



Role of Methanotrophs in Mitigating Global Warming

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Abstract

There is growing concern about global warming worldwide. Greenhouse gases, which absorb the heat energy reflected by the earth's surface, are the main causes of global warming. Carbon dioxide, methane, nitrous oxide, and ozone are the main greenhouse gases. Methane is about 23 times more effective as a greenhouse gas than carbon dioxide. Anthropogenic sources release methane directly or indirectly into the atmosphere account for up to one-third of the global warming currently taking place. Methanotrophic bacteria or methanotrophs may serve as a biofilter and use methane as a source of energy before it is released into the atmosphere. They have been the only recognized major biological sink for atmospheric methane and play a key role in reducing the load of methane up to 15% to the total global methane destruction. Because of its physiologically adaptable nature, methanotrophs exist in diverse habitats and present in a wide range of pH, temperature, oxygen, salinity, and radiation. In this chapter, role of methanotrophs as an effective tool in mitigating greenhouse gas emissions is reported.

Keywords

Carbon dioxide · Global warming · Greenhouse gas · Methane · Methanotrophs

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4.1 Introduction

The atmospheric level of greenhouse gases (GHGs) like carbon dioxide (CO_2), nitrous oxide (N_2O), nitric oxide (NO), methane (CH_4), ozone (O_3), and halogenated hydrocarbons increases considerably with time due to biotic (plants, animals, microorganisms, etc.) and anthropogenic (mining, industry, transportation, deforestation, construction, settlements, etc.) activities. In the future, these gasses will continue increasing with increasing populations, crop production and changing shifting patterns of food consumption, along with increased demand for ruminant meats. Combustion of fossil fuels and industrial procedures are the primary sources contributing to GHGs. A notable contribution is also made by the agricultural sector and land use. The emission of various greenhouse gases and their role to worldwide complete emissions vary with land use. At the global level, N_2O , CO_2 , and CH_4 contribute 8%, 77%, and 15% of total emissions, respectively. The agricultural sector accounts for 32% of total worldwide emissions, of which 6%, 18%, and 8% are N_2O , CO_2 , and CH_4 (De la Chesnaye et al. 2006).

Greenhouse effect is the interaction between incident solar radiation and these gasses to store heat in the atmosphere and stop infrared radiation from escaping. Higher amount of these gasses results in a “radiation drive,” which leads to an increase in temperature. Among them, CO_2 and water vapor dominate (some of which are formed by oxidation of CH_4 by OH radicals in the environment), that together represent approximately 85% of the retention of solar heating (Fig. 4.1).

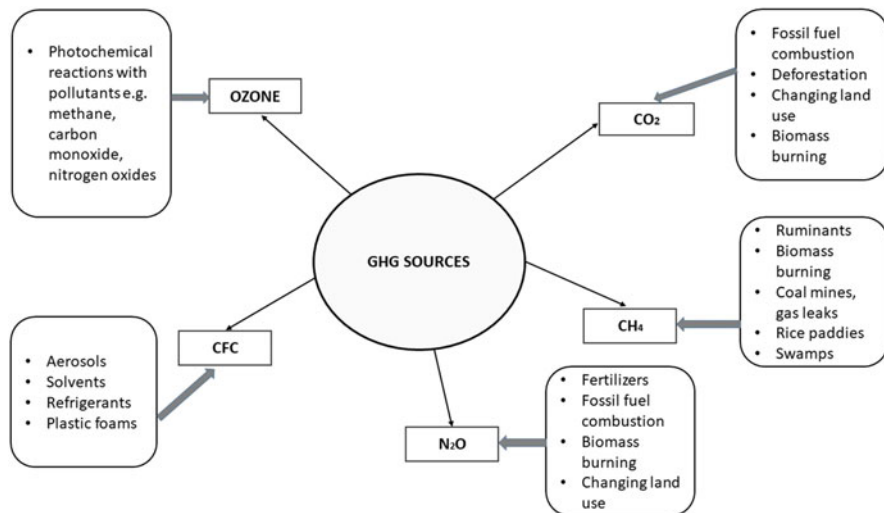


Fig. 4.1 Different sources of greenhouse gases

4.2 Methane and its Sources

Methane is produced during food digestion in ruminants (e.g., buffaloes, goats, camels) through the action of methanogenic archaea and by methanogens in landfills, anoxic sediments (oceans and lakes), wetlands, termite nests, and soils (in particular rice fields) as well as anthropogenic coal mines and natural gas. Methane is the second most prevalent greenhouse gas after CO₂, accounts for around 20% of anthropogenic radiation (Nisbet et al. 2014). Methane contributes the most to the greenhouse effect after CO₂ and is about 23 times more effective than CO₂ on a molar basis as a greenhouse gas (IPCC 2007a, b). It is estimated that microbial production accounts for 70–90% of the atmospheric CH₄ flux. Atmospheric levels of methane increased to 1800 ppb in 2008 from about 715 ppb prior to the industrial revolution (Montzka et al. 2011). CH₄'s potential for global warming is 25 times that of CO₂. Slight alterations in methane levels in atmosphere will therefore make a significant contribution to future worldwide warming (Bridgham et al. 2013). Global climate change like enhanced CO₂ emissions and warming has changed the characteristics and functions of terrestrial ecosystems dramatically (Rosenzweig et al. 2007; Austin et al. 2009; Singh et al. 2010).

4.2.1 Paddy Fields

Agricultural practices also lead to important emissions of methane with rice manufacturing and livestock being the two primary sectors. Rice fields are mainly man-made wetlands and are characterized by elevated concentrations of humidity, lack of oxygen, and elevated concentrations of organic substrates and nutrients. As such, they provide perfect circumstances for methanogenic bacteria and trigger substantial methane emissions of about 40 Mt annually. Populations of methanotrophic organisms that convert methane to carbon dioxide absorb up to 90% of this methane, but the remaining 10% escape to the environment. One kilogram of rice production corresponds to 100 g of methane emission. It should be noted that the precision of methane emission estimates has greatly increased in the last decade, with current figures accounting for almost half of the previous estimates. Rice fields (Yan et al. 2009; Liu et al. 2012), representing 5–19% of worldwide CH₄ emissions, are one of the main sources of atmospheric CH₄ (IPCC 2007a, b). In reaction to high atmospheric CO₂ and high temperatures, an increase in CH₄ emissions from these sources has already been identified resulting in positive feedback from the process of worldwide warming (Allen et al. 2003; Tokida et al. 2010; van Groenigen et al. 2011). Recent proof has shown that high concentrations of CO₂ increased CH₄ emissions from rice soils by an average of 43% (van Groenigen et al. 2011); high soil temperatures (2 °C) resulted in a 42% rise in CH₄ emissions (Tokida et al. 2010). Increased CO₂ is usually considered to encourage photosynthesis, root biomass, and rice exudates (Pritchard, 2011; Okubo et al. 2014), possibly giving more CH₄ substrate (Inubushi et al. 2003).

4.2.2 Methane Hydrates

Although methane hydrates are neither a source nor a sink at the moment, they are by far the biggest methane reservoir on Earth, representing 53% of all fossil fuels on Earth. They are a crystalline solid combination of water and methane (mainly methane trapped in ice) and occur in seabed sediments and permafrost in the Arctic (Kvenvolden 1993, 1998; Kvenvolden 1988; Buffett 2004). The methane in the sediment hydrates of the oceans is trapped deep in the ocean by the high pressure, but is released above a depth of 400 m when the pressure drops. The energy industry strives to exploit these benefits and reduce these occurrences. The methane hydrate melts and releases methane gas into the atmosphere as the temperature increases (Kvenvolden 1993; Shakhova et al. 2007, 2008; Portnoy et al. 2016). Methane release from the Siberian Arctic permafrost can create an important contribution to atmospheric pollution each year (Kvenvolden 1993; Shakhova et al. 2007, 2008; Portnoy et al. 2016). There is concern that as global temperatures rise due to anthropogenic climate change that leads to Arctic permafrost melting, large amounts of methane will be released into the atmosphere, resulting in a catastrophic greenhouse effect even higher than the assumed upper estimate of 5.8 °C going beyond IPCC.

Another unanticipated source of about 4% of overall methane in the atmosphere appears to be aerobic bacterial activity in oceans and precursors like methylphosphonate (Karl et al. 2008). The substituted methane, both chlorine and bromomethane, is also released from marine algae and sources of terrestrial and aquatic bacteria into the atmosphere, contributing to global warming, which has not been quantified sufficiently to date.

4.2.3 Coal Mines

By 2008, 826,001 million tons of coal reserves were estimated globally. World coal consumption reduced in 2008 but continued to rise by 3.1% and stayed for six successive years the world fastest increasing fuel (BP 2009). Methane emissions from coal mines, measured approximately 5–30 Tg annually (Breas et al. 2001), are primarily due to methane desorption during mining, comminution, and inefficient combustion. Coal mine gas can also trigger heavy explosions in relation to the greenhouse effect. For 2004, it is estimated that methane from Chinese coal mines gets into the atmosphere. More than 80% of heavy coal mine accidents in China are caused by coal mine explosions, accounting for one-third of total national deaths (Song and Wang 2005). Therefore, the control of mine gas is important for the reduction of greenhouse gases and for the safe regulation of coal mines for energy industry (<http://www.chinasafety.gov.cn>).

4.3 Methanotrophs Based Mitigation of Methane

Microbial community manipulation provides a possibly efficient means of mitigating global climate change. It is essential to know their ecology and function in order to deal with useful microbial communities. Methane biocycle is more apparent than other GHG cycles because methane pathway is easy and involves advanced microorganisms. The role of methylotrophic groups in mitigating climate change recognized and their position in the global carbon cycle and in decreasing greenhouse gas environmental impacts like methane and indirect carbon dioxide is identified. Methylotrophs are a functional group of microbes that use reduced substrates of carbon comprising of no carbon–carbon bonding. Methylotrophic bacteria include methanotrophs (consuming CH_4) and methylotrophs other than methanotrophs (consuming other substrates of reduced carbon than methane). This functional group can use methanol, methylamine, dimethylamine, formaldehyde, formate, alongside methane as the sole carbon and energy source, and is often involved in the global carbon cycle (Iguchi et al. 2015; Kolb and Stacheter 2013). Methanotrophic biological oxidation of CH_4 accounts for only about 5% of the worldwide sink of atmospheric methane (Hanson and Hanson 1996). Methanotrophs can oxidize as much as 90% of the CH_4 produced in the soil prior it enters into the atmosphere (Oremland and Culbertson 1992). Previously a number of methylotrophic strains are identified as engaging effectively in climate change and reducing greenhouse gas emissions (Kappler and Nouwens 2013; Jhala et al. 2014, 2015; Oshkin et al. 2014; Baesman et al. 2015).

4.3.1 Methanotrophs

It is thought that microorganisms control global methane emissions more directly than CO_2 emissions. Microbial methanogenesis is performed by anaerobic archaea community in oceans, wetlands, rumens, and termite intestines, dominates natural emissions (~250 million tons CH_4 per year). Such biotic sources, however, are surpassed by emissions from human actions (primarily rice farming, fossil fuel extraction, landfilling, and livestock farming) (~320 million tons of methane annually), that, in addition to fossil fuel emissions, also account for emissions primarily from microorganisms.

Methanotrophic bacteria play important role in controlling high levels of methane in some of these environments. Methanotrophs are broadly categorized into two types: Type I methanotrophs or low-affinity methanotrophs primarily belong to gammaproteobacteria class, usually consume large percentage of CH_4 produced in soils prior it escapes in atmosphere. The Type I methanotrophs are present in the Methylococcaceae family that includes the genera *Methylococcus*, *Methylobacter*, *Methylomonas*, *Methylosphaera*, *Methylothermus*, *Methylosarcina*, *Methylocaldum*, *Methylosoma*, and *Methylohalobius*. Methanotrophs can also act as a net CH_4 sink for CH_4 , which is already in the atmosphere. Type II methanotrophs or “high-affinity” methanotrophs belong primarily to Alphaproteobacteria class. They make

an important contribution to the annual reduction of about 30 million tons of CH₄ from the atmosphere. Type II methanotrophs of the genera *Methylosinus* and *Methylocystis* are included in *Methylocystaceae* family. Other Type II genera (in the family *Beijerinckiaceae*) are *Methylocella* and *Methylocapsa*. *Methylocella silvestris* is the first studied facultative methanotroph, which can use either methane or multi-carbon compounds (Dedysh et al. 2005; Theisen et al. 2005). Thermotolerant species of *Methylococcus* and *Methylocaldum* are frequently called as Type X methanotrophs because their biochemical, physiological, and phylogenetic features are different from other Type I methanotrophs.

Our knowledge of the greenhouse gas cycle for CH₄ is more comprehensive than for CO₂ or N₂O because it involves an easy pathway and advanced microorganisms. Since large amount of methane in atmosphere is produced by microorganisms, a large share of CH₄ emissions from terrestrial ecosystems can be regulated technically by the management of microbial community's structure and process. Methanotrophic microbial oxidation of methane is just about 5% of the worldwide sink of atmospheric methane (approximately 30 million tons annually) and thus appears to be less significant. Methanotrophs, however, also oxidize about 90% of methane produced in the soil prior escaping into atmosphere. Transformation of farmland or grassland into forests leads to a significant decrease in CH₄ flux and it is obvious that both the type and frequency of methanotrophs are predictive of CH₄ flux. Future study therefore needs to concentrate on including these information and relationships to enhance CH₄ fluxes in different ecosystems. This understanding can also be used to reduce methane emissions through land use and management modifications. Methanotrophs, for instance, performed a major role in absorbing portion of the generated methane in rice cultivation. This would strengthen the control of flood frequency and duration by increasing the availability of soil oxygen. There is also considerable prospective for using methanogenesis inhibitors, like ammonium sulfate fertilizers, in controlled systems for promoting sulfate reducers growth at the cost of methanogens. Reducing methane emissions from ruminants includes approaches to improve feed quality and inhibit rumen methanogens by employing antibiotics, vaccines, etc.

Microbial use of CH₄ occurs both aerobically and anaerobically. Aerobic methanotrophs can be distinguished in three main groups: Group I (Gammaproteobacteria; also denoted as Type I and Type X) (Anthony 1982; Semrau et al. 2010), Group II (Alphaproteobacteria, also known as Type II and Type III) (Dedysh et al. 2001), and Group III (Verrucomicrobia, frequently known as Type IV) (Murrell and Jetten 2009). They occur in diverse environments and play a significant role in methane oxidation in nature. All recognized aerobic methanotrophs employ methane monooxygenase (MMO) which catalyzes first oxidation step which transforms methane into methanol (Semrau et al. 2010). Methanol is then oxidized to formaldehyde and can then be transformed to biomass or further oxidized to CO₂. Two iso-enzymes of MMO are recognized: cytoplasmic soluble MMO (sMMO), present in only a subset of methanotrophs, and membrane bound (or particulate) MMO (pMMO), that is nearly present in all known methanotrophs. Because of their distinctive microbiological and metabolic characteristics,

methanotrophs are of excellent concern for their industrial application. Research on methanotrophs has enhanced considerably with the fast growth of science and technology since the first methanotroph was isolated in 1906.

4.3.2 Biodiversity of Methanotrophs

In the environment, methanotrophs tend to be ubiquitous and may be isolated from different environments where methane and oxygen are present. Methanotrophs are widely distributed in the environment, including different extreme environments, and can grow at temperatures up to 4 °C (Bowman et al. 1997) or up to 72 °C (Bodrossy et al. 1999). While the first Methanotroph, *Bacillus methanicum* was isolated back in 1906, the basis for the existing classification of these bacteria was not established until Whittenbury et al. (1970) isolated and described over 100 new methane using bacteria. Since 1990s, several extremophilic methanotrophs were discovered from extreme environments, including alkaliphiles (Khmelenina et al. 1997; Sorokin et al. 2000; Kaluzhnaya et al. 2001), acidophiles (Dedysh et al. 2000, 2002, 2005; Dunfield et al. 2007; Pol et al. 2007; Islam et al. 2008), thermophiles (Bodrossy et al. 1999; Whittenbury et al. 1970; Malashenko et al. 1975; Bodrossy et al. 1995, 1997; Tsubota et al. 2005), psychrophiles (Omelchenko et al. 1993, 1996; Bowman et al. 1997), and halophiles (Lidstrom 1988; Fuse et al. 1998; Heyer et al. 2005).

Research has shown a diverse variety of methanotrophs are found in rice fields, comprising *Methylomonas*, *Methylococcus*, *Methylobacter*, *Methylocaldum*, *Methylomicrobium*, *Methylocystis*, and *Methylosinus*. Several factors, like supply of oxygen and rice growth time, influence the diversity of methanotrophs in rice fields (Eller and Frenzel 2001). Several methanotrophs are present in landfills, like *Methylobacter*, *Methylomicrobium*, *Methylocystis*, *Methylosarcina*, and *Methylosinus*. Factors like temperature, oxygen, pH, earthworms, moisture affect the diversity of methanotrophs in landfills (Gebert et al. 2003). A number of methanotrophs have been detected in freshwaters and sediments, mainly Type I methanotrophs like *Methylobacter*, *Methylomonas*, *Methylosarcina*, *Methylosoma*, and *Methylococcus*.

4.3.3 Catalytic Properties of MMOs

Methane monooxygenases (MMOs) catalyze the first oxidation step, i.e., oxidation of methane to methanol, in methanotroph metabolism. While the methane C–H bond is strong and extremely stable, under favorable conditions MMO can break this bond (Dalton 2005). Two genetically unrelated MMOs are produced by Methanotrophs: soluble MMO (sMMO) is expressed by a subset of methanotrophs and membrane bound, particulate MMO (pMMO) is expressed in all methanotrophs with the exception of the genus *Methylocella* (Theisen et al. 2005). Indeed, cultures expressing pMMO usually display greater affinity toward methane in comparison

to cells expressing sMMO. In addition, it is known that cells that use pMMO for growth show higher growth yield, indicating more effectiveness of pMMO for methane oxidation (Leak and Dalton 1986). pMMO is found in complex internal membrane structures, referred as intracytoplasmic membranes (Anthony 1982; Semrau et al. 2010). Both types of enzymes exhibit wide substrate specificities, particularly sMMO, that can oxidize alkanes, alkenes, aromatics, alicyclics, ethers, heterocycles, and ammonia. Several methanotrophs like *Methylobacterium album* BG8 and *Methylomonas methanica* have only the pMMO, *Methylococcus capsulatus* Bath, *Methylosinus trichosporium* OB3b and some others can express both forms (Hanson and Hanson 1996) depending on the amount of copper in the medium. Expression levels are associated with intracellular concentrations of copper in species that have genes for both sMMO as well as pMMO, a mechanism called the “copper switch” where sMMO is produced at low levels of copper while pMMO expression is slightly upregulated when copper is available, sMMO expression is downregulated (Nielsen et al. 1997; Murrell et al. 2000; Kenney et al. 2016). Active sites of the enzymes reveal this metalloregulation: sMMO includes a non-heme diiron active site and pMMO contains a copper active site (Rosenzweig et al. 1993; Lieberman and Rosenzweig 2005; Balasubramanian et al. 2010). While several characteristics of the role of sMMO are known, the current knowledge of pMMO is less known.

4.4 Role of Methanotrophs in Mitigating Methane Emission

Combined with a comparatively brief atmospheric lifetime (about 10 years) compared to CO₂ (50–200 years), the elevated global warming potential of methane demonstrates that mitigation impacts can be accomplished comparatively rapidly (Lelieveld et al. 1998; Breas et al. 2001). Furthermore, the disparity between methane sources and sinks in the worldwide budget accounts for <6% of total global methane sources, that indicates that a slight reduction in methane emissions could stabilize atmospheric methane or possibly lead to substantial stabilization or reduction of concentration of methane in the atmosphere (Lelieveld et al. 1998). Unfortunately, due attention has not yet been paid to control methane emissions, and after nearly a decade of modest changes in atmospheric methane, global atmospheric methane regained growth relative to the mole fraction from early 2007 (Rigby et al. 2008). This will be an important issue for climate change, and this field of research must be given greater attention. The maximum CH₄ released into the atmosphere is around 520 Tg⁻¹ of which 90% oxidizes by photochemical processes in the troposphere and 10% is removed biologically from soil and water, primarily by methanotrophs (Hanson and Hanson 1996; Breas et al. 2001). Approximately 70% of the world’s methane emissions are believed to be anthropogenic (IPCC 1996). Landfill gas and coal mine fuel are two major sources of methane emissions from human activities and therefore considered as significant targets for methane mitigation.

Methanotrophs possess considerable ability for applied microbiology and bioprocess engineering, along with bioremediation of pollutants like halogenated hydrocarbons by co-metabolization by MMOs, biotransformation of various organic substrates, methane assimilation for mitigating greenhouse effects, and production of commercially important compounds (e.g., unicellular protein, astaxanthin, and poly- β -hydroxybutyrate). Therefore, for their industrial applications, engineering on methanotrophs is very essential. Methanotrophs have significant role in the global carbon cycle as they are the only biological methane sink. Methanotrophs can also use several different toxic organic compounds. Such characteristics illustrate that methanotrophs are useful in environmental biotechnology.

4.4.1 Mitigation of Methane Emissions from Landfills

Landfills around the world discharge almost 10% of CH₄ into the atmosphere (Breas et al. 2001) and are considered significant anthropogenic methane sources. In general landfill gas composition involves 30–70% (v/v) methane, 20–50% (v/v) CO₂, nitrogen, oxygen, ammonia, sulfur compounds, hydrogen, carbon monoxide, and trace quantities of various volatile organic compounds like dichloromethane, vinyl chloride, tetrachloroethylene, toluene, benzene, and xylene (Nikiema et al. 2007). Landfills have several advantages, like the ability to oxidize different concentrations of methane and toxic contaminants. Different biological systems are created to decrease methane emissions to landfills by offering optimal circumstances for microbial procedures and effective transport of landfill gas to locations where it is to be produced. Huber-Humer et al. (2008) studied the different bio-based process designs for daily use, comprising transient or long-term biocovers, passively or actively aerated biofilters, biowindows, and biotarps. Biofilters can be more practical owing to their small footprint and elevated removing capability for gases due to the growing use of gas collection systems. Latest IPCC evaluation report identifies biocovers and biofilters as important mitigation techniques that should be commercialized before 2030 (IPCC 2007a, b). Nikiema et al. (2007) reviewed effect of various parameters like different filter beds, operating conditions, inlet loads, elimination capacities, and conversions on methane biofiltration.

There are important variations in methanotrophic reaction to varying levels of methane. Some methanotrophs in upland and forest soils possess strong affinity to methane as they start uptaking methane at low levels and thus consuming methane from the atmosphere (Henckel et al. 2000; Horz et al. 2005). Some low-affinity methanotrophs do not work until the methane amount reaches 0.8–66 $\mu\text{mol l}^{-1}$ (Huber-Humer et al. 2008). Nitrifying bacteria also capable of oxidizing methane, but the removal rates are <5% of that of methanotrophs (Bodelier and Frenzel 1999). Some methanol oxidizing bacterial consortia are also capable of oxidizing methane only when concentration of methane is below 10% (Hughes et al. 2002).

In addition to the biological system configurations and the landfills characteristics, their waste, environmental, and operational parameters also have a major impact on the microbial methane mitigation rates. These parameters are

nutrients (Nikiema et al. 2005; Albanna et al. 2007), filter bed type (Dobbie and Smith 1996; Humer and Lechner 1999; Wilshusen et al. 2004; Haubrichs and Widmann 2006), temperature (Whalen et al. 1990; Cai and Yan 1999; Visvanathan et al. 1999; Borjesson et al. 2004; Kettunen et al. 2006), moisture content (Borjesson et al. 1998; Visvanathan et al. 1999; Albanna et al. 2007), and pH (Bender and Conrad 1994; Syamsul Arif et al. 1996). Therefore, for controlling landfill methane emissions, engineering and standardization of the methanotrophic bioprocess is essential.

4.4.2 Mitigation of Methane Emissions from Coal Mines

Methane is released from operational and inactive underground and surface mines due to post-mining practices such as coal processing, storage, and transport. It has been measured that in the year 2010 global methane emissions from coal mines were approx. 584 MMTCO₂, representing 8% of overall global methane emissions. Coal mine gas is a complex mixture having higher methane concentrations. In addition to the greenhouse effect, coal mine gas may also cause heavy explosions. In China, more than 80% of heavy coal mine accidents are due to coal mine explosions, which account for one-third of total national deaths (Song and Wang 2005). Therefore, the control of mine gas is necessary for the mitigation of greenhouse gases and for the safe coal mining management for the energy sector (<http://www.chinasafety.gov.cn>).

The biological process employing methanotrophs can be more economical and effective in comparison to seam gas drainage and air ventilation that are presently the main efficient approaches for controlling coal mine gas. Biofiltration is a safe and less expensive technology because it uses microorganisms for oxidation of methane at ambient temperature to carbon dioxide and biomass. In the last decade, methane biofiltration approach is considered for treatment of effluent gases produced during landfill and animal husbandry activities, where gas flow levels are relatively low (Cloirec et al. 2001; Nikiema et al. 2005; Gebert and Gröngroft 2006; Dever et al. 2007). Methane removal studies with packaging materials like polypropylene Raschig rings (Apel 1991), glass tubes (Sly et al. 1993), mature compost (Nikiema et al. 2005), gravel (Nikiema et al. 2007; Girard et al. 2011) have shown comparatively slow conversion. A bench-scale bioreactor has been employed to study the ability of *Methylomonas methanica* for removing methane from coal mines, and 90.4% of methane was removed in a 35% methanol-air mix in 24 h (Apel 1991). At flow rates of between 0.2 and 2.4 L min⁻¹ at 30 °C, a laboratory-based coal-packed biofilter has been developed and moderately methane is removed from humidified air. Oxidation of methane was catalyzed by a diverse microbial community, the most prevailing of which was recognized as *Methylocystis* by the 16S rRNA sequencing. Furthermore, *Methylosinus sporium* was also tested in parallel. The results indicate that low-cost coal packaging has a favorable potential as an effective growth surface and contains methanotrophs for methane removal (Limbri et al. 2014).

Detecting the methanotrophs source in the environment is important for basic and applied research. Using culture-independent molecular biological methods, the

diversity of methanotrophs present in alkaline soil of a Chinese coal mine was investigated. A wide variety of methanotrophs along with several uncultivated methanotrophs were reported (Han et al. 2009). The occurrence of viable aerobic methanotrophs in geological coal- and lignite-bearing formations at substantial depths has been proved (Mills et al. 2010; Stepniewska et al. 2013). Such results are significant to the mining industry in particular. Methanotrophs are exceptionally efficient in removal of methane under optimal conditions, for instance, the removal of the methane released in the Gulf of Mexico during the 2010 Deepwater Horizon oil spill (Kessler et al. 2011). A strict control of global emissions of greenhouse gas and further progress in research into methanotrophs can allow these microorganisms to be used in the near future to control coal-gas emissions and explosions.

4.5 Engineered Strategies for Methane Removal

Various strategies may be used, taking into account the stimulation of methanotrophic activity for methane reduction in the atmosphere. The renowned engineered method engaged in this operation is “Biofilters or biocovers,” Biocovers are usually organic substances in which the permeable materials, like compost, sewage sludge, or wood chips, cover the landfill surface. These materials are characterized by effective gas transport for methane coming out from landfills and atmospheric oxygen, as well as the ability to retain water for methanotrophic activity (Huber-Humer et al. 2008; Scheutz et al. 2009).

Knief and Dunfield (2005) isolated two methanotrophic strains, which contributes to the reduction of CH_4 over a 3-month period. However, in the atmospheric concentration of CH_4 , these strains are unable to grow and require unique conditions. Surprisingly, a new strain of *Methylocystis* that can grow at 10 p.p.m.v. CH_4 has been found. Further studies of this strain indicate that this cell has two pMMO isozymes, pMMO1 and pMMO2, resulting in the finding that pMMO2 oxidation of CH_4 is more efficient than pMMO1. twenty-first century scientists are now using biotechnology to effectively improve methanotrophic bacteria, i.e., to control methane emissions, bioremediation, biobleaching, and known methanotrophic biofilters for reducing methane in landfills (Scheutz et al. 2009; Semrau et al. 2011).

Important biotechnological factors, like growth rate, yield, and genetic tractability, were reported for limited methanotrophs. Therefore, today majority of metabolic engineering attempts are focusing on well-known species like *Methylococcus capsulatus* Bath, *M. trichosporium* OB3b, and *Methylocystis* spp. Nevertheless, recent attempts in the cultivation and characterization of new methanotrophic organisms have led to the isolation of a number of strains, which can give a wider variety of biotechnology potential applications. Several number of extremely psychrophilic, thermophilic, alkaliphilic, acidophilic, and halophilic methanotrophs have been identified, thereby increasing the physiological range of aerobic methanotrophy (Kalyuzhnaya et al. 1999; Trotsenko and Khmelenina 2008; Murrell 2010). Growth factors of methanotrophs differ considerably, covering a wide range

of pH (1–10), temperature (4–65 °C), and salinity (0–10%) (Trotsenko and Khmelenina 2008).

While methanotrophs have shown promising potential in methane mitigation and biodegradation of hazardous contaminants, but from a biochemical engineering point of view, still several significant limiting factors remain, like unavailability of desirable methanotrophic strains with better controlled metabolic activity, complexity in the design and control of microbial consortia, low methane and oxygen solubility in aqueous phases resulting in slow growth, competitive inhibition among growth substrate (methane) and co-metabolites. Molecular ecology research indicated that there are far more methanotrophs exist in the ecosystem than documented in culture collections. Enrichment and isolation of novel methanotrophs, especially from more extreme environments, require considerable efforts. It is also necessary to address the nature of non-cultivated methanotrophs that grow in atmospheric methane concentrations. Extremophilic methanotrophs capable of growing at very high or very low pH levels give a means to investigate the mechanisms through which such methanotrophs sustain at extremes.

In technical applications, a microorganism's big biodiversity and exploitability often demonstrate its excellent potential. The earlier results show that for their industrial application we have plenty of methanotrophs available though their diversity is overlooked. New methods are required for better understanding the molecular mechanisms of their adaptation to different stresses such as pH, temperature, salinity, and diverse chemicals. Comparative genomics and proteomics will give insights about their adaptation to extreme situations and on the growth and phylogeny of methanotrophs. However, recent developments in methanotrophic physiology lead to new possibilities for exploiting their machinery. Still there are significant gaps in the fundamental knowledge of methanotrophy. With the development of new approaches several significant breakthroughs in this field will be achieved in the coming years.

4.6 Conclusions

Global warming is seen as a major environmental issue. The increase in the consumption of fossil fuels and deforestation leads to the anthropogenic greenhouse gas emissions contributing to global warming. Methane accounts for approximately 16% of the greenhouse effect among these gases. Methanotrophs possess the remarkable potential to use methane as their only carbon and energy source. Methanotrophs are found in diverse environments and have significant role in methane mitigation. The resources are available for manipulating methanotrophs and new methanotrophic strains are now available to extend the metabolic features for industrial biotechnology. Hence, it is necessary to adopt various strategies that may have practical importance in stimulating the methane sink activities by methanotrophs to mitigate the problem of global warming due to methane.

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