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Insight into Methanobiology and Role of Emerging Technologies in Methane Management

Abhishek Singh ^{a, *}, Karen Ghazaryan ^a, Hasmik S. Movsesyan ^a, Athanasios T. Alexiou ^b, Abdel Rahman Mohammad Al Tawaha ^c, Neha Chakrawarti ^d, Ragini Sharma ^e, Shreni Agrawal ^f, Omkar Singh ^j, Uday Pratap Shahi ^h

^a Faculty of Biology, Yerevan State University, Yerevan, Armenia

^b Novel Global Community Educational Foundation (NGCEF), Hebersham Hebersham, Australia

^c Department of Biological Sciences, Al Hussein bin Talal University, Maan, Jordan

^d Department of Genetics and Plant Breeding, Govind Ballabh Pant University of Agriculture and Technology, Pant-nagar, India

^f Punjab Agricultural University, Ludhiana, Punjab, India

^j Department of Biotechnology, Parul Institute of Applied Science, Parul University, Vadodara, Gujarat, India

^h Department of Soil Science, College of Agriculture, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, India

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Abstract

Methane (CH₄) is produced by a number of natural processes that add to the global CH₄ budget in various ways. A change in the planet's climate can be influenced by CH₄ if there is a surplus or deficit in the CH₄ budget. Major contributors to atmospheric CH₄ levels include wetlands, paddies, animals, industrial facilities, and fossil fuels. CH₄ is emitted from wetland and rice field ecosystems due in large part to the activity of methanogen microbes. CH₄ emission is affected by several variables, including the level of the water table, the average temperature, and the composition of the local vegetation. Understanding the temperature response of microbial methanogenesis in anaerobic soils is crucial for predicting the feedback between this potent greenhouse gas and climate change. It was the bacterial and/or archaeal community structures that determined the methanogenic function of the soil, which in turn was determined by the incubation temperature, albeit to a large extent on an individual basis for each soil. Different taxonomic community structures in the various soils and at various temperatures indicated that there was quite a bit of functional redundancy between them.

Keywords: methane, methanogen, anaerobic soil, temperature, vegetation.

* Corresponding author

E-mail addresses: intmsc.abhi@gmail.com (A. Singh)

1. Introduction

Among the gases contributing to green house effect, methane (CH_4) is a prominent one (Singh, Gupta, 2016). Rice farming, waste management, energy consumption, biomass burning, wetland, livestock, landfills, and others contribute to CH_4 emissions (Dlugokencky et al., 2011; Kirschke et al., 2013; Saunio et al., 2016). Along with this, CH_4 is one of the widely present reducing compounds in the atmosphere, which has significant effects on the carbon cycle of the earth, which is the key to maintaining the balance between inorganic and organic carbon pools in the atmosphere, hydrosphere, terrestrial biosphere, and geosphere (Dean et al., 2018). Marine and terrestrial biosphere can be fixed the oxidized form of carbon which is carbon dioxide (CO_2). Organic material degradation results in the conversion of biomass carbon matter into CH_4 (Bhatla, Lal, 2018). This conversion in turn, depends on environmental conditions. CH_4 gas has the ability to absorb infrared radiation 30 times stronger than other greenhouse gases like carbon dioxide. After becoming stimulated, it absorbs the earth's infrared radiation and begins to release heat into the atmosphere in all directions (Nema et al., 2012). As CH_4 concentration is less than carbon dioxide in the atmosphere, its life span is about 8 years and does not have a prominent effect. However, small changes may significantly affect the greenhouse effect (Tiwari et al., 2020). CH_4 has two major oxidation pathways. In the first one, oxidation of CH_4 occurs photochemically in the atmosphere, and in a second way, oxidation occurs biologically in the terrestrial and aquatic regions. Central biological system on earth, like ocean, grasslands, and desert, acts as a sink for CH_4 , while sources produce CH_4 like, wetlands, rice fields, grazing land of animal and landfills (Ward et al., 2004). Transplanted rice fields and wetlands are significant sources of CH_4 emissions. Some peculiar characteristics like water table, temperature, and organic material aid the CH_4 production in these two areas. Wetland and rice farming is dependent on water. Without water, the existence of wetland ecosystem and rice farming are not possible. The drought-like conditions cause reduced CH_4 production in wetlands (Bubier, Moore, 1994). Environmental temperature has direct effect on wetland and rice field soil temperature. The growth and development of methanogens varies with soil temperature (Lorius et al., 1990; Petit et al., 1999; Quiquet et al., 2015; Renssen et al., 2018). The optimum temperature range for growth of these methanogens can be determined under controlled laboratory conditions for methanogenesis, methanotrophy, and soil respiration but the same temperature-related interpretation is not possible in the field conditions (Serrano-Silva et al., 2014; Singh et al., 2018). The optimum range temperature for the temperate zone lies between 25 °C to 40 °C, and in cold subarctic conditions the growth temperature is between 20 °C to 25 °C (Hanson, Hanson, 1996; Whalen, 2005; Inglett et al., 2011). The availability of carbon, microbial activity, and the respiration of subterranean plant organs all affect how much CH_4 is emitted as a function of temperature (Inglett et al., 2011; Singh et al., 2018). In few cases, the activity of methanogens has been identified at 0 °C, where the earth surface freezes, trapping the expelled gas under or into the ice (Singh et al., 2018; Zheng et al., 2018). Organic material act as the largest store for carbon that affects the soil properties and is also required for the development of microorganisms and plants (Six et al., 2002; Quéré et al., 2018; Wiesmeier et al., 2019). Microbial decomposition of plant, animal cells, and tissues result in the formation of Soil organic matter (SOM) (Singh et al., 2018). Quality and quantity of SOM regulate its decomposition (Dušek et al., 2020). Lack of available atmospheric oxygen in wetlands reduces soil's oxygen rate, which is important for biological and chemical oxidation (Bozkurt et al., 2001; Ulmer, 2002; Reddy, DeLaune, 2008; Duval, Radu, 2018). It has been reported in scientific studies that CH_4 production enhances with increase in soil organic matter concentration. This in turn, allows the potential increase in CH_4 efflux from the soils into the atmosphere (Dušek et al., 2020); water (Crozier, DeLaune, 1996) and rice fields (Annisa et al., 2017) have such type of condition. Methane emission in wetland is also regulated by the presence of vegetation in it (Turetsky et al., 2014). The major biological processes like photosynthesis and decomposition, and accumulated plant biomass are the major sources of carbon compounds which acts as the nutrient for the process of methanogenesis which ultimately leads to CH_4 production (Updegraff et al., 1995; Ström et al., 2012). Vegetation helps in the transport of oxygen via aerenchyma into anoxic soil layers also bypasses oxic soil layers which support rhizosphere CH_4 oxidation (Schimel, 1995; King et al., 1998).

2. Methanobiology

2.1. Methane-producing bacteria

Methanogens are obligate CH_4 -producing Archaea and can't survive in an aerobic environment. Anaerobic respiration culminates in methanogenesis, the final step in the food chain (Figure 1) (Deppenmeier et al., 2002; Hedderich, Whitman, 2006). The methanogens are a single, ancient, monophyletic descendent within the phylum Euryarcheota. There are 3 classes, 6 orders, 12 families, and 35 genera to describe these organisms (Nazaries et al., 2013; Gu et al., 2022). The rice cluster I (RC-I) of methanogens was discovered by culture-independent methods in rice roots (Großkopf et al., 1998; Lueders et al., 2001; Nazaries et al., 2013). Although RC-I is primarily found in tropical forests, its members can be found in a variety of other ecosystems. Genetic analysis of 16S rRNA and *mcrA* (encoding methyl-coenzyme M reductase) revealed that these organisms belong to different clades of the Methanomicrobiales and Methanosarcinales radiation (Großkopf et al., 1998; Lueders et al., 2001; Nazaries et al., 2013). A new order, Methanocellales, was recently established after RC-I members were isolated in pure culture (Sakai et al., 2007). Also, the Methanoregulaceae family was established within the Methanomicrobiales order to include the newly described *Methanoregula*, *Methanosphaerula*, and *Methanolinae* (Großkopf et al., 1998; Lueders et al., 2001; Nazaries et al., 2013).

Although methanogens tend to thrive in moderate temperatures, some methanogen genera can thrive in much more extreme conditions, such as hot springs, hypersaline deposits, and marine geothermal sediments. The major substrates for methanogenesis, acetate, formate, hydrogen (H_2), and carbon dioxide (CO_2), are produced by syntrophic bacteria through the fermentation of simple sugars and fatty acids that are broken down by methanogens into the environment by other anaerobes. When methanogens efficiently use H_2 and formate, they are merged by such a syntrophic consortium that includes acetogens (acetate-producing bacteria) (Stams, 1994; Stams, Plugge, 2009).

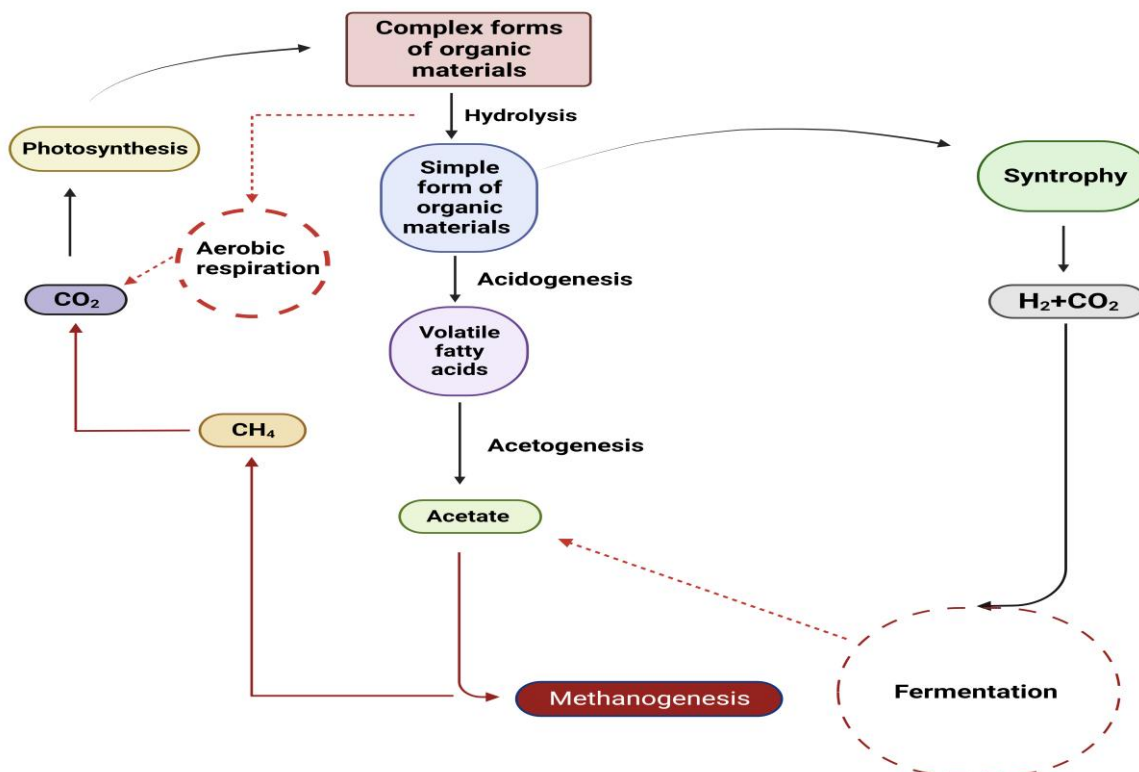


Fig. 1. A biological cycle of natural CH_4 production. The diagrammatic representation shows CH_4 formation through different raw materials in aerobic respiration and fermentation conditions

Due to their need for H₂ as an electron donor and concomitant necessity for intimate interactions with H₂-producing bacteria, most methanogens are H₂-consumers (Stams, 1994; Stams, Plugge, 2009; Renssen et al., 2018).

2.2. Methane formation pathways and key enzymes

The final steps of CH₄ production are similar across the three pathways (Figure 1), despite the fact that the intermediates and enzymatic reactions are distinct for each. A carrier-bound methyl intermediate is formed in both the hydrogenotrophic and acetoclastic pathways (Ferry, 2010; Nath et al., 2021). In the hydrogenotrophic pathway, the carrier protein is H₄MPT; in the acetoclastic pathway, the carrier protein is tetrahydrosarcinapterin (H₄SPT), a derivative of H₄MPT. Methyl-CoM is reduced to CH₄ by the key enzyme methyl-coenzyme M reductase (MCR), and all three pathways require a specific, membrane-bound methyltransferase (MTR) to transfer the methyl group to CoM (Thauer, 1998; Nath et al., 2021). The active site of MCR is a porphinoid nickel (Ni) complex called coenzyme F₄₃₀, which is contained within a dimer of the three subunits a (McrA), b (McrB), and g (McrG) (Nagle, Wolfe, 1983; Zhou et al., 2013). The enzyme appears to have a molecular mass of around 300 kDa, isolated two isoenzymes of methyl-CoM reductase. Methyl reductase two (MRT), also known as methyl reductase, is an enzyme with a unique substrate affinity (Bonacker et al., 1993; Nolling et al., 1995). The *mcrBDCGA* operon encodes MCR activity, while the *mrtBDGA* operon encodes MRT (Thauer, 1998). The *mrt* operon lacks a gene that would normally be located in the *mcrC* locus (Pihl et al., 1994; Nolling et al., 1995). All three proteins encoded by the *mcrC*, *mcrD*, and *mrtD* genes have molecular masses smaller than 20 kilodaltons the reason for this is still a mystery (Reeve et al., 1997; Lever, 2016).

Signal transduction pathways and primary sensors are still poorly understood. Nonetheless, there was proof that the availability of trace elements acted as a regulator (Hedderich, Whitman, 2006). This is because the active site of numerous methanogenesis enzymes includes trace metals (such as molybdenum, tungsten, selenium, and nickel). It was discovered that the production of key enzymes in methanogenesis, such as MRC, is controlled by the availability of the substrate H₂. Isoenzyme I of CH₄ Carbon Reductase (MCR) is highly expressed under H₂-limiting conditions, while isoenzyme II (MCR) is expressed at lower levels (Hedderich, Whitman, 2006). Methanogens' gene expression regulation is poorly understood and requires more research.

2.3. Methane oxidizing methanotrophs

Methanotrophs are gram-negative aerobic bacteria that can exclusively consume methanol or CH₄ as a source of carbon (C) and energy. These organisms, known as methanotrophs, may be found in the anoxic/oxic boundary of many different ecosystems, such as sediments, soils, peat bogs, wetlands, and geothermal reservoirs. Here, they absorb the CH₄ generated by methanogenesis and lower its emissions. (Whalen et al., 1990). These methanotrophs, also known as low-affinity methanotrophs, are able to oxidise extremely high CH₄ (> 100 ppm) concentrations, and many of them are amenable to laboratory cultivation. High-affinity methanotrophs, on the other hand, can oxidise CH₄ even at atmospheric (low) levels (1.8 ppm) (Bender, Conrad, 1992). Although they have been identified in upland soils through molecular and biochemical technique, they have not yet been cultivated (Bender, Conrad, 1992; Lueders et al., 2001). The cultivable methanotrophs (type II and type I) are distantly related to these high-affinity methanotrophs (Pol et al., 2007; Hwang et al., 2008; D'Ambrosio, Harrison, 2022). The carbon and energy need of the vast majority of aerobic methanotrophs are met entirely by CH₄ (obligate methanotrophs). There are also facultative methanotrophs, which can develop on a variety of carbon sources (see below). Most existing aerobic methanotrophs are mesophiles (pH 6.0-8.0) and neutrophiles (pH 6.0-8.0) (Whittenbury et al., 1970). However, numerous methanotroph species have been uncovered in environments with extremely high or low temperatures, pH, or salinity (Op den Camp et al., 2009). Only two phyla, three orders, and four families of aerobic methanotrophs have been identified so far. Exactly 56 species, representing 21 different genera, have been recorded so far. Existing CH₄-oxidizing bacteria have traditionally been split into two groups, type I and type II, according to morphological, physiological, and genetic differences. Now classify them as either Gammaproteobacteria or Alphaproteobacteria. Type I methanotrophs, or gammaproteobacterial methanotrophs, are members of the family *Methylococcaceae* within the Gammaproteobacteria. In this context, we refer to type II methanotrophs (Alphaproteobacteria) as belonging to either the *Methylocystaceae* or the *Beijerinckiaceae* (Op den Camp et al., 2009). Two new *Methylomonas* species, *M. paludis* and *M. koyamae*, as well as the genera *Methylovulum* and *Methylomarinum*,

were recently added to the *Methylococcaceae* (Op den Camp et al., 2009). In addition, *Clonothrix* and *Crenothrix*, two genera of filamentous methanotrophs, are found (Stoecker et al., 2006). Despite being classified as gammaproteobacteria in studies based on 16S rRNA, when compared to other methanotrophs, *Crenothrix* has a highly diverged pmoA gene (encoding for a key polypeptide of pMMO) (Stoecker et al., 2006; Krajewska-Włodarczyk, Owczarczyk-Saczonek, 2022). Recent microbiological discoveries have added to our understanding of the evolutionary history of alphaproteobacterial methanotrophs by naming the genus *Methyloferula* and the family *Methylocystaceae* as the newest members of this group (Vorobev et al., 2011; Belova et al., 2013). Finally, three CH₄-oxidizing bacterial species isolated from geothermal environments in Italy, New Zealand, and Russia were recently identified and placed in the *Methylacidiphilum* genus of the *Methylacidiphilales* order (Dunfield et al., 2007). Methanotrophic verrucomicrobia are a distinct group of methanotrophs due to their ability to thrive in low-Ph environments, despite sharing many features with methanotrophic proteobacteria, especially alphaproteobacterial methanotrophs (Dunfield et al., 2007). Verrucomicrobial methanotrophs have been demonstrated to be able to utilize CO₂ as their only carbon source while converting CH₄ to energy (Hou et al., 2008; Khadem et al., 2010). Recent studies have uncovered more information regarding the biochemistry, physiology, and genetics of CH₄ oxidation by this group. Archaea and the Deltaproteobacteria genus members *Desulfosarcina* and *Desulfococcus* work closely together to carry out the anaerobic oxidation of CH₄ (Khadem et al., 2010). Sulfate is used as an electron acceptor in anaerobic methanogenesis (AOM) to oxidise CH₄ (Hou et al., 2008). The methanogenic Archaea (*Methanosarcinales* and *Methanomicrobiales*) and the three main groups of AMNE Archaea (ANME-1, ANME-2, and ANME-3) are all closely linked. According to a recent study, despite being considered of as obligatory methanotrophs, the ANME-1 group's contribution to the global CH₄ budget should be reevaluated since these organisms may switch to methanogenesis in sediments that produced CH₄. AOM related to denitrification was described from enriched cultures. In instance, the bacteria *Methylomirabilis oxyfera* can generate its own source of oxygen by anaerobically reducing nitrite through a unique intra-aerobic mechanism. Overall, even modest quantities of oxygen inhibit this organism (2-8 %) (Raghoebarsing et al., 2006; Hou et al., 2008). Despite the lack of an apparent intracytoplasmic membrane system, this recently discovered anaerobic denitrifying methanotroph uses a conventional aerobic methanotrophic mechanism to oxidise CH₄, as shown by genomic analysis. It has been discovered that AOM in marine environments requires both manganese and iron in addition to sulphate and nitrite as electron acceptors (Hou et al., 2008).

2.4. Methane Oxidation

Methane monooxygenase (MMO) is an enzyme that catalyses the aerobic oxidation of CH₄, two distinct forms of MMO: soluble (sMMO) and membrane-bound (pMMO) (Hansen, Hansen, 1996; Fatma et al., 2019). The reaction that these two enzymes catalyse is identical, but their mechanisms and evolutionary histories are very different (Ferry, 2010). Methanol dehydrogenase is responsible for converting CH₄ into methanol and then forming formaldehyde (MDH). Formaldehyde is crucial to methanotroph metabolism. Whittenbury and colleagues (1970) utilized two alternative assimilation methods to categorize methanotrophs as type I and type II. Gammaproteobacterial (type I) methanotrophs employ the ribulose monophosphate (RuMP) route, whereas alphaproteobacterial (type II) methanotrophs use the serine pathway (Whittenbury et al., 1970). Only 50% of the carbon at the formaldehyde level is incorporated into cellular biomass (Semrau et al., 2008). The rest is transformed into formate and subsequently into CO₂ to produce reducing power for the first oxidation of CH₄, biosynthetic processes, and energy production. As a third mechanism for carbon absorption, certain verrucomicrobial methanotrophs fix CO₂ through the Calvin-Benson-Bassham (CBB) cycle. No information exists about whether or not naturally occurring methanotrophs (*Methylocaldum*, *Methylococcus*, *Methylogaea*) use the CBB pathway (Semrau et al., 2008).

However, sMMO and pMMO use different electron donor/acceptor systems to oxidize CH₄ (Op den Camp et al., 2009). sMMO is an extremely flexible enzyme that can oxidize a wide variety of alkanes, aliphatics, and aromatic compounds (Op den Camp et al., 2009; Borrel et al., 2016). All methanotrophs, with the exception of *Methylocella* and *Methyloferula* spp., produce CH₄ using pMMO (D'Ambrosio, Harrison, 2022). Many methanotrophs, including those in the genera *Methylomonas*, *Methylomicrobium*, *Methylouvulum*, *Methylococcus*, *Methylocystis*, and

Methylosinus, contain both pMMO and sMMO. Species of *Methylocella* and *Methyloferula*, which use sMMO to grow on CH₄, are facultative methanotrophs that can Obligatory methanotrophy is an unusual metabolic strategy, but its evolutionary roots are unclear (Belova et al., 2013). Other facultative alphaproteobacterial methanotrophs, such as *Methylocapsa aurea* and *M. thermophila*, have been isolated in recent years. *Methylocystis* strain SB2 and the *bryophila* species. Like *Methylocella*, the microorganisms belong to the family *Beijerinckiaceae* and thrive in acidic soils (Im, Semrau, 2011; Belova et al., 2013; Jagadevan, Semrau, 2013). An entire list of recognized facultative methanotrophs, absorption processes for acetate, and ecological applications were identified by various researchers in the past few years (Anderson, Mccarty, 1997; Im et al., 2011; Yoon et al., 2011; Jagadevan, Semrau, 2013).

3. Factors for affecting methane emission

3.1. Hydrology

For studying wetland dynamics, the availability of water is critical. During the growth season, the flooded region would either continuously or intermittently at mean water levels of 6.6 feet or the surface would be having wet soil (Upadhyay et al., 2017). The rate of anaerobic respiration including methanogenesis, iron reduction, denitrification, and sulphate reduction is higher in wet habitats, such as flooded wetlands and aquatic wetlands than aerobic respiration, including nitrification. Oxygen gets depleted in a wetland due to continuous water saturation, causing the microbial community to shift its energy sources to different substrates. Both wet and dry periods occur in the wetland. During dry conditions, oxygen allows the aerobic pathways to work and release energy; in wet conditions, energy is released by anaerobic routes like denitrification.

3.2. Temperature

Temperature is the most crucial factor influencing methanogen activity and the rate of CH₄ emissions (Chin, Conrad, 1995; Liu, Wu, 2004). In CH₄ flow, seasonal and diurnal fluctuations both are positively associated with temperature (Schutz et al., 1989). The ideal temperature for the bulk of methanogens' functioning is between 30 °C and 40 °C (Chamarthi et al., 2011). As the temperature rises from 20-25 °C, CH₄ formation doubles and a 10 °C rise in the temperature leads to a rise in the production of CH₄ by a proportion of 2.5-3.5 (Schutz et al., 1989). As the soil temperature rises, the number of methanogenic bacteria grows slowly. Several studies have linked changes in soil temperature to CH₄ emissions throughout the growing season (Schutz et al., 1989; Tanaka et al., 2006). Methanogenesis has been seen at maxima of three seasons: the first immediately after floods, the second in the growing vegetative phase of plants, and the third is while the maturation and filling of grains. Emission of CH₄ was positively associated with the temperature of soil ($R^2 = 0.281$, $p < 0.05$) and methanogen population with a change in CH₄ flow ($R^2 = 0.82$, $p < 0.05$) in a paddy field that was inundated with N treatment by urea (Zhao et al., 2021).

The rate of CH₄ generation was lowered and the organic material breakdown pathway was altered when the optimum temperature of incubating methanogenic rice soil was decreased from 30 °C to 15 °C. Reduced causes build-up of acetate, lactate, isopropanol, and caproate by lowering the partial pressure of H₂ in steady-state (Chin, Conrad, 1995). Acetate becomes a more essential methanogenic substrate at lower temperatures. This permits the rapid growth of *acetoclastic Methanosarcinaceae*, but on increasing temperatures, fewer acetate concentrations encourage more adapted but slow-developing *Methanosaetaceae* (Chin et al., 1999). The richness of *crenarchaeota* was revealed when anoxic soils of rice fields were incubated for 1 week at 30 °C, but it took further two weeks to raise the relative abundance of *Methanosarcinaceae* (Chin et al., 1999). In a limited population of acetolactic methanogens, methanogenesis was inhibited significantly on incubating rice field soil at 50 °C shortly. A major shift in the microbial population of methanogens was generated on extended incubation at 50 °C which led to CH₄ synthesis from H₂/CO₂ with an increase in Rice cluster I methanogens (Fey et al., 2001).

4. Sources of methane emission

4.1. Wetlands

Wetland cannot be defined easily and is quite complex because wetlands have diverse biotic (for example plants, microbes, animals) and abiotic (for example water, light, radiation, temperature, humidity, atmosphere, acidity, and soil) components (Smardon, 2014). A wetland is

an area where water surrounds the land. Examples of wetlands are ponds, marshes, the edge of a lake or ocean and delta at the mouth of a river, low-lying areas that frequently flood.

Marsh plant photosynthesis plays a significant part in the sequestration of carbon, which begins to build up in plant biomass and wetland soil (Kayranli et al., 2009). Plant growth in wetlands is higher than in terrestrial habitats, although the rate of breakdown is slower (Sun, Liu, 2007). In general, relative to terrestrial ecosystems, wetland ecosystems exhibit rapid plant growth and slower rates of decomposition, both of which promote carbon storage (Sun, Liu, 2007). Wetlands' water tables significantly affect the oxidation and reduction processes, which in turn affect carbon emissions, and in wetlands, the amount of organic matter and redox potential is high (Limpert et al., 2020). The ability of soil carbon to be remineralized by a variety of microbial activities that control carbon storage and release can be facilitated by a change in water level (Olefeldt et al., 2017). This anaerobic condition is most favorable for the multiplication of methanogenic microorganisms in the soil. CH_4 -producing microorganisms are called methanogenic archaea or simply methanogens, and this biological process of CH_4 formation is known as methanogenesis (Cadena et al., 2019). Methanogens are mainly grown where oxygen availability is limited or absent and wetlands are among those places. Methanogens also use CH_4 as a terminal electron acceptor in the metabolic process; the end product of the metabolic reaction is some toxic carbon compound (Bhatla, Lal, 2018). The CH_4 released from wetland to the atmosphere depends on the combined metabolic activities of methanogenic and methanotrophic microbes in the soil (Bhatla, Lal, 2018). In wetlands, CH_4 efflux occurs in the atmosphere through plant aerenchyma, ebullition, and diffusion (Figure 2) (Limpert et al., 2020).

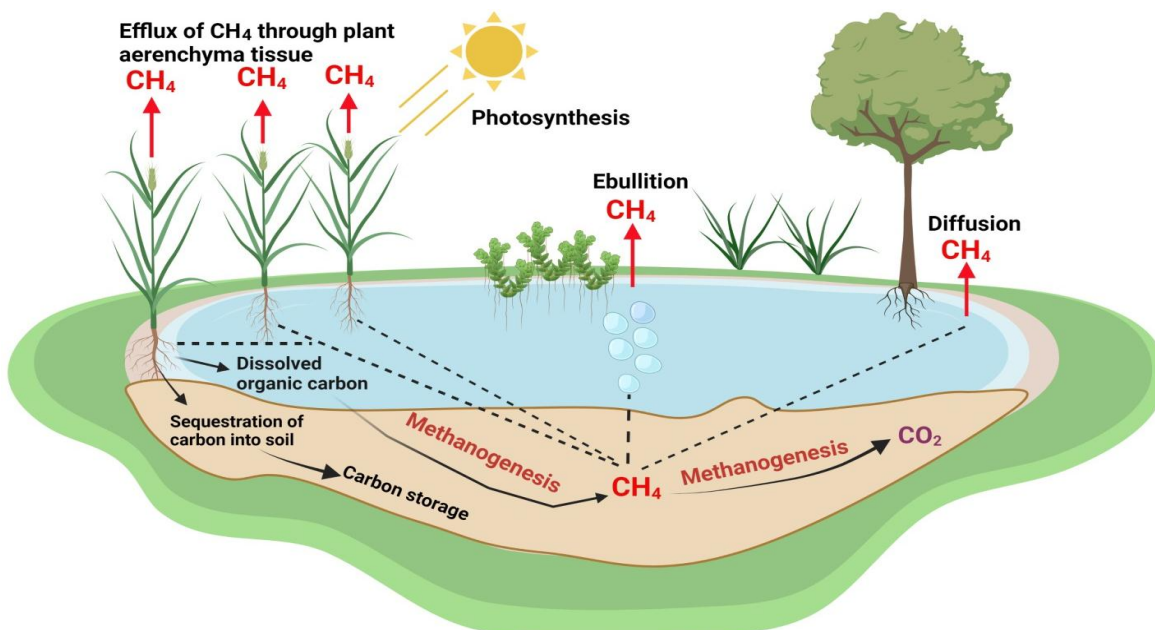


Fig. 2. Biogeochemical cycle of CH_4 emission and formation influences the water table, temperature, and vegetation

Due to the constant waterlogging providing ideal conditions for methanogenic microorganisms, wetland ecosystems are the most prolific natural sources of CH_4 . Wetlands produce 20-39 % of all atmospheric CH_4 , and their contribution could rise by as much as 50-80 % as the planet warms, according to various estimates (He et al., 2015; Koffi et al., 2020). According to the recent reports of NASA, the rates of CH_4 production in atmosphere is 1.7 ppm which vary considerably according to factors such as wetland and vegetation type, acidity, organic matter content, mineral composition, and climate (Kharitonov et al., 2021).

The ability of freshwater wetlands to absorb and store organic carbon has attracted the attention of both government and industry in recent decades due to rising temperatures caused by anthropogenic carbon emissions (Bernal, Mitsch, 2013). In freshwater wetlands, carbon storage and release dynamics can vary depending on environmental factors, making their quantification a

challenging task. Carbon is more easily stored in wetland ecosystems due to their faster plant growth and slower decomposition rates (Ramasamy et al., 2009; D'Ambrosio, Harrison, 2022). CH₄ is a potent greenhouse gas with a radiative forcing that is 87 times stronger than carbon dioxide (CO₂) over 20 years, although it can be produced in more significant amounts under anaerobic marsh conditions (Lever, 2016). However, a lot of freshwater wetlands act as net yearly carbon sinks (Bräuer et al., 2020). Multiple processes and pathways contribute to the equilibrium between carbon sinks and sources in wetland ecosystems. Due to the following factors affecting soil carbon cycling, wetlands have the potential to store significant amounts of soil organic carbon: (1) inundated soils that restrict oxygen (O₂) diffusion into sediment; (2) anaerobic conditions brought on by higher water levels that reduce decomposition rates in comparison to aerobic soils; and (3) the relative decrease in remineralization.

Numerous studies have shown that temperature and water level significantly impact the community and activity of methanogens and methanotrophs in peatlands (Jaatinen et al., 2007; Turetsky et al., 2008; Yrjälä et al., 2011; Peltoniemi et al., 2016). Predicting how warming in different moisture regimes would impact the population and activity of methanogenic and methanotrophic groups is tricky (Peltoniemi et al., 2016). The investigation of 16S-rRNA revealed specific features of the localisation of methanogens and methanotrophs inside a wetland biocenosis (Deppenmeier et al., 2002). Methanogens were more common in sample locations with higher CH₄ production, and their abundance was inversely connected with that of bacteria that reduce nitrate, sulphate, and metals. Microbial phylogeny based on marker genes and quantitative analysis of data collected by shotgun sequencing gave us more insights into the competitive interactions between methanogens and other anaerobic microbes. Anaerobic competitors have been shown to suppress methanogenesis (Krüger et al., 2005).

4.2. Soil

Cell counts in soil samples showed that methanogens and methanotrophs can live together in harmony (Dalal et al., 2008). Due to the coupling of methanogenesis and methanotrophy in aerated soils as well as the great sensitivity of the microorganisms driving these processes to environmental circumstances, there is temporal and geographical variability in the emission or consumption of CH₄ in soils. This fact must be considered when calculating the relative importance of different soil ecosystems in the CH₄ cycle (Semenov et al., 2010). Soil water regime, organic carbon, and total nitrogen are critically important to methanogenesis and methanotrophy (Semenov et al., 2019). Both microbial processes are slowed down by extremely dry soil (Strieg et al., 1992; Bender, Conrad, 1995; Brandt et al., 2015). CH₄ oxidation is inhibited by 1.2-1.3 times when soil moisture is reduced by 10 %, possibly as a result of moisture deficit stress or the accumulation of mineral nitrogen compounds in the soil (Boeckx et al., 1997). Because the size of the soil's aerobic zones is diminished by waterlogging, methanogens thrive and methanotroph populations decline. Maximum CH₄ oxidation rates have been found at moderate moisture in all soil types studied (Torn, Harte, 1996). Lower water table levels increase the CH₄ oxidation rate and, consequently, decrease the CH₄ emission rate of CH₄ oxidation and, consequently, decrease the CH₄ emission rate from the soil (Moore, Dalva, 1993). This negative logarithmic correlation has been observed for a wide range of water table depths. It was found that the CH₄ production rate increased by 12 times in the sites inundated with water as compared to sites where the water level was only 5 centimeters below the soil surface in the Arctic coastal plains (Morrissett, Livingston, 1992). These findings made sense, as a lower water table level is linked to more oxidative conditions in the soil's upper layers and promotes the diffusion of atmospheric oxygen and CH₄ to the soil. Very low soil moisture conditions also drastically reduce the rate of CH₄ oxidation (Morrissett, Livingston, 1992).

The climate in which a given microorganism thrives directly affects a direct bearing on the conditions under which it can most efficiently produce CH₄. This is supported by the fact that, as one travels southward from the north, the optimum temperature rises from 19 °C to 38 °C (Sabrekov et al., 2017). Due to decreased activity of methanogens and other microbial groups comprising the methanogenic community, the rate of CH₄ production slows down at low soil temperatures. Compared to methanogens, methanotrophs appear to be temperature insensitive. It is unclear how soil temperature affects the rate at which CH₄ is oxidized. A definite link between these factors may be seen at temperatures more than or equal to 10 °C or lower than or equal to 40

°C, likely because the activity of mesophilic methanotrophs decreases at these extremes (Castro et al., 1995; Hanson, Hanson, 1996; Semrau et al., 2010).

Because there is no information on the separation of acidophilic methanogens, the prevailing belief that methanogen activity is highest in soil at neutral or slightly alkaline pH and is particularly sensitive to changes in pH values has prevailed (Dalal et al., 2008). Methanogenesis and CH₄ emissions in acidic oligotrophic and mesotrophic bogs and lakes are geographically varied, but not considerably so, according to microbial community adaptations to local average pH (Casper et al., 2003; Horn et al., 2003; Glagolev et al., 2012; Sabrekov et al., 2013).

4.3. Paddy fields

In the world, rice is the largest growing wetland food crop. Rice is one of the most important staple foods worldwide and model species for cereals. *Oryza sativa* (Asian rice) or *Oryza glaberrima* (African rice) are the most cultivated rice. Maize (*Zea mays*) and wheat (*Triticum*) provide more than 50 % of all human calories. The world's annual rice production should have increased from 538 million tons in 1994 to 755 million tons in 2019 (The State of Food and Agriculture 2019 | FAO | Food and Agriculture Organization of the United Nations). Rice-cultivated lands are classified as deepwater, irrigated, and rain-fed upland. A flood-like situation is required in the rice field, so the paddy field requires more water for their growth and development (Singh et al., 2018). Wetland rice field is a seasonal agricultural wetland that is covered with water from sowing to ripening before the harvesting, and the requirement of water depends on growing conditions, especially water availability and solar radiation, normally short-duration varieties take 100–120 days; medium duration 120–140 days, and long duration 160 days (Figure 3) (Wang et al., 2011). Due to flood like conditions in the field from sowing to before the harvesting the diffusion of atmospheric oxygen into the soil drastically reduces. The soil redox potential shows chemical and biochemical oxidations, and reductions in the soil and the greater value of redox potential shows the higher presence of potent oxidizing agents in soil.

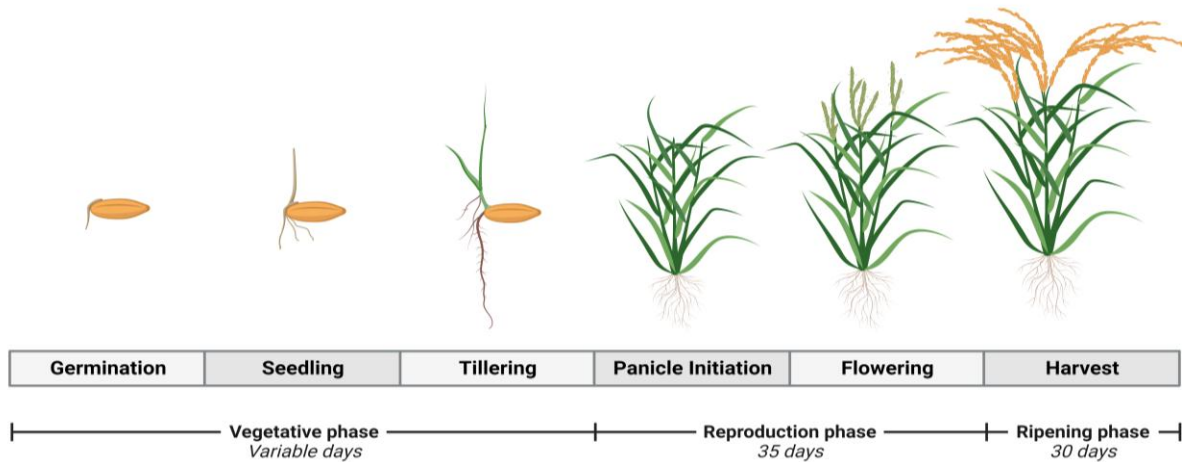


Fig. 3. Life cycle of rice paddy from germination to ripening phase. The germination and ripening days depend on a variety of rice genotypes and climates.

In anaerobic soil conditions, organic matter is degraded by various fermenting microorganisms, mostly bacteria that produce CH₄ (Smartt et al., 2016). This produced CH₄ in rice wetland soil is released into the atmosphere through the diffusion or ebullition of gas bubbles through the aerenchyma tissue of the root of the rice plant (Figure 4) (Hasan, 2013; Bhatla, Lal, 2018). CH₄ emission is directly proportional to plant biomass (Gogoi et al., 2008).

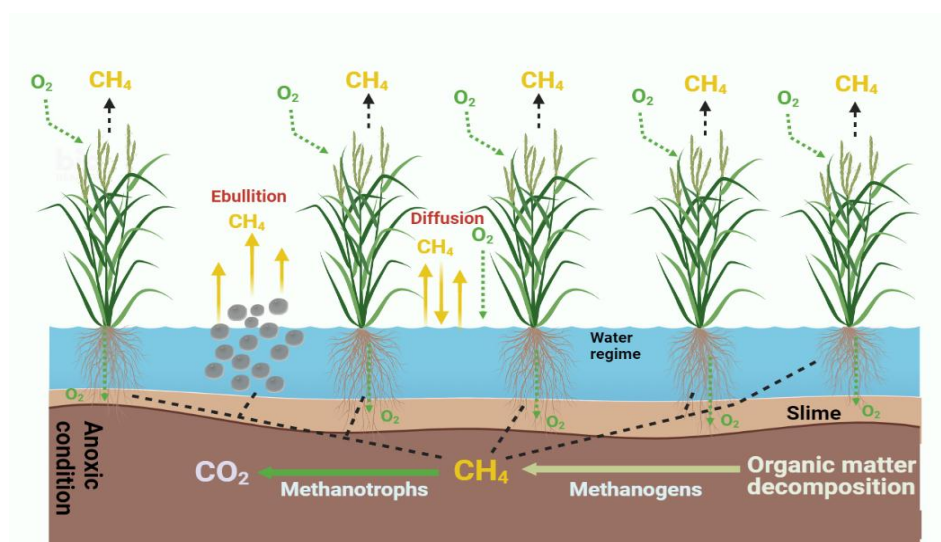


Fig. 4. Methanogens activity forms CH_4 formation in rice fields due to the decomposition of organic matter in anoxic condition. The produced CH_4 in rice wetland soil is released into the atmosphere through the diffusion or ebullition of gas bubbles through the aerenchyma tissue of the root of rice plant.

Rice fields account for 20 % of agricultural CH_4 emissions (Schütz et al., 1990; Datta et al., 2013; Ke et al., 2014; Qi et al., 2021). Fertilizers, soil temperature, redox potential, soil texture, pH, soil organic matter concentration, and soil all play a part in determining the structure of methanogenic and methanotrophic microbial communities in rice fields (Bhatia et al., 2011). The processes of CH_4 emission are affected by management practices such as rice cultivar, fertilizer application, water management, and pesticide application (Smith et al., 2010; Gutierrez et al., 2013; Ke et al., 2014; Jiang et al., 2015; Liang et al., 2016). The same applies to daily and seasonal changes, high ozone, and high CO_2 (Smith et al., 2010). A relationship between CH_4 emission and the makeup of the microbial community has been found for the rice field before its exploitation, at the time of the rice planting, and on day 120 of its growth before maturation (Knief et al., 2011). The examination of 16S rRNA sequences by RT-PCR revealed that methanogens from the genera *Methanosaeta*, *Methanocella*, *Methanosarcina*, and *Methanobacterium* made up between 68.3 % and 86.6 % of the total number of archaea in the microbial community inhabited the rice field (Anesti et al., 2005). On day 90 of the experiment, the abundance of methanogens was at its highest, having increased steadily throughout rice maturity (Brune et al., 2000). Methanotrophs made up only 0.79-1.75 percent of all 16S rRNA gene sequences, making up a much smaller percentage of the microbial community overall. Different patterns of population growth and decline were observed among the various methanotrophic representatives. After the rice was planted, there was a significant drop in the population of *Methylocystis* (type II methanotrophs), while the populations of *Methylosinus* and unclassified type II methanotrophs remained relatively stable. Before rice was sown and during its early stages of growth, type I methanotrophs (genera *Methylocaldum*, *Methylobacter*, *Methyломonas*, and *Methylosarcina*) were rarely detected. However, a peak in the population of all of the aforementioned methanotrophs has been observed between days 90 and 120 of rice development. Meanwhile, anaerobic methanotrophs were scarce, making up only 0.25-3.27 percent of the total 16S rRNA gene sequences, indicating a minor contribution from this process to the rice field soil. A ratio of *mrcA* to *pmoA* emerged as a potential parameter in a multi-factor model for predicting the precise amount of CH_4 released from a rice field.

5 Emerging technologies for management of CH_4 emission from different sources

5.1. Application of Biochar for reduction of CH_4 in rice field

Biochar has been identified as a key player in lowering greenhouse gas emissions from agricultural soils, enhancing pesticide sorption and desorption, reducing leaching loss of nutrients, enhancing soil fertility, and enhancing plant growth and crop yield (Peng et al., 2004). In Eastern Colombian Plains, it has been found that biochar has the potential to fully suppress the CH_4 emission in the soil when amended (20 g kg^{-1}) (Peng et al., 2004). Paddy field soil amendment

with bamboo and straw charcoal reduces CH₄ emissions by 51 % and 91.2 %, respectively, compared to control (Liu et al., 2011). Biochar reduces the CH₄ emission by retarding the methanogenic activity or stimulation of methylophilic activity during incubation. Reduced CO₂ emissions have also been seen in rice fields with biochar addition (Nan et al., 2021). In addition, using biochar made from mango trees resulted in a net decrease in yearly CH₄ emissions and increased soil carbon from non-fertile tropical soil (Shen et al., 2021). The biochar's physicochemical properties regulate biochar's counteraction against CH₄ emissions from paddy fields, soils, microbiological considerations, and water and nutrients management (Chen et al., 2017). Among these, soil pH is one of the most critical factors that affect rate of CH₄ emission from field of rice. Near-neutral pH methanogenic archaea seek (6.5–7.5). Because of this, they are adding bamboo char (pH 9.81) or straw char (pH 10.2) may inhibit the process of CH₄ production and, as a result, the release of CO₂ from the same field. Additionally, slower mineralization rates in soil amended with biochar was obtained due to a higher C/N ratio, which might also retain microbial biomass. Therefore, instead of utilizing straw directly, you may turn it into biochar to reduce CH₄ emissions. Before utilizing this input as a greenhouse gas mitigation approach, it is also necessary to take into account the type of biochar, the local soil quality, and environmental conditions.

5.2. Use nanoparticle base fertilizers

Crop production and bacterial community structure are believed to be primarily influenced by soil organic carbon, accessible NPK, and micronutrients (Smartt et al., 2016). The reactions of several functional microorganisms, including as denitrifiers, methanotrophs, and diazotrophic bacteria, to fertilisation in paddy soil have received attention from some researchers (Aronson et al., 2013). According to reports, paddy fields' long-term fertilization has a considerable impact on the number of diazotrophs and methanotrophs that live freely (Ferry, 2010). Although certain mineral fertilisers have been used, they have helped reduce CH₄ emission from paddy fields, but there is no apparent pattern to them, and different outcomes have been observed. Nitrate, sulphate, and ferric iron favour the corresponding methanogenesis-suppressing nitrate reducers, sulphate reducers, and iron reducers, which successfully compete for the methanogenic substrates.

One of the potential factors causally related to the greenhouse emission in wetlands is attributed to the use of nitrogenous fertilizers (He et al., 2019). Nitrogenous fertilizers are vulnerable for the loss of N due to leaching, volatilization, and immobilization that collectively contribute up to 60%. In order to reduce the loss of N and improve the N use efficiency, nano-fertilizers hold a promise and the benefits of nano-fertilizers have been reviewed (Zulfiqar et al., 2019). Since > 95 % of the Indian soils are deficient in N and the N use efficiency of crops hardly exceeds 30-35%, nano-fertilizers may be ideal for Indian soils to improve the use efficiency while minimizing the greenhouse gas emissions (Conrad et al., 2006). Due to their large surface area, nanofertilizers can retain a large number of nutrient ions and release them gradually and steadily according to the needs of the crop. In order to increase the effectiveness of nutrient utilisation while avoiding nutrient ions from becoming fixed or lost to the environment (Davamani et al., 2020) stated that nano-fertilizers and nanocomposites may be utilised to manage the release of nutrients from fertiliser granules. Nanofertilizers efficiently and with little loss transfer nutrients to rhizospheric targets. Nano-membrane-coated fertiliser particles that enable a gradual release of nutrients. This procedure increases the efficiency with which crops utilise fertiliser while reducing nitrogen loss. It is well known that nano-composites provide plants with all necessary nutrients to achieve balanced fertilisation. Because of their mesoporous structures' capacity to adsorb molecules at relatively low pressure, zeolites are promising adsorbents. They have a long history of usage in the industrial sector as adsorbents, and another intriguing feature of zeolites for the creation of nitrogen nano-fertilizer is the availability of internal space volume (Davamani et al., 2020). The zeolite-based nano-fertilizers are known to increase the efficiency of using macro- and micronutrients (Amira et al., 2015; Kah et al., 2018; Ha et al., 2019; Iqbal, 2019; Rajput et al., 2020, 2021; Zulfiqar and Ashraf, 2021; Verma et al., 2022). It is hypothesized that using nano-fertilizers helps to slow down the release of nitrate and ammoniacal nitrogen, improving rice's ability to utilize nitrogen while lowering the emissions of greenhouse gases.

6. Conclusion

The production and emission of CH₄ from wetlands and paddy fields is affected by a wide range of factors, such as climate, soil physicochemical properties, and cultural practises. The 140

million hectares of rice fields harvested yearly are subject to a wide range of environmental factors, making it impossible to employ uniform approaches to lowering CH₄ emissions. CH₄ emission from water-logged wetlands and paddy fields can be reduced through the implementation of one or more mitigation strategies, such as the management of organic inputs in soil, the prudent use of nitrogen fertiliser, enhanced irrigation practices, the use of improved crop cultivars, and so on. Understanding methanobiology is much more important for better knowledge of the mode of action of CH₄-production microbes in different environments. This scientific knowledge may help in development of more scientific technologies that help manage CH₄ emissions.

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