CHAPTER I: INTRODUCTION

1.1 Metagenomics

The Earth harbors about $>10^{30}$ microbial cells exceeding the number of known stars in the universe by nine orders of magnitude. This huge diversity of single-celled life acts as key functional drivers of biogeochemical processes of our planet's ecosystems. Yet the diversity and interdependencies of these microscopic organisms remain largely unknown and our understanding of the functional potential of most individual microbial taxa residing within any ecosystem is extremely limited (Bardgett and van der Putten, 2014; Hugenholtz and Tyson, 2008).

Metagenomics holds enormous potential for discovering novel enzymes and organisms that are biomarkers or drivers of processes relevant to disease, industry and the environment (Knight et al., 2012; Nesme et al., 2014).

The term metagenomics was coined by Jo Handelsman (Handelsman, 2004; Handelsman et al., 1998; Handelsman et al., 2007). Metagenomics can be defined as a molecular and computational approach for understanding the microbial community structure, and its functional potential associated with any ecosystem (Handelsman, 2007). The earth harbors a huge prokaryotic diversity, and they act as key functional drivers of our planet's ecosystems (Tringe and Rubin, 2005). The diversity and understanding of the functional potential of the most individual microbial taxa residing within any ecosystem are extremely limited because of our inability to isolate and culture them in laboratory conditions (Bardgett and van der Putten, 2014).

As metagenomics seeks to understand microbial ecosystem by studying the genome content of constituent microbes in their natural habitat. It provides a relatively unbiased view not only of the community structure (species richness and distribution) but also of the functional (metabolic) potential in a community (Hugenholtz and Tyson, 2008) (Knight et al., 2012).

There are two advantage of this approach:

1. Microbes that can be identified in laboratory condition using standard culture techniques are less than 1% of total microbes that exist in natural systems (Christensen et al., 1992; Cozzarelli et al., 2000; M., 1999). In Metagenomics, community genomic DNA is directly extracted from the environment, amplified using PCR and sequenced avoiding the need of cultivation (Adrian et al., 1994).

2. Sequences obtained from molecular studies can be aligned with reference sequences available in database and can be used for both microbial identification and inferring phylogenetic history.

In Metagenomics signature sequences like 16S rDNA, 23S rRNA ITS and some functional genes like mcrA gene catalyzing terminal step of methanogenesis, ammonia oxidizing gene AMO etc are used for identification of functional and microbial diversity (Amann et al., 1995b; Muyzer, 1999; Tringe and Rubin, 2005).

1.2. Methanogenic Archaea:

Members of the third domain of life, the Archaea, possess unique structural, physiological, biochemical and genetic features distinct from Bacteria and Eukarya, therefore they have drawn considerable scientific interest. Physiological, biochemical and molecular analyses have revealed many novel biological processes in these important prokaryotes (Rother and Metcalf, 2005). A recent survey of environmental sequences indicates that we know only little of archaeal diversity (Lueders et al., 2001).

Methane of biological origin can be produced from wide range of anaerobic environments, from peat bogs to the digestive tracts of animals and deep-sea hydrothermal vents. Methanogenic archaea serves as methane producer in all these environments (Luton et al., 2002).

Methane flux to the atmosphere is governed by complex communities of diverse microorganisms, including hydrolytic, fermenting, syntrophic, methanogenic and methanotrophic microorganisms (Luton et al., 2002).

Methanogens are of considerable importance because they utilize CO_2 and H_2 as food source and produce methane gas which is 25 times more potent than CO_2 in causing global warming (Bridgham et al., 2013; Cho and Tiedje, 2002; Griggs and Noguer, 2002). They are classified under four phylogenetically divergent orders of the domain Archaea (Phylum Euryarchaeota): Methanobacteriales, Methanopyrales, Methanomicrobiales and Methanosarcinales (Garrity et al., 2004). All of these orders are highly diverse in their morphological and physiological characteristics. However, they all are anaerobic and has the ability to produce methane metabolically.

1.2.1 Morphology:

Morphologically, the methanogens exhibit a wide variety of shapes and sizes, including rods, regular and irregular cocci, long-chained rods, spirilla, sarcina and irregular unusual flattened plates **(Table 1.1)**. Motility is sometimes present. Some species can aggregate in clusters. Several species of *Methanosarcina* and *Methanosaeta* contain gas vacuoles (Garcia et al., 2000).

Order	Morphology	Physiology
Metanobacteriales	Rods or cocci	Hydrogenotrophic, mesophilic or thermophilic
Methanococcales	Irregular cocci	Hydrogenotrophic, mesophilic or thermophilic
Methanomicrobiales	Irregular cocci Small rods Spirilla	Hydrogenotrophic, mesophilic or thermophilic
Methanosarcinales	Rods/filaments Irregular cocci or Sarcina	Acetoclastic/Hydrogenotrophic mesophilic or thermophilic

Table 1.1. Showing morphological feature of methanogens.

1.2.2 Cell envelopes

Although archaea can stain either gram positive or gram negative depending on the thickness and mass of the cell wall, their wall structure and chemistry differ from that of the bacteria (Willey, 2008). There is considerable variety in archaeal wall

structure. Many archaea have a wall with a single thick homogeneous layer resembling gram-positive bacteria and thus stain gram positive. Gram-negative archaea lack the outer membrane and complex peptidoglycan network of gram-negative bacteria. They do not contain the muramic acids and D amino acid characteristic peptidoglycan of Bacteria, which is composed of muramic Acid. Methanogens are therefore insensitive to the antibiotics that inhibit the synthesis of cell walls in Bacteria, such as penicillin, cycloserine, and valinomycin. This feature is utilized for efficient isolation of pure cultures of methanogens (Prescott et al., 2002; Willey, 2008).

Gram-positive archaea can have a variety of complex polymers in their walls. *Methanobacterium* and some other methanogens have walls containing pseudomurein, a peptidoglycan like polymer that has L-amino acids in its cross links, *N*-acetyltalosaminuronic acid instead of *N*-acetylmuramic acid, and β(1→3) glycosidic bonds instead of β(1→4) glycosidic bonds. Methanosarcina lack pseudomurein and contain complex polysaccharides similar to the chondroitin sulfate of animal connective tissue. Gram-negative archaea have a layer of protein or glycoprotein outside their plasma membrane. The chemical content of these walls varies considerably. Some methanogens like *Methanolobus* and several extreme thermophiles have glycoproteins in their walls. In contrast, other methanogens *Methanococcus, Methanomicrobium,* and Methanogenium and the extreme thermophiles *Desulfurococcus* have protein walls (Prescott et al., 2002).

1.3 Pathway for Methanogenesis:

The 83 species of methanogens described so far (including six synonymous) are separated into three main nutritional categories:

(a) 61 species as hydrogenotrophs which oxidize H_2 and reduce CO_2 to form methane and among them 38species are formatotrophs which oxidize formate to form methane.

(b) Twenty species as methanotrophs which use methyl compounds such as methanol, methylamines, or dimethylsulfide and of which 13 species are obligate methylotrophs.

(c) Nine species as aceticlastic (or acetotrophic) methanogens which utilize acetate to produce methane, with two species in this group being obligate acetotrophs (Muyzer, 1999).

As discussed above that methane can be produced by three different pathways, which vary in the carbon compound used as the substrate, as well as the source of the reducing potential, the hydrogenotrophic pathway is the most widespread, being found in all methanogenic orders. It involves the reduction of CO_2 with H_2 as an electron donor, and is composed of seven central steps (Figure 1.1). Formate can also be converted to methane through this pathway, acting as a source for CO_2 as reducing potential.

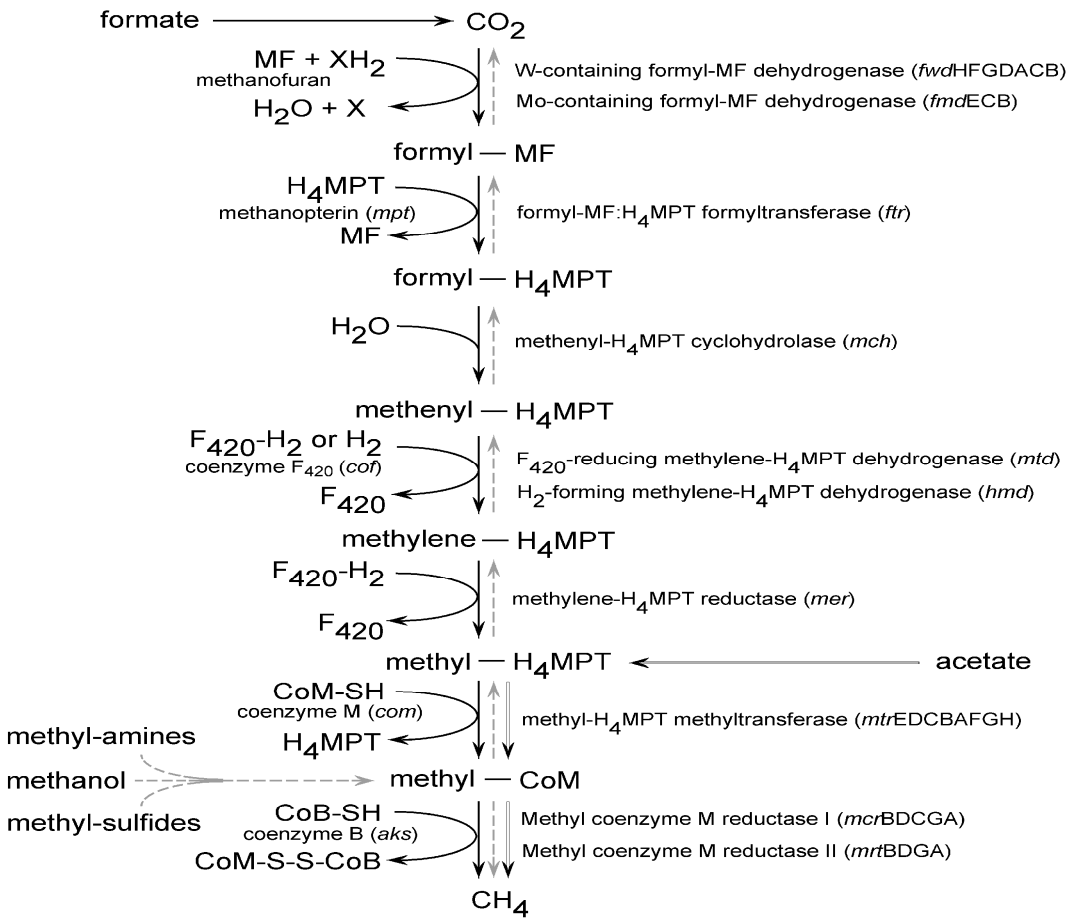

Figure 1.1. Pathways of methanogenesis: (adapted from Garcia et al.2005).

In the acetoclastic pathway, acetate is broken into a methyl group and CO, the latter being subsequently oxidized to provide electrons (Meuer et al., 2002). The methyl group from the splitting of acetate is linked to methanopterin (or sarcinapterin, for Methanosarcina) before being reduced to methane in two enzymatic reactions, homologous to the last two steps of the hydrogenotrophic pathway (Bapteste et al., 2005).

In methylotrophic pathway C-1 compounds such as methylamines or methanol can be used as both an electron donor and acceptor. One molecule of C-1 compound is oxidized to provide electrons for reducing three additional molecules to methane. However, in the presence of methanol and H_2/CO_2, some Methanosarcinales can reduce this C-1 compound using only the last step of hydrogenotrophic methanogenesis (methyl-CoM to CH_4),drawing electrons from H_2 (MacNaughton et al., 1999).

1.4 Ecology of Methanogens:

Methanogens are mostly mesophilic in nature but some methanogens have been found in extreme environments such as marine geothermal sediments and hot springs, as well as in hypersaline sediments (Juottonen, 2008; Maarit Niemi et al., 2001). Mesophilic methanogens are mostly found in anoxic environments, such as rice paddy fields (Iino et al., 2010).,(Yashiro et al., 2011), wetlands (Bridgham et al., 2013; Liu et al., 2011), permafrost (Mondav et al., 2014), landfills (Huang et al., 2003; Laloui-Carpentier et al., 2006), subsurface (Angel et al.2012), subseafloor sediments (Imachi et al., 2011) and ruminants (Cheng et al., 2009; Jeyanathan et al., 2011; Liu et al., 2012a), which are known to be the major sources of atmospheric methane **(Figure 1.2)**.

Takai et al. devloped a new technique for cultivation of chemolithoautotrophs, including methanogens (Takai et al., 2008). By using this technique, growth, survival, and methane production of a newly isolated, hyperthermophilic methanogen *Methanopyrus kandleri* strain 116 was characterized under high temperatures and hydrostatic pressures.

Recently, presence of methanogens and methanogenesis has been reported from oxic marine and freshwater sediments. Methane production in these oxic environments is driven by acetoclastic pathway and it is related with algal dynamics (Bogard et al., 2014).

In addition to such environments, methanogens play key roles in fields of anaerobic digestion technology, which is widely used as a means for treating municipal and industrial waste/wastewater containing high levels of organic compounds (Dunbar et al., 2000; Lee et al., 2008; Lee et al., 2010; Piterina and Pembroke, 2013). Methanogens are often critical components of such bioconversion systems, resulting in the recovery of gaseous methane from those wastes as reusable energy resource.

To better manage the bioconversion systems and achieve a higher efficiency in removing organic compounds from wastes, methanogens in these systems have been extensively studied and the quantitative monitoring of such methanogenic populations in these systems has been conducted (Piterina and Pembroke, 2013).

Because methanogens cannot use complex organic compounds, they need the presence of other microbial guild in the environment which converts high molecular weight compounds into simple sugars and fatty acids. These are further fermented by syntrophic bacteria to form acetate, formate, hydrogen (H_2) and carbon dioxide (CO_2) which constitute the substrates for methanogenesis. Acetogens (acetate-producing bacteria) are part of this syntrophic consortium when methanogens consume H_2 and formate efficiently (Huang et al., 2004).

It has been estimated that the annual global emission of methane is 500–600 Tg, and atmospheric methane concentration has risen threefold over the past 200 years (Laloui-Carpentier et al., 2006). With the increased interests in global climate change and environmental issues, studies on the diversity and ecophysiological functions of methanogens in such environments have been extensively conducted using cultivation-dependent and cultivation-independent approaches.

.

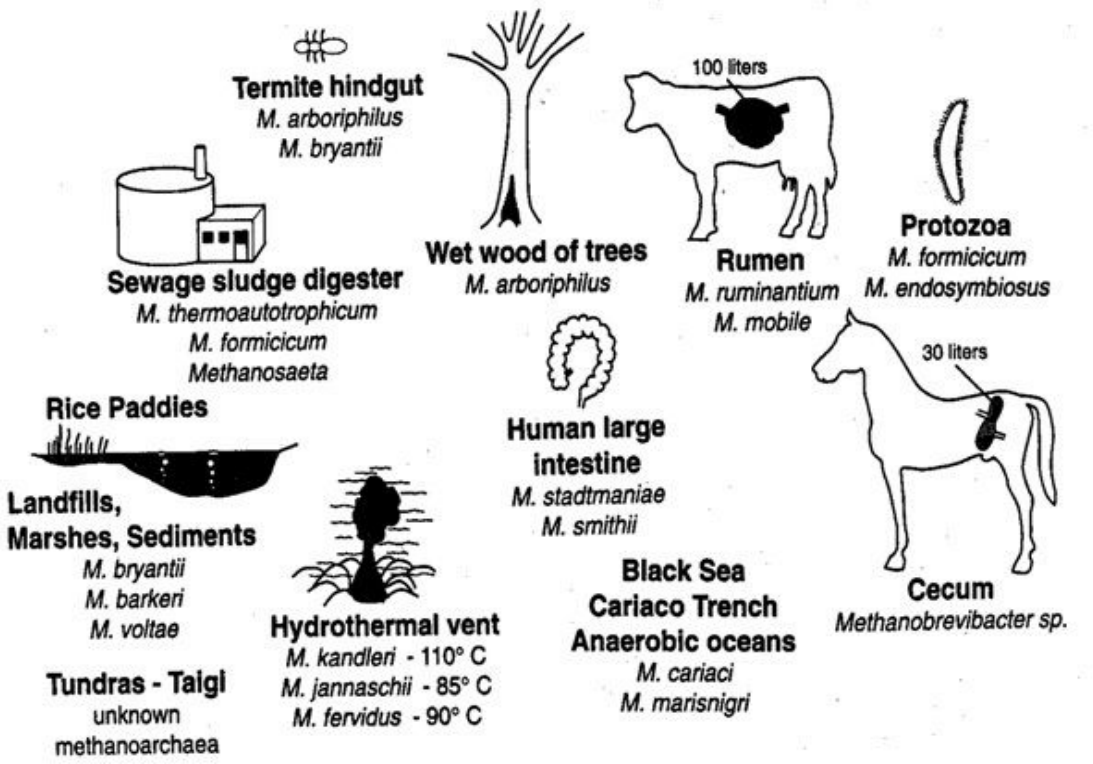

Figure 1.2. Prevalence of methanogens in various habitat.

1.5 Economic importance of methanogens:

When Carl Woese first proposed that the tree of life encompassed three distinct lineages i.e. Archaea, Bacteria and Eukarya, it was hard to imagine, the broad spectrum of novel findings study of these microorganisms would bring to light (Torsvik and Øvreås, 2002,Jarrell et al., 2011).

Studies of archaea have proven to be enormously fruitful: unique traits found nowhere else in nature have been revealed in archaea, and there are many instances of archaeal processes that combine a mosaic of bacterial and eukaryal features with unique archaeal ones to create a third functioning mechanism. Archaea are useful model systems for processes in both Eukarya and bacteria, and their major roles in various ecosystems. They have useful biotechnology/commercial applications, and may affect human health in significant ways (Jarrell et al., 2011).

1. Extremophilic archaea offers potential for many enzymes that can be used for commercial or biotechnology purposes like thermostable DNA polymerase, the only class of molecule which have been utilized for PCR protocols widely and has found commercial and biotechnology importance (Eichler, 2001; Jarrell et al., 2011).

2. The archaeal membranes lipids are extremely chemically stable and offers a novel application as drug delivery system (Jain et al., 2014; Patel and Sprott, 1999) .

3. Self-assembling archaeal components such as the S-layer glycoprotein of archaea have drawn interest for their nanotechnological potential (Sleytr et al., 1997).

4. Methanogenic archaea has gained importance for their capacity to produce methane as clean and inexpensive energy sources from natural as well as anthropogenic sources (Hedlund et al., 2013)

1.6 Molecular Phylogeny:

Molecular phylogeny, branch of science that analyses hereditary molecular differences in DNA sequences to gain an organism evolutionary relationship. The result of molecular analyses is often represented as phylogenetic tree. Every living organism contains DNA, RNA, and proteins. In general, closely related organisms have a high degree of homology in the molecular structure of these substances, while organisms distantly related usually show a pattern of dissimilarity. Conserved sequences like mitochondrial DNA, ribosomal RNA (rRNA) are expected to accumulate mutations over time, and assuming a constant rate of mutation may provide molecular clock for divergence. Molecular phylogeny uses such data to build a "relationship tree" that shows the probable evolution of various organisms.

Prokaryotes represent major portion of earth biota amounting about $4\text{-}6*10^{30}$ cells and total amount of their cellular carbon approx. 350-550 petagrams. Liu and Whitman estimated that amount of carbon stored in prokaryote cells is about 60-100% of total carbon in plants (Whitman et al., 2006). Most of these prokaryotes are found in ocean, soil and terrestrial subsurface. Analysis of microbial diversity over the past few decades has resulted in tremendous increase in new phylotypes.

Prokaryotes both archaea and bacteria have been classified in total 35 Phyla. Bacteria having 30 Phyla and Archaea having 5 Phyla (Parte, 2013). Of the total prokaryotic diversity present on earth bacteria are most ubiquitous and plays important role in functioning of ecosystem. Molecular studies and comparative genomics have shown that archaea are characterized by a combination of unique features like left-handed isoprenoid containing glycerolipids, and mosaic bacterial and eukaryotic features. The study of archaea is essential to understand the history of molecular mechanisms and metabolism diversity on our planet as well as to unravel the mechanisms by which life can prosper in extreme environments (Caetano-Anollés et al., 2014)..

The phylogeny of archaea currently used is based on 16S rDNA sequence comparison (Thauer et al., 2008). Based on 16S rDNA analysis, the archaeal domain is split between five kingdom-level phyla: the Euryarchaeota, which include the methanogenic and the halophilic archaea as well as the Archaeoglobus and the Pyrococcus groups *(Thermococcales*); the Crenarchaeota, which are dominated by thermophilic organisms such as *Sulfolobus and Thermoproteus*; the Korarchaeota (Barns et al., 1996), for which only DNA sequence data exist to date; and the Nanoarchaeota, which are represented to date by only one species, *Nanoarchaeum equitans* (Huber et al., 2003; Rother and Metcalf, 2005) and phylum Thaumarchaeota which is represented by Cenarchaeum (Parte, 2013). Methanogenic archaea mediates all significant biological methane production on earth. Methanogenesis proceeds by conversion of simple C1 and C2 compounds to methane (Matte-Tailliez et al., 2002; Thauer et al., 2008). Methanogens are classified into the phylum *Euryarchaeota* of the domain *Archaea* (Garrity et al., 2004). They are divided into four classes i.e. 'Methanomicrobia', Methanobacteria, Methanococci and Methanopyri. These four classes comprised of 33 genera.

The class 'Methanomicrobia' is the most phylogenetically and physiologically diverse group of methanogens comprising of three orders i.e. Methanosarcinale, Methanomicrobiales and Methanocellales; and 23 genera belonging to seven families. Within the order Methanosarcinales, the genera *Methanosarcina* and *Methanosaeta* are known to play a key role in the conversion of acetate into methane in various anaerobic environments, and the rest are known to metabolize relatively broad ranges of substrates, such as hydrogen, methanol and methylamines (Garrity et al., 2004). Known

members of the order Methanomicrobiales are all hydrogenotrophs, and some of them are found in anaerobic environments where they act as important hydrogen scavengers (Luton et al., 2002). Members of the class *Methanobacteria*, consisting of the families *Methanobacteriaceae* and *Methanothermaceae*, hydrogenotrophic methanogens are also found in diverse anaerobic ecosystems (Garrity and Holt, 2001). *Methanobacteriaceae* comprises four genera, *Methanobacterium*, *Methanosphaera*, *Methanobrevibacter* and *Methanothermobacter*.

The class *Methanococci* includes the families *Methanococcaceae* and *Methanocaldococcaceae*, which are widely distributed in natural ecosystems such as marine sediments and deep sea geothermal sediments (Liu and Whitman, 2008). The class *Methanopyri* consists of single genus *Methanopyrus*, which is a hyperthermophilic, hydrogenotrophic methanogen isolated from the deep-sea hydrothermal field (Takai et al., 2008). The isolation and characterization of novel methanogens from various ecosystems are ongoing, and the descriptions of such methanogens have been carried out at an encouraging rate.

1.7 Methane as greenhouse gas:

Methane is an important greenhouse gas and is produced from both natural (such as wetlands) and anthropogenic activity (such as landfills) (Lelieveld et al., 1998). Landfill sites are estimated to release 40 Tg CH_4 per year, which accounts for 6.7% of global methane emissions. Rice paddy fields cover about 155 million hectares worldwide and contribute approximately 5–19% to annual atmospheric CH_4 emissions and are considered as important anthropogenic CH_4 sources along with landfills, livestock, fossils fuel production and biomass burnings (Ma et al., 2010).

Methane is third most abundant gas in ecosystem after water and CO_2. The global warming potential of methane gas is 25 times more than CO_2. Methane contributes about 18% of the total green house gases in green house effect. It has highest generation (60%) than other gases. Therefore, there is immense concern for its abatement or utilization from landfill areas and marshland. Compared to the western countries, the composition of municipal solid waste (MSW) in developing countries like India has higher (40% - 60%) organic waste. This make it more potential to emit

higher GHG's from per ton of MSW compared to developed world. Beside that landfill areas in India are not planned or engineered generally low lying open areas, where MSW is indiscriminately disposed. This leads to uncontrolled emission of trace gases, foul smell, ground and surface water pollution etc. Due to scarcity of land in big cities, municipal authorities are using same landfill for nearly 10 - 20 years. Hence, the possibility of anaerobic emission of GHG's further increases (Rawat and Ramanathan, 2011).

1.8 Methanogenesis in Landfill and Marshland:

1.8.1 Landfill:

Landfills are site constructed for safe disposal of solid waste either municipal solid waste (household) or hazardous (toxic chemicals etc). Methane emissions from landfill represent the largest source of green house gas (GHG) emissions from the waste sector, contributing around 700 Mt CH_4 (Bogner et al., 2008).

Waste which is used for disposal is rich in organic material such as food, paper and other household products and industrial wastes. Once waste is disposed inside landfill microbes begins to degrade complex polymeric compound into monomers and finally convert them into CO_2-H_2 which is consumed by methane-producing bacteria (Methanogens) to produce methane under anaerobic condition via the process called methanogenesis. Methanogenesis is terminal step of anaerobic digestion (Alvarado et al., 2014).

Anaerobic digestion is a complex series of processes in which microorganisms break down biodegradable materials such as animal manure in the absence of oxygen to methane and byproducts (Gerardi, 2003). It is used for industrial or domestic purposes to manage waste and/or to release energy. The digestion process begins with bacterial hydrolysis of the input materials i.e. break down insoluble organic polymers, such as carbohydrates, proteins lipids and make them available for other bacteria. Acidogenic bacteria then convert the sugars and amino acids into carbon dioxide, hydrogen, ammonia, and organic acids. Subsequently, Acetogenic bacteria then convert these resulting organic acids into acetic acid, along with

additional ammonia, hydrogen, and carbon dioxide. Finally, methanogens convert carbon dioxide or acetate to methane (Figure 1.3). Anaerobic digestion is widely used as a source of renewable energy in the form of biogas (Narihiro and Sekiguchi, 2007; Tabatabaei et al., 2010).

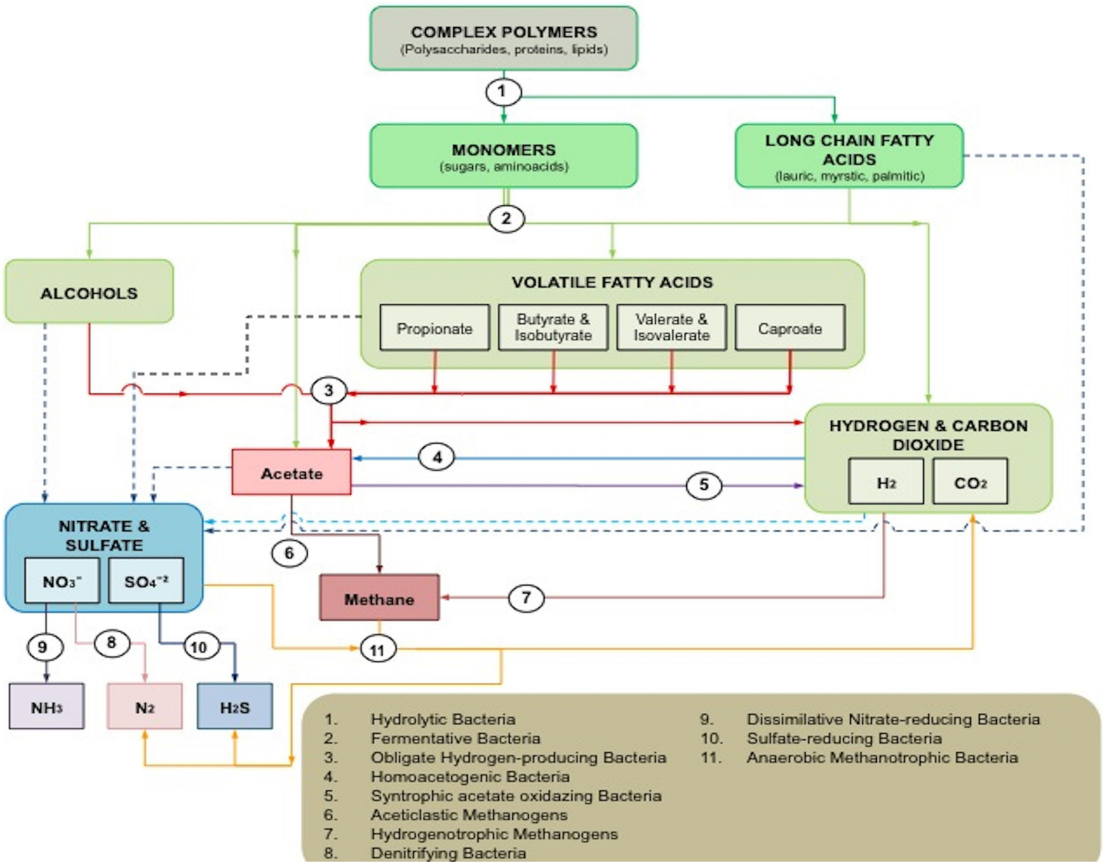

Figure 1.3. Schematic representation of anaerobic digestion and pathway for methane production (Alvarado et al., 2014)**.**

As the microbes gradually decompose organic matter over time, methane (approximately 50%), carbon dioxide (approximately 50%), and other trace amounts of gaseous compounds (< 1%) are generated form landfill. In controlled landfills, the process of burying waste and regularly covering deposits with a low permeability material creates an internal environment that favors methane-producing archaea. As with any ecological system, optimum conditions of temperature, moisture, and nutrient source (i.e. organic waste) result in greater biochemical activity and hence greater generation of landfill gas. This biogas can be used as alternative source of energy.

Methane and CO_2 are two major greenhouse gases whose presence in environment causes global warming and climate change (Trends and Framework, 2010). However, emission of greenhouse gases from landfill site is not the sole cause of concern. Landfills are generally not capped after closure to prevent the formation of leachate by infiltration of rainwater, nor were precautions taken to prohibit spreading of leachate to underlying aquifers. Therefore, release of hazardous chemicals to the environment continues, even if landfilling stops. As a result, leachate plumes forms downstream from many landfill sites with a length up to a few kilometers.

Degradation, sorption, dilution, volatilization, precipitation and ion-exchange are processes which attenuate pollution (Christensen et al., 1992), but only (microbial) degradation really removes the bulk of organic contamination (Adrian et al., 1994). Microorganisms transform organic compounds by means of electron-transfer reactions in order to extract free energy by synthesizing ATP. Usually, organic compounds act as reductor while inorganic compounds (e.g. O_2, SO_2) are used as oxidant(Christenson and Cozzarelli, 1999). Although environmental conditions for degradation of certain organic chemicals may be suitable, presence of specific microorganisms for occurrence of degradation is a pre-requisite. The degradation process occurs in successive stages including hydrolysis, acidogenesis and methanogenesis.

Therefore, molecular microbial community analysis could be a powerful tool for predicting a site's potential for methane production. Culture-independent molecular techniques rapidly advance our understanding of microbial ecology. Ribosomal RNA (rRNA) serves as a gold standard for description of communities of uncultivable microbes without need of cultivation (Amann et al., 1995). Nucleic acid extraction, PCR amplification with domain specific primers, DGGE (Muyzer, 1999) allows the detection and characterization of microbes without need of cultivation.

1.8.2 Marshland/Wetland:

Wetlands can be defined as an areas of marsh, fen, peat land or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six meters (Bassi et al., 2014; Matthews, 1993). Total wetland area estimated is around

15.260 Mha, which is around 4.63 per cent of the geographic area of the country. Assam has 9.74% i.e. 7,64,372 hectare of its land occupied by wetlands and is at 17th position in terms of area in India (SAC, National Wetland Atlas, 2011) (Figure 1.4). India harbours huge diversity of wetlands. Wetlands like peat lands, mires and marshes play an important role in global carbon cycling as they serve both as a sink for CO_2 and source for atmospheric methane (Panigrahy et al., 2012).

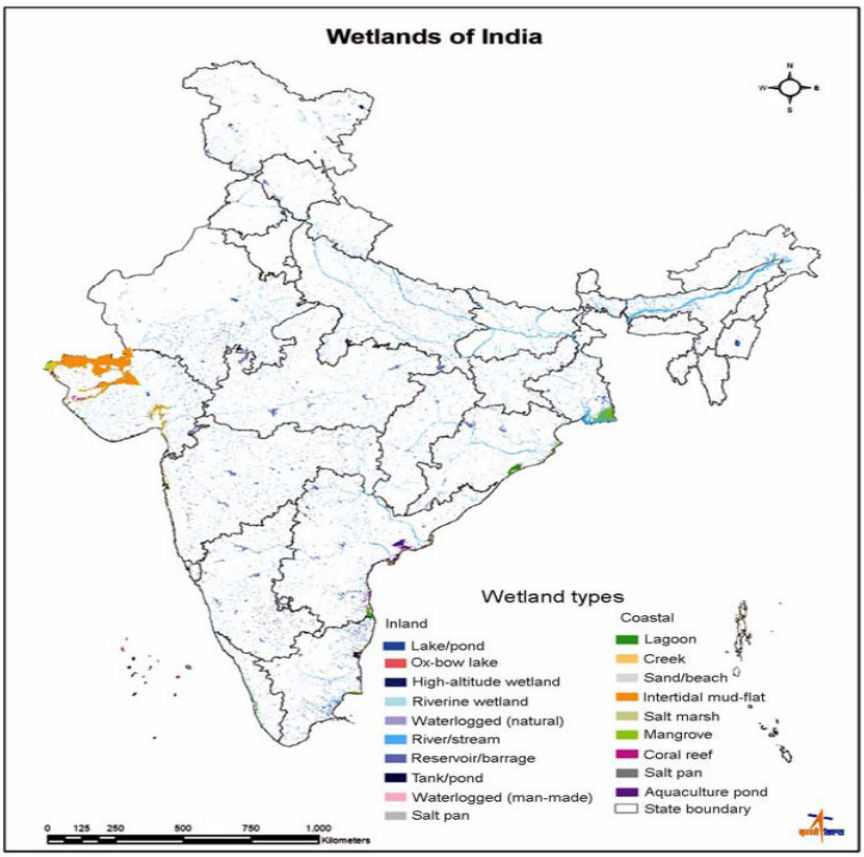

Figure 1.4. Distribution of Wetland in India (SAC, National Wetland Atlas-2011).

Purvaja and co-workers conducted a study to estimate the methanogenic potential of coastal wetland in South India. They found that methanogenesis occurs by both natural and anthropogenic activities in these wetlands. It is affected by factors like organic content, salinity, sulphate concentration. It has been also found that these sites are potential sources of atmospheric methane and it can rise due anthropogenic perturbations (Purvaja and Ramesh, 2001).

The global budget of atmospheric methane is on the order of 500–600 Tg CH_4 per year, is mainly the result of environmental microbial processes, such as archaeal methanogenesis in wetlands, rice fields, and ruminant and termite digestive systems and of microbial methane oxidation under anoxic and oxic conditions (Bogard et al., 2014). Methanogenic archaea and CH_4 production is typically found at those sites where organic matter is decomposed in the absence of oxygen or other oxidants, such as nitrate, sulfate or ferric iron. Wetlands are the largest individual source of microbial CH_4 (Conrad, 2007). Natural wetlands have emitted 100–231Tg CH_4 $year^{-1}$, which accounts for 20–39% of the annual global CH_4 emission (Solomon, 2007). The CH_4 emission from wetlands increased by 7% from 2003 to 2007 (Bloom et al., 2010). Methane (CH_4) emissions from natural wetland ecosystems exhibit large spatial variability at regional, national and global levels related to temperature, water table, plant type and methanogenic archaea etc.

In order to understand the relationship between the population of methanogenic archaea and CH_4 production potential in natural wetlands around China, Liu and co-worker investigated relation between methane production potential and the abundance of methanogenic archaea in vertical soil profiles sampled from the Poyang wetland in the subtropical zone, the Hongze wetland in the warm temperate zone, the Sanjiang marsh in the cold temperate zone, and the Ruoergai peatland in the Qinghai-Tibetan Plateau in the alpine climate zone. They found that top soil layer had the highest population of methanogens in all wetlands except the Ruoergai peatlands (Liu et al 2011).Understanding methanogenesis and methanotrophy is essential to establish new agriculture techniques and industrial processes that contribute to a better balance of GHG. (Serrano-Silva et al.2014).

1.9 National Status:

Landfilling is the most common form of municipal solid waste (MSW) disposal in India. In developing countries like India and other Asian countries, it is considered as a reliable and a cost effective method for disposal of wastes. However, implementation of waste disposal facilities in the country is found to be far below from satisfaction. Most of cities face problem in identification of landfill sites for construction of sanitary landfill sites. This is because of public resistance, rapid growth of urban areas,

escalating land prices and not having proper master plan. During the year 2004-05, Central Pollution Control Board (CPCB) through National Environmental Engineering Research Institute (NEERI), Nagpur conducted survey in 59 cities (35 Metro cities and 24 State Capitals) and estimated 39,031 tons per day MSW generation in these 59 cities/towns. According to annual report of 2010-11, Central Pollution Control Board (CPCB) at present there are 59 landfills constructed in the country, 376 landfills under planned and 1305 landfill sites were identified for future use. The survey conducted by the Central Institute of Plastics Engineering and Technology (CIPET) at the instance of CPCB has reported generation of 50,592 tons of MSW per day in the year 2010-11 in same 59 cities. The national capital Delhi ranked last amongst the four metros in solid waste collection, disposal and recycling, according to a study. A very small fraction of the collected solid waste like paper, plastics, metal and glass can be reused for multiple or substandard purposes.

According to a recent study by the NEERI, Nagpur, the expected quantity of solid waste generated in Delhi would be about 18,000 tonnes per day by 2021(Jhamnani and Singh, 2009). Due to growing pressure on land in Delhi and the projected increase in the quantum of solid wastes, the scope for disposal through landfill sites is limited. Too much land is being consumed accompanied by increasing danger of ground and surface water contamination because Delhi landfill sites are open sites with uncontrolled disposal facility, no base liners and neither gas nor leachate collection facility (Rawat and Ramanathan, 2011; Singh and Mittal, 2011).

1.10 Statement of the problem:

Climate change is one of the most important challenges for humankind in this modern era that is related to forest and land degradation, freshwater shortage, food security and air-water pollution. According to Intergovernmental Panel on Climate Change (IPCC) report the global mean temperature may increase between 1.4 and 5.8°C by 2100 (Mitigation, 2011). The impact of this rise in temperature would be particularly severe in the tropical areas, which mainly consist of developing countries, including India. The climate change issue for developing countries is correlated with pace of sustainable development (Sathaye et al., 2006). At present India's focus and plans for sustainable developmental wherein many climate change concerns requires to be addressed. These

conscious decisions may prove helpful for making climate-friendly sustainable development (Sharma et al., 2006).

Due to higher pace of urbanization, production of waste and size of landfill is also expanding. As mentioned earlier due to growing pressure on land in Delhi and the projected increase in the quantum of solid wastes, the scope for disposal through landfill sites is limited. Too much land is being consumed accompanied by increasing danger of ground and surface water contamination (Kumar et al., 2009).

In metropolitan cities like Delhi groundwater is the major source of drinking and if it will get contaminated by leachate then it will pose a very serious threat to the population lying in its vicinity. River Yamuna receives about 1789 MLD of untreated wastewater from the capital city, Delhi. This is about 78% of the total pollution load that flows in to the river every day. As a result the water quality and hydrological property of Yamuna water in the Delhi is the most polluted in terms dissolved oxygen (DO) and biological oxygen demand (BOD) (Panigrahy et al., 2012).

Landfill generates some other harmful gases like sulfuric acid (H_2S) and nitric acid (HNO_3) which are hazardous for human health. Landfill also contribute significantly in climate change by causing global warming due to emission of methane gas which is 25 times more potent than CO_2 in greenhouse effect. In the similar fashion marshland also contribute to the global warming by generating methane. So, better understanding of microbial communities inhabiting these ecosystem will help us to understand the biochemical processes undergoing and there potentiality in causing climate change and global warming. Secondly, if we manage the disposal of MSW by making bioreactor plant as practiced in some western countries like USA, Netherland and Poland, the emitted methane can be stored and used as an alternative source of energy and leachate can be processed to produce water which can be used for secondary purpose, waste recycling can be enhanced if it will be used for generating electricity.

OBJECTIVES

The aim of this work was to investigate the microbial and methanogenic diversity present in the landfill leachate and marshland. Bacteria and non-methanogenic archaea were assessed because they are potential substrate producers and competitors to methanogens.

The objectives are:

1. **To identify microbial diversity present in the leachate sample of landfill sites, Delhi and Silchar using 16S rDNA sequences.**

2. **Comparative analysis of methanogenic diversity associated with marshlands samples present in Silchar as well as leachate samples of landfill sites present in Delhi using 16S rDNA and *mcrA* sequences.**

3. **Quantification of Methanogens from the samples of marshland Silchar and leachate sample of Delhi landfill sites using 16S rDNA quantification by Real Time-PCR (RT-PCR).**

4. **Molecular Phylogenetic relationship on the basis of Archaeal and Bacterial 16S rDNA sequences.**