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# Diversity of methanogenic bacteria in ecological niches

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## ABSTRACT

*Methanogens are strict anaerobes which share a complex biochemistry for methane synthesis as part of their energy metabolism. Methanogenic bacteria are abundant in habitats where electron acceptors such as O2, NO3−, Fe3and SO42are limiting. Common habitats for methanogens are anaerobic digestors, anoxic sediments, flooded soils, and gastrointestinal tracts. Methanogens are generally absent from the water column of unstratified lakes and rivers because convection currents rapidly aerate the deep waters. However, the diffusion of O2 between the layers of stratified lakes is often too slow to maintain oxic conditions in the lower layers. The methanogens are widely distributed in nature, but confined to strictly anaerobic environments. In addition to aquatic sediments (ponds, marshes, swamps, rice soils, lakes, and oceans), other methanogenic habitats include the intestinal tracts of man and animals (especially the rumen of herbivores), sewage digesters, landfills, heart wood of living trees, hot springs, decomposing algal mats, oil wells, and mid-ocean ridges. In these habitats, the methanogens occupy the terminal niche in the transfer of electrons generated by the anaerobic degradation of organic matter.*

**Key Words:** Methanogens, ecology, review

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## INTRODUCTION

Methanogens are ancient organisms that are key players in the carbon cycle accounting for about one billion tones of biological methane produced annually (1). Methanogens are strict anaerobes which share a complex biochemistry for methane synthesis as part of their energy metabolism. A number of studies have provided evidence that they are of economic value. The successive petroleum crisis since 1973 has led to great interest in alternative forms of energy, including recovery of methane via anaerobic digestion of wastes .Improvements in the design of digestors have been made possible by advances in understanding the ecology and physiology of methanogens. . In the cattle industry, the knowledge of the fermentation processes in the rumen demonstrated a net loss of energy via the methanogenesis, and inhibitors such as Rumens in have been developed to enhance meat yields. Oil companies try to distinguish between natural gas produced by methanogens or by the thermo catalytic reactions associated with petroleum generation. Finally, studies on the global distribution of methane in the earth's atmosphere are increasing due to the sudden awareness of its possible role in the enhancement of the greenhouse effect from $CO_2$ accumulation, and on the reversal of stratospheric ozone depletion. Due to these reasons reviews on their taxonomy and ecology metabolism energetic biochemistry and molecular biology have been published. The most recent compilation on methanogens is the book edited by Ferry This paper summarizes the recent knowledge of methanogenic *Achaea* with emphasis on their taxonomy and ecology(4). Methanosarcina acetivorans, with a genome size of

~5.7 mb, is the largest sequenced arch eon methanogen and unique amongst the methanogens in its biochemical characteristics. By following a systematic workflow we reconstruct a genome-scale metabolic model for M. acetivorans. This process relies on previously developed computational tools developed in our group to correct growth prediction inconsistencies with in vivo data sets and rectify topological inconsistencies in the model. Methanogenic bacteria are abundant in habitats where electron acceptors such as O2, NO3−, Fe3and SO42are limiting. Common habitats for methanogens are anaerobic digestors, anoxic sediments, flooded soils, and gastrointestinal tracts. Methanogens are generally absent from the water column of unstratified lakes and rivers because convection currents rapidly aerate the deep waters. However, the diffusion of O2 between the layers of stratified lakes is often too slow to maintain oxic conditions in the lower layers (4). Interspecies Electron Transfer and Obligate Syntrophy Because of their limited substrate range, methanogens depend on fermentative bacteria to convert a wide range of organic compounds into methanogenic substrates. In environments where organic matter is completely degraded to methane and CO2, the methanogenic precursors are predominantly acetate, formate, and H2، CO2. The organic matter is initially fermented mainly to volatile organic acids, H2, and CO2. Methanogens can directly catabolize H2 ,CO2,formate, and acetate, but longer-chain volatile organic acids (with three or more carbon atoms) such as propionate and butyrate must be metabolized to one or more of these methanogenic precursors by a specialized group of microbes called syntrophs. Habitats of Special Interest. When organic matter is completely catabolized to methane and CO2, the major substrates of methanogens are usually acetate, formate, and H2, CO2. However, in some environments the growth of acetic lacctic methanogens and obligate syntrophs is too slow to maintain a large population in the system. For instance, in the rumen and colon, acetate accumulates to concentrations of 50 to100 mM. Although this is well above the concentration required for acetic lactic growth of methanogens such as *Methanosarcina,* these organisms do not catabolize significant quantities of acetate because their growth rate on this substrate is too slow to maintain the population in a rapid-turnover ecosystem. However, when methylamine or methanol is present, the cell numbers of *Methanosarcina* in the rumen may reach 105 to 106 per milliliter because these substrates support a faster growth rate. Propionate and butyrate are also present in the rumen at significant concentrations, but the slowly growing propionate- and butyrate-degrading organisms are not found in abundance. In the rumen, a wide range of CO2-reducing methanogens may be found, including *Methanobrevibacter ruminantium,Methanobacterium formicicum,* and *Methanomicrobium mobile. Methanobrevibacter* species are the most commonly found CO2-reducing methanogen in no ruminant intestinal tracts. *Methanosphaera* species have also been isolated from colonic environments; they only grow by using H2 to reduce methanol to methane(4).

Hydrogen is, with acetate, one of the most important intermediates in the methanogenic degradation of organic matter and serves as substrate for methanogenic archaea. Hydrogen should theoretically account for 33% of total methanogenesis when carbohydrates or similar forms of organic matter are degraded. Many methanogenic environments show both much lower and much higher contributions of H2 to CH4 production than is considered normal. While the lower contributions are relatively easily explained (e.g. by the contribution of homoacetogenesis), the mechanisms behind higher contributions are mostly unclear. In methanogenic environments H2 is rapidly turned over, its concentration being the result of simultaneous production by fermenting plus syntrophic bacteria and consumption by methanogenic archaea. The steady-state concentration observed in most methanogenic environments is close to the thermodynamic equilibrium of H2-dependent methanogenesis(4).

The threshold is usually equivalent to a Gibbs free energy of −23 kJ mol−1 CH4 that is necessary to couple CH4 production to the generation of 1/3 ATP. Methanogenesis from H2 is inhibited if the H2 concentration decreases below this threshold. Concentrations of H2 can only be decreased below this threshold if a H2-consuming reaction with a lower H2 threshold (e.g. sulfate reduction) takes over at a rate that is equal to or higher than that of methanogenesis. The instantaneous and complete inhibition of H2-dependent CH4 production that is often observed upon addition of sulfate can only be explained if a comparably high sulfate reduction potential is cryptically present in the methanogenic environment.

Methanogenic archaea utilize only a limited number of substrates, the most important ones being acetate and H2/CO2 (or formate). Most methanogenic archaea are able to utilize H2/CO2 and such methanogens can be found in every methanogenic environment. Indeed, H2 is a ubiquitous compound in anaerobic environments where it exhibits a fast turnover but usually occurs at only very low concentration. Low H2 concentrations are a thermodynamic prerequisite for the degradation of alcohols and fatty acids by H2-producing syntrophic bacteria. In methanogenic environments where inorganic electron acceptors other than CO2 are not available, consumption of H2 is only possible by methanogenic archaea and homoacetogenic bacteria. There, degradation of alcohols and fatty acids is

usually accomplished by syntrophy between H2-producing syntrophic bacteria and H2-consuming methanogenic archaea(3). Hydrogen is a product of the anaerobic degradation of organic matter by fermenting and syntrophic bacteria. The most abundant source of dead organic matter in natural environments is usually plant material consisting of lignin and polysaccharides. Some aquatic sediments receive a large input of dead crustaceans consisting of chitin. Lignin is largely recalcitrant under anaerobic conditions, but methanol may be released from the methoxy groups and thus may support methanogenesis to a limited extent. In general, however, we may assume that the anaerobic degradation process is largely driven by carbohydrates as the dominant substrate. This assumption is valid for aquatic sediments, peat, other wetlands, ruminants, arthropods feeding on plant material, and for many types of sewage sludge. There are many studies in the literature which report much higher contributions of H2 than the expected 33%. Conceivable explanations for these exceptions include (i) additional sinks of acetate, (ii) additional sources of H2, or (iii) measurements under non-steady-state conditions. Additional sinks of acetate are not uncommon, e.g. in the rumen, acetate is largely absorbed into the blood stream of the host, leaving H2 as the predominant source for methanogenesis. Similar observations were made in microbial mats where acetate is assimilated by the phototrophs. Transient phenomena must occur when H2 and acetate are sequentially produced or utilized. For example, the low amounts of CH4 produced immediately after flooding of paddy soil are mainly due to H2-dependent methanogens apparently become active before the acetotrophic ones. Eventually, however, steady state is reached and H2 then contributes about 30% to CH4 production as theoretically expected. More than 85% of the ocean's organic carbon is deposited in shallow, anoxic marine sediments. Molecular and isotopic data suggest that most of the methane produced in marine sediments is of biogenic origin(1).

## MATERIALS AND METHODS

***Isolation methods***

Numerous new species of methanogens have been isolated and characterized due to the development of laboratory procedures for culturing strict anaerobes such as a gassing manifold for gassing Hungate tubes and high-pressure aluminium-sealed tubes and serum bottles, and anaerobic glove boxes for transfer of colonies. Details of the laboratory procedures are compiled in the papers of Mah and Smith [20] and Whitman *et al*. It is widely accepted that methanogens are difficult to isolate as some members require long incubation periods for growth and some are sometimes difficult to separate from their syntrophic partners. In addition, a large majority of microbes including methanogens have evaded isolation as they are not amenable to laboratory cultivation due to our incomplete knowledge of their growth requirements. As a consequence, the recent trend is to study their abundance, distribution and biodiversity directly in their ecosystems using molecular tools (e.g. rRNA gene analysis). Data from these studies have been included in the section under Ecology of methanogens (4).

**Ecological of methanogen**

The methanogens are widely distributed in nature, but confined to strictly anaerobic environments. In addition to aquatic sediments (ponds, marshes, swamps, rice soils, lakes, and oceans), other methanogenic habitats include the intestinal tracts of man and animals (especially the rumen of herbivores), sewage digesters, landfills, heart wood of living trees, hot springs, decomposing algal mats, oil wells, and mid-ocean ridges. In these habitats, the methanogens occupy the terminal niche in the transfer of electrons generated by the anaerobic degradation of organic matter.

***1. Soil and aquatic environments.*** Rice fields represent soil areas which are flooded for long periods and where anoxic conditions arise which allow methanogenesis. these soils are similar to the littoral of lakes and are characterized by the presence of plants and the occurrence of oxic and anoxic zones in the sediment. The aerenchyme and intracellular space system of rice plants mediate the transport of $CH_4$ from the anoxic sediment into the atmosphere. In the absence of plants, $CH_4$ is released almost exclusively by emission of bubbles. Factors affecting methane emission from rice fields are strongly correlated with soil properties such as redox value, pH , temperature, and organic content . In planted soils, up to 80% of the methane produced does not reach the atmosphere but is apparently oxidized in the rizosphere. $CH_4$ oxidation activities were also detected in the oxic surface layer of the submerged paddy soil. Methano-genesis is strongly inhibited by brackish water in these soils . Several strains of *Methanobacterium* , *Methanobrevibacter arboriphilus* , and *Methanosarcina* sp. and M. *mazei* have been isolated from rice soils. Adachi provided evidence that hydrogenotrophic methanogens were abundant in subtropical paddy fields that had been amended with organic matter. Diversity and structure of the methanogenic community in

anoxic rice paddy soil microcosms have been examined by MPN counts or direct 16S rRNA gene sequence retrieval . Floodwater management is the best way to minimize methane emission by rice fields. Many workers have illustrated the importance of acetate as a methane precursor in both freshwater and marine sediments, and demonstrated that *Hz* is a rate limiting factor in the process of methanogenesis in sediments. According to Conrad *et al.*, most of the Hy-dependent methanogenesis in these ecosystems occurs as a consequence of direct interspecies hydrogen transfer between juxtapositioned microbial associations within floes or consortia. The importance of methanol and methylated amines as methane precursors in estuarine, intertidal sediments is variable, due to the abundance of decomposing plant materials in the sediment system: algal mat, *Spartina* in salt marsh. Methanogenesis also occurs in the anoxic water columns of meromictic lakes.

Methanogens from the genera *Methanobacterium, Methanosarcina* and other coccoidal morphotypes have been isolated from special sites such as landfills. Recent evidence also suggests that methanogenic *Archaea* are present in deep granitic rock aquifers , in desert soil and other oxic soils and peat bog(4).
***2. Digesters.*** It has been shown by several authors that strictly anaerobic bacteria formed the dominant population in digesters, and that methanogens accounted for about 10% of the total micro flora. Dolfing and Bloemen have presented a rapid and reliable method to assess the potential specific activity of methanogenic sludge, based on the gas analysis for methane in the heads pace of closed vials. The influence of various parameters on the activity of methanogenic sludges was investigated with this method. The potential methanogenic activities of the biomass were shown to reflect the type of waste water on which the organism had been grown. Detection and quantitation of methanogens in digesters or mixed cultures have been proposed by enzyme-linked immunosorbent assay (ELISA method), or immunologic analysis. The latter method established a considerable diversity of methanogens, much larger than previously reported, encompassing at least 14 strains of 11 species. Methods using molecular biology tools such as group specific 16S rRNA hybridization probes are proposed now to describe or quantify natural communities of methanogens(4).

***3. Extreme environments.*** Thermal environments, such as hot springs, solfataras or submarine hydro-thermal vents, are sites of active methanogenesis. A few species of hyperthermophilic methanogens have been isolated. Up to now, *Methanothermus* sp. could only be isolated within solfatara fields in the south-west of Iceland. The authors were unable to obtain it from similar places in Italy, the Azores, and Yellowstone National Park (U.S.A.), and speculated for an endemic growth within Iceland. Dissemination over long distances may not be possible due to their exceptionally high oxygen sensitivity. However, field studies indicate that much lower temperature maxima occur *in situ* Methane emanating from high-temperature (300 to 400°C) submarine 'black smokers' has been suggested to be of bacterial origin. *Methanococcus jannaschii* was isolated from this site. Methanogens have also been isolated from other deep sea hydrothermal vents recently. The experiment of Baross and Deming on vent enrichment cultures grown under high hydrostatic pressure at 250°C was contested by Trent *et al.* who suggested that growth at this temperature may have been due to experimental artifacts. Biogenic methane has also been detected in hyper saline environments. Patterns and rates of methane production in hyper saline algal mats may be determined by a complex interaction between salinity, the use of methylated amines for osmoregulation by algae, and the formation of TMA by fermentation. Methylmercaptans are produced from methionine by halo anaerobic bacteria in these biotopes. Zhilina concluded that methanogenesis may occur in a wide range of salinities up to saturation and that a specific group of halophilic methanogens, which differs from known groups by the diversity of morphological and physiological characters, occupies this peculiar ecological niche. Indeed, all the species isolated until now are methylotrophic methanogens belonging to newly described genera. These new species have been found in salted lakes from Egypt and Kenya *('Methanosalsus zhilinae'*), U.S.A. *(Methanohalophilus mahii*), and Russia *(Methanohalobium evestigatum).* Methanogenesis has been only recently reported from deep oil-bearing strata. Isolation of methanogens has been successful from slightly saline to saline oil well waters in the mesophilic range of temperature. Thermophilic, but not hyperthermophilie isolates have also been reported from hot oil reservoirs. The methanogens so far isolated and characterized include (i) the hydrogenotrophs *Methano bacterium thermoautrophicum*, M. *bryantii* and M. ivanovii, phenotypic variants of *Methanobacterium thermoaggregans* and M. *thermoalcaliphilum*, *Methanococcus thermolithotrophicus* and *Methanoplanus petrolearius* and the recently newly described *Methanocalculus halotolerans*, (ii) two methylotrophs: *Methanohalophilus (Methanococcoides) euhalobius*, and *Methanosarcina siciiiae* and (iii) an acetotroph: *Methanosarcina mazei*.

Several physiological traits from these isolates have demonstrated that virgin oil reservoirs contained an *in situ* methanogenic population(4).

***4. Within living organisms.*** The topic of the relationship that methanogens have with other microorganisms in the rumen is especially important when considering methane mitigation strategies. Methane mitigation is effective in one of two ways: either a direct effect on the methanogens, or an indirect effect caused by the impact of the strategy on substrate availability for methanogenesis, usually through an effect on the other microbes of the rumen. Both approaches will be discussed here with focus on strategies that have shown efficacy in vivo(2). Methanogens are directly involved in the digestive processes of ruminants and other animals including insects. Since the work of Hungate, the activities of methanogenic bacteria have been well studied in the rumen and the cecum, the specialized structures of intestinal tracts of herbivorous mammals. Because they lack cellulolytic enzymes, they have a very large and active anaerobic microbial population which degrades cellulose. But methane production from acetate is not important as compared with other environments, because the animal's nutrition is based on absorption through the intestinal epithelium of the volatile fatty acids produced during the fermentation process. Thus, about 82% of the $CH_4$ formed in the rumen comes from $H_2$ reduction of $CO_2$, while about 18% is derived from formate. However, acetoclastic methanogens are present, and linked to methanogenesis from methylamines or methanol. Recent phylogenetic studies have revealed that taxon-specific association exists between rumen protozoa and methanogen populations. Methanogens are also present in the large bowel of humans, but more research needs to be undertaken to understand better the role of methanogenesis in the physiology of human digestion. The most prominent species of methanogens in the animals' intestinal tract are related to the genus *Methanobrevibacter*, but *Methanosphaera* and *Methanogenium* strains can also be encountered. The same bacteria have been found in the oral cavity of humans associated with dental plaque, but a direct implication in tooth decay has not yet been established. Methanogens have also been identified in the gut of terrestrial arthropods and various insects including termites, where similar species have been observed by epifluorescence microscopy and phylogenetic analysis. The contribution of termites to atmospheric methane is is estimated at 2 to 5 X $10^{12}$ g per year but controversy about this exists. Recent studies have established that $CH_4$ is principally evolved by soil-feeding termites which possess about 10% of methanogens in their gut micro flora.

The heartwood tissues of trees can become infected with soil bacteria and develop conditions for methanogenesis at the expense of the degradation of cellulose and pectins. *Methanobrevibacter arboriphilus* has been isolated from this habitat. Methanogens can also been found as endosymbionts of protozoa, in removing hydrogen produced by the protozoa via interspecies hydrogen transfer, allowing the protozoa to produce more oxidized and energyyielding products such as acetate. These protozoa are sapropelic amoeba from which *Methanoplanus endosymbiosus* has been isolated, or flagellated protozoa from termites hindgut, or free-living anaerobic ciliates. Methanogens have also been observed on the surfaces of ciliated protozoa in the rumen. Methanogens can be easily visualized in these ecosystems by use of epifluorescence microscopy In the oceans, methane evolution is derived from the activities of methanogens located within the intestinal tracts of marine animals (plankton, fishes and also the fore stomachs of baleen whales ) where fermentation of chitin occurs in a situation analogous to the rumen(4).

***5. Atmospheric methane.*** Methane production through enteric fermentation is of concern worldwide for its contribution to the accumulation of greenhouse gases in the atmosphere, as well as its waste of fed energy for the animal. Methane is produced in the rumen and hindgut of animals by a group of Archaea known collectively as methanogens, which belong to the phylum Euryarcheota. Among livestock, methane production is greatest in ruminants, as methanogens are able to produce methane freely through the normal process of feed digestion. Much research has been directed toward methane abatement strategies to be used in ruminants and has been reviewed elsewhere (5-11). Methane production through enteric fermentation is of concern worldwide for its contribution to the accumulation of greenhouse gases in the atmosphere, as well as its waste of fed energy for the animal(2).

The atmospheric methane comes essentially from biological origin. Flooded soils are the main natural sources of $CH_4$ with 100-200 Tg per year. Cattle (65-100 Tg/y) and rice fields (25-150 Tg/y) are responsible of 15 to 45 % of total anthropic methane emission. Global tropospheric methane concentrations average about 1.7 ppm, and there has been a two- to three-fold increase over the past 100-200 years, with an actual increase of 2% per year. Because methane absorbs in the infrared, it plays an

analogous role to carbon dioxide ('Greenhouse' effect) but 20 to 30 times higher. In addition, it is destroyed in troposphere by reaction with hydroxyl radicals, with the production of carbon monoxide and hydrogen. However, methane may react with chlorine or nitrous oxide, derived from chlorofluorocarbons or fertilizer applications, respectively, and helps protect the stratospheric ozone layer from destruction by these compounds]. The residence time of methane in the atmosphere is actually believed to be about 8 years(4).

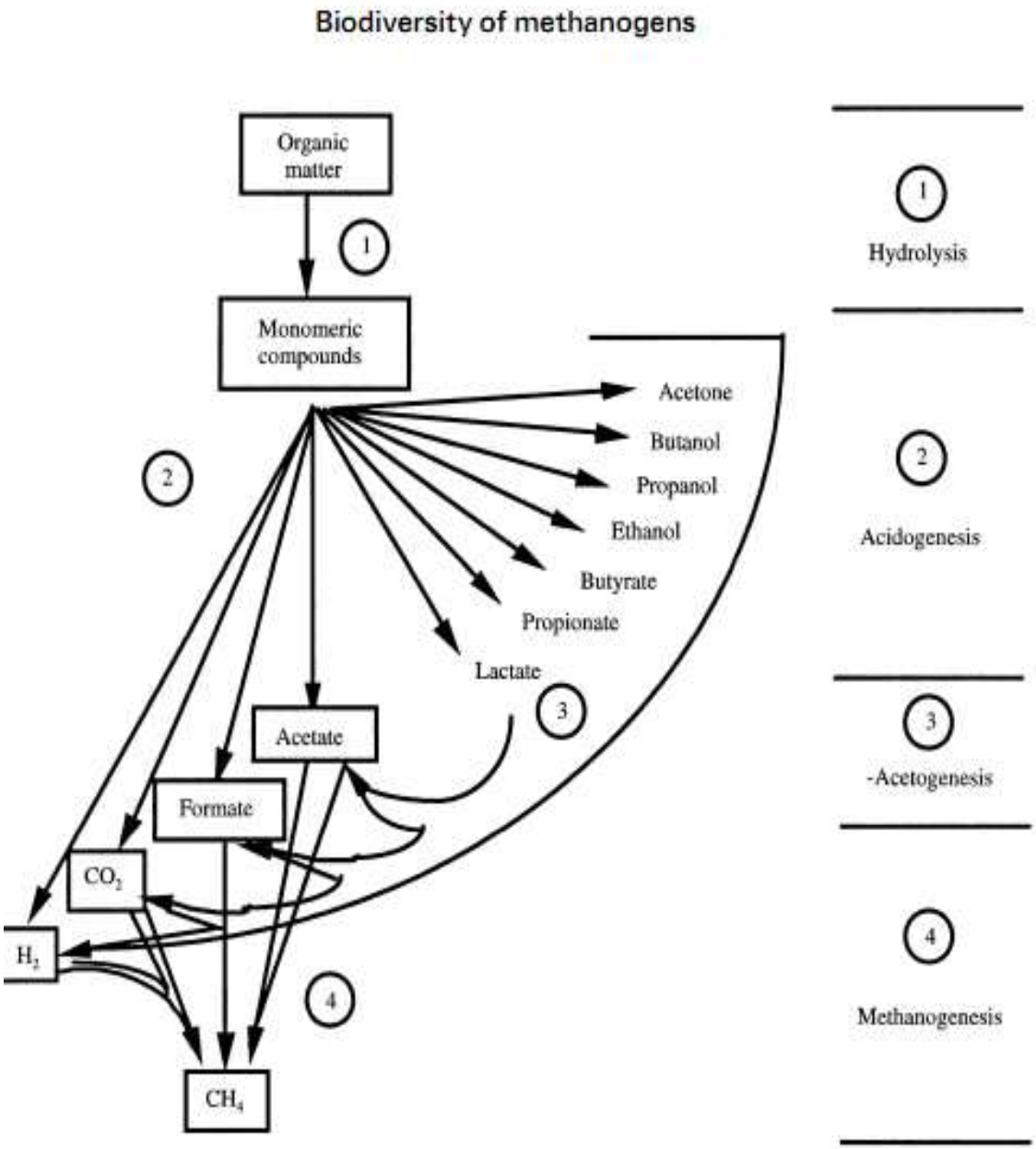


**Figure 2.** Schematic diagram showing anaerobic degradation of organic matter.

## CONCLUSION

In most cases, however, where H2/CO2-dependent methanogenesis dominates (up to 100%) CH4 production in

sediments of lakes, marine bights and peat bogs ,an explanation for elevated contributions of H2 to methanogenesis is more difficult to find. For more than two decades, researchers have been working to identify, quantify, and inhibit methanogens and methanogenesis through various methane mitigation strategies. Although a great deal of information has been gleaned from these experiments, including identification of a number of methanogen strains in the rumens. Important questions concerning diversity and ecophysiology of methanogens remain unanswered. Crucial issues must be clarified before optimization of digesters becomes a reality. It is of interest to establish the variation, if any, of the methanogenic flora in relation to type of waste and digester. Knowledge of the metabolism and biochemistry of pure cultures of the microbes involved in methane fermentation, coupled to studies on defined mixed cultures are essential for future applications of this fermentation. The biochemistry of the methanogens may be exploited, perhaps by manipulation of the physiology of whole cells or by the application of isolated enzyme systems. The genetics of methanogens is likely to be a rewarding area of research to contribute to our understanding of methanogens. Complete genome sequencing of two methanogenic arch eons, *Methanococcus jannaschii* and *Methano bacterium thermoautotrophicum* is currently being carried out. There is also a need for better methods of acquiring data concerning the release of methane from the geosphere in order to obtain accurate estimates of its residence time in the atmosphere.

## REFERENCES

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[1] VS kumar;JG Ferry;CD Maranas.Licensee BioMed Central Ltd, **2011**, Metabolic reconstruction of the archaeon methanogen Methanosarcina Acetivorans.
[2] S E Hook;A Denis; G Wright; B W McBride. Methane Producers of the Rumen and Mitigation Strategies.**2010**.
[3] EC de Macario. Taxa covered by the ICSP Subcommittee on the Taxonomy of Methanogens,2nd ed,NewYork,**2008**.
[4] GL Garcia;BKC Patel;B Ollivier.Anaerobe,**2000**,205-226, Taxonomic, Phylogenetic, and Ecological Diversity of Methanogenic *Archaea.*
[5] E Kebreab, K Clark, C W Riddle, J France, *Canadian Journal of Animal Science*, vol. 86, no. 2, pp. 135–158, **2006**. View at Scopus
[6] K A Beauchemin, T A McAllister, and S M McGinn, *Nutrition and Natural Resources*, vol. 4, no. 9, pp. 1–18, **2009**.
[7] S Kumar, A K Puniya, M Puniya et al., *World Journal of Microbiology and Biotechnology*, vol. 25, no. 9, pp. 1557–1566, **2009**.
[8] B M Buddle, M Denis, G T Attwood et al., "Strategies to reduce methane emissions from farmed ruminants grazing on pasture," *The Vete`rinary Journal*. In press.
[9] R J Eckard, C Grainger, and C A M de Klein, *Livestock Science*, vol. 130, no. 1–3, pp. 47–56, **2010**.
[10] C Martin, D P Morgavi, and M Doreau, *Animal*, vol. 4, no. 3, pp. 351–365, **2010**.
[11] M Shibata and F Terada, *Animal Science Journal*, vol. 81, no. 1, pp. 2–10, **2010**.