University of Natural Resources and Life Sciences, Vienna

in cooperation with

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Microbiology of Landfill Sites

Review of the present State of Knowledge with particular Focus on the Carbon Cycle

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It's not the mountain we conquer, but ourselves.

Nicht der Berg ist es, den man bezwingt, sondern das eigene Ich.

Sir Edmund Hillary

ABSTRACT

The deposition of untreated municipal solid waste (MSW) in landfills exhibits a strong impact on our environment. The main effects are methane release, which is contributing to the greenhouse effect, and contamination of groundwater through leachate. The decomposition of MSW is mediated by the cooperation of several microorganisms and is heavily influenced by substrate composition as well as physical and chemical parameters. In order to accelerate decomposition of waste material and to attain stable conditions with minimized gas and leachate emissions, it is highly important to provide ideal degradation circumstances. Therefore, it is indispensable to identify the participating microorganisms and the required ideal eco-physiological conditions. Nevertheless, there is a lack of knowledge due to the fact that landfill material is extremely heterogeneous and thus difficult to investigate. However, the number of scientific articles about microorganisms in landfills has increased in the recent years. The predominant organisms participating in the degradation process of MSW are members of the class *Clostridia*, of the genus *Fibrobacter* and of the phylum P*roteobacteria*. Methanogenic *Archaea* are dominated by members of the orders of *Methanomicrobiales* and *Methanosarcinales*. The investigations are mainly performed by molecular approaches, whereas the approach of stable isotope probing (SIP) seems to be quite well suited to gain knowledge about microorganisms and substrate preferences. Further, different methods show different methodological biases and thus errors.

ZUSAMMENFASSUNG

Die Ablagerung von unbehandelten kommunalen Siedlungsabfällen auf Deponien hat weitreichende Umweltauswirkungen, wobei es zum einen zu Methanemissionen kommt, welche zum Treibhauseffekt beitragen, und zum anderen kommt es zu Verschmutzungen des Grundwassers durch Sickerwasseremissionen. Der Abbau der Siedlungsabfälle erfolgt durch die Zusammenarbeit verschiedener Mikroorganismen-Gruppen, wobei dieser zum überwiegenden Teil anaerob erfolgt. Der Abbau ist im Wesentlichen von der Substratzusammensetzung und den physikalischen und chemischen Parametern abhängig. Um einen optimalen Abbau zu gewährleisten, ist es unabdingbar, einerseits die beteiligten Mikroorganismen zu kennen und andererseits die idealen ökophysiologischen Bedingungen für diese einzustellen. In beiden Fällen herrscht allerdings ein Mangel an Informationen vor, da es sich bei Deponiematerial um eine äußerst heterogene und schwierig zu untersuchende Matrix handelt. In den vergangenen Jahren wurden jedoch vermehrt wissenschaftliche Publikationen zu der Problematik veröffentlicht, wobei es sich bei den am häufigsten vorgefundenen Mikroorganismen um Vertreter der Klasse der *Clostridia*, der Gattung der *Fibrobacter* und des Phylums der *Proteobakterien* handelt. Bei den methanbildenden *Archaeen* handelt es sich vorwiegend um Vertreter der Ordnungen der *Methanomicrobiales* und der *Methanosarcinales*. Die Untersuchung des Probenmaterials erfolgt vorwiegend durch molekulare/genetische Methoden, wobei sich gezeigt hat, dass der Ansatz der Analyse stabiler Isotopen (SIP) sehr präzise Rückschlüsse auf die Zuordnung bestimmter Mikroorganismen zu bestimmten Substraten erlaubt. Weiters weisen die verschiedenen verwendeten Methoden Vor- und Nachteile auf, die zu eventuellen Verzerrungen der Ergebnisse führen können.

LIST OF ABBREVIATIONS

ADP	Adenosine Diphosphate
ATP	Adenosine Triphosphate
BOD	Biological oxygen demand
cfu	colony forming units
CoA	Coenzyme A
COD	Chemical oxygen demand
CoM	Coenzyme M
DGGE	Denaturing Gradient Gel Electrophoresis
FAD	Flavin Adenine Dinucleotide
FISH	Flueorescence In Situ Hybridisation
GHG	Greenhouse gases
MO	Microorganisms
MOB	Methane-oxidizing bacteria
MSW	Municipal solid waste
NAD	Nicotinamide Adenine Dinucleotide
OM	Organic Matter
PCR	Polymerase Chain Reaction
PLEL	Phospholipid Ether Lipids
PLFA	Phospholipid Fatty Acids
qPCR	Quantitative Polymerase Chain Reaction
s.a.	sine anno
SEM	Scanning Electron Microscopy
SIP	Stable Isotope Probing
T-RFLP	Restriction Fragment Length Polymorphism
TTGE	Temporal Thermal Gel Electrophoresis
VFA	Volatile fatty acids
±	Standard deviation

CONTENTS

1. Introduction

Carbon Dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) are the major greenhouse gases (GHG) contributing to global warming. These gases are often released due to anthropogenic activities such as the burning of fossil fuels and biomass, agricultural or industrial processes (VAN ELSAS et al., 2007). During the processes of waste management and waste disposal, CO_2, CH_4 and N_2O are produced. Amongst those three gases, CH_4 is by far the most important contributor to European GHG emissions mainly caused by waste degradation (SMITH et al., 2001). Methane, which has a greenhouse gas potential 21 times higher than CO_2 (US EPA, 2014a), is released from landfill sites during the decay of biodegradable waste under anaerobic conditions (CONRAD, 1996; SMITH et al., 2001). Landfilling is one out of a few options (like incineration, composting or mechanical biological treatment) for managing municipal solid waste (MSW) and it is defined as follows: "*Landfilling involves the managed disposal of waste on land with little or no pre-treatment. Landfilling of biodegradable wastes results in the formation of landfill gas."* (SMITH et al., 2001).

Dumping waste into uncontrolled open landfills has been the predominant way of waste depositing for a long time (REDDY, 2011) and still is on a global scale. As some components of MSW and subsequently the leachate and gas emissions cause essential health and environmental problems (REDDY, 2011), developed countries became aware of the importance of reducing the amount of MSW that is landfilled and emphasised on certain political and legislative actions that would lead to the desired decline (BLUMENTHAL, 2011). Nevertheless, in the United States about 70 % of MSW are still deposited in landfills (REDDY, 2011). Also in Africa and parts of Asia, landfilling is still the most common way of depositing waste due to very low initial construction costs and low operating costs, leading to serious public health risks, CH_4 and leachate formation (REDDY, 2011; SMITH et al., 2001).

In the European Union, the northern countries deposit less than half of the generated waste in landfills whereas southern countries still use landfills as their major waste system. But one has to take into account that landfills in industrialised countries are mostly controlled and monitored sites with leachate collection systems, leachate recirculation systems and landfill gas recovery for example (REDDY, 2011; SMITH et al., 2001). Throughout the European Union, direct waste disposal to landfills could be estimated with 38 % of MSW in the year 2009 compared

with 68 % in 1995 (also see Table 1). The emphasis of European legislation on reduction of MSW is responsible for that decline (BLUMENTHAL, 2011).

Table 1: Landfilled municipal waste in the EU-27 from 1995 to 2009 (adapted from BLUMENTHAL, 2011)

	1995	**1996**	**1998**	**2000**	**2001**	**2002**	**2003**	**2005**	**2007**	**2008**	**2009**	**Change (2009 compared to 1995)**
Million tonnes	141	138	137	139	135	131	124	109	106	100	96	**-32 %**
Kg per Capita	296	290	285	288	278	269	254	221	213	201	191	**-35 %**

At a global scale, most of the MSW is deposited in landfills and CH_4 is emitted directly to the atmosphere without being used as a renewable energy source for heating or generating electricity (THEMELIS and ULLOA, 2007). Exact data for global landfilling is hard to obtain as there exist only vague and varying numbers but THEMELIS and ULLOA (2007) took the following estimations: in the United States, about 220 million tonnes MSW are landfilled per year. Additionally, the rest of the wealthy western countries (including the European Union) produce approximately 210 million tonnes of MSW that are landfilled eventually. The developing countries produce about 1.08 billion tonnes of landfill material. Adding up those numbers, this means that 1.5 billion tonnes of MSW are globally landfilled per year (THEMELIS and ULLOA, 2007).

To clarify the importance of controlled waste management systems, the main negative environmental impacts (see Figure 1) of landfills are listed below (SMITH et al., 2001; BÄUMLER and KÖGEL-KNABNER, 2008):

- Methane emissions from biodegradable waste
- Local hazards like fires and explosions
- Leachate formation threatening groundwater (e.g. polycyclic aromatic hydrocarbons)
- Non-sustainable use of land resources
- Noise and odour

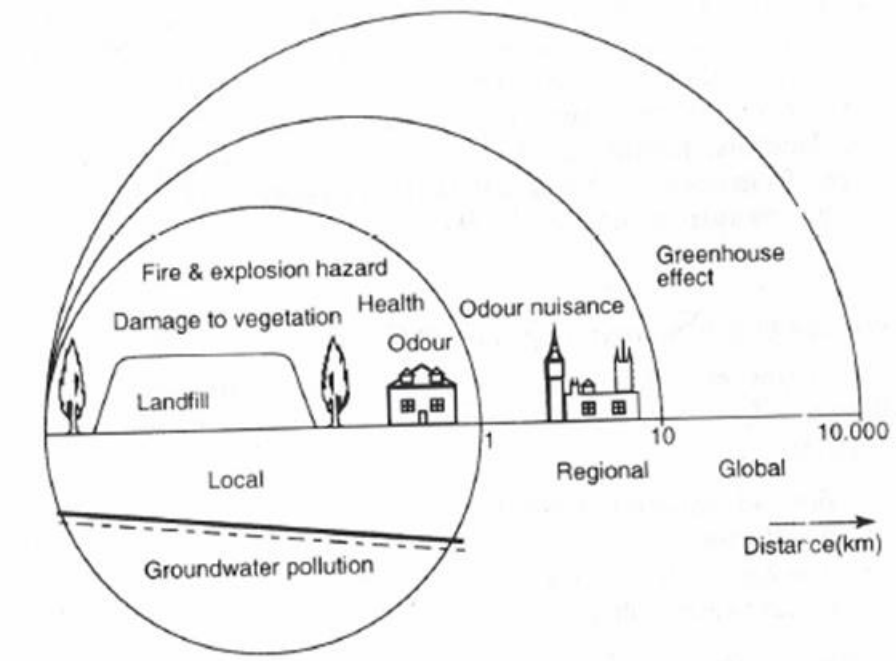

Figure 1: Negative environmental impacts (LUNING and TENT, 1993 cited KJELDSEN, 1996)

The decomposition of MSW is mediated by microorganisms (BARLAZ and HAM, 1993; STALEY et al., 2011) like *bacteria* or *fungi*, whereas several different groups of organisms are responsible for the decay of biodegradable substrates (CYPIONKA, 2010). As explained later on, it is obvious that the major constituents of MSW comprise biodegradable material. During those decomposition processes, leachate and biogas are formed which both can heavily affect our environment (BARLAZ and HAM, 1993). Therefore, it is indispensable to have comprehensive knowledge about fundamental physiological processes of microorganisms to create ideal conditions to accelerate decomposition of waste material and to attain stable conditions with minimized gas and leachate emissions as quickly as possible. At this point it has to be mentioned that a rather fast degradation of MSW leads to a short-term increase and maximisation of CH_4 emissions since decomposition processes lead to the formation of leachate and biogas (BARLAZ and HAM, 1993). However, long-term environmental impacts can be decreased as well as landfilling costs can be reduced by a short-term enhanced degradation (WARITH, 2002). For that reason, it is inevitable to gain further knowledge about the microbially mediated processes in landfills since landfilling is still the major way of depositing waste at a global scale (THEMELIS and ULLOA, 2007).

In reality, conditions for the anaerobic degradation processes inside the landfill body are not ideal, as will be discussed below, resulting in slow degradation of the waste material. This leads in turn to long-term environmental effects (HUANG et al., 2004; EUROPEAN COMMISSION, 2000; HUANG et al., 2005) which can last for several decades (EUROPEAN COMMISSION, 2000; BELEVI and BACCINI, 1992) ending up in high monitoring and management costs of the concerned sites (HUANG et al., 2005). Conventional landfills, which are operated without any control of entering moisture, may require 30 years or even more until they are stabilized (POHLAND, 1973).

The following master thesis is a review about the current state of knowledge in the field of microbial degradation of MSW in landfills, whereas all discussed scientific articles agreed about the 3 following things:

- Knowledge about microbial degradation of MSW is limited so far
- Further research is needed
- Landfills contain a very heterogeneous waste matrix which complicates research about microbial community structures (e.g. HUANG et al., 2005; McDONALD et al., 2012a; STALEY et al., 2011; VAN DYKE and McCARTHY, 2002)

The following questions are supposed to be answered in the present thesis:

(1) Which microorganisms are considered to be responsible for degradation of MSW?

(2) Which conditions are considered the ecophysiological optimum for degrading microorganisms and which conditions can actually be found in landfills?

(3) Which methods are used to investigate microorganisms in landfills? Which methods should be increasingly used for improving our understanding of microbial degradation processes in landfills?

In order to be able to improve the conditions for the responsible microorganisms and subsequently for the waste degradation process, it is indispensable to have detailed insight and comprehensive knowledge about the microbial communities. For that reason, this master thesis provides an overview on the main microorganisms that are currently considered to be responsible for degradation of organic matter in (mainly) municipal solid waste landfills.

Fundamental remarks

It has to be mentioned that this thesis emphasis only on studies that investigated solid or leachate samples that were retrieved from full-scale landfills since artificially constructed landfill conditions in the laboratory are often considered not to represent the heterogeneity and diversity of full-scale landfills (PALMISANO, 1993).

Microorganisms play the decisive role in all material cycles (carbon, nitrogen, phosphorus, sulphur cycle) because they are responsible for the degradation of organic matter to its inorganic components (FUCHS, 2007). It should also be noted, that this thesis mainly outlines the impact of microbial landfill processes on the carbon cycle and the release of CO_2 and CH_4. To give an accurate and comprehensive picture of these processes related to carbon degradation, it is also necessary to discuss biochemical and physiological fundamentals.

2. Material and Methods

This thesis is a review about the microbiology of landfill sites and the present state of knowledge with particular focus on the carbon cycle. Therefore the most important work task was reviewing literature whereas the following sources were primarily used: the Library of the University of Natural Resources and Life Sciences of Vienna, the Public Library of Vienna and several search engines such as Google Scholar, Scopus and ScienceDirect.

3. Microbiology of Landfills

The following chapters will give an overview on the main environmental impacts of landfills, so the reader gets an impression of the urgency and importance of sustainable landfilling. In order to understand the microbially mediated degradation processes, basic information about the physiology and more importantly, the substrate range of certain microorganisms are given. Additionally, fundamentals of the aerobic and anaerobic degradation pathway will be explained. The main part of this thesis, namely the microbiology of landfill sites is also treated in this section. Chapter 3 is completed with the methods that were used in the reviewed papers.

3.1. Environmental Impacts of Landfills

3.1.1. Biogas

Scientific articles basically agree that the main components of landfill gas are CH_4 (55 % ± 5 %) and CO_2 (45 ± 5%) (SENIOR, 1990; Fuchs, 2007). Landfill gas also includes certain trace components depending on the composition of the disposed waste. Three main groups upon those trace components can be distinguished: oxygen compounds, sulphur compounds and hydrocarbons. Landfill gas composition is changing over time and according to BANK (2000), four different phases can be distinguished (see Figure 2).

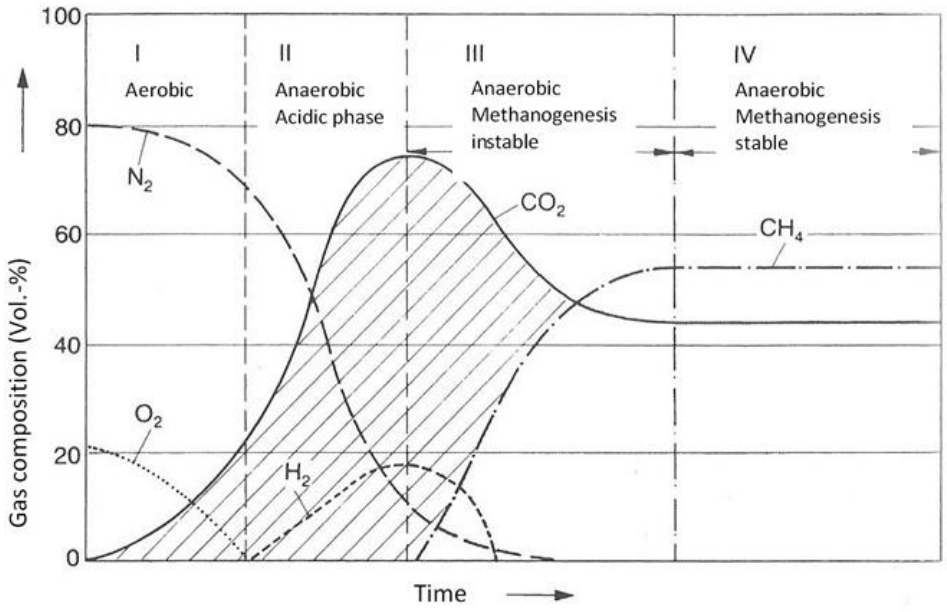

Figure 2: Changes in gas composition in landfills (BANK, 2000)

The phases are briefly explained below in Table 2. The phases can be compared with the four phases of anaerobic degradation (see chapter 3.4.1.2).

Table 2: Changes in landfill gas composition (RETTENBERGER and STEGMANN, 1996; BANK, 2000)

Phase		Changes
Phase 1	Aerobic phase	Depletion of O_2 by converting easily biodegradable organic matter into CO_2; N_2 is also displaced partly
Phase 2	Acidic phase	Volatile fatty acids (mainly CH_3COOH), CO_2 and H_2 are produced under anaerobic conditions; N_2 content in gas decreases during this phase and is nearly completely displaced at the end of the phase; pH is decreasing
Phase 3	Initial methanogenic phase	Increase of CH_4 production associated with a decrease of CO_2 and H_2; pH is slowly increasing
Phase 4	Stable methanogenic phase	50-60 % CH_4; 40 % CO_2 and low concentrations of H_2

In the first phase, big complex biopolymers (carbohydrates, lipids, proteins) are degraded into smaller oligo- and monomers. This aerobic phase corresponds to hydrolysis (DEUBLEIN and STEINHAUSER, 2011) and lasts a few days to a few weeks (KOSTER, 1988 cited KRÜMPELBECK, 1999; BANK, 2000). The second phase, where organic substances are degraded into H_2, CO_2, acetate and other organic substances, can last up to five years (KRÜMPELBECK, 1999). The amount of time that is needed for the phases three and four depends on several conditions, especially waste composition (see 3.3) and consequently gas potential and water balance within the landfill (KRÜMPELBECK, 1999). BANK (2000) indicates that roughly six years are needed before a landfill enters the stable methanogenic phase.

3.1.2. Leachate

Leachate formation is based upon two things: on the one hand, it is a result of the fact that waste itself contains water which is squeezed out with time. On the other hand, leachate arises from rain percolating through the landfill body. It is obvious that landfills containing waste with high water content and landfills placed in rather wet climates with frequent rainfalls show higher leachate emissions (BLIGHT, 2011). The major risk created by leachate is the contamination of groundwater (EUROPEAN COMMISSION, 2000; WOLFGARTEN, 2010; CHRISTENSEN et al., 1992).

The characteristics of leachate are influenced by the composition of the deposited material as well as by various other factors like waste age, design and operation of the landfill as well as climatic conditions like rainfall/moisture and temperature (FELLNER and BRUNNER, 2010;

CAMBA et al., 2013; EUROPEAN COMMISSION, 2000; POHLAND, 1973; HEYER, 2003; ANDREOTTOLA and CANNAS, 1992). Quantity as well as quality of leachate both vary with time, which means that pollutant load reaches a peak after a few years with a subsequent decline (BAGCHI, 2004; CHRISTENSEN and KJELDSEN, 1995 cited EUROPEAN COMMISSION, 2000). Comparable to the biogas composition (see chapter 3.1.1), several different phases for leachate composition can be distinguished. Depending on the literature reference, the stable methanogenic phase is followed by four further phases which are referred to as long-term phases (e.g. RETTENBERGER and MEZGER, 1992) reflected in selected leachate parameters. In Figure 3 the peak of the chemical and biological oxygen demand (COD and BOD), Cl^--concentration and NH_4^+-concentration in the acidic and initial methanogenic phase as well as the decline later on are shown (CHRISTENSEN and KJELDSEN, 1995 cited EUROPEAN COMMISSION, 2000).

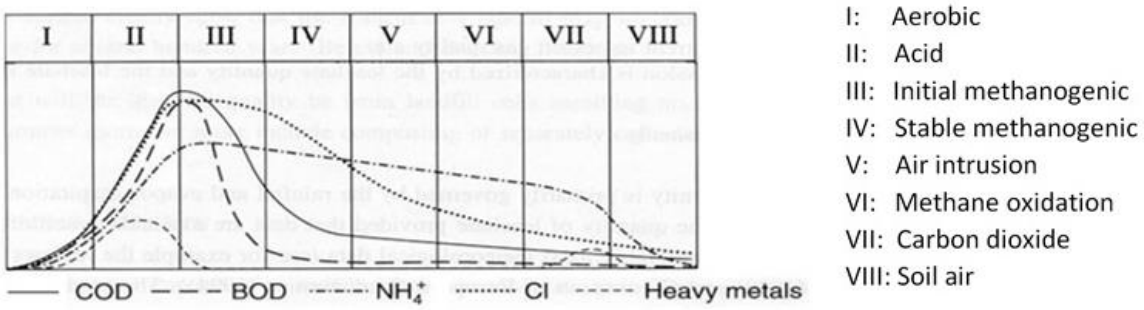

Figure 3: Changes in leachate composition in landfills (CHRISTENSEN and KJELDSEN, 1995 cited EUROPEAN COMMISSION, 2000)

As already mentioned, leachate quality and quantity are influenced by different factors (BAGCHI, 2004; CAMBA et al., 2013) such as temperature and moisture both affecting the growth of microorganisms and in turn the biodegradation of the waste material as well as the leachate production (BAGCHI, 2004). For example CAMBA et al. (2013) confirmed the influence of climatic conditions on leachate production, pointing out that the leachate quantity is significantly higher in areas with high precipitation. Also the moisture content of the waste itself influences the leachate quantity. Dry waste leads to a decrease in leachate formation because unsaturated waste absorbs water until field capacity is reached. The landfill cover can also significantly decrease leachate formation because rainfall cannot penetrate the landfill body (BAGCHI, 2004). Waste composition also influences microbial communities due to the

fact that certain substances can have toxic effects on microorganisms and therefore inhibiting the degradation processes which in turn affect leachate production (ANDREOTTOLA and CANNAS, 1992). Microbial degradation processes include water consumption (anaerobic) and water release (aerobic), also influencing the amount of leachate emitted by the landfill (HEYER, 2003).

3.2. Fundamental Physiological Processes of Microorganisms

Chapter 3.1 provided a short overview of the main environmental impacts of the degradation processes taking place inside landfills. As microorganisms mediate those processes, it is indispensable to have comprehensive knowledge about physiological processes. This thesis emphasizes particularly on physical and chemical parameters that are of major interest referring to landfill sites such as oxygen demand for growth and metabolism, temperature, moisture and pH (see chapter 3.2.2).

3.2.1. Phylogenetic Tree of Life

Basically, living organisms can be divided into 3 domains, namely *bacteria*, a*rchaea* and e*ucarya* (MAIER et al., 2000). In the case of decomposition of MSW, representatives of all 3 domains are present.

Bacteria

According to the List of Prokaryotic names with Standing in Nomenclature" by EUZÉBY and PARTE (s.a.) the domain of *bacteria* currently includes 29 different phyla. Some of them can be phenotypically distinguished because of their special morphology or physiology whereas others are only known because several sequences had been retrieved from natural habitats (MADIGAN and MARTINKO, 2009). The phylogenetic tree of *bacteria* includes, amongst others, the following phyla: *Acidobacteria, Actinobacteria, Chloroflexi, Deferribacteres, Firmicutes, Proteobacteria and Thermotogae* (EUZÉBY and PARTE, s.a.). The domain *bacteria* is very diverse and comprises different phyla with different eco-physiological requirements and metabolic strategies (FUCHS, 2007; MADIGAN und MARTINKO, 2009; FRITSCHE, 2002).

Archaea

Methanogens belong to the domain *archaea* (MAIER et al., 2000). One characteristic feature of *archaea* is their existence in extreme environments like hyperthermophilic *archaea* with a growth optimum at 80 °C or extreme halophilic a*rchaea* which are adapted to high salt concentrations (FRITSCHE, 2002). A high number of *archaea* are chemolithotrophic (see Table 5), which means that they obtain energy (ATP) from the oxidation of H_2 or sulphur (S) (FRITSCHE, 2002).

The main phyla of *archaea* are called *Crenarchaeota* and *Euryarchaeota*. *Crenarchaeota* include mainly hyperthermophilic species. The phylum *Euryarchaeota* also includes lots of species living under harsh conditions like methanogenic *archaea* as well as extremely halophilic *archaea* (class of *Halobacteria*). The first ones are strictly anaerobic whereas the second ones are mainly obligate aerobes which shows the wide range of physiological demands (MADIGAN and MARTINKO, 2009). *Korarchaeota* and *Nanoarchaeota* are two additional phyla which are not yet officially acknowledged (OTTOW, 2011).

Fungi

Among the domain of *eucaryota*, only the kingdom *fungi* is discussed in detail in this thesis. *Fungi* are *eucaryota*, which means that their cells contain a nucleus which is enclosed by a membrane-shell (MADIGAN and MARTINKO, 2009). Fungal growth can either take place through budding or through the formation of a mycelium, which consists of hyphae. Real or true *fungi* have hyphae which are septated and divided into single compartments. Those real or true *fungi* can be further divided into the phyla *Basidiomycota, Ascomycota, Zygomycota, Glomeromycota* and *Chytridiomycota*. *Fungi* can reproduce sexually or asexually, whereas different reproduction possibilities exist. *Fungi* can form important symbiotic relationships with plants (Mycorrhiza) but they can also act as pathogens (FUCHS, 2007). *Fungi* are mainly aerobic organisms but most of them possess the ability for anaerobic respiration (facultative anaerobic – see 3.2.2). Obligatorily anaerobic *fungi* are very rare. For instance cellulose-hydrolysing and cellulose-fermenting fungi from the division of the *Neocallimastigomycotina* are known to be obligatorily anaerobic (OTTOW, 2011).

Bacteria and *archaea* are single-celled, prokaryotic organisms. *Eucaryota* or *eucarya* are more complex and can be multicelled as well (MAIER et al., 2000). The main difference between

prokaryotic and eukaryotic organisms is that the cells of eukaryotic organisms have a nucleus enclosed by a membrane-shell whereas prokaryotic organisms do not have one (MADIGAN and MARTINKO, 2009).

According to SCHLEIFER (2009), there is no official classification system for *bacteria* and *archaea* but since the classification in Bergey's manuel by GARRITY et al. (2004) is used by many microbiologists the taxonomy of this thesis is based upon Bergey's manuel (see Table 3) and EUZÉBY and PARTE (s.a.). However, recent classification systems do not yet include new approaches like 16S rRNA gene sequence analysis. This is caused by tremendous difficulties establishing reasonable phylogenies from DNA sequences but nevertheless classification systems are right now rewritten and in constant flux (YARZA et al., 2008; KLENK und GÖKER, 2010;). The same applies for *Eukaryota* (ADL et al., 2012). The classification system of the kingdom of *fungi* also undergoes enormous changes since molecular techniques like DNA sequence analysis are currently included into modern systematics (GUARRO et al., 1999). Fungal systematics is slightly different than bacterial and archaeal systematics (see Table 4) and the taxonomy used in this thesis is based upon GUARRO et al. (1999) and ROBERT et al. (2014).

Table 3: Hierarchical classification system of bacteria and archaea illustrated using the example of Clostridium thermocellum (own illustration based upon GARRITY et al., 2004; EUZÉBY and PARTE, s.a.)

Taxonomic level	**Example**
Domain	*Bacteria*
Phylum	*Firmicutes*
Class	*Clostridia*
Order	*Clostridiales*
Family	*Clostridiaceae*
Genus	*Clostridium*
Species	*e.g. Clostridium thermocellum*

Table 4: Hierarchical classification system of fungi illustrated using the example of Aspergillus niger (own illustration based upon GUARRO et al., 1999; ROBERT et al. 2014)

Taxonomic level	**Example**
Domain	*Eukaryota*
Kingdom	*Fungi*
Phylum/Division	*Ascomycota*
Subphylum/Subdivision	*Pezizomycotina*
Class	*Eurotiomycetes*
Subclass	*Eurotiomycetidae*
Order	*Eurotiales*
Family	*Trichocomaceae*
Genus	*Aspergillus*
Species	*Aspergillus niger*

3.2.2. Physical and Chemical Requirements

For better distinction and explanation of the metabolism of microorganisms it is common to classify them according to the energy source, the electron donor and the carbon source (FUCHS, 2007). To summarize important living conditions and the associated terms, a short overview is provided in Table 5 followed by a more detailed explanation below.

Table 5: Living conditions and associated terms (adapted from FRITSCHE, 2002)

Energy source	
Electromagnetic radiation (light)	*phototrophic*
Chemical Reactions	*chemotrophic*
Electron donor	
Organic substance	*organotrophic*
Inorganic substance	*lithotrophic*
Carbon source	
Organic	*heterotrophic*
Inorganic (CO_2)	*autotrophic*
Oxygen	
Present (oxic)	*aerobic*
Not present (inoxic)	*anaerobic*
Low O_2 partial pressure required	*microaerophilic*
Temperature	
Low (0-20 °C)	*psychrophilic*
Middle (20-45 °C)	*mesophilic*
High (45-70 °C)	*thermophilic*
Very high (70-110 °C)	*extremophilic/hyperthermophilic*
pH	
Low	*acidophilic*
High	*alkalophilic*
Salt concentration	
High salt concentration	*halophilic*

Energy source: The energy that is needed for synthesizing ATP can be obtained either from electromagnetic radiation (PHOTOTROPHIC) or from chemical reactions (CHEMOTROPHIC). The produced ATP can then be utilized for metabolic processes or growth (FUCHS, 2007; MAIER et al., 2000; FRITSCHE, 2002).

Electron donor: If organic substrates are used as an electron donor, the organisms are called ORGANOTROPHIC. If they use inorganic substances (like H_2, NH_3, H_2S), they are called LITHOTROPHIC (FUCHS, 2007; FRITSCHE, 2002).

Carbon source: The carbon source can either be organic or inorganic. In the first case they are called HETEROTROPHIC, in the second case the can only use CO_2 as their carbon source (AUTOTROPHIC) (FUCHS, 2007; FRITSCHE, 2002). For most microorganisms organic compounds are the major carbon source (FUCHS, 2007).

As already mentioned, oxygen demand, temperature, moisture and pH are important influencing factors for the microbial metabolism and ideal conditions would be necessary to guarantee optimal degradation of MSW.

Oxygen Demand

Concerning oxygen demand for metabolism, several groups of microorganisms can be distinguished (KRÄMER, 2011):

Obligate aerobic microorganisms: Obligate aerobic microorganisms require oxygen (O_2) to gain energy (aerobic respiration). A lot of aerobic *bacteria* are microaerophilic, meaning that O_2 is required for growth but they can't tolerate the O_2 partial pressure of normal air (0.21 atm) and need much lower partial pressures (FUCHS, 2007; KRÄMER, 2011).

Obligate anaerobic microorganisms: Obligate anaerobic microorganisms can only grow in O_2-free environments because of the toxicity of O_2 to those organisms (FUCHS, 2007). They obtain energy solely through fermentation (KRÄMER, 2011).

Facultative anaerobic microorganisms: Facultative anaerobic microorganisms can either grow in presence or in absence of O_2 (FUCHS, 2007) but they prefer to grow under aerobic conditions (KRÄMER; 2011). In this case, two types can be distinguished: Aerotolerant microorganisms (e.g. lactic acid *bacteria*) are able to grow in presence of O_2, but mostly they cannot use the O_2 for gaining energy. Therefore, they have to shift to fermentation. The other type (e.g. *Enterobacteriaceae*) can either gain

energy through respiration (in presence of O_2) or through fermentation (in absence of O_2) (FUCHS, 2007).

Temperature

According to MADIGAN and MARTINKO (2009), temperature is probably the most important influencing factor for growth and survival of microorganisms. Minimum and maximum temperatures necessary for survival vary strongly between different microorganisms. Therefore, ideal temperatures for certain kinds of microorganisms are always indicated as a temperature range. Increasing temperatures enhance microbial growth until an optimum is reached. Above a certain point, membrane structures are irreversible damaged and certain proteins start to denature. As indicated in Table 5, microorganisms can be allocated into four temperature classes whereas the indicated temperatures represent the range in which the concerning microorganisms are able to grow (MADIGAN and MARTINKO, 2009).

As already stated above, ideal temperatures vary widely between different microorganisms, which is why it is hardly possible to indicate an optimum operation temperature for landfills. Not only are landfills very heterogeneous but several different microorganisms with different temperature requirements are involved in the decomposition of MSW. However, for methane formation the optimum temperature was reported as 36-40 °C (SCHULZ et al., 1997).

pH-value

Another important influencing factor for microbial growth is the pH, which is an indicator for the acidity of a habitat. The pH ranges from 0 to 14, whereas a pH of 7 represents neutral conditions (MADIGAN and MARTINKO, 2009). The pH value is defined as the negative decadic logarithm of H_3O^+ ions (BANK, 2000).

$$pH = -\log [H_3O^+] \qquad \text{[Equation 1]}$$

A pH value below 7 indicates increasing acidity, whereas a pH value above 7 indicates increasing alkalinity. Similar to the temperature, different microorganisms show different ideal pH ranges (see Table 5). Most organisms are able to grow within a range of 2 to 3 pH units. A lot of habitats show pH values between 5 and 9 and a lot of organisms show pH tolerance within this range. *Fungi* are more tolerant to acidic conditions than *bacteria*, for instance (MADIGAN

and MARTINKO, 2009). In landfills, an acidic environment is considered to inhibit methanogenesis (e.g. BARLAZ et al. 1990).

Moisture

Data concerning moisture requirements of MO were very hard to find, but FUCHS (2007) made some general statements: the requirements of MO vary considerably in terms of moisture demand. Yeasts like *Saccharomyces rouxii* have very low demands, also the fungi *Aspergillus glaucus* as well as other fungi have rather low demands regarding moisture. But most of the *bacteria* need a very high water activity (FUCHS, 2007). This was also supported by BANK (2000) indicating an optimum water activity of at least 0.94 which equals a relative humidity of 94 %. During anaerobic degradation of solid waste, higher moisture levels are considered to improve degradation (POMMIER et al., 2008).

3.2.3. Substrate Range of Microorganisms

The three main groups of biodegradable organic substances are carbohydrates, lipids and proteins (MUDRACK and KUNST, 1994; CYPIONKA, 2010; REHNER and DANIEL, 2010). These chemical compounds will be explained in detail below.

Carbohydrates consist of hydrogen, oxygen and carbon (molecular formula $C_n(H_2O)_n$) and can be distinguished into further subgroups, namely mono-, di-, oligo- and polysaccharides. The basic units of all carbohydrates are simple sugars (monosaccharides). Those monomers strongly tend to polymerize. Table 6 provides an overview on the most important carbohydrates (REHNER and DANIEL, 2010), whereas the most easily degradable polymeric carbohydrates are starch and glycogen (MUDRACK and KUNST, 1994).

Table 6: Main carbohydrates (REHNER and DANIEL, 2010)

Subgroup of Carbohydrates	Examples
Monosaccharides	**Hexoses:** D-glucose; D-galactose, D-mannose
	Pentoses: D-ribose, D-desoxyribose, D-arabinose (can be found in hemicellulose), D-xylose
Di- and Oligosaccarides	**Disaccharides:** saccharose, maltose, lactose, trehalose, cellubiose (monomer of cellulose)
	Oligosaccharides: Comprise 3 to 9 glycosidically linked monosaccharides

Polysaccharides	**Homoglykanes** (consists of one kind of monosaccharide): glycogen, starch, cellulose, dextran, inulin
	Heteroglykane (consists of carbohydrates and non-carbohydrates): Glykoproteins, peptidglykanes, glykolipids

Cellulose and hemicellulose are known to be the essential carbohydrates in landfills as 2/3 of MSW consists of cellulosic and hemicellulosic material (see chapter 3.3) and are therefore considered to be biodegradable (BARLAZ and HAM, 1993). However, it has to be pointed out that cellulose and hemicellulose can also be lignified – surrounded by lignin – and in that case be inaccessible for anaerobic microorganisms and anaerobic degradation subsequently (BARLAZ and HAM, 1993) (see chapter 3.4).

Lipids (molecular formula: $C_n(H_{2n+1})COOH$) comprise various substances which all have the following in common: they are well soluble in organic solvents (e.g. methanol). Therefore lipids do not or barely mix with water which means that they are hydrophobic. All lipids are synthesized under involvement of Acetyl-CoA and they can be classified into hydrolysable (triglycerides, phospholipids, etc.) and non-hydrolysable lipids (fatty acids) (REHNER and DANIEL, 2010).

Proteins are polymers consisting of amino acids. The formation of proteins occurs due to the connection of the carboxylic group of an amino acid with the α-aminogroup of another amino acid by the formation of peptide bonds (SENIOR, 1990). Bonds with 2 to 9 amino acids are called oligopeptides, 10 to 100 amino acids are called polypeptides and more than 100 amino acids are called proteins. Organisms – from prokaryotes to humans – all share the same 20 L-α-amino acids, for example glycine, valine, leucine or histidine (REHNER and DANIEL, 2010).

The following chapter 3.3 shows the typical chemical constitution of MSW and one can see that the majority of MSW consists of the substrates that are biodegradable by MO, namely cellulose, hemicellulose and proteins.

3.3. Composition of Municipal Solid Waste

The typical chemical constitution of MSW is shown in Table 7 that illustrates that the majority of MSW consists of biodegradable material (like cellulose, hemicellulose and proteins) which is responsible for almost 100 % of methane emissions (BARLAZ, 1989 cited BARLAZ and HAM, 1993). Unfortunately, no details on the waste age at the time of sampling were indicated in the study. Lignin, which comprises 15.2 % of MSW, is not biodegradable under anaerobic conditions (CYPIONKA, 2010). The microbial mediated decomposition of lignin is an aerobic process and mainly done by *fungi* and it is accelerated by a high O_2 content. (HAIDER, 1995). For example LI et al. (2009) and BURRELL et al. (2004) confirmed the results from Barlaz (1989, cited BARLAZ and HAM, 1993) that MSW comprises between 40 to 70 % lignocellulosic material. Hemicellulose, cellulose and lignin are subsumed under the term lignocelluloses (SENIOR, 1990). As cellulose is the major constituent of MSW, it is evident that cellulolytic microorganisms play a pivotal role in waste biodegradation (POURCHER et al., 2001).

Table 7: Composition and methane potential of MSW by chemical constituents (adapted from BARLAZ, 1989 cited BARLAZ and HAM, 1993)

Chemical constituent	% dry weight	Methane potential [% of total methane potential]
Cellulose	51.2	73.4
Hemicellulose	11.9	17.1
Protein	4.2	8.3
Lignin	15.2	0

For the sake of completeness, composition of MSW, differentiated according to material type, should also be mentioned. In the US, representing industrialised countries, more than one quarter comprise paper (28.2 %) followed by food scraps (14.1 %), yard trimmings (13.7 %) and plastics (12.3 %) according to the US EPA (2010).

3.4. Aerobic and Anaerobic Degradation of Biodegradable Material

Degradation of organic substances can take place via the aerobic or the anaerobic pathway or it can be a combination of both (HEYER, 2003). For a better overview of the whole process, the fundamentals of both pathways will be explained in chapter 3.4.1. Chapter 3.4.2 will then show specific characteristics of the degradation processes especially in landfill sites.

3.4.1. Fundamentals

3.4.1.1. Aerobic Degradation

Basically, organic substances are degraded from high-energy, high molecular weight compounds to low-energy, low-molecular compounds. Under aerobic conditions (O_2 is present and enough water is provided), organic substances can be oxidised to inorganic end products (CO_2 and H_2O) in one single metabolism step (FUCHS, 2007). The degradation of organic substances takes place through respiration of *bacteria* and *fungi* (FRITSCHE, 1998). During aerobic respiration, O_2 acts as electron acceptor. If enough O_2 is available and enough water can be provided, this process results in the formation of CO_2, H_2O and mineral constituents (CYPIONKA, 2010; FUCHS, 2007; MADIGAN and MARTINKO, 2009; LIM, 1998; VAN ELSAS et al., 2007). Energy is created during the process which can either be used by microorganisms or it is emitted as thermal energy (HEYER, 2003).

The complete oxidation from glucose to CO_2 can be expressed through equation 2:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O \qquad \text{[Equation 2]}$$

In that case the produced energy is -2870 kJ/mol (FUCHS, 2007).
The energy production during the respiration of *bacteria* is similar to the energy production of *Eucarya* (FUCHS, 2007). A comprehensive explanation of the respiration process is beyond the intention of this thesis but the following paragraph will provide the reader with a short and simplified overview on the main processes of aerobic glucose degradation, associated with ATP production.

The objective of aerobic respiration is the production of energy through the oxidation of appropriate substrates (e.g. glucose), whereas O_2 acts as an electron acceptor. Aerobic metabolism takes plays in the following steps (FUCHS, 2007):

Glycolysis

Even though glycolysis is an anaerobic process, it is the first step of aerobic cellular catabolism and via several intermediates the final product of glycolysis is pyruvate (REHM and HAMMER, 2008). Glycolysis is carried out in the cytosol of cells (GARRETT and GRISHAM, 2012). The whole process is enzymatically controlled. During this step, 2 mol ATP per mol glucose are produced (REHM and HAMMER, 2008). Under aerobic conditions, pyruvate is introduced into the citric acid cycle (KIRCHNER and MÜHLHÄUßER, 2009).

Citric acid cycle

Next, pyruvate gets transformed into Acetyl-CoA through the enzyme pyruvate-dehydrogenase (sometimes also referred to as a separate step: oxidative decarboxylation of pyruvate). The main end products of the citric acid cycle are reduction equivalents (NADH, $FADH_2$), GTP and CO_2. The cycle is inhibited by high amounts of ATP and NADH (REHM and HAMMER, 2008). In eukaryotic cells, the citric acid cycle takes place in the mitochondria and in prokaryotic cells it takes place in the cytosol (GARRETT and GRISHAM, 2012).

Respiratory chain (oxidative phosphorylation)

The respiratory chain consists of four enzyme-complexes, which are necessary for the transfer of electrons and protons from NADH to O_2, whereas H_2O is produced (KIRCHNER and MÜHLHÄUßER, 2009). The electron transport as well as the oxidative phosphorylation is associated to the cell membrane. In eukaryotic cells it is associated to the plasma membrane, whereas in prokaryotic cells these processes take place in the mitochondria (GARRETT and GRISHAM, 2012). The substrates for the respiratory chain are the reduction equivalents (NADH, $FADH_2$) that were produced during the previous steps (glycolysis and citric acid cycle, REHNER and DANIEL, 2010). In this step, the reduction equivalents NADH and $FADH_2$ are oxidised to NAD^+ and FADH and the energy produced during the oxidation is used to create a proton gradient (GARRETT and GRISHAM, 2012). The electrons are transferred from NADH and $FADH_2$ to molecular O_2 (GARRETT and GRISHAM, 2012) which gets then reduced to H_2O (REHNER and DANIEL, 2010). The proton motive force, caused by the created proton gradient is finally used for the synthesis of ATP from ADP and phosphate (REHM and

HAMMER, 2008). Oxidation of one molecule glucose delivers 32 molecules ATP (KÖNIGSHOFF and BRANDENBURGER, 2012).

3.4.1.2. Anaerobic Degradation

In the absence of O_2, organic substances can also be completely oxidised if other electron acceptors than O_2 are available (for example Nitrate NO_3^-; Nitrite NO_2^-; Sulfate SO_4^{2-} or Iron Fe^{3+}) (MADIGAN and MARTINKO, 2009; FRITSCHE, 2002). In the case of methanogenesis, CO_2 acts as final electron acceptor (FUCHS, 2007). Eventually, all biodegradable substances are converted into CO_2 and CH_4, which are known as end products of the degradation of organic matter in the absence of O_2 (VAN ELSAS et al., 2007). Characteristic for the anaerobic degradation pathway is the fact that several groups of microorganisms are working together, converting organic substrates to CH_4 (LYND et al., 2002; CYPIONKA, 2010; SENIOR, 1990). Anaerobic degradation is considered to be a multi-stage process because syntrophic, anaerobic organisms cannot degrade the absorbed substrate completely because they are specialised in several single steps (CYPIONKA, 2010). Those microorganisms that are active first prepare the substrate for the following organisms which in turn improve the situation of the firstly active organisms by removing their generated products (CYPIONKA, 2010; VAN DYKE and McCARTHY, 2002). Degradation of dead plant components also results in the formation of lignin and humic substances such as humic acids and fulvic acids (SANG et al., 2012).

Anaerobic respiration mainly follows the same principles as aerobic respiration with the exception that not O_2 but a different electron acceptor is reduced (FUCHS, 2007). NO_3^-, SO_4^{2-} or CO_2 can act as electron acceptors. For instance, CO_2 is used as electron acceptor by the strictly anaerobic methanogens (see 3.5.3.3). Anaerobic respiration delivers less energy than aerobic respiration depending on the kind of electron acceptor that had been used (FUCHS, 2007; CYPIONKA, 2010). For the energy production of methanogenesis see Table 9.

Another mechanism of anaerobic energy production is fermentation, which starts when there is no O_2 available for aerobic respiration and when there are no alternative electron acceptors for anaerobic respiration (FUCHS, 2007). Depending on which kind of fermentation happens, different amounts of ATP are produced. For instance if glucose is fermented to lactic acid or ethanol, 2 mol ATP are produced (FUCHS, 2007).

The amount of energy that is produced through anaerobic respiration (< 1 mol ATP) or fermentation (2-4 mol ATP) is much less than the amount of energy produced through aerobic respiration (32 mol ATP) (see Table 8) (KÖNIGSHOFF and BRANDENBURGER, 2012).

Table 8: Net energy gain for different microbial respiration pathways (own illustration based upon ANTRANIKIAN, 2006; KÖNIGSHOFF and BRANDENBURGER, 2012; NELSON and COX, 2010)

	Microbial respiration pathways					
	Aerobic (electron acceptor: O2)		**Fermentation (no alternative electron acceptor available)**		**Anaerobic Respiration (electron acceptor: CO_2)**	
Oxygen conditions	aerobic		anaerobic		anoxic	
	ATP	Net Energy [kJ/mol glucose]	ATP	Energy [kJ/mol glucose]	ATP	Energy [kJ/mol]
Glycolysis	2	61.2	2-4	200-300	--	--
Citric Acid cycle	2	61.2	--	--	--	--
Respiratory chain	28	856.8	--	--	--	--
Methanogenesis	--	--	--	--	<1	131 kJ/mol CO_2 27.5 kJ/mol CH_3COOH
Total	32	979.20	2-4	200-300	<1	131 kJ/mol CO2 27.5 kJ/mol CH^3COOH

Notes: For the aerobic case, one produced mol of ATP was considered as an energy equivalent of 30.5 kJ/mol. The production of reduction equivalents was taken into account for the respiratory chain whereas one mol of NADH was considered equivalent to 2.5 mol of ATP and the production of $FADH_2$ as equivalent to 1.5 mol of ATP. The conversion of the reduction equivalents NADH and $FADH_2$ into ATP is also responsible for different ATP amounts indicated in different literature references (KÖNIGSHOFF and BRANDENBURGER, 2012). For example FUCHS (2007) stated 38 ATP. In the case of anaerobic respiration, the example of "CO_2-respiration" (methanogenesis) had been chosen.

In chapter 3.4.1.1, an energy gain of 2870 kJ/mol glucose was indicated for aerobic respiration. The difference to the 979.20 kJ/mol glucose indicated in Table 8 is a result of the rather bad efficiency of glucose utilization and losses during degradation of glucose to CO_2 and H_2O, respectively (BISCHOFSBERGER et al., 2005; KÖNIGSHOFF and BRANDENBURGER, 2012).

Anaerobic degradation of organic substances is traditionally divided into 4 phases (BISCHOFSBERGER et al., 2005) which are explained in more detail below:

- **1st phase – Hydrolysis:** Decomposition of high-molecular substances into low-molecular compounds
- **2nd phase – Acidification**: Formation of organic acids (propionic acid, butyric acid, lactic acid, acetic acid), ethanol, H_2 and CO_2
- **3rd phase – Acetogenesis:** Formation of H_2, CO_2 and acetic acid
- **4th phase – Methanogenesis**: Formation of CH_4 and CO_2

1st phase: Hydrolysis

The initial substrate comprises water-insoluble, big, complex biopolymers (carbohydrates, lipids, proteins) which cannot be incorporated into microbial cells. During this step the above mentioned substrates are broken down into smaller, water-soluble oligo- and monomers through exoenzymes. Expressed in simplified terms, this leads to the following products:

- Long-chain carbohydrates are converted into short-chain sugars through enzymes called hydrolases
- Lipids are converted into fatty acids and glycerine through lipases
- Proteins are converted into amino acids through proteases

(HEYER, 2003; FUCHS, 2007; LIM, 1998; BARLAZ and HAM, 1993; DEUBLEIN and STEINHAUSER, 2011)

O_2 will be used up by facultative anaerobic MOs during this step which in turn leads to the low redox potential (see Figure 5 on page 27) necessary for obligatorily anaerobic MOs which are performing the next steps (DEUBLEIN and STEINHAUSER, 2011).

2nd phase: Acidification

During the 2nd phase the products of the 1st phase are turned into organic acids (such as acetic acid, propionic acid, butanoic acid or lactic acid), ethanol, CO_2 and H_2 depending on the concentration of H_2. High partial pressure of H_2 mainly leads to the formation of organic acids and ethanol whereas low partial pressure of H_2 mainly leads to formation of acetic acid, CO_2 and H_2 (HEYER, 2003; LECHNER, 2004; DEUBLEIN and STEINHAUSER, 2011).

3rd phase: Acetogenesis

The products of the 2nd phase are then used by acetogenic *bacteria* which turn the organic acids into H_2, CO_2 and acetic acid (HEYER, 2003). This step requires energy. For instance, turning propionic acid into acetic acid and CO_2 requires $\Delta G' = +74$ kJ/mol. This reaction only works at very low hydrogen partial pressure. Therefore H_2 has to be withdrawn constantly by H_2-using *bacteria* which in turn deliver the energy necessary for the acetogenic reaction. To maintain ideal hydrogen partial pressure conditions, acetogenic *bacteria* enter a symbiosis with methane-producing *archaea* which, unlike acetogenic *bacteria*, require high hydrogen partial pressure (HEYER, 2003; DEUBLEIN and STEINHAUSER, 2011).

As a summary, acetogenic *bacteria* require low hydrogen partial pressure to transform organic acids into H_2, CO_2 and acetic acid, but if the hydrogen partial pressure is rather high, acetogenic *bacteria* predominantly turn the initial products into butyric, capronic (hexanoic), propionic and valeric acid as well as ethanol (DEUBLEIN and STEINHAUSER, 2011).

4th phase: Methanogenesis

The 4th step is carried out by strictly anaerobic methane-producing *archaea* which can only survive in anaerobic environments. During this step CO_2 and CH_4 are produced (DEUBLEIN and STEINHAUSER, 2011).

Methane can either be built from H_2 and CO_2 through hydrogenotrophic methanogens or from CH_3COOH through acetoclastic methanogens (see Table 9) (STAFFORD et al., 1980). In both cases energy is produced during the reduction of the initial products to CH_4 (DEUBLEIN and STEINHAUSER, 2011).

The substrate range of methanogens is rather tight and limited to C1-compounds (formate, CO, methanol, formaldehyde, methylamine) and only one C2-compound, namely acetate (FUCHS, 2007). Quantitatively C1-compounds play a minor role, except for CO_2 (CYPIONKA, 2010). Many species also have the ability to grow autotrophic by usage of H_2 and CO_2 (see hydrogenotrophic methanogens).

Even though CH_4 can be built from various C1-compounds and from CH_3COOH (FUCHS, 2007), the main substrates for methanogenesis are H_2 and CO_2 and CH_3COOH (BANK, 2000):

Table 9: Substrates for methane production (own illustration based upon DEUBLEIN and STEINHAUSER, 2011; HEYER, 2003)

	Substrate	**Energy yield**	**Percentage of CH_4 production**
Acetotrophic methanogens	$CH_3COOH \rightarrow CH_4 + CO_2$	- 27.5 kJ/mol	70 %
Hydrogenotrophic methanogens	$4H_2 + CO_2 \rightarrow 2H_2O + CH_4$	- 131 kJ/mol	30 %

The following paragraph intends to provide a detailed overview on the fundamental principles of the complex biochemical methanogenesis pathways.

Hydrogenotrophic methanogens use H_2 and CO_2 as their main substrate, they do not possess the ability of using acetate for instance. The chemical process from H_2 and CO_2 to CH_4 is summarized as follows: First, CO_2 binds to methanofuran (coenzyme) which is then reduced to a formyl-group. The formyl-group is then transferred to the next coenzyme called tetrahydromethanopterine which is then methylated. The methyl-group is then transferred to the thiol-group of the coenzyme M and the product methyl-CoM (Coenzyme M) is formed. Methyl-CoM is then transformed into CH_4 through methyl-CoM-reductase. The enzyme ATP-Synthase is responsible for the conversion of energy; this form of methanogenesis can also be called anaerobic "CO_2-respiration" (FUCHS, 2007; CYPIONKA, 2010; MADIGAN and MARTINKO, 2009).

Acetotrophic methanogens use acetate as a substrate for methanogenesis, whereas the methyl-group (CH_3) is reduced to CH_4 and the carboxy-group (COOH) is oxidised to CO_2. The biochemical processes from CH_3COOH to CH_4 are as follows: First, acetate is included into the microbial cells and activated to Acetyl-CoA. Acetyl-CoA is broken down into CoA, CO-units and methyl-units. Carbon oxide is oxidised to CO_2. The methyl-group of acetyl-CoA is then transferred to CoM. The last steps (transformation into CH_4 via methyl-CoM-reductase) are identical with the respective steps of hydrogenotrophic methanogenesis (FUCHS, 2007; CYPIONKA, 2010).
Along both pathways, the ATP production rate is very low. In the case of hydrogenotrophic methanogens, the whole reaction has to run three or four times to produce one mol of ATP (see Table 8) (FUCHS, 2007).

Comparing the energy gain from aerobic degradation (see 3.4.1.1) to anaerobic degradation it is obvious that the energy balance of aerobic degradation is clearly more beneficial (see Table

8) (HEYER, 2003). At this stage, it is important to mention once again that the main degradation processes in landfills are anaerobic (HEYER, 2003). Figure 4 illustrates the overall degradation of organic substances in the absence of O_2.

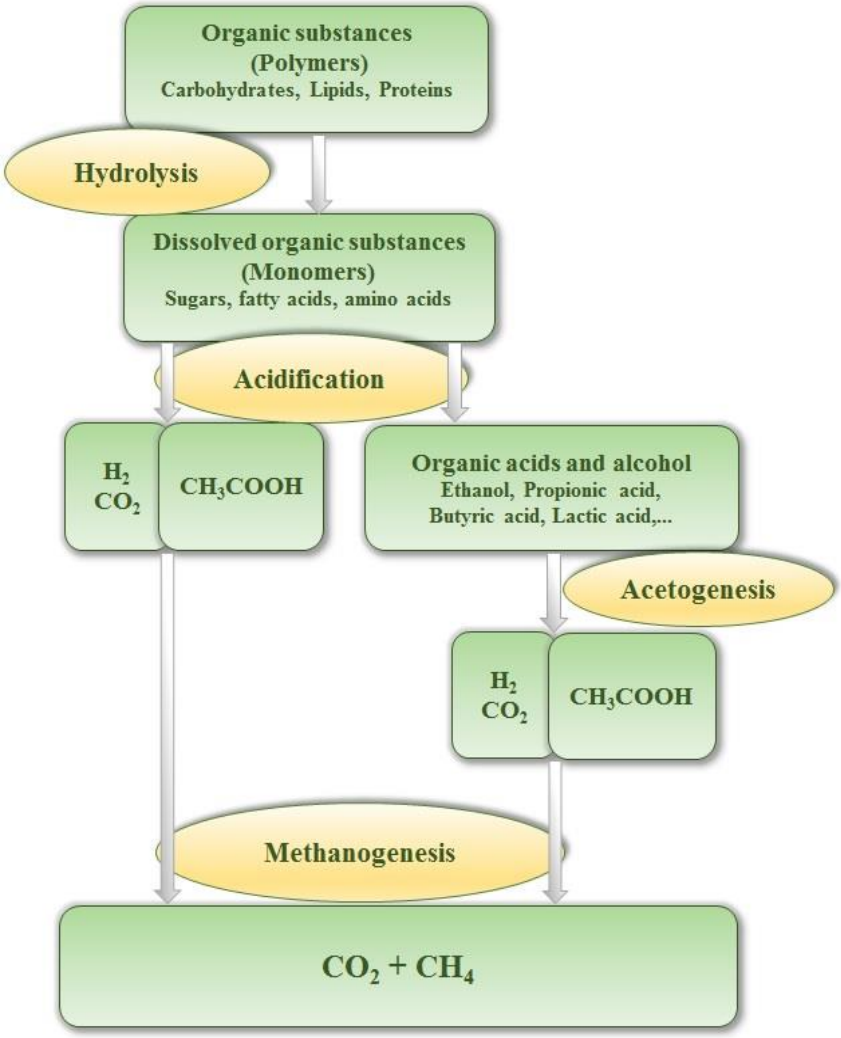

Figure 4: Anaerobic degradation of organic substances (adapted from CYPIONKA, 2010; HEYER, 2003 and LECHNER, 2004)

3.4.2. Degradation Processes in Landfills

Shortly after depositing the material, landfill sites are considered to be aerobic but anaerobic conditions evolve quickly resulting in huge anaerobic areas inside the landfill body (VARNAM and EVANS, 2000; STALEY et al., 2012; LALOUI-CARPENTIER, 2006). Therefore, the major degradation pathway of MSW takes place under anaerobic conditions and the participating microorganisms are primarily anaerobic (HEYER, 2003).

At this point, it is important to mention that one has to distinguish between anaerobic and anoxic conditions, whereas anoxic means that O_2 is only present in a non-dissolved, bonded form (like in nitrate NO_3^-). Anaerobic means, that no O_2 is present at all (UNIVERSITÄT BREMEN, s.a.). As already stated above, in aerobic environments O_2 acts as the electron acceptor (CYPIONKA, 2010; FUCHS, 2007). As the term anoxic hardly ever occurred during my research and as all scientific articles referred to conditions in landfills as anaerobic conditions, I will further on only use this term. The term anoxic only occurred associated with the processes of denitrification, or sulphur reduction being not an object of this thesis since I concentrated on processes related to carbon.

In order to make the difference between aerobic, anaerobic and anoxic more clear, Figure 5 shows the predictable sequence of electron acceptors during the microbial degradation processes of biodegradable material (FLYHAMMAR and HÅKANSSON, 1999).

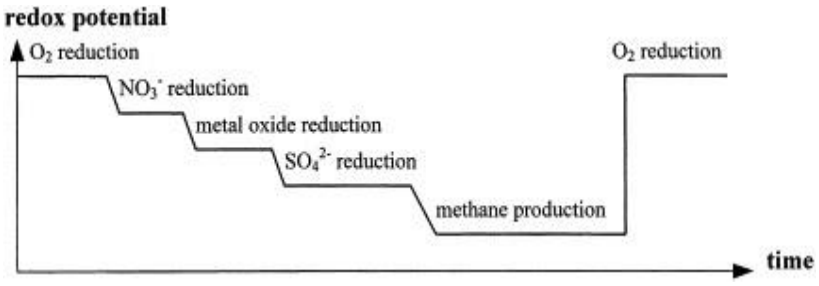

Figure 5: Electron acceptors in the natural succession of redox reactions (FLYHAMMAR and HÅKANSSON, 1999)

Based on Figure 5 and the literature cited above (e.g. HEYER, 2003; FUCHS, 2007), the four phases of anaerobic degradation can be linked to the prevailing O_2 conditions as follows:

Table 10: Phases of anaerobic degradation linked to the oxygen conditions (based upon literature cited above)

Phase	Oxygen conditions
1st phase: Hydrolysis	rather aerobic to anoxic
2nd phase: Acidification	rather anoxic to anaerobic
3rd phase: Acetogenesis	rather anaerobic
4th phase: Methanogenesis	anaerobic

As illustrated in chapter 3.3 the composition of landfill material is characterized by a great variety. Subsequently this material has to be decomposed through a great variety of degradation processes. As different authors classify the phases of CH_4 production differently, various classification systems can be found in the scientific books and papers (see Figure 2 and Table 2). For instance, CHRISTENSEN et al. (1996) use eight different phases. But the most common description of the phases used in the scientific articles referring landfills is the following:

1st phase: Hydrolysis

Scientific articles agree that cellulose hydrolysis is the slowest and rate-limiting step of anaerobic degradation of waste (e.g. McDONALD et al., 2012b; LYND et al., 2002; HEYER, 2003; VAN DYKE and McCARTHY, 2002; LAI et al., 2001) which in turn led to the conclusion that increasing the rate of hydrolysis will increase the efficiency of the whole anaerobic degradation process (BURRELL et al., 2004). The first step of anaerobic degradation lasts a few days to a few weeks (KOSTER, 1988 cited KRÜMPELBECK, 1999).

According to LYND et al. (2002) various different rate-limiting factors for hydrolysis of cellulose exist in nature: From the view of the cellulase enzymes, the following factors are suggested to be important: crystallinity, degree of polymerization, particle size, pore volume and accessible surface area. From the view of the microorganisms that are synthesizing the necessary cellulase enzymes, physical and chemical parameters are important; above all temperature has dominant effects on degradation processes.

2nd phase: Acidification & 3rd phase: Acetogenesis

The phases of acidification and acetogenesis are often summed up with the term "acidic fermentation" (e.g. BANK, 2000; LECHNER et al., 2004).

The degradation process of organic substances into H_2, CO_2, acetic acid and other organic acids can last up to 5 years according to KRÜMPELBECK (1999) and takes approximately 4 years according to BANK (2000).

4th phase: Methanogenesis

The amount of time needed for this step depends on several conditions, especially waste composition (see 3.3) and consequently gas potential and water balance within the landfill (KRÜMPELBECK, 1999). According to BANK (2000), approximately six years are needed until the rather stable gas composition of CH_4 (55 % ± 5 %) and CO_2 (45 ± 5%) is reached (SENIOR, 1990).

At this point it is indispensable to point out that the strict division of the degradation pathway into four phases taking place one after the other is rather of theoretical nature. Due to the stratification of the landfill material, different deposition dates and different conditions inside the landfill body, all four phases can happen at once in the landfill (LAMPERT and SCHACHERMAYER, 2008; RETTENBERGER and MEZGER, 1992).

The following section 3.5 will give a detailed description of relevant taxonomic groups being involved in biodegradation.

3.5. Microorganisms Responsible for Biodegradation

3.5.1. Overview

Table 11 is supposed to give an overview on the involved microorganisms that are responsible for biodegradation of MSW. Table 12 should provide the reader with an overview on the methods that had been used in the reviewed studies. A more detailed and comprehensive picture on the reviewed studies is given in chapter 3.5.3.

Table 11: Overview on microorganisms in landfills

	Phase / Substrate		Microorganisms			Conditions in the landfill			Oxygen conditions		Sampling			Source
			Phylum	Genus	Species examples	pH	Temperature [°C]	Moisture [%]	anaerobic	aerobic	Leachate	Solid / Sediment	Artificially simulated landfill conditions in the laboratory	
1	No specific phase indicated/Cellulose		Ascomycota	Trichoderma	T. harzianum	--	--	--	--	--	--	x	--	BARI et al., 2007
2	Hydrolysis/Cellulose		Firmicutes; Bacteroidetes	Clostridium (group III); Bacteroides	C. stercorarium; C. thermocellum; B. cellulosolvens	--	--	10	x	--	--	--	Anaerobic bioreactor with crystalline cellulose and sterile landfill leachate	BURRELL et al., 2004
	Acidogenese/Glucose fermenters		Firmicutes; Bacteroidetes	Clostridium (group XIVa)	--									
	Methanogenesis		Euryarchaeota	Methanosaeta	--									
3	No specific phase indicated		Firmicutes; Proteobacteria; Bacteroidetes; Actinobacteria; Thermotogae; Lentisphaerae; Acidobacteria; Chloroflexi; Cyanobacteria; Synergistetes; Spirochaetae; Deferribacteres; Deinococcus-Thermus	Lactobacillus; Clostridium; Sporanaerobacter; Thermacetogenium; Leuconostoc; Bacillus; Lactobacillus; Providentia; Klebsiella; Schineria; Escherichia; Enterobacter; Empedobacter; Flavobacterium	--	between 6.5 and 7.5	35	--	x	--	--	--	Inoculation of organic household waste with landfill leachate	CARDINALI-REZENDE et al., 2009
	Methanogenesis		Euryarchaeota	Methanoculleus; Methanosphaera; Methanobacterium; Methanofollis	Methanoculleus bourgensis; Methanospaera stadtmanae; Methanofollis formosanus									
4	Methanogenesis	3m	Euryarchaeota	Methanosarcina; Methanoculleus; Methanobacterium	Methanosarcina thermophila	8.81	--	28.12	x	--	--	x	--	CHEN et al., 2003a
		1 m				9.03	--	16.35						
5	Methanogenesis	10 m	Euryarchaeota	Methanothermobacter; Methanosarcina; Methanosaeta; Methanoculleus	Methanothermobacter thermautotrophicus; Methanosarcina thermophila	8.5 ± 0.1	40-50	42.87 ± 2.36	x	--	--	x	--	CHEN et al., 2003b
		20 m				8.5 ± 0.1		46.83 ± 3.18						
		30 m				8.3 ± 0.1		50.81 ± 1.21						
6	Methanogenesis		Euryarchaeota; Crenarchaeota	Families: Methanobacteriaceae; Methanosarcinaceae; Desulfurococcaceae; Methanomicrobiaceae	--	--	--	23	x	--	--	--	MSW samples incubated in laboratory bioreactor landfill simulators	FEI et al., 2015
7	No specific phase indicated/Cellulose		Ascomycota	Trichoderma; Aspergillus; Curvularia; Fusarium	T. viride; A. niger; A. fumigatus; Curvularia sp.; Fusarium sp.	between 6.16 ± 0.21 and 7.65 ± 0.21	--	between 35.3 and 57.4	--	x	--	x	--	GAUTAM et al., 2011
8	No specific phase indicated	0 m	--	--	--	--	--	34.2	--	--	--	x	--	GOMEZ et al., 2011
		10 m	--	--	--	--	--	22.15						
		20 m	Proteobacteria; Actinobacteria; Firmicutes	Alpha-; Beta- and Gamma-Proteobacteria; Bacillus sp.	Brevundimonas sp. and Thalossospira (α); Burkholderia and Ralstonia sp. (β); Pseudomonas and Acinetobacter sp. (γ); Arthrobacter	--	--	17.1						
		30 m	Proteobacteria; Actinobacteria; Firmicutes	Alpha-Proteobacteria; Beta-Proteobacteria; Gamma-Proteobacteria; Bacillus sp.	Brevundimonas sp. and Thalossospira (α); Burkholderia and Ralstonia sp. (β); Pseudomonas and Acinetobacter sp. (γ); Arthrobacter	--	--	Saturated conditons						

	Phase / Substrate	Microorganisms			Conditions in the landfill			Oxygen conditions		Sampling			Source
		Phylum	Genus	Species examples	pH	Temperature [°C]	Moisture [%]	anaerobic	aerobic	Leachate	Solid / Sediment	Artificially simulated landfill conditions in the laboratory	
9	No specific phase indicated	Firmicutes; Actinobacteria; Proteobacteria; Bacteroidetes; Ascomycota; Basidiomycota	Bacillus; Cellulomonas; Staphylococcus; Acinetobacter; Chromobacterium; Enterobacter; Flavobacterium; Moraxella; Proteus; Pseudomonas; Shigella; Yersinia; Candida; Cryptococcus	Bacillus azotoformans; B. mycoides; B. pasteurii; Cellulomonas turbata; Staphylococcus capitis capitis; S. epidermidis; S. lentus; S. pasteuri; Acinetobacter lwoffi; Chromobacterium violaceum; Enterobacter agglomerans; Flavobacterium multivorum; Moraxella sp.; Proteus mirabilis; Pseudomonas aeruginosa; P. putida; P. maltophilia; P. paucimobilis; P. pseudomallei; Shigella sp. Yersinia enterocolitica group; Y. pseudotuberculosis; Y. ruckeri; Candida albicans; Candida tropicalis; Cryptococcus terreus	--	--	--	--	x	--	x	--	HALE BOOTHE et al., 2001
		Firmicutes; Actinobacteria; Proteobacteria;	Bacillus; Cellulomonas; Staphylococcus; Acinetobacter; Actinobacillus; Alcaligenes; Citrobacter; Enterobacter; Klebsiella; Moraxella; Pasteurella; Proteus; Pseudomonas; Serratia; Shigella; Yersinia	Bacillus brevis; B. megaterium; Cellulomonas cellulans; Staphylococcus delphini; Acinetobacter lwoffi; Actinobacillus actinomycetemcomitans; Actinobacillus lignieresii; Alcaligenes sp.; Citrobacter freundii; Enterobacter agglomerans; Enterobacter cloacae; Klebsiella pneumoniae; Moraxella sp.; Pasteurella haemolytica; P. multocida; P. ureae; Proteus vulgaris; Pseudomonas cepacia; P. paucimobilis; P. pseudoalcaligenes; P. vesicularis; Serratia rubidaea; Shigella sp.; Yersinia enterocolitica group						x	--	--	
10	No specific phase indicated	Firmicutes; Actinobacteria	Bacillus	--	--	--	--	--	--	--	x	--	HE et al., 2014
	Methanogenesis	Euryarchaeota; Crenarchaeota	--	--	--	--	--	x	--				
11	Methanogenesis	Euryarchaeota	Methanoculleus; Methanocorpusculum; Methanospirillum; Methanogenium; Methanosarcina	Methanosarcina siciliae	Between 6.8 ± 0.5 and 8.6 ± 0.4	--	--	x	--	x	--	--	HUANG et al., 2002
12	Methanogenesis	Euryarchaeota; Crenarchaeota;	Methanoculleus; Methanospirillum; Methanobacterium; Methanosaeta; Thermoplasma	Methanoculleus oldenburgensis; M. chikugoensis; M. marisnigri; Methanospirillum hungatei; Methanobacterium oryzae; Methanosaeta concilii; Thermoplasma acidophilum	8.2 ± 0.2	--	--	x	--	x	--	--	HUANG et al., 2003
13	No specific phase indicated	Firmicutes; Chlamydiae; Verrucomicrobia; Bacteroidetes; Planctomycetes; Spirochaetae; Proteobacteria; Actinobacteria	Clostridium; Eubacterium; Synthrophomonas; Chlamydiae/Verrucomicrobia group; Cythophaga-Flexibacter-Bacteroides group	C. stercorarium; Desulfuromonas acetoxidans; Desulfuromonas thiophila	--	--	--	x	--	x	--	--	HUANG et al., 2004
14	No specific phase indicated	Firmicutes; Proteobacteria; Bacteroidetes; Spirochaetae; Cyanobacteria	Bacillus; Clostridium; Pseudomonas; Dechloromonas sp.; Thauera; Arcobacter; ; Cythophaga-Flexibacter-Bacteroides group	Deinococcus thermus; Dehalococcoides ethenogenes; Bordetella petrii	--	--	--	x	--	x	--	--	HUANG et al., 2005

	Phase / Substrate	Microorganisms			Conditions in the landfill			Oxygen conditions		Sampling			Source
		Phylum	Genus	Species examples	pH	Temperature [°C]	Moisture [%]	anaerobic	aerobic	Leachate	Solid / Sediment	Artificially simulated landfill conditions in the laboratory	
15	Hydrolysis/different substrates	Firmicutes; Actinobacteria; Proteobacteria	Bacillus; Aerococcus; Brevibacillus; Cohnella; Lysinibacillus; Oceanobacillus; Ornithinibacillus; Paenibacillus; Paenisporosarcina; Staphylococcus; Streptococcus; Desulfotomaculum; Clostridium; Actinomadura; Arthrobacter; Corynebacterium; Dietzia; Kocuria; Microbacterium; Micromonospora; Mycobacterium; Nocardia; Rhodococcus; Microvirga; Pseudomonas; Roseomonas; Stenotrophomonas	M. immunditiarum; Paenisporosarcina macmurdoensis; Solibacillus silvestris; Terribacillus goriensis; Psychrobacillus insolitus; P. psychrodurans; P. psychrotolerans	--	30	--	x	x	--	x	Culture-dependent approach	KRISHNAMURTHI and CHAKRABARTI, 2013
	No specific phase indicated	Firmicutes; Gemmatimonadetes	Bacillus; Clostridium	Solibacillus silvestris; Filibacter limicola; Sporosarcina psychrophila; Bacillus sp.; Paenibacillus sp.; Psychrobacillus psychrodurans	--	--	--	--	--			Culture-independent appraoch	
	Methanogenesis	Euryarchaeota	Methanosarcina; Methanoculleus	Methanoculleus bourgensis	--	--	--	x	--				
16	Hydrolysis/LDPE	Ascomycota	Aspergillus sp.; Fusarium sp.	A. niger	--	--	--	--	--	--	x	--	KUMAR et al., 2013
17	Methanogenesis	Euryarchaeota; Crenarchaeota	Methanoculleus; Methanofollis; Methanosarcina; Methanosaeta	Methanoculleus palmeoli; Metanofollis liminatans; Methanosarcina barkeri; M. semesiae; Methanosaeta concilii	Between 7.5 and 8.0	--	--	x		x	--	--	LALOUI-CARPENTIER et al., 2006
18	No specific phase indicated	Bacteroidetes; Chloroflexi; Firmicutes; Spirochaetes; Thermotogae; Actinobacteria; Proteobacteria	--	--	--	37	--	x	--	--	--	Organic household samples incubated in anaerobic bioreactors	LEVÉN et al., 2007
		Thermotogae; Firmicutes; Bacteroidetes	--	--	--	55	--						
	Methanogenesis	Euryarchaeota; Crenarchaeota	Methanospirillum; Methanoculleus; Methanosarcina; Methanomethylovorans; Thermoplasma	--	--	37	--						
		Euryarchaeota	Methanosarcina; Methanoculleus; Methanobacterium	--	--	55	--						
19	Hydrolysis/Cellulose	Firmicutes	Acetivibrio; Clostridium; Porphyromonadaceae (family)	--	--	mesophilic	--	x	--	--	--	incubation of waste samples with ^{13}C-cellulose; ^{13}C-glucose and ^{13}C-acetate	LI et al., 2009
	Acidogenesis/Glucose fermenters	Firmicutes; Bacteroidetes	Clostridium; Porphyromonadaceae (family)	--									
	Acetate degraders	Proteobacteria	Pseudomonadaceae (family)	--									
	Methanogenesis/Acetate degraders	Euryarchaeota	Methanoculleus; Methanosarcina	--									
20	No specific phase indicated/Cellulose	Neocallimastigomycota	Neocallimastigales	--	--	--	--	x	--	x	x	--	LOCKHART et al., 2006
21	Hydrolysis/Cellulose	Fibrobacteres	Fibrobacter	F. succinogenes	--	--	--	x	--	x	--	--	McDONALD et al., 2008

	Phase / Substrate		Microorganisms			Conditions in the landfill			Oxygen conditions		Sampling			Source
			Phylum	Genus	Species examples	pH	Temperature [°C]	Moisture [%]	anaerobic	aerobic	Leachate	Solid / Sediment	Artificially simulated landfill conditions in the laboratory	
22	Hydrolysis/Cellulose		Fibrobacteres; Firmicutes	Fibrobacter; Clostridium	--	--	--	--	x	--	x	--	--	McDONALD et al., 2012a
	Hydrolysis/Cellulose		Firmicutes; Neocallimastigomycota; Fibrobacteres	Clostridium; Neocallimastigales; Fibrobacter	--	--	Incubation at average room temperature	--	x	--	--	--	Incubation of dewaxed cotton strings with landfill leachate	
23	No specific phase indicated		Proteobacteria; Acidobacteria; Actinobacteria	Sphingosinicella; Rhizobium; Microvirga; Mesorhizobium; Herminiimonas; Dervosia; Herbaspirillum; Amaricoccus; Skermanella; Masilia; Conexibacter; Arthrobacter; Modestobacter; Couchioplanes; Cryobacterium; Marmoricola; Pseudonocardina; Microbacterium	--	between 7.58 ± 0.37 and 7.8 ± 0.39	--	--	--	--	--	--	x	--	PÉREZ-LEBLIC et al., 2012
24	Hydrolysis/Cellulose	1 yr old refuse	Firmicutes; Actinobacteria;	Bacillus; Promicromonospora; Cellulomonas; Microbacterium; Lactobacillus; Enterococcus	Bacillus licheniformis; B. subtilis; B. amyloliquefaciens; B. pabuli; Paenibacillus amylolyticus; P. polymyxa; P. alginolyticus; P. chondroitinus; P. macerans; Bacillus circulans; Promicromonospora citrea; P. Sukumoe; Cellulomonas cellulans; C. hominis; C. turbata	6.4 ± 0.6	--	48.3 ± 4.7	--	x	--	x	--	POURCHER et al., 2001
		5 yr old refuse		Bacillus; Promicromonospora; Cellulomonas;		7.9 ± 0.35	--	46.7 ± 5.0						
25	No specific phase indicated	3m	Aquificae; Bacteroidetes	--	--	--	--	5	x	--	--	x	--	SAWAMURA et al., 2010
		7 m	Thermotogae; Firmicutes; Aquificae	Fervidobacterium; Clostridium;	--	--	--	30						
		11.5 m	Firmicutes	Clostridium	--	--	--	20						
		15 m	Firmicutes	Thermoanaerobacterium	--	--	--	10						
		17.5 m	Proteobacteria; Firmicutes	Gamma-Proteobacteria: Pseudomonas; Enterobacteriaceae (family); Halorhodospira; Thermoanaerobacter	--	--	--	25						
26	Acidification		Proteobacteria	Pseudomonas	P. toyotomiensis; P. alcaliphila; P. oleovorans	--	--	--	x	--	x	--	--	TAO et al., 2014
27	Methanogenesis		Euryarchaeota	Methanoculleus; Methanofollis; Methanosaeta; Methanosarcina	Methanoculleus marisnigri; M. olentangyi. M. thermophilicus; Methanofollis liminatans; Methanosaeta concilii; Methanosarcina barkeri	--	--	--	--	--	--	x	--	UZ et al., 2003
28	Hydrolysis/Cellulose; Proteolysis		Firmicutes	Clostridium; Eubacterium; Ruminococcus; Sporobacter	C. thermocellum; C. leptum; C. cellulosi; R. albus; R. flavefaciens; E. plautii; S. termitidis	--	Mesophilic	--	x	--	x	--	--	VAN DYKE and McCARTHY, 2002

	Phase / Substrate	Microorganisms			Conditions in the landfill			Oxygen conditions		Sampling			Source
		Phylum	Genus	Species examples	pH	Temperature [°C]	Moisture [%]	anaerobic	aerobic	Leachate	Solid / Sediment	Artificially simulated landfill conditions in the laboratory	
29	Hydrolysis and Proteolysis	Firmicutes	Lactobacillus; Butyrivibrio	Lactobacillus parabrevis; Lactobacillus spicheri; Butyrivibrio sp.	--	55	--	x	--	--	--	Thermophilic municipal biogas plant	WEISS et al., 2008
	Acidification/Fermentation	Firmicutes; Synergistetes	Lactobacillus; Anaerobaculum	Lactobacillus fuchuensis; Lactobacillus sakei; Lactobacillus hammesii; Anaerobaculum mobile									
	Acetate oxidation	Firmicutes	Thermacetogenium; Clostridium	Thermoacetogenium sp.; Clostridium ultunense									
	Acetogenesis	Firmicutes	Sporanaerobacter; Synthrophococcus; Pseudoramibacter	Sporanaerobacter acetigenes; Syntrophococcus sucromutans; Pseudoramibacter alactolyticus									
	Methanogenesis	Euryarchaeota	Methanobrevibacter; Methanoculleus; Methanosphaera; Methanimicrococcus	Methanobrevibacter sp.; Methanoculleus bourgensis; Methanosphaera stadtmanae; Methanimicrococcus blatticola									

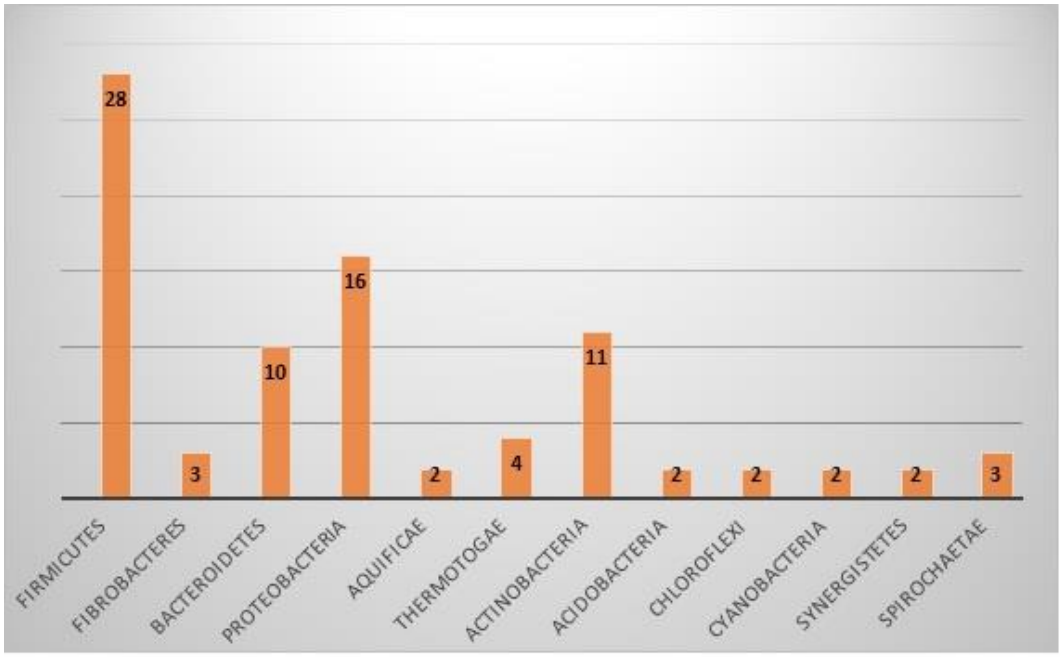

Figure 6: Number of occurrence of bacterial phyla in the reviewed studies (own illustration)

All phyla that only appeared once, such *as Lentisphaerae, Deferribacters, Deinococcus-Thermus, Chlamydiae, Verrucomicrobia, Planctomycetes* and *Gemmatimonadetes,* are not indicated in the graph. *Firmicutes* was by far the most retrieved phylum (28 references), followed by *Proteobacteria* (16 references), *Actinobacteria* (11 references) and *Bacteroidetes* (10 references).

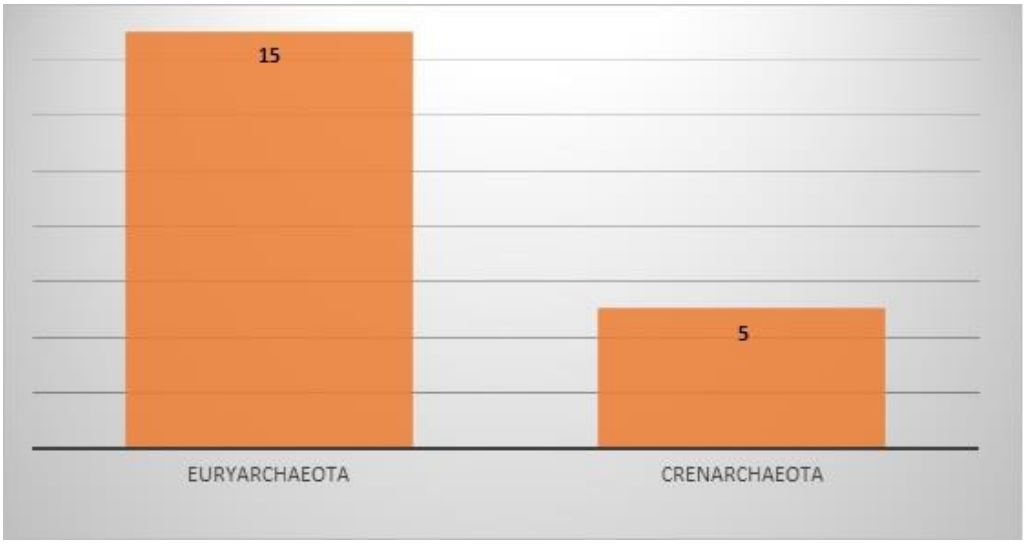

Figure 7: Number of occurrence of archaeal phyla in the reviewed studies (own illustration)

The number of *Euryarchaeota* retrieved in the above mentioned scientific studies was 3 times higher than the number of *Crenarchaeota*.

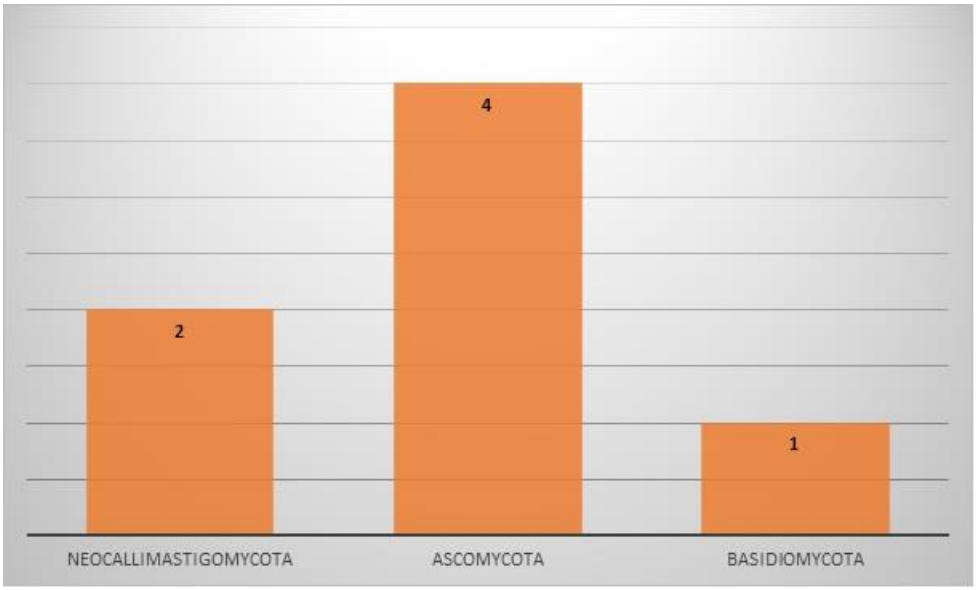

Figure 8: Number of occurrence of fungal phyla in the reviewed studies (own illustration)

Regarding fungal phyla, *Ascomycota* was the most abundant retrieved phylum (4 references), followed by *Neocallimastigomycota* (2 references) and *Basidiomycota* (1 reference).

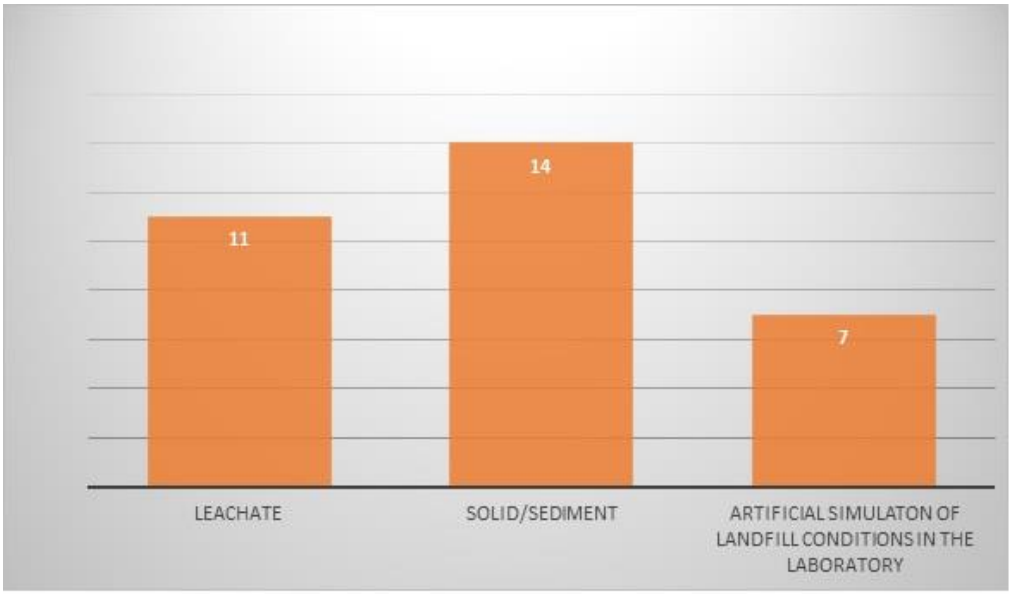

Figure 9: Overview on the sampling methods (own illustration)

The majority of the reviewed studies used solid waste samples for the investigation (14 references). Leachate had been used in 11 studies and 7 scientific investigations had been carried out in the laboratory, where landfill conditions had been artificially simulated. This number exceeds the 29 observed studies, since some authors took samples from both solids/leachate and/or conducted laboratory experiments in addition to landfill sampling.

Table 12: Overview on the used methods

	Phase/Substrate	Microorganisms		Method	Source/Paper
		Phylum	Genus		
1	Hydrolysis/Cellulose	Ascomycota	Trichoderma	Culture plate and suspension	BARI et al., 2007
2	Hydrolysis/Cellulose; Acidogenesis/Glucose fermenters; Methanogenesis	Firmicutes; Bacteroidetes; Euryarchaeota	Clostridium; Bacteroides; Methanosaeta	DNA extraction; Cloning; DNA sequencing; Phylogenetic analysis; FISH	BURRELL et al., 2004
3	No specific phase indicated; Methanogenesis	Firmicutes; Proteobacteria; Bacteroidetes; Actinobacteria; Thermotogae; Lentisphaerae; Acidobacteria; Chloroflexi; Cyanobacteria; Synergistetes; Spirochaetes; Deferribacteres; Deinococcus-Thermus; Euryarchaeota	Lactobacillus, Clostridium, Sporanaerobacter, Thermacetogenium, Leuconostoc, Bacillus, Lactobacillus; Providentia, Klebsiella, Schineria, Escherichia, Enterobacter; Empedobacter; Flavobacterium; Methanoculleus; Methanosphaera; Methanobacterium; Methanofollis	DNA extraction; PCR amplification, gene sequencing and phylogenetic analysis	CARDINALI-REZENDE et al., 2009
4	Methanogenesis	Euryarchaeota	Methanosarcina; Methanoculleus;Methanobacterium	DNA extraction; PCR amplification and cloning; Sequencing and phylogenetic analysis; FISH	CHEN et al., 2003a
5	Methanogenesis	Euryarchaeota	Methanothermobacter; Methanosarcina; Methanosaeta; Methanoculleus	DNA extraction; PCR amplification and cloning; Sequencing and phylogenetic and chemical analysis	CHEN et al., 2003b
6	Methanogenesis	Euryarchaeota; Crenarchaeota	Families: Methanobacteriaceae; Methanosarcinaceae, Desulfurococcaceae; Methanomicrobiaceae	DNA extraction, PCR amplification, pyrosequencing,	FEI et al., 2015
7	Hydrolysis/Cellulose	Ascomycota	Trichoderma; Aspergillus; Curvularia; Fusarium	Isolation of Fungi, Culture plate and suspension	GAUTAM et al., 2011
8	No specific phase indicated	Proteobacteria; Actinobacteria; Firmicutes	Alpha-, Beta- and Gamma-Proteobacteria; Bacillus sp.	DNA extraction; PCR amplification; 16S TTGE, T-RFLP	GOMEZ et al., 2011
9	No specific phase indicated	Firmicutes, Actinobacteria, Proteobacteria (β and γ), Bacteroidetes, Ascomycota, Basidiomycota	Bacillus, Cellulomonas, Staphylococcus, Acinetobacter, Actinobacillus, Alcaligenes, Chromobacterium, Citrobacter, Enterobacter, Flavobacterium, Klebsiella, Moraxella, Pasteurella, Proteus, Pseudomonas, Serratia, Shigella, Candida, Cryptococcus, Yersinia	Culture-dependent approach (utilization of agar media; Determination of colony forming unites in solid samples; Biolog system for identification of gram+ and gram- bacteria)	HALE BOOTHE et al., 2001
10	No specific phase indicated; Methanogenesis	Firmicutes, Actinobacteria; Euryarchaeota, Crenarachaeota	Bacillus	DNA extraction, 16S rRNA gene amplification, DGGE, DNA sequencing and phylogenetic analysis	HE et al., 2014
11	Methanogenesis	Euryarchaeota	Methanoculleus; Methanocorpusculum; Methanospirillum; Methanogenium; Methanosarcina	DNA extraction, PCR amplification and cloning; DNA sequencing and phylogenetic analysis	HUANG et al., 2002

	Phase/Substrate	Microorganisms		Method	Source/Paper
		Phylum	Genus		
12	Methanogenesis	Euryarchaeota; Crenarchaeota;	Methanoculleus; Methanospirillum; Methanobacterium; Methanosaeta; Thermoplasma	DNA extraction and sequencing, phylogenetic analysis	HUANG et al., 2003
13	No specific phase indicated	Firmicutes; Chlamydiae/Verrucomicrobia; Bacteroidetes; Planctomycetes; Proteobacteria, Actinobacteria	Clostridium, Eubacterium, Synthrophomonas; Chlamydiae/Verrucomicrobia group; Cythophaga-Flexibacter-Bacteroides group; Delta-Proteobacteria; Actinobacteria	DNA extraction; PCR amplification and cloninig; RFLP; DNA sequencing and phylogenetic analysis	HUANG et al., 2004
14	No specific phase indicated	Firmicutes; Firmicutes; Proteobacteria; Bacteroidetes; Spirochaetae;	Bacillus; Clostridium; Gamma-Proteobacteria; Cythophaga-Flexibacter-Bacteroides group;	DNA extraction and amplification; DNA sequencing and phylogenetic analysis	HUANG et al., 2005
15	Hydrolysis/different substrates	Firmicutes; Actinobacteria; Proteobacteria	Bacillus; Aerococcus; Brevibacillus; Cohnella; Lysinibacillus; Oceanobacillus; Ornithinibacillus; Paenibacillus; Paenisporosarcina; Staphylococcus; Streptococcus; Desulfotomaculum; Clostridium; Actinomadura; Arthrobacter, Corynebacterium; Dietzia; Kocuria; Microbacterium; Micromonospora; Mycobacterium; Nocardia; Rhodococcus; Microvirga, Pseudomonas; Roseomonas; Stenotrophomonas	Culture-dependent approach (utilization of different agar media; phenotypic characterisation and determination of colony forming unites in solid samples)	KRISHNAMURTHI and CHAKRABARTI, 2013
	No specific phase indicated	Firmicutes; Gemmatimonadetes	Bacillus, Clostridium	Culture-independent approach (DNA extraction; PCR amplification and sequencing; Phylogenetic analysis)	
	Methanogenesis	Euryarchaeota	Methanosarcina; Methanoculleus;Methanobacterium		
16	Hydrolysis/LDPE	Ascomycota	Aspergillus sp.; Fusarium sp.	Isolation and screening of LDPE degrading fungi from MSW samples; Identification of fungal strains by Lactophenol cotton blue staining, colony morphology and miscroscopic examination	KUMAR et al., 2013
17	Methanogenesis	Euryarchaeota; Crenarchaeota	Methanoculleus, Methanofollis, Methanosarcina, Methanosaeta	DNA extraction, PCR amplification, Cloning and sequencing, Sequence analysis	LALOUI-CARPENTIER et al., 2006
18	No specific phase indicated; Methanogenesis	Bacteroidetes, Chloroflexi, Firmicutes, Spirochaetes, Thermotogae, Actinobacteria, Proteobacteria; Euryarchaeota, Crenarchaeota	Methanospirillum, Methanoculleus, Methanosarcina, Methanomethylovorans, Thermoplasma, Methanobacterium	DNA extraction, PCR amplification, cloning and sequencing, sequence analysis	LEVÉN et al., 2007

	Phase/Substrate	Microorganisms		Method	Source/Paper
		Phylum	Genus		
19	Hydrolysis/Cellulose; Acidogenesis/Glucose fermenters; Acetate degraders; Methanogenesis/Acetate degraders	Firmicutes; Bacteroidetes; Proteobacteria; Euryarchaeota	Acetivibrio; Clostridium; Porphyromonadaceae (family); Pseudomonadaceae (family); Methanoculleus; Methanosarcina	SIP (stable isotope probing); DNA extraction; PCR amplification; cloning; sequence analysis; FISH	LI et al., 2009
20	No specific phase indicated/Cellulose	Neocallimastigomycota	Neocallimastigales	DNA extraction; Nested PCR	LOCKHART et al., 2006
21	Hydrolysis/Cellulose	Fibrobacteres	Fibrobacter	DNA extraction; Nested PCR; qPCR; Cloning and sequencing; TTGE; phylogenetic analysis	McDONALD et al., 2008
22	Hydrolysis/Cellulose	Fibrobacteres, Firmicutes, Neocallimastigomycota	Fibrobacter, Clostridium, Neocallimastigales	DNA extraction; qPCR; PCR; SEM	McDONALD et al., 2012a
23	No specific phase indicated	Proteobacteria; Acidobacteria; Actinobacteria	Sphingosinicella, Rhizobium, Microvirga, Mesorhizobium, Herminiimonas, Dervosia, Herbaspirillum, Amaricoccus, Skermanella, Masilia; Conexibacter, Arthrobacter, Modestobacter, Couchioplanes, Cryobacterium, Marmoricola, Pseudonocardina; Microbacterium	DNA extraction; PCR amplification; DGGE	PÉREZ-LEBLIC et al., 2012
24	Hydrolysis/Cellulose	Firmicutes; Actinobacteria	Bacillus; Promicromonospora; Cellulomonas; Microbacterium; Lactobacillus; Enterococcus;	16S rDNA sequence analysis	POURCHER et al., 2001
25	No specific phase indicated/Cellulose	Aquificae; Bacteroidetes; Thermotogae; Firmicutes	Fervidobacterium; Clostridium; Thermoanaerobacterium; γ-Proteobacteria; Thermoanaerobacter	Carbon utilization tests; DNA extraction; real-time PCR; T-RFLP	SAWAMURA et al., 2010
26	Acidifiation	Proteobacteria	Pseudomonas	16S rRNA sequence analysis	TAO et al., 2014
27	Methanogenesis	Euryarchaeota	Methanoculleus, Methanofollis; Methanosaeta; Methanosarcina	DNA extraction and PCR, RFLP, sequencing and phylogenetic analysis	UZ et al., 2003
28	Hydrolysis/Cellulose; Proteolysis	Firmicutes	Clostridium; Eubacterium; Ruminococcus; Sporobacter	DNA extraction; PCR amplification; TTGE; sequence analysis	VAN DYKE and McCARTHY, 2002
29	Hydrolysis, Acidification, Acetate oxidation, Acetogenesis, Methanogesis	Firmicutes, Synergistetes, Euryarchaeota	Lactobacillus, Butyrivibrio, Anaerobaculum, Thermacetogenium, Clostridium, Sporanaerobacter, Synthrophococcus, Pseudoramibacter, Methanobrevibacter, Methanoculleus, Methanosphaera, Methanimicrococcus	DNA extraction, DGGE	WEISS et al., 2008

Figure 10 summarizes the methods used in the reviewed studies. The methodology is specified in chapter 3.5.2. Culture-independent approaches like TTGE, DGGE, SIP, FISH, RFLP and T-RFLP had been used noticeably more than culture-dependent approaches. From the 29 reviewed scientific studies, only five used a culture-dependent approach (GAUTAM et al., 2011; BARI et al., 2007; KUMAR et al., 2013; KRISHNAMURTHI and CHAKRABARTI, 2013 and HALE BOOTHE et al., 2001). KRISHNAMURTHI and CHAKRABARTHI (2013) used culture-dependent as well as culture-independent approaches.

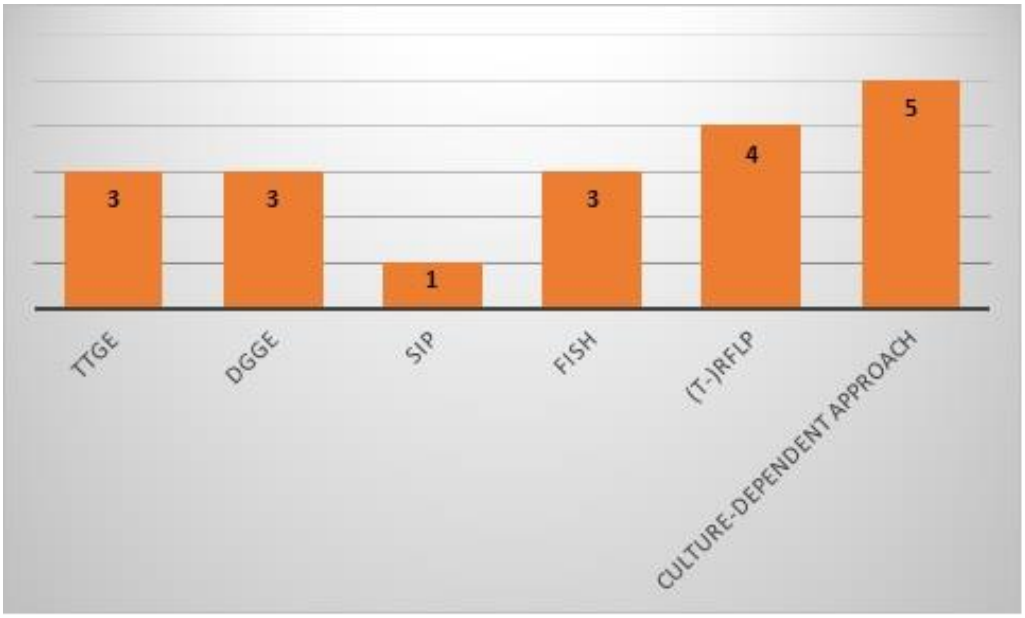

Figure 10: Overview on used methods in the reviewed studies (own illustration)

3.5.2. Methods

A roughly summarized methodology for performing microbial analyses in landfills followed a typical sequence in the observed literature: Sampling (leachate or solid samples); DNA extraction, PCR amplification and cloning, DNA sequencing and phylogenetic analysis. Various studies also used techniques like FISH, DGGE, SIP or TGGE. The following chapters will provide a short overview on the used methods and on related errors or biases.

Generally, for material from natural sites (like landfills) the microbial abundance is difficult to quantify because for instance bacteria often tend to form aggregates. Additionally the cultivation of MOs is necessary for e.g. sequencing (FUCHS, 2007), whereas environmental microorganisms are mostly not cultivable (FUCHS, 2007; SAWAMURA et al., 2010).

Therefore mainly molecular approaches based on 16S rRNA sequences are used for the investigation of microorganisms (SAWAMURA et al., 2010; HUANG et al., 2005), since they are culture-independent. For the sake of completeness, an overview on non-molecular approaches like dying techniques, microautoradiography, chemical methods and cultivation of bacteria is given at the end of the chapter (FUCHS, 2007).

PCR (Polymerase Chain Reaction)

The polymerase chain reaction is one of the basic analysing methods in molecular microbiology with various ways of application (FRITSCHE, 2002). The PCR method can briefly be explained as in-vitro amplification of DNA whereas DNA molecules can be multiplied by a billion times with the intention to gain a huge amount of specific genes for several applications. The enzyme DNA-Polymerase is being used to copy the DNA molecule (MADIGAN and MARTINKO, 2009). The PCR cycle consists of three steps (FRITSCHE, 2002), which are explained more detailed below. This cycle is repeated about 25 to 30 times and at each cycle the DNA target amount is doubled, which leads to an exponential increase (MAIER et al., 2000).

In the first step (**denaturation**) the DNA double-strand is denatured at high temperatures (LIM, 1998) into two single strands (MAIER et al., 2000). The second step is called **primer annealing**, where primers are annealed to the DNA sequence of interest (DNA target or DNA template) at low temperatures (LIM, 1998; MAIER et al., 2000). In the third step (**extension**), DNA polymerase synthesizes a complementary strand to the original two single strands (MAIER et al., 2000). These three steps are forming a cycle which is then repeated several times (MADIGAN and MARTINKO, 2009). The amplified DNA can then be run out on agarose gel for gel electrophoresis as will be explained below.

Several modifications of PCR are valuable tools in molecular biological experiments (MARLOWE et al., 2000). The following section gives a brief explanation of two PCR modification types: Real-time PCR and Nested PCR.

Real-time PCR

Real-time PCR (also called quantitative polymerase chain reaction qPCR) is a highly sensitive and specific PCR modification (DORAK, 2007), where the production of amplification products in each cycle is quantified (BUSTIN, 2005). Different to the conventional PCR, where

DNA can be made visible on agarose gel (PRIMER DESIGN, s.a.), fluorescence signals which are generated proportional to the DNA template are used (BUSTIN, 2005). The whole qPCR can be watched in "real-time" with a camera or a detector already during the process. The amount of gene copies produced during the repeated PCR cycles, are related to the fluorescence signal (PRIMER DESIGN, s.a.). One of the major achievements of qPCR is being quantitative, whereas conventional PCR is only considered semi-quantitative (PRIMER DESIGN, s.a.). McDONALD et al. (2008, 2012a) suggest that using reverse-transcribed RNA instead of DNA leads to better and more accurate results when studying the occurrence of *Fibrobacter* in landfills because qPCR performed with DNA samples showed lower abundance of *Fibrobacter* than using RNA for qPCR.

Nested PCR

Another important modification of PCR is called nested PCR, which is – similar to qPCR – characterized by an extremely high sensitivity and specificity. First, the DNA of interest is amplified in up to 30 cycles. Afterwards further PCR cycles are performed with the end product of the first step, but this time with a primer pair that is specific for the DNA sequence of interest. The end product can then be examined by agarose gel electrophoresis (LIM, 1998).

McDONALD et al. (2012a) pointed out that the usage of nested PCR show a significant improvement when it comes to the detection of *Fibrobacter*. They compared the results of direct PCR with the results of nested PCR, where they used specific primer sets for *Fibrobacter*. Whereas nested PCR confirmed 21 out of 22 leachate samples to contain *Fibrobacter*, via direct PCR only one positive leachate sample could be obtained. But the detection of *Fibrobacter* via direct PCR clearly indicated that *Fibrobacter* were quite abundant in the area where they extracted the positive sample.

Gel Electrophoresis

Gel electrophoresis (for instance performed on agarose gel) is a fundamental method for the analysis of nucleic acids (e.g. deoxyribonucleic acid DNA). First, DNA is injected into agarose gel (MAIER et al., 2000). Afterwards voltage is applied to this gel and DNA (negatively charged) starts to migrate through the gel. Migration is influenced by the charge of the molecule, by their size and structure. Smaller and more compact fragments migrate faster through the gel whereas bigger molecules tend to move at lower speed. After a certain amount of time, the gel

can be dyed, usually with ethidium bromide. The DNA can then be viewed under ultraviolet light. By comparing the DNA with DNA strands of known size, the size of the examined DNA can be determined (MAIER et al., 2000; MADIGAN and MARTINKO, 2009). TGGE (temperature gradient gel electrophoresis) and DGGE (denaturing gradient gel electrophoresis) are both approaches for the examination of complex microbial communities because temporal and spatial distribution of bacterial populations can be examined perfectly according to MUYZER and SMALLA (1998) and VAN DYKE and McCARTHY (2002). Similar to the above explained gel electrophoresis, DNA is migrating through a gel, separated either by a chemical gradient (urea/formaldehyde) at DGGE or a temperature gradient at TGGE (MAIER et al., 2000).

DNA Sequencing

The determination of DNA sequences of certain DNA segments of any organism is one of the major intentions in molecular biology (FRITSCHE, 2002). DNA sequencing requires utilization of pure cultures (CYPIONKA, 2010). Various different techniques exist which are characterized by constant further development, whereas according to FRITSCHE (2002) the three main goals are to automatize the process, high speed and high precision. The aim of DNA sequencing is to determine the exact sequence of the nucleotides in a DNA molecule (MADIGAN and MARTINKO, 2009).

SIP (Stable Isotope Probing)

Stable isotope probing (SIP) is a method for investigating substrate usage (FUCHS, 2007). The technique allows for the examination of metabolic abilities of non-cultivable microorganisms on the basis of a substrate that is marked with stable isotopes (e.g. ^{13}C) which is then incorporated into the biomass of the respective microorganisms (JEHMLICH, 2011). In a subsequent step, DNA of all microorganism is being extracted (FRIEDRICH, 2006). Afterwards the heavier, marked ^{13}C DNA is separated from the lighter non-marked ^{12}C DNA by density gradient centrifugation (FUCHS, 2007; FRIEDRICH, 2006). Via cloning and sequencing, microorganisms can then be identified (FRIEDRICH, 2006).

FISH (Fluorescence In Situ Hybridisation)

Fluorescence in situ hybridization (FISH) made it possible to visualize selected DNA sequences with fluorescence dye. This approach is based on the property of single stranded nucleic acids

(like deoxyribonucleic acid DNA) to attach to complementary base sequences (CREMER et al., 1995; SAVIC and BUBENDORF, 2007). In the case of FISH, the DNA strand is marked and can bind to the specific target sequence. If the marked DNA strand is chosen wisely, microorganisms can be identified at species, genus or family level. An important advantage of this approach is the fact that this method is being applied in-situ, which allows real conditions being represented (Steinbeis Transferzentrum Technische Chemie, s.a).

RFLP (Restriction Fragment Length Polymorphism)

Restriction Fragment Length Polymorphism (RFLP) is a molecular fingerprinting method. First, DNA is extracted from the concerning sample (MAIER et al., 2000). Afterwards the prepared DNA samples are cut into smaller pieces by so called restriction enzymes (e.g., specifically restriction endonucleases (MAIER et al., 2000; FUCHS et al., 2007). Those enzymes cut the DNA at specific points. The DNA fragments are then separated by gel electrophoresis. The pattern of the DNA fragments allow a characterisation of the microorganisms of the original sample where the DNA had been extracted from (MAIER et al., 2000).

Further Methods and concluding remarks

Several different approaches have been developed to visualize DNA of microorganisms. For instance, fluorescent markers attach to microbial DNA and dye DNA which can then be counted. Microautoradiography is used after spreading radioactive substrates which are then incorporated into microbial cells. Those approaches do not allow the identification of microorganisms or any allocation to specific metabolic performances (FUCHS, 2007).

Chemical indicators can be used to quantify total biomass of a certain material. For example, via the total amount of ATP or DNA, conclusions about the viable biomass can be drawn. Another important method I mainly found in studies about methane oxidation, is the determination of fatty acids. Different microorganisms have different structures of their fatty acid chains (FERREIRA FERNANDES, 2007) and based on fatty acid patterns, information about specific groups of organisms and partly about their metabolic performances can be obtained (FUCHS, 2007). One way of analysing fatty acid patterns of *bacteria* is to determine bacterial phospholipid fatty acids (PLFAs) (FERREIRA FERNANDES, 2007; HANIF et al., 2012). For a*rchaea*, the structure of fatty acids can be investigated by determining archaeal phospholipid ether lipids (PLELs) (HANIF et al., 2012).

Several authors of the observed articles agreed that during different methodological steps (e.g. DNA extraction or PCR amplification), different biases and mistakes can occur (LALOUI-CARPENTIER et al., 2006; CHEN et al., 2003a, b). Also the number of clones being obtained at the end of the respective methodological approach might not be proportional or representative for the actual abundance in the landfill. This is related to the fact that certain microorganisms are underrepresented in clone libraries (CHEN et al., 2003a, b). GOMEZ et al. (2011) also point out that different DNA extraction kits used for DNA extraction can lead to different results. Further on, the utilization of reverse-transcribed RNA (see glossary) in PCR was considered to be more efficient than the utilization of DNA e.g. when it comes to the detection of *Fibrobacter* (RANSOM-JONES et al., 2012; McDONALD et al., 2008).

The cultivation of bacteria, only possible for a very small part of organisms, can be carried out for aerobic and anaerobic organisms. On the surface of solid culture media or within the solid culture media (like agar), bacteria can be quantified. Not only solid media, but also liquid media can be used for the quantification of cultivable bacteria. Nevertheless this approach shows quite a lot of uncertainties because bacteria have the tendency of forming aggregates (FUCHS, 2007).

3.5.3. Selection of Reviewed Papers

The following chapter will provide more detailed information on a selection of the reviewed literature. For reasons of better readability, participating microorganisms are allocated according to their domain (*bacteria*, *archaea*) and their kingdom (*fungi*). Within domain or kingdom, microorganisms are roughly allocated to the phase they appear in, whereas certain microorganisms play a role in more than one phase. Within the phases, they are sorted roughly in frequency of mention in the literature. Most of the reviewed studies occur more than once in the following part of the thesis. The applied methodology is explained in chapter 3.5.2.

At the end of this chapter, a short introduction about methanotrophic *bacteria* is given (see 3.5.3.4). However, they are not part of the reviewed studies but since they diminish landfill-induced CH_4 emissions, methanotrophs should be explained as well.

3.5.3.1. *Bacteria*

Chapter 3.5.3.1 is further divided into the class *Clostridia*, the phylum *Fibrobacteres*, the phylum *Proteobacteria*, the Family *Porphyromonadaceae* and the family *Pseudomonadaceae*.

Clostridia

Members of the class of the gram+ *bacteria Clostridia* (phylum: *Firmicutes*) were detected in several studies (Table 11). They are considered to be the most important actors in cellulose-degradation and hydrolysis (McDONALD et al., 2012a). A few relevant studies have been chosen to be explained in more detail to show the importance of *Clostridia*. Members of the phylum *Firmicutes* comprise microorganisms that are common for anaerobic habitats, and they are known to decompose complex organic macromolecules like proteins and carbohydrates (WIEGEL et al., 2005 cited LALOUI-CARPENTIER et al., 2006).

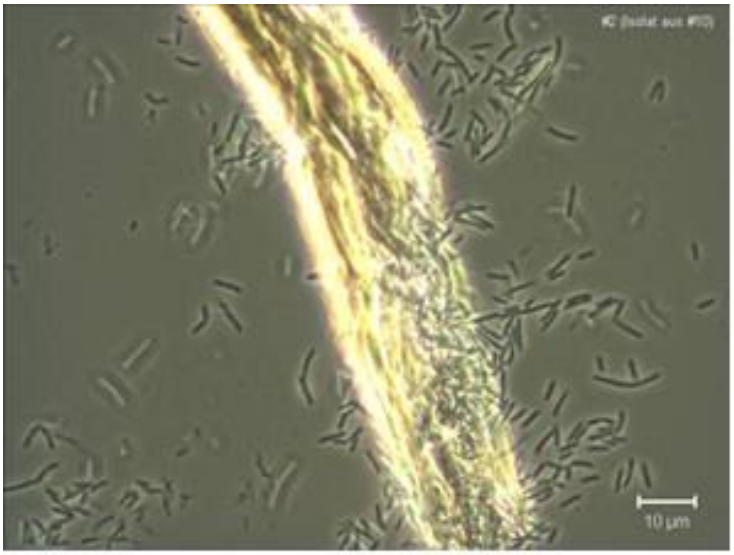

Figure 11: Degradation of cellulose fibres by C. thermocellum 1000x enlargement (KÖLLMEIER, 2011)

VAN DYKE and McCARTHY (2002) detected representatives of the species *Clostridium thermocellum* (see Figure 11) and *C. leptum* in leachate samples of a MSW landfill in England by applying a molecular approach. They used specific polymerase chain reaction (PCR) amplification, temporal thermal gel electrophoresis (TTGE) and sequence analysis for the detection of C*lostridia*. In this study, four subgroups of *Clostridia* were examined, namely subgroup I, III, IV and XIVab. *Clostridium* Cluster III solely contains cellulose-degrading C*lostridia*. The appearance of Clostridium subgroup I, IV and XIVab might be an indication for saccharolytic and proteolytic degrading microorganisms. *Clostridium thermocellum* and *C. leptum* are assigned to cluster III and cluster IV, respectively, and they were the most dominating among the investigated groups in this study. *Acetivibrio cellulolyticus*, which is a species that belongs to the genera of *Acetivibrio* (class *Clostridia*), was also retrieved. The results of the study showed *C. thermocellum* (cluster III) and *C. leptum* (cluster IV) to be predominant and the most ubiquitous groups which is an evidence for cellulolytic, saccharolytic and proteolytic organisms. The authors also mentioned the close relationship of these two groups which led to the conclusion that they might descend from the same precursor organism (VAN DYKE and McCARTHY, 2002).

Clostridia spp. were also detected by BURRELL et al. (2004), who investigated the microbial population in a landfill leachate bioreactor by incubation of crystalline cellulose using the method fluorescence in situ hybridization (FISH). The 16S rRNA gene clone libraries, which were generated from the extracted DNA after performing PCR, were dominated by sequences of the phylum *Firmicutes*. Representatives of *Clostridium* group III were mostly related to the three following *Clostridium* species: *C. stercorarium, C. thermocellum* and *Bacteroides cellulosolvens*. Furthermore, representatives of *Clostridium* group XIVa and VI were found. It is important to mention that PCR primers were specified for *Clostridium* group I, III, IV and

XIVab, indicating that other cellulolytic microorganisms could have been present in the landfill as well but could not be detected because of the specified PCR primers. BURRELL et al. (2004) further on suggest that the three species of *Clostridium* group III are highly important in cellulose hydrolysis, whereas representatives of *Clostridium* group XIVa might play a role in glucose fermentation (Acidification – 2nd phase) resulting in the production of acetate and propionate.

LI et al. (2009) also detected *Clostridia*. They applied DNA stable isotope probing (DNA-SIP) on MSW samples under mesophilic conditions. The samples were incubated with ^{13}C-cellulose, ^{13}C-glucose and ^{13}C-acetate to analyse the relationship between hydrolytic, fermentative and methanogenic microorganisms. Their study confirmed present knowledge about the importance of several phyla in anaerobic biodegradation of MSW, like *Firmicutes* or *Bacteroidetes*. They detected various representatives of *Firmicutes* like *Clostridia*, which constituted 10.3 % of the *Firmicutes* phyla. The results of the ^{13}C-cellulose experiment lead to the conclusion that *Clostridia* are important cellulose hydrolysers. The obtained sequences were related to the following cultivable species: *C. propionicum*, *C. tetani* (see Figure 12) and *C. quinii* and to the not cultivable *Clostridium spp*.

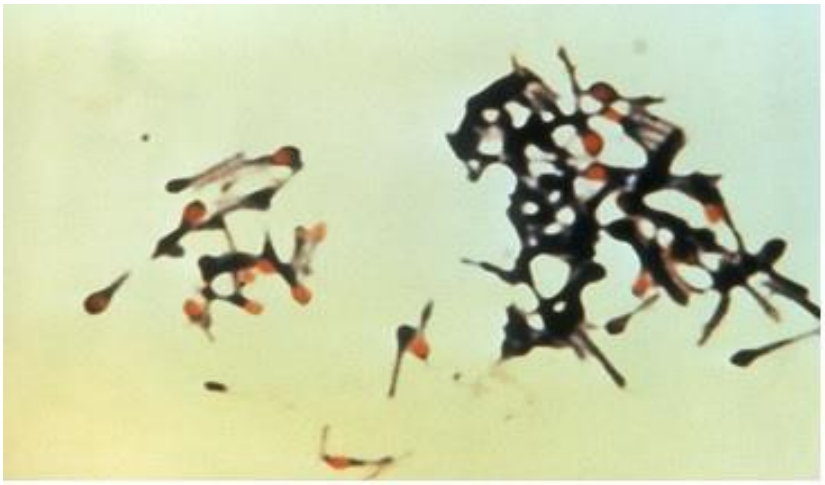

Figure 12: C. tetani (CENTERS FOR DISEASE CONTROL AND PREVENTION, 1995)

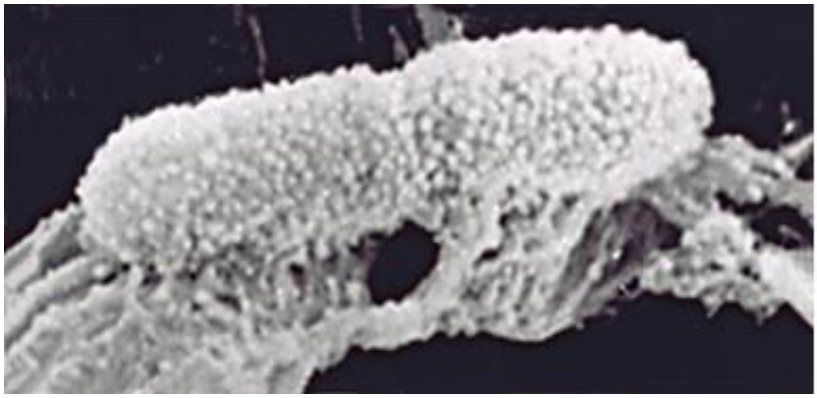

Figure 13: Surface of the bacterium Acetivibrio cellulolyticus (NATIONAL RENEWABLE ENERGY LABORATORY, s.a.)

In addition, LI et al. (2009) examined the functional role of the retrieved microorganisms with the expected result that different MO have been found depending on the substrate that had been used for the incubation. The genera *Acetivibrio* (class *Clostridia*) was identified to be responsible for cellulose hydrolysis next to other unidentified members of the class of *Clostridia* because FISH analysis had revealed *Acetivibrio* to be closely spatially attached to cellulose fibres. A distant relationship of the retrieved sequences to the known mesophilic cellulolytic species of *Acetivibrio cellulolyticus* (see Figure 13) could be determined. Based on FISH, LI et al. (2009) concluded that cellulose degradation was mainly carried out by the genus *Acetivibrio* in this study whereas species of the genus *Clostridium* were mainly performing "saccharide fermentation".

SAWAMURA et al. (2010) examined microbial distribution at different depths using carbon-utilization patterns by using Biolog-MT plates as well as T-RFLP. Five waste layers in different depths were investigated which were considered to cover a time period of 14 years of dumping between bottom and top layer. *Clostridia*, amongst other microorganisms, could be found at 7.0 m, 11.5 m and 17.5 m depth, indicating anaerobic conditions in these deep layers (SAWAMURA et al., 2010). However, *Clostridia* can also be tolerant to O_2 to some extent (BAHL and DÜRRE, 2011). At this point, it should be mentioned that at a depth of 17.5 m, *Gamma-Proteobacteria* were found to be dominant (Relative abundance of *Gamma-Proteobacteria* with Hha I 40.8 %). In this study, they also collected data about organic matter (OM) and moisture content. Table 13 is supposed to associate the relative abundance of *Clostridia* with OM, moisture and monosaccharide content. A high monosaccharide (> 5 %) content could indicate an active (hemi-) cellulose degradation, since monosaccharides are the known end products of hydrolysis. Furthermore they investigated utilization patterns of several substrates in different depths. Protein and lipid contents were rather high in 7 m, which is consistent with a high *Clostridia* population in this depth. Volatile fatty acid (VFA) contents (like lactate, acetate, butyrate, valerate), which are products from the acidification phase carried

out by fermentative *bacteria*, were generally low or not detected at all. This correlates with the small population of volatile fatty acid utilizing *bacteria*, which were also examined. According to SAWAMURA et al. (2010) the low VFA content might be an indication that high molecular compounds were hardly transformed into low molecular compounds and that the degradation of solid waste material is not yet completed.

Table 13: Organic matter and moisture contents at different depths (SAWAMURA et al., 2010)

	Depths [m]		
	7.0	**11.5**	**17.5**
Organic matter content [%]	37.0	10.0	10.0
Moisture content [%]	30.0	20.0	25.0
Monosaccharide content [%]	> 5	> 5	< 5
Relative abundance of *Clostridia* with Hha I [%]	6.4	9.4	2.1

NOTE: In the depth of 17.5 m, the authors found indications for extreme halophilic conditions and high temperatures.

SAWAMURA et al. (2010) also applied T-RFLP analysis which showed that at each depth unique microbial community structures are formed and that the waste layers are rather isolated from each other.

McDONALD et al. (2012a) detected *Clostridia* in landfill leachate samples as well as in two leachate microcosms where they incubated dewaxed cotton strings with landfill leachate. They applied PCR, qPCR and scanning electron microscopy (SEM). In the leachate samples, representatives of *Clostridium* clusters III and XIV were the most abundant, but also *Clostridium* cluster IV was present in a noteworthy quantity. The results of the in-situ microcosms experiment, where they intended to investigate colonization and degradation of cellulolytic substrates (de-waxed cotton strings incubated with landfill leachate) at average room temperature, corresponded with existing knowledge; The microcosm with high abundance of *Clostridia* (in that case *Clostridium* cluster III representatives) and *Fibrobacter* spp. showed high cellulose degradation rates, whereas the second microcosm with very low abundance of *Clostridium* cluster III and *Fibrobacter* spp. hardly showed any cellulose degradation. The authors of this study investigated samples of five different landfills in the United Kingdom. The relative abundance of occurring microorganisms varied significantly

between the different landfills and also within samples taken from the same landfill. Once again this is an indication for the heterogeneity of landfill material and the high impact of the material composition on the microbial structure.

Clostridia were also detected by KRISHNAMURTHI and CHAKRABARTI (2013) through culture-dependent methods and culture-independent methods. According to the culture-dependent approach, *bacteria* were clearly dominated by members of the *Firmicutes* phylum especially by the family of *Bacillaceae*. From the family of *Clostridiaceae*, three isolates showed high sequence similarities with *C. histolyticum*, *C. butyricum* and *C. sporogenes* according to 16S rRNA gene sequencing. Species of the genus *Bacillus* as well as species of the genus *Clostridium* are well known for their hydrolytic and fermentative capabilities. In this study, the authors also investigated substrate utilization patterns through oxidation of carbon sources in three different depths (0.91 - 1.68 m), whereas metabolic diversity of the microbial community was much higher at a depth of 0.91 m compared with the two deeper waste layers because far more substrates could be oxidised in the shallow waste layer. The authors point out that this might be biased because of the aerobic cultivation procedure, favouring aerobic and facultative anaerobic microorganisms. A culture independent approach showed a different bacterial composition in the depth of 0.91 m and 1.68 m. At 0.91 m the clones could mainly be assigned to the genus *Bacillus*, whereas at 1.68 m the clones could predominantly be assigned to the genus *Clostridium*.

Fibrobacter

The phylum *Fibrobacteres* only contains one genus, namely *Fibrobacter*, which in turn contains two species: *F. succinogenes* and *F. intestinalis*. *Fibrobacter* are often considered to be major cellulose degraders in landfills (McDONALD et al., 2012a; McDONALD et al., 2008; RANSOM-JONES et al., 2012), next to various *Clostridium and Bacillus* species.

McDONALD et al. (2008) used a *Fibrobacter* specific primer-set for quantitative PCR (qPCR) for the detection of the genus *Fibrobacter* spp. in landfill sites. As already mentioned, so far there are two known species: *F. succinogenes* and *F. intestinalis*. Quantitative PCR revealed a varying abundance of *Fibrobacter* spp. from 0.2 % to 40.0 % relative to the total number of *bacteria* in landfill leachate samples indicating that *Fibrobacter* spp. can be considered a significant part of the community of cellulose degraders. The study also gave evidence for the potential existence of novel lineages that can be affiliated to the *Fibrobacter* genus. 58 clone sequences had been analysed with TTGE and only two out of these 58 sequences could be related to already known *Fibrobacter* species, namely *F. succinogenes*. The remaining 56 sequences are considered to represent novel variations of the *Fibrobacter* genus.

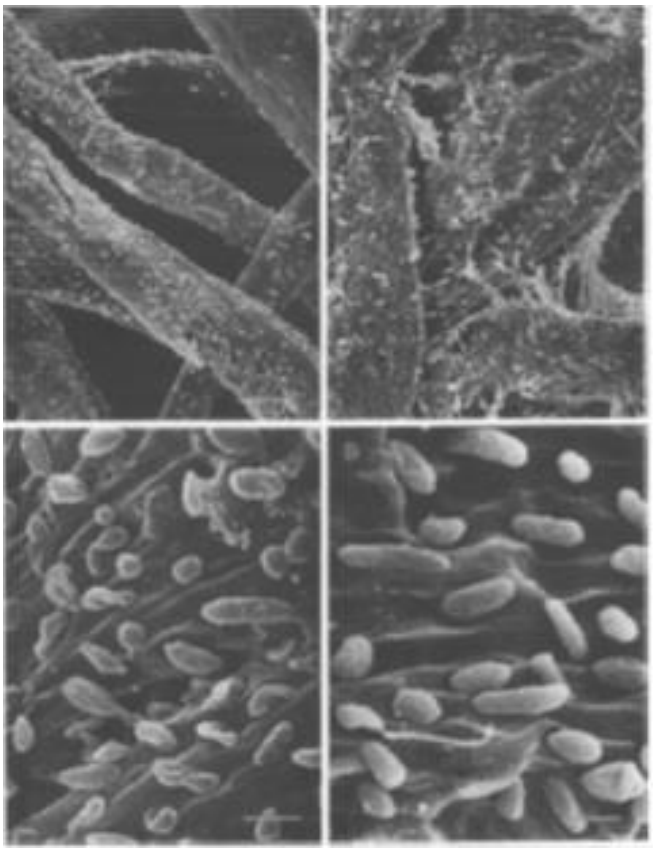

Figure 14: F. succinogenes on cellulose fibers (DONG BAE, 1993)

McDONALD et al. (2012a) detected the genus *Fibrobacter* spp. by applying traditional and nested PCR (see methods chapter 3.5.2) by using a *Fibrobacter*-specific primer set. *Fibrobacter* spp. are known to be able to degrade cellulose under anaerobic conditions and McDONALD et al. (2012a) draw the conclusion that *Fibrobacter* spp. are common degrading microorganisms in active landfills. In their experiment, they examined microbial populations in landfill leachate samples and in two leachate microcosms. *Fibrobacter* spp. could be detected in almost all leachate samples via nested PCR whereas via traditional PCR only in one out of 22 leachate samples *Fibrobacter* spp. could be detected. This was considered as a clear indication that nested PCR increases the chance to detect existing organisms. However, the fact that *Fibrobacter* spp. had been detected in one sample via traditional PCR is a clear evidence for *Fibrobacter* spp. to be abundant in a high number in the areas where this leachate sample had

been taken. One of the two artificially constructed landfill leachate microcosms where they incubated dewaxed cotton strings with landfill leachate showed high cellulose degradation rates caused by *Fibrobacter* spp. and *Clostridium* cluster III (see chapter 3.5.3.1). The second group of microcosms where the abundance of *Fibrobacter* spp. (and *Clostridium* cluster III) was less than 0.1 %, showed no cellulose degradation. The authors concluded that *Fibrobacter* spp. play an important role in cellulose degradation and confirmed the major role of members of the class of *Clostridia*, specifically *Clostridium* cluster III.

A recently published study from RANSOM-JONES et al. (2012) also indicated the importance of the phylum of *Fibrobacteres* for cellulose degradation. Former, there had been two known ways of microbial degradation of cellulolytic material, but RANSOM-JONES et al. (2012) detected a third way which could explain the highly efficient hydrolysis of cellulolytic material under anaerobic conditions.

Proteobacteria

HUANG et al. (2005) investigated the bacterial community in leachate samples of a closed municipal solid waste landfill without leachate recirculation. The phylum *Proteobacteria* showed a relative abundance of 35.5 %, which means that *Proteobacteria* were the second most abundant determined type of *bacteria* only outnumbered by gram+ *bacteria* with low G + C content. Amongst *Proteobacteria*, members of the class *Gammaproteobacteria* were found to be dominant. Further analyses showed similarities to the genera of *Pseudomonas* (*Gammaproteobacteria*) (see Figure 15) and *Dechloromonas* (*Betaproteobacteria*), whereas *Dechloromonas* are known to be able to reduce perchlorate. Within the class *Epsilonproteobacteria*, sequences of the genus *Arcobacter* could be retrieved, which are known for the oxidation of acetate with Mn-oxide. *Deltaproteobacteria*, known to be often present in anaerobic environments, were only present in a very low number.

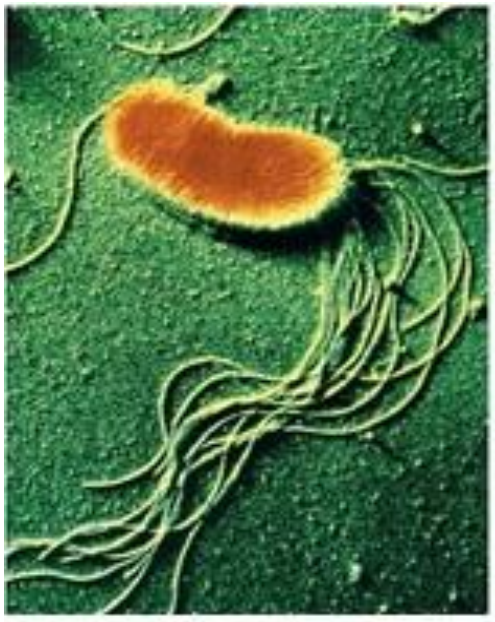

Figure 15: Pseudomonas fluorescens as a representative for the glass of Gamma-Proteobacteria (MICROBIOLOGY GLOSSARY, s.a)

HUANG et al. (2004) investigated a landfill with leachate recirculation were the abundance of *Proteobacteria* was very low (approximately 1 % of the analysed sequences). Due to the fact that the same analysing methods have been used, it is highly impossible that these significant differences could be a result of methodological biases but rather a result of the different microbial community composition present in the landfills.

CARDINALI-REZENDE et al. (2009) detected *Proteobacteria* in an anaerobic digestion reactor operated with organic household waste. Analysis of bacterial clone libraries revealed the presence of all classes of *Proteobacteria,* with *Gammaproteobacteria* being the dominating class. Analysed sequences were related to the following genera: *Providentia, Klebsiella, Schineria, Escherichia* and *Enterobacter*. Representatives of *Betaproteobacteria* were also found in small numbers and it is noteworthy that members of that group are responsible for the fixation of nitrogen and the oxidation of ammonia (BOND et al., 1995).

SAWAMURA et al. (2010) detected *Proteobacteria* while investigating solid samples of a landfill at various depths. The structure of the microbial community was analysed with T-RFLP revealing that different microbial patterns were present at different depths. At a depth level of 17.5 m, the *Gammaproteobacteria* were dominating. Further information about general properties (OM content, moisture) of this study can be found in chapter 3.5.3.1 were the occurrence of *Clostridium* is described.

GOMEZ et al. (2011) investigated a contaminated landfill site that contained phenols, benzene and heavy metals like chromium and cadmium. They applied the methods TTGE and T-RFLP for determining the microbial community structure at different depths (0, 10, 20 and 30 m). Comparing the results of the different depths, a variation of both abundance and diversity could be observed. More complex structures and higher microbial diversity have been found in deeper waste layers, which also correlated with higher organic carbon concentrations at 20 m (11 %) and 30 m (5.9 %) in comparison to 0 m (3 %) and 10 m (4.8 %). Microbial structures were dominated by members of the phylum *Proteobacteria*, with *Alpha-*, *Beta-* and *Gammaproteobacteria* being most abundant. *Acinetobacter* sp. (class *Gammaproteobacteria*) was the most abundant genus amongst those three divisions, followed by the genus *Bacillus* sp. (phylum *Firmicutes*). *Gammaproteobacteria* and members of the phylum *Firmicutes* represented hydrocarbon-degrading microorganisms. Sites contaminated with hydrocarbons are

known to be dominated by members of the phylum *Proteobacteria*, which is also confirmed by other studies (e.g. PÉREZ-LEBLIC et al., 2012). GOMEZ et al. (2011) also stated that once the intensity of the contamination decreases, *Proteobacteria* are replaced by members of the phylum *Actinobacteria*.

The aim of the study of PÉREZ-LEBLIC et al. (2012) was to investigate the influence of contaminants like hydrocarbons, polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB) on microbial activity. The results showed that the highest number of viable *bacteria* and *fungi* as well as high microbiological enzyme activities could be observed in areas with the lowest concentrations of hydrocarbons, PAHs and PCBs. These results were supported by the finding that highly polluted areas showed low diversity of microorganisms and also that enzymatic activity significantly decreased with an increasing concentration of hydrocarbons. Clone libraries based on 16S rRNA revealed the dominating abundance of *Proteobacteria* (followed by *Acidobacteria* and *Actinobacteria*) in both highly contaminated areas and low contaminated areas whereas *Alpha- and Betaproteobacteria* were the most abundant classes in all areas.

According to KRISHNAMURTHI and CHAKRABARTI (2013), young and active landfills do not offer ideal conditions for gram- *bacteria* like *Proteobacteria*. However higher numbers of *Proteobacteria* have been found in an old landfill by HUANG et al. (2005). A possible explanation from KRISHNAMURTHI and CHAKRABARTI (2013) is the fact that members of the phylum *Firmicutes* are responsible for the initial phases of decomposition (like in young and active landfills) and prepare the substrate for following microorganisms like *Proteobacteria* which appear later on in older landfills as has been shown by HUANG et al. (2005).

Porphyromonadaceae

LI et al. (2009) discovered that the family of *Porphyromonadaceae* play a part in glucose fermentation (2nd phase – Acidification). The used method was FISH, which revealed *Porphyromonadaceae* to be abundant (next to members of the genus *Clostridium).* This study also indicated that the family of *Porphyromonadaceae* (phylum *Bacteroidetes*) could be involved in the degradation of cellulose as well.

Pseudomonadaceae

The family of *Pseudomonadaceae* (phylum *Proteobacteria*) was discovered by usage of FISH and they were identified as acetate degraders by LI et al. (2009). The combination of *Pseudomonadaceae* and hydrogenotrophic methanogens (like the strictly hydrogenotrophic genera of *Methanoculleus* or the genera *Methanosarcina*, which is able to use both, acetate or H_2 and CO_2 as substrate – see chapter 3.5.3.3) was a rather surprising finding. The authors suggest that acetate was not used directly as the carbon source but for instance CO_2 was used instead to assimilate carbon. A possible explanation for this finding, was that the acetate oxidation would be carried out by a cooperation between the family *Pseudomonadaceae* and the genera *Methanoculleus* (LI et al., 2009).

3.5.3.2. *Fungi*

LOCKHART et al. (2006) investigated the role of anaerobic gut *fungi* in landfills with the result that members of the order *Neocallimastigales* were considered to contribute to the degradation of cellulolytic material. *Neocallimastigales* are known to play an important role in cellulose degradation in the rumen of mammalian species even though they are outnumbered by *bacteria*. For that reason, LOCKHART et al. (2006) used a sediment/leachate mix in order to investigate anaerobic gut fungi via nested PCR (see chapter 3.5.2) and used specific primers to improve the detection probability in leachate samples that have been taken from a waste disposal containing low-level radioactive waste.

GAUTAM et al. (2011) also studied the role of fungi in landfills and discovered that total colony-forming units (cfu) were correlated with different habitat characteristics like pH (ranging from 5 to 7) and moisture conditions (between 35 and 57 %). High moisture contents provided the best conditions for fungal populations; ideal moisture content for aerobic fungi was determined with 50-75 %. OM, phosphorus (P) and potassium (K) contents were also determined and a positive correlation between those parameters and fungi population could be detected (see Table 14). Amongst others, the following species were obtained from different samples and cultured at large scale: *Trichoderma viride* (see Figure 16), *Aspergillus niger, Aspergillus fumigatus, Curvularia sp.* and *Fusarium sp.* (see Figure 17). They used different growth media for culturing of the fungi, whereas potato dextrose provided growth conditions for *T. viride, A. fumigatus, A. niger* and *Curvularia sp.* The species *Fusarium sp. and Curvularia sp*. were best suited to Czapeck Dox growth medium. Ideal pH ranges for fungal growth were between 7 and 8 and highest growth rates of all 5 species were found at 40-60 °C (see Table 14).

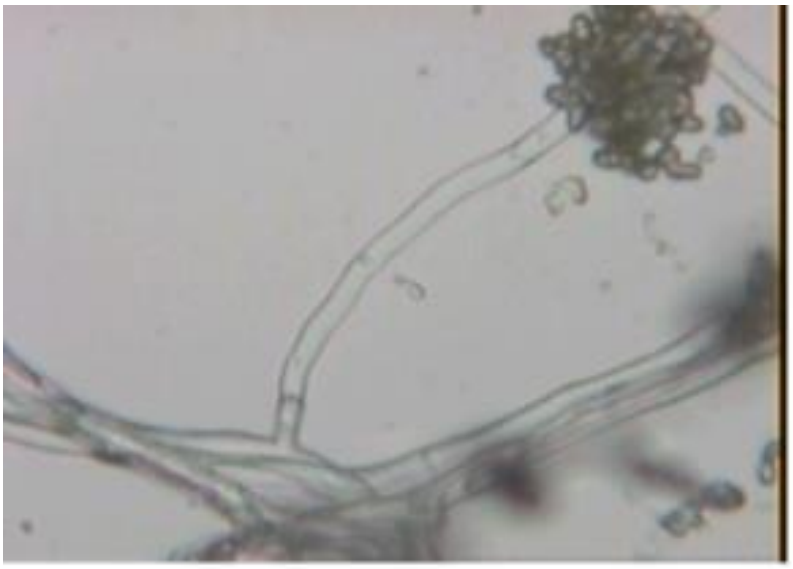

Figure 16: Trichoderma viride (GAUTAM et al., 2011)

Table 14: Important parameters for fungi in landfills (GAUTAM et al., 2011)

Parameter	Correlation	Growth conditions for the selected fungi
pH	Positive correlation	5-7
Moisture [%]	Positive correlation	35-57
OM [%]	Positive correlation	24.2-34.7
P [mg/kg]	Positive correlation	0.12-0.54
K [mg/kg]	Positive correlation	5-9
Cfu (10^{-6}/gm)	--	152-225
Temperature	--	40-60 °C

Volume loss and weight loss of waste were investigated and based on volume and weight loss, *T. viride* was found to be the most effective *fungi* for degradation of waste material (GAUTAM

et al., 2011). The reason for that could be that *T. viride* is known to produce enzymes for the effective degradation of polysaccharides and, according to ZHENG and SHETTY (1998), especially for long chain carbohydrates.

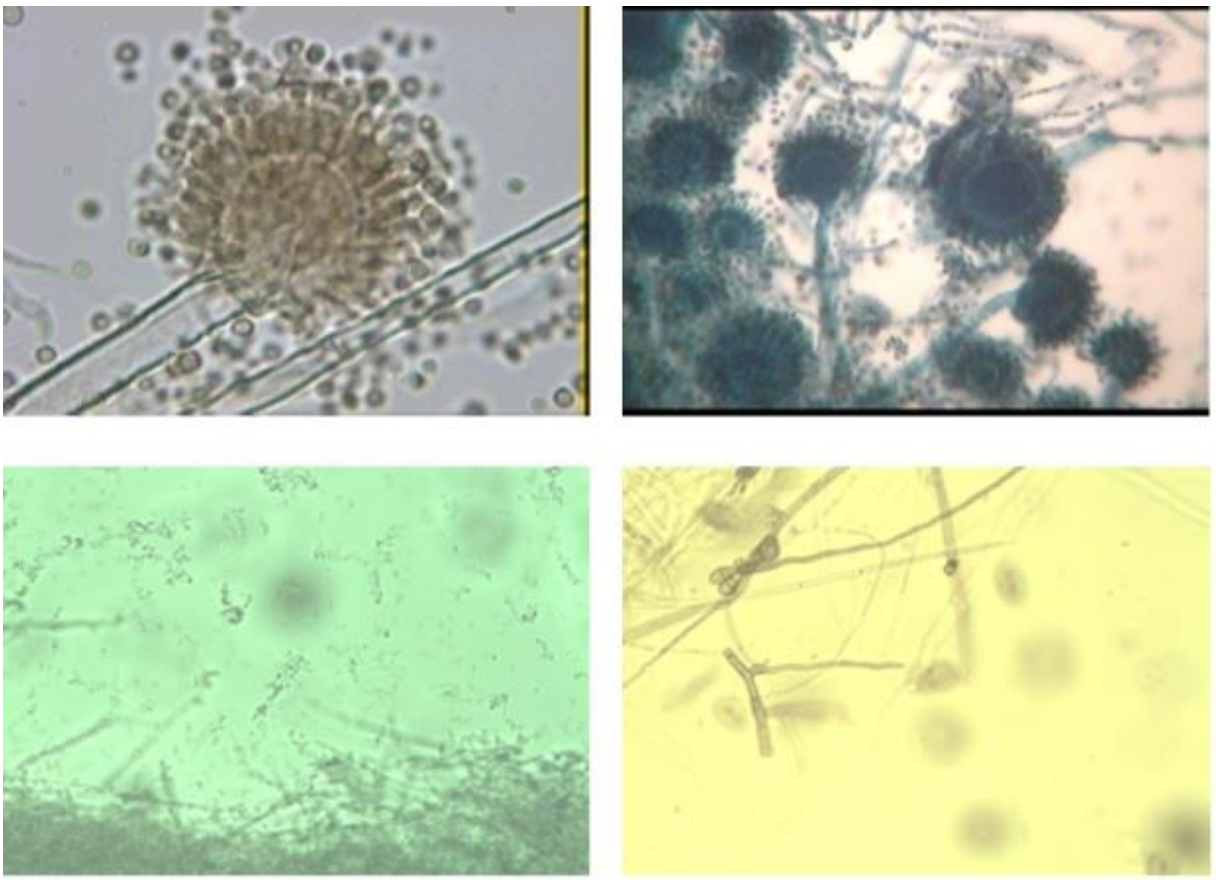

Figure 17: Top left: Aspergillus fumigatus; Top right: Aspergillus niger; Bottom left: Fusarium sp.; Bottom right: Curvularia sp. (GAUTAM et al., 2011)

3.5.3.3. *Archaea*

Various studies have shown that the last step of anaerobic degradation, namely methanogenesis, is mainly performed by members of two methanogenic orders, *Methanomicrobiales* and *Methanosarcinales* (KRISHNAMURTHI and CHAKRABARTI, 2013; LALOUI-CARPENTIER et al., 2006). Most studies recovered the genera *Methanosarcina* and *Methanoculleus* (HUANG et al., 2002; CHEN et al., 2003a and 2003b; UZ et al., 2003). The genus *Methanosaeta* could also be recovered in various studies (e.g. HUANG, 2003), however it has to be mentioned that HUANG et al. (2002) did not retrieve any sequences that could be affiliated with *Methanosaeta* from the full-scale leachate recirculating landfill. The following section is supposed to present a selection of recent studies that examined methanogenic populations in landfills, which are commonly considered to show a limited diversity (LI et al.,

2009; LALOUI-CARPENTIER et al., 2006). The limited diversity can be explained by the limited number of substrates that are used by methanogens and by the fact that methanogens are slow-growing organisms (HUANG et al., 2002). About 1 % of the total number of detected cells could be identified as archaeal cells according to CHEN et al. (2003a) and about 2 % by HUANG et al. (2003, 2005).

Table 15 is supposed to give an overview on the substrate utilization patterns of important methanogenic *archaea*. All of the named genera belong to the phylum Euryarchaeota

Table 15: Important Methanogenic archaea and their substrate utilization patterns (own illustration based upon the literature cited in chapter 3.5.3.3)

Genus	Family	Order	Class	Substrate
Methanobacterium	*Methanobacteriaceae*	*Methanobacteriales*	*Methanobacteria*	$H_2 + CO_2$; Formate
Methanoculleus	*Methanomicrobiaceae*	*Methanomicrobiales*	*Methanomicrobia*	$CO_2 + H_2$; Acetate
Methanofollis	*Methanomicrobiaceae*	*Methanomicrobiales*	*Methanomicrobia*	$H_2 + CO_2$; Formate
Methanosaeta	*Methanosaetaceae*	*Methanosarcinales*	*Methanomicrobia*	Acetate
Methansarcina	*Methanosaetaceae*	*Methanosarcinales*	*Methanomicrobia*	Acetate; $CO_2 + H_2$; Methanol; Methylamine

HUANG et al. (2002, 2003) carried out two studies comparing the structure of methanogenic *archaea* in the leachate of a full-scale recirculating landfill with the structure of methanogens in a closed MSW landfill. In the landfill without recirculation of leachate two archaeal phyla could be detected: *Euryarchaeota* and *Crenarchaeota*. The majority of the analysed clones was related to the orders *Methanomicrobiales* (substrate: H_2 and CO_2) and *Methanosarcinales* (substrate: methyl compounds or acetate). The genera *Methanosaeta* spp. showed high abundance, whereas *Methanosarcina* spp. (both belonging to the order *Methanosarcinales*) showed low abundance. This could be explained by the low concentration of volatile fatty acids in the leachate. *Methanosarcina* would be favoured in systems with a high organic matter input because this would lead to an accumulation of acetate. On the contrary, *Methanosaeta* are favoured in more stable environments, being consistent with the fact that this landfill was considered to be at a stable stage already since this landfill was closed years before investigation. Moreover they detected *Thermoplasma*, being considered ubiquitous in anaerobic

environments. The detected sequences could be distinctly related to *Thermoplasma acidophilum,* which is considered to be thermoacidophilic (HUANG et al., 2003).
In the full-scale recirculated landfill HUANG et al. (2002) retrieved methanogens that could be related to the following genera: *Methanoculleus, Methanocorpusculum, Methanospirillum, Methanogenium* (all belonging to the order of *Methanomicrobiales*) and *Methanosarcina* (order: *Methanosarcinales*). In comparison with the closed landfill where *Methanosaeta spp.* was present at a high level, this genus could not be observed in the landfill with leachate recirculation.
Quite similar to the closed landfill, *Methanomicrobiales* and *Methanosarcinales* were the most abundant orders. The genera *Methanosaeta* and *Methanosarcina* are both known to be able to use acetate as a substrate whereas *Methanosaeta* has a disadvantage over *Methanosarcina* at a high acetate level. The full-scale recirculating reactor was operated with a high organic matter input which in turn led to high concentrations of acetate and hydrogen. This is consistent with the observation that *Methanosarcina* have an advantage in such environments with high acetate concentrations. Therefore, they are considered mainly responsible for acetate and hydrogen removal in that case (HUANG et al., 2002).

CHEN et al. (2003a) investigated solid landfill waste samples at 1.0 m and 3.0 m depth with the aim to gain insight into communities of methanogenic *archaea* and their methane producing potential by using FISH. The sample at 1.0 m depth (see Table 16) showed the highest methane producing potential and DNA sequences isolated at 1.0 m depth were mainly closely related to the genera of *Methanosarcina*, *Methanoculleus* and *Methanobacterium*. The phylogenetic identification of methanogenic archaea through archaeal 16S rDNA cloning analysis was only conducted with the 1.0 m sample since this depth showed the highest methane production activity. The fact that the genus *Methanosaeta*, which uses acetate as a substrate, could only be detected at low frequency in this study is an evidence for higher hydrogenotrophic methanogenic activity through *Methanosarcina*, *Methanoculleus* and *Methanobacterium*. In accordance with investigations of CHEN et al. (2003b), differences in the methanogenic community structure in old and young aged landfill samples could be observed. The genus *Methanosarcina* seems to dominate young aged landfills whereas *Methanosarcina* plays a minor role in older landfills.

Table 16: Selected parameters at different depth (adapted from CHEN et al., 2003a)

	1 m	**3 m**
pH	9.03	8.81
water content [%]	16.35	28.12
Methane production activity [mmol g^{-1} dry wt d^{-1}]	H_2/CO_2: 2.08 CH_3COOH: 0.17	H_2/CO_2: 0.09 CH_3COOH: 0.16

The composition of a*rchaea* at different depths was investigated by CHEN et al. (2003b) whereas samples were taken at 10 m, 20 m and 30 m considering to represent different aged MSW (3 years, 6 years and 9 years). Solid waste samples were taken from the landfill, DNA was extracted and PCR amplification and cloning of archaeal 16S rDNA was conducted before sequencing and phylogenetic analysis. According to the investigation water content was highest at 30 m, whereas pH was slightly lower at the bottom. The 30 m sample was also considered to be most stabilized, since the volatile solids were lowest at this depth. High sulphate concentrations might be an indication for a negative influence on methanogenesis (Table 17).

Table 17: Selected physical and chemical parameters at different depth and corresponding age of MSW (adapted from CHEN et al., 2003b)

	10 m (3 years)	**20 m (6 years)**	**30 m (9 years)**
pH	8.5 ± 0.1	8.5 ± 0.1	8.3 ± 0.1
water content [%]	42.87 ± 2.36	46.83 ± 3.18	50.81 ± 1.21
Volatile solids [%]	36.31 ± 0.06	25.39 ± 4.65	24.43 ± 1.46
Sulphate (SO_4^{2-}-S) [mg g dry $solid^{-1}$]	0.15 ± 0.01	0.14 ± 0.02	0.17 ± 0.06

The methane production activity was also measured at different temperatures (25 °C and 55 °C) and showed no significant difference along with the depth with both CH_3COOH and $H_2 + CO_2$ as substrates (see Table 18). Using H_2 and CO_2 as substrate, methanogenic activity was approximately 3 orders higher at 55 °C than at 25 °C. Using acetate as substrate, the differences between 55 °C and 25 °C were much lower. According to CHEN et al. (2003b) these results were in accordance with the finding that 16S rDNA cloning analysis revealed thermophilic hydrogenotrophic methanogens to be dominating.

Table 18: Methane production activity [µmol g^{-1} dry solid d^{-1}] at different depths with different substrates (CHEN et al., 2003b)

		10 m (3 years)	**20 m (6 years)**	**30 m (9 years)**
$H_2 + CO_2$	55 °C	$6.16 \times 10^{-3} \pm 0.11 \times 10^{-3}$	$5.48 \times 10^{-3} \pm 0.06 \times 10^{-3}$	$5.64 \times 10^{-3} \pm 0.06 \times 10^{-3}$
	25 °C	$3.37 \times 10^{-3} \pm 0.73 \times 10^{-3}$	$2.3 \times 10^{-3} \pm 0.59 \times 10^{-3}$	$2.56 \times 10^{-3} \pm 0.06 \times 10^{-3}$
CH_3COOH	55 °C	$7.2 \times 10^{-3} \pm 0.28 \times 10^{-3}$	$7.9 \times 10^{-3} \pm 1.69 \times 10^{-3}$	$8.2 \times 10^{-3} \pm 1.98 \times 10^{-3}$
	25 °C	$6.99 \times 10^{-3} \pm 0.17 \times 10^{-3}$	$5.8 \times 10^{-3} \pm 1.17 \times 10^{-3}$	$10.59 \times 10^{-3} \pm 1.74 \times 10^{-3}$

Analysis of 16S rDNA sequences showed a very low archaeal diversity which was also detected by various other studies (e.g. CHEN et al., 2003a; HUANG et al., 2003; HUANG et al., 2005). Even though sample depth and thus sample age differed, the archaeal structure showed a high degree of similarity and the community structure seemed to be quite stable over the time period from 3 to 9 years. However, the authors suggested that significant structure changes might happen during earlier stages, shortly after waste burial. Detected methanogenic genera were *Methanosarcina*, *Methanoculleus*, *Methanosaeta* and *Methanothermobacter*. The most abundant clones were closely related to thermophilic, hydrogenotrophic methanogens like *Methanothermobacter thermautotrophicus* but also similarities to e.g. *Methanosarcina thermophila* could be detected, being thermophilic as well. Since relatively high temperatures (40-50 °C) were measured, the authors concluded that thermophilic species are favoured. The observed high temperatures might also be the reason for the rather low archaeal diversity which is in accordance with other studies drawing the conclusion that high temperatures might be linked to low microbial diversity (Chin et al., 1999; Lapara et al., 2000).

UZ et al. (2003) investigated methanogenic *archaea* in a MSW landfill by taking samples of two different areas inside a landfill with varying age. Younger Samples showed acetoclastic, hydrogenotrophic and formate-using methanogens whereas older samples only showed hydrogenotrophic and formate-using methanogens. Sequence analysis showed relationships to the following genera: *Methanoculleus, Methanofollis, Methanosaeta* and *Methanosarcina*.

BURRELL et al. (2004) detected members of the family *Methanosaetaceae* (Order: *Methanosarcinales*) in a landfill leachate bioreactor, whereas most of the detected archaeal sequences belonged to the genus *Methanosaeta* which was determined by FISH. *Methanosaeta* is known to use acetate as a substrate.

LALOUI-CARPENTIER et al. (2006) examined the diversity and activity of methanogenic *archaea* in leachate samples of a MSW landfill. The majority of the analysed sequences belonged to the order *Methanosarcinales* whereas almost two third of the clones were affiliated to the family *Methanosaetaceae* which is known to solely use acetate as substrate. About 18 % of the clones were related to the family *Methanosarcinaceae* which is known to be able to use various substrates quite in contrary to the strictly acetoclastic *Methanosaetaceae.* Furthermore

a few members of the order *Methanomicrobiales* (substrate H_2 + CO_2) could be retrieved, such as the genera *Methanoculleus* and *Methanofollis.*

WEISS et al. (2008) investigated microorganisms in a thermophilic municipal biogas plant and detected the following archaeal methanogens: the hydrogenotrophic *Methanobrevibacter sp.*, the acetogenotrophic *Methanoculleus bourgensis* as well as the methylotrophic *Methanosphaera stadtmanae* and *Methanimicrococcus blatticola* and some uncultured *Methanomicrobiales*.

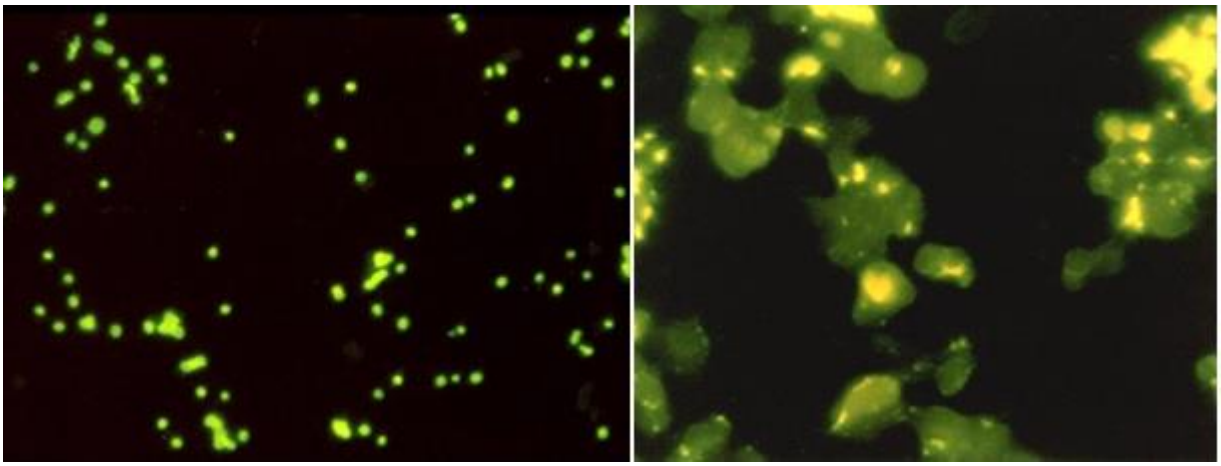

Figure 18: left: Methanoculleus marisnigri; right: Methanosarcina mazei (DE MACARIO AND MACARIO, s.a.)

LI et al. (2009) detected sequences which could be linked to the genera of *Methanoculleus* (Order: *Methanomicrobiales*) and *Methanosarcina* (Order: *Methanosarcinales*) under mesophilic conditions. Certain sequences showed relatively high identity with the species of *Methanoculleus marisnigri*, (see Figure 18) *Methanoculleus bourgensis* and *Methanosarcina mazei*. *Methanoculleus* is known to be hydrogenotrophic, which means that this genus is only able to use CO_2 and H_2 as substrate. The second detected genus, *Methanosarcina*, is able to perform Methanogenesis either by using acetate (acetoclastic) or by using CO_2 and H_2 as a substrate.

CARDINALI-REZENDE et al. (2009) set up a waste reactor loaded with organic household waste, where (landfill) leachate was being used as a start-up inoculum. The leachate was collected from an anaerobic organic household waste reactor after 90 days of treatment. They detected two archaeal orders, namely *Methanomicrobiales* and *Methanobacteriales*, both belonging to the group of hydrogenotrophic methanogens. All of the sequences affiliated to

Methanomicrobiales showed a relationship to the genus *Methanoculleus*. Sequences of the order *Methanobacteriales* were related to the genera *Methanosphaera* and *Methanobacterium*. Acetoclastic *Methanosaeta* and *Methanosarcina,* often detected in anaerobic environments as being responsible for methanogenesis (e.g. UZ et al., 2003; CHEN et al., 2003b), were not detected at all in this study. The reason for the lack of acetoclastic methanogens might be the accumulation of ammonium and VFA (CARDINALI-REZENDE et al., 2009).

KRISHNAMURTHI and CHAKRABARTI (2013) also found the two rather common orders *Methanosarcinales* (the clones showed sequence similarities with the genus *Methanosarcina*) and *Methanomicrobiales* (the clones showed sequence similarities with the species *Methanoculleus bourgensis)* in solid samples of a MSW landfill. Similar to HUANG et al. (2003), they detected the thermoacidophilic *Thermoplasma acidophilum* whereas the role of this organisms in the anaerobic environment is still unknown. The genus *Methanosaeta* was not detected, like in the study from HUANG et al. (2002) in a landfill with leachate recirculation. It has to be mentioned that various other studies, like HUANG et al. (2003) or LALOUI-CARPENTIER et al. (2006), who investigated older landfills, confirmed the presence of *Methanosaeta*. One possible explanation might be the age of the landfill, because *Methanosaeta* are known to prefer environments with a low acetate concentration which is typically found in older or closed landfills.

The authors confirmed existing knowledge in this study when they found *Methanosarcina* in the upper waste layer samples (0.91 m depth) and *Methanoculleus* in the deeper waste layer samples (1.68 m). *Methanosarcina* are known to use acetate as substrate which is present in higher amounts in the upper waste layers, whereas *Methanoculleus* are known to be strictly hydrogenotrophic (substrate: H_2 and CO_2) and was therefore present in the deeper waste layers where lower acetate levels could be observed.

Table 19 shows important features (leachate recirculation, waste age, sampling depth) related to methanogens that were found in the discussed studies.

Table 19: Important features related to the methanogens found in landfills (own illustration based upon the literature cited above)

Feature	Orders	Genera	Interpretation	Source
Full scale recirculating landfill	*Methanosarcinales*, *Methanomicrobiales*	*Methanosarcina* (amongst others)	High acetate level → advantage for *Methanosarcina*; no detection of *Methanosaeta*	HUANG et al., 2002
Old, closed landfill	*Methanosarcinales*, *Methanomicrobiales*	High abundance of *Methanosaeta*	Low concentration of volatile fatty acids (e.g. acetate); stable environment; no more input of organic matter	HUANG et al., 2003 KRISHNAMURTHI and CHAKRABARTI, 2013
Top layers, young material	*Methanosarcinales*, *Methanomicrobiales*, *Methanobacteriales*	*Methanosarcina*, *Methanoculleus*, *Methanobacterium*	High CH_4 production activity (2.08 mmol g^{-1} dry weight d^{-1}); higher amount of acetate in the upper waste layers; low abundance of *Methanosaeta*; domination of *Methanosarcina* in young landfills; acetoclastic, hydrogenotrophic and formate-using methanogens	CHEN et al., 2003a; UZ et al., 2003 KRISHNAMURTHI and CHAKRABARTI, 2013
Deep waste layers	*Methanomicrobiales*	*Methanoculleus*	Lower acetate levels	KRISHNAMURTHI and CHAKRABARTI, 2013
Investigation of different depths (3m, 6m, 9m)	*Methanosarcinales*, *Methanomicrobiales*, *Methanobacteriales*	*Methanosarcina*, *Methanoculleus*, *Methanosaeta*, *Methanothermobacter*	Stable community structure and high similarities of archaeal structure for all depths; structure changes might have happened shortly after waste burial; high abundance of thermophilic, hydrogenotrophic methanogens	CHEN et al., 2003b

Table 20 is supposed to summarize the above explained studies and to provide the reader with an overview on the detected archaeal genera.

Table 20: Overview on methanogenic archaea in landfills (own illustration based upon the literature cited above)

Author	Year of Publication	Genera
HUANG et al.	2002	With leachate recirculation: Methanoculleus, Methanocorpusculum, Methanospirillum, Methanogenium, Methanosarcina
HUANG et al.	2003	Without leachate recirculation: Methanosaeta, Thermoplasma
CHEN et al.	2003a	Methanosarcina, Methanoculleus, Methanobacterium
CHEN et al.	2003b	Methanosarcina, Methanoculleus, Methanosaeta, Methanothermobacter
UZ et al.	2003	Methanoculleus, Methanofollis, Methanosaeta, Methanosarcina
BURRELL et al.	2004	Methanosarcina
LALOUI-CARPENTER et al.	2006	Methanoculleus, Methanofollis
WEISS et al.	2008	Methanobrevibacter, Methanoculleus, Methanosphaera, Methanomicrococcus
LI et al.	2009	Methanoculleus, Methanosarcina
CARDINALI-REZENDE et al.	2009	Methanoculleus, Methanosphaera, Methanobacterium
KRISHNAMURTHI and CHAKRABARTI	2013	Thermoplama acidophilum, Upper waste layers: Methanosarcina Deeper waste layers: Methanoculleus

3.5.3.4. Methanotrophic *bacteria*

For the sake of completeness, I also want to mention the existence and importance of methanotrophic *bacteria* (MOB). Their ability to oxidise the produced CH_4 to CO_2, vastly diminishing greenhouse gas potential (US EPA, 2014b), is often used in landfill cover soils to reduce the CH_4 emissions of landfills (SPOKAS and BOGNER, 2011; CÉBRON et al., 2007). On the contrary to the methane producing, strictly anaerobic methanogens (see 3.5.3.3), which belong to the domain of *archaea* (see 3.2.1), methane-oxidizing *bacteria* are aerobic and belong to the domain of *bacteria* (HENNEBERGER et al., 2013). Methane is their only carbon and energy source (see 3.2.2) and they are situated not only in landfill cover soils, but also in other environments being potential sources of CH_4 like wetlands or rice paddies (HENNEBERGER et al., 2013). Landfill soil covers are a traditional method of a microbially mediated reduction of CH_4 emissions. However, as researchers became aware of the potential of landfill soils covers, the method has been developed further. For instance, the use of organic rich soils increases the rate of methane oxidation (SADASIVAM and REDDY, 2014). Still, there are

promising but yet not well enough developed approaches for further improvements of landfill soil covers (CAO and STASZEWSKA, 2013). MOB can be divided into type I and type II methanotrophs. The typology is primarily based on different carbon assimilation pathways and different intracellular membrane typology (BODROSSY et al., 1997). The type I group comprises members of the family of *Methylococcaceae* (BOWMAN, 2000 cited STRALIS-PAVESE et al., 2006): *Methylomonas, Methylobacter, Methylomicrobium; Methylosarcina, Methylosphaera, Methylococcus* (BOWMAN et al., 1993, 1995 and 1997 cited WISE et al, 2001), *Methylocaldum* (BODROSSY et al., 1997) and *Methylothermus* (BODROSSY et al., 1999), all belonging to *Gammaproteobacteria* (STRALIS-PAVESE et al., 2006). The type II group comprises members of the family *Methylocystaceae* (BOWMAN, 2000 cited STRALIS-PAVESE et al., 2006), namely *Methylosinus, Methylocystis* (BODROSSY et al., 1999), *Methylocapsa* (DEDYSH et al., 2002) and *Methylocella* (DEDYSH et al., 2000), all belonging to *Alphaproteobacteria* (STRALIS-PAVESE et al., 2006).

3.5.4. Ecology of common Microorganisms in Landfills

The following chapter summarizes important ecological requirements of common microorganisms found in landfills (see Table 21). At the end of this chapter, the results of a study conducted by BÄUMLER and KÖGEL-KNABNER, where landfill parameters like temperature, pH and moisture were investigated, are indicated. Table 23 in chapter 4 compares the optimum conditions for microorganisms with actual conditions found in landfills.

Ecology of *Clostridia*

Clostridia are known to be a very heterogeneous and diverse class of *bacteria*, belonging to the phylum *Firmicutes* (BAHL and DÜRRE, 2001; VAN DYKE and McCARTHY, 2002). But they all share two common characteristics: they are anaerobic and gram+. Even though *Clostridia* are considered to be anaerobic organisms, some *Clostridia* are known to be tolerant to oxygen to some extent. The pH optimum is mainly ranging from 6.5 to 7.0; however some *Clostridia* are able to survive under higher or lower pH values. The pH can heavily affect the capability of metabolizing sugars. The temperature optimum is very wide, ranging from psychrophilic to thermophilic (see Table 5). *Clostridia* are able to use a wide range of substrates like sugars, organic acids, alcohols, amino acids, amines, purines and pyrimidines. A wide

range of carbohydrates (see Table 6) can be used by *Clostridia* – monosaccharides as well as large polymers (e.g. cellulose, xylan, starch) (BAHL and DÜRRE, 2001).

Ecology of *Fibrobacter*

Researching the ecology of the phylum *Fibrobacteres*, I mainly came across studies performed on the herbivore gut (e.g. ATASOGLU et al., 2001; MONTGOMERY et al., 1988). As both landfills and gut systems are widely comparable (BURRELL et al., 2004) due to the fact that in landfills as well as in gut systems cellulose degradation takes place under anaerobic conditions (LOCKHART et al., 2006), I included the outcomes of those gut studies into my thesis as well. The phylum *Fibrobacteres* only comprises two cultivable species, namely *F. succinogenes* and *F. intestinalis* (RANSOM-JONES et al., 2012). Ever since the first isolation of *F. succinogenes* from the bovine rumen by Hungate in 1940, *Fibrobacter* are known to be cellulolytic organisms, contributing to cellulose degradation in gut systems (HUNGATE, 1947 cited by RANSOM-JONES et al., 2012). Both, *F. succinogenes* and *F. intestinalis* are gram-, obligate anaerobes and show the capability to degrade lignocellulosic material (MONTGOMERY et al., 1988). *Fibrobacteres* are able to use the following substrates (amongst others) for fermentation: xylan (MIRON and BEN-GHEDALIA, 1993), glucose, cellobiose and cellulose (MONTGOMERY et al., 1988). *Fibrobacteres* are known to degrade cellulolytic material to succinic acid as the main product of the fermentation process, next to acetic acid (McDONALD et al., 2008; MONTGOMERY et al., 1988). Nitrogen sources can be formic acid, NH_3 (MONTGOMERY et al., 1988), peptides and amino acids (LING and ARMSTEAD, 1995). Data from a study investigating the degradation of lignocellulosic feed stock material by *F. succinogenes* suggest ideal fermentation temperatures of 39 °C and ideal pH conditions of 6 (MUTALIK et al., 2012).

Ecology of *Proteobacteria*

The gram- *Proteobacteria* represent the biggest phylum within the bacterial domain with a very extensive ecological range from chemoorganotrophic, phototrophic and chemolithotrophic species (see Table 5) with a wide dispersion of temperature and pH optimums. Also disease-causing agents (like *Pseudomonas*) belong to the phylum *Proteobacteria*. Proteobacteria can be divided into 5 classes – *Alpha-*, *Beta-*, *Gamma-*, *Delta-* and *Epsilonproteobacteria* (MADIGAN and MARTINKO, 2009). For instance *Pseudomonas* (*Gammaproteobacteria*), detected in a landfill by HUANG et al. (2005), are not very demanding regarding nutrients, they

are chemoorganotrophic and they need pH values around 7 and mesophilic temperatures (20 to 45 °C) (MADIGAN and MARTINKO, 2009).

Ecology of methanogenic *Archaea*

Methanogenic *archaea* are strictly anaerobic, meaning that they are living under anoxic conditions present in sediments, paddy fields or swamps. The end product of their metabolism is CH_4 and CO_2, whereas approximately 60 % are accounted for CH_4 and 40 % are accounted for CO_2. Methanogenic *archaea* are a huge and varied group. They belong to the phylum of *Euryarchaeota* and show a very specific metabolism which is unique amongst prokaryotes (FRITSCHE, 2002). On the basis of physiological characteristics and 16S rRNA-sequences, methanogens can be divided into the following classes according to EUZÉBY and PARTE (s.a.): *Methanobacteria, Methanococci, Methanomicrobia, Methanopyri.*

Substrate range: The substrate range of methanogens is rather tight and limited to C1-compounds (formate, CO, methanol, formaldehyde, methylamine) and only one C2-compound, namely acetate (see acetotrophic methanogens) (FUCHS, 2007). C1-compounds play quantitatively a minor role (CYPIONKA, 2010). A lot of species have the ability to grow autotrophic with H_2 and CO_2 (see hydrogenotrophic methanogens) (FUCHS, 2007).

Salinity: Methanogenic a*rchaea* are able to survive in hypersaline water as well as in freshwater. Freshwater and marine methanogens are much more frequent than extremely halophilic ones. All the halophilic *archaea* belong to the family *Methanosarcinaceae* (ZINDER, 1993).

Temperature: The temperature range of methanogens is very wide – they can be found in marine sediments with average temperatures around 2 °C as well as in geothermal areas with around 100 °C. Both psychrophilic and thermophilic (see Table 5) *archaea* are characterized by a great diversity. In general thermophilic *archaea* show a higher growth rate than mesophilic ones (ZINDER, 1993).

pH: Most methanogens show a pH optima around 7 (neutrality) (JONES et al., 1987). Some species also survive at pH values around 4, whereas other species have their optimum at a pH level of 8 or 9 (ZINDER, 1993).

Oxygen: Methanogens are considered to be among the strictest anaerobic organisms. However, some can also be tolerant to O_2 exposure and they are able to adapt to O_2 conditions without being able to produce CH_4 under such conditions (ZINDER, 1993).

Table 21: Environmental requirements of common microorganisms in landfills (own illustration based upon the indicated literature)

Parameter	Optimum conditions for the concerned MO		Source
	Genus	**Optimum conditions**	
Temperature	*Clostridium*	Wide range from psychrophilic to thermophilic	BAHL and DÜRRE, 2001
	Methanogens	Wide range from psychrophilic to thermophilic	ZINDER, 1993
	Trichoderma, Aspergillus, Curvularia, Fusarium	50-60 °C	GAUTAM et al., 2011
	Fibrobacter	30 °C	MUTALIK et al., 2012
	Proteobacteria	Mesophilic temperatures	MADIGAN and MARTINKO
Moisture content	*Methanogens*	Between 500 and 600 g*kg^{-1} waste	LOPEZ ZAVALA and FUNAMIZU, 2005; KELLY, 2006
pH-value	*Methanogens*	Around 7.0, but able to survive under extreme conditions	JONES et al., 1987; ZINDER, 1993
	Trichoderma, Aspergillus, Curvularia, Fusarium	Between 7.0-8.0	GAUTAM et al., 2011
	Fibrobacter	Around 6.0	MUTALIK et al., 2012
	Proteobacteria	Around 7.0	MADIGAN and MARTINKO, 2009

Typical physical and chemical parameters in landfills

BÄUMLER and KÖGEL-KNABNER (2008) characterized solid waste organic matter with spectroscopy and wet chemical analysis methods and they investigated parameters like temperature, moisture and pH found in landfills. Increasing temperatures were measured with increasing depth. In the first 2 meters average temperatures of less than 20 °C were measured. The properties of this shallow part of the landfill body were considered independent of waste age and deposition time but stronger influenced by climatic conditions. With increasing depth, waste age increasingly influenced temperature conditions. Young waste showed higher temperatures that could rise up to 50 °C. This temperature range was ideal for thermophilic microorganisms (see Table 5). Old waste tends to show lower temperatures, which might be caused by a decreasing microbial activity.

Ideal moisture conditions for methanogenesis are between 500 and 600 g per kg waste (LOPEZ ZAVALA and FUNAMIZU, 2005; KELLY, 2006). BANK (2000) indicate a water content of the landfill body of at least 40 % to guarantee ideal anaerobic processes. At water contents

lower than approximately 30 % anaerobic degradation is inhibited and below 15 %, degradation process comes to a halt. However, mean values are often far below the indicated optimum values, indicating that moisture is the limiting factor of biodegradation (LOPEZ ZAVALA and FUNAMIZU, 2005). Ideal moisture conditions necessary for microbial decay are linked to the oxygen requirements of microorganisms, resulting in a trade-off of moisture and oxygen content (HAUG, 1993 cited MADEJON et al., 2002). Mean pH values varied in the study of BÄUMLER and KÖGEL-KNABNER (2008) between 7.1 and 8.4 which are considered to be typical for methanogenesis.

Data about moisture demands of specific MO were hard to obtain, so I needed to rely on general data based upon data of FUCHS (2007) stating that *bacteria* need a rather high water activity which was also supported by BANK (2000) indicating an optimum water activity of at least 0.94 which equals a relative humidity of 94 %.

4. Discussion

The chapters 3.5.1 and 3.5.3 were supposed to provide the reader with an overall picture of the microorganisms that were retrieved in several selected scientific studies by various authors. I have deliberately chosen to focus on scientific articles that were published in the recent years from 2002 to 2015 because I aimed at drawing a picture of the present state of knowledge. Furthermore, the amount of published studies in the field of microbiology of landfills has drastically increased only within the last few years in connection with the methodological advance in microbial ecology.

As already mentioned in chapter 1, the thesis emphasis mainly on studies that examined solid or leachate samples retrieved from full-scale landfills in order to reflect actual landfill conditions. Artificially constructed landfill conditions reconstructed in the laboratory – also referred to as anaerobic digesters – are often considered not to represent complex full-scale landfills (PALMISANO, 1993). However, after the evaluation of the discussed scientific literature, the lack of scientific studies investigating full-scale landfills became obvious. According to HUANG et al. (2003), size, complexity and heterogeneity of landfills make it highly difficult to obtain representative information about the ecology of microbial communities. The authors also stated that even though landfills and anaerobic digesters are similar, comparison of these two environments is not as simple as one might think since landfills are mainly operated without any control mechanisms (see chapter 1) unlike anaerobic digesters, which are operated under controlled conditions. Five studies that reconstructed landfill conditions in the laboratory were also included in the thesis (see Table 11 and Table 12).

Referring to chapter 1, this thesis is supposed to answer and discuss the following questions:

(1) Which microorganisms are considered to be responsible for degradation of MSW?

(2) Which conditions are considered the eco-physiological optimum for degrading microorganisms and which conditions can actually be found in landfills?

(3) Which methods are used to investigate microorganisms in landfills? Which methods should be increasingly used for improving our understanding of microbial degradation processes in landfills?

Based on the results of chapter 3.5, the above mentioned questions will be discussed in this particular chapter.

4.1. Degrading Microorganisms

The majority of the articles covered the genera *Clostridium* and methanogenic *archaea* (e.g. VAN DYKE and McCARTHY, 2002; HUANG et al., 2002 and 2003). This might be due to the fact that the genera *Clostridium* as well as methanogenic *archaea* are quite well known, which is for instance proven by the number of known species (204 *Clostridium* species and 188 methanogenic archaeal species) described in the "List of Prokaryotic names with Standing in Nomenclature" by EUZÉBY and PARTE (s.a.). Furthermore they are well represented in clone libraries according to various studies cited above. Moreover the phylum *Proteobacteria* (e.g. HUANG et al., 2005) and the genus *Fibrobacter* (McDONALD et al., 2012a; McDONALD et al., 2008) were retrieved by various authors and are considered to be important members of the microbial community in landfills.

Anaerobic, gram+ *Clostridia* spp. appeared in the majority of the studies which might be a consequence of their wide ecological range in terms of pH and temperature (BAHL and DÜRRE, 2001). *Clostridia* spp. are considered to be the most important actors in cellulose-degradation and hydrolysis (1st phase) (McDONALD et al., 2012a) and they also play an important role in glucose fermentation (2nd phase) (BURRELL et al., 2004; LI et al., 2009).

Proteobacteria, a phylum with a very broad ecological range, were also detected by various studies (e.g. LI et al., 2009; SAWAMURA et al., 2010; GOMEZ et al., 2011; CARDINALI-REZENDE et al., 2009; PÉREZ-LEBLIC et al., 2012) and *Gammaproteobacteria* were found to be the most dominant class (e.g. HUANG et al., 2005; CARDINALI-REZENDE et al., 2009). HUANG et al. (2004 and 2005) showed that *Proteobacteria* are very abundant in landfills without leachate recirculation, whereas the class of *Gammaproteobacteria* was again dominating. On the contrary, in landfills with leachate recirculation, *Proteobacteria* are not very frequent (HUANG et al., 2004). The authors stated that this phenomenon is probably a result of the different microbial community composition present in the landfills. LI et al. (2009) detected *Proteobacteria* – more specifically the family *Pseudomonadaceae* – to play an important role in acetate degradation. GOMEZ et al. (2011) and PÉREZ-LEBLIC et al. (2012) both retrieved *Proteobacteria* from contaminated landfills.

Methanogenic *archaea* (4th phase) present in the vast majority of the reviewed scientific studies (see Table 11), whereas the genera *Methanoculleus* (Order: *Methanomicrobiales*) and *Methanosarcina (Order: Methanosarcinales),* followed by *Methanosaeta* (Order: *Methanosarcinales*) were discovered the most (see Figure 19). Twelve studies determining the archaeal community on genus level and *Methanoculleus* could be retrieved in all of these studies; *Methanosarcina* could be retrieved in three-quarters of the reviewed studies. Several genera, like *Methanothermobacter* or *Methanogenium* could only be retrieved once and are thus not included in Figure 19.

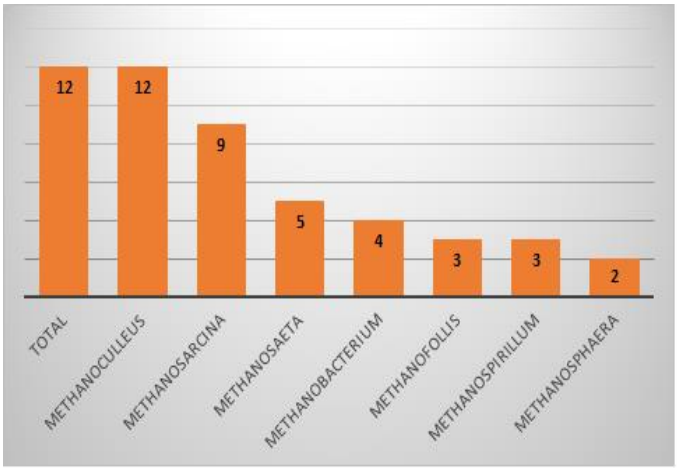

Figure 19: Number of occurrence of methanogenic genera in the reviewed studies (own illustration)

Interestingly, no scientific article specifically addressed or named members of the 3rd phase (acetogenesis), where organic acids and ethanol are turned into H_2, CO_2 and acetic acid. According to DRAKE et al. (2008), 22 acetogenic genera are known, such as *Acetobacterium, Clostridium, Eubacterium, Ruminococcus, Sporomusa* or *Syntrophococcus*. However, most papers did not specifically allocate the retrieved microorganism to the phase they appear in or they did not indicate any details about substrate utilization patterns. Therefore, retrieved microorganism might as well appear in the 3rd phase.

4.2. Comparison of Ecological Requirements and actual Conditions in Landfills

Anaerobic, gram+ *Clostridia* spp. appeared in the majority of the studies which might be a consequence of their wide ecological range in terms of pH and temperature (BAHL and DÜRRE, 2001). In the reviewed studies, the genus *Clostridium* mainly occurred in mesophilic environments (see Table 5 as well as VAN DYKE and McCARTHY, 2002; McDONALDS et al., 2012a; LI et al., 2009 and CARDINALI-REZENDE et al., 2009). WEISS et al. (2008) conducted a study about a thermophilic municipal biogas plant which was operated at 55 °C and they also retrieved *Clostridium*. For instance VAN DYKE and McCARTHY (2002) retrieved *C. thermocellum* from a landfill. *C. thermocellum* is known to be thermophilic, anaerobic and able to degrade lignocellulosic material into ethanol (CHINN et al., 2008). The temperature conditions under which VAN DYKE and McCARTHY (2002) detected *C. thermocellum* (20-45 °C – see Table 5 on page 13) are therefore in accordance with the optimum conditions of *C. thermocellum.* Unfortunately, the majority of the studies did not indicate temperatures, which would support the interpretation of ecological aspects of the respective microbes. *Clostridia* spp. are capable of using a broad spectrum of substrates (e.g. cellulose, sugars, organic acids, amino acids) (BAHL and DÜRRE, 2001) which also contributes to their ubiquitous abundance. LI et al. (2009) named *Clostridium* as Cellulose hydrolysers (1st phase) and as Glucose fermenters (2nd phase).

Several studies (LI et al., 2009; LALOUI-CARPENTIER et al., 2006) stated that methanogenic a*rchaea* only show limited diversity since they can only utilize a few substrates – unlike *Clostridia* – and they are classified as slow-growing organisms (see Table 15; HUANG et al., 2002). Similar to *Clostridia*, they also show a very broad temperature range – from mesophilic to thermophilic – which is in accordance with the reviewed studies. Methanogenic *archaea* were retrieved under mesophilic conditions (LI et al., 2009; CHEN et al., 2003b; CARDINALI-REZENDE et al., 2009 and LÉVEN et al., 2007) as well as under thermophilic conditions (CHEN et al., 2003b; WEISS et al., 2008 and LÉVEN et al., 2007). Again, the lack of information about temperature at the time of sampling was apparent. Table 22 compares optimum pH values indicated by JONES et al. (1987) and ZINDER (1993) (see also 3.5.4) with actual pH values found in landfills.

Table 22: Comparison of actual pH values and optimum pH values of methanogenic archaea (own illustration based upon the literature cited above)

Study	Retrieved genera	Actual pH-values	Optimum
CHEN et al., 2003a	*Methanosarcina*, *Methanoculleus*, *Methanobacterium*	8.81 (3 m below surface) 9.03 (1 m below surface)	Around 7.0 (JONES et al., 1987) Around 8 or 9 (ZINDER, 1993)
CHEN et al., 2003b	*Methanothermobacter*, *Methanosarcina*, *Methanosaeta*, *Methanoculleus*	8.3 ± 0.1 (30 m below surface) 8.5 ± 0.1 (20 m below surface) 8.5 ± 0.1 (10 m below surface)	
CARDINALI-REZENDE et al., 2009	*Methanoculleus*, *Methanosphaera*, *Methanobacterium*, *Methanofollis*	Between 6.5 and 7.5	
HUANG et al., 2003	*Methanoculleus*, *Methanospirillum*, *Methanobacterium*, *Methanosaeta*, *Thermoplasma*	8.2 ± 0.2	
HUANG et al., 2002	*Methanoculleus*, *Methanocorpusculum*, *Methanospirillum*, *Methanogenium*, *Methanosarcina*	Between 6.8 ± 0.5 and 8.6 ± 0.4	
LALOUI-CARPENTIER et al., 2006	*Methanoculleus*, *Methanofollis*, *Methanosarcina*, *Methanosaeta*	Between 7.5 and 8.0	

As shown in Table 22, the optimum pH values are in accordance with the pH values at the time of sampling indicated in the reviewed studies.

Comparing the ecological optima of *Proteobacteria* with the actual conditions in landfills, the broad ecological spectrum of *Proteobacteria* can be confirmed. However, the above mentioned lack of information about pH, temperature and moisture complicates analysis of ideal ecological conditions.

Table 23 provides an overview on the actual conditions found in landfills compared with the optimum conditions of microorganisms that had been retrieved from the landfills (see also 3.5.4).

Table 23: Comparison of actual conditions found in landfills and the optimum conditions for microorganisms

Parameter	Actual conditions found in landfills	Optimum conditions for the concerned MO		Source	
		Genus	Optimum conditions	Actual conditions found in landfills	Optimum conditions
Temperature	< 2m depth: <20 °C			BÄUMLER and KÖGEL-KNABNER, 2008	
	> 2m depth: in young landfills up to 50 °C				
	> 2m depth: in old landfills < 30 °C				
	Mesophilic (20-45 °C)	*Clostridium*	Wide range from psychrophilic to thermophilic	VAN DYKE and McCARTHY, 2002; LI et al., 2009	BAHL AND DÜRRE, 2001
	Average room temperature			McDONALD et al., 2012	
	Thermophilic (45-70 °C)	Methanogens	Wide range from psychrophilic to thermophilic	CHEN et al., 2003b	ZINDER, 1993
	40-60 °C	*Trichoderma*, *Aspergillus*, *Curvularia*, *Fusarium*	50-60 °C	GAUTAM et al., 2011	GAUTAM et al., 2011
	30 °C	*Clostridium*	Wide range from psychrophilic to thermophilic	KRISHNAMURTHI and CHAKRABARTI, 2013	BAHL and DÜRRE, 2001
Moisture content	Between 60 and 420 g*kg^{-1} waste		Between 500 to 600 g*kg^{-1} waste for methanogenesis	LOPEZ ZAVALA and FUNAMIZU, 2005; KELLY, 2006	
	28.12 %	Methanogens	Between 500 to 600 g*kg^{-1} waste for methanogenesis	CHEN et al., 2003a	LOPEZ ZAVALA and FUNAMIZU, 2005; KELLY, 2006
	16.35 %				
	Between 5 and 50 %	*Clostridium*		SAWAMURA et al., 2010	
	34.2 % (0 m)	*Proteobacteria*		GOMEZ et al., 2011	
	22.15 % (10 m)				
	17.1 % (20 m)				
	Between 35 to 57 %			GAUTAM et al., 2011	

pH value	Between 7.1 and 8.4			BÄUMLER and KÖGEL-KNABNER, 2008	
	8.81	Methanogens	Around 7.0, but able to survive under extreme conditions	CHEN et al., 2003a	JONES et al., 1987; ZINDER, 1993
	9.03			CHEN et al., 2003a	
	Between 4.0 and 8.0	*Trichoderma, Aspergillus, Curvularia, Fusarium*	Between 7.0-8.0	GAUTAM et al., 2011	GAUTAM et al., 2011
	Between 7.58 ± 0.37 and 7.8 ± 0.39			PÉREZ-LEBLIC et al., 2012	
	8.2 ± 0.2	Methanogens	Around 7.0, but able to survive under extreme conditions	HUANG et al., 2003	JONES et al., 1987; ZINDER, 1993
	Between 6.8 ± 0.5 and 8.6 ± 0.4			UZ et al., 2003	

Two problems are evident when it comes to determining the ecological requirements of microorganisms in landfills. First, data about ecological requirements of microorganisms are either missing at all or the information about the optimum conditions are rather of general nature and not very specific. Second, information like temperature, pH and moisture conditions at the time of sampling are either not collected or not indicated in the published studies. As already indicated in chapter 1, knowledge about the present physical and chemical conditions in landfills is of major importance because pH, temperature and moisture – next to other factors like waste composition (ANDREOTTOLA and CANNAS, 1992) – are highly influencing the degradation process. In order to be able to adapt the actual landfill conditions to the ecological requirements of the responsible microorganisms, it is inevitable to obtain specific insights into pH, temperature and moisture conditions under which the microorganisms had been retrieved.

However, from the 29 studied that had been reviewed for this thesis, only CHEN et al. (2003b), who conducted a study about the archaeal community composition at different depths of a municipal solid waste landfill, indicated pH, temperature and moisture at the time of sampling. Apart from that, nine studies indicated information about pH, eight studies indicated temperature values and eight studies indicated moisture content (see Figure 20).

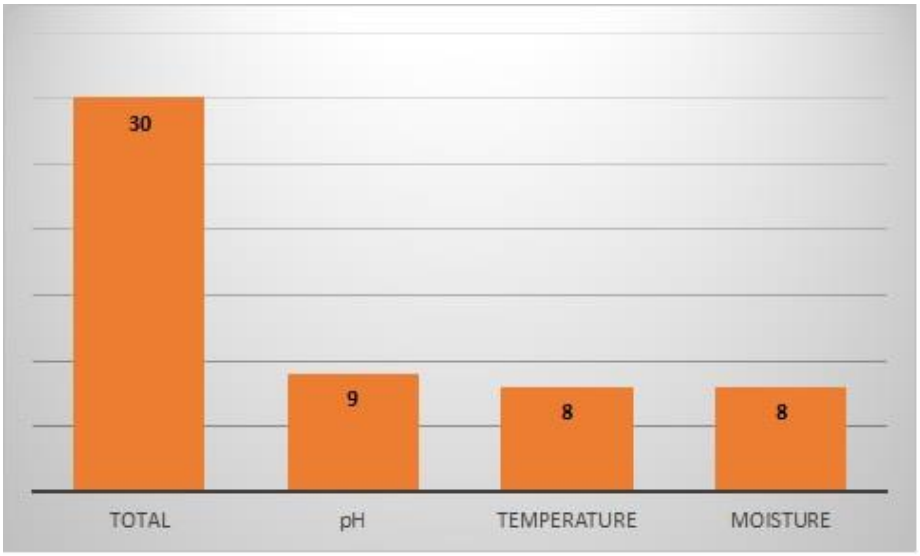

Figure 20: Overview on physical and chemical parameters (own illustration)

Finally, it should be stated that indicating physical and chemical parameters in the published scientific studies is urgently recommended since this information is highly needed to adapt actual landfill conditions to the ecological requirements of degrading microorganisms. However, it also has to be said that in those cases where pH, temperature and moisture contents

had been indicated, the optimum values were in accordance with the actual values at the time of sampling. Nevertheless, the more precise knowledge we have about physical and chemical conditions in landfills, the better we can adapt to the optimum conditions. Optimum conditions are necessary in order to accelerate decomposition of waste material and to attain stable conditions with minimized gas and leachate emissions as quickly as possible (BARLAZ and HAM, 1993). One adaption mechanisms might be leachate recirculation because several studies named this technical measure as one opportunity to increase degradation intensity (e.g. SANG et al. 2012; WARITH, 2002). However, it is very difficult to draw conclusions about optimum degradation conditions, given the fact that the landfill body consists of different materials with different chemical composition. Further, it has to be considered that the degradation process includes various groups of organisms depending on the state of degradation. Nevertheless, it is very promising that the number of scientific articles covering the issue of microbial decay of landfill material is strongly increasing in the recent few years.

4.2.1. Microbial Structures connected with Landfill Features

An overview of the complex connections of microbial structures with specific landfill features like sample age, depth, pH, OM concentration, operation type, temperature and site contamination is provided in Table 24.

Table 24 shows different microbial structures of old and young refuse samples. Aged refuse in comparison with young refuse is characterised by a lower concentration of acetate, a lower microbial acitivity and a lower number of cellulolytic *bacteria*. The authors cited in Table 24 showed that old refuse samples are dominated by members of the family *Bacillaceae* and especially the genus *Bacillus.* According to POURCHER et al. (2011), the family *Bacillaceae* might be favoured due to the fact that lower contents of carbohydrates (6.4 ± 1.6 % dry weight) and higher pH values (7.9 ± 0.35) can be found in old refuse samples than in young refuse samples (1 year). *Bacillus* was also the dominating genus in a study conducted on incineration ash deposited in landfills. Incineration ash is characterised by extremely high pH values around 12.8 (MIZUNO et al., 2008). Moreover, the conditions in aged landfills are favourable for *Proteobacteria* und *Methanosaeta*.

Young refuse samples, characterised by a high concentration of acetate, high microbial activity and a higher number of cellulolytic *bacteria*, provide unfavourable conditions for

gram- *bacteria*, but favourable conditions for *Methanosarcina*. CHEN et al. (2003b) stated that significant changes of the archaeal community happen in the earliest stages.

Referring to different sampling depths, deeper waste layers with a lower concentration of acetate favour *Methanoculleus* (KRISNAHMURTHI and CHAKRABARTI, 2013). Upper waste layers with a higher acetate level, provide favourable conditions for *Methanosarcina* (KRISHNAMURTHI and CHAKRABARTI, 2013) und moreover a higher methane production activity was noticed by CHEN et al. (2003a).

Landfills with leachate recirculation are characterised by a high input of OM and an accumulation of acetate which favours *Methanosarcina* (HUANG et al., 2002; 2003). However, these conditions are disadvantageous for *Proteobacteria* and *Methanosaeta* (HUANG et al., 2002; 2003; 2004). Lower temperatures generally lead to higher microbial diversity (LÉVEN et al., 2007). Contaminated sites are dominated by *Proteobacteria* (GOMEZ et al., 2011; PÉREZ-LEBLIC et al., 2012). McDONALD et al. (2012a) stated that fungi need high cellulose contents in order to encourage fungal growth.

The discovery from UZ et al. (2003) that older refuse samples show favourable conditions for hydrogenotrophic and formate-using methanogens is not in accordance with the discovery of KRISHNAMURTHI and CHAKRABARTI (2003). The latter stated that *Methanosaeta* are favoured in old refuse samples but *Methanosaeta* are known to be acetoclastic, which means that they use acetate as their only substrate (e.g. BURRELL et al., 2004).

LI et al. (2009) stated that the genera *Methanoculleus* is strictly hydrogenotrophic, however, WEISS et al. (2008) stated in their study that *Methanoculleus* can also use the acetoclastic (acetogenotrophic) pathway. The two studies were roughly conducted at the same time and *Methanoculleus* is in general referred to as being hydrogenotrophic (e.g. KRISHNAMURTHI and CHAKRABARTI, 2013). The genus *Methanoculleus* belongs to the order *Methanomicrobiales* commonly known as being hydrogenotrophic (e.g. HUANG et al., 2002; 2003) and not to be able to use acetate as substrate (GARCIA et al., 2006).

Table 24: Overview on important landfill features (own illustration)

Feature		Note	Characteristic	Source
SAMPLE AGE	Old refuse samples	Lower concentration of acetate Lower microbial activity Lower number of cellulolytic bacteria	Domination of the family *Bacillaceae*	POURCHER et al., 2001 KRISHNAMURTHI and CHAKRABARTI, 2013 HUANG et al., 2003
			Domination of the genus *Bacillus*	HE et al., 2014
			Identification of extremophiles (alkaliphiles and halophiles)	
			High abundance of *Bacillus*-like DNA sequences	HUANG et al., 2005
			Proteobacteria are favoured	HUANG et al., 2005
			Favourable conditions for *Methanosaeta*	KRISHNAMURTHI and CHAKRABARTI, 2013
			Favourable conditions for hydrogenotrophic and formate-using methanogens	UZ et al., 2003
	Young refuse samples	Higher concentration of acetate Higher microbial activity Higher number of cellulolytic bacteria	Unfavourable conditions for gram- bacteria like *Proteobacteria*	KRISHNAMURTHI and CHAKRABARTI, 2013 POURCHER et al., 2001 HUANG et al., 2003
			Favourable conditions for Methanosarcina	CHEN et al., 2003b
			Favourable conditions for acetoclastic, hydrogenotrophic and formate-using methanogens	UZ et al., 2003
	--	--	Significant structure changes of the methanogenic community happen shortly after waste burial	CHEN et al., 2003b
DEPTH	Deeper waste layers	Lower concentration of acetate	Favourable conditions for *Methanoculleus*	KRISHNAMURTHI and CHAKRABARTI, 2013
	Upper waste layers	Higher concentration of acetate	Favourable conditions for *Methanosarcina*	KRISHNAMURTHI and CHAKRABARTI, 2013
			Higher methane production activity	CHEN et al., 2003a
HIGH pH	Incineration ash layer in landfill	--	Dominated by *Bacillus*	MIZUNO et al., 2008

OM concentrations	High organic matter concentrations	--	High diversity of microbial communities	GOMEZ et al., 2011
LEACHATE	Full-scale recirculation of leachate	High input of OM associated with leachate recirculation; Accumulation of acetate	Favourable conditions for *Methanosarcina* No detection of *Methanosaeta*	HUANG et al., 2002 HUANG et al., 2003
			Very low abundance of Proteobacteria	HUANG et al., 2004
	Closed landfill	Stable conditions	Favourable conditions for *Methanosaeta* Low abundance of *Methanosarcina*	HUANG et al., 2003
			High abundance of *Proteobacteria*, mainly *Gammaproteobacteria*	HUANG et al., 2005
TEMPERATURE	Lower Temperature	--	Higher microbial diversity	LÉVEN et al., 2007
	High Temperature	40-50 °C	Low archaeal diversity	CHEN et al., 2003b
CONTAMINATED SITES	Pollutants: Hydrocarbons, PAH, PCB, Heavy metals	--	Domination of *Proteobacteria*	GOMEZ et al., 2011 PÉREZ-LEBLIC et al., 2012
	Contamination intensity		Lowest microbial diversity and lowest enzymatic activity in areas with highest pollutant concentrations	

4.3. Analysis of used Methods

While reviewing the scientific articles, I also focused on the used methods (see chapter 3.5.2). Along with it, methodological advantages and disadvantages reported by the authors of the studies were evaluated. Unfortunately, most authors did not address advantages and disadvantages of the used methods or explained possible biases caused by the used methods. Nevertheless, some authors (e.g. McDONALD et al., 2012a; RANSOM-JONES et al., 2012) named important methodological aspects which are briefly summarized in Table 25.

Table 25: Methodological aspects (own illustration)

Result	**Author**
Nested PCR (specific primer sets are used) shows higher sensitivity than direct PCR	McDONALDS et al. (2012a)
Representativeness of leachate samples might not be given	McDONALDS et al. (2012a)
Under-estimation of *Fibrobacters* due to the fact that they are underrepresented in clone libraries	McDONALDS et al. (2008)
Utilization of reverse transcribed RNA shows better results than the utilization of extracted DNA in PCR	RANSOM-JONES et al., (2012); McDONALD et al., (2008)
Mistakes during certain steps of examination (eg. DNA extraction, PCR amplification) can lead to biases → utilization of different DNA extraction kits lead to different results	LALOUI-CARPENTIER et al., 2006; CHEN et al., 2003; GOMEZ et al. (2011)
Results of experiments carried out in bioreactors resemble results of experiments carried out with corresponding leachate samples	McDONALDS et al. (2012a)

An important aspect that needs to be discussed further, is the fact that the approach of stable isotope probing was only used in one scientific study, namely by LI et al. (2009) which might be caused by high costs and advanced methodological knowledge necessary for the application of SIP. A specialised search, where I focused only on studies using the SIP approach, mainly showed results for the investigation of methanotrophic microorganisms (e.g. HENNEBERGER et al., 2013; CÉBRON et al., 2007). To me, the approach of stable isotope probing seemed to be one of the most promising methods because it demonstrated which groups of microorganisms use which substrates. The study performed by LI et al. (2009) was the only one I found during my research, where the interconnection between substrate type and associated microorganisms was demonstrated quite obviously. Intensifying the usage of the SIP approach,

more information about substrate utilization patterns of different landfill-typical microorganisms could be derived, which might support establishing optimum ecological conditions for the main waste degrading microorganisms.

As Figure 10 on page 40 illustrates, culture-independent approaches like TTGE, DGGE, SIP, FISH, RFLP and T-RFLP had been used noticeably more than culture-dependent approaches. From the 29 reviewed scientific studies, only five used a culture-dependent approach (GAUTAM et al., 2011; BARI et al., 2007; KUMAR et al., 2013; KRISHNAMURTHI and CHAKRABARTI, 2013 and HALE BOOTHE et al., 2001). KRISHNAMURTHI and CHAKRABARTHI (2013) used culture-dependent as well as culture-independent approaches. Figure 9 on page 36 shows that the majority of the reviewed studies used solid waste samples for the investigation (14 references). Leachate had been used in 11 studies and 7 scientific investigations had been carried out in the laboratory where landfill conditions had been artificially simulated.

McDONALD et al. (2012a) point out that due to the heterogeneity of landfill sites, representative solid samples cannot be obtained. Therefore the authors of this study claim that leachate, which is percolating through the landfill body, is the only available landfill sample type that can be used for studying microbial ecology directly. SANG et al. (2012) also stated that analysis of leachate samples is easier for investigation of microbial communities but they also highlight the problem of uncertain representativeness of leachate samples. McDONALD et al. (2012a) also raise the question whether landfill leachate samples could be considered representative for microbial communities, because the existence of preferential flow pathways of the leachate through the landfill body is well known (FELLNER and BRUNNER, 2010). As already mentioned, McDONALD et al. (2012a) stated that representative solid waste samples cannot be obtained and therefore lab-scale bioreactors are used to examine microorganisms in landfills. In that specific study the results of the bioreactor-experiment resembled the results of the corresponding experiment performed with leachate samples. But as already mentioned in the beginning of chapter 4, PALMISANO (1993) and HUANG et al. (2003) stated that anaerobic digesters might not be representative for full-scale landfills.

5. Conclusion

Degradation of MSW is mediated through microorganism and the microbial community structures are still considered to be a "black box". Anaerobic waste degradation is performed via cooperation of several microbial groups in order to degrade organic matter to the final products CH_4 and CO_2. The main microorganisms found being present in the 1st phase of degradation were *Clostridia*, *Bacillus* and *Fibrobacter* which are known for their hydrolytic and fermentative capabilities. *Clostridia* and *Porphyromonadaceae* are known to play an important part in the 2nd phase (acidification) where monomers are degraded into H_2, CO_2, organic acids and ethanol. The 4th phase (methanogenesis) is mainly performed by members of the orders *Methanomicrobiales* and *Methanosarcinales*, whereas *Methanoculleus* and *Methanosarcina* are the most important genera.

Most reviewed articles showed a lack of information about crucial parameters like temperature, pH and moisture which complicates drawing conclusions about the ecology of certain taxonomic groups. Thus it is highly necessary to include this important information about the sampling conditions in future studies.

The application of SIP approach, where waste samples are incubated with ^{13}C-cellulose, ^{13}C-glucose and ^{13}C-acetate, should be used more frequently to gain improved knowledge of the relationship between hydrolytic, fermentative and methanogenic microorganisms.

Finally, it has to be mentioned that the whole process of microbial degradation in landfills is still poorly understood but the rising number of publications in the recent years indicate great effort in the field of anaerobic degradation of MSW.

Glossary

16S rRNA:	Best known DNA sequence of Prokaryotes; consists of approximately 1500 base pairs
Acetyl-CoA:	Important intermediate or end product in several metabolism processes (e.g. in the citric acid cycle); also called activated acetate
ADP:	Adenosine-diphosphate; Hydrolysis of ATP results in the production of ADP and inorganic phosphate (P_i)
Aerobic:	An environment with oxygen
Agarose gel:	Growth medium for microorganisms containing important nutrients; Polysaccharide made of red algae
Anaerobic:	An environment where there is no oxygen present
Anoxic:	An environment where there is only non-dissolved, bonded oxygen available
ATP:	Adenosine-triphosphate; Most important chemical compound; the main task of metabolism is the consumption and regeneration of ATP; "energy currency" of the cell
Biolog MT plates:	Substrate utilization test plates
Budding:	Asexual, vegetative reproduction
Cellulolytic:	Cellulose degrading microorganisms like *bacteria* or *fungi*
Cytoplasm:	Internal structure of eukaryotic and prokaryotic cells
Cytosol:	Liquid component of cytoplasm of eukaryotic and prokaryotic cells
Denature:	Separation of the double stranded DNA through heat exposure (e.g. during PCR)
Electron acceptor:	Is reduced during a redox reaction by accepting electrons that are transferred to it from another compound
G + C content:	Characteristic feature of DNA molecules; Percentage of the bases guanine or cytosine on a DNA molecule
Gram+:	Gram staining; a possibility to divide *bacteria* by different physical and chemical properties of their cell walls into two groups: gram+ and gram-
Gram-:	see gram+
GTP:	Guanosine-triphosphate; High energy chemical compound like ATP
Hydrolysis:	Cleavage of chemical compounds by the addition of water

Hypha:	Filamentous structure of fungi; A form of fungal growth; Hyphae form the mycelium
Fermentation:	Anaerobic metabolic pathway which results e.g. in the formation of lactic acid
Field capacity:	Water saturated soil/waste which can hold water for a few days against gravity
Lignin:	Highly stable compound; e.g. in the cell wall of plants
Methanogens:	Methane producing *archaea*
Methanotrophs:	Methane oxidising *bacteria*
Monomer:	low molecular; can form polymers together with other molecules
Mycelium:	see Hypha
Oxidation:	Chemical reaction, where one electron is given up. The electron has to be taken over by another e.g. atom or molecule (Reduction). The process of Oxidation and Reduction is called redox reaction.
Phenotype:	Visible characteristics of an organism
Phylogenetic:	Evolutionary caused relationships between organisms
Polymer:	high molecular; consists of monomers
Pure Culture:	Culture consisting of descendants of one single cell, clone
Primer:	Starting point for DNA synthesis (e.g. used in PCR)
Redox potential:	Measures the tendency of chemical agents to aquire or give off electrons; measured in V or mV
Reduction:	see Oxidation
Reduction equivalent:	Electrons or protons; Vectors are e.g. NAD or FAD
Restriction enzymes:	Enzymes which cut DNA at specific positions (e.g. Hha I)
Volatile fatty acid:	Short-chain fatty acid with less than six carbon atoms (e.g. acetic acid)
Water activity:	Relation between the partial vapor pressure of water in a certain substance and the standard state partial vapor pressure of water

Glossary is entirely based on the literature cited in this thesis.

Figures

Tables

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