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Methanogenesis: Are ruminants only responsible: A review

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Abstract

Methane is a potent greenhouse gas with a global warming potential 25 times more than carbon dioxide. Methanogenesis is a multi-step process involving different group of micro-organisms like hydrolytic, fermentative, acetogenic and above all methanogenic micro-organisms. Methanogenesis is the biological production of methane mediated by anaerobic microorganisms from the domain Archaea commonly called methanogens. These methanogens are organism carrying out methanogenesis, requiring completely anaerobic conditions for growth. These methanogens are different from bacteria and eukarya as they lack peptidoglycan in their cell wall, which is present in bacteria and eukarya. Methane is produced by three major pathways on the basis of substrate utilized for methane production: (1) hydrogenotropic (2) acetoclastic and (3) methylotropic. Out of these, hydrogenotropic and acetoclastic is the predominant pathway. All three pathways have in common the demethylation of methyl-coenzyme M to methane and the reduction of the heterodisulfide of coenzyme M and coenzyme B catalysed by methyl-coenzyme M and heterodisulfide reductases. There are two major sources of methane i.e. natural and anthropogenic. The natural sources include wetlands, termites and oceans, whereas, the anthropogenic sources include fossil fuel transport and distribution, livestock, rice fields, landfills etc.

Keywords: Acetoclastic, anthropogenic, hydrogenotropic, methane, methanogenesis

Introduction

Greenhouse gases such as carbon dioxide, methane, nitrous oxide, moisture and ozone contribute to climate change and global warming by absorbing light energy in visible and infrared region ^[1]. Among green house gases methane is the second most important gas after CO₂ which contributes to global warming ^[2]. Methane makes up 16% of total global GHG emissions having 23 times more global warming potential than carbon dioxide ^[2]. Methane emissions from agriculture represent around 40% of those produced by human-related activities ^[3] and is the single largest source (25%) in enteric fermentation in livestock, mainly ruminants ^[4].

Methanogens belong to the domain Archaea under the phylum Euryarchaeota. Unlike Bacteria, methanogens lack peptidoglycan in the cell wall, replaced by pseudomurein in *Methanobrevibacter* and *Methanobacterium*, heteropolysaccharide in *Methanosarcina*, and protein in *Methanomicrobium*. Apart from cell wall there are certain other characteristics that differentiate archaea from bacteria and eukarya. Antibiotic sensitivity is absent in archaea which is present in bacteria and eukarya. The cell membrane in archaea have branched carbon chains linked with an ether linkage to the respective alcohols whereas bacteria and eukarya have unbranched carbon chains linked with ester linkage to the respective alcohols.

Sources of methane emission

There are both natural and human sources of methane emission as shown in fig 1. The main natural sources include wetlands, termites and the oceans. Natural sources create 36% of methane emissions. Important human sources come from landfills, livestock farming, as well as the production, transportation and use of fossil fuels. Human-related sources create the majority of methane emissions, accounting for 64% of the total ^[5]. Methane levels have more than doubled over the last 150 years because of human activities like fossil fuel use and intensive farming. Before the Industrial Revolution, the atmospheric concentration of methane was maintained in a safe range by natural sinks. But for a long time now human activities have been creating methane emissions much more rapidly than the Earth can remove them, increasing global methane levels.

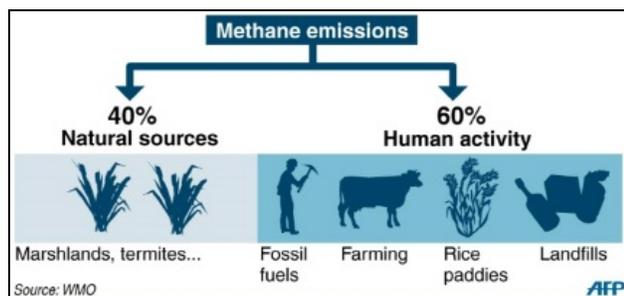


Fig 1: sources of methane emission

According to NRC (1994), methanogenesis is the production of methane by bacteria. Because they thrive without oxygen, methanogenic bacteria have an important role in the subsurface, where oxygen is often absent. It is the bacterial conversion of methanogenic substrates (acetate, formate, hydrogen, carbon dioxide) into methane. Methanogenesis is a multistep process carried out by different groups of microorganisms. The various processes and microorganisms included are:

(1) Hydrolysis

Hydrolysis is used to break down larger polymers *i.e.* it involves the breakdown of large polysaccharides. Through hydrolysis large polymers, namely proteins, fats and carbohydrates, are broken down into smaller molecules such as amino acids, fatty acids, and simple sugars. While some of the products of hydrolysis, including hydrogen and acetate, may be used by methanogens later in the anaerobic digestion process, the majority of the molecules must be further broken down in the process of acidogenesis to be used to create methane. eg *Fibrobacter succinogens*, *Ruminococcus albus* etc

(2) Acidogenesis

Acidogenesis is the next step of anaerobic digestion in which acidogenic microorganisms further break down the product of hydrolysis. These fermentative bacteria produce an acidic environment forming ammonia, H₂, CO₂, H₂S, shorter volatile fatty acids, carbonic acids, alcohols, as well as trace amounts of other byproducts. e.g. *Lactobacillus spp.*

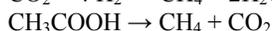
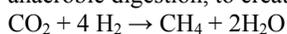
(3) Acetogenesis

Acetogenesis is the creation of acetate from carbon and energy sources by acetogens. These microorganisms catabolize many of the products created in acidogenesis into acetic acid, CO₂ and H₂. Acetogens break down the biomass to a point to which methanogens can utilize much of the remaining material to create methane. e.g. *Clostridia spp*

$$\text{CO}_2 + 3 \text{H}_2 \rightarrow \text{CH}_3 \text{COOH}$$

(4) Methanogenesis

Methanogenesis constitutes the final stage in which methanogens create methane from the final products of acetogenesis as well as from some of the intermediate products from hydrolysis and acidogenesis. There are two general pathways involving the use of acetic acid and carbon dioxide, the two main products of the first three steps of anaerobic digestion, to create methane in methanogenesis:



Based on the substrate utilized these methanogenic bacteria can be classified as:

1. Hydrogenotropic: utilizing hydrogen and formate as substrate e.g. *Methanobrevibacter ruminantium*.

2. Acetoclastic : utilizing acetate as a substrate e.g. *Methanosaeta*
3. Methylotropic: utilizing methyl groups as a substrate e.g. *Methanosarcina barkeri*.

Coenzymes for methanogenesis

Methanogenesis pathways utilize several coenzymes of which methanofuran (MF), tetrahydromethanopterin (H₄MPT), tetrahydrosarcinapterin (H₄SPT) and coenzyme M (or HS-CoM) carry the carbon moiety destined to generate methane, while coenzyme F₄₂₀, coenzyme B (HS-CoB), methanophenazine and coenzyme F₄₃₀ transfer electrons that are used in carbon reduction.

Methanogenesis pathway

The production of methane is through various pathways. The important ones include

- The predominant pathway is the hydrogenotrophic using CO₂ as the carbon source and H₂ as the main electron donor [6].
- Methane is also produced from acetate via the acetoclastic pathway eg *Methanosarcina* and *Methanotrix*.
- Formate is also an important electron donor used by many rumen hydrogenotrophic methanogens and may account for up to 18% of the methane produced in the rumen [7].
- Methylamines and methanol produced in the rumen can also be used by methylotrophic methanogens of the order *Methanosarcinales* and *Methanobacteriales*.

All the pathways have in common the demethylation of methyl-coenzyme M to methane and the reduction of the heterodisulfide of coenzyme M and coenzyme B catalysed by methyl-coenzyme M and heterodisulfide reductases.

(1) Hydrogenotrophic pathway

In this pathway carbon dioxide is captured by methanofuran (MF) to form an unstable compound called carboxy-MF which is reduced by formyl-MF dehydrogenase in an energy-dependent manner to formyl-MF with a low-potential ferredoxin serving as electron carrier. Formyl-MF dehydrogenase exists in two forms, one of which contains molybdenum and the other tungsten [8]. At the next step the formyl group is transferred to H₄MPT by a transferase enzyme to form formyl-H₄MPT. From this stage H₄MPT carries four forms of the fixed carbon representing three oxidation states. First formyl-H₄MPT is dehydrated by methenyl-H₄MPT cyclohydrolase to form methenyl-H₄MPT which in turn is reduced to methylene-H₄MPT by the action of one of the two enzymes, F₄₂₀-dependent methylene-H₄MPT dehydrogenase and a Fe-containing hydrogenase. Methylene-H₄MPT is reduced with F₄₂₀H₂ and by the action of F₄₂₀-dependent methylene-H₄MPT reductase (Mer) providing the last H₄MPT derivative on the pathway, methyl-H₄MPT. The transfer of the methyl group from methyl-H₄MPT to coenzyme M is catalyzed by a membrane-bound sodium ion (Na⁺)-pumping enzyme complex called methyl-H₄MPT: coenzyme M methyl transferase. This complex not only yields methyl-coenzyme M but also generates a Na⁺-gradient that is used for energy production [9]. The next step in the sequence yields methane. This last carbon-reduction reaction is catalyzed by CH₃-CoM reductase with coenzyme B serving as an electron source, resulting in a heterodisulfide, CoM-S-S-CoB, as product in addition to methane. The heterodisulfide is reduced by a reductase to regenerate HS-CoM and HS-CoB.

Hydrogen-oxidizing methanogens often carry two $\text{CH}_3\text{-CoM}$ reductase isozymes (McrI and McrII) one of which is effective under high hydrogen availability and the other under low hydrogen conditions.

(2) Acetoclastic pathway

About 70% of the biologically produced methane originates from acetate. The methyl group of acetate is reduced to methane and the carboxyl group is oxidized to CO_2 . The process begins with the activation of acetate by the action of acetate kinase generating acetyl-CoA. At first there is generation of ADP that is converted back to ATP via electron transport phosphorylation at an ATPase. The next step is the breakage of the carbon-carbon bond of the acetate moiety in acetyl-CoA catalyzed by an acetyl-CoA decarbonylase/synthase-carbon monoxide dehydrogenase complex. The carbonyl group of acetyl-CoA is oxidized to CO_2 by the CODH component and the two electrons generated by this process help to reduce ferredoxin. The methyl group of the acetyl group is transferred to H_4MPT via a corrinoid cofactor of the CODH complex, producing $\text{CH}_3\text{-H}_4\text{MPT}$. The methyl group of $\text{CH}_3\text{-H}_4\text{MPT}$ leads to methane via the actions of methyl- H_4MPT : coenzyme M methyl transferase and methyl-CoM reductase^[10].

(3) Methylotropic Pathway

Methanogenesis from methanol involves the formation of methyl-CoM as an intermediate. Methanol provides both carbon and reductant for methanogenesis and this process consumes four moles of methanol for every three moles of methane generated^[11]. FEMS Microbiology Reviews 23: 13-38. Of these, one mole of methanol is oxidized to CO_2 , generating six-electron equivalents of reductant, which are then used to convert three moles of methanol to three moles of methane.

The oxidation of methanol to CO_2 involves a part of the CO_2 reduction pathway, but in the reverse direction. The methyl groups enter this oxidation process at the methyl-coenzyme M stage by the action of two methyl transferases, MT1 and MT2. MT1 is a two-subunit enzyme (MtaBC) and MT2 has one subunit (MtaA). The first reaction involves transfer of the methyl group of methanol by MT1 to the corrinoid co-factor of its MtaC subunit; this is an automethylation process. Then MT2-M or MtaA transfers the methyl group from MtaC to HS-CoM, generating methyl-coenzyme M. The methyl groups destined for oxidation are transferred from $\text{CH}_3\text{-CoM}$ to H_4MPT by the membrane-bound methyl- H_4MPT : coenzyme M methyl transferase. This endergonic reaction is assisted by a Na^+ -gradient and generates $\text{CH}_3\text{-H}_4\text{MPT}$ which then leads to methane production similar to other pathways.

Pathways of Methane Emission

(1) Diffusion

Diffusion through the profile refers to the movement of methane up through soil and bodies of water to reach the atmosphere. The importance of diffusion as a pathway varies per wetland based on the type of soil and vegetation. For example, in peatlands, the amount of dead, organic matter results in relatively slow diffusion of methane through the soil. Additionally, because methane can travel more quickly through soil than water, diffusion plays a much bigger role in wetlands with drier soil.

(2) Aerenchyma

Plant aerenchyma refers to the vessel-like transport tubes

within the tissues of certain kinds of plants. Plants with aerenchyma possess porous tissue that allows for direct travel of gases to and from the plant roots. Methane can travel directly up from the soil into the atmosphere using this transport system.

(3) Ebullition

Ebullition refers to the sudden release of bubbles of methane into the air. These bubbles occur as a result of methane building up over time in the soil, forming pockets of methane gas. As these pockets of trapped methane grow in size, the level of the soil will slowly rise up as well. This phenomenon continues until so much pressure builds up that the bubble "pops," transporting the methane up through the soil so quickly that it does not have time to be consumed by the methanotrophic organisms in the soil.

Factors affecting methane production

Temperature: It plays a very important role in methane production. At 40-50°C methanogenesis is done only by hydrogenotrophic methanogens^[12].

Stage of plant: The stage of plant decides which form of methanogenic dominates at a particular stage. However at flowering stage both acetoclastic and hydrogenotrophic methanogens are higher^[13].

Soil depth: Depth of the soil is important for survival of methanogens. Methanogens usually occur at a higher population in the top soil^[14].

pH: The optimum pH for growth of these methanogens is 6.5-6.9, therefore it is important to maintain pH of soil at this range.

Addition of nitrates and sulphates: Addition of nitrates and sulphates in the soil in form of fertilizers help reduce methane production, as these act as alternate sink to use hydrogen.

Mechanism of methane formation

The organic matter undergoes hydrolysis, acidogenesis, acetogenesis and ultimately methanogenic archaea act to produce methane. These landfills receiving a wide variety of solid waste, generally carries a fairly low pH level. The low pH level makes it difficult for most methanogens to survive. It was found that *Methanosarcina barkeri* can survive at this low pH levels. *M. barkeri* consumes the acids in its environment, producing methane and increasing the pH levels in its immediate area. This, in turn, makes that area more amenable for other methanogens. As moisture leaches through the landfill, it disseminates that high pH level, making other parts of the landfill habitable for *M. barkeri* and other methane producing microbes. *M. barkeri* then moves in and repeats the process, leaving neutral pH levels and healthy populations of other methanogens. Since *M. barkeri* and its methanogen cousins produce large quantities of methane. This methane is often collected at landfill sites and used for power generation. Furthermore, methanogens break down solid waste as they go compacting it so that it takes up less space. Methane production due to landfills is around 25 Tg^[15].

Sources of methanogenesis

Methanogenesis from formate

The carbon transfer and reduction steps in this process are similar to those described above for methanogenesis from H_2+CO_2 . Both the CO_2 and reducing power are derived from

formate by the action of an F_{420} -dependent formate dehydrogenase (FdhABC); FdhAB subunits form the enzyme that produces CO_2 and reduced F_{420} or $F_{420}H_2$ and FdhC is thought to import formate into the cell. CO_2 is converted to methane using the CO_2 -reduction pathway.

Methanogenesis in rice fields and wetlands

Wetlands and rice fields are characterized by water-logged soils and distinctive communities of plant and animal species that have evolved and adapted to the constant presence of water. Due to this high level of water saturation as well as warm weather, wetlands and rice fields are one of the most significant natural sources of atmospheric methane. Anaerobic decomposition of organic material in flooded rice fields produces methane. Anaerobic conditions occur in rice field as a result of soil submergence. Water saturation of soil limits the transport of oxygen in soil resulting in higher activity of methanogens to produce methane. Under anaerobic and reduced conditions methanogens produce methane either by using CO_2 and H_2 or by using acetate. Under steady conditions methanogenesis by acetoclastic pathway predominates and accounts for 75-80% of total methane emitted.

Methanogenesis in termites

The termite gut consists of foregut (which includes the crop and muscular gizzard), the tubular mid gut (which as in other insects is a key site for secretion of digestive enzymes and for absorption of soluble nutrients) and relatively, a voluminous hindgut (which is also a major site for digestion and for absorption of nutrients). Some bacteria colonize the foregut and midgut, bulk of intestinal microbiota is found in the hindgut, especially in the paunch. Termites have good sources of wood degrading enzymes such as xylanases, laccases; as their main dietary component is wood^[16]. Alongwith bacterial spp like *Bacteroides Cellulomonas*, *Spiromusa termitida* etc termites harbour flagellate protists that fill up the bulk of the hindgut paunch. The gut flagellate includes *Trichonympha*, *Calonympha* which degrade the lignocellulosic feed with formation of excess hydrogen as intermediate. This large number of bacterial, archaeal and protozoal population inhabit the gut of termites. This partnership with a diverse community of bacterial, archaeal and eukaryotic gut symbionts break down the plant fibre and ferment the products to acetate and variable amounts of methane, with hydrogen as a central intermediate.

The fermentation of wood polysaccharides by the gut flagellates yields acetate and other short-chain fatty acids, which are resorbed by the host. Hydrogen is an important intermediate that drives the reduction of CO_2 , which yields additional acetate and some methane. Although H_2 may strongly accumulate at the gut centre, most of it is consumed before it can escape from the gut. *Methanogenic archaea* (methanogens) that inhabit the gut of termites generate enormous amount of methane that adds to the global atmospheric methane (CH_4). The predominant species is *Methanosarcina barkeri* using acetate as major source of methane production. The total methane contribution due to termites is probably less than 15 Tg per year^[15].

Methanogenesis in landfills

A mixture of organic and inorganic wastes is disposed at a landfill with varying humidity and much heterogeneity. Approximately 75% of municipal waste is biodegradable organic material. Substances in waste have various decomposition rates. Food waste is most readily degraded.

Garden waste forms a group with medium half life (5 years). Paper, cardboard, wood and textile waste decomposes slowly (half-life of 15 years), while plastics and rubber are not degraded at all. A number of factors affect the quantity of gases formed at landfills and their composition, such as waste type and age, quantity and type of organic components, waste humidity and temperature.

Methanogenesis in oceans

A large amount of methane is generated in ocean sediments which travel a long way to get to the surface and escape to the atmosphere. While travelling through the oceans this generated methane gets eaten up by other microorganisms termed methanotrophs (methane eaters) prior to being released. But inspite of this the methane production from oceans is very high. This is because a high concentration of methane is also found in surface water which is oxygenated. It is reported that Methane from the aerobic oceans accounts for up to 4% of global methane production. A group of very simple and tiny bacteria help produce methane. Usually these bacteria don't produce methane but they can produce the gas as a byproduct of their natural metabolism when they are starved for phosphorus. In phosphate-depleted waters, aerobic microbes could metabolise a compound called methylphosphonic acid (MPn) as a source of phosphate, releasing methane as a by-product. These micro organisms in marine areas had the biosynthetic apparatus for MPn, suggesting that the molecule is widespread in ocean environments. One of these organisms is a marine microbe called *Nitrosopumilus maritimus*, part of a group^[17] whose members are among the most abundant microorganisms in marine surface waters. Contribution of oceans to total methane production is 10 Tg.

Methanogenesis from fossil fuels

Methane is naturally present in fossil fuels due to long term decomposition of organic matter. Methane from fossil-fuel production is primarily emitted through:

- 1) The combustion of extracted fossil-fuels;
- 2) The industry practices of venting, or intentionally releasing excess gas, and flaring, or intentionally burning excess gas.
- 3) Fugitive emissions, which include unintentional leakage from the transportation, storage, and distribution of fossil fuels. The combustion of fossil fuels mined, drilled, and otherwise extracted on federal lands and waters contributed approximately 62,000 metric tons (U.S.A) of methane to the atmosphere in 2012 alone, or more than 1.5 million metric tons of carbon dioxide equivalent. While venting and flaring are only two of the many root causes of GHG emissions from public lands, the amount of methane these practices emit remains significant and, according to new estimates, has steadily increased over the past five years. The increases in venting and flaring levels are likely in part due to rising oil and natural gas production as well as the lack of adequate infrastructure to capture and process natural gas released during production.

Methanogenesis in herbivores

Emissions from enteric fermentation of pigs and horses are of minor importance but not negligible. Methane production is influenced in particular to diet composition and feeding practices. The formation of CH_4 in the digestive system (enteric fermentation) of pig and horse is mainly centred in the hind gut (colon). Here, bacterial action degrades those organic species that passed the digestive tract undigested,

mainly cellulose, hemi-cellulose and pectin which are summed up as bacterially fermentable substrates (BFS). Bacterial action converts these substrates to volatile fatty acids, CH₄ and carbon dioxide. The fatty acids play an important role in the energy supply of pigs. In experiments with sows, about half the cellulose and about 90 % of the sugar (xylose), starch and cellulose (pectin) as well as the protein casein that were applied to the animals intracaecally were degraded in the hind gut. The gross energy loss of feed in the form of methane is very less around 0.1%-1% GE of feed intake. The emission of methane from horse and pig is 0.14 Tg and 1.7 Tg respectively. [18].

Methanogenesis in ruminants

Methane is produced in the rumen as a product of normal fermentation of feedstuffs. Although methane production can also occur in the lower gastrointestinal tract, as in non-ruminants 89% of methane emitted from ruminants is produced in the rumen and exhaled through the mouth and nose. Globally, ruminants produce 80 MMT of methane annually. India has largest livestock population & emits about 10.8 MMT of CH₄ annually from enteric fermentation. Energy loss ranges from 4 to 12% of GE intake in cattle [19].

Methanogenic strains in rumen

In ruminants the major strains found are *Methanobrevibacter ruminantium*, *Methanosphaera*, *Methanomicrobium*, *Methanobacterium formicicum* followed by *Methanosarcina barkeri* [20].

Methane production

The methanogens in the bovine rumen utilize hydrogen and carbon dioxide to produce methane. Methanogens of the genus *Methanosarcina* grow slowly on hydrogen and carbon dioxide and therefore utilize methanol and methylamines to produce methane. Formate, which is formed in the production of acetate, can also be used as a substrate for methanogenesis. Volatile fatty acids (VFA) are not commonly used as substrates for methanogenesis as their conversion into carbon dioxide and hydrogen is a lengthy process, which is inhibited by rumen turnover. By removing hydrogen from the ruminal environment as a terminal step of carbohydrate fermentation, methanogens allow the microorganisms involved in fermentation to function optimally and support the complete oxidation of substrates. The fermentation of carbohydrates results in the production of hydrogen and if this end product is not removed, it can inhibit metabolism of rumen microorganisms.

Relation with other microbes

Methanogens are known to have symbiotic relationships involving interspecies hydrogen transfer with rumen microorganisms, especially with rumen protozoa where the methanogens can be associated intracellularly and extracellularly. Common protozoa in the bovine rumen found to have such a relationship are from the genera *Entodinium*, *Polyplastron*, *Epidinium*, and *Ophryoscolex*, while the methanogens most often associated with protozoa are from the orders *Methanobacteriales* and *Methanomicrobiales*. Anaerobic fungi, such as *Neocallimastix frontalis*, have also been found to have a relationship with methanogens involving interspecies hydrogen transfer whereby the fungi's enzymatic activity has increased and metabolism has shifted towards acetate production.

Importance of methanogenesis in ruminants

It helps in the removal of excess hydrogen. A high hydrogen level will cause:-

1. Decrease in overall degradation of carbohydrates.
2. Decrease in rate of microbial growth
3. Decrease in synthesis of microbial protein

Conclusion

Atmospheric methane is currently increasing at a rate of about 30 to 40 Tg (10¹² g) per year. Stabilising global methane concentrations at current levels would require reductions in methane emissions or increased sinks for methane of approximately the same amount. This reduction represents about 10% of current anthropogenic sources (of which ruminants contribute about 30%). This is much less than the percentage reduction necessary to stabilise the other major greenhouse gases. Additionally, because methane has a shorter atmospheric lifetime and greater radiative absorption capacity than carbon dioxide, methane reduction strategies offer an effective means of slowing global warming in the near term.

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