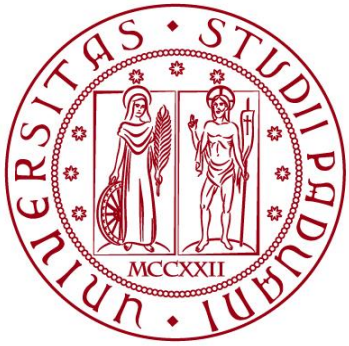
UNIVERSITÀ DEGLI STUDI DI PADOVA
DIPARTIMENTO DI INGEGNERIA CIVILE, EDILE E AMBIENTALE
Department Of Civil, Environmental and Architectural Engineering

Corso di Laurea Magistrale in Environmental Engineering



TESI DI LAUREA

Investigation of aerobic and anaerobic oxidation of methane in hotspots of microbial methane oxidation systems

Relatore:
Chiar.mo PROF. ROBERTO RAGA
Relatori esteri:
Chiar.mo Univ.Prof. Ing. Dr.nat.techn. MARION HUBER HUMER
Chiar.mo Dipl. Ing. Dr. MARLIES HRAD

Laureando: IRENE ZILIO
2061897

ANNO ACCADEMICO 2022-2023

To my grandmother,

la dolce nonna Rina

ABSTRACT

Landfill gas, in which methane is included, is produced by the biological degradation of untreated organic waste, and if released in the atmosphere can contribute to global climate change. As a complementary or replacement measure to gas extraction systems, microbial methane oxidation systems (MMOS) can control methane emissions from landfills.
This thesis work focuses on the research to improve MMOS performance; both aerobic and anaerobic methane oxidation in landfill covers (LC) are investigated. In order to do so, both literature review and laboratory experiments have been performed. Particularly, anaerobic methane oxidation in landfill covers is presented, as it is a novel research field; indeed, to date, very few studies have been performed on this topic.

The laboratory experiments performed are mainly two, in order to determine aerobic and anaerobic methane oxidation potential of a compost material, which was sampled in a biowindow eight years after the installation. The material was sampled at two different depths: the shallow part – which represents the main methane oxidation horizon – and the deeper part – which might have been exposed to anaerobic methane oxidation – at a hotspot area, where the MMOS was temporarily overloaded with methane. The first experiment was performed under aerobic conditions in four soil columns. Two of them were filled with soil samples from the shallow part of a biowindow hotspot, while the other two with the deeper part of the same hotspot. Pure methane was supplied from the bottom of the columns and air from the top; three different gas phases were investigated, in which both air and methane flow rates were changed. The second experiment was performed under anaerobic conditions, using two of the columns of the aerobic test, one filled with the shallow hotspot material and the other with the deeper samples. To simulate anaerobic conditions pure nitrogen was supplied at the top, instead of air, and methane was provided from the bottom. The parameters monitored during the tests were the composition (CH_4, CO_2 and O_2) of the exhaust gas and of the column interior gas at various depth levels (10 cm, 20 cm, 30 cm and 40 cm), together with chemical, physical and maturity parameters, such as pH, organic content, water content etc., checked at the beginning and end of both experiments.
Concurrently with the main experiments, Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed in order to compare the IR spectrum of biowindow field samples with material from column experiments, under aerobic and anaerobic conditions.

Results from the columns under aerobic conditions show the high oxidation rate (> 90% for a methane load of 200 Nl CH_4 /m^2d, and > 70%, for a methane load of 300 Nl CH_4 /m^2d) of the material, even

after eight years of temporal methane overload, under optimised laboratory conditions. A slight difference is found between the material coming from the shallow part of the hotspot and the deeper one. In particular, only when the methane flow rate was increased, in the second phase of the experiment, the material of the shallow part of the hotspot shows a bigger decrease in the methane oxidation rate, with respect to the deeper part, indicating a higher sensitivity to methane load, perhaps due to the formation of extracellular polymeric substances (EPS) – execrated by the microorganisms under stress conditions – or to the higher adaptation to methane load of the microbial community present in the lower layer.

Regarding the columns under anaerobic conditions, just three measurements are reliable, due to technical problems during the experiment. Nonetheless, these results show a decreasing profile of methane concentration from the inlet to the outlet gas. This could imply the presence of methane oxidation also under anaerobic conditions, even if at significantly lower rates with respect to the one observed under aerobic conditions. This experiment should be performed for a longer period, with a higher number of samples, in order to have a statistically significant number of measurements to evaluate anaerobic methane oxidation process in microbial methane oxidation systems.

The results from the FTIR comparisons, show no differences in the composition of the compared samples. The only differences found were in the concentrations of ammonium ion, organic sulphates and EPS, which poorly varied between the analysed samples. Also in this case the comparison should be performed with a substantially higher amount of samples, to obtain statistically significant results.

Highlights and take-home points of this Thesis are:

- Methane oxidation is existent in both aerobic and anaerobic conditions
- Hotspot material is capable to oxidise methane with a high rate, even after eight years of methane overload, if put in proper texture, temperature and humidity conditions
- Anaerobic methane oxidation in microbial methane oxidation systems is still an open topic, worth to be explored in future research, to finally improve the knowledge of the processes ongoing in the deeper layers of an MMOS
- FTIR spectroscopy can be a useful tool to assess the behaviour of different MMOS parameters, if performed on a statistically significant number of samples

List of contents

Chapter 1: INTRODUCTION

Methane is the second most abundant anthropogenic greenhouse gas (GHG), after carbon dioxide. Its global warming potential is 81.2 over 20 years and 27.9 over 100 years (IPCC, 2021). Landfills and the waste sector is responsible for ~20% of anthropogenic methane emissions (United Nations Environment Programme and Climate and Clean Air Coalition, 2021); particularly, in Europe the landfill and waste sector produced 13388 kt of methane, in 2022 (International Energy Agency, 2023). In landfills, whenever economically feasible, gas collection systems are recommended for landfill gas emissions control and recovery. Nevertheless, collection systems are not 100% efficient, and emissions may also escape preferentially from and around wells and along the routes of installed landfill equipment. Furthermore, once methane concentrations in landfill gas, fall below 35–40% v/v and total gas production rates are 30–50 m^3 h^{-1}, energy recovery becomes technically and economically infeasible; when methane concentrations reach 20–25% v/v and landfill gas flowrates fall to 10–15 m^3 h^{-1}, the most suitable treatment method becomes high-temperature flares (Huber-Humer et al., 2008b). Below these values, the treatment of poor landfill gas becomes more expensive and complex, and the most promising technologies to control methane emissions from landfills are microbial methane oxidation systems (MMOS) (Huber-Humer et al., 2008b).

Microbial methane oxidation in landfills has been studied since the ‘80s of the last century, but a proper MMOS design has been recommended in 2002, by the Consortium for Landfill Emissions Abatement Research (CLEAR). Since then, numerous studies have been performed on this topic, and these systems have increasingly been implemented in different landfills.

Usually, MMOS are studied for aerobic methane oxidation; however, due to the limitation of oxygen diffusion, this process is only active in the top layer of the system. This leads to the fact that more than half of the technologies is under anaerobic conditions, therefore anaerobic methane oxidation could represent a potential methane removal process in landfill covers (Xu and Zhang, 2022).

This thesis is focused on research to improve the performance of microbial methane oxidation systems.

Both aerobic and anaerobic methane oxidation in landfill covers (LC) are investigated and the research questions, that this thesis work targets to answer are:

1. To what extent does anaerobic oxidation of methane (AOM) play a role in hotspot areas of microbial methane oxidation systems?
    - Is it possible to detect/measure/ quantify the effects of AOM in LC?

2. Which are the differences between oxidation efficiencies, in aerobic and anaerobic methane oxidation?
3. Which are the responses of different depth samples, under aerobic conditions, to different methane flow rates?
4. Can a MMOS still reach high methane oxidation rates after eight years of methane overload?
5. Is it possible to use Fourier Transform Infrared Spectroscopy (FTIR) to assess the differences between samples of different MMOS materials (aerobic versus anaerobic conditions), and to predict their methane oxidation capacity?

Chapter 2: BACKGROUND AND STATE OF KNOWLEDGE – LITERATURE REVIEW

2.1 European waste production and disposal

Waste is in everybody's lives. The production of waste in European countries by all economic activities and households amounted to 2153 million tonnes or 4813 kg per capita, in 2020. The sector that contributes the most to this production is the construction sector, with the 37.5% share of total waste produced, and households represent the fifth sector, with 9.4%, as can be seen in Figure 2.1(Eurostat, 2023).

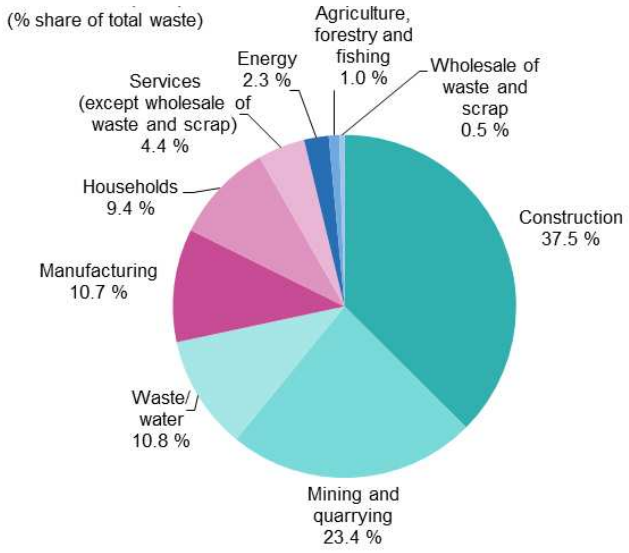


Figure 2.1 Waste generation by economic activities and households, EU, 2020 (Eurostat, 2023)

The production amount of major mineral waste – waste from mining and quarrying and from construction and demolition – is considerably different between EU Member States, this may reflect different economic structures. Indeed, for Finland, Sweden, Bulgaria and Luxembourg major mineral waste accounted for between 84% and 89% of all waste generated; the first three countries are characterised by having relatively sizeable mining and quarrying activities, while Luxembourg is characterised by construction and demolition. If major mineral waste, are not taken into account, the production of waste in the EU, in 2020, amounted to 775 million tonnes, equivalent to 36% of the total waste generated, or, on average, 1.7 tonnes per inhabitant, as reported in Figure 2.2 (Eurostat, 2023).

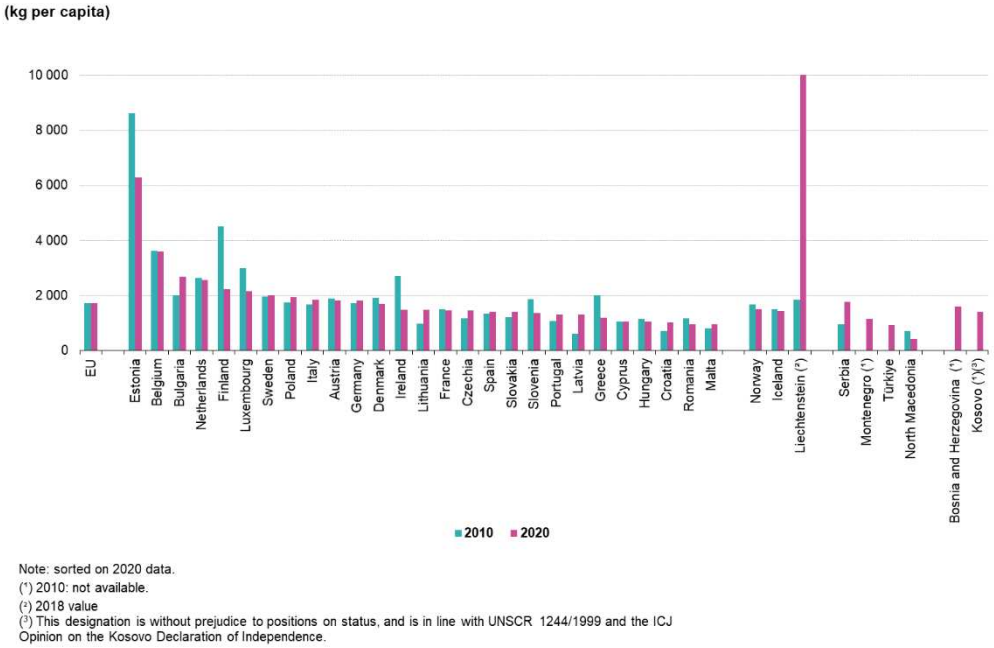


Figure 2.2 Waste generation excluding major mineral waste, EU, 2010 and 2020 (Eurostat, 2023)

In the EU in 2020, 59.1% of the total waste produced was treated in recovery operations, such as recycling (39.9% of the total treated waste), backfilling (12.7%) – the use of waste in excavated areas for the purpose of slope reclamation or safety or for engineering purposes in landscaping – and energy recovery (6.5%) (Eurostat, 2023). The remaining 40.9% was either landfilled (32.2%), incinerated without energy recovery (0.5%) or disposed otherwise (8.2%). The differences in recovery and disposal shares, between EU Member States are reported in Figure 2.3 (Eurostat, 2023).

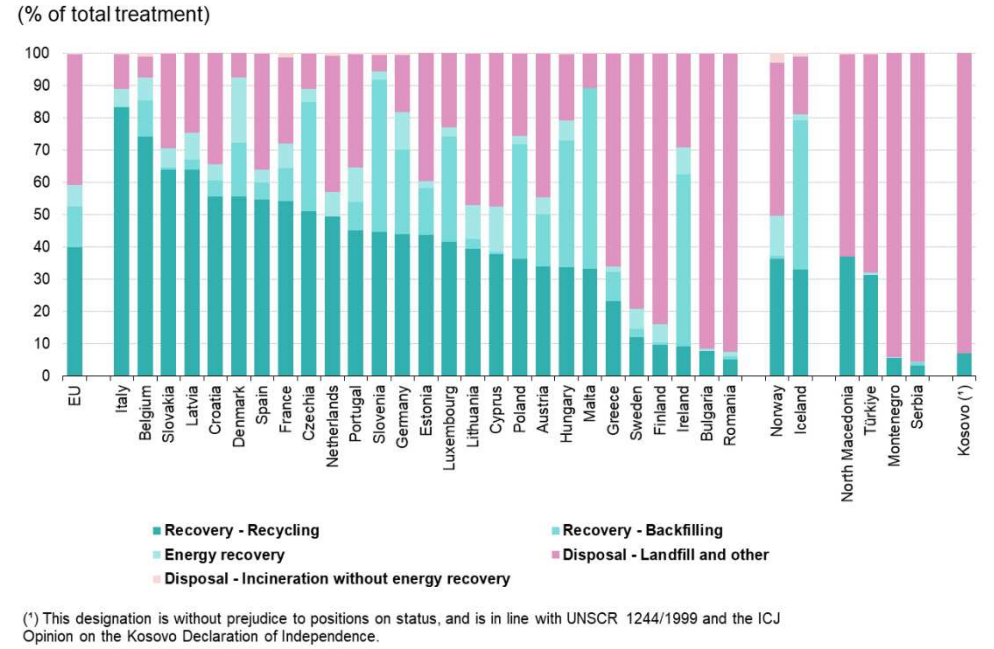


Figure 2.3 Waste treatment by type of recovery and disposal, EU, 2020 (Eurostat, 2023)

Focusing on municipal solid waste, the development in landfill rate of municipal waste in European countries in 2010 and 2020 is reported in Figure 2.4(European Environment Agency, 2022).

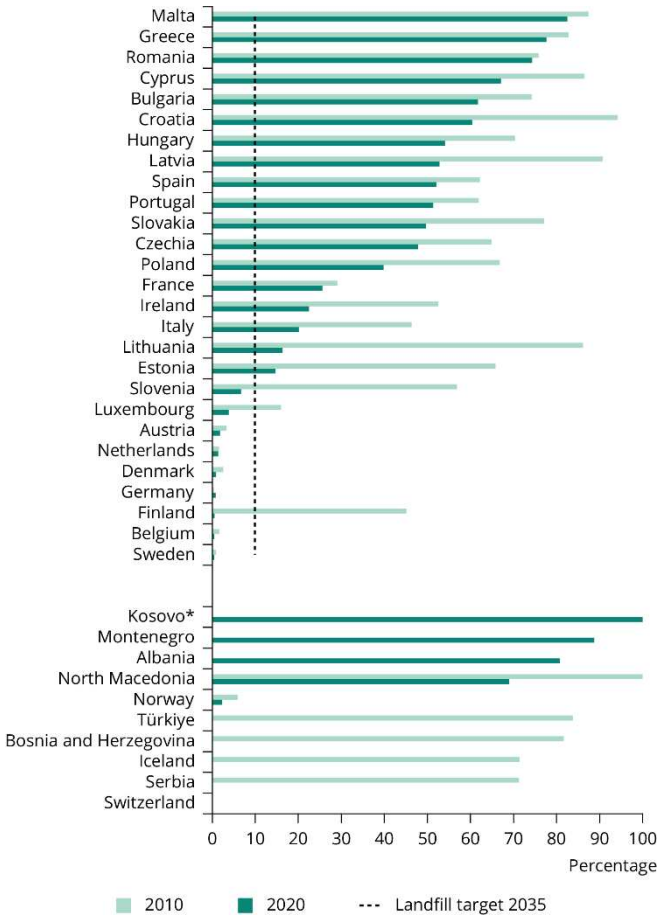


Figure 2.4 Municipal waste landfill rates by country, EU, 2010 and 2020 (European Environment Agency, 2022)

It is interesting to notice the difference between Figure 2.3 and Figure 2.4, regarding the percentage of different kinds of waste sent to landfill, in Italy and Austria. From this comparison it is noticeable that Italy has a higher percentage of municipal solid waste, sent to landfill, with respect to the one of total waste; vice versa for Austria, which has a significantly higher percentage of total waste sent to landfill with respect to municipal solid waste. This is due to the prohibition of directly landfilling municipal solid waste, in Austria, that are pre-treated, with mechanical-biological treatment, and then landfilled (Bundesministerium für Klimaschutz, Umwelt, Energie, Mobilität, Innovation und Technologie, 2022).

As can be seen from the above graphs, landfills still have an important role in waste disposal and cannot be removed from the equation, even if landfilling is progressively banished as an obsolete, hazardous and polluting system. Furthermore:

- not all wastes can be recycled or cannot be recirculated endlessly, unavoidably producing residues by the recycling activities;

- there are risks, for human health and the environment, correlated to the use and final disposal of hazardous substances, contained in the original products, that accumulate in the residues and in the recycled materials;
- the market instability does not assure the continuous allocation of recycled material (Cossu et al., 2022).

Landfilling can have a fundamental role: to provide a final sink to residual waste; following the concept of Back to Earth Alternatives (BEA). BEA implies that the residues, after proper treatment, should be returned to their non-mobile state, as they were before they were extracted (Cossu et al., 2022).

The landfills should follow the principle of environmental sustainability, adopting measures to control the waste stability and to immobilise contaminants, such as:

- specific pre-treatments, to minimize the quantities of contaminants to be landfilled and their potential emissions;
- maximisation of the controlled removal of mobile contaminants, by means of biogas and leachate generation and extraction;
- in situ treatments, to maximise waste stabilisation;
- physical barriers to control and remove mobile contaminants (bottom liner, drainage, emissions collection, etc.,) (Cossu et al., 2022).

2.2 Landfills: basic concepts and legislation

The term landfill stands for the place where waste is disposed in large quantities.
Before 1960, the disposal of waste was characterized by the phenomenon of uncontrolled dumping, landfills were just convenient holes where waste was filled in, without infrastructures, nor environmental control (Lavagnolo, 2022). Uncontrolled dumps have significant environmental impacts, such as: leachate percolation that can reach underground water and contaminate the drinking water, and the release of explosive and flammable gases, with consequent random combustions, and of greenhouse gases (GHG) (US EPA, 2002). Leachate is a liquid formed when rainwater filters through wastes placed in a landfill, drawing out, chemicals or constituents from those wastes (OLEM US EPA, 2016).
Between 1960 and 1980 controlled tipping/burial method was performed. This method consists of the disposal of solid waste into a dug pit, that is regularly covered (Lavagnolo, 2022; "OLCreate: HEAT_HEH_ET_1.0 Hygiene and Environmental Health Module: 22. Solid Waste Management," n.d.).
After 1980, landfilling became more engineered with the application of contained landfilling method, in these years were added: lining systems, leachate and biogas management, waste compaction and a low permeability surface capping (Lavagnolo, 2022).
The next step took place in 2000, when the waste started to be treated before the disposal in the landfill, through mechanical biological treatment (MBT), the multi-barrier approach has been introduced, together with environmental monitoring.
As latest development in landfilling, from 2010, the concept of sustainable landfilling was introduced (Lavagnolo, 2022). A landfill is considered sustainable when the emissions after 30 years do not have any significant environmental impact (Cossu et al., 2022).

In Figure 2.5 a timeline of the development of landfilling is reported.

Figure 2.5 Landfill evolution (Lavagnolo, 2022)

Contamination from landfills can derive mainly from leachate and landfill gas.

The composition of leachate varies depending on waste composition, age and landfilling technology; nevertheless, considering municipal solid waste, landfill leachate can be described as a water-based solution of four groups of pollutants:

1. Dissolved organic matter, including CH_4, volatile fatty acids, and refractory compounds as fulvic-like and humic-like compounds;
2. Inorganic macro components, such as Ca, Mg, Na, K, NH_4^+, Fe, Mn, Cl, SO_4^{2+} and HCO_3^-;
3. Heavy metals, such as Cd, Cr, Pb, Ni and Zn;
4. Xenobiotic organic compounds (XOCs), including aromatic hydrocarbons, phenols and chlorinated aliphatics. (Christensen et al., 2001)

The contamination from landfill leachate can lead to a contamination of surface and groundwater, but also to a reduction of soil permeability and to a modification of soil chemical and physical characteristics (Mukherjee et al., 2014).

Landfill gas is described in detail in the following section, as it is the focus of this thesis work.

2.2.1 Landfill gas and methane

The biogas produced in a landfill derives from the biological degradation of the organic fraction of waste, if untreated. Initially the organic fraction present is decomposed by aerobic bacteria and the gases present in the landfill are oxygen (O_2), and nitrogen (N_2), while carbon dioxide (CO_2), together with hydrogen (H_2), are produced. At this moment nitrates and sulphates are reduced following the reactions:

$$6\,NO_3^- + 5\,CH_3OH \xrightarrow{bacteria} 3\,N_2 + 5\,CO_2 + 74\,H_2O + 6\,OH^-$$

$$S0_4^{2-} + c.o \xrightarrow{bacteria} S^{2-} + H_2O + CO_2$$

$$S^{2-} + 2H^+ \rightarrow H_2S$$

After the oxygen has been depleted, the acid phase begins, characterised by hydrolysis and acidogenesis reactions, operated by anaerobic bacteria, and the presence of CO_2 and H_2. The organic acids, such as acetic acid (CH_3COOH), fulvic acid, and other more complex acids, and the hydrogen are then converted into carbon dioxide and methane (CH_4), by methanogenic bacteria (Babikova, 2018). A scheme of the landfill gas evolution during these different phases is reported in Figure 2.6.

Therefore landfill gas is composed of CO_2 (40–45 % v/v) and CH_4 (55–60 % v/v), as main components, together with minor amounts (<5% v/v) of carbon monoxide, nitrous oxide, oxygen, sulphides, hydrogen, and a large number of trace (<1% v/v) non-methane volatile organic compounds (NMVOCs) (Hanson et al., 2023).

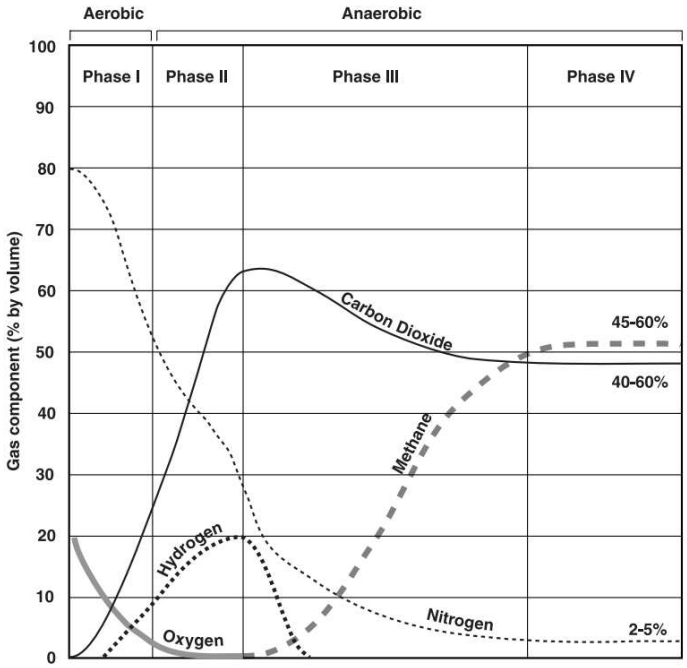


Figure 2.6 Production phases of landfill gas (OAR US EPA, 2016a)

Biogas production depends on different factors:

- Waste's characteristics: composition, granulometry, density, pre-treatments;
- Environment's characteristics: precipitations, humidity and temperature;
- Landfill management: cover materials, waste deposition modality, and leachate recirculation. (Babikova, 2018)

Carbon dioxide and methane are the two most abundant anthropogenic greenhouse gases (GHG), accounting for 65% and 16% respectively, as reported in Figure 2.3 (OAR US EPA, 2016b). Furthermore, their concentration in the atmosphere is increased since 1850, as shown in Figure 2.8 (IPCC, 2023).

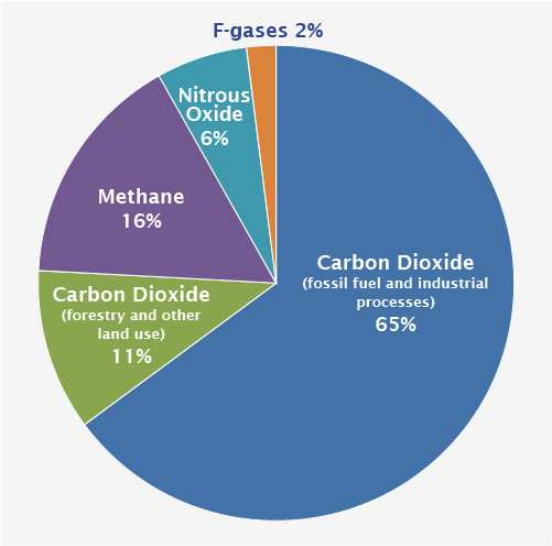


Figure 2.7 Global greenhouse gas emissions by gas in 2010 (OAR US EPA, 2016b)

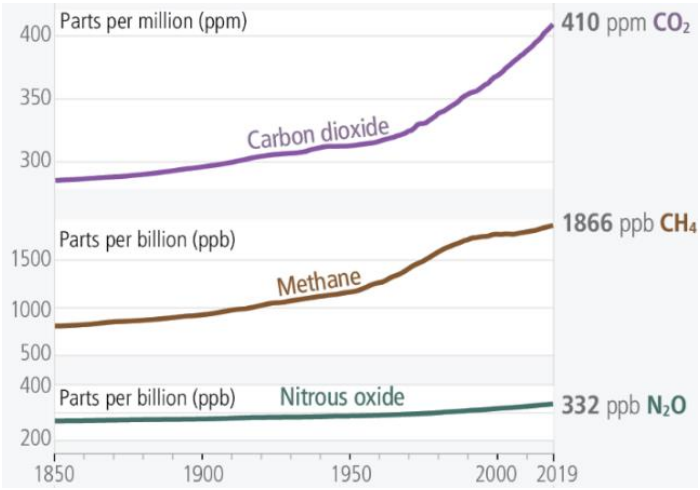


Figure 2.8 Increasing of GHGs concentrations in the atmosphere, 1850-2019 (IPCC, 2023)

In particular, methane has a global warming potential (GWP) of 81.2 over 20 years and 27.9 over 100 years (IPCC, 2021), and anthropogenic emissions represent roughly 60% of the total methane emissions. Anthropogenic methane emissions, in the world, come primarily from three sectors: fossil fuels (~35%); agriculture (~40%); and landfill and waste (~20%) (United Nations Environment Programme and Climate and Clean Air Coalition, 2021).

Globally is estimated that the landfill and waste sector produced 68 Mt/yr of methane, in 2017, as reported in Table 2.1(United Nations Environment Programme and Climate and Clean Air Coalition, 2021); while in Europe, in 2022, the landfill and waste sector produced 13388 kt of methane, as can be seen in Figure 2.9 (International Energy Agency, 2023).

Table 2.1 Estimated natural and anthropogenic sources and sinks of methane, 2017 (United Nations Environment Programme and Climate and Clean Air Coalition, 2021)

NATURAL SOURCES	MAGNITUDE (MT/YR)	ANTHROPOGENIC SOURCES	MAGNITUDE (MT/YR)	SINKS	MAGNITUDE (MT/YR)
Wetlands	145 [100–183]	Coal mining	44 [31–63]	Soils	40 [37–47]
Termites	9 [3–15]	Oil and gas industry	84 [72–97]	Total chemical loss	531 [502–540]
Oceans	6 [4–10]	Landfill and waste	68 [64–71]	Total loss	571 [540–585]
Geological	45 [18–65]	Ruminants	115 [110–121]		
Wild animals	2 [1–3]	Rice cultivation	30 [24–40]		
Freshwaters	159 [117–212]	Biomass burning	16 [11–24]		
Permafrost soils	1 [0–1]	Industry	3 [0–8]		
		Biofuels	13 [10–14]		
		Transport	4 [1–13]		
Total natural	367 [243-489]	Total anthropogenic	380 [359–407]		
Total natural (top-down)	232 [194-267]	Total anthropogenic (top-down)	364 [340-381]		

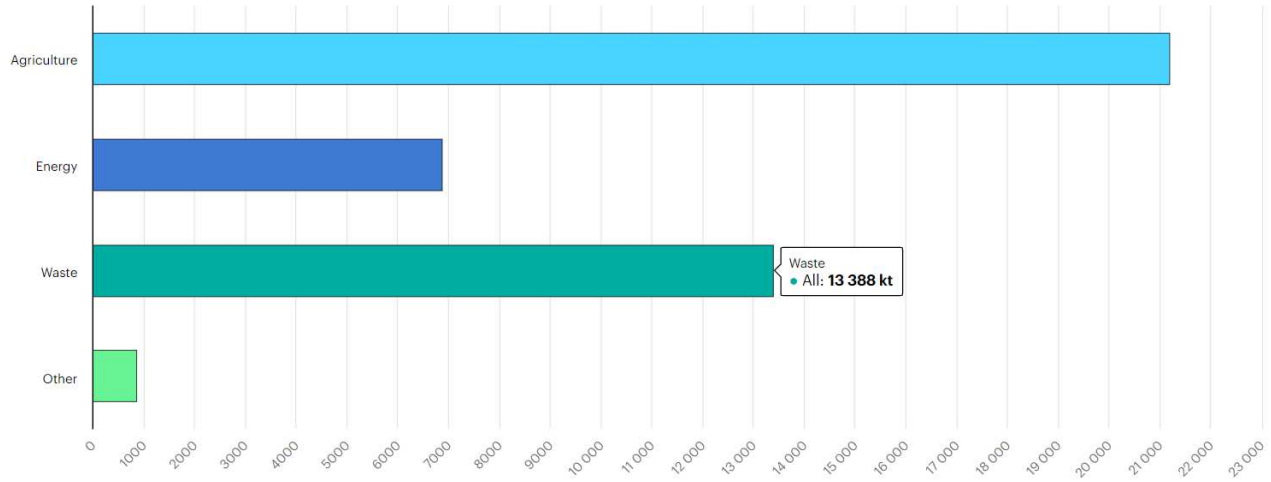


Figure 2.9 Europe methane emissions from all sources (in kilotons), IEA estimate from available datasets (International Energy Agency, 2023)

In Italy, methane emission from the waste sector comes, for 76% in 2020, specifically from the management of landfills (ISPRA, 2022). While in Austria landfills are responsible for 37% of greenhouse gas emissions in the waste sector (Bundesministerium für Klimaschutz, Umwelt, Energie, Mobilität, Innovation und Technologie, 2022).

The trends of emissions of CH_4 per capita, for the waste sector, of Austria and Italy, between 1990 and 2019, are reported in Figure 2.10.

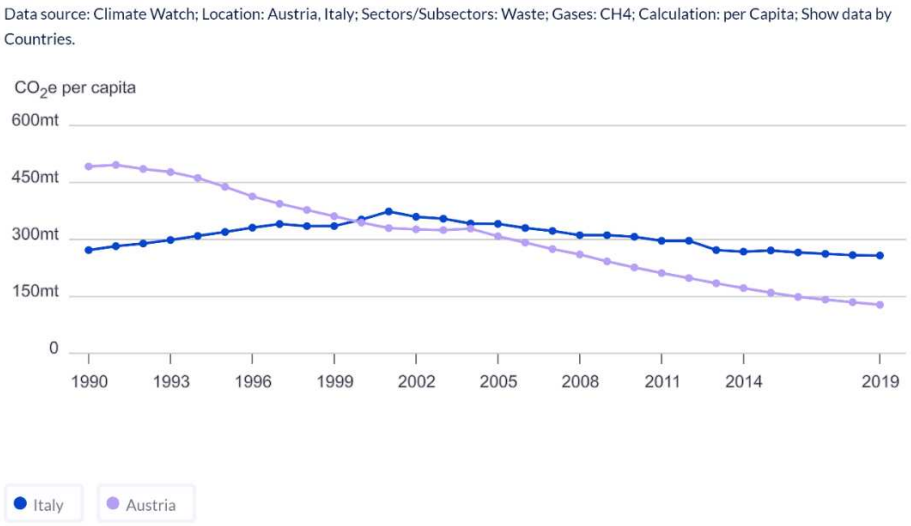


Figure 2.10 Comparison between Austria and Italy's methane emissions per capita, waste sector, from 1990 to 2019

For these reasons landfill gas emissions, and more specifically methane emissions from landfills, represent an important environmental challenge, to be addressed in the perspective of containing climate change.

Uncontrolled emissions have to be avoided, therefore for the treatment of landfill gas three options can be individuated:

- Energy recovery, this is the most preferable option to reduce methane emissions and to produce electricity from a renewable source; this process starts from the pre-treatment of the biogas, that after is burned to produce thermal energy, that is used to heat fluids for heat distribution, or that is converted firstly to mechanical energy and then to electricity;
- Flare combustion, this is the case of landfills where an energy recovery line cannot be installed or is out of service; biogas combustion requires methane concentration higher than 25%, and during this process methane and other combustible gases are converted to water vapour, CO_2 and sulphur and nitrogen's oxides;
- Microbial methane oxidation systems (MMOS), used in closed and old landfills, where the production and the quality of landfill gas are not economically worth it to be used to recover energy; this system is based on the capacity of microorganisms to metabolise compounds present in the biogas. (Babikova, 2018)

2.2.2 Landfill legislation

Waste and landfilling are regulated by specific legislation in each country, to prevent pollution in different environmental compartments.

Besides current regulations, Austria and Italy are both participants in the Global Methane Pledge, launched at COP 26 in November 2021 in Glasgow. The Pledge is led by the United States and the European Union, but now has more than 110 country participants who together are responsible for 45% of global human-caused methane emissions (IEA, 2022). Participants joining the Pledge agree to take voluntary actions to contribute to a collective effort to reduce global methane emissions by at least 30% from 2020 levels by 2030, which could eliminate over 0.2˚C warming by 2050 ("Global Methane Pledge - Homepage," 2021).

2.2.2.1 European regulation

In the European Community, the regulations in force are the Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste and repealing certain Directives, also called the Waste Framework Directive, and the Directive 1999/31/EC on the landfill of waste.

The Waste Framework Directive sets the basic concepts and definitions on waste management. "Waste" means any substance or object which the holder discards or intends or is required to discard; "recovery" means any operation the principal result of which is waste serving a useful purpose by replacing other materials which would otherwise have been used to fulfil a particular function, or waste being prepared to fulfil that function, in the plant or in the wider economy; and "disposal" means any operation which is not recovery even where the operation has as a secondary consequence the reclamation of substances or energy (*Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste and repealing certain Directives (Text with EEA relevance)*, 2008).

Regarding landfilling, the Directive 1999/31/EC on the landfill of waste aims to prevent, or reduce as much as possible, the negative impact of landfill on surface and groundwater, soil, air or human health. It divides landfill sites into 3 categories:

- landfills for hazardous waste;
- landfills for non-hazardous waste, where municipal waste can be disposed; and
- landfills for inert waste (waste which will not decompose or burn, such as gravel, sand and stone).

Some important concepts introduced are:

- the obligation to progressively reduce the amount of biodegradable waste sent to landfills, by 2024;
- only waste that has been treated may be landfilled;
- landfill facilities may not accept used tyres or waste which is liquid, flammable, explosive or corrosive, or from hospitals and medical and veterinary practices.

Further directives were implemented, after 1999: Decision 2003/33/EC lays down the criteria and procedures for the acceptance of waste at landfills, and the Directive (EU) 2018/850 that amends Directive 1999/31/EC. The latter in particular:

- introduces restrictions on landfilling from 2030 of all waste that is suitable for recycling or other material or energy recovery;
- seeks to limit the share of municipal waste landfilled to 10% by 2035; ("Landfill of waste directive," 2020)
- requires landfill operators to manage landfill gas by using it to produce energy or by burning it in a flare.

Further, in the review of the landfill directive, planned for 2024, the European Commission will consider other actions to improve landfill gas management, minimizing its harmful effects on the climate and exploiting all its potential energy benefits. These changes are expected to produce a further reduction of methane emissions from landfills (COMMISSIONE EUROPEA, 2022).

2.2.2.2 Italian regulation

In Italy, the current legislation is regulated by the D. Lgs. n. 152/2006 – Testo Unico Ambientale, which gathers all the regulations in the environment field. In particular:

- the fourth part – regulations on waste management and remediation of contaminated sites – reports all the definitions, authorizations and procedures for waste management; and
- the fifth part – regulations on air protection and emissions reduction in the atmosphere – states the limits for emissions in air for VOC, dust and combustion plants (*DECRETO LEGISLATIVO 3 aprile 2006, n. 152. Norme in materia ambientale.*, 2006)

Regarding specifically landfills, the current regulation in Italy is the D. Lgs n. 121/2020 – Attuazione della direttiva (UE) 2018/850, che modifica la direttiva 1999/31/CE relativa alle discariche di rifiuti, that implements the Directive (EU) 2018/850, which amends directive 1999/31/EC on landfills. This

regulation traces the European one before mentioned, and it does not give any limit values regarding air emissions from landfills (*DECRETO LEGISLATIVO 3 settembre 2020, n. 121*, 2020).
These limits are set case to case, for landfills, which receive more than 10 Mg of waste per day or with a total capacity of more than 25000 Mg, excluding the landfills for inert waste, as reported in the Directive 2008/1/EC of the European Parliament and of the Council of 15 January 2008 concerning integrated pollution prevention and control (*Directive 2008/1/EC of the European Parliament and of the Council of 15 January 2008 concerning integrated pollution prevention and control (Codified version) (Text with EEA relevance)*, 2008).

2.2.2.3 Austrian regulation

In Austria, the "Abfallwirtschaftsgesetz" (Waste Management Act) (AWG 2002), is the most important regulation regarding waste management. In addition to the national provisions, there is a large number of European regulations, that are implemented in Austria, such as the Waste Framework Directive.

The AWG 2002 concepts relate to the avoidance, preparation for reuse, recycling, other utilization and disposal of waste, obligations of people working in waste management and specifications for waste treatment plants (Bundesgesetzblatt, 2002).

Regarding specifically landfills, the "Deponieverordnung" (Landfill Ordinance) (DVO 2008) is the current regulation, and states:

- the basic definitions;
- landfill classes and the respective waste allocations;
- treatment obligations;
- waste acceptance procedure and limits;
- landfill site and technology requirements;
- landfill operation.

In addition, the landfill ordinance provides requirements for temporary covers, that act as MMOS (biocovers) for a period of maximum 20 years, minimising methane emissions from landfills with high amounts of biodegradable waste. Particularly appendix 3, section 6.1, provides specifications regarding biocover's effectiveness and a monitoring program for temporary covers. Furthermore, limits about gaseous emissions from a landfill site are set: they must not exceed an average of 5 kg CH_4/m^2year and values from hotspots – small areas of the landfill cover characterised by relatively high methane emissions in comparison to its surroundings – may exceed this up to a maximum of 10 kg CH_4/m^2year (Bundesgesetzblatt, 2008).

2.3 Aerobic methane oxidation

Methane is aerobically oxidised according to the following equation:

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O\ (+ biomass + energy) \quad \text{(Kriipsalu et al., 2023)}$$

With $\Delta G° = -780$ kJ mol^{-1} CH_4. This reaction is carried out by methanotrophic bacteria, and happens in the biosphere wherever methane and oxygen are present at the same time (Scheutz et al., 2009). Specifically, in landfills methane is oxidised in landfill covers, where CH_4 is present due to emission from the waste and O_2 to the diffusion from ambient air.

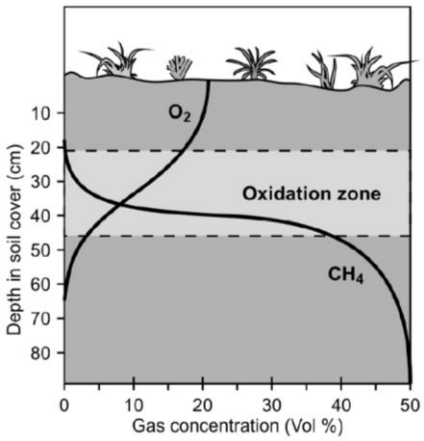


Figure 2.11 Idealized gas concentration profile in landfill cover (Pedersen, 2010)

Figure 2.11 shows that the highest oxidation potential is found in the upper part of the landfill cover soil, where both CH_4 and O_2 are present. In general, the methanotrophic active zone occurs in the first 30–40 cm below the soil surface, with maximal oxidation activity between 15–20 cm. At depths greater than 60 cm aerobic methane oxidation is limited by low O_2 concentrations (Scheutz et al., 2009). The diffusion of oxygen into the CH_4 aerobic oxidation zone depends on the total and the gas-filled porosity of the porous medium; while the depth of the aerobic methane oxidation zone varies depending on the flow of landfill gas, when it increases the zone is pushed upwards, towards the surface and becomes shallower (Pedersen, 2010).

The aerobic methane oxidation kinetics of methanotrophic cultures and soils is well described by Michaelis-Menten kinetics:

$$r = \frac{V_{max}[CH_4]}{K_m + [CH_4]}$$

Where r is the methane oxidation rate, V_{max} the maximum methane oxidation rate, K_m the Michaelis-Menten constant, given as the methane concentration where the CH_4 oxidation rate is half of V_{max}, and [CH_4] the methane concentration (Scheutz et al., 2009).

Landfill cover soils exhibit high methane oxidation capacities, as demonstrated both under laboratory and field conditions:

- >100 μg CH_4 g^{-1} h^{-1} in batch experiments; and
- >200 g CH_4 m^{-2} day^{-1} in column experiments (Cossu and Stegmann, 2018)
- In field conditions different oxidation efficiencies are reported:
  - A large-scale passive biocover implemented at Aikkala landfill, in Finland, revealed > 25–46% CH_4 oxidation of the input fluxes;
  - Two biocover systems, including an actively loaded biofilter and a passively loaded biowindow, in Italy, revealed 58% and > 95% oxidation efficiencies;
  - Different biofilters, in Denmark, showed oxidation efficiency of 55% and > 95% (for a biofilter inlet landfill gas flowrate of 20 m^3h^{-1}), while the whole-site CH_4 emission measurements showed lower methane reductions before and after implementing the biocovers (29–72%) (Duan et al., 2022); and
  - Different biocover systems, in Austria, revealed methane oxidation reduction between 75% and 99%, depending on the substrate utilised and its depth (Huber-Humer et al., 2008a).

2.3.1 CH_4 mass balance in landfills

In landfills, methane is object of many processes and to deeply understand its oxidation and the quantities emitted from a landfill it is useful to investigate methane mass balance, schematised in Figure 2.12.

CH_4 mass balance in a landfill can be described by the following relationship (Scheutz et al., 2009):

$$CH_4 \text{ production [mass } t^{-1}] = CH_4 \text{ recovered [mass } t^{-1}] + CH_4 \text{ emitted [mass } t^{-1}]$$
$$+ \text{ Lateral } CH_4 \text{ migration [mass } t^{-1}]$$
$$+ CH_4 \text{ oxidized [mass } t^{-1}]$$
$$+ \Delta CH_4 \text{ storage [mass } t^{-1}]$$

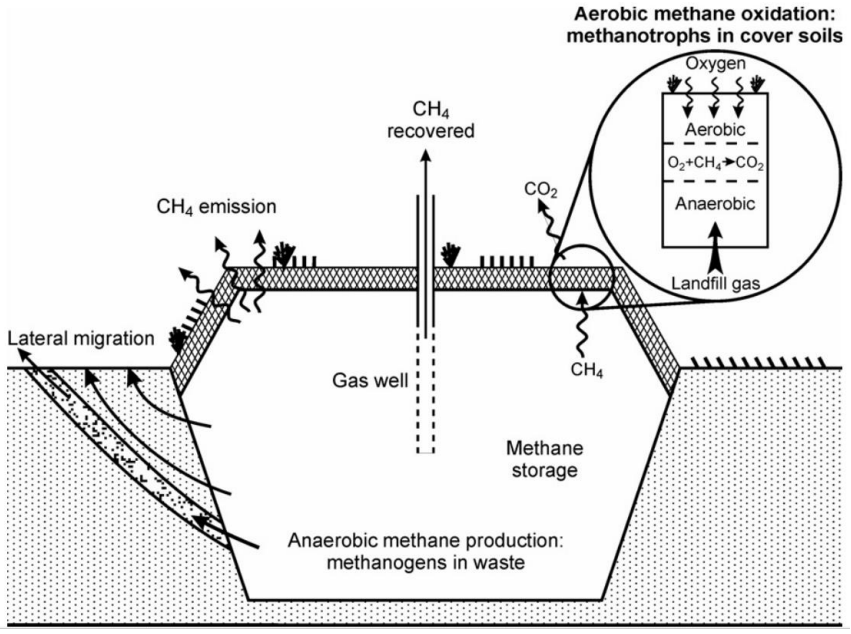


Figure 2.12 Landfill CH_4 mass balance (Scheutz et al., 2009)

Methane is produced by the degradation of the organic fraction of waste. Therefore, the production depends on landfilled waste volume, content of organic waste fractions and their degradability, waste age and environmental factors, such as temperature, moisture content, nutrients, inhibiting compounds, etc (Scheutz et al., 2009).

The recovery of methane occurs by the gas extraction system, and then is reutilised for energy purposes or burned in flares. The gas extraction system's efficiency is stated to be averagely 50-60% (Duan et al., 2022), with the remaining landfill gas escaping into the atmosphere or to be intercepted with other methods.

CH_4 emissions can occur by the following mechanisms (Scheutz et al., 2009):

- Diffusion, the major process, caused by variation of the gas component concentration between soil and ambient air, where the concentration is low due to dilution;
- Advection via Darcy flow, caused by pressure gradient due to changing of barometric pressure or by the pressure deriving from the landfill gas generation; and
- Wind-induced advection, caused by pressure gradient due to wind.

The lateral migration of methane occurs especially when cover soils are saturated, in wet seasons. In this case, diffusive methane flux to the atmosphere is limited, the internal gas pressure rises and the gas is driven to the sides of the landfill.

Temporary CH_4 storage is linked to lateral migration because it occurs in the same situation. During periods characterised by high precipitation quantity, the moisture content in cover soil increases,

leading to a decrease in emissions and therefore to the methane temporarily stored inside the landfill. This can happen also due to changes in barometric pressure.

From methane mass balance in landfills, can be understood that methane emissions can be controlled firstly via gas extraction systems, but with methane oxidation in landfill covers as well. Microbial methane oxidation systems can act as a secondary biological treatment for the gas share that is not collected by the active extraction system (Scheutz et al., 2009).

2.3.2 Methanotrophic bacteria

Methanotrophic bacteria oxidize methane as a carbon source and for energy generation. They are a subset of a physiological group of bacteria known as methylotrophs, those aerobic bacteria that utilize one-carbon compounds more reduced than formic acid as sources of carbon and energy and assimilate formaldehyde as a major source of cellular carbon (Hanson and Hanson, 1996).
Aerobic methanotrophs belong to the Gammaproteobacteria (Type I, with families *Methylococcaceae* and *Methylothermaceae*), Alphaproteobacteria (Type II, with families *Methylocystaceae* and *Beijerinckiaceae*), and the Verrucomicrobia phyla (family *Methylacidiphilaceae*) (Guerrero-Cruz et al., 2021). Type I methanotrophs use the ribulose monophosphate pathway for formaldehyde assimilation, while type II the serine pathway, as specifically described in the next section. Another difference is that most of type I bacteria are not able to fixate nitrogen and are typically associated with low CH_4 concentrations and high O_2 and nutrients concentrations; while type II methanotrophs are capable of fixating nitrogen and can thrive with high CH_4 concentrations and low O_2 and nutrient concentrations (Pedersen, 2010). In landfill cover soils, high methane concentrations are typical in the soil gas phase and therefore type II bacteria are prevalent (Röwer, 2014).

2.3.2.1 Aerobic methane oxidation pathway

The aerobic methane oxidation pathway, reported in Figure 2.13, is initiated by the methane monooxygenases (MMOs), which requires both oxygen and reducing equivalents for activity (Conrad, 1996). The MMOs are classical monooxygenases that utilize two reducing equivalents to split the O-O bonds of dioxygen; one of the oxygen atoms is reduced to H_2O, and the other is incorporated into methane to form methanol (CH_3OH). Methanol is oxidized to formaldehyde (HCHO) by a periplasmic methanol dehydrogenase (MDH) in methylotrophs.

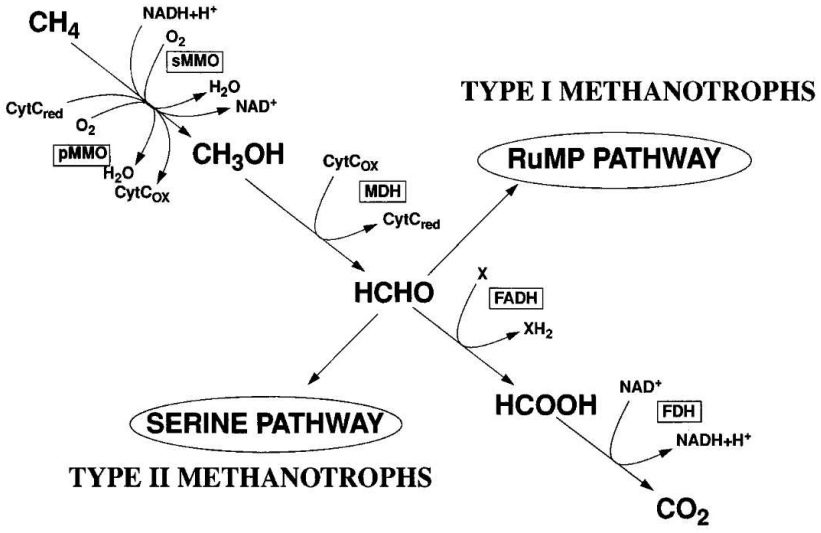


Figure 2.13 Pathways for the aerobic oxidation of methane and assimilation of formaldehyde. Abbreviations: CytC, cytochromec; FADH, formaldehyde dehydrogenase; FDH, formate dehydrogenase (Hanson and Hanson, 1996).

HCHO is assimilated by methanotrophic bacteria to form intermediates of the central metabolic routes, that are subsequently used for biosynthesis of cell material. The pathways to synthetise formaldehyde are (Hanson and Hanson, 1996):

- The serine pathway, used by bacteria of type II and reported in Figure 2.14, in which formaldehyde and carbon dioxide are utilized to form a three-carbon intermediate; and
- The ribulose monophosphate (RuMP) cycle, used by bacteria of type I and reported in Figure 2.15, in which formaldehyde is utilized to form a three-carbon intermediate. In this case, all cellular carbon is assimilated at oxidation level of formaldehyde.

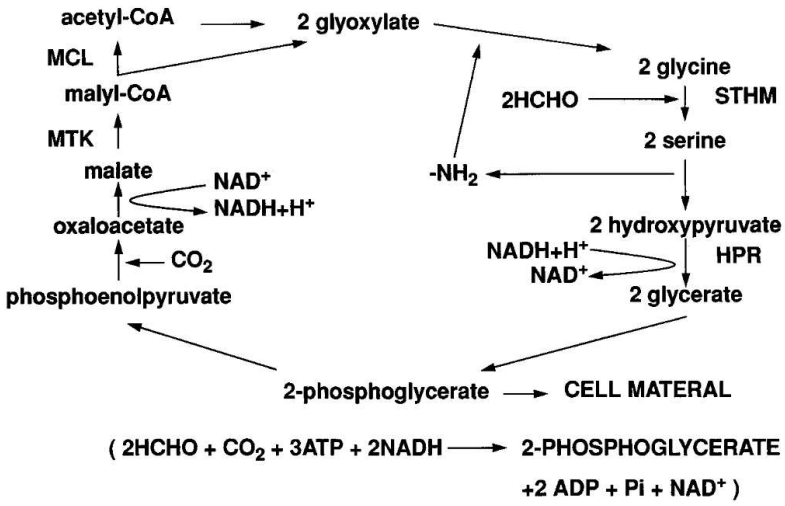


Figure 2.14 Serine pathway for formaldehyde fixation (Hanson and Hanson, 1996)

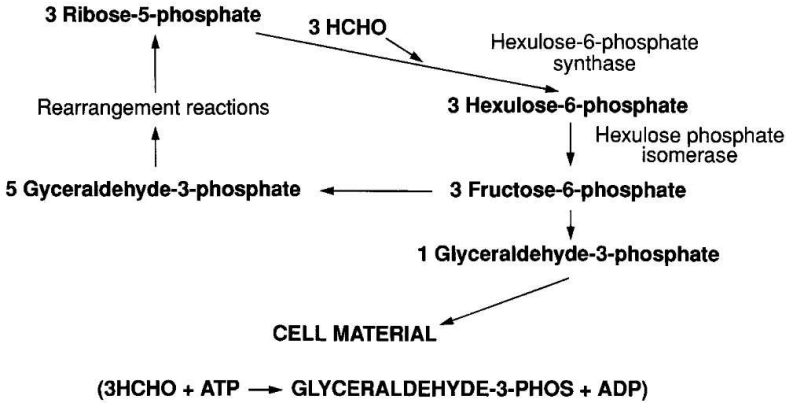


Figure 2.15 RuMP pathway for formaldehyde fixation (Hanson and Hanson, 1996)

Aerobic methane oxidation ends with the oxidation of formaldehyde to carbon dioxide, done by a NAD-dependent formate dehydrogenase (FDHs) in methanotrophs (Hanson and Hanson, 1996).

2.4 Methane oxidation systems and their guidelines

Aerobic methane oxidation is concretely applied in landfills through Microbial Methane Oxidation Systems (MMOS). MMOS are considered key technologies for landfill emissions abatement, indeed they have been indicated as promising mitigation strategies to reduce greenhouse gas emissions from the waste sector, in the 4th IPCC Assessment Report. They are applied when gas extraction becomes technically or economically unfeasible, but methane generation is still expected to continue for decades, as can be seen in Figure 2.16.

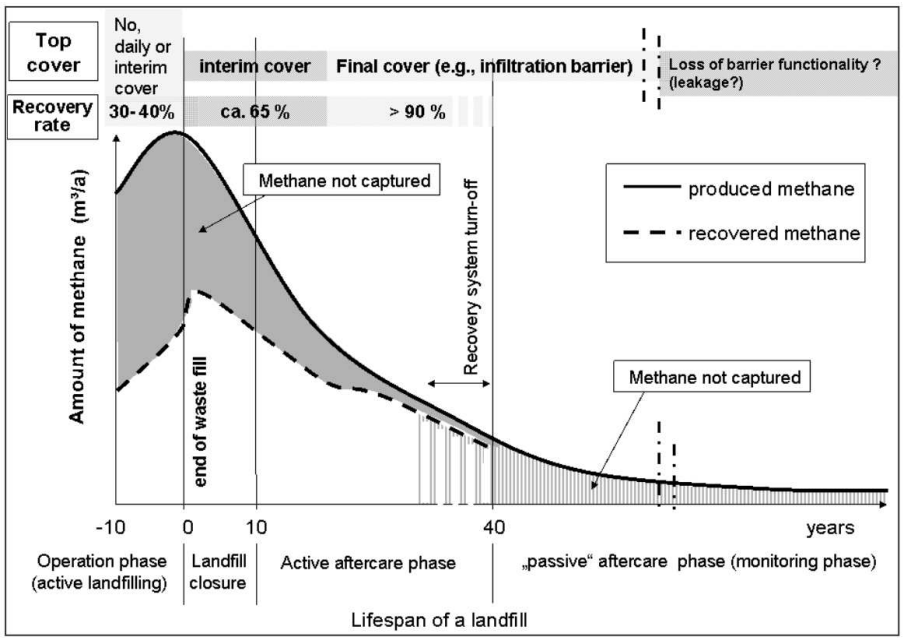


Figure 2.16 Time-dependent methane production and recovery over a landfill lifetime. Methane emissions (methane not captured) are shown as a function of the cover type and do not include methane oxidation removal) (Huber-Humer et al., 2008b)

The main challenging design aspects of these technologies are (Gebert et al., 2022a):

- Selection of the material, it impacts MMOS performance because of parameters such as porosity, water holding capacity, gas permeability, texture and compaction, and has to provide the following services:
  - advective-diffusive gas transport, an adequate O_2 supply in relation to CH_4 load is needed for methanotrophic activity, and adequate aeration is needed for plant root respiration
  - Water retention, to favour the conditions for plant and methanotrophic activity
  - Physical attachment of methane oxidising bacteria (MOB), to reach high amounts of them
  - Temperature insulation, to ensure a suitable temperature range for methanotrophic activity
  - Geochemical environment, to favour the conditions for microbial and plant activity
  - Nutrients, to favour the conditions for plant and methanotrophic activity

- Ensuring uniform spatial distribution of methane load, in order to improve emissions abatement. In fact, the formation of preferential pathways, caused by the unequal distribution at the base of the MMOS, or by the formation of cracks and fissures, within the MMOS, or from spatially uneven compaction or high moisture content of the MMOS material, ultimately results in "hotspots" – areas of high CH_4 fluxes or uncontrolled emissions – at the surface of the landfill cover.

The size of methane oxidation systems base (area) is determined by either the known methane load or the surface emission (in case of hotspot) and the oxidation capacity (OC), following the relationship (Gebert et al., 2022a):

$$Area\ [m^2] = \frac{Emission\ or\ load\ \ [g\ CH_4\ h^{-1}]}{OC\ \ [g\ CH_4\ m^{-2}h^{-1}]}$$

If the methane load/surface emission is known or estimated, the oxidation capacity can be determined in the laboratory, requiring time and space, or alternatively approximated using specific tools.
Particularly, OC and consequently the area depend on the thickness of the MMOS, which is stipulated to be minimum 1 m, in many national regulations.

MMOS can be of three different types, that differ in geometric characteristics and methane supply, but share the same layering layout: biocovers, biofilters and biowindows (Gebert et al., 2022a).

2.4.1 Typical MMOS' layering

2.4.1.1 Foundation layer

The foundation layer, or MMOS bed, is the layer placed on the waste mass, or the base of the structure that supports the methane oxidation layer. This difference depends on the type of microbial methane oxidation system, the first is the case of biowindows, while the second is referred to biocovers and biofilters (Gebert et al., 2022a).

2.4.1.2 Gas distribution layer (GDL)

The gas distribution layer is placed on top of the foundation layer, and its functions are to distribute the methane load as uniformly as possible to the base of the methane oxidation layer (MOL), and to drain away water that leaks through the MMOS. In biowindows, landfill gas is captured by the GDL directly from the waste body or through a dedicated gas supply pipe, whereas in biofilters only

dedicated gas supply pipes are used. GDL material has to own certain characteristics (Gebert et al., 2022a):

- long-term stability;
- inertia to any biogeochemical reactions, in order to avoid changes in its pore structure;
- very low, or null, organic and inorganic carbon content, in order to avoid the precipitation of carbonates, due to the increase of partial pressure in carbon dioxide, and hence in the reduction of pore volume available for gas transport;
- free of dissolvable iron, in order to avoid mobilisation and precipitation of iron oxides/hydroxides, that would clog the pore space;
- particle diameters in the range of coarse sands or greater;
- not be prone to cracking;
- adequate to maximize the horizontal flow of gas in the gas distribution layer; and
- adequate to minimize the effect of capillary barrier at the interface of different materials.

Suitable materials for GLD are: carbonate-poor or carbonate-free gravels, crushed glass and coarse quartz sands, as well as recycled coarse materials, if carbonate-free. Lastly, the thickness of the GDL is typically recommended to be >0.1–0.3 m, but depends on the constructability, hence on the available construction equipment (Gebert et al., 2022a).

2.4.1.3 Filter layer (FL)

The filter layer is meant to separate the GDL and the MOL, in order to avoid the clogging at the interface due to the capillary barrier effect, which can occur between materials with specific particle size distribution, however, this FL is not necessary in any case.

Clogging is due to the difference between the particle diameters. In particular, particles of the MOL could migrate into the GDL causing mechanical clogging, and ultimately leading to hotspots.

The capillary barrier is a zone where the degree of water saturation in the MOL is high enough to cause occlusion of the pores by water at its lower boundary. It is due to the hydraulic contrast between the two layers, and leads to gas blocking and ultimately to preferential flow formation. Besides the installation of a filter layer, that is not required in each case, also a sloped interface could reduce the negative impact of the capillary barrier effect.

The most suitable materials for the FL are granular materials, it allows to prevent erosion of the MOL material, while maintaining the permeability and diffusivity of water and gas. Lastly, the filter layer thickness depends on constructability (Gebert et al., 2022a).

2.4.1.4 Methane oxidation layer (MOL)

The methane oxidation layer is where CH_4 oxidation happens. Due to environmental conditions, accumulation of landfill gas and the depth of ingress of atmospheric air, the methane oxidation area may vary over time. The MOL has to (Gebert et al., 2022a):

- Offer a suitable geochemical and physical environment for methanotrophic activity and their attachment;
- Supply nutrients to sustain microorganisms and plants' activity;
- Provide enough water retention to sustain microorganisms and plants' activity;
- Permit advective and diffusive gas transport up (CH_4 and CO_2) and down (O_2) to maintain methane oxidation capacity; and
- Offer sufficient insulation effect, to maintain temperature within a suitable range for methanotrophic activity.

In case mineral soils are used, the MOL can be formed by two sublayers: the vegetated top part, composed of humic topsoil (where roots are present), and the subsoil.

MOL's material has to own the following physical, hydraulic and chemical properties (Gebert et al., 2022a):

- Long-term stability, for this purpose natural humus is favoured, but most topsoils can support microbial activity and sustain a vegetated cover. Furthermore, to guarantee MMOS functionality materials with minimal degradable organic matter contents has to be considered, to avoid biological degradation;
- A suitable volumetric air content at occlusion, and the associated volumetric water content at occlusion, to avoid water clogging at MOL/FL or MOL/GDL interfaces;
- The gas conductivity coefficient (or coefficient of gas permeability) of the MOL, must be lower than the one of the GDL. This ensures that landfill gas does not enter the MOL right above the gas feed lines or through cracks. Nevertheless, the difference between the coefficients forces pressure loss to be homogenized over all path lengths, near or far to the gas inlet point;
- The air capacity – share of oxygen ingress and freely draining pores available for gas transport – has to be high, even under a condition of maximum water retention. A minimum amount of interconnected water-free pores must be maintained, but the required air capacity depends on the CH_4 flux to be treated and the expected methane oxidation capacity;
- Available field capacity, the water demand by vegetation and the potential evapotranspiration under the given climatic conditions, has to be balanced;

- The susceptibility to compaction has to be considered, to prevent a decrease in air capacity. Indeed, compaction should be kept to a minimum, but MOL material is usually slightly compacted to create a contrast in the gas conductivity coefficients between MOL and GDL, or to prevent unwanted post-construction settlement;
- The susceptibility to crack formation has to be considered to avoid preferential pathways. For this purpose, fine grained loamy textures are not suitable for methane oxidation layers, because of their clay content the leads to aggregation;
- The pH value has to stay between 5.5 and 8.5, which is the optimum pH for aerobic methanotrophs;
- The organic matter content has to be between 2 and 8% (with respect to dry weight) for mineral soils forming the topsoil, and less than 1% for the subsoil; with the only exception of composts;
- The electric conductivity has to be lower than 4mS/cm. At higher values, the methanotrophic activity decreases significantly due to osmotic stress;
- The ammonium, NH_4^+, content has to be low, due to CH_4 oxidation inhibition.

Considering the above mentioned properties, the suitable materials for the methane oxidation layer are mineral and organic materials. (Gebert et al., 2022a).

2.4.2 Biocovers

A methane oxidation cover, or biocover, is an engineered soil cover, designed for biological CH_4 consumption (in addition to other purposes, such as water infiltration control and protection of the surface liner), that covers an entire landfill or a big part of it (Röwer, 2014).

In biocovers, landfill gas generally is passively loaded. It reaches the gas distribution layer directly from the underlying waste or through the former cover, that in this case is partly excavated, as schematised in Figure 2.17. If the biocover is installed in an old landfill, and an impermeable liner is present or existing layer of compacted soil caps the waste mass, the landfill gas has to be conducted in the biocover through gas supply pipes. Where an active gas extraction system is present, the biocover can be connected to it.

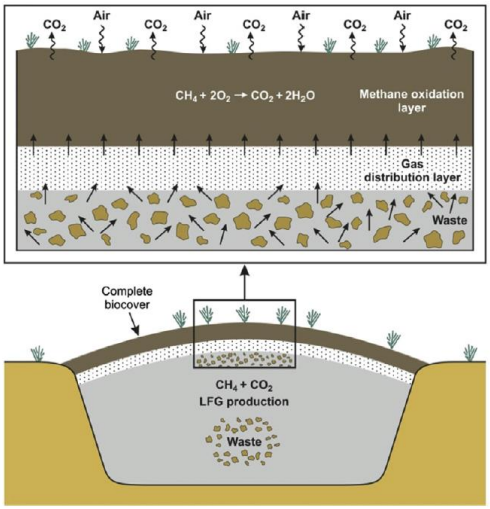


Figure 2.17 Biocover conceptual scheme (Cossu and Stegmann, 2018)

One of the major challenges of this kind of MMOS is to guarantee the uniformity of spatial distribution of methane load. The difficulty lies in cases in which leaks of landfill gas from the waste mass are present and their extent is unknown, and in cases of potential accumulation of moisture at the interface between the GDL and the MOL, particularly in downslope areas. The latter can be overcome by the insertion of drainage spots along the interface, to evacuate accumulated moisture. On the other hand, biocovers' advantage is to have at disposal a large area for methane treatment, and therefore a low load each m^2. Lastly, it is also to consider the economic side of constructing an entire landfill cover: large amounts of suitable materials are indeed required, which can represent a considerable cost factor or even a limiting issue concerning availability (Gebert et al., 2022a).

2.4.3 Biofilters

Methane oxidation filters, or biofilters, are self-contained systems placed on top of, or integrated into the landfill cover (Röwer, 2014), as schematised in Figure 2.18. Landfill gas is supplied either passively or actively, in the latter case it is provided by a gas collection or drainage system (Cossu and Stegmann, 2018).

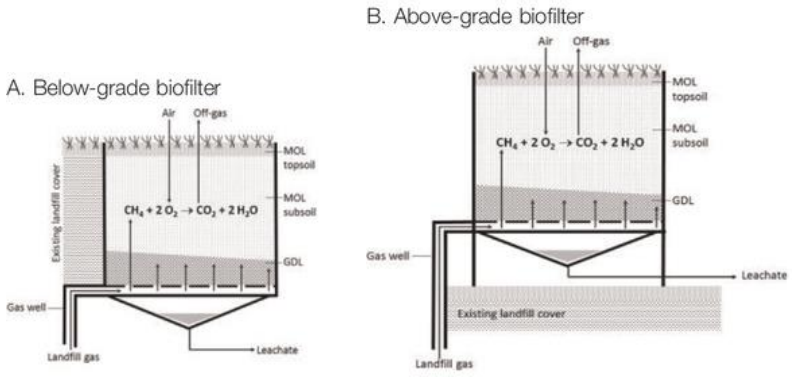


Figure 2.18 Biofilters conceptual scheme (Gebert et al., 2022a)

Biofilters can be open or closed bed. In the first case, oxygen is diffused directly from the atmosphere, and it is particularly suited for passive operation, in which the gas flow through the filter following the pressure gradient between the waste mass and the atmosphere; parameters such as temperature, humidity and pressure are controlled by the local weather and are thus subject to seasonal variability, but the extremes are mitigated by the presence of vegetation on the top, that also protect the system from erosion.

In closed biofilters the gas supplied should contain both CH_4 and O_2, therefore oxygen must be either previously admixed or supplied through aeration of the filter bed. The only case these procedures are not needed is when landfill gas comes from a landfill under in situ aeration, where the loaded gas mixture already contains enough oxygen. In this case, temperature, humidity and pressure, can be controlled (Gebert et al., 2022a). Lastly, the use of closed bed biofilters may be constrained by the total gas load; indeed, at high gas loading rates, the total size of a biofilter has to be quite large, which may also give rather costly solutions (Cossu and Stegmann, 2018).

2.4.4 Biowindows

Methane oxidation windows, or biowindows, are parts of the landfill cover optimised for methane oxidation (Röwer, 2014). They are open and do not require an active gas supply, as can be seen in Figure 2.19, therefore oxygen is diffused from the atmosphere. A biowindow system is most relevant at reduced landfill gas generation rates and at landfills, which are finally covered with relatively gas impermeable materials (Cossu and Stegmann, 2018), and therefore in cases of old landfills with lacking gas extraction systems.

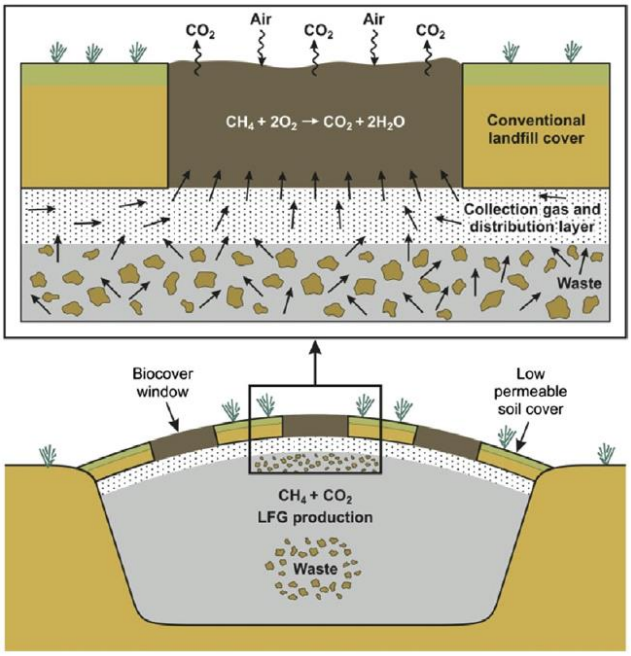


Figure 2.19 Biowindows conceptual scheme (Cossu and Stegmann, 2018)

Biowindows can be planned from the landfill's design phase or inserted later, for example to remediate a hotspot which developed, or to replace gas wells if the active gas extraction system on closed landfills is to be turned off. If planned, the landfill gas flows to the engineered window due to its higher permeability compared to that of the surrounding landfill cover. If it is inserted later and a geosynthetic liner is present in the landfill cover, landfill gas can be conducted into the window using existing gas wells (Gebert et al., 2022a).

2.4.5 Guidelines

In order to design, construct and control microbial methane oxidation systems, a science-based guidance should be written and then followed. The first review on microbial CH_4 oxidation processes and technologies for landfill methane emission abatement, was published by CLEAR (Consortium for Landfill Emissions Abatement Research) working group in 2009, presenting the state-of-the-science of the fundamental processes controlling CH_4 oxidation in soils and landfill settings, and reporting the results of small/lab-scale experiments and the first engineered field systems for mitigating landfill methane emissions. Since 2009, further research has been done, expanding the knowledge of MMOS and reaching large and full scales, including field trials. Nonetheless, science-based guidelines on MMOS are still missing, few countries have developed just brief technical information for biocovers, biofilters and biowindows; thus no more than general recommendations, for example on quality criteria to select suitable materials for the construction of methane oxidation layers, are commonly found (Gebert et al., 2022a).

Specifically, Austria and Italy were published two technical documents on biocovers, biofilters and biowindows, the content is summarized in Table 2.2, and further described in the following sections.

Table 2.2 Summary of Austrian and Italy technical documents on MMOS (Gebert et al., 2022b)

	Austria	**Italy**
System considered		
Biocover	Yes	No
Biofilter	No	Yes
Biowindow	Partially	Yes
Design		
Layering	Yes	Yes
Material properties	Yes (focus on compost)	Yes (focus on compost and sand)
Dimensioning	Yes	Yes
Construction practice		
Method of construction, machinery	Recommendations regarding compaction, water content	Yes, recommendations regarding gas distribution system, compaction and water content
Monitoring of performance		
Monitoring interval	Yes	Yes
Method surface concentrations	Yes	Yes
Method emission measurement	Yes	Yes
Method soil gas concentration	Yes	Yes
Method CH_4 oxidation performance	No	Yes
Limit values		
Surface concentrations	No	Yes
Surface emissions	Yes	No
Oxidation rate	No	Yes
Oxidation efficiency	No	Yes
Action plan		
What action if limit values are exceeded?	Maintenance / remediation measures	Maintenance
Other issues		
Further recommendations and tests	Extensive recommendations on pre-investigation, including decision tree	Assessments of the applicability of biofiltration systems; Consideration on odours and VOCs reduction
Further measurements and inspections	Measure soil temperature	Control filter media temperature
Cost estimations	Exemplary costs for practical applications	Estimated cost for construction

2.4.5.1 Austrian MMOS' guidelines

In Austria, the current document is "Leitfaden Methanoxidationsschichten", created by ÖVA-Arbeitsgruppe "Leitfaden Methanoxidationsschichten" and published in October 2008 (Huber-Humer et al., 2008a). In this guide, the state of knowledge and technology are presented, based on research results and practical experience, as well as the design, technical structure and monitoring of methane oxidation layers.

The document opens with basic definitions of methane oxidising material, extracellular polymeric substances, biocover, etc. Principles of methane oxidation follow, with the state of the art and the existing standards and regulations. Then the inventory of waste disposal, the areas of application and conditions of use are presented. In this part it is recommended to do: measurements of gas concentration and emissions, collect information on gas migration, and to analyse a representative sample of waste, to evaluate different characteristics, such as the organic matter and water contents. Furthermore, a decision tree, is proposed to assess whether the application of a MMOS would be effective or not. As reference value of methane emissions is reported 0,5 l CH_4/m^2h, if the values measured in situ exceed this reference, the landfill gas extraction is not possible/valuable, and if the landfill conditions allow it, MMOS might be installed.

The document proceeds with the description of the suggested preliminary test to perform on methane oxidant substrate, such as pH value, water holding capacity, nitrogen and phosphorus contents, gas permeability etc., and the method for estimating the gas production, that uses FID (Flame Ionization Detector).

Afterwards, the procedure description is given, together with the requirements for the gas distribution layer and the oxidation layer. In particular, the depth of the latter has to be >1,2 m, with a maximum height of 2 – 2,5 m, for mature composts with a rich structure, and significantly lower for fine-grained materials or immature composts with higher oxygen consumption (breathability); the depth of the gas distribution layer instead should be 0,5 m. Furthermore, the methane oxidation layer, if made of compost, should have an ammonium content <350 ppm, no detectable nitrite and a respiration activity (AT7) < 8 mg O_2/g DM; the compost should also contain around 30% by volume of wood chips.

The document further describes critical areas, maintenance measures, vegetation, costs, monitoring and international applicability (Huber-Humer et al., 2008a).

2.4.5.2 Italian MMOS' guidelines

In Italy, the current document is a draft of the "Guidelines for the design, construction, operation, monitoring and maintenance of the biofiltration systems", created by the RE Mida LIFE project – innovative methods for residual Landfill gas emissions mitigation in Mediterranean Regions, and published in December 2018 (Caselli et al., 2018). The aim of these guidelines is to provide to the operators of landfill facilities and the competent authorities with a practical guide for the development of technologies for the treatment of residual landfill gas with low calorific power produced predominantly in landfills in the post management phase and/or subject to processes of reinstatement, in order to minimize or mitigate methane emissions. In particular, the explored topics are:

- basic information about:
  - the management of landfill gas,
  - current European and national legislation,
  - the biological methane oxidation process,
  - the state of the art concerning technologies used to reduce the emission of methane from landfills;
- description of the biofiltration systems as technologies used to optimize the biological oxidation process and reduce landfill methane emissions,
- discussion of issues influencing the biological oxidation process,
- basic information on:
  - construction of biofiltration systems,
  - management, monitoring and maintenance of biofiltration systems,
  - specific emission limits;
- costs for the construction and management of a biofiltration system.

For different stages of a landfill's life are assigned different biological oxidation technologies, as reported in Table 2.3. And the decision is furthermore guided by a flowchart, to better understand when to install a MMOS, and of which type.

Regarding oxidation efficiency, related to methane load, it is suggested that for low values of the load less than 5 g/m^2h, the oxidation efficiency is always maintained above 85%, while it is very variable if the load is between 5-15 g/m^2h and drastically decreases in performance if the methane to be treated is greater than 15 g/m^2h.

Table 2.3 Biological oxidation technologies to be applied to reduce emissions from landfill facilities (Caselli et al., 2018)

Phase I – Active landfill	**Phase II – Post-management start**	**Phase III – 30 years from the start of the post-management phase**
Large plants (100,000-200,000 t/year)		
1. Temporary biocover 2. Passive biofilters are connected to the gas extraction system 3. Active extraction system and biofiltration system	1. Final biocover, to supplement the of extraction and treatment system	1. Active biofiltration 2. Passive drainage trenches and passive biofiltration system 3. Biocover 4. Biowindows (sites being reinstated)
Small/medium-sized plants		
1. Temporary biocovers and biotarps (paragraph 2.4.1) 2. Temporary passive biofilters connected to a passive biofiltration system 3. Final biocover 4. Passive drainage trench and biofiltration system	1. Final biocover, to supplement the of extraction and treatment system	1. Drainage trenches and passive biofiltration 2. Final biocover 3. Biowindows (sites being reinstated)

The reported requirements for the methane oxidation layer are a minimum thickness between 0,8-1,2 m, and a maximum thickness of 1,5 m; the optimal composition is compost mixed with sand, with a compost/sand ratio individuated to be 5:1. The thickness of the gas distribution layer, instead, should be 30-50cm. Other reference values for biofiltering material are reported, such as pH, conductivity, porosity, humidity, etc.

Regarding parameters for the dimensioning of a MMOS, the reference values are reported in Table 2.4.

Table 2.4 Reference values of sizing parameters of a MMOS (Caselli et al., 2018)

Sizing Parameters	**Unit of Measurement**	**Reference value**
Specific volumetric load	[Nm^3CH_4 /m^3h]	0,049
Average residence time	[min]	9
Oxidation rate	[GCH_4 /m^2h]	5
Oxidation Efficiency	[%]	80
Volumetric load VOC	[Mg/cu.mhr]	Active biofilter 1.18 Biowindows 0.73
Height of the filtering bed	[m]	1.2÷1.5

Furthermore, the list of the steps to follow while implementing the biofiltration system is reported, together with the potential criticalities. Management, monitoring and maintenance of the MMOS are then explained, particularly dividing them into routine monitoring and occasional monitoring, ordinary maintenance activities and extraordinary maintenance activities. Afterwards, the costs are estimated for the construction, maintenance and management of both biofilters and biowindows.
In the end, other four guide values are defined:

- CH_4 surface concentration = 2500 ppm
- CH_4 surface flow = 2.9 $NlCH_4$/sq.m h
- Oxidation rate = 5 g CH_4/sq.m h
- Oxidation efficiency = 80%

(Caselli et al., 2018)

2.5 Anaerobic methane oxidation

As already discussed, in landfill cover methane is conventionally converted to carbon dioxide by microorganisms, through aerobic processes. However, these processes take place usually within 30–40 cm from the surface of the cover, with the highest oxidation rate at a depth of 10–20 cm from the surface; while at depths greater than 55 cm, oxygen availability is limited and aerobic oxidation of methane is almost zero. For this reason, utilizing anaerobic oxidation can potentially decrease overall methane emissions into the atmosphere (Parsaeifard et al., 2020).

Anaerobic oxidation of methane (AOM) is not deeply investigated in landfill covers, but it has been in marine and freshwater sediments (Parsaeifard et al., 2020); it has been observed that AOM is catalysed by microorganisms related to several clusters of anaerobic methanotrophic (ANME) archaea, more specifically phylum Euryarchaeota (namely ANME-1, ANME-2, ANME-3), and a bacterium belonging to phylum NC10 (NC10 bacteria) (Cai et al., 2021).

2.5.1 AOM's metabolic pathways

The mechanisms to oxidise methane, used by ANME archaea and NC10 are completely different (Cai et al., 2021).

ANME archaea oxidises methane through the process of reverse methanogenesis.
This process is made of seven steps, as can be seen in Figure 2.20, where enzymes in blue are the ones present in all ANME archaea, enzymes in green (Nar, nitrate reductase; Nrf, nitrite reductase) are present in ANME archaea that mediate nitrate-dependent AOM, orange enzymes (MP, methanophenazine) are used as in-membrane electron carrier by ANME archaea mediating sulphate-dependent AOM or metal-dependent AOM, while those mediating nitrate-dependent AOM use menaquinone (MQ), in green.
Methane is activated and transformed into methyl-coenzyme M, by methyl-coenzyme M reductase (Mcr), then converted in methyl-H_4MPT, by methyl-H_4MPT coenzyme M methyltransferase (Mtr), and finally turned into CO_2 as the final product (Cai et al., 2021).

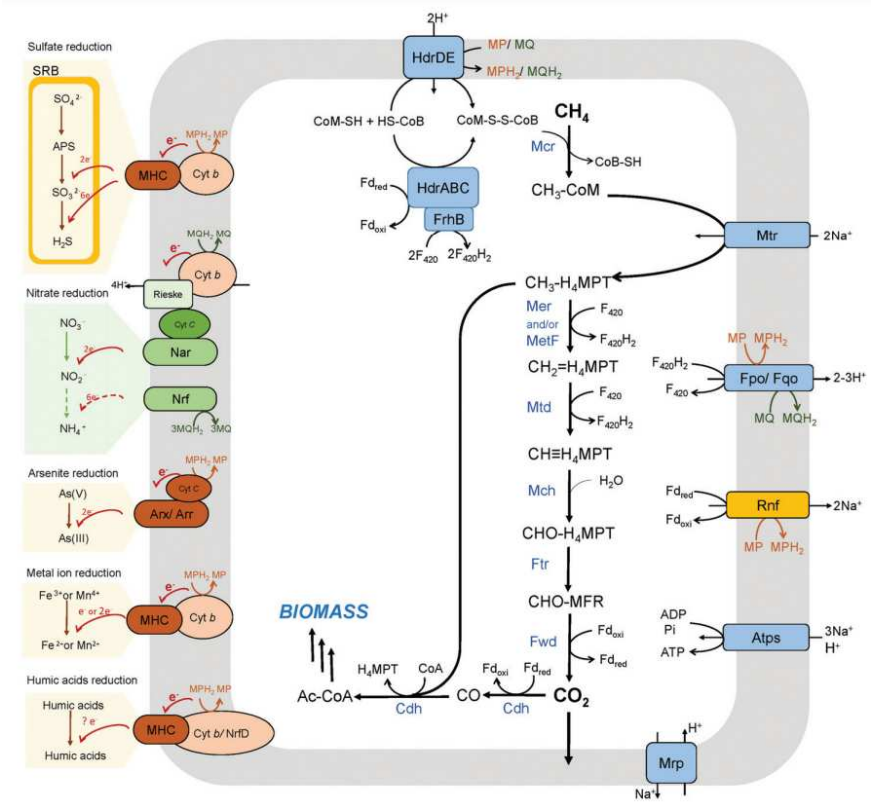


Figure 2.20 Reverse methanogenesis pathway of ANME archaea with central carbon and energy metabolisms (Cai et al., 2021)

NC10 bacteria oxidises methane through the process of intra-aerobic methane oxidation, reported in Figure 5.5.

This process is similar to the one performed by aerobic methanotrophs, described in chapter 2.3, with a substantial difference: NC10 bacteria, that are obligate anaerobic microorganisms, produce their own oxygen for methane oxidation. This is why this pathway is called 'intra-aerobic methane oxidation' (Cai et al., 2021).

2.5.2 Terminal electron acceptors coupled to AOM

Microorganisms gain energy from AOM by coupling it to the reduction of an external electron acceptor, different from oxygen. The substances at issue are: sulphate, nitrate, nitrite, and Fe(III)/Mn(IV) oxides, for which it is demonstrated; while it has been hypothesized that also humic acids, selenate, chromium(VI), arsenate, elemental sulphur, and CO_2, could act as electron acceptor for anaerobic methanotrophs (Cai et al., 2021). In Figure 2.21, the main connection network between different AOM processes is reported, with the following abbreviations: DNRA, dissimilatory nitrate reduction to ammonium; SAD, sulphide-oxidizing autotrophic denitrifier; SRB, sulphate-reducing bacteria.

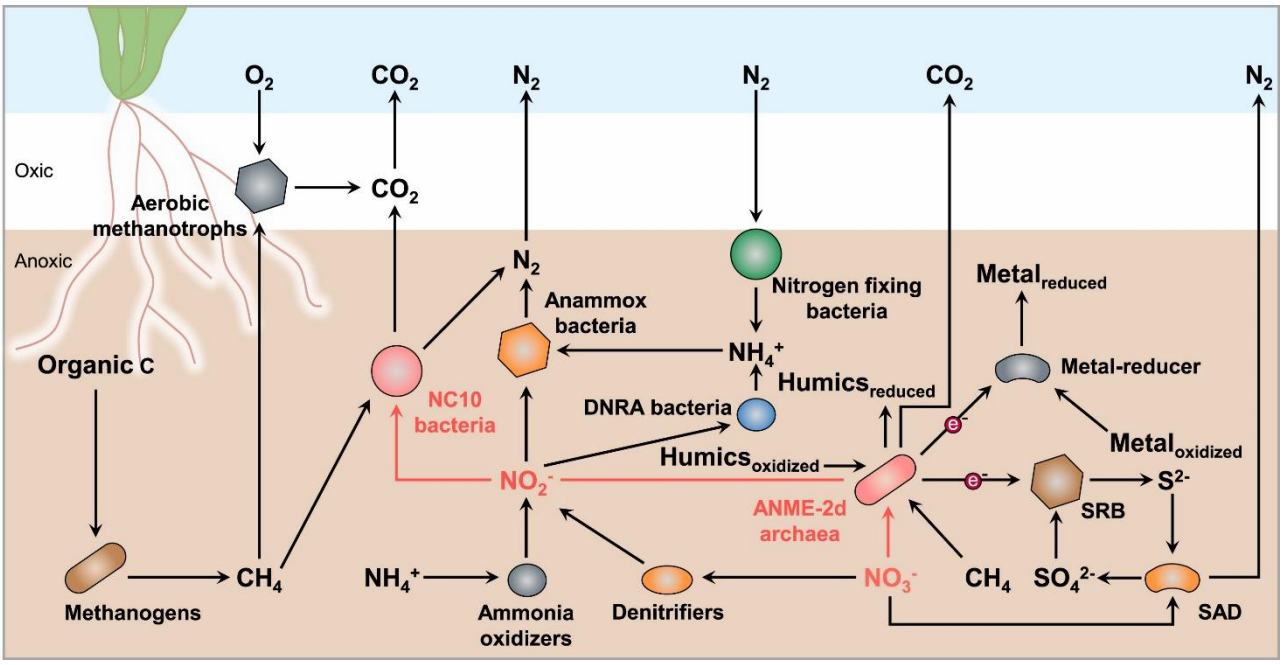


Figure 2.21 Main connection networks among the different AOM processes and other relevant microbial processes (Yang et al., 2023)

It is important to highlight that, to date, there still is uncertainty regarding the AOM driven by various electron acceptors in the environment. This is a reason to carry out further research on this topic.

2.5.2.1 Sulphate dependent AOM

The sulphate dependent anaerobic oxidation of methane (S-AOM), consumes sulphate and methane:

$$CH_4 + SO_4^{2-} \rightarrow HS^- + HCO_3^- + H_2O \qquad (\Delta G^{\circ\prime} = -21 \text{ kJ/mol})$$ (Zhang et al., 2022)

This process, schematised in Figure 2.22, is performed by ANME-2d, and it is believed that they collaborate with sulphate-reducing bacteria (SRB), because of the absence of the genes for dissimilatory sulphate reduction (Yang et al., 2023).

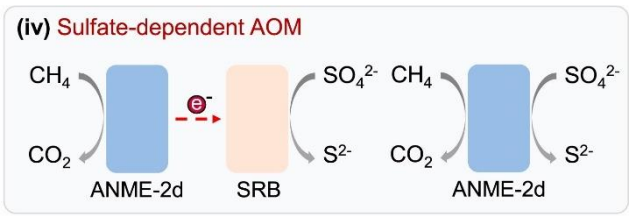


Figure 2.22 ANME-2d-mediated sulphate-dependent AOM pathway (Yang et al., 2023)

In marine sediments, 90% of methane and 70% of sulphate are consumed by S-AOM (La et al., 2022).

2.5.2.2 Nitrate and nitrite dependent AOM

The nitrate/nitrite dependent anaerobic oxidation of methane AOM (N-AOM), is performed by ANME-2d that mediates an incomplete denitrification pathway in which nitrate is reduced to nitrite, afterwards NC10 reduce some of the nitrites to nitric oxide (NO), two molecules of NO are then split into N_2 and O_2, and the latter is used for the intra-aerobic methane oxidation process (Cai et al., 2021). The remaining nitrite is used to complete the denitrification.

$$CH_4 + 4NO_3^- \rightarrow CO_2 + 4NO_2^- + 2H_2O \qquad (\Delta G^{\circ\prime} = -503 \text{ kJ/mol}) \text{ (Zhang et al., 2022)}$$

$$3CH_4 + 8NO_2^- + 8H^+ \rightarrow 3CO_2 + 4N_2 + 10H_2O \quad (\Delta G^{\circ\prime} = -928 \text{ kJ/mol}) \text{ (Zhang et al., 2022)}$$

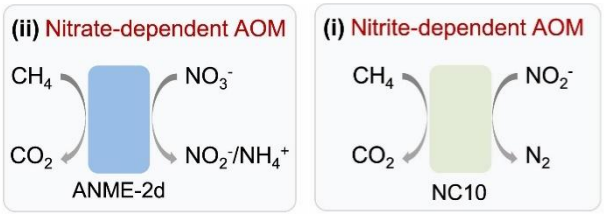


Figure 2.23 ANME-2d and NC10 mediated nitrate and nitrite dependent AOM pathway (Yang et al., 2023)

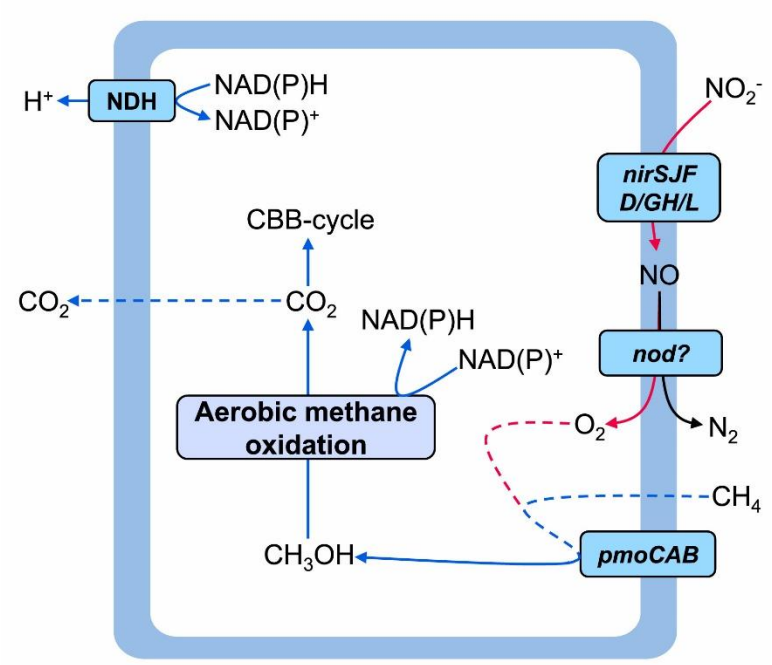


Figure 2.24 Predicted mechanisms of nitrite-dependent AOM by NC10 bacteria (Yang et al., 2023)

N-AOM is schematised in Figure 2.23, and the detailed mechanism of anaerobic methane oxidation coupled to the reduction of nitrite by NC10 bacteria is reported in Figure 2.24, with the following abbreviations: *nirSJFD/GH/L*, nitrite reductase; nod, nitric oxide dismutase; *pmoCAB*, particulate

methane monooxygenase; CBB-cycle, Calvin–Benson–Bassham cycle; NDH, NAD(P)H dehydrogenase complex.

2.5.2.3 Metal dependent AOM

The metal dependent anaerobic oxidation of methane, performed mainly by Fe (III) and Mn (IV), is still an unclear process in the environment, although the ANME-2d archaea have been confirmed to catalyse it in enrichment cultures (Yang et al., 2023).

$$CH_4 + 4MnO_4 + 7H^+ \rightarrow HCO_3^- + 4Mn^{2+} + 5H_2O \quad (\Delta G^{\circ\prime} = -494 \text{ kJ/mol})$$ (Zhang et al., 2022)

$$CH_4 + 8Fe^{3+} + 2H_2O \rightarrow CO_2 + 8Fe^{3+} + 8H^+ \quad (\Delta G^{\circ\prime} = -455 \text{ kJ/mol})$$ (Zhang et al., 2022)

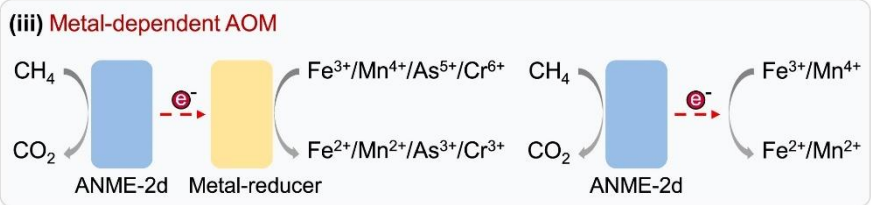


Figure 2.25 ANME-2d-mediated metal-dependent AOM proposed pathway (Yang et al., 2023)

Figure 2.25 reports the pathway's scheme of metal dependent AOM, in which can be noticed that the electrons are transferred from ANME-2d archaea to dissimilatory metal-reducing bacteria that use them to produce siderite ($FeCO_3$). The secondary mineral blocks, then, could cover the cell surface and passivate metal oxides, preventing further reactions. This is one hypothesis for the uses of insoluble metal electron acceptors, by ANME-2d archaea, and their electron transfer pathways. The other hypothesis is that ANME-2d archaea can independently mediate iron-dependent AOM via direct interspecies electron transfer (DIET) (Yang et al., 2023) – a pervasive strategy for respiring solid electron acceptors (Cai et al., 2021).

2.5.3 Applications in landfill cover soils

Anaerobic oxidation of methane applied in landfill cover soils is not deeply studied. The first mention has been made in "*Extended effects of methane oxidation and their relevance for biocover application in the field*" published by Huber Humer (2005). In this article, methanotrophic "side-effect" are observed, one of them is the indications of anaerobic methane oxidation processes taking place at hotspots, in compost landfill covers. In particular, a general overview of the process is given, with a

focus on previous studies on anaerobic methane oxidation in marine habitats, and is made the hypothesis that such processes occurred also in the investigated hotspots in compost covers. This hypothesis was justified by the facts that: Archaea were probably existent in the sewage sludge compost that was used as substrate for the landfill covers; high concentrations of sulphate in the sewage sludge compost were found (necessary for anaerobic methane oxidation); and carbonate precipitants formed at the interface layer between aerobic and anaerobic zone and in the anaerobic/anoxic layer of the compost (HCO_3^- is formed during anaerobic methane oxidation, and accordingly carbonate precipitants) (Huber-Humer, 2005).

Furthermore, to date, two studies have been fully dedicated to the topic:

"*Enhancing anaerobic oxidation of methane in municipal solid waste landfill cover soil*" – Parsaeifard N. et al., published in 2020. Had as objectives:

1. to evaluate the ability of alternate electron acceptors (besides oxygen) to facilitate anaerobic methane oxidation in clay soil, using batch tests;
2. to study the effect of conditions (with/without nutrient addition, with/without methane generation inhibitor, and different initial methane concentrations) on AOM through batch tests; and
3. to use the most promising electron acceptor concentrations determined from the first objective, to measure rates of AOM in clay landfill covers via column tests, which include realistic conditions of gas flow, cover thickness, and cover compaction.

The results of batch tests showed that sulphate, nitrate, and the combination of sulphate and hematite were the most effective electron acceptors in facilitating anaerobic oxidation of methane. Methane generation inhibitor did not affect net methane removal. Adding nutrients to the soil significantly enhanced methane removal only in the case of soil without electron acceptors. Greater methane removal was observed for reactors with higher initial methane concentration.
The results of column reactors showed that methane removal in the column amended with sulphate and hematite had the highest (around 10%) removal of methane in the anoxic zone, followed by the column that contained sulphate. Adding iron oxide to the soil sample that contained sulphate promoted AOM by sulphate-reducing bacteria; as a result, methane removal in the anoxic zone of the column reactor containing iron and sulphate was higher compared to the reactor that was amended with just sulphate (Parsaeifard et al., 2020).

"*First evidence for anaerobic oxidation of methane process in landfill cover soils: Activity and responsible microorganisms*" – Xu S., Zhang H., published in 2022. Had the objectives:

1. to track the activities of AOM in landfill cover soil (LCS), through microcosm incubation with $^{13}CH_4$; and
2. to reveal the responsible microorganisms, through quantitative PCR.

It was hypothesized that AOM could occur in LCS and different groups of anaerobic methane oxidizing microbes might occupy specific depths within LCS, as can be seen in Figure 2.26.

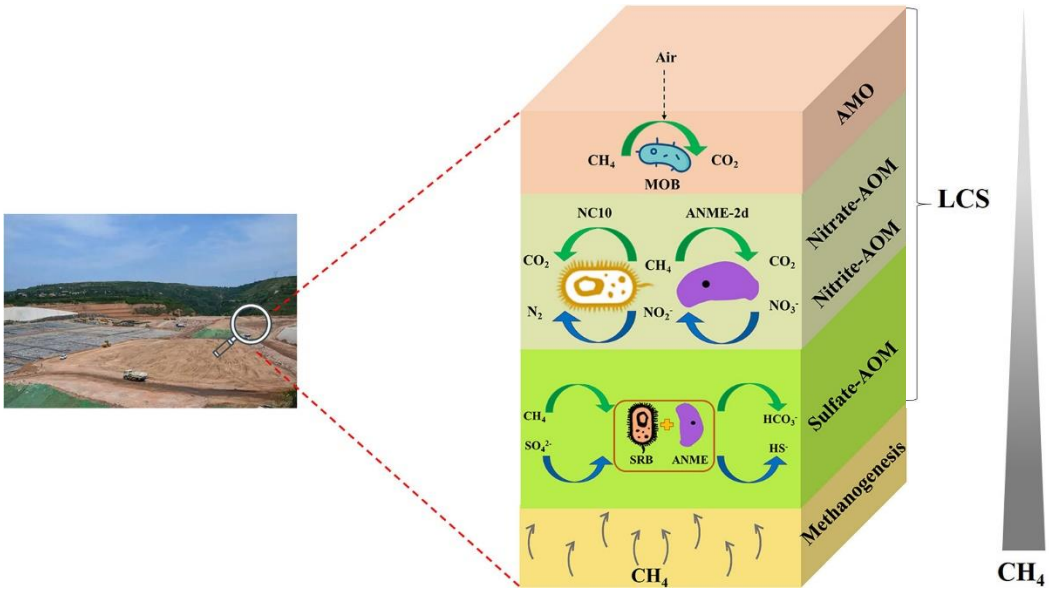


Figure 2.26 The hypothesized spatial segregation of methanotrophs in LCS.
(AMO: aerobic methane oxidation; SRB: sulphate-reducing bacteria) (Xu and Zhang, 2022)

The results of both activity tests and microbial community analysis, indicated a spatial segregation of anaerobic methane oxidizing microbes in LCS. ANME archaea (without ANME-2d), responsible for sulphate-AOM, occupied the bottom layer of LCS, while ANME-2d archaea and NC10 bacteria, that mediate respectively nitrate-AOM and nitrite-AOM, are present mainly in the middle layer. The abundance of anaerobic methane oxidizing microbes in the top layer of LCS was relatively low, which might be dominated by MOB (Xu and Zhang, 2022).

These studies provided evidence that AOM can occur also in landfill cover soils, thus there could be the possibility that microbial methane oxidation systems could be improved by enhancing anaerobic oxidation of methane in the lower depths. Nevertheless, further investigations on this topic are needed and worth it to explore.

2.6 Influencing factors

Methane oxidation, both aerobic and anaerobic, and consequently microbial methane oxidation systems (MMOS), are influenced by external environmental factors, e.g. water, oxygen and methane availability; as well as influencing factors deriving from the physical and biological processes, such as those related to the impact of the material properties, the gas transport, and others. Generally, the most relevant environmental factors and conditions are interdependent; for example, soil moisture depends on prevailing climatic conditions, geometry of the MMOS, and on the texture and compaction of the material constituting the methane oxidation layer (Gebert et al., 2022a).

The factors influencing methane oxidation successively described are common for the aerobic process and the anaerobic process, and therefore for the different bacteria performing those, except for the production of extracellular polymeric substances, which is studied only for aerobic methanotrophs.

2.6.1 Temperature

Most aerobic methanotrophs available in pure culture are mesophiles. The optimum temperature for methane oxidation is around 25°C in most peat soils, although oxidation can also occur at 0 to 10°C (Hanson and Hanson, 1996). Different soils exhibit different methane oxidation responses with respect to temperatures, this indicates that populations of methanotrophs in nature adapt to different temperatures (Hanson and Hanson, 1996). In particular, temperature could exhibit a selecting effect that determines which of the two main types of methanotrophs will predominate in a given environmental system. In fact, all of the bacteria found in low temperature environments belong to type I methanotrophs, and it appears that in landfill conditions type I methanotrophs tend to have a lower temperature optimum than type II methanotrophs; consequently, type I methanotrophs are more dominant at 10 °C than at 20 °C. In cold areas or during winter season with temperatures below 5–10°C the CH_4 oxidation might be significantly reduced or even come to standstill (Scheutz et al., 2009). Overall temperature is an important controlling factor for the methane oxidation process.

Anaerobic methanotrophic bacteria are able to adapt to a vast range of temperatures. Indeed, ANME-1 archaea have been found in environments with a temperature range between 3 and 95 °C, and were active in the range 5-70°C. Contrarily, ANME-2d and NC10 bacteria, derived from freshwater environments, are found in conditions of 22 to 35°C, as preferred range, but may also survive in colder environments (until ~10°C) (Cai et al., 2021).

2.6.2 pH

The optimum pH values for the growth of aerobic methanotrophs, and CH_4 oxidation, in soils generally lie between 5.5 and 8.5. Specifically, in landfill covers it depends on the characteristics of the soil material used; for example, if decalcified or sand-dominated natural substrates are used, pH values can be far below 7 (down to 4.5). Thereby pH limitation of aerobic methanotrophs is unlikely to occur in natural soil substrates, due to the wide pH range in which methanotrophs operate and their capability to adapt to the prevailing environmental conditions. (Scheutz et al., 2009)

The optimum pH values for the growth of anaerobic methanotrophs varies with the species. ANME-3 archaea are active in pH range of 7.7 to 7.9. ANME-2 archaea were found to have an optimum pH between 7 and 7.5, from *in vitro* tests, and a value of 8.3 *in situ*. ANME-1 archaea have a wider optimum pH range, that goes from 6.8 and 8.1, with higher values not tested. Concluding with NC10 bacteria, that exhibit AOM activity over a pH ranging from 6.0 to 9.0, with the optimal value at 7.6. (Cai et al., 2021)

2.6.3 Inorganic nitrogen content

Inorganic nitrogen (ammonium [NH_4^+]/nitrate [NO_3^-]) might stimulate or inhibit CH_4 oxidation in soils, depending on: species of N and their concentrations, CH_4 concentrations, pH, and type of methanotrophs present. Methanotrophic bacteria have a relatively high nitrogen demand: for every mole of assimilated carbon, 0.25 mole of N is required. Therefore, limitation of inorganic nitrogen may occur, especially in environments in which the molar ratio of CH_4 to N is higher than 10 (assuming 40% assimilation of every CH4 mole consumed), such as landfill soils. If nitrogen is depleting in a long-term scenario, bacterial growth and protein synthesis decrease, leading to reduction or cessation of methane consumption. On the other hand, high NH_4^+ concentrations in soils tend to inhibit aerobic CH_4 oxidation, due to NH_4^+ acting as a competitive inhibitor towards MMO enzymes (Pedersen, 2010). The same stands for high NO_3^- concentrations, that have proven to be inhibitory through osmotic effects (Scheutz et al. 2009, X. Zhang et al. 2014).
The opposite happens for the N-AOM process, indeed in this case, incrementing nitrogen input, enhances the activity and the abundance of nitrate-dependent methanotrophs in the environment (Yang et al. 2023, Cai et al. 2021).

2.6.4 Soil physical parameters

The physical properties of the microbial methane oxidation system (MMOS) material, that influence methane oxidation are: porosity, permeability, diffusivity, particle size distribution, and water holding capacity (Pedersen, 2010). Indeed, the porosity of the MOL material, its water holding capacity after placement, and its gas permeability have a crucial effect on the performance of the MMOS (Gebert et al., 2022a).

Soil permeability includes both liquid and gaseous fluxes in the material. Particularly, gas permeability gives information on the materials' ability to support advective flux. In fact, the transport of the landfill gas to the CH_4 oxidation zone is mainly advective and therefore this is an important parameter. A material with homogeneous gas permeability will minimize the formation of hotspots and minimize the risk of the landfill gas escaping through lateral sides of the landfill and installations, such as leachate and gas collection wells (Pedersen, 2010). In addition, a small-scale patterned gas permeability of the soil causes preferential pathways, in which the landfill gas flows causing a selection in the composition of the microbial community (Gebert and Perner, 2015), exceeding the oxidation capacity and ultimately ending up in higher methane emission in the atmosphere.

Regarding liquid fluxes in the soil, permeability depends on soil particle size, porosity and degree of saturation. Water content, addressed also as soil moisture content, is essential to the life of microorganisms. In fact, it provides both the supply of nutrients and the removal of residual metabolic compounds, as transport medium (Scheutz et al., 2009). Regarding anaerobic methane oxidation, water content provides more substrate for ANME-2d archaea as a consequence of promoting methanogenic activity for CH_4 production (Yang et al., 2023) (Cai et al., 2021). At soil's degree of saturation higher than 85% (volume of water/volume of voids), the air-filled voids are no longer interconnected and the gases have to diffuse in the liquid phase, this leads to a substantial decrease of CH_4 and O_2 availability, therewith limiting methane oxidation. This is a crucial point for both aerobic and anaerobic CH_4 oxidation; in the first case because of the needs of both gases (methane and oxygen), and in the latter one because of the needs of CH_4 solely. Moreover, it is also important to consider that water saturation of the soil can lead to increased lateral gas transport, causing emissions adjacent to the landfill or to a pressure build-up creating the necessary driving force for advective transport through the soil (Scheutz et al., 2009). On the contrary, a soil moisture content of less than 5% (dry weight basis) leads to no methane oxidation activity, and the desiccation of the CH_4 oxidation layer leads to the formation of cracks and fissures, causing hotspots in the cover with relatively high emissions of methane (Cossu and Stegmann, 2018). The optimum soil moisture content is reached when both maximum gas phase diffusion and sufficient soil moisture content to achieve microbial activity to oxidize the methane delivered. For landfill cover soils, the optimum soil

moisture content ranges between 10 and 20% w/w; in general, the specific value is dependent on soil texture, precisely on the specific pore size distribution, that determines the pore volume available for water retention and gaseous transport (Scheutz et al., 2009).
Another important physical property, connected to temperature, is the thermal conductivity of soils. The thermal conductivity is the capacity of a material to conduct heat, and consequently, it affects the temperature in the biocover materials. If the thermal conductivity is high, a good insulation is provided.
In the end, also the specific surface area of materials has a role. Methanotrophs live adhered to the surface, therefore materials with high specific surface allow to have a high density population of methanotrophs (Pedersen, 2010).

2.6.5 Availability of electron acceptors and oxygen exposure

Methane oxidation processes are accomplished only in presence of an electron acceptor, which can be oxygen in case of aerobic CH_4 oxidation, or sulphate, nitrate, nitrite, and Fe(III)/Mn(IV) oxides, for anaerobic methane oxidation.

Aerobic methanotrophic bacteria can achieve optimum CH_4 conversion rates even at very low oxygen concentrations; in pure methanotrophic cultures the maximum methane oxidation rates, in both type I and II bacteria, are reached with O_2 concentrations ranging from 0.45 to 20%, while in biofilter material with approximately 9%. Furthermore, in landfill cover soils, CH_4 oxidation starts at oxygen concentrations above 1.7–2.6%. (Scheutz et al., 2009)

Anaerobic methanotrophic bacteria may survive in environments with infrequent oxygen exposure, depending on the species. For example, ANME-2 archaea are more robust than ANME-1 archaea in withstanding oxygen stress. Nonetheless, it is still unclear how these species survive in aerobic environments.
In marine sediment, the predominant electron acceptor is sulphate, used by ANME-1, ANME-2 and ANME-3 archaea to carry out anaerobic methane oxidation. However, some anaerobic methanotrophs are able to use also other electron acceptors, as described in chapter 5, because of the thermodynamical advantage (Fe(III)/Mn(IV) or nitrate/nitrite are more thermodynamically favourable than sulphate), or in order to cope with varying environmental conditions. In consequence, the availability and type of electron acceptor may regulate metabolic pathways of anaerobic methanotrophs; specifically, in absence of nitrate or sulphate, ANME-2 species can alter their metabolic pathways to respire metal oxidants. However, over a long-term period, anaerobic methanotrophs prefer to use the primary electron acceptor when it is available. (Cai et al., 2021)

2.6.6 Inhibitory substances

Methane oxidation can be inhibited by specific substances, and the extent of inhibition depends on the concentration of the inhibitor and of CH_4 in the landfill gas, as well as on the methanotrophic community composition. Some inhibitory substances for aerobic methane oxidation are: difluoromethane, dichloromethane, methyl fluoride, acetylene, ethylene, NH_4^+, methanethiol, carbon disulphide, and hydrochlorofluorocarbons (HCFCs). (Scheutz et al., 2009)

2.6.7 Extracellular polymeric substances (EPS)

Extracellular polymeric substances, initially addressed as exopolymeric substances (EPS), are polymers biosynthesized by several strains of microorganisms. EPS is composed mainly of polysaccharides, proteins, and DNA, and the production is triggered primarily by environmental signals, such as variations of temperature and pH (Costa et al., 2018), or nitrogen limiting conditions – in fact, type I aerobic methanotrophs are not able to fixate nitrogen from the atmosphere, but it is believed that they respond to nitrogen limiting conditions by producing EPS, which has a higher C/N ratio than bacterial cell tissue (Pedersen, 2010). Since extracellular polymeric substances' biosynthesis is energetically expensive, they should generate some kind of advantage for the producer microorganism; indeed, the matrix produced by EPS around microbial cells has different functions, reported in Figure 2.27: capability of shielding them against antimicrobial compounds and heavy metals, retain water, protecting microbes and the environment against drought, adhesion, communication with other microbes and plants, antioxidant, aggregation, carbon storage, and entrapment of nutrients (Costa et al., 2018).

Methanotrophs, both type I and type II (Scheutz et al., 2009), are known to produce EPS in the form of capsules and copious slime (Hilger et al., 2000). The amount of EPS production per unit of carbon substrate is higher in type I methanotrophs, using the RuMP pathway, compared with type II methanotrophs using the serine pathway for carbon assimilation (Wei et al., 2015).

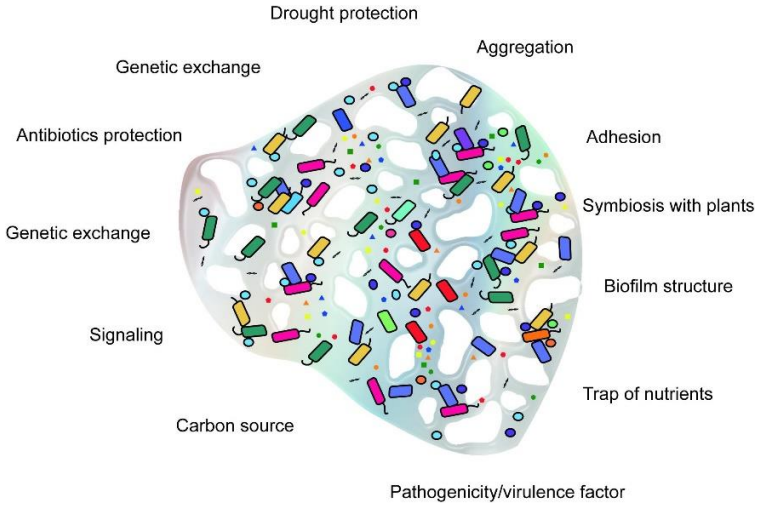


Figure 2.27 Conceptual framework of the functions of EPS in soil (Costa et al., 2018)

EPS is produced at high CH_4 flux rates, as a metabolic mechanism to prevent formaldehyde accumulation when carbon is in excess, but also as a means to protect the cells from O_2 stress. Extracellular polymeric substances accumulation has several consequences: a decrease in diffusivity, and therefore O_2 limitation and, after prolonged gas exposure, has been hypothesized to cause methane oxidation rates decline after a peak (Wei et al., 2015).

Under field conditions, EPS formation may be lower with respect to laboratory tests, or it may be removed by dissolution and other processes soon after formation. In addition, its accumulation is reduced by fungi, that grow on exopolymeric slimes and sugar crystals. EPS formation in landfills is mainly linked to hotspots with high methane emissions due to inhomogeneous gas release. (Huber-Humer, 2005)

2.7 Methane oxidation potential: measuring techniques in laboratory pre-tests

To assess the methane oxidation potential of the methane oxidation layer of MMOS, two main laboratory tests can be performed, prior to the installation of MMOS:

- incubation experiments – batch tests, the batch reactors are closed systems (impermeable for gas), where initial gas concentrations can be adjusted, together with a constant temperature, and the actual gas concentrations are measured regularly (Sindern et al., 2013). These are technically simple, have a lower cost and are less laborious to conduct compared to the second type. Therefore, also due to their small dimensions, they are used in case of high number of samples, or furthermore, when the goal of the experiment is to determine the impact of different environmental parameters, because of batch conditions that can be easily manipulated. On the other hand, in this kind of test is not possible to simulate the dynamic gas transport, typical of landfill covers, or to evaluate the consequences of long-term gas exposure (Scheutz et al., 2009); and
- continuous gas flow systems – packed soil column test, these are more suitable for a higher mass input and in the presence of heterogeneous materials, such as compost. Column tests are able to simulate the dynamic gas transport that occurs in landfill soil covers and the effects of long-term gas exposure. In general, aerobic conditions are performed, with defined air supplied at the top and a controlled methane influx from the bottom, and the conditions are kept constant for the whole experiment – for example room temperature. In these long-term laboratory column experiments, CH_4 oxidation rates often exhibit a peak followed by a decrease to a lower steady state value (Scheutz et al., 2009). The advantage of such kind of experiments is that the results are more representative of real landfill covers with respect to results derived from batch-tests; on the other hand, it is more difficult to unravel the diverse processes and conditions of such complex column microcosm tests (Huber-Humer, 2004). A typical column setup is reported in Figure 2.28.

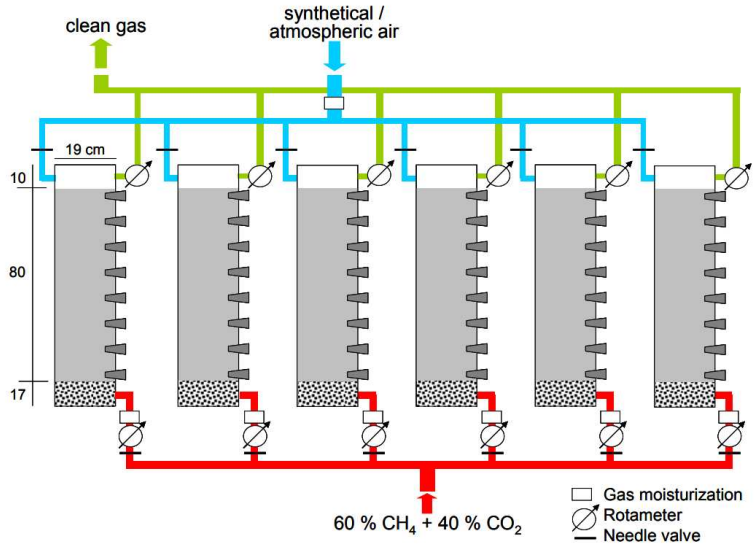


Figure 2.28 Schematic column setup (Gebert et al., 2008)

It was observed that columns are usually operated with CH_4 inlet concentrations of 50 or 100% v/v and CH_4 loads between 200 to 300 g CH_4 $m^{-2}day^{-1}$, which is in the middle to high range of reported landfill methane fluxes. Indeed, active landfills can have gas fluxes up to 1300 g CH_4 $m^{-2}day^{-1}$, but in old landfills or sites with gas collection systems fluxes of approximately 85 g CH_4 $m^{-2}day^{-1}$ are typical. In general, steady state methane oxidation rates for landfill cover soils are between 30 to 60% removal with maximum rates up to 80 to 100% removal (Scheutz et al., 2009).

In Table 2.5 is presented a brief overview of the results of CH_4 oxidation capacity obtained from soils with different organic content (Majdinasab and Yuan, 2017).

Table 2.5 Methane oxidation capacity in landfill cover soils (Majdinasab and Yuan, 2017)

Landfill cover content	CH_4 oxidation capacity		CH_4 loading ($g\,CH_4\,m^{-2}\,d^{-1}$)	Ref.
	Oxidation yield (%)	Oxidation rate ($g\,CH_4\,m^{-2}\,d^{-1}$)		
Landfill cover soil	20–100		35.3–84.7	Gebert et al. (2011)
Four earthly mineral soilssediment containing high organic matter			25–100	Rachor et al. (2011)
Control (without compost content)	63	19.5	29.4	Abichou et al. (2009)
Cover with compost material	100	2.69	2.69	
Garden refuse composts Waste activated sludge compost	23–56	45–112	179–201	Pedersen et al. (2011)
Mixture of soil and earthworm cast	99–100	232	233.6	Park et al. (2008)
Mixture of soil and PAC*	99–100	232	233.6	
Landfill cover soil	51	118	233.6	
Biologically-mechanically treated MSW		22–82	30–78	Einola et al. (2008)

2.7.1 Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectroscopy is the measurement of different infrared frequencies (IR) by a sample located in the path of an IR beam. Spectrometers can be used with a wide range of sample types such as gases, liquids, and solids. The principle behind IR spectroscopy is the Beer-Lambert law, defined in 1852, which relates the absorption of light to the properties of the material through which the light is travelling. The first spectrophotometers, also called dispersive spectrometers, were invented at the beginning of the 20th century, and, due to the rapid technological progress, in the mid-1960s Fourier transform infrared (FTIR) spectrometer was conceived. Compared with the first generation of spectrometer, the FTIR instrument has several advantages (Aroui et al., 2012):

- "Felgett advantage" → a complete spectrum is obtained during a single scan of the moving mirror, while the detector observes all frequencies simultaneously;
- "Jaquinot advantage" → increased optical throughput. FTIR systems use circular optical aperture is used, and the beam area of a Fourier transform instrument is about 100 times larger than the one of a dispersive spectrometer; thus, more radiation energy is available;
- "Connes advantage" → use of a helium neon laser as internal reference. This source radiation provides an automatic and stable calibration for all wavelengths. This eliminates, to some extent, the need for external calibration sources;
- Computerized data system (Fourier transform algorithm) → providing a wide variety of data processing tasks, such as baseline correction, smoothing and integration; and reducing the computation time.

FTIR spectroscopy is accomplished using an interferometer, which allows the scanning of all the frequencies present in the IR radiation generated from the source. Scanning is possible thanks to a movable mirror which, by moving, introduces an optical path difference, which gives rise to constructive or destructive interference with the beam reflection from a fixed mirror. In this way, the representation of the intensity, in the time domain, is obtained. Then the representation of intensity in the frequency domain, that is the infrared spectrum, is acquired by applying the Fourier transform. FTIR is used for the study of soil chemical processes. Indeed, in the mid-infrared (mid-IR) range, vibrations arise from many environmentally relevant molecules, such as organic acids, soil organic matter, and mineral phases (Peak, 2005).

Soil samples can be analysed by FTIR spectroscopy using different methods. The most common are: transmission, diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS), and attenuated total reflectance (ATR). In particular, ATR-FTIR spectra provide information on functional groups near the surface (approximately 1 μm) of an internal reflection element (IRE), and one of its key

advantages is the ability to collect accurate spectra of samples in the presence of water. Overall, this technology furnishes a high degree of experimental and analytic flexibility, indeed there is a wide variety of methods for collecting spectra, and soil components and processes are particularly suitable for this kind of molecular analysis (Margenot et al., 2017).

This leads to the possibility to apply FTIR spectroscopy also to MMOS, in order to predict a specific methane oxidation rate of a potential biocover material. To date, just one study has been made on this topic: "*Scrutinizing compost properties and their impact on methane oxidation efficiency*" by Huber-Humer et al., published in 2011. The aim of this paper was to delineate and discuss the main properties and routine parameters of composts, which may be fundamental in defining and assessing the suitability of these materials for biocover construction, and therefore to support material selection. FTIR spectroscopy was used to characterise compost material as support media in methane oxidation by means of a more comprehensive approach. The spectral characteristics, derived using FTIR spectroscopy and multivariate data analysis, can indicate different chemical properties of composts, such as total organic carbon (TOC), organic matter content (LOI), C, total and available N, respiration activity (RA_7), and humic acids (HA), as well as other parameters like plant suppression and phytotoxicity. Thus, crucial parameters determining methane oxidation capacities can be derived from FTIR spectra. Although the data used were not enough to be statistically relevant, the conclusions, regarding the use of this technology, were that FTIR spectroscopy may represent a promising tool for the characterisation and selection of methane oxidation substrates in the future (Huber-Humer et al., 2011).

Chapter 3: LABORATORY EXPERIMENTS TO ASSESS AEROBIC AND ANAEROBIC METHANE OXIDATION POTENTIAL

3.1 Materials and methods

To answer the research question reported in the introduction of this thesis, two main laboratory tests were conducted. The first with the goal to observe the responses of microbial activity in different depths of a hotspot to different methane flow rates, under aerobic conditions; and the second with the goal to observe methane oxidation under anaerobic conditions.
A graphical description of the experiments performed is reported in Figure 3.1.

Concurrently with the first two laboratory tests, another analysis was performed, using Fourier Transform Infrared Spectroscopy (FTIR). The goal of this analysis is to compare the spectrum of the previously investigated material, in different conditions: from the landfill, after the aerobic columns experiment and after the anaerobic columns experiment; to search for differences which could help having a more comprehensive understanding of the process occurred during the experiments, or even lead to the prediction of the methane oxidation capacity of the specific material. Indeed, the spectrum gives information on the chemical composition of the sample, like a chemical ‘‘fingerprint”, from which the sample’s specific characteristic could be derived.

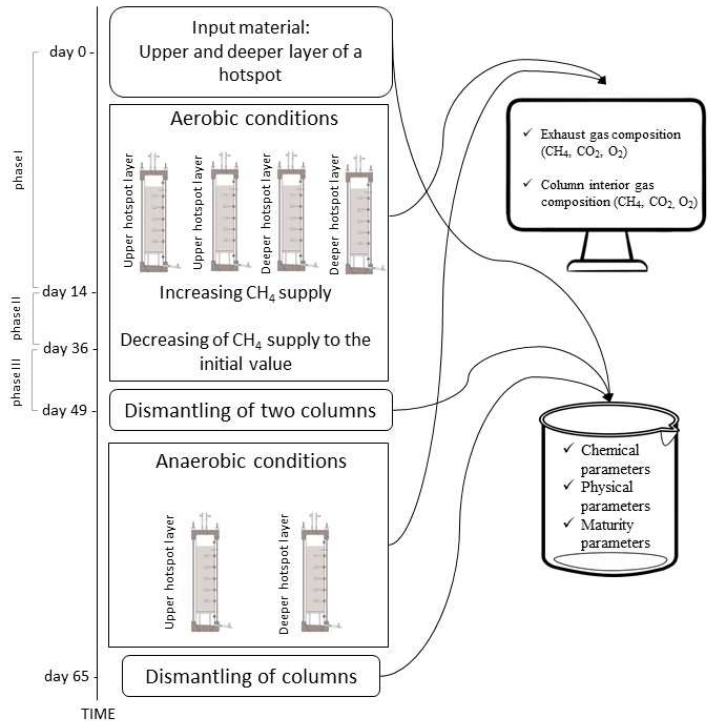


Figure 3.1 Schematic diagram of the column experiments (unscaled axis)

3.1.1 Soil samples characteristics

The material used for these two laboratory tests comes from biowindow G19 (see 3.1.1.1) installed at the "Landfill Allerheiligen", located in Styria, Austria. The active landfill gas collection system – consisting of gas collection points, compressor station and gas flare – was shut down, because of the fluctuating gas qualities, decreasing gas production, and the consequent impossibility of sufficiently treating the landfill gas. It is converted to a passive landfill gas treatment via methane oxidation windows, after the dismantling of the gas wells.

3.1.1.1 Landfill site and biowindow description

"Landfill Allerheiligen" was built in 1978 for the disposal of municipal solid waste after mechanical-biological pre-treatment. The total capacity is 540,000 m^3 approximately, and the total area is 10 ha (100000 m^2). The landfill is divided into four sections, three of which are closed (sections I-III) and one is still in operation (section IV), which were adapted to the state of the art in accordance with the Landfill Ordinance in the years 1993-1999. Between 1994 and 2014, landfill gas was collected through the active collection system. The latter was composed of vertical gas wells (steel, DN 800) and horizontal gas collection pipes (HDPE, DN 63), both surrounded by gravel, and by eight gas collection stations in two main lines – one directed to the high-temperature flare, and the second to the biofilter. The landfill gas was extracted using two compressors, each with a capacity of 300 Nm^3/h. The landfill sections I-III are currently covered with a 1 m thick mixture of soil and compost.

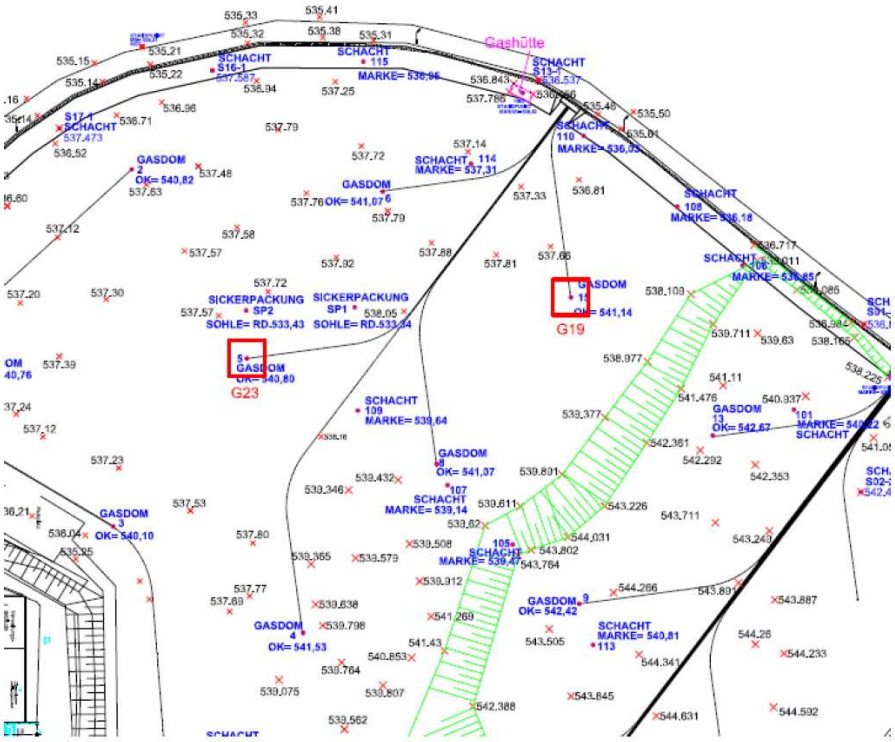


Figure 3.2 Section of the Allerheiligen landfill plan – location of the biowindows G23 and G19 (Hrad and Huber-Humer, 2015)

In 2014, two pilot methane oxidation windows (G23 and G19) were installed on landfill sections I-II, as shown in Figure 3.2 Windows G23 and G19 both covered an area of 6 x 6 m at the base, which was enlarged to 7 x 7 m for window G19 in April 2015. Both biowindows consist of a gas distribution layer (GDL), characterised by a depth of 0.5m and made of basalt gravel with a grain size between 32 and 64mm, and an overlying methane oxidation layer (MOL) of mature compost of 1.4m depth, after the settlement. The compost material was positioned approximately 1 - 1.5m over each side, in order to prevent undesired leakage of landfill gas from the edges of the biowindow. Furthermore, it was added with structural material in the form of wood chips (volume ratio 70:30) prior to installation, and its basic suitability as a carrier substrate for methane oxidation was confirmed in advance in column tests under standardized conditions, in which complete methane degradation was achieved with a methane load of approximately 200 Nl CH_4/m^2d, which is the double of the value specified in the Austrian MMOS guidelines (96 Nl CH_4/m^2d) (Huber-Humer et al., 2008a). The design of the biowindows G23 and G19 is shown in Figure 3.3, while Figure 3.4 and

Figure 3.6 depict the conditions of the gas distribution layer and the methane oxidation layer, respectively, during the installation of the biowindows, and Figure 3.5 and Figure 3.7 respectively show windows G23 and G19 after the enlargement works in 2015 (Hrad and Huber-Humer, 2015).

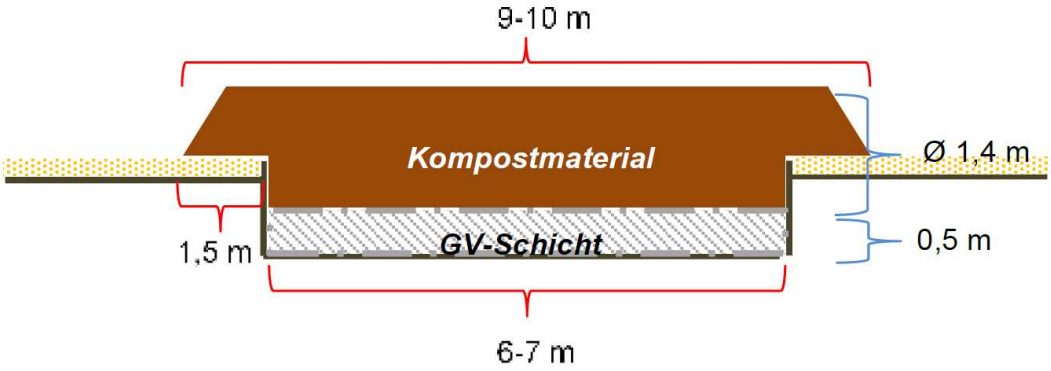


Figure 3.3 Cross-section of biowindows G19 and G23 (Kompostmaterial means compost material, and GV-Schicht stands for GDL) (Hrad et al., 2022)

Figure 3.4 Photo of the biowindows' GDL (Hrad and Huber-Humer, 2015)

Figure 3.6 Photo of the biowindows' MOL (Hrad and Huber-Humer, 2015)

Figure 3.5 Photo of biowindow G23 after the enlargement (Hrad and Huber-Humer, 2015)

Figure 3.7 Photo of biowindow G19 after the enlargement (Hrad and Huber-Humer, 2015)

After the construction of G19 biowindow, it was evident that the landfill gas was not homogeneously distributed and that there was a temporal methane overload, especially in the upper – north-west – edge area; the limit value of gaseous emissions for hotspots (< 10 kg CH_4/m^2year), according to the Landfill Ordinance (DVO 2008), was exceeded both in 2014 and 2015, with a significant decrease in methane emissions in 2015, due to the enlarging of the biowindow itself. Therefore, to be able to implement further adaptation measures and rehabilitation options in this hotspot area, the gas flows and the framework parameters are under examination.

This thesis work happens in this investigation framework. The material used comes from different depths of window G19, sampled in October 2022 at the hotspot area, eight years after the installation. Specifically, the samples were taken from the upper layer – 10-20 cm – and from the deeper part – 90-115 cm, as depicted in Figure 3.8 and Figure 3.9 respectively. The upper layer could be visually identified as a methane oxidation horizon, for the presence of dried EPS slime and the clogging of the material. The material sampled was used also for another master thesis, focused on reviewing methane oxidation performance after 8 years of field use.

Figure 3.8 Sampling of the upper layer

Figure 3.9 Sampling of the deep layer

3.1.1.2 Samples characteristics analysis

Before the installation of the tested columns, the samples were examined to evaluate the essential parameters for methane oxidation. The same examinations are repeated after the completion of the test. During the dismantling of the columns, the substrates were removed in layers; and the sampling in layers was carried out according to the classification: from the methane oxidation horizon (0-15 cm depth in the columns) and below the methane oxidation horizon (15-40 cm depth in the columns). The following parameters were analysed:

- Physical parameter: water content;
- Chemical parameters: pH, conductivity, ammonium, nitrate, sulphate, total phosphorus, total nitrogen, organic content (loss on ignition at 550°C), total organic carbon (TOC) and total inorganic carbon (TIC);
- Maturity parameters: respiration index at 4 days (RA_4) and 7 days (RA_7).

The initial analysis results are summarized in Table 3.1. For the final samples, the analysis of total phosphorus, and respiration activity, were not performed. The parameters analysed show a higher ammonium content in the upper layer, that is where the aerobic methane oxidation occurs, with respect to the lower one; nevertheless, the value does not exceed the one indicated in the Austrian guidelines on MMOS (Huber-Humer et al., 2008a). A significant difference instead is found in the water content values, which is lower in the upper layer, showing that the zone was dry. The value of nitrate, as well, is significantly higher in the upper layer, this could be linked to the natural cycle of nitrogen, and to the presence of vegetation. Nonetheless, observing the notable difference with the lower layer, another correlation could be hypothesised. Indeed, in the deep layer a higher presence of anaerobic methanotrophic bacteria could occur, as one of their systems to gain energy is to consume nitrates, as reported in literature. The contrary happens for the value of sulphates, which is higher for the deep layer, also this could be linked to the presence of different microbial communities in the two layers of the MMOS. In addition, the higher presence of organic content and TOC in the upper layer, could be due to a higher existence of microbial biomass. This could be confirmed also by the values of the respiration activity, which is significantly higher in the upper layer with respect to the lower one.

Table 3.1 Original material characteristics

Parameter	Unit	G19 10-20cm	G19 90-115cm
Water content	% w/w	25,9%	49,1%
Ph	-	7,4	8,2
Conductivity	mS/cm	0,68	0,52
NH_4^+ -N	mg/kg DM	74	39
NO_3 -N	mg/kg DM	269	50
SO_4^{2-} -S	mg/kg DM	60,4	92,8
P total	% DM	0.4%	0.35%
N total	% DM	2,5%	1,6%
Organic content	% DM	41,5%	31,7%
TOC	% DM	23,3%	17,5%
TIC	% DM	1,2%	1,3%
RA_4	mgO_2 /g oDM	4,0	1,4
RA_7	mgO_2 /g oDM	6,1	2,1

3.1.2 Column experiment – aerobic conditions

In order to simulate as closely as possible landfill cover conditions, soil columns (microcosms) were used to test the various samples. The experiment was carried out at a temperature of 22°C ±1°C, in a dark climate chamber, using four transparent plastic columns made of gas-tight acrylic glass, which schematic diagrams are illustrated in Figure 3.10 and Figure 3.11. In each of the four columns of height 70 cm, and inner diameter 19 cm, a perforated PVC plate with a nylon mat was placed to distribute more homogeneously the gas and for a potential water drainage. Underneath this plate, a volume of height 5 cm, approximately, and inner diameter 19 cm, was left free for the gas entry, where pure methane was continuously added. In addition, air was provided from the top of the columns. The sampling ports, sealed with rubber septa, were placed every 10 cm in the columns as well as in the gas input tube at the bottom and in the effluent tube at the top; the outlet tube was linked to a gas buffer bag, used to measure the gas composition of the effluent. The gas composition (CH_4, CO_2 and O_2) of the exhaust air and of the column interior air at various depth levels (10 cm, 20 cm, 30 cm and 40 cm), was measured twice a week, using a landfill gas measurement device. The measurement of carbon dioxide and methane is based on the principle of non-scattered infrared absorption (measurement accuracy: 0.05%), while oxygen is measured using a special galvanic sensor (measuring range: 0–25 vol.%). Together with the gas composition, also the inlet flow rates were checked, to verify methane and air supply values.

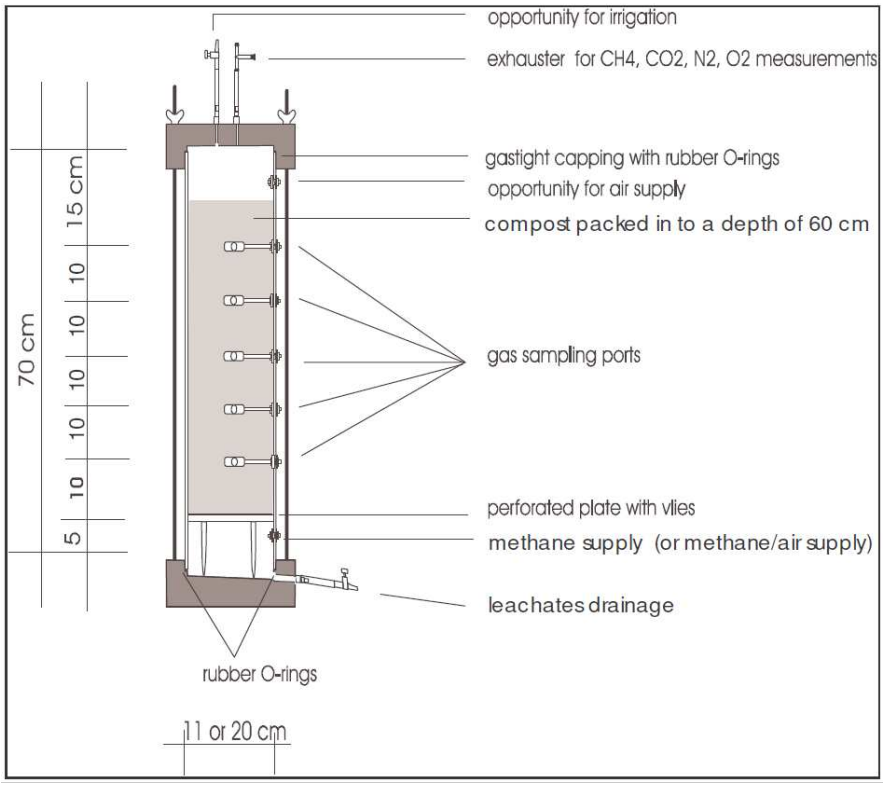


Figure 3.10 Schematic diagram of a microcosm-column used in the laboratory tests (Huber-Humer, 2004)

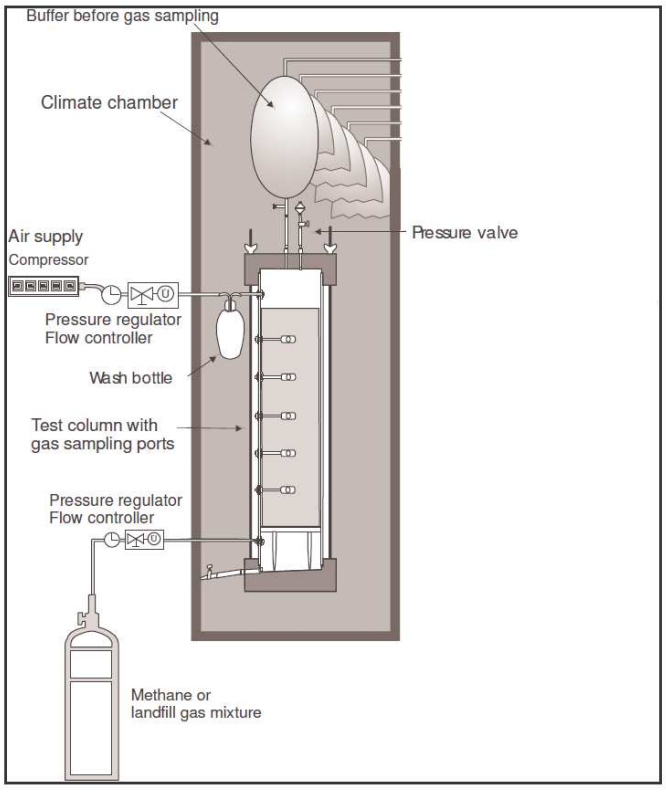


Figure 3.11 Schematic design of experimental setup of laboratory tests (adapted from Huber-Humer, 2004)

Each test sample was investigated in a duplicate approach, this means that two test columns were used for each of the two depths (10-20 cm, and 90-115cm), with a total of four columns. In order to ensure comparable and optimised conditions for all test columns, the material was firstly homogenized, particularly optimizing the water content of the upper layer sample, and then placed inside the columns divided into 5-6 layers of 1-2 kg each, that were put in and slightly compacted using a 2 kg weight, dropped twice from a height of around 10 cm.

After the installation of the material, the columns were hermetically sealed and continuously charged with methane – synthetic gas from a gas bottle, 100% by volume CH_4 – from below. The amount of methane supplied was regulated using a digital mass flow controller. The air was supplied via a compressor in the upper part of each column, above the built-in material, so that the air had to diffuse into it, which best simulates natural landfill conditions; and its flowrate was regulated by flowmeters with needle valves or by digital mass flow controllers. In addition, the columns with the material coming from the deeper part of window G19 were humidified by feeding the methane through a bottle filled with water, during phases II and III.

The columns' technical specifications are given in Table 3.2.

Table 3.2 Columns' technical specifications

Column name	Material origin	Number of layers	Tara [kg]	Sample weight [kg]	Total weight [kg]	Sample weight [kgDM]	Height [cm]	Volume [l]	Density [kg/l]
S1a	G19 Hotspot 10-20 cm	5	9,4	9,5	18,9	5,9	47,1	13,4	0,7
S2a	G19 Hotspot 10-20 cm	5	9,3	9,2	18,5	5,7	45,6	12,9	0,7
S3a	G19 Hotspot 90-115 cm	6	10,2	11,0	21,2	5,8	44,7	12,7	0,9
S4a	G19 Hotspot 90-115 cm	6	10,1	11,0	21,1	5,8	44,9	12,7	0,9

To test the maximum methane oxidation capacity of the samples, the test procedure was divided into three phases with different methane loads. In the beginning, the methane supplied was 200 $NlCH_4/m^2d$, which represents the double of the value of the surface load for a methane oxidation layer (96 Nl CH_4/m^2d), specified in the Austrian MMOS guidelines (Huber-Humer et al., 2008a), which can usually be reduced without any problems by suitable materials; the air supply was calculated to be the optimum value for microbial activity – methane to oxygen ratio 1:2.9 in accordance with (Huber-Humer, 2004) – and its flowrate was 56 ml/min. Afterwards, in phase II, the methane flow

rate, and consequently the air flow rate, were increased to 300 Nl CH_4/m^2d and 64 ml/min respectively; and in the end, during phase III, they were brought back to the initial values.
Trial framework conditions are reported in Table 3.3.

Table 3.3 Aerobic trial framework conditions

	Phase I	Phase II	Phase II
Ambient temperature		22 °C	
Methane supply [ml/min]	4	6	4
Methane supply [Nl/m²d]	200	300	200
Air supply [ml/min]	56	64	56
Duration	14 days 06.03.2023 - 20.03.2023	22 days 20.03.2023 - 11.04.2023	13 days 11.04.2023 - 24.04.2023

3.1.3 Column experiment – anaerobic conditions

The setup of the soil columns test under anaerobic conditions was the same as the one under aerobic conditions, as well as the parameters monitored. One column for each depth (10-20 cm and 90-115cm) was used, from the test under aerobic conditions (columns S1a and S4a), but instead of air, pure nitrogen was provided from the top. The columns' technical specifications are given in Table 3.2, referring only to columns S1a and S4a; and Figure 3.12 shows the setup of the columns under anaerobic conditions.

Figure 3.12 Columns under anaerobic conditions

To observe anaerobic methane oxidation a methane load of 100 Nl CH_4/m^2d was provided, corresponding to the value of the surface load for a methane oxidation layer, specified in the Austrian MMOS guidelines (Huber-Humer et al., 2008a). The test should have been run for 21 days (from 24.04.2023 to 15.05.2023), but due to the inspected ending of the nitrogen bottle, it had to be stopped at day 16 (24.04.2023 - 10.05.2023). The quantity of nitrogen in the bottle started to be significantly low after ten days from the start of the experiment, and it finished around day 14. Consequently, the columns were dismantled two days after. Trial framework conditions are reported in Table 3. 3.4.

Table 3. 3.4 Anaerobic trial framework conditions

Ambient temperature	**22 °C**
Methane supply [ml/min]	2
Methane supply [Nl/m²d]	100
Nitrogen supply [ml/min]	48
Duration	16 days 24.04.2023 - 10.05.2023

3.1.4 FTIR analysis

In this thesis mid infrared spectroscopy (MIR), using the attenuated total reflection (ATR) technique, was applied to investigate the fingerprint of the samples used in the columns experiments. ATR spectra were collected with a Bruker Alpha, shown in Figure 3.13 Bruker Alpha FTIR spectrometer, in the wavenumber range from 4000 to 400 cm^{-1}. The milled sample is directly applied on the ATR reflection module containing a diamond crystal and pressed by a pressure applicator. The measured area was approximately 4 mm^2. Twenty-four scans per spectrum were collected at a resolution of 4 cm^{-1}, a zero-filling factor of 2 and corrected against ambient air as background. Three spectra per sample were averaged for data analysis. The maximum deviation of the three spectra from the average spectrum was lower than 5%.

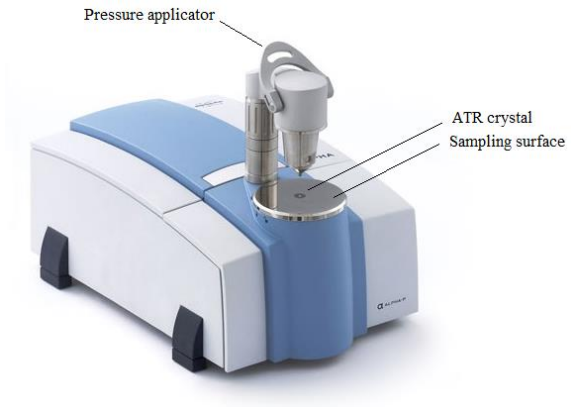


Figure 3.13 Bruker Alpha FTIR spectrometer

The material analysed came from:

- Hotspot G19, 10-20 cm depth
- Hotspot G19, 90-115 cm depth
- Column S1a under anaerobic conditions, 0-15 cm depth – input material 10-20 cm hotspot
- Column S1a under anaerobic conditions, 15-40 cm depth – input material 10-20 cm hotspot
- Column S2a under aerobic conditions, 0-15 cm depth – input material 10-20 cm hotspot
- Column S2a under aerobic conditions, 15-40 cm depth – input material 10-20 cm hotspot
- Column S3a under aerobic conditions, 0-15 cm depth – input material 90-115 cm hotspot
- Column S3a under aerobic conditions, 15-40 cm depth – input material 90-115 cm hotspot
- Column S4a under anaerobic conditions, 0-15 cm depth – input material 90-115 cm hotspot
- Column S4a under anaerobic conditions, 15-40 cm depth – input material 90-115 cm hotspot

Before the analysis with the spectrometer, the samples were prepared. The first step of the sample preparation was to "air drying" them in the oven at 30°C, for 4-5 days until all the liquids inside were evaporated. Secondly, the dry samples were milled using in sequence Retsch SM 2000 cutting mill, Retsch ZM 200 ultra centrifugal mill, and Retsch RS100 vibratory disc mill.

3.2 Results

3.2.1 Sample characteristic analysis

The final sample characteristics analysis results, after the column test in aerobic and anaerobic conditions, are reported in Table 3.5 and Table 3.6, respectively.

If the initial samples from the biowindow, reported in Table 3.1, and the samples from the aerobic columns test are compared, most of the values don't exhibit any variation, such as conductivity, total nitrogen and TIC; while the organic content and TOC show slightly higher values after the aerobic columns test, probably indicating a higher microbial biomass in the methane oxidation horizons. Instead, values of pH, ammonium, nitrates and sulphates present some variations. The pH value has a stabilization at ~7.7, for both the upper and lower layers. The ammonium values present an increase for both types of samples, reaching the same value (~77 mg/kg DM) for the upper part of the columns, and differing in the lower zone: while a slight increase – reaching a value in between of the initial condition and the upper layer of the column – occurs in column S3a (containing the lower layer of hotspot G19), the deeper part of column S2a (containing the upper layer of hotspot G19) shows a significant increase. The nitrate values show an accumulation in the upper layer of hotspot G19, and lower values in all columns, showing a dynamic behaviour. The sulphate values show a decrease in all samples from field to column conditions, except for the deep part of the column containing the lower layer of hotspot G19, which value increased.

Table 3.5 Final sample characteristics - aerobic conditions

Parameter	Unit	S2a (G19 10-20cm) 0-15cm	S2a (G19 10-20cm) 15-40cm	S3a (G19 90-115cm) 0-15cm	S3a (G19 90-115cm) 15-40cm
Water content	% w/w	34,6%	35,9%	47,6%	49,5%
pH	-	7,7	7,7	7,6	7,7
Conductivity	mS/cm	0,5	0,47	0,56	0,46
NH_4^+ -N	mg/kg DM	78	160	77	64
NO_3 -N	mg/kg DM	32	38	53	49
SO_4^{2-} -S	mg/kg DM	18,7	17	22,6	123,3
N total	% DM	2,7%	2,6%	2%	1,6%
Organic content	% DM	42,9%	40,3%	33,5%	26,5%
TOC	% DM	23,6%	22,4%	19,3%	15,4%
TIC	% DM	1,2%	1,3%	1,2%	1,5%

Moreover, if anaerobic conditions are compared with both samples from aerobic and field conditions, the only characteristic with a significant variation is the one of ammonium, which is significantly higher in both columns under anaerobic conditions; furthermore, its value is considerably higher with respect to the one of nitrates. Another interesting value is the sulphate of the deep part of the column containing the lower layer of hotspot G19, indeed it decreases again after the material was put under anaerobic conditions. Overall, it can be noticed that the difference between the values of the two depths is less pronounced under anaerobic conditions compared to aerobic ones. Additionally, is important to take also in consideration that the anaerobic columns were previously exposed to aerobic conditions, which might have influenced the characteristics' values of the final samples.

Table 3.6 Final sample characteristics - anaerobic conditions

Parameter	Unit	S1a (G19 10-20cm) 0-15cm	S1a (G19 10-20cm) 15-40cm	S4a (G19 90-115cm) 0-15cm	S4a (G19 90-115cm) 15-40cm
Water content	% w/w	37,1%	37,5%	49,6%	48,3%
pH	-	7,5	7,7	7,8	7,7
Conductivity	mS/cm	0,67	0,47	0,56	0,49
NH_4^+ -N	mg/kg DM	147	152	114	80
NO_3 -N	mg/kg DM	26	52	45	57
SO_4^{2-} -S	mg/kg DM	21	18,7	23,2	25,8
N total	% DM	2,7%	2,5%	1,7%	1,5%
Organic content	% DM	42,6%	40,1%	31,2%	29,5%
TOC	% DM	23,3%	21,8%	18,0%	17,7%
TIC	% DM	1,3%	1,3%	1,5%	1,5%

For a better understanding of the latter comparisons in Figure 3.14, the values are graphically compared.

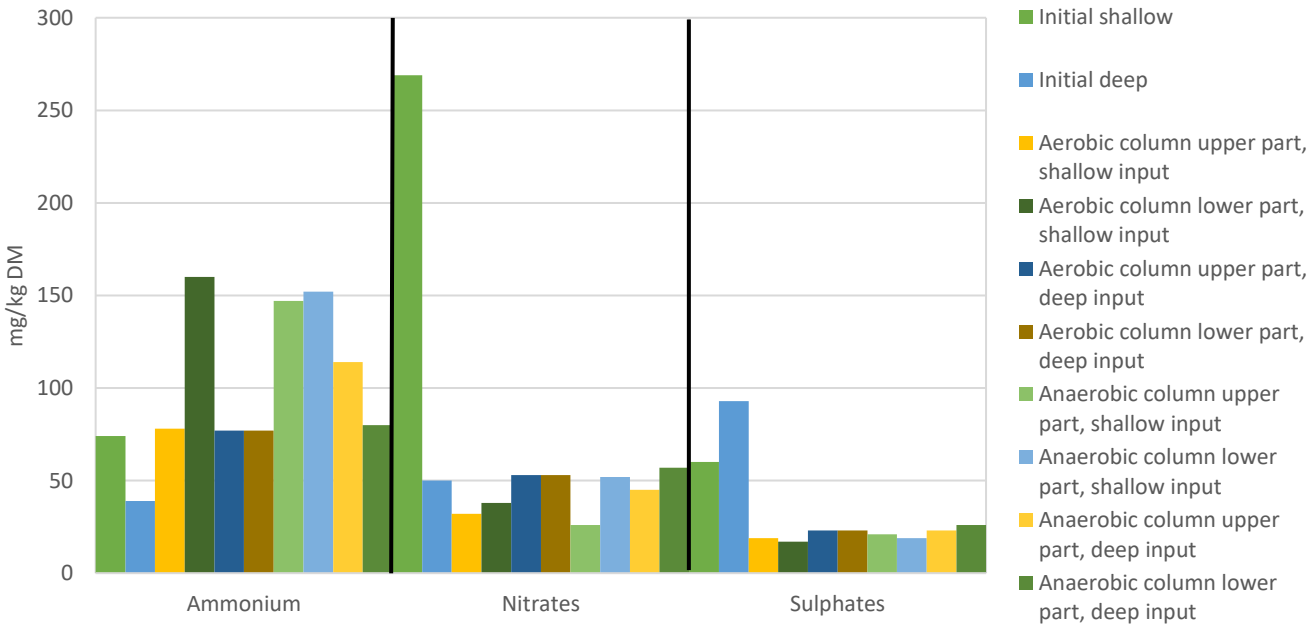


Figure 3.14 Graphical comparison of initial and aerobic and anaerobic ammonium, nitrates and sulphates values

3.2.2 Column experiment - aerobic conditions

To calculate the methane oxidation rates a mass balance was applied, as a consequence of knowing the flow rates in the input and output, as well as the methane concentrations in the inlet and outlet of the tested columns. The methane oxidation rates are presented as relative (percentages) values, related to the charged methane loads, because of the sometimes unstable methane supply flow.

In Table 3.7 the results of the aerobic columns test are presented. The term "shallow" refers to the samples taken from the upper layer of hotspot G19, specifically from 10-20 cm depth, while the term "deep" refers to the samples taken from the deep layer of hotspot G19, of 90-115 cm depth. As can be observed, the values of the shallow columns, S1a and S2a, and of the deep columns, S3a and S4a, are overall comparable in between them, showing similar behaviours.

Table 3.7 Results of tested samples under aerobic conditions

Day	**% CH_4 in exhaust gas [%vol]**				**% CH_4 at 40 cm depth [%vol]**				**% CH_4 oxidation rate**				**CH_4/Air [ml/ml]**			
	Shallow		Deep		Shallow		Deep		Shallow		Deep		Shallow		Deep	
	S1a	S2a	S3a	S4a	S1a	S2a	S3a	S4a	S1a	S2a	S3a	S4a	S1a	S2a	S3a	S4a
1st phase – "medium" methane supply																
3	0,9	2,7	0	0	13,6	18,7	16,1	15,7	88%	75%	100%	100%	8,0	11,0	9,8	9,2
7	0	0	0	0	11,9	14,7	18,1	10,3	100%	100%	100%	100%	7,3	8,4	8,4	6,0
10	0	0	0	0	11,8	14,5	15,6	9,6	100%	100%	100%	100%	7,7	8,9	7,9	5,5
14	0	0	0	0	11,2	13,5	14,6	11,9	100%	100%	100%	100%	7,6	9,1	8,1	7,8
2nd phase – "high" methane supply																
17	0,1	0,8	0,4	0,1	20,8	23,1	26,5	22,2	99%	90%	95%	99%	8,5	9,1	9,2	7,3
21	0,6	1,8	0,5	0,4	22,0	24,5	28,2	26,4	93%	80%	94%	94%	9,4	9,7	8,8	7,3
24	1,5	1,1	0,1	0,1	18,6	22,0	27,3	27,1	77%	84%	98%	98%	7,0	7,3	6,6	6,4
29	1,8	1,9	0,4	0,2	22,5	22,8	28,6	28,5	74%	73%	96%	98%	7,5	7,5	10,6	9,5
31	2,2	2,3	0,2	0,0	23,3	23,8	28,2	27,9	71%	70%	97%	100%	8,1	8,3	6,3	6,3
36	1,8	2,0	1,3	0,3	22,5	23,3	28,9	30,0	79%	77%	87%	97%	9,4	9,5	10,7	9,5
3rd phase – "medium" methane supply																
38	1,5	1,7	1,7	0,1	17,4	18,0	23,9	21,8	84%	82%	83%	99%	10,1	10,5	11,3	9,3
42	0,0	1,0	0,9	0,9	19,5	23,8	17,4	16,9	100%	85%	89%	87%	6,8	6,5	8,5	7,0
45	0,8	0,8	0,2	0,0	16,9	17,3	22,0	17,3	89%	89%	95%	100%	7,0	7,3	3,8	4,1
49	0,7	0,8	0,7	0,0	16,1	17,0	23,7	11,9	90%	89%	89%	100%	6,9	7,2	6,4	3,6

From Figure 3.16 to Figure 3.21 are reported the gas concentration profiles of the two kinds of samples, shallow and deep, in the three different phases of the experiment. While in Figure 3.22 and Figure 3.23 the methane oxidation rate of the shallow and the deep material are respectively shown, and in Figure 3.24 their comparison. Furthermore, the values reported are a mean of the results of the two columns having the same samples inside. Therefore, the values reported under "shallow" are the

mean values of the results from columns S1a and S2a, and the values reported under "deep" are the mean values of the results from columns S3a and S4a.

In order to be able to better understand the interpretation of the gas profiles, a few brief explanations of a typical one is summarized in advance: atmospheric air penetrates the methane oxidation layer from above, while landfill gas, or – in the laboratory column tests – pure methane, rises from the bottom to the top. This results in a mixing and dilution effect of the rising gas with the air penetrating from above. Where the mixing ratio between oxygen and methane is most suitable for the methane oxidizing microorganisms (depending on the soil-physical conditions and the impact rate), a main active layer, called methane oxidation horizon, forms (vertical extent usually of 15-30 cm). There the oxygen is consumed very quickly by the microbial activity, for both methane oxidation and basic respiration of the material itself; therefore, it decreases significantly and is usually hardly detectable in that area and below anaerobic conditions mostly prevails. Furthermore, in the methane oxidation layer the highest concentration of CO_2 is usually found. If all these phenomena are considered, it can be deduced that the gas concentration profiles show the position of the methane oxidation horizon, which is the intersection of methane and oxygen profiles, and the CO_2 peak (Hrad and Huber-Humer, 2014). In the case of the samples investigated, the horizon occurred between 10 and 25 cm depth, during phase I, and it grew during phase II, reaching approximately 20 cm depth (from 5 to 25 cm), as can be observed comparing Figure 3.15, depicting column S3a (containing deep hotspot material) during phase II, where the horizon is clearly visible (white stripe and light brown colour of the soil), and Figure 3.19.

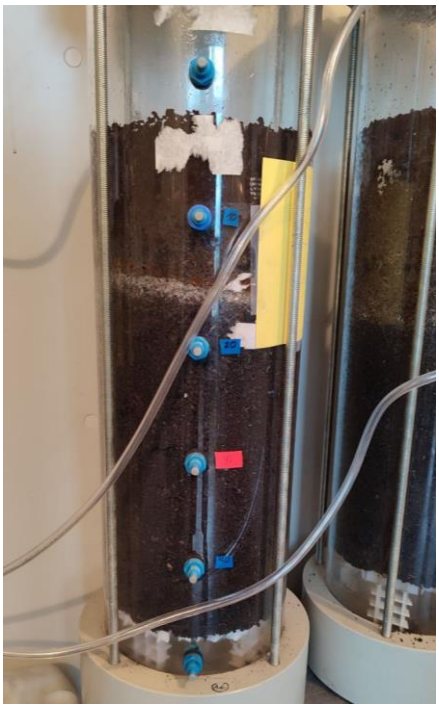


Figure 3.15 Methane oxidation horizon, during phase II, in column S3a (containing deep hotspot material) (the blue and red labels on the column indicate the depth)

Overall, the gas concentration profiles revealed a similar behaviour for all columns, during all three operational phases. Oxygen decreases with depth's increase, and it is not present in depths bigger than 20 cm in all columns. The only variation is in the concentration at depths between 0 and 20 cm, where the main microbial activity happens, between the different phases in the shallow material: in phases I and III, the concentration in the upper part of the columns is lower with respect to the concentration during phase II. Methane and carbon dioxide showed the same profile through the different phases, for both studied materials; with a slight difference in the outlet concentration – at 0cm depth – of methane for the shallow material, during phase II, a higher concentration of CH_4 in the exhaust gas was observed, which is in accordance with the lower methane oxidation rate reported in those conditions.

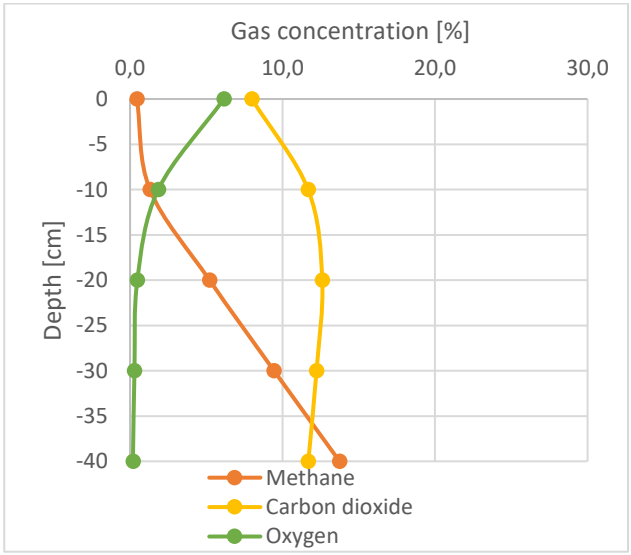


Figure 3.16 Gas concentration profile – shallow, phase 1

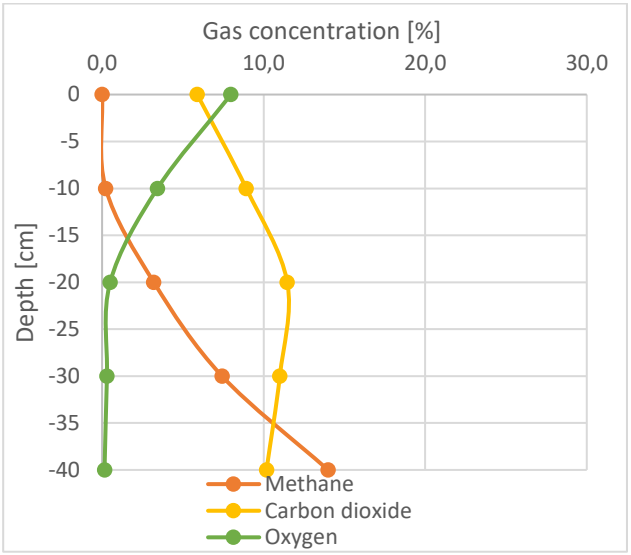


Figure 3.18 Gas concentration profile – deep, phase 1

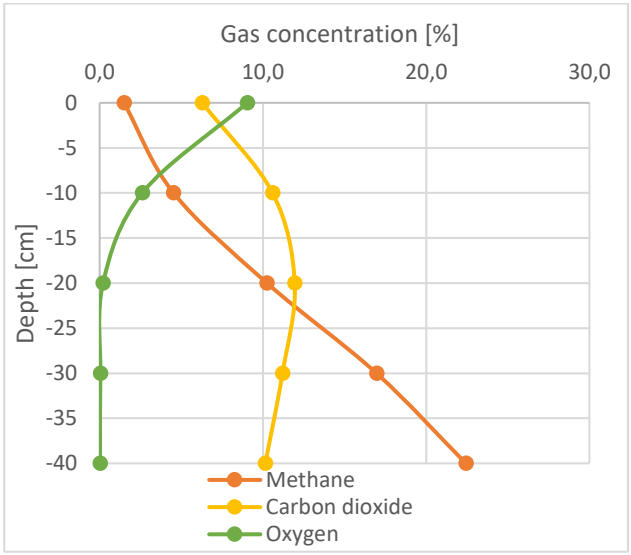


Figure 3.17 Gas concentration profile – shallow, phase 2

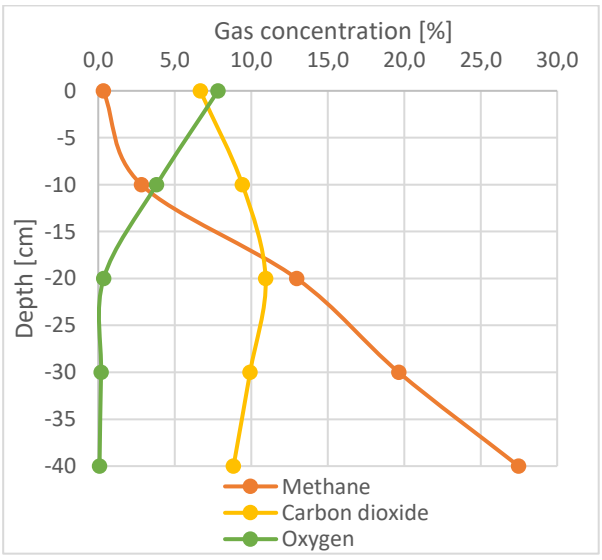


Figure 3.19 Gas concentration profile – deep, phase 2

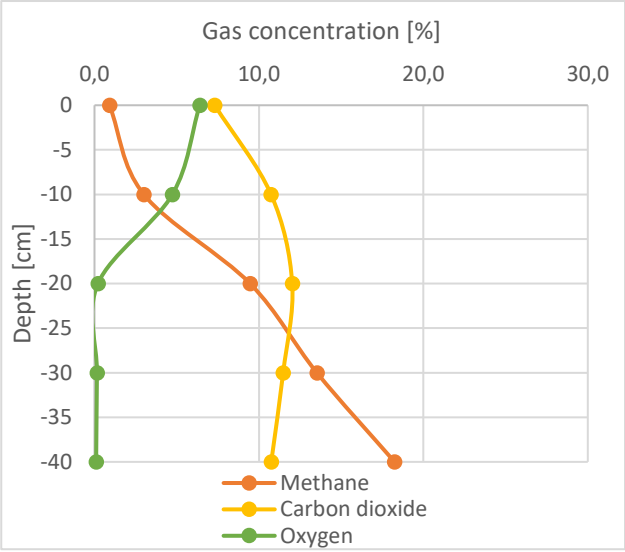


Figure 3.20 Gas concentration profile – shallow, phase 3

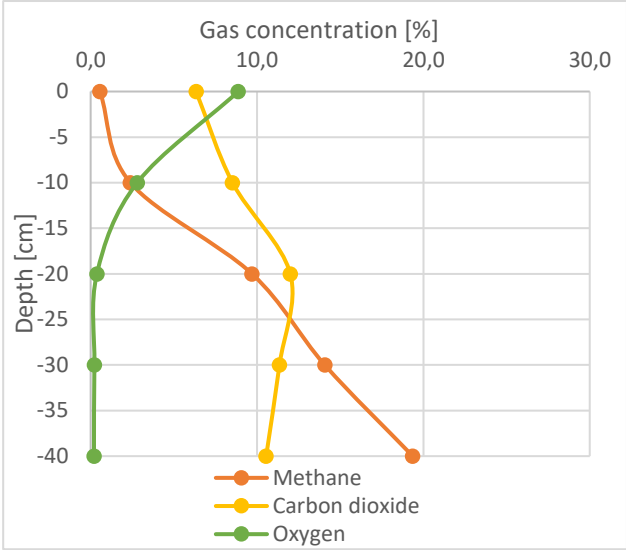


Figure 3.21 Gas concentration profile – deep, phase 3

Methane reduction took part in all investigated columns during the entire period of the experiment under aerobic conditions, as illustrated in Figure 3.22 and Figure 3.23. However, the performance of the different samples varied, over time as a result of changing the inlet methane flux, as well as between columns containing shallow and deep material. The initial inlet methane flux was completely oxidised by all columns, during phase I, with a small lag period, of less than one week, present only in the shallow material columns. In general, the performance of the columns decreased during phase II, as a consequence of the increase of methane flow rate in the inlet. In particular, the deep material showed a slight decrease, maintaining the methane oxidation rate higher than 90%; while the decrease of the oxidation rate of shallow material was more pronounced, reaching at the lowest point 70% of methane oxidation, followed by an increase in the performance. During phase III, the oxidation rate of both materials recovered, but without reaching 100% of oxidation of the fed CH_4.

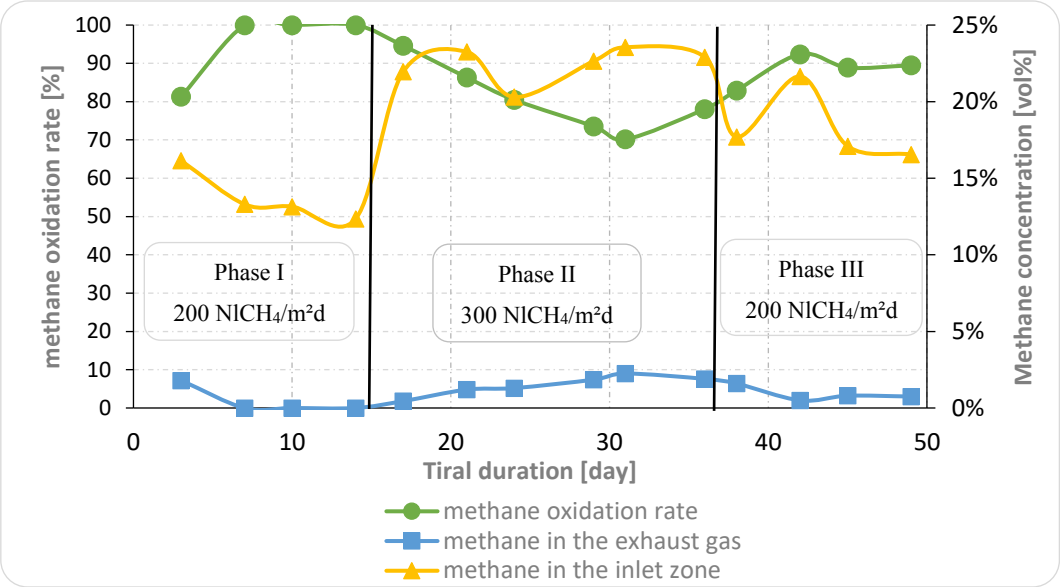


Figure 3.22 Aerobic methane oxidation rate – shallow material

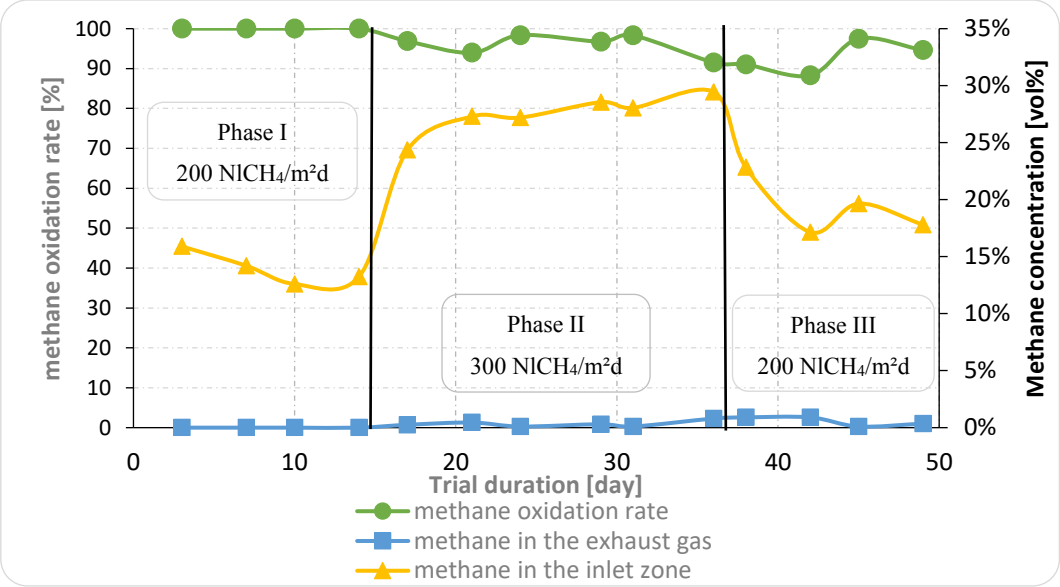


Figure 3.23 Aerobic methane oxidation rate – deep material

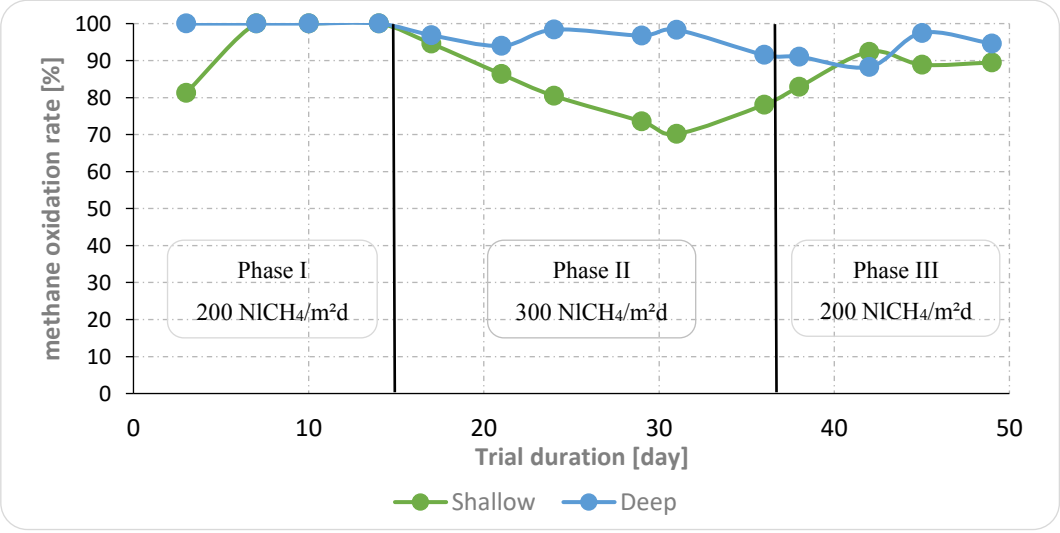


Figure 3.24 Aerobic methane oxidation rate – shallow and deep comparison

3.2.3 Column experiment - anaerobic conditions

Similar to the column experiment under aerobic conditions a mass balance was applied to calculate the methane oxidation rates, as a consequence of knowing the flow rates in the input and output, as well as the methane concentrations in the inlet and outlet of the tested columns. The methane oxidation rates are presented as relative (percentages) values, related to the charged methane loads, because of the sometimes-unstable methane supply flow.

In Table 3.8 the results of the anaerobic columns test are presented. The term "shallow" refers to the samples taken from the upper layer of hotspot G19, specifically from 10-20 cm depth, while the term "deep" refers to the samples taken from the deep layer of hotspot G19, of 90-115 cm depth.

Furthermore, in this case the values reported are not a mean of the results of the two columns having the same samples inside, but the direct measurements taken during the experiment, because only two columns, one for the shallow material and the other for the deep material, were used to assess anaerobic methane oxidation.

Table 3.8 Results of tested samples under anaerobic conditions

Day	Note	% CH_4 in exhaust gas [%vol]		% CH_4 at 40 cm depth [%vol]		% CH_4 oxidation rate	
		Shallow	Deep	Shallow	Deep	Shallow	Deep
3	--	5	4	14	15	9%	7%
8	--	4	3	13	12	13%	13%
10	--	4	5	14	21	2%	6%
14	N_2 bottle finished	11	12	21	25	--	--
15	N_2 bottle finished	21	24	37	42	--	--
16	N_2 bottle finished	29	28	41	50	--	--

In Figure 3.25 and Figure 3.26 the gas concentration profiles of the two kinds of samples, shallow and deep, are depicted. While in Figure 3.27 and Figure 3.28 the methane oxidation rate of the shallow and the deep material are respectively shown, together with the profile of methane concentration in the exhaust gas and in the inlet zone (40cm depth in the columns).

The reported values for the methane oxidation rate are just three, because of the impossibility to provide nitrogen earlier than expected. Therefore, the three values reported are the only reliable data present in this experiment in order to assess the methane oxidation rate profile under anaerobic conditions. Certainly, it is not possible to have statistically significant results, but anaerobic methane oxidation could be observed. As expected, the detected oxidation rate of methane is not comparable to the one under aerobic conditions, and results from shallow and deep materials show no relevant differences between one another. Indeed, the methane oxidation rates for the first week were less than 15%. On the other hand, the methane concentration profiles in the exhaust gas and in the inlet zone

revealed an interesting behaviour. The initial values are consistent with the ones reported under aerobic conditions (methane concentration in the outlet between 0 and 10% [vol%] in both cases), and the concentrations started to increase when nitrogen was no longer provided due to the missing dilution. Another interesting aspect is the formation of carbon dioxide, as reported in literature, CO_2 is formed during both aerobic and anaerobic methane oxidation processes; indeed, this could be a sign of microbial activity, but unfortunately it cannot be confirmed if aerobic or anaerobic methanotrophic bacteria.

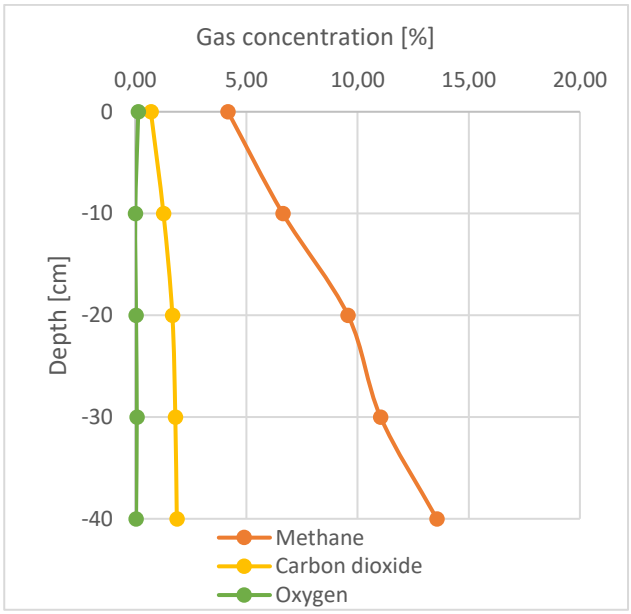


Figure 3.25 Gas concentration profile – shallow (column S1a)

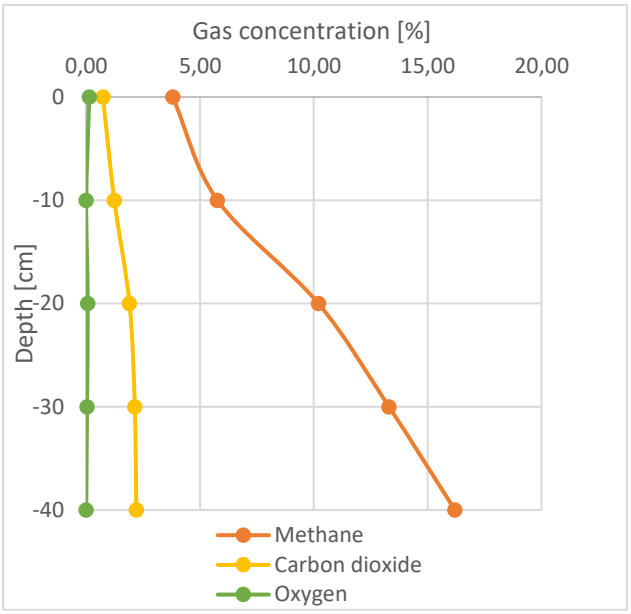


Figure 3.26 Gas concentration profile – deep (column S4a)

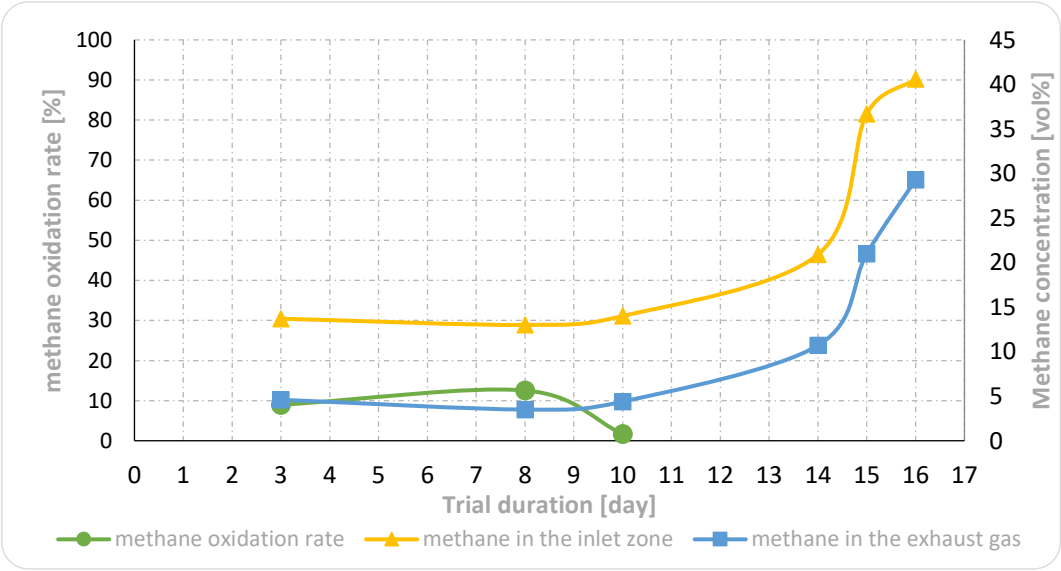


Figure 3.27 Anaerobic methane oxidation rate – shallow

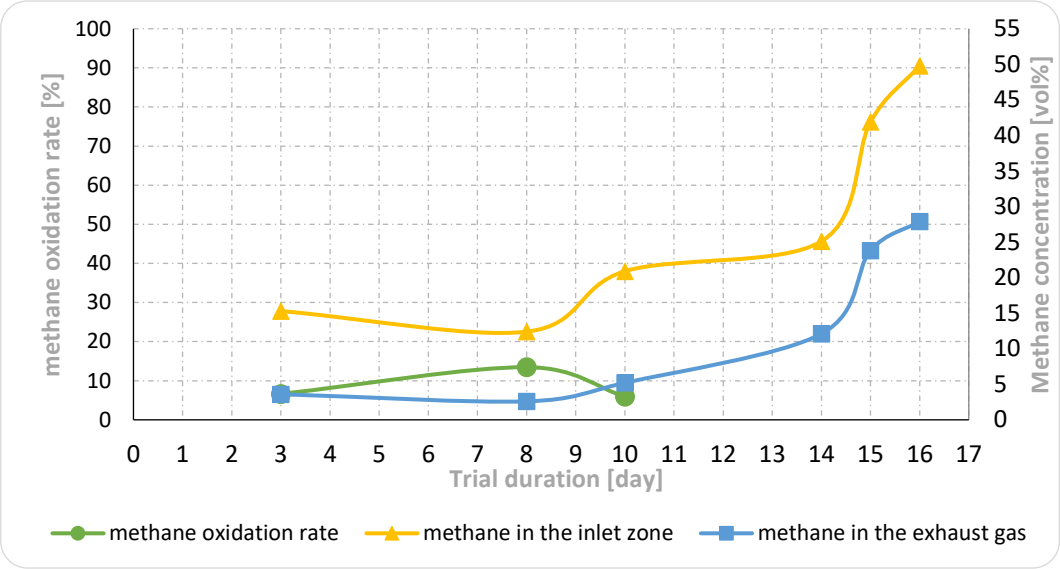


Figure 3.28 Anaerobic methane oxidation rate – deep

3.2.4 FTIR analysis

The analysed samples were compared in different combinations. They were firstly analysed all together, as reported from Figure 3.29 to Figure 3.32, to compare the spectrum shape. If Figure 3.30 is observed no difference can be noticed between the shape of the analysed samples. The composition can be derived by the following bands: the aliphatic methylene bands at 2920 and 2850 cm^{-1} represent the skeleton of many biomolecules; the amide II band at approximately 1540 cm^{-1}, the (aromatic) amine band at 1320 cm^{-1}; the band at 1260-1240 cm^{-1} (C–O vibration of carboxylic acids and C–N of amide III) can be assigned to readily degradable organic matter; the band at 1510 cm^{-1} represents lignin; the band at 1640 cm^{-1} cannot be unequivocally assigned due to overlapping of aromatic C=C vibrations, functional group vibrations such as C=O (carboxylates and amides), C=C (alkenes) and OH from water, all contributing to this strong band; bonded and non-bonded hydroxyl groups and water (O–H stretch) are reflected by the band at about 3400 cm^{-1}; furthermore, inorganic components of clay minerals (1030 cm^{-1}) and carbonates (2520, 1780, 1430 and 875 cm^{-1}) can be found in all samples; the bands at 3690 and 3620 cm^{-1} can be attributed to kaolin (Huber-Humer et al., 2011).
However, the differences are in the peaks' height, which occur in bands 1010-1030 [cm^{-1}], 1420-1430 [cm^{-1}], and 2850-2950 [cm^{-1}]. Nevertheless, only the last two bands are taken into consideration, because they correspond to compounds relevant to this thesis topic, which are organic sulphates and ammonium ion (1420-1430 [cm^{-1}] band) and methylene (2850-2950 [cm^{-1}] band) (Nandiyanto et al., 2019), that can be linked to sugars produced by microorganisms (EPS) under stress conditions.
The colour legend for all the following figures is reported in Table 3.9.

Table 3.9 Spectrum legend

Colour	Sample code	Sample type	Input material
Bright red	CS-an	Column - anaerobic condition	10-20cm depth G19 hotspot
Dark red	CD-an	Column - anaerobic condition	90-115cm depth G19 hotspot
Light blue	CS-a	Column - aerobic condition	10-20cm depth G19 hotspot
Dark blue	CD-a	Column - aerobic condition	90-115cm depth G19 hotspot
Light green	FS	Field	10-20cm depth G19 hotspot
Dark green	FD	Field	90-115cm depth G19 hotspot

The figures firstly reported in each of the following comparisons (Figure 3.29, Figure 3.33, Figure 3.36, Figure 3.39, Figure 3.42, Figure 3.45), are shown to give an overview of the spectra analysed, not for results' interpretation; while the figures that depict the details show the analysed spectra overlapped in specific points, to have a better understanding of their profiles in the analysed bands. From Figure 3.31 and Figure 3.32, where bands peaks are reported in detail, can be observed that for band 1420-1430 [cm^{-1}] the highest peak corresponds to CS-an, and the lowest to CD-an; and for band 2850-2950 [cm^{-1}], the highest peak is reported by the CS-an, and the lowest by CD-a.

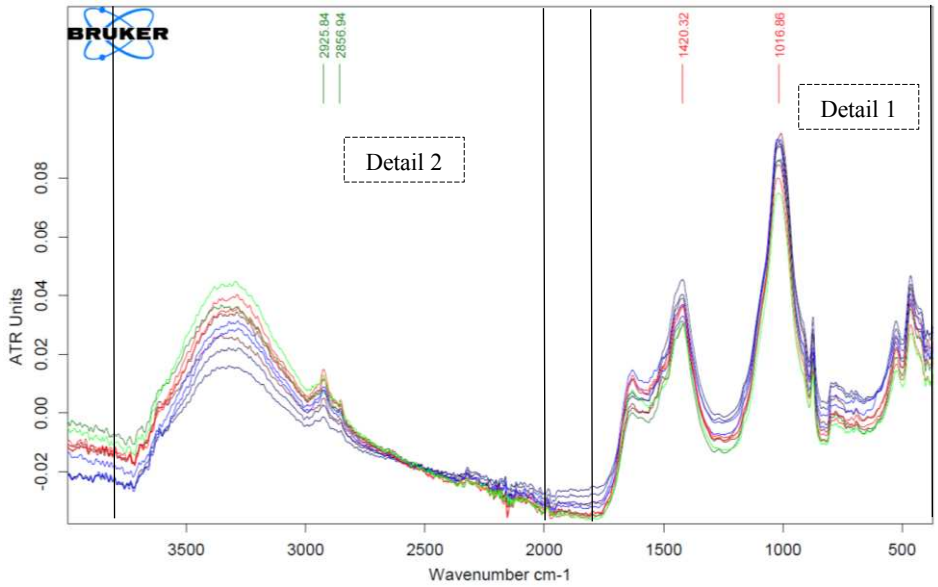


Figure 3.29 Spectra of all samples

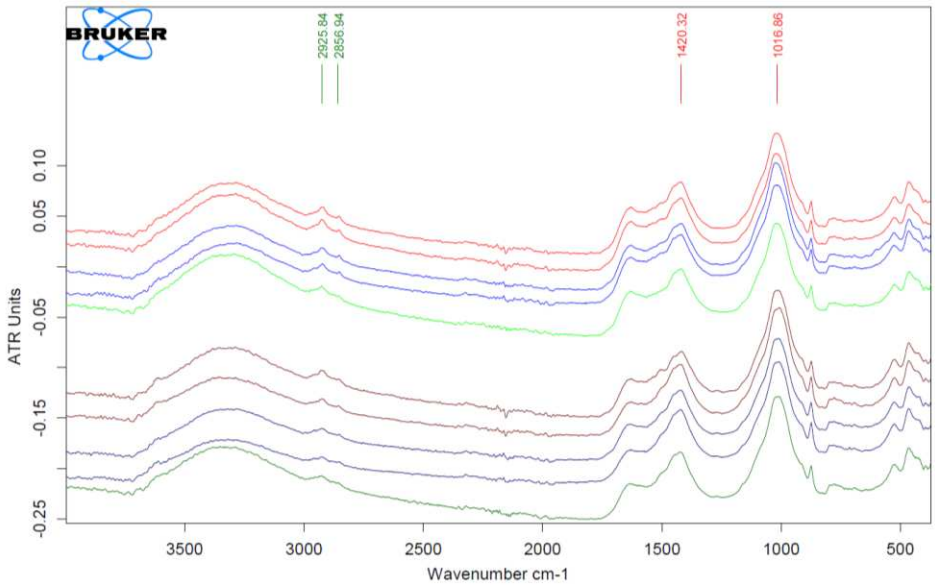


Figure 3.30 Shape comparison of all samples

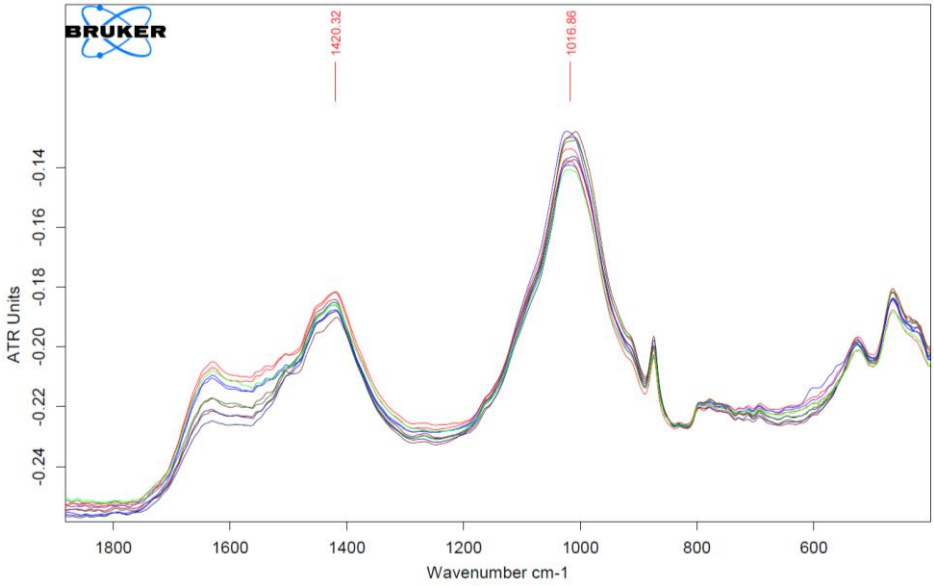


Figure 3.31 All samples – detail 1 (organic sulphates and ammonium ion)

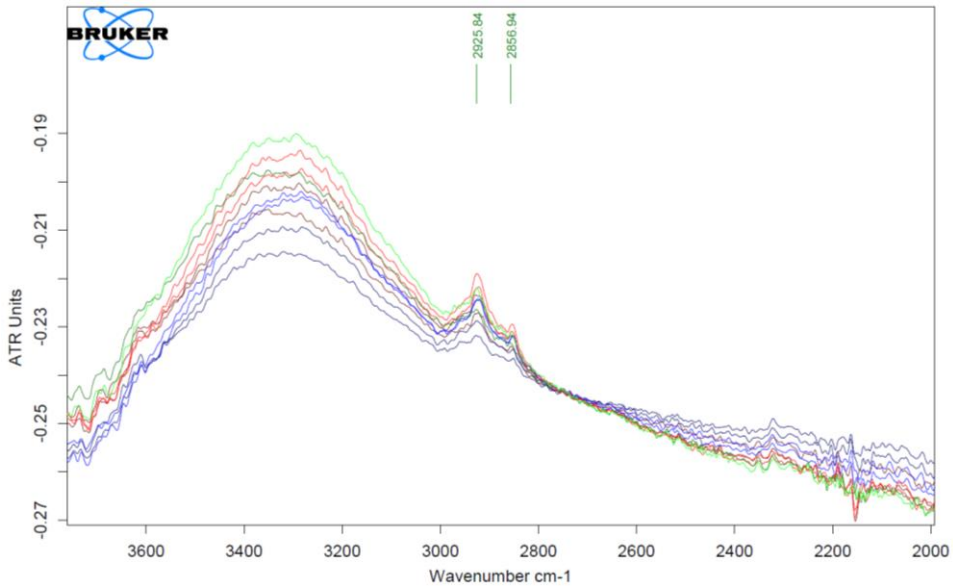


Figure 3.32 All samples - detail 2 (methylene – EPS)

The second comparison reported from Figure 3.33 to Figure 3.35 was made between the field reference samples, taken from 10-20 cm and 90-115 cm depths in the G19 hotspot. From Figure 3.34 can be observed that in band 1420-1430 [cm^{-1}] the spectra overlap; and for band 2850-2950 [cm^{-1}], reported in Figure 3.35, the highest peak is reported by FS, and the lowest by FD.

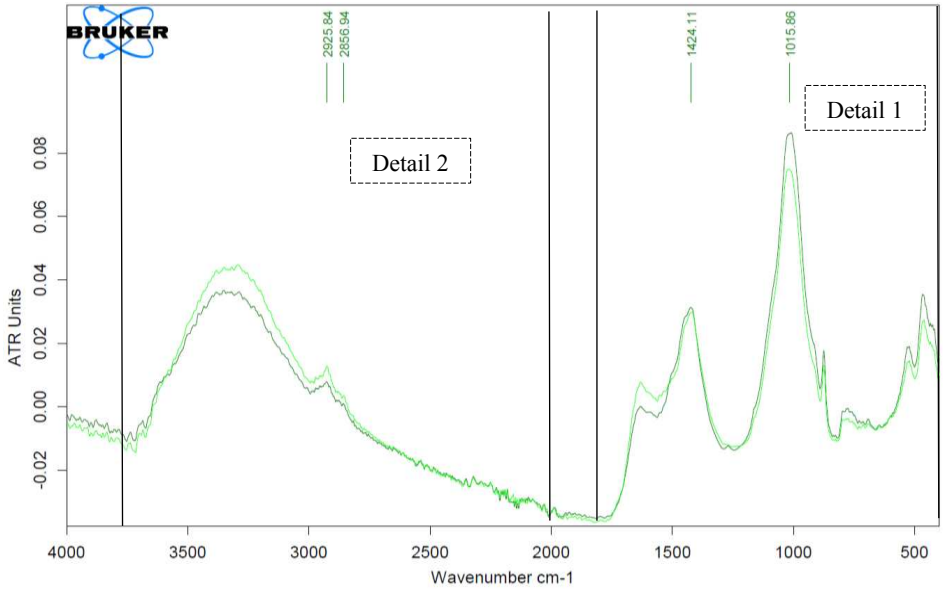


Figure 3.33 Field - shallow and deep

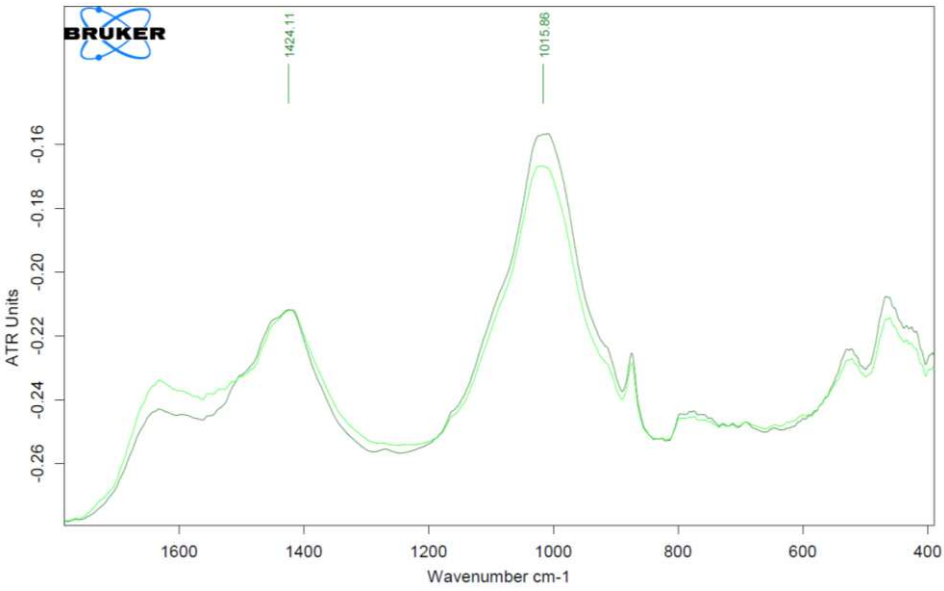


Figure 3.34 Field - shallow and deep, detail 1 (organic sulphates and ammonium ion)

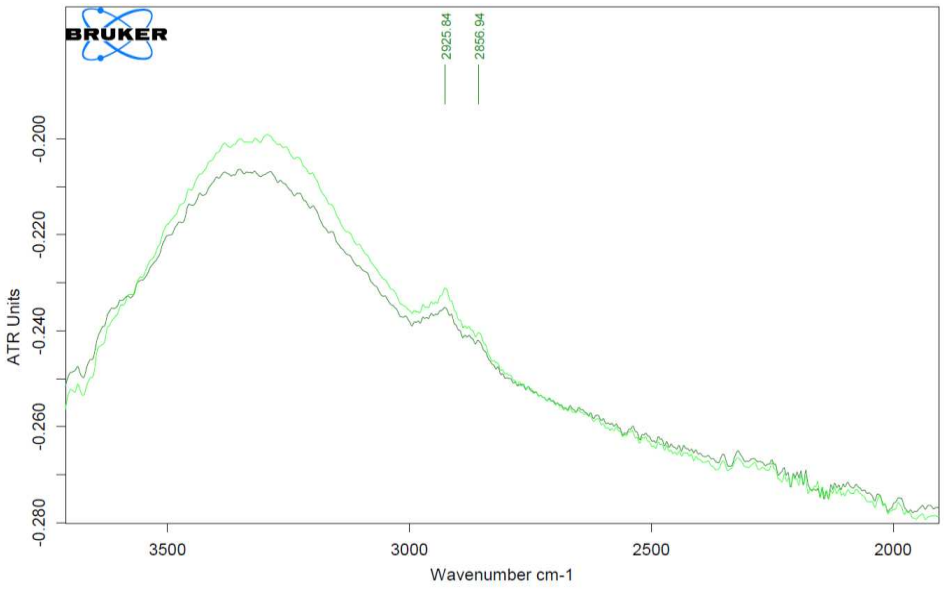


Figure 3.35 Field - shallow and deep, detail 2 (methylene – EPS)

The third comparison reported from Figure 3.36 to Figure 3.38 was made between the columns under aerobic conditions comparing shallow and deep material (as done in the columns experiment under aerobic conditions), with the field spectra as reference. From Figure 3.37 can be observed that in band 1420-1430 [cm^{-1}], the highest peak is reported by CD-a, and the lowest by the CS-a; and band 2850-2950 [cm^{-1}], reported in Figure 3.38, presents the same behaviour.

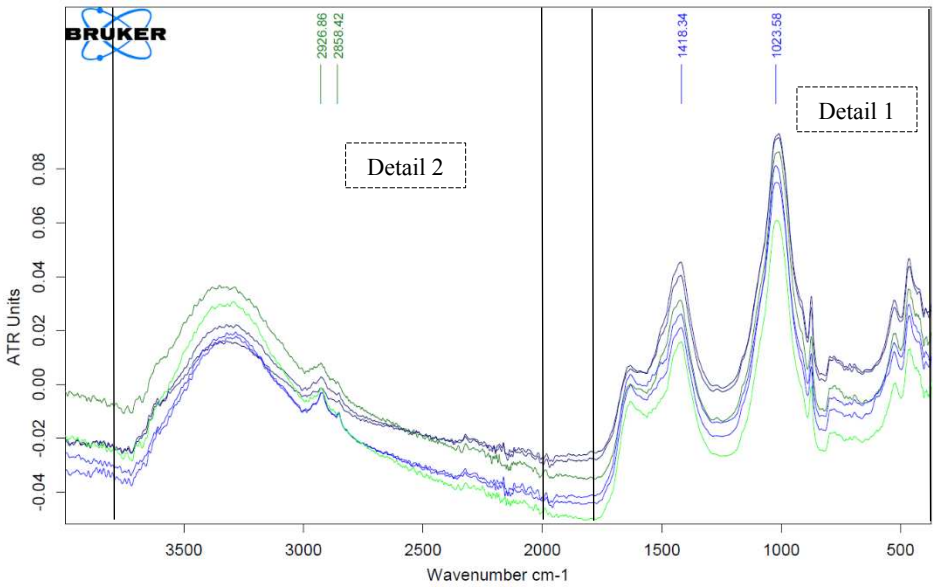


Figure 3.36 Aerobic conditions - shallow and deep

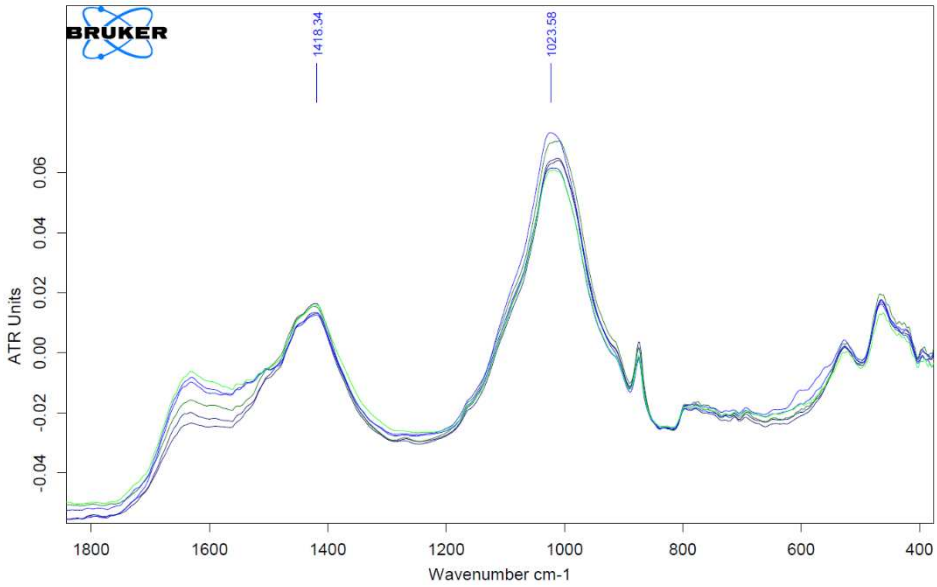


Figure 3.37 Aerobic conditions - shallow and deep, detail 1 (organic sulphates and ammonium ion)

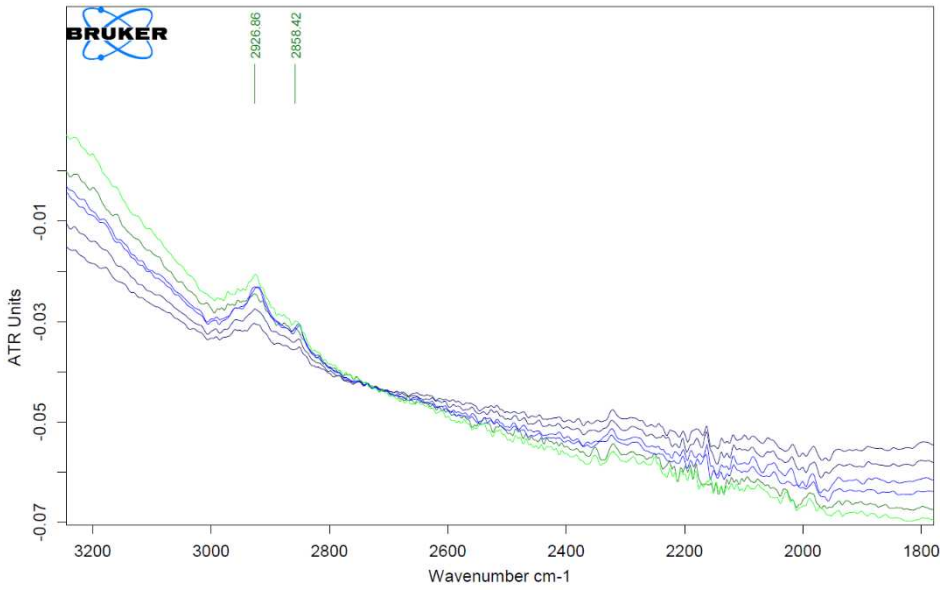


Figure 3.38 Aerobic conditions - shallow and deep, detail 2 (methylene – EPS)

The fourth comparison reported from Figure 3.39 to Figure 3.41 was made between the columns under anaerobic conditions containing shallow and deep input material, with the field spectrum as reference. From Figure 3.40 can be observed that in band 1420-1430 [cm^{-1}] the highest peak is reported by CS-an, and the lowest by CD-an; and in band 2850-2950 [cm^{-1}], reported in Figure 3.41, the behaviour is the same.

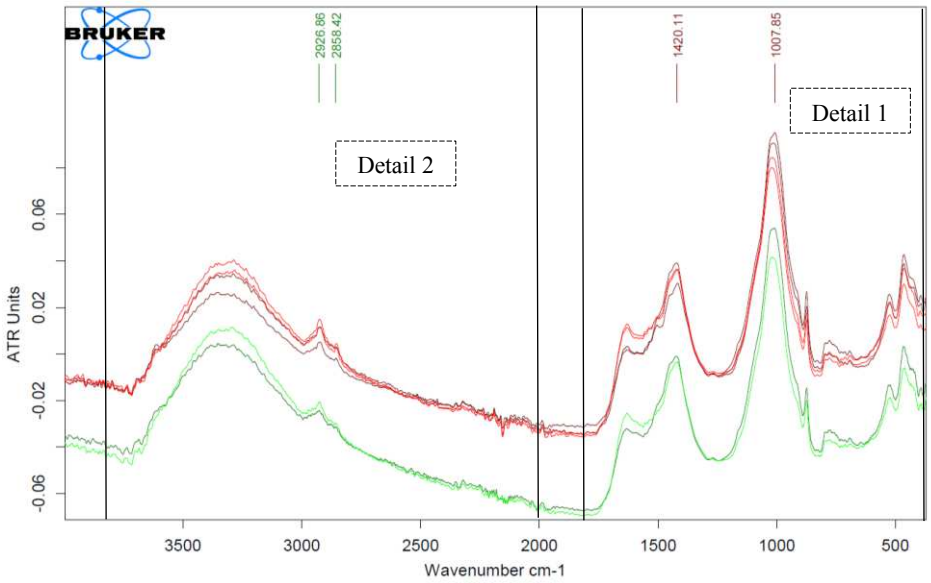


Figure 3.39 Anaerobic conditions - shallow and deep

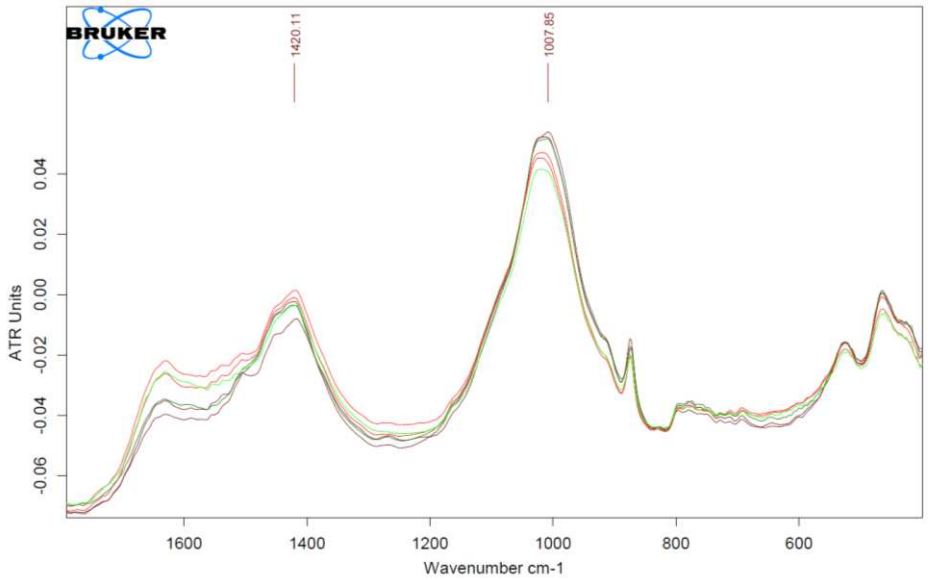


Figure 3.40 Anaerobic conditions - shallow and deep, detail 1 (organic sulphates and ammonium ion)

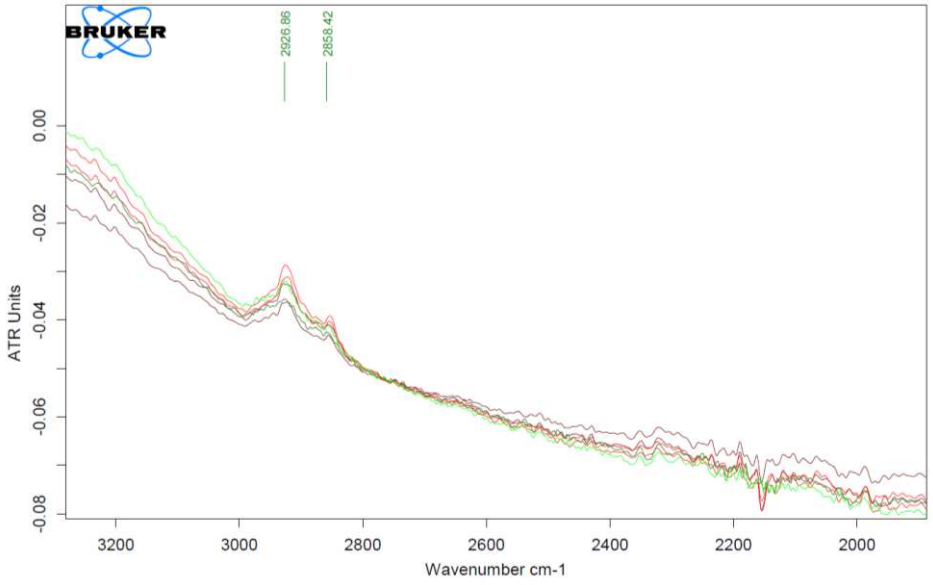


Figure 3.41 Anaerobic conditions - shallow and deep, detail 2 (methylene – EPS)

The fifth comparison reported from Figure 3.42 to Figure 3.44 was made between the columns under aerobic and anaerobic conditions containing shallow input material, with the field spectrum as reference. From Figure 3.43 can be observed that in band 1420-1430 [cm^{-1}] the highest peak is reported by CS-an, and the lowest by CS-a; and band 2850-2950 [cm^{-1}], reported in Figure 3.44, presents the same behaviour.

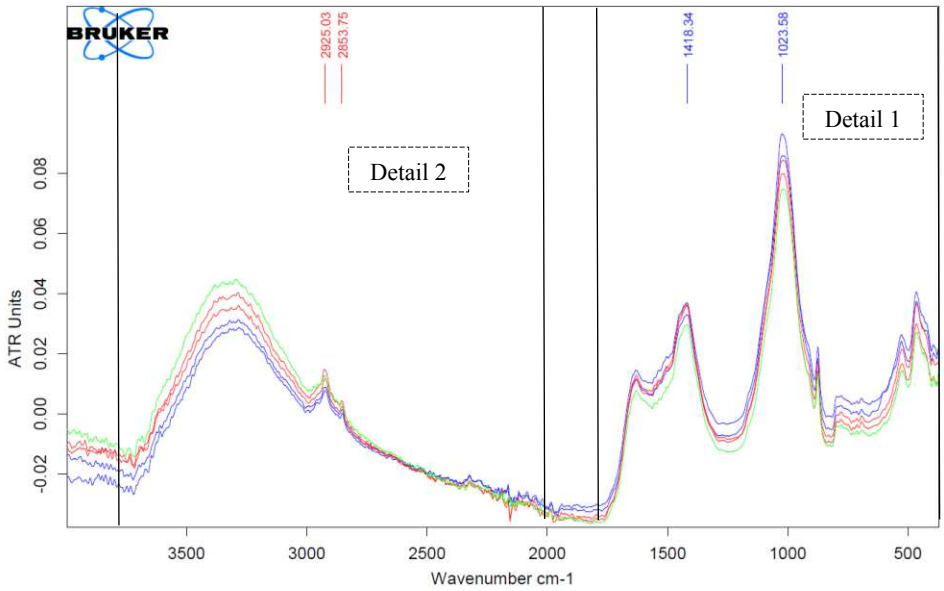


Figure 3.42 Aerobic and anaerobic conditions – shallow material

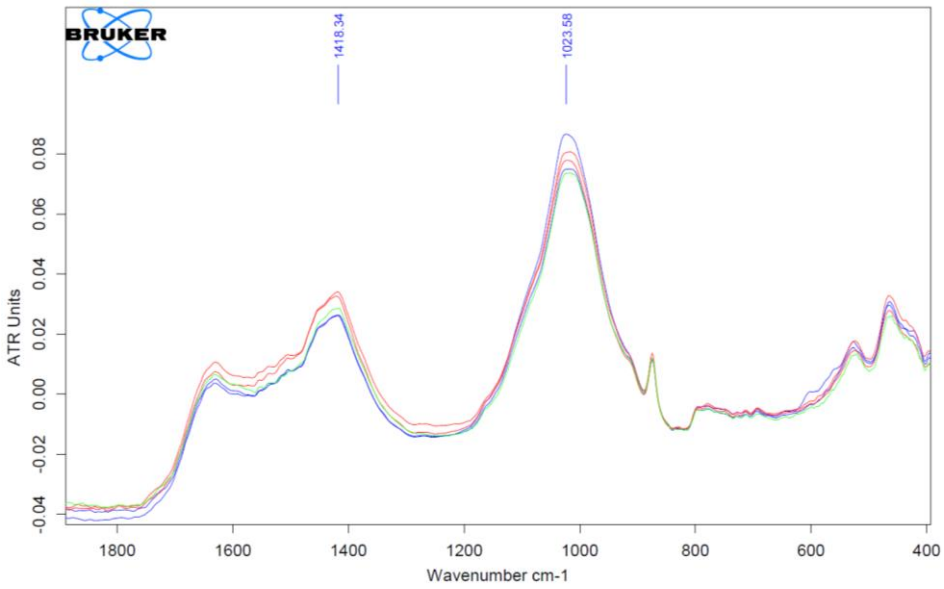


Figure 3.43 Aerobic and anaerobic conditions – shallow material, detail 1 (organic sulphates and ammonium ion)

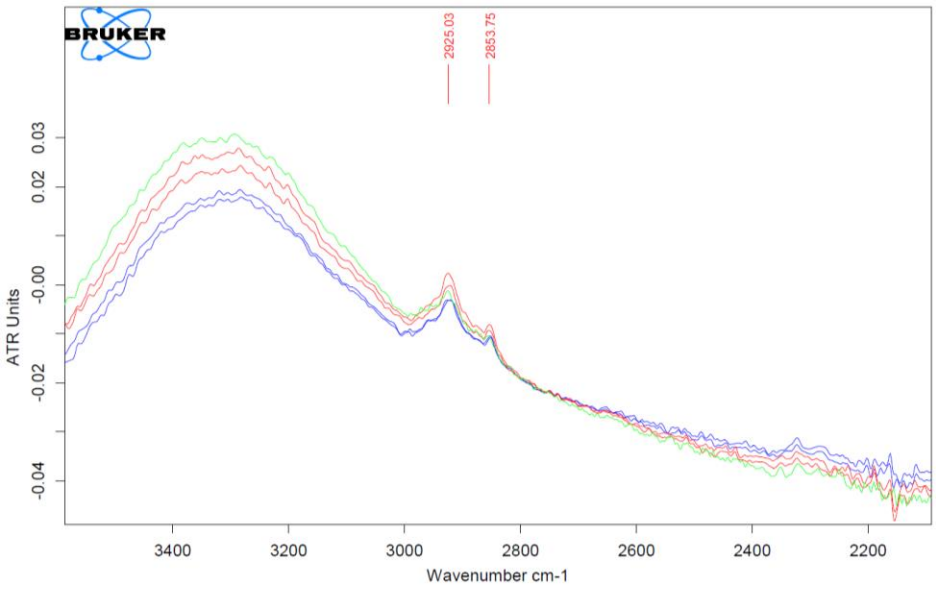


Figure 3.44 Aerobic and anaerobic conditions - shallow material, detail 2 (methylene – EPS)

The sixth comparison reported from Figure 3.45 to Figure 3.47 was made between columns under aerobic and anaerobic conditions containing deep input material, with the field spectrum as reference. From Figure 3.46 can be observed no clear behaviour in band 1420-1430 [cm^{-1}]; while for band 2850-2950 [cm^{-1}], reported in Figure 3.47, the highest peak is reported by CD-an, and the lowest by CD-a.

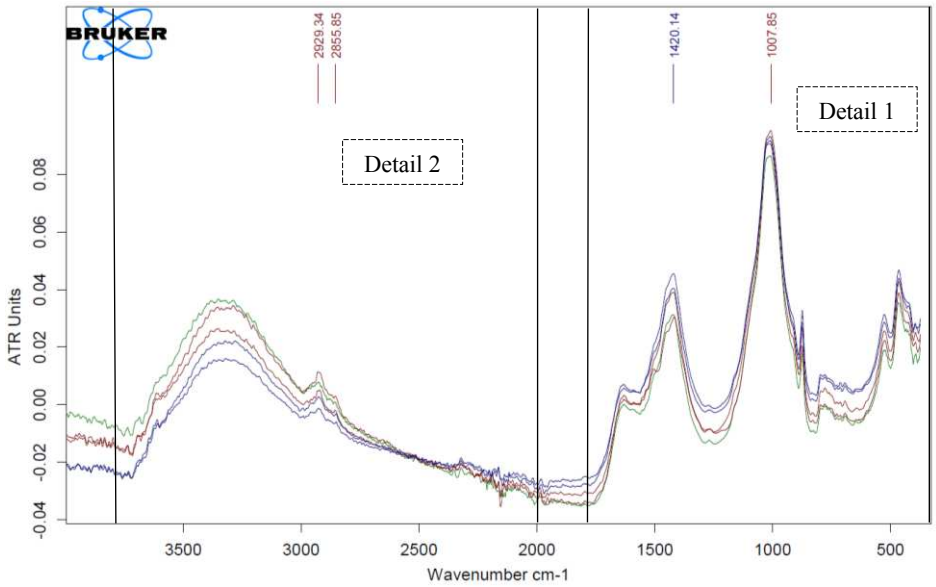


Figure 3.45 Aerobic and anaerobic conditions - deep material

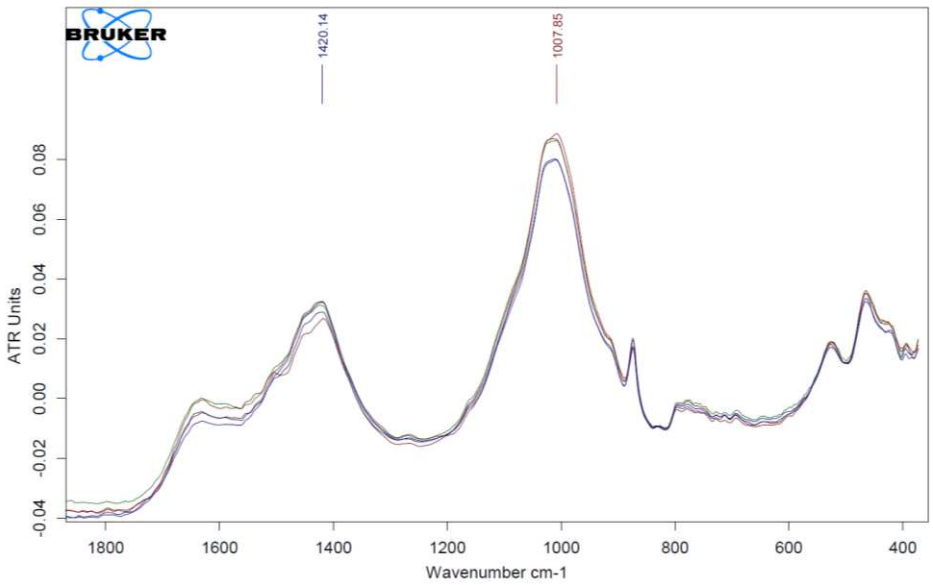


Figure 3.46 Aerobic and anaerobic conditions - deep material, detail 1 (organic sulphates and ammonium ion)

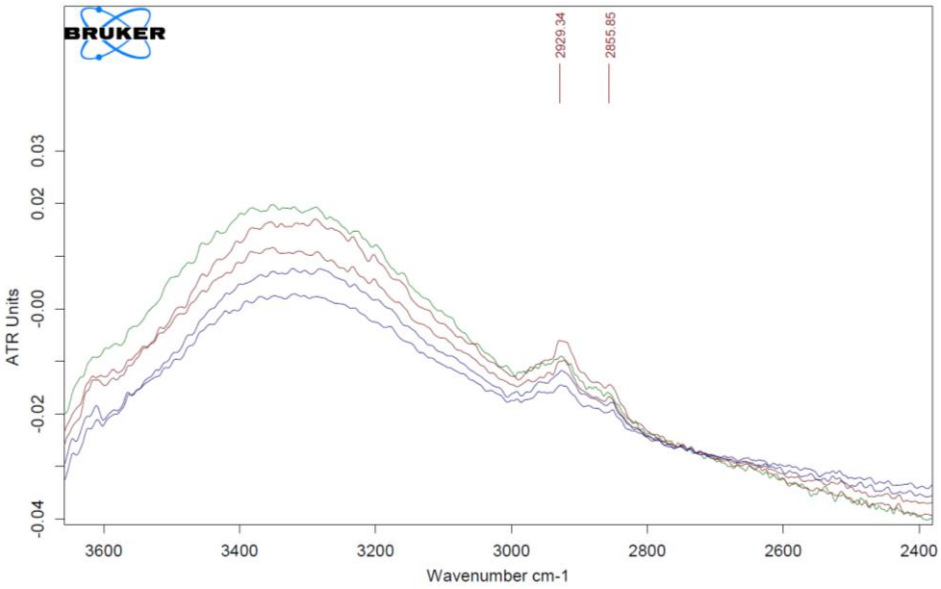


Figure 3.47 Aerobic and anaerobic conditions - deep material, detail 2 (methylene – EPS)

3.3 Discussion

3.3.1 Column experiment – aerobic conditions

In order to try to answer the research questions, reported in the introduction – "which are the responses of different depth samples, under aerobic conditions, to different methane flow rates?" and, "a MMOS can still reach high methane oxidation rates after eight years of methane overload?" – the comparison between the shallow and deep hotpot materials was performed. Overall, the column experiment under aerobic conditions has demonstrated that the compost material of the hotspot area, has a high methane oxidation potential (methane oxidation rates > 70% – higher than values generally measured in columns containing landfill cover soils, that are between 30 to 60% removal (Scheutz et al., 2009)), even after 8 years of temporal methane overload, if put under optimised laboratory conditions. Nevertheless, as shown in Figure 3.24, the profile of methane oxidation rates varies for the tested samples. In particular, it can be observed that the shallow material has an overall difficulty to oxidise methane – although keeping a methane oxidation rate higher than 70% – with respect to the deep material; especially when the methane inlet flow rate is increased, in phase II. This could be explained by the profile of the oxygen concentration, reported in Figure 3.17 for the upper layer, and in Figure 3.19 for the deep layer. As already pointed out in the results chapter, the concentration of oxygen during phase II is similar between the investigated upper and deep hotspot layers, except for the outlet concentration, which was higher for the shallow material. This means that the columns were in the same conditions (same profile shape), but it could be an index of a slower microbial activity of the shallow material. Nevertheless, it is known from literature that in the upper layer of MMOSs oxygen is available, while in the deeper part (from depth > 55cm) it is not, this means that the microorganisms of the deep sample are already used to anaerobic conditions, more than the ones in the shallow sample. Furthermore, the different samples could contain different microbial communities, as discussed in the theoretical background. For this purpose, a fluorescence in situ hybridization (FISH) test could be performed. FISH is a laboratory technique used to detect and locate a specific DNA sequence on a chromosome, that could help understand the kind of microorganisms present in the different samples, to confirm or disprove the previous hypothesis. Unfortunately, in this thesis work it was not possible to conduct it due to time constraints, but it certainly is an interesting side to investigate. Nevertheless, another phenomenon could cause the observed difference in the methane oxidation rates: a higher extracellular polymeric substances (EPS) formation in the methane oxidation horizon of the columns containing the upper layer of hotspot G19, with respect to the deep one; indeed the excreted EPS clog the pore spaces, making it more difficult for air to penetrate or completely preventing it over time, and therefore inhibits aerobic methane oxidation (see 2.6.7). The presence of EPS could also explain

why after phase II the complete methane degradation was no longer achieved. Indeed, under these conditions only the first centimetres of soil in the columns were available for methane degradation.

Furthermore, the results are comparable to a former study, and literature. The former study was conducted in 2014 by the Institute of Waste Management of BOKU University (Wien), investigating three gas phases, in which methane flow rate was increased from 102 Nl CH_4/m^2d, I phase I, to 203 Nl CH_4/m^2d, in phase II, and to 305 Nl CH_4/m^2d in phase III. The results showed a methane oxidation rate of 100% in the first two phases, with a decrease in the third (with values higher than 80%) (Hrad and Huber-Humer, 2014); which reflect the behaviour obtained in the column test under aerobic conditions performed in the framework of this thesis. A study, about revisiting the passive biocover system of a landfill after six years from the construction, was found in literature. Both the sample framework and the results are comparable to the one of this thesis; indeed, also in that case laboratory tests showed methane oxidation potential approximately equal to former tests (Scheutz et al., 2022).

In addition, the variation between the sample characteristics before and after the aerobic column experiment could be explained in the following ways. The pH stabilization at ~7.7, for both the upper and lower layers, could be a consequence of the addition of water in the upper layer sample and to the stabilisation of temperature in the climate chamber. The slight increase of ammonium values for both types of samples – reaching ~77 mg/kg DM – for the upper part of the columns is probably due to adaptation to the environment; while the significant increase of ammonium in the deeper part of the column containing the upper layer of hotspot G19, could be explained by the anaerobic condition that occurred in that part of the column and the possible consequent change in the nature of methanotrophic bacteria. The nitrate accumulated in the upper layer of hotspot G19, highlights a condition of stress, and potentially explains the high methane emission (due to the inhibition of aerobic methane oxidation derived from the high presence of nitrates); indeed, the lower values in the columns could be due to denitrification that may have occurred in them (depending on environmental conditions), or to the fixing of atmospheric nitrogen the methanotrophic biomass. And the increasing of sulphate values in the deep part of the column containing the lower layer of hotspot G19, with respect to field conditions, could be explained by a higher presence of sulphur oxidising bacteria; which are able to oxidise several sulphur compounds and form sulphate as the end product, they indeed require an environment rich in CO_2 and poor of O_2, which is occurring in both the initial and the column conditions; while in the other conditions (upper layer in the field and in the columns) methanotrophic bacteria growth is favourited, and their presence could inhibit the sulphur-oxidising bacteria (Huber-Humer, 2004).

3.3.2 Column experiment – anaerobic conditions

From the comparison of the sample characteristics between anaerobic conditions and both aerobic and field conditions, the only characteristics with a significant variation are ammonium and sulphate contents. Ammonium is significantly higher in both columns under anaerobic conditions, due to the injection of nitrogen; and its value is considerably higher also respect to the one of nitrates; one possible explanation could be that the nitrogen supply was too much with respect to the amount needed by the microorganisms, that were not able to convert it to nitrates and use it as electron acceptor for anaerobic methane oxidation, in a short period of time, such as the one of this experiment. While the decrease of sulphate value in the deep part of the column containing the lower layer of hotspot G19, after the material was put under anaerobic conditions, could be linked to sulphate dependent anaerobic methane oxidation.

In order to try to answer the research questions, reported in the introduction, and specifically to try to understand the role of anaerobic methane oxidation in MMOS, methane oxidation rates are reported in Figure 3.27 and Figure 3.28, for the two samples investigated, displayed together with the profile of methane concentration in the exhaust gas and in the inlet zone. After the nitrogen bottle was finished the methane oxidation was not calculable with a mass balance, because the input flow rate was missing and the potential dilution was unknown. Furthermore, no other comparable method could be used due to the presence of too many uncertainties. Indeed, the exact day when the bottle ran out of nitrogen was unknown, as well as the gases present inside the columns, and even a column leakage was hypothesized. In addition, when interpreting the results it is important to take also in consideration that the anaerobic columns were previously exposed to aerobic conditions, which might have influenced them.

The anaerobic oxidation rates in landfill cover soils are not deeply discussed in the literature, but two studies, already discussed in paragraph 5.3, reported the following methane oxidation rates. For (Xu and Zhang, 2022) the highest potential methane oxidation rate was found for sulphate-AOM, with a value of 7.44–9.88 nmol CO_2 g^{-1} d^{-1}, Fe-AOM was observed to be the second most active process with a potential methane oxidation rate of 3.60 ± 0.30 – 2.41 ± 0.25 nmol CO_2 g^{-1} d^{-1}, respectively, and nitrate-AOM, nitrite-AOM, and Mn-AOM showed relatively low methane oxidation rates < 2 nmol CO_2 g^{-1} d^{-1}. While for (Parsaeifard et al., 2020), sulphate+iron-AOM had the highest methane removal in the anoxic zone, with a value of 10.68%, sulphate-AOM was the second, with a value of 0.97% (in that column CH_4 and CO_2 removal were equal, indicating removal due to dilution), and nitrate-AOM showed negative methane removal of -3.72% (methane generation was greater than methane oxidation in the column).

Nevertheless, because of the few reliable values of methane oxidation rates the interesting parameters are the methane concentration in the exhaust gas and in the inlet zone. Their profiles show a logical behaviour: at the beginning of the experiment the values were slightly decreasing, according to the methane oxidation rate, and when the nitrogen bottle was finished the values started to increase, possibly showing that the process of methane oxidation stopped in absence of electron acceptors, although also this affirmation could be affected by the possible – unknown – dilution effect. Therefore, even if it is not possible to properly answer the initial research questions – "to what extent does anaerobic oxidation of methane (AOM) play a role in hotspot areas of microbial methane oxidation systems?" and, "is it possible to measure/detect/quantify the effects of AOM in LC?" – it can be affirmed that methane oxidation takes place also under anaerobic conditions in MMOS, even with an oxidation efficiency significantly lower than the one of the aerobic process. Nevertheless, anaerobic methane oxidation in microbial methane oxidation systems is worth to be explored. In order to do so, columns tests under anaerobic conditions have to be long-term, due to the needed adaptation phase of the microorganism and the slow process; also an additional initial phase, where the columns are flushed with nitrogen – to ensure anaerobic conditions – could avoid unwanted uncertainties regarding the nature of the gas inside the columns.

3.3.3 FTIR analysis

In this paragraph the code reported in Table 3.10 is used to address the samples.

Table 3.10 Sample's name legend

Sample name	Sample type	Input material
CS-an	Column - anaerobic condition	10-20cm depth G19 hotspot
CD-an	Column - anaerobic condition	90-115cm depth G19 hotspot
CS-a	Column - aerobic condition	10-20cm depth G19 hotspot
CD-a	Column - aerobic condition	90-115cm depth G19 hotspot
FS	Field	10-20cm depth G19 hotspot
FD	Field	90-115cm depth G19 hotspot

The spectra reported in 3.2.4 show that the samples analysed have the same composition, this affirmation is a consequence of the shape of the spectra themselves, as they are equal.
As the shape of the spectra is the same, the focus of the comparisons is the heights of the peaks. For the Lambert-Beer law the absorbance, and therefore the height of the spectra peaks, is proportional

to the concentration of the absorbent species contained in the sample. Consequently, observing and comparing the spectra could give information on the quantity of specific compounds contained in the analysed samples. For this reason, the samples are compared focusing on the height of the spectra peaks, and in particular on the peaks of bands, 1420-1430 [cm^{-1}], and 2850-2950 [cm^{-1}] where the differences in the peaks' height are showing. Unfortunately, due to the low amount of samples, it was not possible to do a quantitative analysis, but only a qualitative one. The 1420-1430 [cm^{-1}] band corresponds to organic sulfates and ammonium ion, while the 2850-2950 [cm^{-1}] band to methylene (Nandiyanto et al., 2019), which can be linked to sugars produced by microorganisms (EPS) under stress conditions.

From the previously reported spectra can be derived that CS-a has an equal amount of organic sulphates and ammonium ion and a higher amount of sugars with respect to CD-a; these results are not reflected by the corresponding values obtained by the laboratory analysis, reported in Table 3.5.

From both the comparisons between CS-a and CS-an, and CD-a and CD-an, is detected a higher quantity of sugars in samples under anaerobic conditions. Both the comparisons between FS and FD, and between CS-an and CD-an show higher quantities of sugars in the upper layer, as expected; indeed, EPS were found during the sampling in the upper layer of the G19 hotspot. Another expected behaviour is confirmed in both the comparisons between CS-a and CS-an, indeed the content of organic sulphates and ammonium ion are found higher under anaerobic conditions, as reported in literature, and reflected by the results of the laboratory analysis assessing the same parameters (see Table 3.6). However, the previous discussion could be affected by the fact that anaerobic columns were previously exposed to aerobic conditions. Furthermore, it has to be pointed out that the difference in the peaks' height is overall not significantly pronounced, which might derive from a slight difference in the concentration of the studied compounds in the sample.

Unfortunately, no statistically significant results can be deducted by the analysis made, due to the small number of samples available. Therefore, it looks promising to give a positive answer to the initial research question – "is it possible to use Fourier Transform Infrared Spectroscopy (FTIR) to assess the differences between samples of different MMOS materials, in order to predict their methane oxidation capacity?" – but a higher number of samples should be used, to confirm their spectrum behaviour.

Chapter 4: CONCLUSIONS AND OUTLOOK

This thesis work highlights the benefits of microbial methane oxidation systems, related to global warming abatement. Indeed, the application of MMOS is beneficial for old landfills, where the low landfill gas production and the declining methane concentrations, make landfill gas recovery no longer virtuous; but also if paired with active gas collection systems. In fact, they can capture methane that escapes collection and limit oxygen inflow into the landfill or into the gas collection system (Huber-Humer, 2004).

The first laboratory experiment conducted has investigated aerobic methane oxidation in hotspot areas of MMOS, and its results emphasize the capacity of the compost material, coming from a hotspot area in "Landfill Allerheiligen", to oxidise methane with a relatively high rate (> 70%, under aerobic conditions), even after eight years of temporal methane overload, if put under optimised laboratory conditions. Three different gas supplies have been investigated, to observe the response of samples containing different depths of the hotspot to the increase and decrease of methane supply. The results show differences in the methane oxidation rates, in particular when the methane inlet was increased. Indeed, the shallow material presented a lower methane oxidation rate with respect to the deeper layer. Furthermore, after the methane flow rate was brought back to the initial value, after the increase, complete methane degradation was no longer achieved. A possible cause could be a higher formation of extracellular polymeric substances (EPS) in the methane oxidation horizon of the columns containing the material from the upper layer of the hotspot, with respect to the deep one. EPS clogged the pore spaces, impeding the air to penetrate the soil, and therefore inhibiting aerobic methane oxidation. Further investigations could be performed to better understand this behaviour. One of them could be the DNA analysis of the samples, to investigate the magnitude of the hypothesis that different microorganisms were present in the deep sample, already used to anaerobic conditions, more than the ones in the shallow sample, and therefore needed less time to adapt and showed an overall higher, and more stable, oxidation rate.

In a following experiment, also anaerobic methane oxidation potential was investigated, showing the possibility to detect it, even if its rate is not comparable with the aerobic one. Nevertheless, improvements have to be considered regarding the performing of a column experiment under anaerobic conditions. Such as, flushing the column with nitrogen, before starting the operation, to assure anaerobic conditions inside the column; mixing sulphate ion, iron (III) hydroxide, hematite, or nitrogen as electron acceptors in the tested soil; and performing it long-term, with a high number of samples, in duplicate approach. In addition, performing FISH analysis on both samples under aerobic

and anaerobic conditions, before and after the experiments could help providing a more comprehensive vision of the involved microorganisms and the processes happening inside the columns. This kind of analysis could be considered also to be applied in the field, to understand what kind of methanotrophic bacteria are present in which depth of a MMOS.

Furthermore, a third analysing approach was applied, using Fourier Transform Infrared Spectroscopy (FTIR) spectra to search for differences which could help having a more comprehensive understanding of the processes that occurred during the experiments, or even lead to the prediction of the methane oxidation capacity of the specific material. Indeed, the spectrum gives information on the chemical composition of the sample, like a chemical ‘‘fingerprint”, from which the sample’s specific characteristic could be derived. The spectra of the previously investigated material were compared in different conditions: from the landfill, after the aerobic columns experiment and after the anaerobic columns experiment. It is found that the main composition of the samples is the same, therefore the comparison is focused on the peaks of bands 1420-1430 [cm^{-1}], and 2850-2950 [cm^{-1}] respectively corresponding to the presence of organic sulfates, ammonium ion, and of methylene, that can be linked to sugars (EPS) produced by microorganisms under stress conditions. The only behaviours that reflect laboratory and literature values are: (1) the organic sulphates in the comparison between columns under aerobic conditions containing shallow and deep input material; (2) the organic sulphates and ammonium ion in the comparison between columns under aerobic and anaerobic conditions containing shallow input material; and (3) the sugars in the comparisons between shallow and deep layer of the hotspot, and between columns under anaerobic condition containing shallow and deep input material. Future studies could use a higher number of samples, to also perform a quantitative analysis and to have statistically significant results.

In conclusion, hotspot material in MMOS can still be considered suitable for methane oxidation – when put under optimised conditions, particularly a restored suitable texture and water content of the material – but further analyses have to be made to assess a more comprehensive understanding of the action to be performed in landfill conditions. As well as further improvements have to be applied to assess the extent of anaerobic methane oxidation in MMOS and to achieve statistically significant results using FTIR spectroscopy.

Chapter 5: BIBLIOGRAPHY

Aroui, H., Orphal, J., Tchana, F.K., 2012. Fourier Transform Infrared Spectroscopy for the Measurement of Spectral Line Profiles.

Babikova, K., 2018. LA PRODUZIONE DI PERCOLATO E DEL BIOGAS NELLE DISCARICHE CONTROLLATE: ANALISI CON MODELLI MATEMATICI E FISICI. Università degli studi di Palermo.

Bundesgesetzblatt, 2008. Verordnung des Bundesministers für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft über Deponien (Deponieverordnung 2008 – DVO 2008).

Bundesgesetzblatt, 2002. Bundesgesetz über eine nachhaltige Abfallwirtschaft (Abfallwirtschaftsgesetz 2002 – AWG 2002).

Bundesministerium für Klimaschutz, Umwelt, Energie, Mobilität, Innovation und Technologie, 2022. Klimaschutzbericht 2022.

Cai, C., Zhang, X., Wu, M., Liu, T., Lai, C.-Y., Frank, J., He, B., Marcellin, E., Guo, J., Hu, S., Yuan, Z., 2021. Roles and opportunities for microbial anaerobic oxidation of methane in natural and engineered systems. Energy Environ. Sci. 14, 4803–4830. https://doi.org/10.1039/D1EE00708D

Caselli, R.L., Spadi, S., Paggetti, G., Zipoli, L., Meneghetti, F., Bucci, L., Pecorini, I., Rossi, E., Bacci, R., Frasi, N., 2018. Guidelines for the design, construction, operation, monitoring and maintenance of the biofiltration systems.

Christensen, T.H., Kjeldsen, P., Bjerg, P.L., Jensen, D.L., Christensen, J.B., Baun, A., Albrechtsen, H.-J., Heron, G., 2001. Biogeochemistry of land®ll leachate plumes. Appl. Geochem.

COMMISSIONE EUROPEA, 2022. REGOLAMENTO DEL PARLAMENTO EUROPEO E DEL CONSIGLIO sulla riduzione delle emissioni di metano nel settore dell'energia e che modifica il regolamento (UE) 2019/942 [WWW Document]. URL https://eur-lex.europa.eu/legal-content/IT/TXT/PDF/?uri=CELEX:52021PC0805R(01)&from=FR (accessed 3.28.23).

Conrad, R., 1996. Soil microorganisms as controllers of atmospheric trace gases (H2, CO, CH4, OCS, N2O, and NO). https://doi.org/10.1128/mr.60.4.609-640.1996

Cossu, R., Grossule, V., Lavagnolo, M.C., 2022. Role and Design of Sustainable Landfilling as Final Sink in Circular Economy Cycle.

Cossu, R., Stegmann, R., 2018. Solid Waste Landfilling: Concepts, Processes, Technology. Elsevier.

Costa, O.Y.A., Raaijmakers, J.M., Kuramae, E.E., 2018. Microbial Extracellular Polymeric Substances: Ecological Function and Impact on Soil Aggregation. Front. Microbiol. 9.

DECRETO LEGISLATIVO 3 aprile 2006, n. 152. Norme in materia ambientale., 2006.

DECRETO LEGISLATIVO 3 settembre 2020, n. 121, 2020.

Directive 2008/1/EC of the European Parliament and of the Council of 15 January 2008 concerning integrated pollution prevention and control (Codified version) (Text with EEA relevance), 2008. , OJ L.

Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste and repealing certain Directives (Text with EEA relevance), 2008. , OJ L.

Duan, Z., Kjeldsen, P., Scheutz, C., 2022. Efficiency of gas collection systems at Danish landfills and implications for regulations. Waste Manag. 139, 269–278. https://doi.org/10.1016/j.wasman.2021.12.023

European Environment Agency, 2022. Municipal waste landfill rates in Europe by country [WWW Document]. URL https://www.eea.europa.eu/data-and-maps/figures/municipal-waste-landfill-rates-in-1 (accessed 3.26.23).

Eurostat, 2023. Waste statistics 2020 [WWW Document]. URL https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Waste_statistics (accessed 3.29.23).

Gebert, D.J., Rachor, I., Gröngröft, D.A., 2008. Column Study for Assessing the Suitability of Different Soil Substrates for CH4 oxidation in Landfill Covers.

Gebert, J., Huber-Humer, M., Cabral, A.R., 2022a. Design of Microbial Methane Oxidation Systems for Landfills. Front. Environ. Sci. 10.

Gebert, J., Huber-Humer, M., Cabral, A.R., 2022b. Design of Microbal Methane Oxidation Systems for Landfills. Supplementary Material 1 - Overview of national guidelines on methane oxidation systems.

Gebert, J., Perner, M., 2015. Impact of preferential methane flow through soil on microbial community composition. Eur. J. Soil Biol. 69, 8–16. https://doi.org/10.1016/j.ejsobi.2015.03.006

Global Methane Pledge - Homepage [WWW Document], 2021. URL https://www.globalmethanepledge.org/#pledges (accessed 3.28.23).

Guerrero-Cruz, S., Vaksmaa, A., Horn, M.A., Niemann, H., Pijuan, M., Ho, A., 2021. Methanotrophs: Discoveries, Environmental Relevance, and a Perspective on Current and Future Applications. Front. Microbiol. 12.

Hanson, J.L., Manheim, D.C., Yeşiller, N., 2023. Geoenvironmental assessment of climate impacts from landfill gas emissions. Soils Found. 63, 101279. https://doi.org/10.1016/j.sandf.2023.101279

Hanson, R., Hanson, T., 1996. Methanotrophic bacteria. https://doi.org/10.1128/mr.60.2.439-471.1996

Hilger, H., Cranford, D., Barlaz, M., 2000. Methane oxidation and microbial exopolymer production in landfill cover soil. Soil Biol. Biochem. - SOIL BIOL BIOCHEM 32, 457–467. https://doi.org/10.1016/S0038-0717(99)00101-7

Hrad, M., Huber-Humer, M., 2015. BOKU internal report.

Hrad, M., Huber-Humer, M., 2014. BOKU internal report.

Hrad, M., Wechselberger, V., Huber-Humer, M., 2022. BOKU internal report.

Huber-Humer, M., 2005. Extended effects of methane oxidation and their relevance for biocover application in the field.

Huber-Humer, M., 2004. ABATEMENT OF LANDFILL METHANE EMISSIONS BY MICROBIAL OXIDATION IN BIOCOVERS MADE OF COMPOST. University of Natural Resources and Applied Life Sciences Vienna.

Huber-Humer, M., Amann, A., Bogolte, T., Dos Santos, M., Hagenauer, I., Pauliny, W., Reichenauer, T., Watzinger, A., Wimmer, B., 2008a. Leitfaden Methanoxidations-schichten.

Huber-Humer, M., Gebert, J., Hilger, H., 2008b. Biotic systems to mitigate landfill methane emissions. Waste Manag. Res. J. Int. Solid Wastes Public Clean. Assoc. ISWA 26, 33–46. https://doi.org/10.1177/0734242X07087977

Huber-Humer, M., Tintner, J., Böhm, K., Lechner, P., 2011. Scrutinizing compost properties and their impact on methane oxidation efficiency. Waste Manag., Landfill Gas Emission and Mitigation 31, 871–883. https://doi.org/10.1016/j.wasman.2010.09.023

IEA, 2022. The Global Methane Pledge [WWW Document]. IEA. URL https://www.iea.org/reports/global-methane-tracker-2022/the-global-methane-pledge (accessed 3.28.23).

International Energy Agency, 2023. Methane Tracker Database [WWW Document]. IEA. URL https://www.iea.org/data-and-statistics/data-tools/methane-tracker-data-explorer (accessed 3.28.23).

IPCC, 2023. SYNTHESIS REPORT OF THE IPCC SIXTH ASSESSMENT REPORT (AR6) - Longer report [WWW Document]. URL https://report.ipcc.ch/ar6syr/pdf/IPCC_AR6_SYR_LongerReport.pdf (accessed 3.29.23).

IPCC, 2021. Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. https://doi.org/10.1017/9781009157896

ISPRA, 2022. Le emissioni di gas serra in Italia alla fine del secondo periodo del Protocollo di Kyoto: obiettivi di riduzione ed efficienza energetica.

Kriipsalu, M., Somani, M., Pehme, K., Tamm, O., Truu, J., Truu, M., Orupold, K., 2023. Performance of biocover in controlling methane emissions from landfill: A decade of full-scale investigation. Process Saf. Environ. Prot. 172, 486–495. https://doi.org/10.1016/j.psep.2023.02.048

La, W., Han, X., Liu, C.-Q., Ding, H., Liu, M., Sun, F., Li, S., Lang, Y., 2022. Sulfate concentrations affect sulfate reduction pathways and methane consumption in coastal wetlands. Water Res. 217, 118441. https://doi.org/10.1016/j.watres.2022.118441

Landfill of waste directive [WWW Document], 2020. URL https://eur-lex.europa.eu/EN/legal-content/summary/landfill-of-waste.html (accessed 3.27.23).

Lavagnolo, M.C., 2022. Module5a SW Landfill Mass Balance.pdf.

Majdinasab, A., Yuan, Q., 2017. Performance of the biotic systems for reducing methane emissions from landfill sites: A review. Ecol. Eng. 104, 116–130. https://doi.org/10.1016/j.ecoleng.2017.04.015

Margenot, A., Calderón, F., Goyne, K., Mukome, F., Parikh, S., 2017. IR Spectroscopy, Soil Analysis Applications, in: Encyclopedia of Spectroscopy and Spectrometry. pp. 448–454. https://doi.org/10.1016/B978-0-12-409547-2.12170-5

Mukherjee, S., Mukhopadhyay, S., Hashim, M.A., Gupta, B.S., 2014. Contemporary environmental issues of landfill leachate: assessment & remedies.

Nandiyanto, A., Oktiani, R., Ragadhita, R., 2019. How to Read and Interpret FTIR Spectroscope of Organic Material. Indones. J. Sci. Technol. 4, 97–118. https://doi.org/10.17509/ijost.v4i1.15806

OLCreate: HEAT_HEH_ET_1.0 Hygiene and Environmental Health Module: 22. Solid Waste Management [WWW Document], n.d. URL https://www.open.edu/openlearncreate/mod/oucontent/view.php?id=209#downloads (accessed 3.22.23).

Parsaeifard, N., Sattler, M., Nasirian, B., Chen, V.C.P., 2020. Enhancing anaerobic oxidation of methane in municipal solid waste landfill cover soil. Waste Manag. 106, 44–54. https://doi.org/10.1016/j.wasman.2020.03.009

Peak, D., 2005. Fourier transform infrared spectroscopy. Encycl. Soils Environ.

Pedersen, G.B., 2010. Processes in a compost based landfill biocover; methane emission, transport and oxidation. Technical University of Denmark.

Röwer, I., 2014. Reduction of methane emissions from landfills: processes, measures and monitoring strategies. Hamburg.

Scheutz, C., Kjeldsen, P., Bogner, J.E., De Visscher, A., Gebert, J., Hilger, H.A., Huber-Humer, M., Spokas, K., 2009. Microbial methane oxidation processes and technologies for mitigation of landfill gas emissions. Waste Manag. Res. J. Sustain. Circ. Econ. 27, 409–455. https://doi.org/10.1177/0734242X09339325

Scheutz, C., Olesen, A.O.U., Fredenslund, A.M., Kjeldsen, P., 2022. Revisiting the passive biocover system at Klintholm landfill, six years after construction. Waste Manag. 145, 92–101. https://doi.org/10.1016/j.wasman.2022.04.034

Sindern, A., Ricken, T., Bluhm, J., Widmann, R., Denecke, M., 2013. Bacterial methane oxidation in landfill cover layers - a coupled FE multiphase description. PAMM 13, 193–194. https://doi.org/10.1002/pamm.201310092

United Nations Environment Programme, Climate and Clean Air Coalition, 2021. Global Methane Assessment: Benefits and Costs of Mitigating Methane Emissions.

US EPA, 2002. What Are the Options For Waste Disposal?

US EPA, OLEM, 2016. Municipal Solid Waste Landfills [WWW Document]. URL https://www.epa.gov/landfills/municipal-solid-waste-landfills (accessed 3.22.23).

US EPA, OAR, 2016a. Basic Information about Landfill Gas [WWW Document]. URL https://www.epa.gov/lmop/basic-information-about-landfill-gas (accessed 3.26.23).

US EPA, OAR, 2016b. Global Greenhouse Gas Emissions Data [WWW Document]. URL https://www.epa.gov/ghgemissions/global-greenhouse-gas-emissions-data (accessed 3.26.23).

Wei, X.-M., Su, Y., Zhang, H.-T., Chen, M., He, R., 2015. Responses of methanotrophic activity, community and EPS production to CH4 and O2 concentrations in waste biocover soils. Waste Manag. 42, 118–127. https://doi.org/10.1016/j.wasman.2015.04.005

Xu, S., Zhang, H., 2022. First evidence for anaerobic oxidation of methane process in landfill cover soils: Activity and responsible microorganisms. Sci. Total Environ. 841, 156790. https://doi.org/10.1016/j.scitotenv.2022.156790

Yang, W., Shen, L., Bai, Y., 2023. Role and regulation of anaerobic methane oxidation catalyzed by NC10 bacteria and ANME-2d archaea in various ecosystems. Environ. Res. 219, 115174. https://doi.org/10.1016/j.envres.2022.115174

Zhang, K., Wu, X., Wang, W., Chen, J., Luo, H., Chen, W., Ma, D., An, X., Chen, F., Cheng, L., Mo, Y., Wei, Z., Zhang, X., 2022. Anaerobic oxidation of methane (AOM) driven by multiple electron acceptors in constructed wetland and the related mechanisms of carbon, nitrogen, sulfur cycles. Chem. Eng. J. 433, 133663. https://doi.org/10.1016/j.cej.2021.133663

Zhang, X., Kong, J.-Y., Xia, F.-F., Su, Y., He, R., 2014. Effects of ammonium on the activity and community of methanotrophs in landfill biocover soils. Syst. Appl. Microbiol. 37, 296–304. https://doi.org/10.1016/j.syapm.2014.03.003

ACKNOWLEDGEMENTS

Concluding this thesis, this Erasmus, and this chapter of my life, I would like to express my deepest gratitude to my supervisors, both Italian and Austrian. Univ. Prof. Roberto Raga, who made this journey possible, and followed the writing of this thesis, even if remotely, with interest and courtesy. Univ. Prof. Ing. Dr.nat.techn. Marion Huber Humer, and Dipl. Ing. Dr. Marlies Hrad. whom guided me with kindness, professionalism, knowledge and expertise; showing commitment and patience; and making me feel welcomed in a foreign country. I would like to extend my sincere thanks also to the *Universität für Bodenkultur Wien*, and in particular to the *Institut für Abfall- und Kreislaufwirtschaft*, for their inclusiveness and willingness, owned by every person I had the opportunity to collaborate with.