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Methanotrophic Oxygen Dependency and Availability for Sustained Oxidation

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Abstract

The oxidation of landfill methane is dependent upon a multitude of factors, some of which have been researched in-depth, while others require further investigation. One of the factors, that has not been carefully looked into, is the time factor for soils to rejuvenate and start oxidising methane efficiently. Using a batch reactor, soil samples, having no or little exposure to methane, were compared with other samples that had continuous methane exposure, in terms of the time they took to allow efficient methanotrophic mitigation of methane. In addition, the effect of oxygen availability and continuity to supply nourishment to the methanotrophic bacteria in relation to the soil types and the conditions of the soil's exposure to methane was also investigated. The results showed that acclimation time was an important factor in establishing high methane oxidation activity, with up to four days of lag time being observed before the methane oxidation could commence, as was the case for soil samples, previously exposed to methane. This is particularly important, since active land filling could last for twenty years of active operations, and would release up to an estimated 2.1 to 2.8 x 10^4 $MtCO_2$-eq per day per landfill of methane into the atmosphere, globally, if the time lag were not controlled. Most importantly however, was the oxygen availability in landfill cover layers. The study showed that physical mixing of samples by mechanical agitation during incubation could allow higher concentrations of oxygen to permeate into the soil, increasing methane oxidation rates, which were approximately doubled due to this action. Furthermore, a linear relationship was found to form between methane consumption and the time when oxygen concentration was not rate limiting to the bacteria.

Keywords: Landfill gas control; Landfill cover design; Methane assimilation; Oxidation lag time; Oxygen sustainability in soils

Introduction

Landfills are one of the highest pollution production sources among all of human activities, including those from agriculture, coal mining, biomass burning, gas and petroleum production, wastewater treatments, and industrial processes. Moreover, landfills range from the managed sanitary landfills in developed economies to simple open landfills (waste dumps) in developing economies. However, in all cases, the biological waste components in them ferment to produce large quantities of methane gas. Based on the 1996 and 2006 IPCC estimates and guidelines, the global production of methane from landfills, as a result of biodegradation, is estimated to reach 500-800 million metric tons of carbon dioxide CO_2 equivalent per year ($MMTCO_2$-eq), and is projected to reach up to 2900 MMT CO_2-eq/year in 2050. This can amount to methane gas emissions at the range of 10-20% [1], or 24-30% [2,3] of the total from all human activities combined. These quantities are equivalent to 20-70 Tg of CH_4 released into the atmosphere annually. In addition, escalating methane emissions have caused atmospheric concentrations to rise from 700 ppb recorded in 1750 to more than 1774 ppb in 2005 [4]. Other studies had also estimated that annual methane production from human activities accounts for more than 300-400 Tg of CH_4 globally, from all sources [5]. These alarming indicators are increasing, despite all efforts to utilise, contain, or reduce methane in many processes, designed to limit their negative effects on the atmosphere. The concern about this rise in methane release into the atmosphere is that, methane can be 23 times more harmful to the environment than CO_2 over a 100-year time span, as a study has suggested. Thus, in the light of recent international laws of taxation on carbon emissions into the atmosphere, landfill gases are now even more important to control.

Typical landfill sites are filled up daily with municipal wastes, deposited from collection trucks into landfill cells, with each cell being sealed at the end of the day with earth's soil covers (Figure 1). These earth's soil covers of 6-12 inch thick, are placed one on top of another, or side-by-side to prevent odour and health hazard from the surrounding environment. To manage and control these landfills, methane collection systems are installed, and the gas is utilized for heat or electricity. However, it is the economical viability and not the environmental benefit that is normally considered as the driving factor that influences, whether such systems can actually be built and operated. Nonetheless, in cases of low economical feasibility, flaring landfill gases could be an alternative, or if not attainable (due to a decline of gas production, or for other reasons), landfills are then closed and abandoned. When landfills are closed, gases are released into the atmosphere unabated for many decades. Figure 2 shows a general trend of methane emission from landfill using a computer model, indicating the life cycle of landfills, which can take different shapes and forms for each particular landfill [6]. For these typical landfills, 30% of gas emissions can occur during the operation of the land active fillings, occurring prior to the closure, and that active filling could take up to 20 years. Typically, only 50% of the gas can be produced during the first 30 years after closure, and the rest of it, approximately 20%, is produced afterwards, continually for the next 100 years of the life of the landfill. The remainder of the gas will be emitted continuously, and which is not recoverable. This

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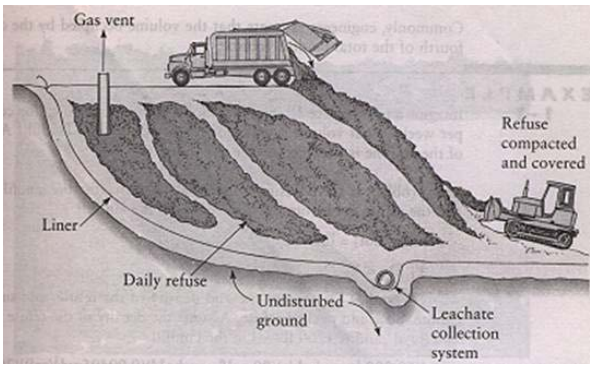

Figure 1: Schematic diagram of typical landfill cells and coverings [55].

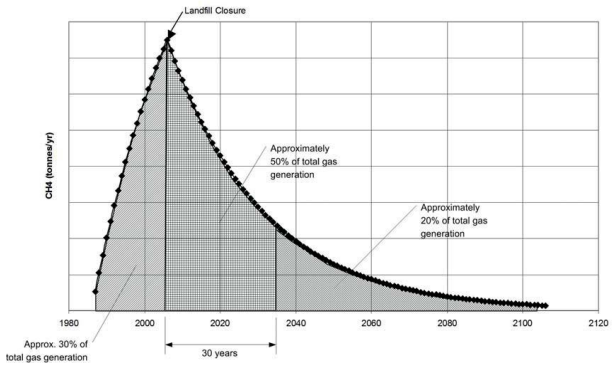

Figure 2: Indicative methane generation curve of landfills before and after closure [6].

means that only 50% of gas is available for collection, regardless of the efficiency of the installed collection systems [7]. This high proportion of wasted gas has triggered research works on determining other approaches to control and transform methane gas into the less harmful gas, CO_2, and to reduce the carbon budget of the process. In addition, more methane could also freely escape unchecked during the active filling of the landfill before closure, if wastes were uncovered for some time or covered with unexposed methane cover materials, during the 20 years of active fillings.

Methane gas production in landfills is attributed to anaerobic microbial digestion and degradation of organic matter, ultimately by methanogens growing under suitable conditions of p^H, temperature, and humidity [8,9]. Other microorganisms however, mostly methanotrophic bacteria (methanotrophs) can oxidise methane biologically into CO_2 and other by products. These methanotrophs are gram-negative bacteria [10] and are able to utilise methane as their sole source of carbon and energy. They were first identified by Sohngen [11], but the detailed identification, classification, and characterisation of more than 100 organisms had not been done until after 1970 [12], Thereafter, it was recognised by researchers and waste managers alike, that methanotrophs could offer a possible solution to the control of fugitive methane emissions from landfills. Subsequent research studies on the methanotrophs have classified them into three main categories, namely, Type I, Type II, and Type X [12]. The first and third types use one pathway and sometimes, they are classified into the same group; while Type II uses a different pathway. These pathways involve complex enzyme processes and have been described in detail by Hanson and Hanson [12]. Hilger and Humer [13] defined the stoichiometry of methane oxidation by two different biochemical pathways as follows:

- Ribulose monophosphate pathway (RuMP):

$$CH_4 + 1.5\ O_2 + 0.118\ NH_4^+ \rightarrow 0{,}118\ (C_4H_8O_2N) + 0.529\ CO_2 + 1.71\ H_2O + 0.118\ H^+ \quad (1)$$

- Serine pathway:

$$CH_4 + 1.57\ O_2 + 0.102\ NH_4^+ \rightarrow 0.102\ (C_4H_8O_2N) + 0.593\ CO_2 + 1.57\ H_2O + 0.102\ H^+ \quad (2)$$

where $(C_4H_8O_2N)$ represents the biomass produced by the bacteria.

Through biochemical reactions initiated by the bacterial enzyme, methane monooxygenase enzyme (MMO), energy is released by first converting methane to methanol, and then to other carbon compounds as intermediates [14]. Although Type X methanotrophs can utilise the ribulose monophosphate (RuMP) as the primary pathway; albeit, they also possess a low level of enzymes from the serine pathway. Subsequently, they can grow at higher temperatures, a characteristic that has made them distinct from Type I methanotrophs.

Methanotrophs are a unique kind of bacteria that are capable of utilising methane from a variety of environments, under a wide range of conditions. They are present in sediments [15], groundwater, seawater [16], peat bogs [17], hot springs [18], salt storage, and in the Antarctic [19].

Therefore, the process of oxidising methane by methantrophic bacteria in all of these environments could be considered as a carbon sink process. This unique potential of methanotrophic bacteria for the remediation of the environment has encouraged researchers to focus on optimising the oxidisation processes of these bacteria and exploiting this biological activity on engineering remediation systems.

Methane Oxidation Processes in Landfills

Gases emitted by landfills are produced anaerobically under specific soil conditions, where methane and carbon dioxide are the two main gases produced (among other trace gases) in a ratio of 55-60% v/v to 40-45% v/v, respectively [20]. However, the oxidation of this methane by methanotrophic bacteria in the topsoil layer of the landfills is dependent upon many factors, which can be summarized as follows:

- Landfill disposed waste factors:
  - Amount and composition of waste
  - Waste fermentation time
  - Humidity and moisture contents
  - Acidity of the waste and the soil
  - Methanotrophic bacteria culture type and concentration
  - Air permeability and aggregate porosity
  - Soil compaction and soil types
  - Mineral presence, contents, and concentrations
  - Methane production rates, gases concentrations, and gases proportions
  - Inhibiting substances
  - Accumulation of bacteria's biomass, the extra cellular substance (EPS).

- Landfill site location and surroundings:
  - Site barriers
  - Site geological formation, voids, and cracks
  - Site porosity and diffusivity
  - Site water contents and seepage
  - Water table depth and water salinity
  - Landfill design and construction
  - Landfill covers and irrigation.
  - Meteorological and atmospheric:
  - Barometric pressure
  - Site temperature
  - Site wind speed and directions
  - Amount of rain and frequencies
  - Site location and latitude.

These categories are the main factors that influence the oxidation process of methane by the methanotrophic bacteria within landfills and the methane production by methanogens. This can either be a result of an influence of each factor individually, or by an influence of combined factors working collectively, that affects the oxidisation and production process of methane [20]. Notwithstanding, engineers, waste planners, and researchers are interested only in those factors that can be managed, changed, and modified within landfill wastes and the environment of the containments.

In terms of exploring landfill factors in general, existing research has focused on the effects of soil conditions [21], moisture content [22], methane oxidation [23], biomass accumulation [24], physical determination of methane oxidation [25], landfill cover materials [26,27], landfill containments, inhibiting substances [28,29], soil temperature [30], gas diffusivity, soil capacity and methane diffusivity [31], and the methanotroph community structure in landfills. Additionally, oxygen availability has been identified as the most important factor affecting the growth of methanotrophic bacteria in the top cover layer (dependent on porosity) [30,32]. However, other factors are also important, such as landfill waste content and methane production rate, structure and location of landfills, p^H of cover soil, and soil mineral composition, all of which are difficult to manage and control from an engineering standpoint. Consequently, research has been focusing on identifying factors that are most effective in reducing methane emissions and those most readily manageable in stimulating an increase in methanotrophic activities.

Concepts and Methane Oxidation Technologies

Flaring or using methane as an energy source is one of the well-known conventional processes for methane oxidation for decades. Conversely, and in light of recent discoveries, researchers have started to employ aerobic reactions as a way of methane elimination, through the use of methanotrophic process, which is regarded both as an economical and an environmentally viable elimination process. Taking all these into account and the knowledge that approximately 85% of produced methane gas from conventional uncontrolled landfills escaping into the atmosphere, have prompted researchers to explore other means of enhancing methane oxidation [33].

Increasing number of investigators have concentrated more of their efforts on the redesign of the top cover soil of landfills, showing a potential of eliminating higher percentage of produced methane. The most commonly redesigned system of landfill's top cover soils is the arrangement of different layers on top of each other, in which an oxidation layer, typically compost material, is placed over a gas distribution layer, made up of a material, such as gravel, that has the features of high permeability [7]. This arrangement, known as a bio-cover system, is intended to encourage the homogenisation of gas and air fluxes together, and therefore, could have a higher potential for methane oxidation. Bio-covers are more effective when used on a large scale, in order to cover more of the area of the landfills for higher rate of oxidation, making it necessary to use large amounts of structural support materials. Thus, even though bio-cover systems are relatively an efficient way of eliminating methane, they could also prove to be a potentially expensive undertaking.

Another methane oxidation enhancing method is the bio-filtration system. This gas capture system is constructed by digging a small area of space in the top cover soil, then, the space is filled with biomaterials for purposes of capturing the gases produced from bacteria degrading the waste. Three different bio-filtration design systems have been used, such as bio-windows, which are cells of spaces, cut into the cover soil and filled with support mediums and the bio-filters, which differ from bio-windows in that, they are contained in the cover layer of the landfill [7]. The third system is the bio-tarp cover, which is a temporary system made of a thick film, infused with methanotrophic bacteria, and placed daily over an on going operation of filling an active landfill site. The inducement of bacteria is done, so that the bio-tarp could immediately consume the escaped methane gas reaching the top soil, thereby, reducing fugitive gases while operating on the site. These systems are designed so that they can create a favourable environment for the methane capture and elimination [34,35]. Moreover, by utilising these types of systems, the parameters for oxidation, such as methane and oxygen loadings, moisture content, temperature, filter material composition, and layer arrangements become more obtainable and measurable. In comparison to the active gas management systems, such as the active collection and flaring of the gas, the use of bio-filters has been determined to be economically more viable, particularly for smaller landfills [36].

The implementation of bio-cover and bio-filter systems has been examined in greater detail, where their arrangement and filtration settings have been investigated by addressing the favourable settings for the methanotrophic bacteria to work more efficiently. Table 1 illustrates some of the design concepts for these systems, with corresponding references.

It is interesting to note from the design of these filtration systems, that the designers have attempted to strike a balance between numbers of conflicting parameters. For instance, as the gas rate increases in landfills due to the high rate of waste degradation, as a result of high organic composition and quantity, the flux of methane upward tends to replace oxygen in the soil's pores, causing less oxygen to be available for the methanotrophs. Additionally, if the landfill covers have had high permeability characteristics due to these pore spaces; then, it is more likely that moisture, leachate, and fine soil particles would fill into these spaces. Another conflicting parameter that must be considered into the designs is the increased level of nutrients within the top cover layer (i.e., immatured compost). When used for increasing oxidation, while this is beneficial for the methanotrophs to be fully active, it may also lead to production of more methane by other microorganisms present

Bio-cover Design	Purpose	Source
A bio-cover constructed from a gravel layer, under a 1.2-m compost layer and over landfill waste	To distribute gas loading into compost cover layer	Humer and Lechner [47]
A bio-cover made of an oxidation enhancing material over coarse material as a bio-cover over landfill waste	To enhance methane oxidation with minimum cost of construction and maintenance	Huber -Humer et al. [7]
Capillary barrier layer under a bio-cover layer fed by pipe system removing gas from below the barrier into the bio-cover layer	To activate oxidation without allowing water and leachate to seep through to the ground water layer	Etalla and Vaisanen [57]
A constructed passive bio-filter within a landfill-capping layer with a cross-connected piping system to landfill drainage	To oxidise methane gas without allowing leachate contamination and to improve oxidisation	Dever et al. [58]; Cabral et al. [59]
A passive bio-filtration and drainage system, consisting of a compost layer placed over a geo-textile layer and in turn, placed above coke filling in a contained box. This allows gas to filter from below, through the drainage systems and into three design concepts, such as pile, middle sunk, and counter sunk.	Passive system for small landfills and cost-effective when compared to active gas collection system	Straka et al. [36]
A construction of a bio-window containing a compost medium, erected within existing conventional landfills and through the capping layer, allowing gas to migrate into this higher permeability window.	Useful for old landfill, when drainage and infrastructures are not in existence	Kjeldsen et al. [60]
A bio-filter gas collection system constructed from a box containing bio-medium, a geo-textile separation layer above a high permeability aggregate layer, and with landfill gas entering into this aggregate layer	To be used for point gas emission from leachate drain dumps, uncapped monitoring walls and/or for a temporary bio-filter for a landfill	Dever et al. [61]
A vertical bio-filtration trench surrounding the landfill site, consisting of a bio-medium and above a geo-textile separation layer. This is all placed above a high permeability aggregate layer. The filter is built vertically and continues down to the bottom of the landfill or water table.	To capture lateral gas migration, or escaped gas through the landfill lining	Dever et al. [58]
A distribution bio-cover layer of a bio-char material as an amendment to the landfill, in order to passively oxidise landfill gases.	To encourage more diffusion of oxygen through landfill layers, hence oxidising more methane.	Yaghoubi [44]

Table 1: Passive biofilter design concepts used in experiments.

in the soil, feeding abundantly on the nutrients, and producing their own methane, therefore, causing an added source of methane escaping from the landfill.

In order to analyse the methanotrophs' oxidation processes, it is a standard procedure to experiment with either a batch test or a column test within a laboratory environment. Researchers have conducted a number of experiments using these methods, and have introduced within each method a variety of materials and arrangements to calculate the methane oxidation rates, under different methane loading conditions. Table 2 presents a number of these selected materials with the observed oxidation rates [20]. From this table, it may be noted that the oxidation rate from one set of experiments is different from another. For instance, when using the sandy loam material in the experiment, the rate of oxidation was 19%, with a loading of 281-g CH_4 m-2d-1; whereas, Humer and Lechner [37] observed for the same material a higher rate of oxidation reaching 42%, with a lesser loading of 180-g CH_4 m-2d-1, over a shorter period of time. The same observation of different results was noted from the experiments conducted by Stein and Hettiarachi [38] as regards the landfill loam material. Similarly, the data in Table 3 show estimated rates for methane oxidation from field test experiments with fractional oxidation of the total flux of methane reaching the cover layer itself. The data also indicated varying methane rates from one study to another, showing an oxidation rate of 60.7-g CH_4 m-2d-1 for the sandy loam cover material in the study of Borjesson et al. [23], in contrast to only 7.3-g CH_4 m-2d-1 from the study of Abichou et al. [39].

Interesting results were obtained by Pawlowska and Stepniewski [40] which showed methane oxidation rate to have increased in a continuous fashion in an experiment, with the increase of methane loading to a certain constant level, then, gradually leveling off. This result however contradicts the findings of other studies found in Tables 2 and 3. These results further highlighted the complexities involved when exploring landfill cover oxidation processes, particularly when there is a lack of any standard setup or comparative system having put in place for reference [32].

Soil Material	CH_4 Loading (g CH_4 / m^2d)	CH_4 Oxidation Rate (%)	Period (days)	References
Fine Sand Clay Top Soil Corse Sand	266 266 266	41 40 61	180 180 180	Kightley et al. [41]
Sandy Loam	281	19	120	Hilger et al. [24]
Sandy Loam Sand Sand	180 94 216	42 96 97	51 84 84	Humer and Lechner [37]
Landfill Loam Landfill Loam	186 319	50 32 – 38	260 260	Stein and Hettiarachi [38]
Soil - agricultural (Rocky View Dark)	310	32	260	Stein and Hettiarachi [38]
Laomy Sand	525	83	314	Park et al. [62]
Landfill Laom	250	81	30	Scheutz and Kjeldsn [63]

Table 2: Loading and oxidation rates of methane in soil, columns experiments[1] . **Note**: [1] Selected from Scheutz et al. [20].

Cover Layer Material Amendments

Material amendments to landfill covers are an added aspect in the design of viable bio-cover methane reduction systems. These amendments are used to support the activity of the methanotrophic bacteria and reduce the methane from escaping to the atmosphere. This can be seen when comparing the estimated 85% of methane generated from conventional landfills, naturally escaping to the atmosphere when not controlled, with a significant reduction of that percentage to only 14% that could escape when using composite covers on landfills [33]. Thus, amendments of materials to the soil of the top cover layer are essential for catalysis, and therefore, researchers have been in search to find an optimal material, albeit, with mixed outcomes. For their experiments, they used varieties of soil amendments, such as

Cover Material	CH_4 Oxidation Rate (g CH_4 / m^2d)	Fractional Oxidation[2] (%)	References
Sand – Clay Loam	14.5	-	Czepeil et al. [64]
Mulch and Topsoil	26.8	26	Chanton and Liptay [65]
Sandy Loam	60.7	26	Borjesson et al. [23]
Compost	0.7	55	Barlaz et al. [66]
Sandy Loam	7.3	25	Abichou et al. [39]
Yard Waste Compost	1.7	38	Stern et al. [48]
Mulch and Topsoil	26.8	26	Stern et al. [48]

Table 3: Estimated CH_4 oxidation and fractional oxidation from field studies[1] .
Note: [1] Selected from Chanton et al. [31]. [2] % of oxidised CH_4 over the total flux into the cover layer.

agricultural soil [38], fine and coarse sand and clay topsoil [41], silty clay soils [42], matured and immatured composts [24], earthworm cast and activated carbon soil [43], bio-char (carbon-rich material obtained as a result of heated plant-based biomass in a free oxygen container) [44], crushed glass and ceramic [45], spent grain [46], and porous mulch [47]. Subsequently, a notable amendment material came out from these investigations that had drawn the interest of researchers and engineers alike. Compost material was found to be the most suitable for a landfill cover amendment. This material has been known to possess permeability properties, sufficient moisture retention characteristic, providing sufficient number of pores at high moisture content, fine texture, and is biologically stable. However, this material can produce methane by its own, particularly when used in an immature state [7,30,32,48]. Nevertheless, researchers and engineers have sought to use it as an additive to landfill covers, having shown its potential for higher methane oxidation.

Even though compost material is the choice of researchers for testing for top cover layers, there are a number of issues related to the use of this material that should be highlighted. For instance, compost material could produce methane under anaerobic conditions if little oxygen were available in the soil. This process of producing methane can inhibit the activity of the methanotrophic bacteria, particularly if high concentration of nitrogen is present in the compost material in the form of ammonium. The ammonium and the methane are therefore in competition for oxygen, leaving the bacteria with little oxygen [49]. However, to mitigate this problem, the compost material can be left to mature for some time, before using it as an amendment. In addition, compost contains high amount of nutrients, and as previously explained, it could allow other microorganisms to grow and compete for oxygen as well. Table 3 shows a higher percentage (55%) of fractional methane oxidation when using compost material as an amendment cover material in comparison to all the other materials that were tried on field tests. These fields tests further affirmed that compost is indeed a better material for cover use; although, this could also be attributed to the lower loading rate.

Methane Oxidation

Methanotrophic bacteria are very delicate microorganisms that are, on average, present at the top 20-35 cm of soil [50]. These microorganisms evolve in accordance with their surrounding environments, particularly where there is an abundance of methane. These bacteria metabolise methane at their own pace to produce CO_2 and other products, according to the aforementioned Equations 1 and 2. As with all living organisms, this surrounding environment can affect their activities and existence in ways that are hard to measure, given the varying makeup and composition of the waste materials from one site to another, particularly in terms of the types of nutrient, chemical, and mineral combinations. Among these combinations are various factors that can suppress their activities, such as the presence of copper in excess of 4.3 mM (60 mg/kg of soil), a pH value that is outside the range of 2.0-7.65, a presence of inhibiting elements, such as ammonium, diffusion of nitrogen and methane concentrations [21,29]. Another important factor to note is the latitude location of the landfill site itself. This factor is temperature-dependent and can determine the whereabouts of the bacteria within the landfill, as well as the dominant type of bacteria present in the soil. In this regard, the bacteria behave in a way that they protect themselves from unfavourable heat or cold fluctuations by coagulating up or down the top layer, in accordance with this fluctuation [23]. At the same time, this is met by the distribution of oxygen and methane along the depth of the top layer, which is a gradient distribution that runs counter and opposite of each other [51]. Hence, at the top of the soil cover, the methanotrophs will have ample supply of oxygen, but insufficient supply of methane. On the other hand, at the bottom of the soil cover, the case was found to be opposite, i.e., ample supply of methane, but insufficient amount of oxygen [52]. This kind of a situation can deprive the bacteria from the needed elements at their temperature-bound location at the top cover layer.

Oxygen is an essential element needed for methanotrophs to metabolise methane for survival. Oxygen is found in landfill soils through the process of diffusion into soil pores by the action of atmospheric wind, molar, and barometric pressures. Therefore, the rate at which it diffuses through the soil is dependent upon the mechanism of the atmosphere, as well as upon the permeability of the soil and the type of the cover materials present [20]. As stated earlier, large pore sizes will allow moisture, leachate, and other fine particles to fill in the pore gaps, forcing oxygen out; although too little will not provide adequate space for oxygen to diffuse in sufficient amounts, subsequently, preventing oxygen from diffusing into the pore spaces. This fact has motivated researchers to explore other option to optimise landfill materials for oxygen availability, in order to maintain sufficient levels of methanotrophic activities. Suitable materials for that purpose should not only be environmental-friendly, but also should be made of readily available and cost-effective materials. All efforts to find such material have been tried with varying successes. As yet, only compost material has been found to stand out to meet the criteria. However, with the aforementioned drawbacks, it is imperative that this compost material must be fully matured in order to lessen its own production of methane.

The availability of oxygen in landfill covers was investigated, indicating that the oxygen concentration rate can gradually increase the activities of the methanotrophs, specifically when oxygen rates increase from 2.5% to 15%. Henceforth, the rate can provide a constant level of activities. This highlights the importance of oxygen concentrations and its availability, for the bacteria to consume methane and indicates the capacity of the bacteria to consume oxygen, as shown in Figure 3.

Oxygen availability and sustainability are essential factors and can increase methane oxidation rate significantly, only limited by the type of material amendments and the passive design concepts employed, as discussed previously. Due to the importance of delivering oxygen to the inside of the top soil, sometimes, active systems are employed as alternative to the passive systems and are operated by actively installing pumps to force the delivery of oxygen inside the soil [53]. However, these active systems are difficult to operate and maintain and also have their own drawbacks. For the passive systems, increasing the pore size in the soil to optimise porosity of the top cover soil or in the bio-filters for the

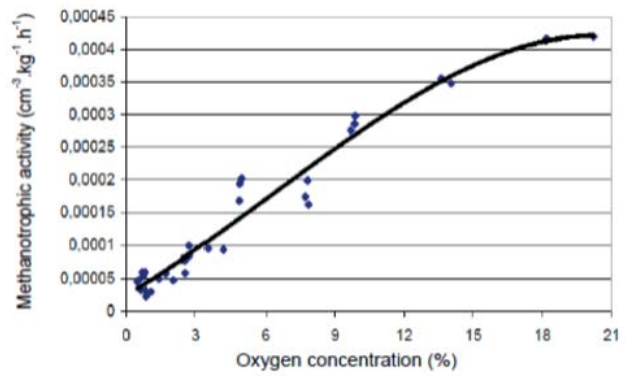

Figure 3: Effect of oxygen concentration on methanotrophic Activities [56].

purpose of holding more oxygen is a temporary solution, because the pores will soon be filled with silt, water, or leachate, and will close due to compaction. Moreover, these trapped oxygen moles found within the pores of the soil, trapped when the layers were first constructed, would also be depleted by methanotrophic bacteria consumption, with limited means of replenishment. From that point on, pore spaces will be filled with carbon dioxide and methane gas as a result of consumption and movement of the gas from the bottom of the layer to its top. Besides, methane production in landfills occurs almost immediately when materials of organic nature are deposited in a landfill. The reason is that the organic waste has already been decomposing during the transport by some degree, depending on several factors, such as time, humidity, and temperature inside the transporting trucks. This transport time of waste materials from households and various industries to the landfills, would certainly have effects on generating some gases, no matter how minimal. Cumulatively, they would have significant atmospheric effects. Unfortunately, this part of the process of methane generation has not been researched well, so far. More importantly however, is the time that is spent in filling landfills with wastes, which could last for years (Figure 2), leaving a significant amount of gas to escape prior to actively extracting gas by any controlling methods. This is because, traditionally, landfills are kept in a 'wait mode' until landfill sites are filled with wastes, covered, and then, controlling methods are put in place after some time.

Experimental Setup and Results

Methanotrophic activity in different soil types

Of utmost importance is the time for the methanotrophs to regenerate and start digesting methane in landfills, as it can affect the amount of pollutants emitted into the atmosphere. Therefore, it is essential to investigate the time for the methanotrophic bacteria to commence the degradation of methane, in addition to the importance of asserting oxygen availability in the soil, and more importantly, its sustainability in relation to various soil types. With these two objectives in mind, an experimental batch setup was prepared.

A batch reactor experiment was set up on a bench in the laboratory, in which samples of several 1000 ml, and 160 ml bottles, filled with different landfill top soils and leachates samples, selected from low and highly exposed methane locations, were taken from Coxhoe landfill, Durham, UK. All of these samples were obtained for experiments in sealed containers with tight caps carried away to the laboratory to compare with other samples that were taken from other places, as prescribed in Table 4.

The samples were all measured, and then a 20-ml portion was extracted from each, placed in the batch reactors and mixed with 10 ml of nutrients media solution (Table 5), which made the samples consistent. The remaining volume space of the reactors was filled with 30% methane and 70% of room air (having approximately 21% oxygen); then, the reactors were left to incubate on a bench. Methane percentage content in each of the incubators was measured continuously by drawing gas sample from each, using a syringe for a one-month duration. This kind of measurement was used as a way of measuring methane consumption. The results are shown in Figure 4.

These results presented in the figure simulated conventional and non-biologically active landfill covers, and showed little or no methanotrophic activities; therefore, another set of samples, with more added nutrient media solution, was prepared, as indicated in Table 6. The samples were again placed in batch incubators and subjected to the same conditions as in the previous set, except, that only 1 ml of collected subculture samples was mixed with 10 ml of nutrients media solution. The results of the observed activities that lasted about another month are plotted in Figure 5. The results shown in the figure indicated that methanotrophic bacteria present in the soil samples were active and responded well to dilution of the samples with the nutrients; even though each sample behaved differently in response to the different soil structure.

To investigate the behavior of the bacteria present in the soils, exposed to high or low methane loadings, a third set of samples were collected from different location sites in the landfill. Some were collected near the methane collection pipes, while some were collected away from them. In addition to these samples, a set of samples were also collected from Newcastle University grounds, which had little or no methane exposure. Table 7 shows these collected soil samples. Batch incubation tests of these samples, (all tests were done with duplicates to ensure accuracy, and all measurements were analysed twice), introducing approximately 22% v/v methane in each batch test; while the rest was filled with room air, with all other conditions kept the same as those of the previous set of samples. The results of the observations are plotted in Graph 6.

Bacterial response to oxygen availability

The effect of oxygen on the methanotrophic activities is known and documented in the literature; however, the presence of oxygen

Sample No.	Soil type	Added solution
Sample test 1	Landfill top soil samples in 160-ml bottles, from different locations (taken from Coxhoe landfill, Durham, UK)	10 ml of Media solution
Sample test 2		
Sample test 3		
Sample test 4		
Sample test 5		
Sample test 6		
Sample test 7		
Sample test 8	Landfill top soil with leachate sample in 1000-ml bottles 10/7/2012	
Sample test 9	River Tyne (UK) sample in 1000-mL bottle 10/7/2012	
Sample test 10	Pure culture sample in 1000-ml bottle*	
Sample test 11		
Sample test 12	Leachate sample 1 in 160-ml bottles, active landfill	
Sample test 13	Leachate sample 2 in 160-ml bottles, old landfill	
Sample test 14	Leachate Sample mix of 1 and 2 in 160- ml bottle	

Table 4: Landfill soil samples with added media solution. *Kept in the care of Dr. Angela of the Civil Engineering Geosciences Department, Newcastle University.

	Salt solution (g/ L)	Phosphate solution (g/ L)	Trace metal solution (mg/l)	Iron solution (g/L)	Sulfuric Acid
Solutions	$NaNO_3$ 85 K_2So_4 17 $MgSO_4$-$7H_2O$ 3.7 $CaCl_2$-$2H_2O$ 0.7	KH_2PO_4 53.0 NO_2HPO_4 86.0	$ZnSO_4$-$7H_2O$ 288.0 $MnSO_4$-$7H_2O$ 233.0 H_3BO_3 62.0	$FeSO_4$-$7H_2O$	H_2SO_4
Amount	100	100	500	1000	5- ml/100-ml Iron solution

Table 5: Commercially available media solution for cultures used in batch experiment.

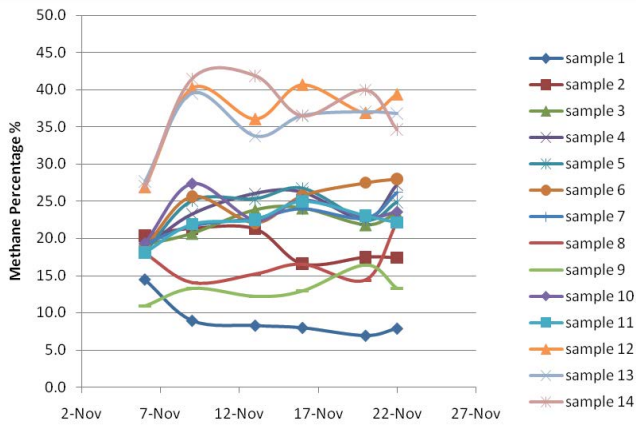

Figure 4: Soil samples with added media solution.

New samples No.	Amount from Earlier Sample	Earlier Samples	Added Media
sample 20	1 ml of soil samples	sample 1	in 10 ml of media solution
sample 21		sample 2	
sample 22		sample 3	
sample 23		sample 4	
sample 24		sample 5	
sample 25		sample 6	
sample 26		sample 7	
sample 27		sample 8	
sample 28		sample 9	
sample 29		sample 10	
sample 30		sample 11	
sample 31		sample 12	
sample 32		sample 13	
sample 33		sample 14	

Table 6: Soil sub-culture samples with increased media solution.

and its penetration into the soil of various types are in need of further investigation. When oxygen is diffused via the atmospheric and molar diffusion mechanisms, it tries to overcome the soil's microstructure obstacles to reach the methanogenic bacterial groups present in the lower layers of the soil. For this set of experiments, the reaction of

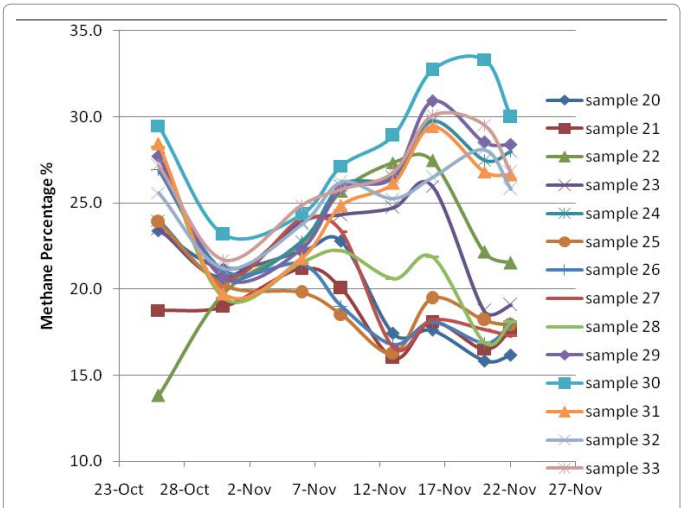

Figure 5: Soil sub-culture samples in an increased media solution.

Sample No.	Soil Type	Added Media
Sample 1a	Near top gas collection pipe	One millilitre of sample added into 10 ml of media solution
Sample 1b		
Sample 2a	Landfill lower side	
Sample 2b		
Sample 3a	Near methane collection pipe	
Sample 3b		
Sample 4a	Near Leachate collection pipe	
Sample 4b		
Sample 5a	Garden soil	
Sample 5b		

Table 7: Low and highly exposed methane soils.

methanotrophs in the soil samples that were collected previously to oxygen availability is in need of further investigation. Thus, a new set of soil samples were obtained from Newcastle University grounds, the samples were then mixed with distilled water and nutrients, and all were prepared as samples for batch incubation investigation in duplicates. These sets were split into two groups of the same composition; one group was tested while still on a bench, and the other group was tested on a continuously shaking platform. The shaking condition was intended to enhance oxygen penetration. These two groups of samples were compared with the earlier samples that were collected from landfill locations, taken from specific points in the landfill, as described in Table 6. All were tested under the same set of constants, both of temperature and nutrients, as those of the previous sets of tests. These new sets of samples are shown in Table 8. In each of these sets, methane was introduced as a percentage of volume per volume by replacing a 10 ml of air volume with the same volume of methane gas using a syringe. All observation results were plotted for methane oxidation in relation to time, as indicated in Figures 6,7, 8, and 9.

In addition to the standard air volume existing in the batch reactors, an injection of oxygen was also introduced into all of the samples in this set of experiment, using a syringe. Ten millilitres of oxygen was introduced once, and then again, at intervals shown in Figures 7, 8, and 9, for all of the samples described in Table 8. This was done in order to understand the effects of oxygen availability, if it were to be available to the bacteria in intermittent or continuous presence, besides the existing static oxygen in the reactors. The results are shown also in these figures. The shaking condition subjected to the incubation

Sample No.	Status	Soil Mix.
Sample 1a	Placed on shaking platform	University ground garden soil Sample
Sample 1b		
Sample 2a		University ground garden soil with Sand
Sample 2b		
Sample 3a		University ground garden with distilled water
Sample 3b		
Sample 4a		University ground garden with nutrients
Sample 4b		
Sample 6a	Placed on a still bench	University ground garden soil sample
Sample 6b		
Sample 7a		University ground garden soil with sand
Sample 7b		
Sample 8a		University ground garden soil with distilled water
Sample 8b		
Sample 9a		University ground garden soil with nutrients
Sample 9b		
Landfill Sample 1, Near top gas collection pipe. (LFS 1)	Placed on shaking platform	with nutrients
Landfill Sample 2, Lower side location. (LFS 2)		
Landfill Sample 3, Near methane collection pipe. (LFS 3)		
Landfill Sample 4, Near leachate collection pipe. (LFS 4)		
River Tyne sample, taken for river bank. (Tyne S1)		

Table 8: Soil sample types and condition comparisons.

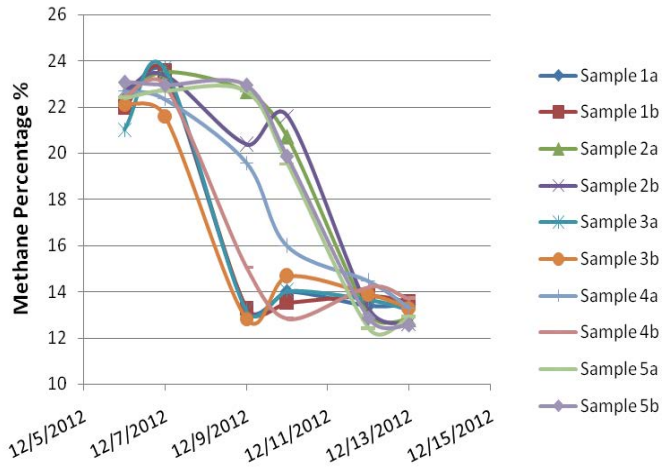

Figure 6: Comparison between low and highly exposed methane samples.

reactors, was intended to allow further air and oxygen to penetrate into the body of the samples.

Discussion

Data collected from the batch reactors experiment, as previously indicated, would suggest that an added nutrient to dilute the soil samples to allow better oxygen penetration, could be an important factor for the methanotrophs to assimilate methane, as shown in Figures 4 and 5; however, this fact is linked directly to the availability

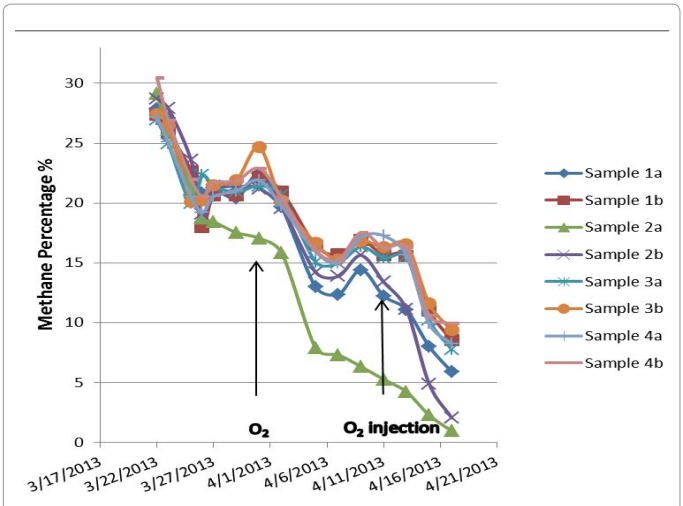

Figure 7: Soil samples under shaking condition and after the introduction of oxygen.

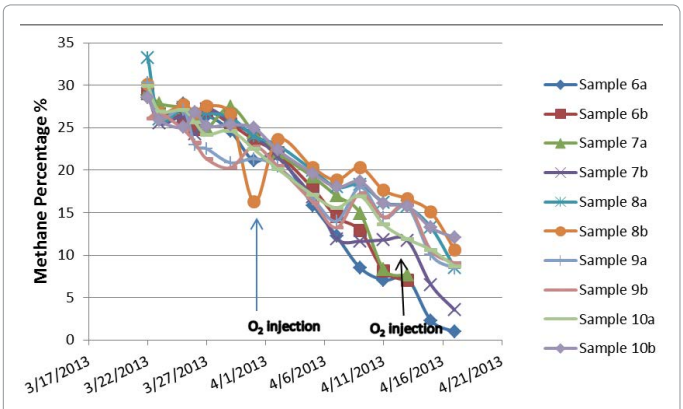

Figure 8: Soil samples under still conditions and introduction of oxygen.

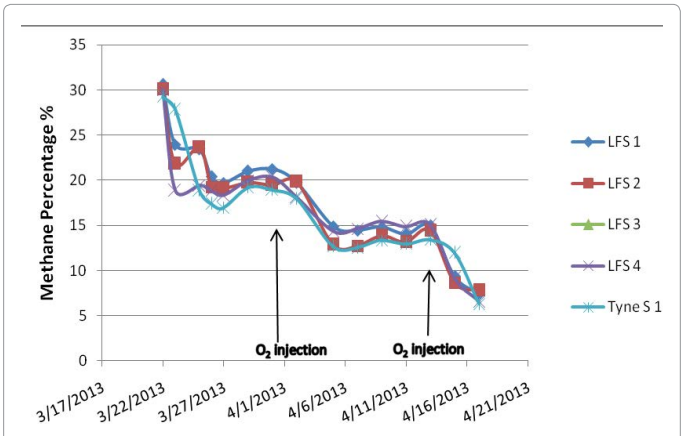

Figure 9: Landfill samples under shaking conditions and after the introduction of oxygen.

of oxygen. Then, when oxygen was made more available by introducing the shaking mechanism to allow air penetration further into the soil, uniform activities of methane consumption were observed in the presence of nutrients. Increasing nutrient amounts as prescribed in Table 6 produced mixed results, as indicated in Figure 5, where methane

consumption increased by approximately 30% in the 10-day interval for most of the samples tested; afterwards, began to decrease continuously, except for samples 20, 21, 25, 28, and 32. These results can be explained by the makeup of the soil samples that have porous grains and holding more oxygen, allowing the bacteria to consume more methane and continuing for a longer time span until reaching constant methane percentage average. As of the latter samples, they held less oxygen, and thus, allowed poor oxygen consumption performance. These findings confirm the well known fact, that in choosing the types of soil to be used in landfill cover, porosity of the soil is important to fully utilise the full potential of the aerobic bacteria.

When designing landfill bio-covers, little has been known about the effect of soil types on the time period that it will take methanotrophs to be activated after the immediate installation of the cover. Because of the high amount of methane emitted into the atmosphere, estimated to reach an average of 760 MMT of CO_2 equivalent ($MMTCO_2$-eq) from landfills alone [4], and given the ever increasing human and land filling activities, the choice of the type of soil for bio- covering or bio-filtration, that will enhance methane consumption without delay, is essential and of great importance. Therefore, a comparison was done by using low and high methane-exposed soils to understand the time effect of choosing soils for biologically active and non-active soils, simulating the active and non-active covers, as described in Table 7. The results of these observations are plotted in Figure 6, for soils obtained from active landfill and non-active garden grounds (university ground). The time variation between active and non-active soils, when used in batch reactors, is quite clear, as it took approximately 4 days for the methanotrophic bacteria to regenerate and be active in consuming methane compared to non-active soil. The lower side location of the landfill sample, taken a distance away from the methane collection pipe and the clay and sandy soil sample taken from Newcastle University grounds, both showed to have reduced methane from approximately 22% v/v methane to approximately 13% v/v of methane. Both exhibited a time delay of 4 days for the bacteria to be fully active. Conversely, the other samples, taken near and on top of the methane collection pipe of the landfill, have consumed the same rate of methane consumption; however, they exhibited an immediate response of consumption. In contrast, the samples collected from near the leachate pipe had an intermediate response. This latter behavior could be due to the unfavourable toxic habitat to the methanotrophic bacteria by the leachate section of the landfill.

It was also noted from the shape of the curves 5a and 5b of Figure 6, that once the bacteria had been activated, the rate of consumption of approximately 7.15 v/v of methane per day in the figure was the same for both the high and the low exposed samples. This would imply that the bacteria type and the quantity had reached the same rate of activities once the environments of both were the same, with only a time delay. Typical landfills in Figure 1 have earth's soils used as covering on cells, which are soils of having little or no methane exposure at the time of the operation; meaning in that, some time must have passed before the methanotrophic bacteria could start assimilating methane. In a year, approximately 300 working days would allow the production of 300 cells, typically covered with unexposed earth's soils, which could let unassimilated methane to escape to the atmosphere. Each cell, and according to Figure 6, would allow a time of 3-4 days of unchecked methane to migrate from cell to other cells and , to the atmosphere. Given the fact that global estimation of landfill gas production is an imperfect science, usually based on the amount of biodegradation of the biological component of the waste, with date used to be simulated by employing theoretical models [54], an estimate of methane escaping due to this lag of methanotrophic activity could be calculated. Taking the global estimated methane production to be of 760 $MMTCO_2$-eq per year [4], and since there would approximately be 300 landfill cells created during the working days; then, this could translate into 2.53 $MMTCO_2$-eq of methane production per cell per year, globally. Consequently, 3–4 days of unassimilated methane would produce 7.6 to 10.1 $MMTCO_2$-eq a year, or 2.1 to 2.8 x 10^4 $MtCO_2$-eq of methane per day on a global basis, which would escape unoxidised to the atmosphere. For landfills to operate for approximately 20 years before closure, according to the US-EPA landfill model in Figure 2; then, this produced amount of methane per day globally would constitute an important factor for landfill managers to consider when covering cells on a daily basis. Figure 2 shows a graph generated from the US EPA LandGem model to estimate methane generation through the life cycle of a landfill. The figure shows that landfill gas production increases continuously and incrementally up to closure time, after which landfill gas production would decrease rapidly. If control methods were to be installed for landfill gas recovery, the curve would take different shapes. This lag time, if not addressed, could result in the escape of methane into the atmosphere, and consequently, could translate into either carbon international taxation costs, or cover redesign costs to readjust landfill covers.

As shown in Figures 7 to 9, the shaking action performed on the samples, regardless of their soil types, had profound difference on the consumption of methane, and in essence, allowing oxygen to penetrate through the soil particles and distribute the oxygen moles directly to the methanotrophic bacteria to a wider range. However, this mechanism of shaking did not help much after oxygen was depleted. It is worth noting that after the depletion of oxygen, the anaerobic action took effect, and methane started to rise again for almost all the samples, and more so, under the shaking and less, under the still conditions. To offset this behavior, an oxygen dose was injected into the reactors for all samples, for all conditions of the shaking and the still samples, at the time intervals indicated in Figures 7, 8, and 9. The oxygen dose was seen to last for approximately five days for both conditions, before it started to rise again. Another dose of oxygen was again injected which helped more in the reduction of methane, for both conditions. This dramatic reduction of methane when oxygen was introduced is clearly an important observation. In addition, the process of continuously dosing oxygen into the reactors in a sustained measure, has produced a continuous linear relationship in the reduction of methane with time. Worth mentioning is that, this linear relationship had the same methane rate of consumption, estimated at 0.90% v/v per day, for both shaking and still conditions in graphs 7, 8, and 9. However, the rate of consumption was much higher for samples under shaking condition, estimated to equal 2.0% v/v per day, before oxygen depletion, in comparison to only 0.8% v/v per day for the samples under still condition, a rate of which was seen to have doubled for the shaking condition, at the same time interval.

Conclusion

The batch reactor experiments in this investigation showed that three to four days of lag time would result before an active oxidation could occur, when using typical earth's soil for covering on a landfill. It was estimated that 2.1 to 2.8 x 10^4 $MTCO_2$-eq CH_4 per day for every landfill globally, would escape from the landfill before the methanotrophic bacteria could have the time to regenerate and take hold in the cover soil. If this amount of landfill gas were not to be controlled, fugitive methane would escape into the environment unabated within an active lifetime of a landfill, which could last up to 20 years of the filling. To

combat this time lag, a cover soil would have to be impregnated with active methanotrophs and used, instead of the common practice of using the site's earth's soil for covering. However, this step had to be weighed carefully, since immature and highly exposed soils could produce their own methane through anaerobic action, and could inhibit the methanotrophic bacteria, particularly when nitrogen present in the cover is high [49], producing more methane into the environment. The solution for these two extremes is to have an appropriate cover material that is matured, one that has been previously exposed to methane, and does not produce its own methane.

The experiments also showed that oxygen is an essential element in catalysing methane through the methanotrophic process, in which the bacteria metabolise and generate their own energy through the breakup of the methane in the presence of oxygen. This is, as recognized, a well-established process. However, the most important element in this process is the sustainability of oxygen in the cover soil compared to many of the other factors. Also, the experiments showed a dramatic increase in the consumption of methane by bacteria, when the soils were put on shaking platforms, allowing oxygen to penetrate into the soil, and hence, providing oxygen and nutrients to the bacteria. Moreover, almost two folds of methane consumption could result from this action compared to the batch incubators placed on still and unshaken conditions. Further, when the batch samples were dosed with oxygen in two separate time intervals, the consumption of methane went almost in a linear relationship behavior with time, suggesting that methane could be consumed totally, if oxygen were to be introduced continually. This indicated the importance of sustainability of providing oxygen into the soil cover. Likewise, almost all soil samples, regardless of their physical and chemical compositions, reacted in a very similar way under oxygen availability, again implying that oxygen is an important and is the dominant factor to be considered when designing a bio-cover system, in relation to the types of soils, micro environmental conditions, and the degree of exposure to methane.

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