNA isolation for methanogens and methanotrophs identification

Figure 3.9 Met 27F primer (c) + E. coli control (d)
Bacterial observation
Some bacterial isolates were found to produce slimy colonies especially on the soil colonies (fig. 3.5 b). The isolates have cocci and short rods, most stained gram negative (fig. 3.5 c and d).

Having said that, the numbers varied with introduction of different treatments. The separate treatments supposedly introduced more bacterial numbers in the system. However, this was not the case with sewage. Although the conditions were not changed, addition of sewage averaged the counts to 7.5 log cfu/ml. Maybe the low levels of methane were not enough to sustain the population growth.

Leachate observation
It was further observed that there is some fermentation taking place in the leachate as there were bubbles forming on the surface. This is further confirmed by the presence of yeast in leachate (figure 3.8 [a and b])

Figure 3.10 Darker leachate showing surface layer and bubbles forming (fermentation)
Discussion

The purpose of this study was to investigate the methane oxidising capabilities by bacteria from soil. The ability of microorganisms to oxidize methane has been studied and documented. Sohngen (1906) first isolated an organism capable of growing on methane as a sole carbon source. These types of organisms are difficult to isolate and grow in pure culture under methane atmosphere (Rocco et al. 1981).

Methane generated by methanogenic bacteria within sanitary landfills has been well documented and has been implicated in several fires, explosions and deaths (First et al. 1966. Martyny et al. 1979). These problems have made it necessary for governments to determine the amount of methane in and around closed sanitary landfills, a very difficult task because methane content in soil varied erratically with time and conditions, for example, temperature, pressure moisture content and organic matter in soil (Davis, 1967). The soil analysis indicate that the bacterial numbers of methane oxidizing bacteria do not vary as much as the methane concentration produced. This was indicated by Study done by Rocco (1981) where he showed that the methanotrophs numbers do not vary with methane concentration except when methane reaches 50% concentration where the flow is hydrodynamic (gas saturation in water and moves the water upwards to lesser concentration). Smirnova (1971) and Nesterov et al. (1977), showed that, these bacteria are sensitive to high concentration of oxygen which, although is needed for oxidation to occur, prolonged exposure will affect these bacteria in soil. Some bacteria produces exopolysaccharides (EPS) which in return reduces methane permeability. Although moisture is needed for oxidation to occur, the EPS increases air travelling resistance therefore, poor oxidation (Hilger et al., 1999: Hummer and Lechner. 1997).

From the data collected, it has seen that the counts increase with exposure time. The counts from waste reactors averaged 5 log cfu/ml in the first week increasing gradually to log 8cfu/ml in 6 weeks. This is related to the decaying of the waste. As the waste decayed, bacteria increased in numbers. The first
week of decaying is aerobic due to the presence of oxygen during closure. As the waste matured the oxygen got depleted and the reactor turned anaerobic. Therefore, the cultures isolated after two weeks, were presumed to be anaerobic which meant that they could be methanogens as this was seen with increase in methane production. The increase in bacterial numbers can be explained as thus, the bacteria present in sewage could not compete when simple sugars are produced during degradation. This is also seen in leachate produced. In the first week, the leachate is straw yellow and as the waste gets older or more degradation occurs, it turns to be dark (figure below and fig. 3.6 (c,d and e)). It was observed that there was activity occurring in the leachate as there were bubbles on the surface of the leachate. This to some extent the leachate was further degraded. This however, was not seen in the results as the volatile fatty acids were fluctuating within the experiment period. There is no conclusive data on the fatty acids production and degradation.

It was expected that the addition of sewage and cow-dung would increase the bacterial numbers significantly which would shorten the decaying time and increase gas (methane) production, because they habitated anaerobic cultures. But, it was found that they did not have any effect on gas production although the numbers were not significantly shifted. The gas was therefore, related to the composition of the waste and the time taken to decay it. The gas concentration was not affected by all these treatments.

Figure 3.6 (c, d and e) is comparison of leachate at different times in experiment. There is colour change as the leachate gets older. It is straw yellow in the first week and turns reddish-brown in two to three weeks running the experiment. Lastly, it turned blackish-brown in the last week (week 6).

This then, can be due to some metal degradation which lowers the pH resulting in differentiation of electron transfer. The pH was controlled by adding sodium hydroxide in leachate, this maintained the growth condition of methanogens as they die in lower pH, resulting in the reactor becoming sour.
In soil environment, the bacteria seem to oxidise the gas (methane) as expected to combat emissions which cause greenhouse effect. The bacterial numbers were slightly increased with different treatments. The original counts were averaging log 6, 4.5 and 6 cfu/ml, in garden soil, roadside and veld soil respectively. The average was taken on six week period. Addition of compost gave a log 2 cfu/ml increase in all soils. Looking at the results, this showed more oxidation methane. Addition of compost increased the quality of soil to manage emissions of the gas. Lastly, there was another log increase of bacterial numbers when adding isolated cultures. However, the increase was dominant in the first three weeks and later stabilised. The soil grew fungi (fig. 3.5 e and d) on the surface during the first run of the experiment. The fungi disappeared as the soil was more exposed to the landfill gas. The soil was reused in the alternative experiments to decrease the stabilisation time. Although the soil was reused, the same procedure was followed that is, air drying after every run and re-hydrating with 135 ml water. In the aerobic reactor (soil) the bacterial numbers increased slightly due to addition of compost and isolated cultures. However, as the methane concentration was little it is presumed that the methanotrophic bacteria isolated was type I which has high affinity to less concentration of methane.

The isolated colonies varied in morphology. But as they were purified, they changed their colour and growth morphology. Prolonged sub-culturing also changed their gram staining and some mucoid colonies did not develop the mucoid surface when grown in pure cultures.

The bacterial community appears to be sensitive to substrate changes in the environment. This community change varies with subtle environmental change. The bacterial numbers did not change with depth on the soil, but do change with temperature change as the waste gets older. However, (Jakel, Thummes and Kampfer, 2005) showed that methane production increased with temperature. Due to high microbial activity the easily available organic compounds are converted into biomass and heat. The temperature rises dramatically, as generally observed in waste heaps (Jakel and Kampfer, 2003). This high temperature is maintained due to periodic turning or use of
controlled air flow (during compression). The heat dissipation at the surface explains the decreasing temperature from the heap centre to the surface. After easily degradable compounds are metabolised, the temperature is decreased because heat dissipation surpasses the heat production and the number on bacterial stabilises. Jakel et al. (2005) observed that methane oxidation was at maximum between 45 – 55 °C. This proves that at high substrate concentration and under certain environmental conditions, a moderate thermophilic methanotrophic community can be developed. This was surprising as methane solubility in water decreased with increasing temperature and thus limits methanotrophic growth (Tratseko and Kmelinina, 2002). Addition of compost increased methane oxidation potential in landfill cover soils to which it was added (Humer and Lechner, 1999; De Vissher et al., 2001)

Production of methane is carried by a consortium of microorganisms present in soil, cowdung and farmyard manure. The organic matter present is degraded to organic acids which are finally metabolised to methane (Zeikus, 1997; Steiner et al., 1987). Addition of cowdung/farmyard manure increased methanogenic population and adds required nutrients for methane production, although this was not the case in this study. Micronutrients like Fe, Mn, Zn and Ni are present in cowdung/farmyard manure all essential for the growth and metabolic activities of the methanogens (Hoban and Vandenburg, 1979; Jarrel et al., 1987; He et al., 1989).

Molecular ecological studies have adopted two separate approaches for the identification of methanotrophs in the environment. The first indirect approach relies on enrichment and isolation with subsequent characterisation of methanotrophs. The second direct approach relies of polymerase chain reaction (PCR) technology and the use of functional gene probes (figure below)
Figure 3.11 A strategy for the molecular ecological analysis of environmental sample. Murrell, et al. (1998)

Whittenbury and colleagues isolated and characterised over 100 methanotrophs isolates and grouped them into five proposed genera. Application of molecular biology techniques to methanotrophs has been aided considerably by the sequencing of 16s rRNA genes from a number of methanotrophs and methylotrophs reviewed by Hanson and Hanson, (1996). Most of the work done on methanotrophs is on sMMO gene.

The reactor might not have totally finished acidogenic stage although some methane was produced, hence low concentration of methane. On the soil samples, there are methane oxidising bacteria which are not methanotrophs. Therefore, oxidation occurred but not because of methanotrophs presence but due to the presence of other bacteria. They found out that methanogens population was less in leachate which is another reason for less DNA in samples as sample was collected in leachate. In our study we saw yeast from scanning electron microscope (SEM). The physico-chemical parameters of
the leachate (little concentration of organic pollutants) indicate that the waste stabilization process has occurred.

Concerning methanotrophs, high concentration (production of methane) of methane had to occur to produce good numbers of methanotrophs. Due to their different kinetic parameters (e.g. km value), methanotrophs would be favoured under conditions in which a high input of methane would lead to accumulation in numbers and thereby decreasing competition with other methane oxidisers which are not archaea.

In regard to cell lysis and DNA extraction are especially worth considering since methanogens have different outer cell layer (Balch, Fox, Magrum Woese and Wolfe, 1979) that might lead to difficulties in nucleic acid extraction.

To conclude, there is degradation occurring as the waste volume decreased (fig. 3.7 a and b). The mass of waste decreased but when left for longer periods the fungi grew and increased the dry mass of the waste. The general feeling is that, although the waste used decreased in volume (figure 3.7) there was still organic matter left to sustain other growth which increased the biomass resulting in mass gain.
Future prospects

While molecular microbial ecology is being used with considerable success, there are limitations to these techniques (Lidstrom, 1996), including DNA extraction and PCR bias. Increasing database of methanotroph DNA and RNA sequences enables a variety of molecular ecological techniques to be applied directly to these bacteria. Some work has to be done on methanotrophs in environments such as peat bogs, agricultural and forest soils, effects of short and long term ammonium fertilizers on the capacity of these soils. Which organisms in soils are responsible for methane oxidation at low temperatures and low concentration of methane. The so called “high affinity methanotrophs”.

In this study, the experiment could be left for a longer period to see any effect of prolonged degradation in relation to concentration of gas produced. Further studies and better techniques (e.g. DGGE technique for population identification) can be coupled with methane oxidation measurements, this can allow the detection and isolation of methanotroph with unique physiological and biochemistry from the natural environment.
CHAPTER 4

Methane production and oxidation in landfills in relation to soil properties
Abstract

Methane is one of the by products formed in anaerobic environments. This methane produced is known to cause environmental problems in relation to global warming. In this chapter, we studied the effect of soils on methane oxidation. The soils compared are three; garden soil which has compost rich in minerals for the plants, roadside (sandy) was used for paving and veld (uncultivated soil dug about 30 cm from the surface to eliminate dead plants organics). The landfill soil was collected from Springfield landfill and it was used as a control to monitor the oxidation effect of methane. It was observed that the soils vary in their coarse properties. The stones vary with soil quality, whereas the finer particles are almost the same. This helps to keep soils together as in bulk density and plasticity. There are other factors that are seen to influence the oxidation of methane in soils. Porosity, as one of the factors enables the emission of gas into the atmosphere. The bigger the particles the more porous it becomes hence the more emission of gas.

It was observed that the veld soil had more oxidative power as it compressed more than other soils hence less air spaces. Moreover, it is seen to hold moisture more than the two soils which emission is dependent on moisture content of the soil. Although sandy soil has some clay properties in it, it was concluded that it dries faster and is prone to wind blowing. The garden and sandy soil do not compact well which might cause land slide and expose the waste that we are trying to control (dispose).

The soils were exposed to methane generated from the simulated landfill in the lab. It was found that there is some oxidation of methane by all different soils. Although the methane generated was less that expected (50%) we managed to get at the most (8%), the soils oxidised methane to 4%. Some treatments were introduced to soils, e.g. addition of compost and hydrating the soil with isolated cultures. The compost addition proved to reduce methane even further to 0.2%. Methane oxidation has already reached 0.0% in some soils varying with time (weeks) therefore; addition of isolated cultures
confirmed a 0% methane emission. The difference between the soils was negligible therefore can be concluded that there are methanotrophs naturally occurring in soils which is why the soil acts as a sink for atmospheric methane.
Soil

Introduction

There is an urgent need to possess better knowledge on the soil system in order to practice sustainable and healthy management, to protect the soil itself as well as the entire environment including hydrosphere and biota (Doran and Jones. 1996). One of such possibility is through management of soil biophysical properties. In order to do it, a classification of soil properties and processes from the physical, chemical and biological point of view was proposed. An example of the effect of soil biophysical properties of the efficiency of biological CH$_4$ oxidation in the landfill re-cultivation soil layer constituting a methanotrophic biofilter reducing the amount of CH$_4$ emitted to the atmosphere was studied (Stepniewski, et al., 2002). Such soil layer usually cover municipal solid waste in closed landfill.

Improvement over this in which each day’s waste deposited is covered with a layer of soil (U.S. Department of Health, Education and Welfare. 1970). After completion of the landfill, the site becomes usable for recreation and eventually for construction. This simple and inexpensive disposal technique, however, has several disadvantages. The limited number of suitable disposal sites available in urban areas is rapidly becoming filled, necessitating longer hauling of the solid waste to more distant sites. The organic content of the landfill undergoes slow, anaerobic decomposition over a period of thirty to fifty years. During this period, the landfill slowly subsides, and methane (CH$_4$) is produced. Premature construction of the landfill site may result in structural damage to the buildings and explosion hazard due to CH$_4$ seeping into basements and cellars. CH$_4$ seepage may also damage planting on the disposal site.
Soil
Lithosphere habitats occur as land masses, consisting of rocks and soil and sediments. The soil, which arises from weathering of parent rock materials, is by definition capable of acting as a habitat for biological organisms (Brady, 1984). The weathering of rocks that results from physical, chemical and biological forces reduces rock first to regolith (rock rubble) and then to soil (Atlas and Bartha, 1993). It is then, divided into layers (horizons) as the soils gets older or develops into different categories.

Soil horizon
When the soil forms from dead plants, a series of distinct horizons develops, as a result of the weathering process (Brady, 1984). The O-group (organic horizon), develop above the mineral sink and contain the soil organic matter known as the humus; these horizons are formed from plant and animal materials deposited on the surface. The O-horizon is divided into an O₁ horizon, where the plant and animal forms are recognisable, and O₂ horizon, where the plant and animals have decayed to a point of no recognition. The A-horizon or (eluvial), is a mineral horizon that lies near the soil surface. The A-horizon is characterised as a zone of maximal leaching of silicate clays, iron oxides and aluminium oxides, and an A₃ horizon is a transition of underlying B-horizon. The B or (illuvial) horizon is where deposition has taken place and there is a maximal accumulation of materials such as iron oxides, aluminium oxides and silicate clays. The combined A and B horizons are known as solum. Biological activities do not greatly affect the C-horizon, beneath the solum; the C-horizon may contain accumulation of calcium and magnesium carbonates. Beneath the C-horizon lie the regolith and bed rock.

Soil texture
Soils may also be classified by the relative portions of clay, silt and sand particles they contain. Soils that are dominated by one size class of particles are named according to that class, that is, as sand soil silt soil or clay soils. Soils that are not dominated by any specific particle size are called loams. Intermediate classes of soil texture are recognized for an example, sandy
loam soils. Soil texture is an important descriptive measure of the microbial habitat, as it describes in part the spatial interactions that can occur among microorganisms occupying that habitat. Soils are frequently described by their mixture and location, such as, a New Jersey sandy loam or Georgia clay. When considering soil particles as habitats for microorganisms it is also important to consider the nature of the clay particles (Marshall, 1980). Clay colloids differ in their physical and chemical properties (table 2.1).

**Table 4.1: Selected properties of major clay colloids**

<table>
<thead>
<tr>
<th>Properties</th>
<th>Montmorillonite</th>
<th>Illite</th>
<th>Kaolite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (µm)</td>
<td>0.01 – 0.1</td>
<td>0.1 – 2.0</td>
<td>1.0 – 5.0</td>
</tr>
<tr>
<td>Surface (m²/g)</td>
<td>700 – 800</td>
<td>100 - 200</td>
<td>5 - 20</td>
</tr>
<tr>
<td>Cation exchange capacity (meq/100g)</td>
<td>80 – 100</td>
<td>15 - 40</td>
<td>3 - 15</td>
</tr>
</tbody>
</table>

The differences influence how many and what type of microorganisms can occupy the particular soil habitat (Hattori and Hattori, 1976). Organic matter (humus) is an important constituency of soils. It acts as a nutrient reserve, increases ion exchange capacity, and loosens the structure of the soil. When the virgin lands are put to agricultural use, their humus content decreases for the first forty to fifty years, eventually equilibrating at a much lower value.

The mechanism or granular composition (texture) of soil is the number and size of its mechanical elements or particles after all the compounds holding them together have been destroyed. There are two soil fractions, namely fine earth particle sizes under 2 mm and coarse or skeleton earth, which includes particles above 2 mm in international classification or under 1 mm in Kachiskii classification, and coarse or skeleton earth, which includes particles above 2 or 1 mm respectively (refer to table 4.2).
Table 4.2: Classification of Mechanical Elements of Fine Earth

<table>
<thead>
<tr>
<th>Size of particle</th>
<th>Name of fraction</th>
<th>Size of particle</th>
<th>Name of fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 – 0.2</td>
<td>Coarse and medium sand</td>
<td>1 – 0.5</td>
<td>Coarse sand</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5 – 0.25</td>
<td>Medium sand</td>
</tr>
<tr>
<td>0.2 – 0.02</td>
<td>Fine sand</td>
<td>0.25 – 0.05</td>
<td>Fine sand</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.05 – 0.01</td>
<td>Coarse silt</td>
</tr>
<tr>
<td>0.02 – 0.002</td>
<td>Silt</td>
<td>0.01 – 0.005</td>
<td>Medium silt</td>
</tr>
<tr>
<td>Under 0.002</td>
<td>Mineral colloid (clay)</td>
<td>0.005 – 0.001</td>
<td>Fine silt</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Under 0.001</td>
<td>Clay</td>
</tr>
</tbody>
</table>

Soil differentiation into fractions of different sizes is based on experimental data on changes in the physical properties of diverging in their size, porosity, moisture capacity and water permeability. Thus, sand can readily let water pass; consequently, it can not properly retain its clay particles, on contrary, clay filter water very slowly and absorbs it in large quantities.

Soil texture is determined by the physical and physicochemical properties of soil. Soils with sandy or loamy sand texture are mainly made from quartz particles. Sandy soil, have non-capillary rocks and loose structures. They are thus poor water retainers. They are poor in nutrients, therefore, need additional clay and mineral and organic fertilisers. Soils with clay texture have a densely packed structure and capillary pores. They retain large qualities of water due to small diameter capillaries, therefore, get plugged and swell. The composition of clay minerals is important in that it governs the formation of micro and macro-aggregates and the degree of swelling. Although they have unfavourable physical properties; clay soils can contain large quantities of nutrients. To improve their physical properties large amounts of organic substances are frequently applied for loosening or sanding.
Soil structure

In order to understand soil processes and visible profile differentiation, it is necessary to consider the various phases existing in the soil. Thus, in the gas phase, the biophysical fluxes concern CH$_4$, CO$_2$, O$_2$, NO$_2$ etc... There are two mechanisms of gas transportation in soil, that is, diffusive flow in which the driving force of the flux are the concentration changes in space and time ($\delta C/\delta x$, $t$), and mass flow, where the driving force is the pressure change in space and time ($\delta p/\delta x$, $t$).

The diffusive flow is described by the first Fick’s law:

$$ F_d = -D \frac{\Delta C}{\Delta x} $$

Where $F_d$ is the volumetric gas flow through a unit surface and during a unit time, $\delta C/\delta x$ the concentration gradient of the diffusing gas and, $D$ the gas diffusion coefficient characterising the soil medium.

Figure 4.1  Soil structure as a function of colloid state

A – Granular structure
B – Separate-particle structure
1 – Flocculated colloid
2 – Dispersed colloid

(Ducanfour; 1970)
The aggregation of soil depends on its composition, content and soil colloids (particles under 0.0001). The more a soil can coagulate into a larger particles, the more expressed is its aggregation (fig. 4.1). A soil’s structure greatly influences the migration of substances, the water properties of soils, and their water accumulation.

**Soil porosity**
The total porosity is the sum of the non-capillary porosity, which is the volume of the pores filled with air after atmospheric precipitation has filtered through and capillary porosity, which is the volume of pores that retain water due to gravity forces. The capillary porosity corresponds to the volume of water that fills the capillaries (or pores) of a soil when it is saturated from beneath. To non-capillary porosity is the difference between the total and the capillary porosities (Kachinskii. 1986)

**Soil chemistry**
With its relative content of individual chemical elements, soil is in many ways similar to the lithosphere (table 4.3)

**Table 4.3 Elements in soil**

<table>
<thead>
<tr>
<th>Element</th>
<th>Lithosphere</th>
<th>Soil</th>
<th>Element</th>
<th>Lithosphere</th>
<th>Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>47.20</td>
<td>49.00</td>
<td>Mg</td>
<td>2.10</td>
<td>0.60</td>
</tr>
<tr>
<td>Si</td>
<td>27.60</td>
<td>33.00</td>
<td>Ti</td>
<td>0.60</td>
<td>0.46</td>
</tr>
<tr>
<td>Al</td>
<td>8.80</td>
<td>7.13</td>
<td>H</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>5.10</td>
<td>3.80</td>
<td>C</td>
<td>0.10</td>
<td>2.00</td>
</tr>
<tr>
<td>Ca</td>
<td>3.60</td>
<td>1.37</td>
<td>S</td>
<td>0.09</td>
<td>0.08</td>
</tr>
<tr>
<td>Na</td>
<td>2.64</td>
<td>0.63</td>
<td>P</td>
<td>0.08</td>
<td>0.09</td>
</tr>
<tr>
<td>K</td>
<td>2.60</td>
<td>1.36</td>
<td>N</td>
<td>0.01</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Ronald and Richard (1992)

Compounds of iron and aluminium in tropical soils lose their mobility and begin accumulating due to the solution and washout of other elements. In the arid tropics, on the contrary, all elements, except silica, reveal minimum
mobility. Therefore, the chemical composition of soils, as a rule, is much diversified. This diversity can result from the composition of rocks which formed the particular soils; from the climatic conditions that may accelerate or slow the rates of soil weathering and soil evolution; from the nature of vegetation yields acidic products which differ in their quantities and qualities.

**Biological factors of soil**

The soil has abundance of bacteria, however, the surface area which has viable microbes is about $10^{-6}\%$ (Crawford, 2004). These microbes are important in agriculture, waste management and water industry. The physical structure of soil is likely to have an impact on diversity of microbial environments. Older geological feature (sandstone and granite) differs with younger soil system in that the latter shows pore structures that are not defined by nature of their chemical material but by life itself and thus, experience significant biophysical and biochemical changes over relatively short spatial and temporal scales. The capacity of soil to absorb chemicals from solutions (the cat-ion exchange capacity) regulated the movement of pollutants to the atmosphere and waterways. It controls the absorptive properties of nutrients through interaction between cat-ions and clay particles (Young and Crawford, 2004). An important step toward integrating the biology and physics of the soil ecosystem is to allow microbes to be observed in their natural physical habitat. Foster (1988) has investigated the organization of soil nature of some organisms allows physical adhesion between organisms and charged clay platelets.

Compared soil barriers are components of essentially all caps placed on closed waste disposal sites. The intended function of soil barriers in waste facility caps include restricting infiltration of water and release of gases and vapours, either independently or in combination with synthetic membrane barriers, and protecting other man-made or natural barrier components. Review of the performance of installed soil barriers and of natural processes affecting their performance (Suter et al. 1993) indicates that compacted soil caps may function effectively for relatively short periods (years to decades),
but natural physical and biological processes can be expected to cause them to fail in the long term (decades to centuries).

Although the atmospheric concentration of CH$_4$ has more than doubled since pre-industrial times, its rate of increase has slowed considerably over the last few decades (Steel et al. 1992). It has been suggested by Prim et al. (1992) that one of the major cause of the slowdown is the increasing magnitude of CH$_4$ oxidation by methanotrophic bacteria is in the aerobic zones of soils. Hence Tamai et al. (2003) note, “this biological sink plays in important role in modulating global warming”. It can be appreciated that carbon dioxide (CO$_2$) induced global warming, would produce two biological-mediated negative feedback to counter the increase in temperature: a warming-induced increase in CH$_4$ uptake from the atmosphere by essentially all soils. An increase in soil CH$_4$ uptake from the atmosphere that is produced by landfills increases in plant litter C/N ratio (Tamai et al. 2003).

**Physical processes influencing barrier integrity.**

Landfill capping is a containment technology that forms barrier between the contaminated media and the surface, thereby, shielding humans and the environment from the harmful effects of its contents and perhaps limiting the migration of the contents. A cap must restrict surface wear infiltration into the contaminated subsurface to reduce the potential for contaminants to leach from the site. Cap performance varies, depending upon its function and where it is used, for an example, compacted clay liners are effective if they can retain moisture contents, but they are susceptible to crackling of the clay material is dried out, several alternative design enhancement are being tested. ([http://www.sandia.gov/subsurface.factshts/ert/alcd/pdf](http://www.sandia.gov/subsurface.factshts/ert/alcd/pdf))

The compaction of soil barriers in shallow burial and remediation sites is affected by physical conditions such as temperature cycles, for example; diurnal which is hourly changes, precipitation and evapo-transpiration, occurring in few days to months and lastly annual which is seasonal. The hourly change varies the soil temperature gradient to the roots which influences root water uptake. The wet and dry cycle changes soil water matrix
and the seasonal changes have wider range of temperature variation. This have a smaller effect as the conditions are fairly constant for a period of time (Smith et al. 1994).

Normally, a landfill cover requires several layers or clay, soil and plastic liners that are sloped to allow precipitation to drain off the top. Clay layers are at an effective mechanism in arid climate because clay tends to dry and shrink and crack in dry weather creating potential pathways for water to migrate to landfill materials. A better cover for landfills in arid climates is an evapo-transpiration cap, which is made of local and native vegetation. When it rains or snows, the soil layer acts like a sponge, holding all the moisture. The moisture then evaporates from the layer’s surface or transpires through the vegetation. During transpiration, moisture is pulled out of the soil and up through the shallow root systems of the vegetation to its leaves, where it is released onto the atmosphere. Therefore, a cover promoting a combination of both evaporation and transpiration—evapo-transpiration—moves moisture up instead of down, naturally limiting percolation to landfill materials (O’Neil. 2000).

Definition of processes and functions on soils as the basis for further soil management strategies.

In order to define biophysical aspects of soil processes more precisely and to discuss in more detail various consequences of soil management, it is necessary to determine strict categories of data and dimensions related to the soil. The capacity properties are related to the soil volume, e.g. they define the amount of soil mass over volume (bulk density) or the amount of water per volume (volumetric water content), but not the arrangement of the mass (soil particles and soil water) in the volume. This means that they cannot be used for the definitions of any site soil specific process such as conductivity, diffusivity and permeability.
It should be noted that, if the soil CH$_4$ oxidation is of biophysical character as it contains a physical term related to the properties of the medium (diffusivity, conductivity and permeability) and the gradient of the transported agent (concentration C, pressure P and temperature T) being the resultant effect of the existence of a biological sink/source term (oxygen uptake, CH$_4$ production and water uptake). Main groups of soil properties according to the proposed classification presented in figure 4.2.

**Soil classification**

Comprise biological, chemical and physical as well as biophysical, biochemical and physicochemical parameters. The physical properties are further subdivided into capacity and intensity properties (Stepniewski *et al.* 2002).
Figure 4.2 Soil analysis classifications

Managing soil biophysical properties for environmental protection (Georg and Werner, 2001)
Generation and uptake of other gases in soil

Soil is a sink and source of other gases such as nitrogen (N\(_2\)), CO\(_2\), Dinitrogen oxide (N\(_2\)O) and CH\(_4\), which are of high importance from the environmental point of view. All the fluxes of these gases at the soil atmosphere interface are of biophysical character. N\(_2\) is produced in the denitrification process, but it is also fixed by some microbes; both processes being strongly depended on the O\(_2\) status in soil. In the case of N\(_2\)O and CH\(_4\), which are considered greenhouse gases, soil can produce and absorb these gases dependently of the redox and pH conditions. This capability can be used to control inputs of these gases onto the atmosphere, to modify absorption of these gases from the atmosphere and to construct soil biofilters for gases.

More precise description of functioning of particular environmental applications of soil as a biofilter is beyond the scope of this paper. Consequently, only the example of CH\(_4\) oxidation by bacteria will be presented here.

Landfill cap enhancement

The purpose of landfill cover enhancement is to reduce or eliminate contaminant migrating (e.g. percolation). Water harvesting and vegetation cover are two ways for landfill cover enhancements. Water harvesting uses run off enhancement to manage landfill waste water balance. This enhancement can be achieved by simply covering landfill cover with metal rain gutter placed parallel to the slope. Vegetation reduces soil moisture via plant uptake and evapo-transpiration. Plant cover also limits soil erosion. Vegetative cover is more stable because it emphasizes use of natural materials and configurations, which implies longevity (Remediation technologies; version 40 landfill cap enhancement).
**Purpose and function of cover system**
Various types of covers are caused to close municipal solid waste landfills, hazardous waste landfills and other types of hazardous waste sites. The primary purpose of a landfill cover is to isolate waste materials from the environment by minimizing the infiltration of surface water, preventing human and animal contact with the waste materials, and controlling landfill gases. A landfill cover will also control surface runoff to minimize erosion and adverse impacts of adjacent waterways and properties.

**Alternative covers types**
The cover system depends on its component such as function, environment, applicable regulations and site-specific conditions. Many government and local environmental agencies have criteria for cover systems that are rigid. The most strict guidelines usually govern the design of cover, in most cases, cover systems depend on processes to isolate waste material and they are therefor, known as “Alternative covers”. They vary in structure and configuration and are known as evapo-transpiration (ET) caps. For arid and semi-arid climates, infiltration control caps have been considered. ([http://wwwrtdf.org/public/phyto/minutes/altocov/default.htm](http://wwwrtdf.org/public/phyto/minutes/altocov/default.htm))

**Hazardous waste landfill covers**
The components of a hazardous waste landfill cover consist of a protective cover layer, a drainage layer, a low-permeability layer and random fill overlying the waster. Optional layers include a gas collection system and a biotic barrier. Bacterial covers are placed between cover materials to prevent methane escaping from the landfill, animals and plant are prevented from this to safe guard damage of the biotic barriers. However, they are rarely used. Site-specific physical conditions such as topography, material availability and cover stability affect the design and material selection of the cover components. Some soils have been manually designed e.g. N-Vitro soil (NVS). This is a valuable and versatile product manufactured from a combination of municipal wastewater biosolids and mineral by-products. As a
landfill cover material, it is another high volume product market that has shown much growth over the past few years. Large cost savings are experienced by using NVS instead of expensive top soil. The use of biosolids as landfill cover has been a positive experience in Greenville, South Carolina (www.nviro.com infor@nviro.com). It provides beneficial alternative for managing solids produced by Western Carolina Region Sewer Authority and managing wastes received by Greenville County’s Enoree Landfill.

Pre-design investigation
Prior to preparing a design analysis or plans or and specifications for a landfill cover, it is necessary to conduct pre-design surveys and investigations to fill data gaps. The existing database available from the remedial investigation (RI), feasibility studies (FS) and any other documents must be reviewed before scooping a pre-design investigation.

Field survey and record searches
i) Aerial photography: Historical aerial photographs can be used to preliminary define the mature and extent of a landfill.

ii) Design and operational data: Design and operational information obtained from as-built drawings, specifications, design analyses, and interviews of people associated with the site may help in identifying the nature and extent of a landfill.

iii) Map database figure 4.3
Figure 4.3 Alternative covers that can be used (Picture from Atlas and Bartha, 1992)

**Topographic survey**

There is a strong link between soil biological and physical properties. In fact, most fluxes in soil are of biophysical character e.g. emission. They are connected with the purity of the atmospheric air and of water quality. Better understanding of the inter-relation among biophysical properties on soils offer a possibility of their management towards environmental protection. A possibility of mitigation of CH$_4$ emissions from landfills to the atmosphere through use of soil re-cultivation layer (fig.4.4).
After the existing database has been reviewed, geological investigations can then be scoped.

- **Landfill limits;** it is imperative that the depth of the waste materials and the limits of the landfill or contaminates are to be determined. Surface depressions and stressed vegetation along with historical aerial photographs can also help in delineating approximate landfill boundaries.

- **Existing cover;** material should be evaluated to determine cover thickness, material types and overall conditions.

- **Landfill gas;** empirical methods estimates from soil gas survey can also be used to estimate landfill gas emission rate.

- **Leachate may migrate literally and exit at the surface of the existing landfill.** Drainage to collect this seepage may need to be installed below the landfill cover before it is constructed (fig. 4.5).

- **Ground-water,** it is necessary to define levels of water gradients, flow direction and ground water chemistry in all water-bearing units in the vicinity. The wells are used to determine if the site is contaminating the water. Monitoring well date should be reviewed during the pre-design phase to see if additional wells are needed.

- **Foundation of soils,** samples should be collected to determine specific geotechnical engineering properties (fig. 4.5).
Geotechnical laboratory tests are required to assess the suitability of barrow source and to establish soil properties for use in the stability, settlement and drainage analysis. They include:

- Classification testing which is sieve sand hydrometer analysis and moisture content testing. They are used to select borrow site for cover materials and to design filter and drainage layers.
- Standard of modified proctor to develop compaction for all material requiring compaction.
- Hydraulic conductivity, these are done on all barriers and drainage layer soils when a site is identified and available.
- Density to establish the existing condition of foundation and barrow soils. It is later used to estimate swell and shrinkage potential attributed to excavations and compaction.
- Dispersive clay. This, though not common is used to determine adorability of soil to avoid air erosion and clogging of drainage layers.
- Consolidation measures foundation soil to estimate settlement resulting from placement of the landfill cover.
• Shear strength, this is done to establish stability of cover soil and foundation.
• Direct shear test is conducted to all interface soils to determine interface friction values for use in stability analysis.

Chemical data requirements this is to test for the contaminants of various cover features like landfill gas composition, barrow soil testing, leachate, ground water, landfill limits handling of contaminated materials and disposal, air monitoring and surface and table water. These will show how the soil was before working on it and how much contamination has been brought as means of disposal or containment.

**METHANE**

**Anaerobic digestion**
Anaerobic digestion is a process carried out under non-sterile conditions by a consortium of naturally occurring anaerobic bacteria. This consortium can be developed from bacteria in for instance, faecal wastes or organic matter decaying anaerobically, by providing suitable conditions of anaerobiosis, temperature and feed stock. Once developed consortium is very stable and the digesters are run as continuous cultures for periods of years. Such digesters settle down to a steady state in so far as such can be obtained with a mixed culture carrying out complex series of reactions on an inhomogeneous substrate. Most importantly, anaerobic decomposition products, heavy metals and a variety of hazardous pollutants may seep from the landfill site into underground aquifiers, polluting much-needed urban water resources.

Large amounts of CH₄ are produced during the anaerobic decomposition of organic materials, but this energy resource normally is re-oxidized, as in many aquatic habitats, or lost to the atmosphere, as in most terrestrial habitats (Hauser *et al.* 2001). CH₄ production can be based on the decomposition of
waste materials or on the conversion of biomass produced as a step in the conversion of solar energy to usable chemical energy.

CH$_4$ emission sources associated with agriculture include livestock husbandry (enteric fermentation in ruminants), anaerobic respiration in soils associated with wet rice agriculture especially in the eastern hemisphere and combustion of biomass (wood fuel, harvest residues and cleared forests) (Hellebrand et al. 2003). Many soil organisms consume O$_2$ and produce CO$_2$ and other gases. Depending on soil conditions, some bacteria can also utilize H$_2$, CO$_2$ or CH$_4$ as an energy source. It is estimated that about 50% of carbon from weeds, straw, roots and aquatic biomass is returned back to soil yearly (Neue and Roger, 1993). Particularly in anoxic natural environments such as hydromorphic soils (wetland soils), methanogens produce CH$_4$ and methanotrophs consume CH$_4$ (Conrad. 1989; Conrad.1996; Galchenko et al. 1989). This may result in either the emission or uptake of CH$_4$. The transport of CH$_4$ between atmosphere and soil is usually controlled by convection and diffusion (Striegel. 1993). In the soil, the pore volume the pore structure and the water content are important as they determine the diffusion resistance. Furthermore, the cultivation practice and the cultivars have an influence in the gas exchange between soil and atmosphere and thus on the CH$_4$ flux rates (Ball et al. 1997; Ball et al. 1999). Particularly in forest soils and other non-fertilized soils, CH$_4$ oxidation rates are higher compared to cultivated soils (Keller et al. 1990; Dobbie and Smith. 1996; Smith et al. 2000).

**Methane in agriculture**

The rice plants do not produce methane but their vascular system acts as transport channels of methane dissolved in water (Cicerone and Shetter, 1981; Nouchi et al., 1990). Animal dung produced large amounts of methane. Apart from methanogens being present in cow-dung and farmyard manure, the organic matter present therein is degraded by a consortium of microorganisms of the soil which are capable of producing methane (Stanier et al., 1987). In India (Banik et al., 1995), cow dung and farmyard manure have been used as organic manure even long time ago. The methanogens are still active as some are encapsulated deep in the manure (Nayer and
Conrad, 1990). With monsoon rains or irrigation water, there is a possibility that the soils are enriched with methanogens in cow dung (Hobson, 1982).

Methane in other environments

CH$_4$ is a common constituent on the deep subsurface. As much as 20% of the world’s natural gas resources are estimated to have been generated by microbes (Rice, 1993). Subsurface coal deposits, oil wells, natural gas storage in carbonated shelves, coal swamps, coastal plains, deep sea up swellings and hydrocarbon deposits are a major sources of ‘biogenic’ CH$_4$ (Katelivkova, 2002). Microbial CH$_4$ oxidation is a biogeochemical process that limits the release of CH$_4$ from anaerobic environments (Hanson and Hanson, 1996). The contribution of marine systems to the global methane cycle is generally poorly understood (McDonald et al., 2005). Concentration and oxidation of methane has been determined in many oceanic sites and these have shown that the upper ocean is usually saturated with methane. Ward et al. (1987) and Conrad and Seiler (1988) have shown biological oxidation of methane at the air-sea interface. Characteristics of methane oxidation in a fresh water lake have been previously reported by Kuivila et al. (1988) and Lidstrom and Somer (1984).
Table 4.4: Gas yields from anaerobic digestion wastes

<table>
<thead>
<tr>
<th>Nature of solid waste</th>
<th>Total gas yield (m³/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Municipal sewage sludge</td>
<td>0.43</td>
</tr>
<tr>
<td>Municipal garbage only</td>
<td>0.61</td>
</tr>
<tr>
<td>Municipal paper only</td>
<td>0.23</td>
</tr>
<tr>
<td>Municipal refuse combined</td>
<td>0.28</td>
</tr>
<tr>
<td>Dairy wastes, sludge</td>
<td>0.98</td>
</tr>
<tr>
<td>Yeast wastes, sludge</td>
<td>0.49</td>
</tr>
<tr>
<td>Brewery wastes, hops</td>
<td>0.43</td>
</tr>
<tr>
<td>Stable manure, with straw</td>
<td>0.29</td>
</tr>
<tr>
<td>Horse manure</td>
<td>0.40</td>
</tr>
<tr>
<td>Cattle manure</td>
<td>0.24</td>
</tr>
<tr>
<td>Pig manure</td>
<td>0.26</td>
</tr>
<tr>
<td>Beet leaves</td>
<td>0.46</td>
</tr>
<tr>
<td>Maize tops</td>
<td>0.49</td>
</tr>
<tr>
<td>Grass</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Source: Imhoff et al. 1971

The evolution of CH₄ from anaerobic digesters and other bioconversion processes occurs simultaneously with the evolution of CO₂. The ratios of CH₄ to CO₂ depend on the chemical composition of the substrate and the environmental conditions under which the bioconversion is carried out. Biotechnological processes can, and must, be adjusted to maximize the proportions of CH₄ in the evolved gases. The CH₄ must be trapped and separated from other gases to be a useful energy resource that can supplement and/or replace natural gas as a fuel. This makes it expensive therefore other separation techniques are looked into.

Covers and compaction lead to lower penetration rate for gases onto the waste and thus limit the availability of O₂ (Borjesson et al. 1995). The anoxic conditions result in the development of an anaerobic fermentative population of microorganisms, which is close interaction hydrolyses organic polymers and ferment the hydrolysed matter to products which form the substrates for
the organisms responsible for CH₄ formation. Without compaction or covers, more O₂ is available, which leads to an extended oxic degradation of the waste. This is what happens on the so-called “open dumps”, which is the case for half of the world’s landfills (Moore et al. 1998). Such landfills run a high risk of spontaneous combustion due to heat development during the aerobic degradation. Normally, a steady rate of CH₄ is reached after 80 – 500 days and is then maintained for 10 – 20 years (Moore et al. 1998). The time required for degradation of waste on landfills and the amount of gas formed depends on a number of factors, such as type and amount of waste, water content, compaction, leachate treatment etc. (Farquhar and Rovers. 1973, Rees. 1980). The composition of waste differs considerably between different parts of the world. While the waste in developing countries is dominated by garden material the waste, the waste in industrialized countries is dominated by paper (Moore et al. 1998). Paper has a higher content of degradable organic carbon, corresponding to a higher CH₄ generation potential. The water content is of the utmost importance and the effect can be both suppressive (Grischek et al. 1999) and promotive of CH₄ production in dry waste (Vroon et al. 1998).

Table 4.5 Idealized reaction of bacteria in presence and absence of methanogens

<table>
<thead>
<tr>
<th>Reaction</th>
<th>ΔG°°(kJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>i. Hydrogen used by methanogens</td>
<td></td>
</tr>
<tr>
<td>4H₂ + HCO⁻₃ + H⁺ ↔ 3H₂O</td>
<td>-135.6</td>
</tr>
<tr>
<td>ii. Without hydrogen using methanogens</td>
<td></td>
</tr>
<tr>
<td>Glucose + 2H₂O ↔ acetate + 2HCO⁻₃ + 4H⁺</td>
<td>-191.9</td>
</tr>
<tr>
<td>Glucose + H₂O ↔ propionate⁻ + acetate⁻ + HCO⁻₃ + H₂ + 3H⁺</td>
<td>-282.4</td>
</tr>
<tr>
<td>Glucose + 2acetate ↔ 2 butyrate + 2HCO⁻₃ + 2H⁺</td>
<td>-302.9</td>
</tr>
<tr>
<td>iii. With hydrogen-using methanogens</td>
<td></td>
</tr>
<tr>
<td>Glucose + H₂O ↔ 2 acetate + HCO⁻₃ + CH₄ + 3H⁺</td>
<td>-342.3</td>
</tr>
</tbody>
</table>

Thauer et al. (1977). Calculations are based on H₂ and CH₄ in gaseous state.
Models for prediction of landfill gas (LFG) production

There are many methods of estimating CH$_4$ production from the landfill. To mention some which will not be dealt with in this study are:

- **Static models:** IPCC and other sources, show that CH$_4$ emission was estimated on laboratory results and degradation on few different wastes. This however, has been modified to larger experiments, the model is simple to use but is “static”. The waste that was put in landfill for one year is assumed to be converted to its full potential of LFG and the CH$_4$ produced is assumed to be released the same year. Thus, if the amount of waste put in landfill is increasing, it will have an immediate effect on the model, despite the fact that it may take some years before the CH$_4$ production reaches its maximum. This approach may be desirable from a political point of view, since it offers a strong incentive for the installation of gas recovery systems. In order to adjust the model towards more real conditions, several conversion factors have been added to late versions of the IPCC model. However, these factors have tended to become more difficult to motivate and use (e.g. 1.0 for managed site; 0.8 for unmanaged deep and 0.4 for unmanaged shallow site etc.)

- **First order kinetics:** a decline of CH$_4$ production according to first order kinetics was assumed in 1996 (Aitchson et al.). Similarly, some kind of decline mode has been introduced in other national models e.g. Denmark and Norway (Froiland Jensen. 1999). In Sweden, both an earlier Swedish Environmental Protection Agency (SNV; Montelius. 1997) and in the on-going work at statistics Sweden (Rolf Adolfsson, per. Comm. 2000.02.06) consideration has been given to a decline. It is assumed that the gas production is proportional of organic matter along first order kinetics as described by Gendebien et al. (1992; p.352)

\[
C_t = C_0 e^{-kt}
\]

Where $C_t$ = the concentration of organic matter at time t, $C_0$ = the initial concentration of organic matter, and $k$ = a

indicating the half-life ($=\ln0.5/-k$)
Degradation sequence (Warith and Sharma, 1998)

This is a five stage sequence which assumes homogenous waste, constant age and easily degradable waste. Landfills, however, are complex and highly variable biological system and therefore, a high degree of uncertainty exists in estimation of gas production (Gregory, 1998). Landfill cell will vary in age and composition and will not follow degradation sequence exactly as some organic matter releases simpler sugars faster.

a) Short period of aerobic decomposition as soon as the waste is deposited in the landfill (O\textsubscript{2}) still available. Most easily degraded materials are consumed first and only carbon dioxide is released.

b) First intermediate anaerobic phase, aerobic bacteria declines as oxygen is used and the action of fermentative and acetogenic bacteria increase. Fermentative bacteria hydrolyse organic matter into volatile acids, alcohols hydrogen and carbon dioxide. These are consumed by acetogenic bacteria producing acetic acid and more hydrogen and carbon dioxide.

c) Second intermediate phase, this is the first where methanogenic appear. High volume of methane production is delayed due to competition with sulphate reducing bacteria (SRB) because they consume the same substrate.

d) Steady production of methane. Methane inhibit SRB and the gas (CH\textsubscript{4}) totals 50% of the gas generated in the cell (landfill)(Morgan and Yang, 2001; Warith and Sharma, 1999)

e) Decomposition of most recalcitrant waste, little methane is produced.

Methane emission estimation

Methane emission from landfills is a major contributor to the greenhouse effect. Regulators throughout the world are implementing waste management strategies, policies and regulations aimed at reducing methane emission from landfills. Landfills are the main source of methane diffusing into the atmosphere therefore; it is not easy to measure its emissions. Quantification of emissions either per country or per landfill is essential. Several models to
predict methane emissions originating from landfills have been proposed or are recommended by national governments. The most commonly used model is a first order kinetic model that describes the biodegradation of waste and production of methane. Production and oxidation of methane at the cover enables calculation of emission. The problem emerges when landfill operators has differentiated waste into categories so that the biodegradable waste equation can be applied.

The first order model implies the depletion of carbon in the waste through time (Oonk et al., 1994). Gas formation from certain waste is assumed to decay exponentially in time; represented as:

\[
\alpha_i = \zeta 1.87 \ \hat{A} C_o k_i e^{kt} \quad \text{(Oonk et al.,1994)}
\]

The first order model is based on degraded organic carbon in the waste. For example; the biodegradation of simple sugars can be chemically described as:

\[
C_6H_{12}O_6 \rightarrow 3CH_4 + 3CO_2
\]

\[
180g/mol = 72gC/mol \quad 48g/mol \quad 132g/mol
\]

Therefore, methane production per kgM degraded 48/180 = 0.3m³

\[
CH_4 = 0.75m^3
\]

Methane production per kgC degraded 48/72 = 0.7 \quad CH_4 = 1.87m^3

In order to derive emission based upon the production estimate, a very straight forward calculation is used;

\[
CH_4 \text{ (emi)} = CH_4 \text{ (prod)} - CH_4 \text{ (recov)} - CH_4 \text{ (oxid)}
\]

Digester design

“Anaerobic digestion” is a process carried out under non-sterile conditions by a consortium of naturally occurring anaerobic bacteria. This consortium can be developed from bacteria in, for instance, faecal wastes or organic matter.
decaying anaerobically, by providing suitable conditions of anaerobiosis, temperature and food stock (Hobson and Shaw 1973). Once developed consortium is very stable and the digesters are run as continuous cultures for periods of years. Such digesters settle down to a steady state in so far as such can be obtained with a mixed culture carrying out complex series of reactions on a homogenous substrate.

Organic matter in feedstock (including pollutants) is converted to “biogas”, a mixture of methane (50%) and carbon dioxide (30%). The methanogenic bacteria can utilize only a limited number of substrates, principally hydrogen and carbon dioxide and acetic acid. Higher fatty acids are converted to methane via degradation to acetic acid and hydrogen with, according to chain length, propionic acid which can also be converted to methane (carbon dioxide is formed in some of these reactions) (Hobson et al., 1984).

The maximum growth rates of the methanogenic bacteria are low and methanogenesis is the rate limiting reaction in digestion of dissolved sugars. However, the vegetable feedstuffs of animals have been subject to gastric and intestinal digestion or to microbial digestion in rumen or caecum, or both. Therefore, what remains is lignified and ordered cellulose or hemicellulose which has restricted degradation in the animal. The rate and extent of hydrolysis and subsequent fermentation of fibrous feedstock can be improved by chemicals or physical treatment e.g. alkali or autoclaving which loosens the fiber structure. The final stages take 10, 20 or more days in anaerobic digester, and thus fiber degradation becomes the rate limiting step in the conversion of the feedstock to methane (Houser et al. 2001).

The type of apparatus which can be used for anaerobic digestion is limited by the particular feedstock and by economic considerations. Basically, anaerobic digestion has to be a low-cost process. Anaerobic digesters, like other fermentors, can be run at temperatures suitable for mesophilic (25 – 44°C) or thermophilic (50 – 65°C) bacteria. Since bacterial reactions are anaerobic and slow, metabolic heat generation in digesters is low and in both cases heat must be supplied to the digester (Hobson et al., 1981). Heating is
usually by an interval hot water system. The biogas produced, is measured by displacement unit connected at the roof of the digestor.

Soil is the most abundant material available. It is therefore used carelessly and without consideration to the next specie. This however, can be changed to benefit all using it. In this study, we looked at the different kinds of soils to be used as landfill cover material.
Materials and methods

Soil analysis: Two 1 kg soil samples were dried; 1 kg air dried on a bench, 1 kg oven dried at 105°C. Both dried for 24 hours, after drying they were sieved with .0425 mm sieve and the finer soil was collected for further analysis. The course soil was discarded.

- Sieve analysis: A dish and soil were weighed. The soil was washed through a 0.075mm mesh till no colour came with the effluent. The soil was dried over night at 105°C. After cooling the soil was sieved in a column of sieves, this measures particle size.

- Air dried sample: With this soil plastic index, liquid limit and linear shrinkage were determined. The soil was mixed with water to a muddy texture. The mud was placed on Atterberg (Bosch, Germany) machine and split using a fork. The number of blows were counted to pull the mud together. For softer mud, the number of blows should be between 7 and 11. The soil was added to stiffen the mud, which then increased the number of blows. This determined the liquid limit of soil. When the blows are between 18 and 22 the soil was used to determine plastic index. The mud was rolled in small pellets, 2 grams of this was oven dried overnight. For linear shrinkage, however, a fairly hard dough-like mud where the blows are over 25 was put in a 150 mm rail and air dried for two hours. The rails were put in the oven for the night. This avoids shock drying as this increases shrinkage.

- Specific gravity: Oven dried soil sieved with 0.425 mm mash was used, split in two parts for hydrometer analysis and specific gravity.

- SG: Volumetric flask and approximately 10 grams of soil were weighed. The flask was half filled with distilled water and vacuumed for 20 minutes. When the vacuuming procedure was finished the flask was cleaned and distilled water was put in and weighed.
- Hydrometer analysis: About 50 grams of soil was weighed and mixed with a dispersant (calgon solution). The blender was rinsed with water to avoid sample loss and made up to 1000ml with distilled water. A few drops of methylated spirit was added to kill foam forming. The readings were taken at 30 seconds followed by 1, 2, 4, 10, 15 and 60 minutes. This method also determines the finer particle size (clay particles). (These methods were designed by Wits University, department of environmental engineering, 1992)

In this experiment waste was collected and weighed as in chapter 2. Due to less methane production of gas and longer degradation of some materials in waste reactor for example, paper, glass etc., the recipe was altered to the one below (table 4.6).
Figure 4.6  Apparatus set for this study (a) as set for the experiment, (b) experiment running
Table 4.6 Modified recipe of waste for quick rotting and faster acetogenesis

<table>
<thead>
<tr>
<th>Components</th>
<th>Mass (g) {actual mass (g)}</th>
<th>Sub-components</th>
<th>% mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper</td>
<td>45 {22.5}</td>
<td>Newspaper</td>
<td></td>
</tr>
<tr>
<td></td>
<td>{7.5}</td>
<td>Cardboard</td>
<td></td>
</tr>
<tr>
<td></td>
<td>{7.5}</td>
<td>Office paper</td>
<td></td>
</tr>
<tr>
<td></td>
<td>{7.5}</td>
<td>Magazine</td>
<td>10</td>
</tr>
<tr>
<td>Textile</td>
<td>15</td>
<td>Clothing material</td>
<td>3.3</td>
</tr>
<tr>
<td>Plastic</td>
<td>25 {8.6}</td>
<td>Shopping bag</td>
<td></td>
</tr>
<tr>
<td></td>
<td>{7.8}</td>
<td>Polystyrene</td>
<td></td>
</tr>
<tr>
<td></td>
<td>{8.6}</td>
<td>PVC, Perspex</td>
<td>5.6</td>
</tr>
<tr>
<td>Metal</td>
<td>19 {9.5}</td>
<td>Aluminium cans</td>
<td></td>
</tr>
<tr>
<td></td>
<td>{9.5}</td>
<td>Steel shaving</td>
<td>4.2</td>
</tr>
<tr>
<td>Putrecible</td>
<td>250 {35.7}</td>
<td>Meat</td>
<td></td>
</tr>
<tr>
<td></td>
<td>{35.7}</td>
<td>Bones</td>
<td></td>
</tr>
<tr>
<td></td>
<td>{35.7}</td>
<td>Bread</td>
<td></td>
</tr>
<tr>
<td></td>
<td>{35.7}</td>
<td>Fruit/veg.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>{35.7}</td>
<td>Rice</td>
<td></td>
</tr>
<tr>
<td></td>
<td>{35.7}</td>
<td>Noodles</td>
<td></td>
</tr>
<tr>
<td></td>
<td>{35.7}</td>
<td>oats</td>
<td>55.6</td>
</tr>
<tr>
<td>Other</td>
<td>39 {15}</td>
<td>Soap</td>
<td></td>
</tr>
<tr>
<td></td>
<td>{15}</td>
<td>Wax</td>
<td></td>
</tr>
<tr>
<td></td>
<td>{9}</td>
<td>Tyre</td>
<td>8.7</td>
</tr>
<tr>
<td>Ash</td>
<td>57</td>
<td>Ash</td>
<td>12.7</td>
</tr>
</tbody>
</table>

Treatments included addition of sewage and cow-dung as an inoculum of the reactor before closure to allow aerobic bacteria to use all available oxygen. Gas was collected in a gas bomb and sent for gas analysis. Gas was analyzed with gas chromatography (Varian GC, 3700. USA).
Gas collection;
As gas was generated in the waste reactor, it passed through the gas bomb to the soil reactor where it was oxidized. In this gas bomb the amount of gas produced is equal the gas escaping to the soil reactor. The emitted gas was collected from the top of the soil reactor. To collect this gas, the bomb was pumped to create a vacuum. The outlets on the reactor were closed for an hour to remove surface oxygen. The gas bomb was plugged in and the tap opened, this will suck in gas as the bomb has negative pressure. The tap will be closed and sample taken for analysis.
The gas was analyzed with gas chromatography flame ionization detector (FID) while hydrogen was analyzes on thermal conductivity detector (TCD) (Varian GC 3700, USA)
Statistical evaluation

Using Statistica 6.0 programme, (Wilcoxon test) where the P-value was 0.05. Value to be used was 5% confidence. These soils were evaluated against landfill soil as it was more exposed to methane and therefore, had established microbial growth.

Methane oxidation in veld (uncultivated) soil was less than 5%, while in sandy soil was at 8% and lastly, the garden soil was also less than 5%. This occurred in all the tested parameters e.g. addition of compost.

The Z-test was at 5, 15 and 4% respectively. This Z-test determines the viability of the test.

Z-test shows if the test is viable, in this case whether the soil bacteria can be used to oxidise methane. Whereas the P-value tells us that the soil bacteria is effective. This therefore, can be concluded as thus, the soils tested can be used as landfill cover and the available bacteria will oxidise the methane with the other parameters used in soils e.g. addition of compost.
Results

Soils:

![Soil samples](image)

**Figure 4.8** Test soils

A: Garden soil (Wits Library gardens)

B: Sand soil (from roadside used for paving)

C: Veld soil (collected 30cm from uncultivated land)

D: Landfill soil (Collected from Springfield landfill)

**Example** Springfield soil

<table>
<thead>
<tr>
<th></th>
<th>Liquid Limit (LI)</th>
<th>Plastic Limit (PI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of blows</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td>Container number</td>
<td>48</td>
<td>21</td>
</tr>
<tr>
<td>Wet mass sample +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>container (a)</td>
<td>41.165</td>
<td>35.285</td>
</tr>
<tr>
<td>Dry mass sample +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>container (b)</td>
<td>36.654</td>
<td>33.149</td>
</tr>
<tr>
<td>Mass of container</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c)</td>
<td>25.268</td>
<td>27.208</td>
</tr>
<tr>
<td>Mass of water (a-b)</td>
<td>4.511</td>
<td>2.136</td>
</tr>
<tr>
<td>Mass of dry soil (b-</td>
<td>11.386</td>
<td>5.941</td>
</tr>
<tr>
<td>c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moisture content %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[(a-b)/(b-c)* 100)</td>
<td>39.62</td>
<td>35.95</td>
</tr>
</tbody>
</table>
Sample description and comments: The soil was collected from the landfill and slightly moist from rotting organics. The colour was brick red and had stone bigger than 19 mm rubble.

<table>
<thead>
<tr>
<th>Linear Shrinkage (Ls). Trough No. E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of dry soil (a) 146</td>
</tr>
<tr>
<td>Total shrinkage (b) 4</td>
</tr>
<tr>
<td>Linear shrinkage % [(100/150)*(b)] (2.67)</td>
</tr>
<tr>
<td>Plastic index (lp) = Li – Pi = 8</td>
</tr>
</tbody>
</table>

Sieve analysis

<table>
<thead>
<tr>
<th>Sieve No.</th>
<th>Sieve size (mm)</th>
<th>Mass retained</th>
<th>% retained</th>
<th>% passing</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.00</td>
<td>12.5</td>
<td>4</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>13.20</td>
<td>16.9</td>
<td>9.5</td>
<td>90.5</td>
<td></td>
</tr>
<tr>
<td>9.50</td>
<td>15.9</td>
<td>14.7</td>
<td>85.3</td>
<td></td>
</tr>
<tr>
<td>¼ in</td>
<td>6.70</td>
<td>17.8</td>
<td>20.4</td>
<td>79.6</td>
</tr>
<tr>
<td>4</td>
<td>4.750</td>
<td>11.7</td>
<td>24.2</td>
<td>75.8</td>
</tr>
<tr>
<td>8</td>
<td>2.360</td>
<td>31.1</td>
<td>34.3</td>
<td>65.7</td>
</tr>
<tr>
<td>16</td>
<td>1.180</td>
<td>28.1</td>
<td>44.4</td>
<td>56.6</td>
</tr>
<tr>
<td>28</td>
<td>0.600</td>
<td>21.9</td>
<td>50.4</td>
<td>49.6</td>
</tr>
<tr>
<td>40</td>
<td>0.425</td>
<td>10.7</td>
<td>53.9</td>
<td>46.1</td>
</tr>
<tr>
<td>50</td>
<td>0.300</td>
<td>12.6</td>
<td>58</td>
<td>42.0</td>
</tr>
<tr>
<td>100</td>
<td>0.150</td>
<td>27.8</td>
<td>65</td>
<td>35.0</td>
</tr>
<tr>
<td>200</td>
<td>0.075</td>
<td>16.8</td>
<td>70.5</td>
<td>29.5</td>
</tr>
<tr>
<td>Pan</td>
<td>6.6</td>
<td>72.6</td>
<td>27.4</td>
<td></td>
</tr>
</tbody>
</table>

Pan Number 28

Mass of pan and dry material before washing 485.5
Mass of pan 176.4
Mass of pan and dry material after washing 392.2
Specific Gravity:

<table>
<thead>
<tr>
<th>Bottle number</th>
<th>5</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass of bottle</td>
<td>w1</td>
<td>28.575</td>
</tr>
<tr>
<td>Mass of bottle and dry soil</td>
<td>w2</td>
<td>38.700</td>
</tr>
<tr>
<td>Mass of bottle soil and water</td>
<td>w3</td>
<td>85.595</td>
</tr>
<tr>
<td>Mass of bottle and water</td>
<td>w4</td>
<td>78.990</td>
</tr>
<tr>
<td>Specific gravity Gs</td>
<td></td>
<td>2.876</td>
</tr>
<tr>
<td>Average Gs</td>
<td></td>
<td>2.878</td>
</tr>
</tbody>
</table>

\[ Gs = \frac{(w2 - w1)}{(w4 - w1)} - w3 - w2 \]

Hydrometer analysis

<table>
<thead>
<tr>
<th>Time</th>
<th>Hydro reading</th>
<th>Temp. Hydro</th>
<th>Adjusted hydro</th>
<th>Corrected reading</th>
<th>% passing</th>
<th>Approx particle mm</th>
<th>Actual particle mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 sec</td>
<td>49</td>
<td>19.4</td>
<td>50</td>
<td>42.7</td>
<td>84.5</td>
<td>0.075</td>
<td>0.0681</td>
</tr>
<tr>
<td>40 sec</td>
<td>47</td>
<td>19.4</td>
<td>48</td>
<td>40.7</td>
<td>80.6</td>
<td>0.050</td>
<td>0.0490</td>
</tr>
<tr>
<td>2 min</td>
<td>42</td>
<td>19.4</td>
<td>43</td>
<td>35.7</td>
<td>70.7</td>
<td>0.040</td>
<td>0.0286</td>
</tr>
<tr>
<td>5 min</td>
<td>38</td>
<td>19.4</td>
<td>39</td>
<td>31.7</td>
<td>62.8</td>
<td>0.026</td>
<td>0.0185</td>
</tr>
<tr>
<td>15 min</td>
<td>32</td>
<td>19.2</td>
<td>33</td>
<td>25.7</td>
<td>50.9</td>
<td>0.015</td>
<td>0.0112</td>
</tr>
<tr>
<td>30 min</td>
<td>27</td>
<td>19.0</td>
<td>28</td>
<td>20.7</td>
<td>41.0</td>
<td>0.010</td>
<td>0.0083</td>
</tr>
<tr>
<td>60 min</td>
<td>24</td>
<td>18.8</td>
<td>25</td>
<td>17.7</td>
<td>35.0</td>
<td>0.0074</td>
<td>0.0059</td>
</tr>
<tr>
<td>250 min</td>
<td>18</td>
<td>18.0</td>
<td>19</td>
<td>11.5</td>
<td>22.8</td>
<td>0.0036</td>
<td>0.0042</td>
</tr>
<tr>
<td>1440 min</td>
<td>13</td>
<td>17.0</td>
<td>14</td>
<td>6.3</td>
<td>12.5</td>
<td>0.0015</td>
<td>0.0013</td>
</tr>
</tbody>
</table>

pH of the soils

- Library garden: 6.92
- Road side: 6.98
- Uncultivated soil: 7.23
- Landfill soil: 6.74
### Table 4.7 Compiled results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Li</th>
<th>Pi</th>
<th>Ls</th>
<th>Ip</th>
<th>Sg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Springfield (Landfill)</td>
<td>33.15</td>
<td>27</td>
<td>2.67</td>
<td>8</td>
<td>2.88</td>
</tr>
<tr>
<td>Uncultivated land (grazing area)</td>
<td>17.55</td>
<td>13</td>
<td>3.3</td>
<td>10</td>
<td>2.71</td>
</tr>
<tr>
<td>Library gardens (fertilized soil)</td>
<td>17.83</td>
<td>17</td>
<td>3.2</td>
<td>8</td>
<td>2.57</td>
</tr>
<tr>
<td>Road side (Sandy)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.69</td>
</tr>
</tbody>
</table>

### Table 4.8 Particle size: determined by % passing through the mash

<table>
<thead>
<tr>
<th>Sieve size (mm)</th>
<th>Springfield landfill</th>
<th>Uncultivated soil (Veld)</th>
<th>Library garden</th>
<th>Road side (sand)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.00</td>
<td>96</td>
<td>97.7</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>13.20</td>
<td>90.5</td>
<td>96.5</td>
<td>100</td>
<td>99.3</td>
</tr>
<tr>
<td>9.50</td>
<td>85.3</td>
<td>94.7</td>
<td>100</td>
<td>98.9</td>
</tr>
<tr>
<td>6.70</td>
<td>79.6</td>
<td>88.8</td>
<td>99.9</td>
<td>97.7</td>
</tr>
<tr>
<td>4.750</td>
<td>75.8</td>
<td>82.2</td>
<td>98.5</td>
<td>96.0</td>
</tr>
<tr>
<td>2.360</td>
<td>65.7</td>
<td>67.7</td>
<td>89.8</td>
<td>84.9</td>
</tr>
<tr>
<td>1.180</td>
<td>56.6</td>
<td>59.7</td>
<td>77.9</td>
<td>73.1</td>
</tr>
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<td>0.600</td>
<td>49.6</td>
<td>56.5</td>
<td>64.0</td>
<td>64.5</td>
</tr>
<tr>
<td>0.425</td>
<td>46.1</td>
<td>53.7</td>
<td>55.4</td>
<td>61.0</td>
</tr>
<tr>
<td>0.300</td>
<td>42.0</td>
<td>49.3</td>
<td>47.3</td>
<td>57.4</td>
</tr>
<tr>
<td>0.150</td>
<td>35.0</td>
<td>34.7</td>
<td>33.7</td>
<td>31.9</td>
</tr>
<tr>
<td>0.075</td>
<td>29.5</td>
<td>48.8</td>
<td>25.4</td>
<td>15.8</td>
</tr>
<tr>
<td>0.050</td>
<td>80.6</td>
<td>44.8</td>
<td>26.3</td>
<td>15.8</td>
</tr>
<tr>
<td>0.040</td>
<td>70.7</td>
<td>42.9</td>
<td>26.3</td>
<td>9.9</td>
</tr>
<tr>
<td>0.026</td>
<td>62.8</td>
<td>33.7</td>
<td>26.3</td>
<td>7.9</td>
</tr>
<tr>
<td>0.015</td>
<td>50.9</td>
<td>29.7</td>
<td>24.4</td>
<td>5.9</td>
</tr>
<tr>
<td>0.010</td>
<td>41.0</td>
<td>27.7</td>
<td>24.4</td>
<td>5.9</td>
</tr>
<tr>
<td>0.0074</td>
<td>35.0</td>
<td>25.7</td>
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</tr>
<tr>
<td>0.0036</td>
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<td>21.8</td>
<td>16.4</td>
<td>4.0</td>
</tr>
<tr>
<td>0.0015</td>
<td>12.5</td>
<td>20.4</td>
<td>12.5</td>
<td>4.5</td>
</tr>
</tbody>
</table>
Graph 4.1  Soil particle size

Graph 4.2a  Methane production after increasing organics (putrecibles)
Methane production when sewage and cowdung added

Graph 4.2b Methane production

Methane oxidation by soil when compost was added

Graph 4.3a Methane oxidation is soils
Graph 4.3b Methane oxidation in different variables

Graph 4.2 shows different methane production when sewage and cow dung was added. Graph 3.3 is methane oxidation in composted soils and when isolated cultures were added. Note; all the materials were the same running at the same temperature. For control landfill soil was collected in a litre Schott bottle and a 50%:50% gas (CO₂:CH₄) was pumped in and incubated at 25°C. After 7 days a gas sample was taken from the head space and analysed. It was observed that methane was reduced to 5%. 
Figure 4.9 Gas analysis electrographs
(i) Calibration curve; (ii) Gas from waste reactor; (iii) Gas after passing through soil
Calculation of oxidation efficiency

Methane oxidation rate can be calculated as thus:

\[
\% \text{ Oxidation} = \left( \frac{[Q_{CH4}]_{in} \times 100\% - [Q]_{out} \times [C_{CH4}]_{out}}{[Q_{CH4}]_{in} \times 100\%} \right)
\]

Where

- \([Q_{CH4}]_{in}\) = flow rate of CH\(_4\) entering at the column’s base
- \([Q]_{out}\) = flow rate of column’s effluent
- \([C_{CH4}]_{out}\) = CH\(_4\) concentration in column’s effluent

(Stein and Hettiaratchi, University of Calgary, Alberta. Dep. of civil engineering)

This equation is used in a known gas (methane) supply. This however, can be altered to:

\[
\% \text{ Oxidation efficiency} = \left( \frac{[CH4]_{in} - [CH4]_{out}}{[CH4]_{in}} \right) \times 100
\]

For example: waste 2, and road side soil

\[
= \frac{4.2 - 0.4}{4.2} \times 100
\]

\[
\% \text{ Oxidation efficiency} = 90.48\%
\]

The original recipe produced less methane and only started to produce detectable amounts about 4 % in the second week. The inoculums added did not have significant effect on gas production but increased the rate of reaction. The acetogenesis stage was reduced and the detectable gas was at approximately 3 %. This was due to increased anaerobic cultures collected from sewage and in cow-dung. Increase bacterial numbers were seen to speed up the rate of gas production.
Discussion

Methane

Organic matter in feedstock (including pollutants) is converted to biogas, a mixture of methane (50%) and carbon dioxide (45%). The methanogenic bacteria can utilize only a limited number of substrates, principally hydrogen and carbon dioxide and acetic acid (Hobson, 1984). Higher fatty acids are converted to methane via degradation to acetic acid and water with, according to chain length, propionic acid which can also be converted to methane (carbon dioxide is formed in some of these reactions).

Biological methane production is an anaerobic process, while methane consumption occurs predominantly under aerobic conditions. However, both processes can occur simultaneously in soil. Thus, the field measurements of methane flux reflect the net result of both consumption and production (Chan and Parkin, 2000).

The maximum growth rates of the methanogenic bacteria are low and methanogenesis is the rate limiting reaction in digestion of dissolved sugars. However, the vegetable feed stuffs of animals have been subject to gastric and intestinal digestion or to microbial digestion in rumen or caecum, or both. Therefore, what remains is lignified and ordered cellulose of hemi-cellulose which has restricted degradation in the animal. The rate and extent of hydrolysis and subsequent fermentation of fibrous feedstock can be improved by chemical or physical treatment, e.g. alkali or autoclaving which loosens the fibre structure. The final stages take 10, 20 or more days in an aerobic digester, and thus fibre degradation becomes the rate limiting step in the conversion of the feedstock to methane.

Landfill gas production and migration is controlled by microbial activity and soil physical properties such as gas (air) permeability, gas diffusivity and atmospheric pressure variations.
With about 40 – 60 mega tons (Mt) of methane emitted each year worldwide, landfills are important global source of this greenhouse gas. These emissions are especially caused by inadequate gas extraction facilities, missing gas collection systems at old dumpsites and landfills, or unauthorised open dumping. A low cost alternative method or an additional measure to the conventional degasification systems is the application of the natural potential of microbial methane oxidation in landfills in a suitable cover layers. By creating optimal ambient conditions for methanotrophic bacteria in cover layers, it is possible to foster the microbial activity and to attain very high oxidation rates. The results derived from laboratory experiments as well as first experience from a large scale field investigation show that waste components are suitable carrier substrates for methanotrophic bacteria (Humer and Lechner, 2003). Even at higher methane supply the compost (organic matter in soil) proved to remove more methane than natural soils. This was observe in the experiments because addition of compost reduced emission of methane (graph 4.3a)

From the results, in this study it is seen that the oxidation of methane is 90 % efficient and this is increased when compost is added to the soil. This organic matter binds with gas and slows it to have more contact with the bacterial population hence oxidation occurs.

The phenomenon of greenhouse gases being oxidised in cover material has been established, but need more economic strategies. In previous studies it was seen that addition of chemicals (as trace elements [nitrates and phosphates]) increased oxidation of the gases (Muthraparsad, 2005). The compost as fertiliser has trace elements and nutrients to sustain this theory.

Visscher and Van Cleemput (2003) modelled methane oxidation rate using mathematical simulation based on bacterial growth rate. They found out that the oxidation rate of the laboratory soil column was 82.8 % when $K_m$ was set to zero and the $V_{max}$ was set to one. Under field conditions the prediction was at 60 %. The effect of increased bacterial numbers is observed in this experiment as well. As the original soil still emitted more methane at about 3
% whereas the subsequent experiment was run using the same soil with the intention to establish the methanotroph bacteria in soil. The bacteria would have grown in the presence of methane therefore, multiplied. This resulted in the decreased emission of methane to 1.5% on average. Haubrich (2006) reported that addition of compost to soil improved oxidation of methane to 0.0% after 31 days of equilibrating with air. It was mentioned that drying of material i.e. soil reduced effect of oxidation. In this study, the soil was kept moist except at the start of a new run when it was air dried.

A study done by Chan and Parkin (2000) showed that some of the domestic waste emitted chemicals like acetylene, ethane, ethane methyl chloride and methyl fluoride. These in nature are toxic due to the effect that they inhibit some metabolic reactions, which in turn inhibit oxidation of landfill gases. Their inhibition however, varies with concentration.

Any change in variation of oxygen concentration in soil will affect the numbers of methanotrophs. In addition, methane flow and oxygen diffusion in soil is affected by soil type, for example, sand is gas permeable whereas clay is more impermeable. The concentration of gas produced was very low where the bacteria in the study soil showed more efficiency in methane oxidation. In reality the gas produced from the waste tend to be higher. The un-oxidised gas was emitted because the methanotrophs cannot optimally use all the gas produced. The flow of gas and availability of oxygen in the soil are the limiting factors to oxidation. This is equated to the enzymatic graph where substrate is in excess and the enzyme activity is maximal ($\lambda$ max). This also proves the importance of methanotrophic activity on the methane emission from anthropogenic sources.

In long term, landfills produce gas which is low in energy values. Using the gas produced in landfills as power supply would be expensive when the gas volume or energy value diminishes as it is limited for a limited period. Composting can be used as a method of reducing methane emissions from landfills. Nevertheless, composts to some extent are the source of
atmospheric methane, but mixed with covering material can enhance the oxidation effect.

The intention of the estimates is to disclose data to public and regulators. This has been welcomed in Europe as a positive development. It is good criteria such as timeless, completeness, certainty, comparability, consistency and transparency are set for admission of emission data in a public accessible database. Scharff and Jacobs (2006), have made comparison in three landfills (although not representative) emission. It was recommended that more data should be generated for comparison before proposal is made in countries that do not have guideline models. A large number of data should be generated to enable a fair representation of the future methane emission data reported to UN.

**Influence of environmental factors**

Although external factors have been linked to methane oxidation, in this study the temperature was set to 25°C and moisture content of the soil was at 25%. Temperature was predicted to have a very pronounced influence on methane oxidation using mathematical model by De Visscher *et al.* (2003). Increase in temperature increased oxidation of methane, but above temperatures of 35 denaturation occurred and a drop in efficiency was observed. They used a $Q_{10}$ value of 2.8. Methane oxidation efficiency decreased rapidly with increasing moisture content as proven by De Visscher *et al.* (2003). This is because moisture reduces the diffusion of oxygen into the soil. It illustrates the importance of oxygen diffusion for methane oxidation in landfill cover soils.

Influence of methane concentration in the landfill gas on methane oxidation depends on the partial pressure of the gas and its diffusability in soils. Increasing methane concentration as a contact leads to increased methane flux. As a result a smaller amount of methane is oxidized the soil bulk density plays little role in methane oxidation. But, the water filled pore space, can influence migration of methane hence alter oxidation rate. The more filled the
pore space the less migration of the gas and the more contact time with the bacteria. And therefore, more oxidation occurs.

Soil

The soils were collected from three different sites, namely the garden, unused land (uncultivated), road side and the landfill itself. Only the physical and microbial analyses were done on soil, the chemical analysis was not done. The landfill soil has been taken from Springfield landfill about 6 km south of Johannesburg. The landfill has been covered for approximately ten years and the soil was moist at some areas wet as if there was a leaking pipe underneath. The garden soil was collected before the compost was added, bearing in mind that the garden (soil) is watered and taken care of weekly although the compost was not added weekly. The road side soil was taken during construction of the pavement. It was collected in the morning hours at a busy road therefore, there might be some chemical imbalance. The uncultivated soil was an open land, which was under construction. The soil was taken approximately 45 cm from the foundation trench. The soils were kept in a dark plastic to keep moisture at room temperature for six months (July to January) for methane oxidation and microbiology. Stones bigger than 13 mm were removed to maintain uniformity of the soil. They (soils) were selected by visual appearance and texture so as to compare the different properties in the soil as a landfill cover.

The road side sample was sandy and therefore, did not have most of the properties analyzed for, for example, it did not have liquid limit, plastic index, plastic limit and linear shrinkage. These factors are all required in soil to be used or the cover will lose its integrity, thereby not perform what is designed for. The plastic limit and the plastic index are the measure of the malleability of the soil. These on the other hand, will determine how much shrinking and stretching of the soil when it dries that is in dry areas. The holding together of soil is important in that it will show how much it will hold in stressful conditions. This was a normal sand use in paving and plastering. In building industry,
cement is added as means of adding finer particles to hold together; although cement chemically has gluing effect.

The problem with sand soil is that it loses the moisture and this implies that the porosity of the soil is high therefore; it will not hold the methane and the odour produced in the landfill. Moreover, as the water is filtered through it will sip through the waste to the table water used in the urban areas. Although there is some clay found in this sand, it is not strong enough to hold the sand in the cover state as it will be blown with heavy winds. This therefore, does not give this type of soil the characters of being a cover coil, as more treatment will be applied. This then makes it more expensive for a landfill cover.

The uncultivated soil has some dead root as its organic matter. Physically it showed easy compaction due to moisture levels, and finer grains no lumpy stones. During different treatments, it showed to have finer particles although comparatively they all showed some degree of clay presence. However, this when used as a cap it will absorb more water and keep it resulting in a landslide as the weight of water will cause the mud to slide. This is seen in water affinity (plastic limit)

It has been stated that some factors that influence microbial CH₄ oxidation includes temperature and moisture (Jones and Nedwell. 1993). Before packaging, the soil was air dried for 24 hours and hydrated with 135 ml of water (Wits procedure, 1992). The difference with previous researchers is that I warmed the soil to 25 degrees for 72 hours in a column before packing the waste column.

Addition of compost increased porosity of the soil, which resulted in greater contact with bacteria. It however, had little effect on moisture content of the soil. Increased pore size increase air-flow in the soil. This improves oxidation of methane by bacteria.
Landfill caps, does not lessen toxicity or volume of hazardous waste but they limit migration. They are estimated to last from 50 to 100 years. In areas prone to earthquakes they should be designed appropriately. Change in conditions, such as soil moisture and movements should be monitored as indicators of potential problem. Nearly all currently used landfill covers (caps) employ barrier-type systems. They consist of a layer of soil covered by native grasses to control infiltrating precipitation as follows;

a) the soil store infiltrating water

b) natural evapo-transpiration empties the soil water reservoir.

The vegetative cover concept has been extensively verified in the field (Houser et al., 2001). Because they are natural, they should perform better than conventional covers over decades or centuries and they are less expensive to build and maintain. The goal is to design the cover that it never reaches the saturation point. Engineers use climatic data and the soil characteristics to predict infiltration rates and determine the necessary cover thickness (Civil Engineering News. 1998). It is believed that management strategies in the unsaturated soil zone will offer the best opportunities for preventing or limiting pollution, or for remediation of ongoing pollution problems. This is so, because chemical residence times in ground-waste aquifers can range from years to thousands of years, so that once contaminants have entered the ground-water, pollution is essentially irreversible in many cases. Therefore, prevention or remediation of soil and ground-water contamination starts with proper management of the unsaturated zone (van Genuchten. 1994).

There is a strong link between soil biological and physical properties. In fact, most fluxes in soil are of biophysical character (e.g. emission of CO₂, CH₄, NO₂). They are connected with the purity of atmospheric air and of water quality. Better understanding of the inter-relations among biophysical properties on soils offers a possibility of their management towards environmental protection.
Alternative capping

Plastic geomembranes were suggested in Germany as they have a service life of 100 years. These would be water tight and make it easier to collect gas (Finsterwalder, 2003). But it has been abandoned since, due to expense and the functional of the landfill and installation of the geomembranes would affect the design of the landfill, also the aging of the membrane was not incorporated in the landfill design. It has been discovered by Melchior (2001), that the vegetation would destroy the plastic if used below the soil and the plastic will not hold the temperature produced during fermentation. In the long run processes of soil formation will transform the conventional geomembrane into a part of the restoration layer depending on local conditions such as weather.

Clay liners are prone to cracks as they dry out. The clay is compacted with high water content to achieve low hydraulic conductivity. However, small change in moisture content caused soil to absorb water from the clay cover which leaves it to crack. In addition, heat generated in waste will cause evaporation from the clay, which will reduce the water content resulting in cracks formation. Desiccation effect is caused by plant transpiration as a result the cycle repeats itself. In this study the evaporation effect was limited as the soil was in a reactor, with limited air circulation. Some plastic geomembranes have been studied by Muller and Jakob (2003) and concluded that if properly installed the pollutants can be controlled depending on the nature of the chemical (leachate). Since the shelf life of the membrane is estimated to be over 100 years. The plastics to be used should be inert (impervious) to heavy metals and inorganic compounds (cations and anions in aqueous solution (Muller et al. 1997). The performance of the membrane depends on quality during installation and damage that occurs due to stress and other variable. This requires expertise of technical staff which is another hindrance. We were looking at the soil as the main cover material in landfills.
Synthetic covers

Synthetic clay material is spread between two textile materials. This is more robust than the plastic and can withstand changes in moisture as it cannot expand more than the textile which means, during shrinkage it goes to original length (size). This phenomenon is called “self-healing” capacity therefore can withstand ion-exchange and desiccation effect (Kiditz et al. 2004 and Schick and Schmittz, 2004). Desiccation effect is virtually eliminated when rewetted due to large swelling capacity of bentonite (synthetic clay). On the other hand, concrete barriers have been studied by Burkhardt and Egloffstein (1995). The problem is sand and cement mixing to hold the compression strain.

Alternative barriers

Instead of clay, which displays a valuable resource, especially in mixed grained material, it can be used in landfills. Other materials like soil are cheap and readily available. Capillary barriers, as they are called do not depend on the mechanism used in synthetic covers. They do not allow water leakage into the waste but at the same time allow diffusion of gasses. Well adjusted grains distribution of layers in relation to slope will control water permeability (Melchoir and Steinert, 2001). Capillary barriers are applied on the sloped section of capping system. The advantage compared to synthetic clay is that there is no desiccation and cracking, it acts as drain because of the finer particles used.
Conclusion

There is a relation in methane oxidation by soil and this is further seen in addition of organics (compost). It has been stated by Conrad (2003) that moisture plays a vital role as well. However, in this study a uniform moisture level was established. Veld soil proved to be the best methane oxidising as compared to the other soils. The experiment can be left for longer periods to establish gas production to better concentrations.
Chapter 5

SUMMARY OF CONCLUSION
Municipality and industry and their waste source; the effect of waste in the environment

The impact of waste management on the environment has been widely recognized, the most important progress in the increased level of awareness among the public and politicians, ISWA; Industry as a partner for sustainable development (Waste management, UK). As a result, municipal waste generation and its management and associated externalities have been addressed as one of the key target in environmental policies (Ayalon et al., 1999). Externalities are different social or class of people having impact on other people. These may include waste generation, transportation and disposal to landfills, which is costly or beneficial on labour generation. They (externalities) creates many global, regional and local disruption such as pollution to soil air and water resulting in climate change, poor health and damage to crops and buildings. All alternative strategies of waste management result in externalities that are generated at collection, transportation and disposal stages. They are influenced by composition of waste treatment process (land filling or incineration) and age of the site to some extent geographic location.

Land filling; this is associated with landfill gas that contributes to global warming and leachate that contaminate table water. Occasionally, benefits come through energy generation from methane.

Incineration; this is costly as it generates air pollution from burning these materials; it has no benefits as it takes in energy and the ash produced will need to be cleaned (land filled). Other means of waste management are item specific and therefore, do not have much impact on environment e.g. separation for recycling.

It is known, that a landfill is a kind of a bioreactor generating biogas containing different amounts of methane and other gases. Because of its methane content, landfill gas has a fuel value of 18-22 MJ/m^3 (Spokas et al. 2006). Landfill methane is used to fuel industrial boilers or commercial boilers to generate electricity using internal combustion engines or gas turbines. In
addition to providing a local source of energy, the commercial recovery of landfill methane decreases a source of atmospheric methane, the second most important greenhouse gas after carbon dioxide and responsible foe approximately 40% of global warming (Hansen et al., 1998). Since methane has an atmospheric lifetime of about 10 years and global warming potential 23 times higher than carbon dioxide, reduction in individual methane sources can decrease atmospheric concentrations within a decade (Houghton et al., 2001). Currently, estimates indicate that commercial landfill gas recovery projects recover more than 5Tg worldwide, thus reducing atmospheric methane contribution from landfill (Willumsen, 2003 and Bogner et al., 2003). In developing countries, the annual volume of landfill methane generated will increase significantly over the next decade as more controlled Land filling practices are expanded to deal with large quantities of solid waste in an environmentally acceptable manner, especially in rapidly growing mega-cities (Spokas et al., 2006).

**Estimates of methane emitted**

Methane generated in landfills is partitioned into methane recovered, emitted to the atmosphere, oxidized by methanotrophs, lateral migrated and internally stored in the landfill volume (Bogner and Spokas, 1993). They came to this equation;

\[
\text{CH}_4 \text{generated} = \text{CH}_4 \text{emitted} + \text{CH}_4 \text{oxidized} + \text{CH}_4 \text{recovered (flared)} + \text{CH}_4 \text{migrated} + \Delta \text{CH}_4 \text{storage} \quad (\text{unit} = \text{mass/time})
\]

In this study; \( \text{CH}_4 \text{generated} = \text{CH}_4 \text{oxidized} + \text{CH}_4 \text{emitted} \)

As it can be seen from the simulation, a gas bomb was connected before the soil reactor, which gives the total gas produced. From this, it was channelled to the soil reactors where oxidation took place. However, the emitted gas was collected in a separate gas bomb for analysis to monitor how much of the produced gas has been oxidized. Hence, our equation omitted other factors like storage because it was assumed that the gas pressure was high enough to push the gas out of the reactor.

Therefore, \( \text{CH}_4 \text{generated} = \text{CH}_4 \text{collected (before soil)} + \text{CH}_4 \text{oxidized} + \text{CH}_4 \text{emitted} \)
The term storage is defined as the gas collected in void space created by degradation of organic matter and fluctuation of leachate drainage, which sometimes is drained faster (all) creating negative pressure on gas emission. From the study, the gas is collected vertically as upward movement of the gas. The flow rate varies as it is seen using the water displacement unit, therefore, the recovered gas cannot be quantified accurately. Most of the fluxes were calculated on assumption that all the gas produced will be transported to the surface where oxidation will take place in relations to oxygen availability. Borgner et al. (1997) developed a model for landfill gas transport and methane oxidation in landfill cover soils in terms of molecule collisions with the soil matrix. Hilger et al. (1999) improved on Stefan-Maxwell equation on using a thick biofilm model. Oxygen in these simulations was the only limiting substrate because the depth of the active methanotrophic bacteria, are limited by oxygen penetration.

Gas emission could be controlled by active gas recovery or by increasing naturally occurring methanotrophs. However, gas also migrated laterally (in landfills) which created problems when trying to get accurate measures. This was controlled by artificial covers (synthetic plastics or clays). Finally, oxidation occurred in presence of oxygen, the diffusion potential of oxygen in the barrier will determine how much methane will be oxidised and at what level. That is, the depth of free oxygen in the cover material is indirectly proportional to bacterial numbers, which oxidises methane. This phenomenon is proven when there is a decline in methane production in the landfill where bacteria uses atmospheric methane as it diffuses in the soil (Bogner et al., 1995). De Visscher and Cleemput, (2003) stated that if 50% of methane is pumped into the soil and 50% of it will be incorporated into biomass. However, on long time scale it can be assumed that biomass will decay and eventually mineralise into carbon dioxide, leading to;

\[
\text{CH}_4 + 2\text{CO}_2 \rightarrow \text{CO}_2 + 2\text{H}_2\text{O}
\]

However, due to large oxygen requirements, oxygen penetrates less deep into soil, which causes the decrease of the modelled methane degradation.
Spokas et al. (2006) stated that the fraction of oxidized methane by bacteria in aerated soils ranges from 10% to 50%, we found that the oxidation efficiency is more than 95% although the gas production was not high enough. Our highest percentage production was 8%, which had more contact with bacteria hence better oxidation. From this, experiments as little as 2.5% was emitted to the atmosphere and this was further decreased when adding compost and further inoculating the soil with isolated cultures to increase bacterial numbers in the soil.

**Soil as a biofilter for liquids**

Utilization of soil as a biofilter changes purification of liquids (for example, of wastewater) has a long tradition. Its efficiency is determined strictly by biophysical properties of soil (Stepniewski et al., 2002). A key role is played here by oxygen status in soil that is very important for the plant and other biota, root penetration depth, absorption of phosphorus, nitrates and other nutrients, oxidation of organic matter, emission of gases etc. Possibility of biological methane oxidation is the use of the landfill cap (soil) as methane oxidation layer; on the other hand, they (landfill covers) are exposed to climatic changes and may show uneven flow distribution (Borjesson et al., 2001). It has been shown that woodchips as cover material is not good as there is more are flow nor fine grained material as there will be clogging formed by extra-cellular polymeric substances (ESP) produced by bacteria that will reduce oxidation rate in a long run. The choice of material would enhance methanotrophic growth in presence of methane and have large surface area ratio to allow mass transfer.

The soil was given six week to stabilise in landfill gas. The original soil was put in the bioreactor as a filter and was exposed for six weeks. There was oxidation observed in these soils although the rate was slow. It was later decided to introduce compost 65g compost to 150g soil.
Influence of environmental factors

Under field conditions, a high efficiency is obtained for methane fluxes, up to 50g/m²/d. Whereas, under laboratory conditions high efficiency are possible for methane fluxes up to 200g/m²/d, (De Visscher and Van Cleemput, 2003). Temperature is redetected to have a very pronounced influence on methane oxidation. This was related to Q10 value of the bacteria. Borjesson et al. (2004) found that low temperature influenced certain volatile fatty acids especially produced by type I methanotrophs, on the other hand, type II methanotrophs were increased with temperature change to 20°C. Whereas, in this study the variance was observed in prolonged degradation. There was a shift in cell population from cocci to rods as the reactors were left over extended period.

The influence of soil moisture content on model predictions.

Although, it is said that increased moisture content increases methane oxidation. However, very high moisture content is detrimental to oxidation of methane. This is because the increased moisture content reduces diffusion of oxygen into the soil. This clearly illustrated the importance of oxygen diffusion for methane oxidation in landfill cover soils. Landfills contain a considerable gas volume which is compressed and can expand as the air pressure increased resulting in a pumping effect that might enhance gas transport.

The influence of methane concentration in the landfill gas of methane oxidation depends on total methane flux. Increasing methane concentration at constant flux leads to increased oxidation until $V_{\text{max}}$ is reached which also is controlled by oxygen transport into the soil.

The influence of soil bulk density has little effect except when the pore space is filled with water. But if (bulk density) is kept constant the diffusion of gases will not be affected hence oxidation occurs.

Simulations models have been built to (Liptay et al., 1998; Chanton and Liptay, 2000; Borjesson et al., 2001; Borgner et al., 1997 and Hilger et al., 1999) understand gas transportation in soil, methane oxidation and study seasonal variations. They concluded that the calculations and gas oxidation
is sensitive to $V_{\text{max}}$. Therefore, more work should be done on maximum methanotrophic activity that can be reached in soil. It has been shown that temperature and moisture are factors that influence methane oxidation in soil the most. Most of the simulated studies are related to steady state conditions which may not be reached in real landfill as biotic and abiotic factors change within a given time and type of waste and environment.

In spite of the considerable progress that has been made, over the years, quantification of cost and impact related to municipal solid waste (MSW) are still associated with large uncertainties. Estimates are used to give some figure of how much is needed and the value of damage done by the improper waste control. For impacts of pollution and for disamenities, there are relatively large number of studies and estimates, whereas for pollution to soil and water, studies are still going on.

**Other International work**

Consortium for landfill emissions abatement research (CLEAR) as an international working group of the international waste working group (IWWG), is setting up standards and trying to implement rules of adherence to the emissions that can be allowed in relation to the damage of the environment. They include academics and industrial researchers (Scientist) coming mostly from the first world countries including Europe, United State, Canada and Australia, they encompass broad range of scientific discipline including microbiology, soil science, chemistry, geochemistry, civil and environmental engineering and waste management. They initially focused on natural biological processes to reduce missions of methane and non-methane organic compounds and improve on methodologies to measure and model landfill gas emissions. As research is coordinated, new data is compiled and stored to serve as a clearinghouse, a point of contact to those seeking information. Landfill operators, legislators’ industry groups and citizen groups will be able to access information through publications and international journals. Some of the works being addressed currently are;

- Landfill gas generation and emission (control and mitigation)
• Prediction and modelling on regional, national and global basis
• Evaluation of methane as a greenhouse gas to climate change
• Microbial methane oxidation and biodegradation of organics in soils

Their current activities include standardization of methods in biotic methane oxidation, improving engineering systems to enhance methane oxidation in landfill cover soils and setting up a database summarizing international methane emissions, and publications (Huber-humer, 2004).

Finally, the methane oxidizers convert methane to water, carbon dioxide and microbial biomass. Many other organic compounds in landfill gas such as aromatic and halogenated hydrocarbons, have also been shown to be partially or wholly degraded in such aerobic environments, this is because methanotrophs are able to co-metabolize substrates other than methane. Evidently, the enzyme that catalyzes the addition of an oxygen atom to the methane molecule can also catalyze similar reaction involving more complex organic substrates. Moreover, the transformed organic compound can, be further degraded to carbon dioxide and water, by a consortium of other microbes in the landfill cover soils (Scheutz and Kjeldsen, 2002).

**Issues raised by the media**

The unpleasant aromas that hover over refuse bins left at the side of the road for collection by pick it up trunks makes one feel sorry for the people loading and unloading sorting the foul smelling domestic waste that emerges from otherwise spotless homes.

Handling waste is a world wide problem. Consumers often spend time concentrating more on the packaging than the actual contents of the items they buy. This packaging ends up in being tossed into refuse bins or on pavements, in parks around picnic sites.
Many countries are making efforts to recycle packaging and other waste. In Sweden, for instance, customers can place empty plastic cold drink bottles into a machine that coughs out refund vouchers. In Germany, glass sorted by colour and collected outside apartments. In Portugal, there are shops that sell products by mass or volume (Kg or litre). A customer brings his own container. South Africa (Mondi), is delivering special bags to people’s homes (Durban, Pretoria and Johannesburg) to put in paper for recycling, which is collected in fortnight (Lyde, 2005).

Where does all the waste go?
Most people have no idea what happens to the waste in their bins once it has been collected. Pik-it-up (South Africa), which is owned by the city council but runs as a private concern, dumps the waste in five landfill sites. Informal recyclers collects the reusable waste from various landfills sites and sell it to the third party contractors approved by pik-it-up (Melanie, 2005).

Mandisi Majavu (Star, 2002), writes; “Health risks imposed by illegal dumping are frightening. We (Pik-it-up) believe children who play in open areas containing dumped waste are at risk if being injured by broken glass, sharp piecing metal, used hypodermic needles or other dangerous items in the waste. Also perishable waste like discarded food creams an ideal breeding ground for bacteria. To deal with this predicament, pik it up has trained staff and Metro police officers to conduct a one on one interviews with community on spread awareness (change your attitude: world summit on sustainable development), and information about illegal dumping. At the same time the educators are informing residence about the range of waste management services offered by us (pik it up). These include a wide range, from domestic waster and street sweeping to the collection and destruction of animal carcasses and bulk services to industry” (environment news service [ENS]; 2002).
New Research

China has developed worms to eat all organic (food) waste. This eliminates the organic matter put in landfill. However, in all this, the people should be made aware. The environmental issues are slowly being seen and the people are trying to understand and work hand in hand with the government.
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