Anaerobic methane oxidation and methane formation in marine environments

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Introduction:

Anaerobic methane formation and methane oxidation are important processes in marine environments. Presently much research is done to get insight into anaerobic methane oxidation coupled to sulfate reduction, which seems to be performed by syntrophic microbial associations of bacteria and archaea. It is still unclear how this syntrophy functions. Methanogenesis, which has been well studied in freshwater environments, is still poorly understood in marine environments. Though some marine methanogens have been isolated and characterized, the bacteria that provide the methanogens with substrates like hydrogen, formate and acetate, are largely unknown.

Methane as fuel and as greenhouse gas:

Methane (CH_4) is a colorless and odorless gas. It has a relatively low solubility in water of about 1.4 mM at 20ºC and a methane gas pressure of 0.101 MPa. About 2.7 million years ago, methane was a major component in the earth's atmosphere, but since then the atmosphere became more oxidized. Two facets of methane are of environmental importance. On the one hand, methane is a strong greenhouse gas; methane is about 20 times more effective in trapping infrared radiation than carbon dioxide. On the other hand, methane is a fuel, and controlled anaerobic decomposition of organic matter in bioreactors or landfills is a sustainable way of waste treatment and energy conservation. Compared to other alkanes, methane has an unusually high C-H bond strength, which makes it more resistant chemical oxidation. Methane is the most reduced form of carbon (oxidation state -4), carbon dioxide (CO_2) is the most oxidized form (oxidation state +4). Methane is the main component of natural gas (70-90%) and biogas (50-70%). The energy yield per carbon during oxidation of methane is higher than for other hydrocarbons or coal. Therefore, less CO_2 is produced when natural gas or biogas is used as fuel or as energy source for microorganisms.

Methane formation:

Biogas, with methane as the major reduced component, is produced during the biological degradation of organic matter in the absence of inorganic electron acceptors like oxygen, nitrate, iron (III), manganese (IV) and sulfate. Natural methanogenic environments can be encountered in both marine and freshwater sediments that are rich in organic matter, in wetlands and in the intestinal tract of ruminants and insects. Engineered methanogenic systems are anaerobic bioreactors used to treat solid organic waste or to treat wastewaters from agro-industry, like food and beverage industry, paper industry, agriculture and households Anthropogenic methane emissions arise from agriculture and waste disposal, enteric fermentations, animal and human wastes, rice paddies, biomass burning and open landfills.

Anaerobic degradation of organic matter proceeds via a number of microbial processes, which include hydrolysis, acidogenesis and acetogenesis, by which complex organic matter is degraded to hydrogen and CO_2, formate, acetate and ammonium The final step is methanogenesis. Methanogens are strict anaerobes and belong to the archaea. Methanogenesis proceeds via a number of unique coenzymes, which are mainly or exclusively found in methanogenic and methanotrophic archaea. Three methanogenic pathways can be distinguished: The hydrogenotrophic pathways, in which hydrogen and CO2 or formate are converted to CH_4 and H_2O. The aceticlastic pathway, in which acetate is converted to CH_4 and CO_2. In the methylotrophic pathway methanol or other methylated compounds (methanethiol, dimethyl sulfide, or methylated amines) are fermented to methane and carbon dioxide.

Seawater contains much sulfate. Therefore, anaerobic organic matter degradation in marine sediments is for a large part coupled to sulfate reduction. However, when the organic matter input is large enough, sulfate will be depleted in the top part of the sediment and organic matter degradation will result in methane production. Though some marine methanogenic archaea are isolated and described, remarkably little is known about the microbiology of methanogenesis in marine environments. The highest marine methane production rates can be found near the continental margins, because the primary production

in the overlying surface waters and thus also the organic matter deposition is largest in relatively shallow waters. This methane production by organic matter degradation results in a very diffuse source for methane.
There are also some less diffuse sites where methane is traveling up by convection along cracks and faults. These are called cold seeps or methane vents, in which pore water or fluid with dissolved methane seeps up from deeper sediment layers, or in which gaseous methane vents up. These seeps can occur in many forms, e.g. as cold mud volcanoes and brine pools and hydrothermal vents. The methane from these vents and seeps can be produced biologically, but can also be produced geochemically or thermogenically from organic matter. Methane seeps and vents occur above fossil fuel fields or gas hydrates. Gas hydrates are ice-like structures in which a gas, mostly methane, is incorporated. The earth's gas hydrates are an enormous reservoir of organic carbon. These hydrates are stable at low temperatures (below 15°C), high pressures (above 50 bars) and in the presence of dissolved methane.

Methane oxidation:

Described methanotrophs are aerobic bacteria that can use methane as electron donor and carbon source. Aerobic methane oxidation proceeds according to equation 1. Methanotrophic bacteria are found in many environments like swamps, rivers, rice paddies, soils, deciduous woods and sewage sludge as well as sea water and sea sediments. The oxidation proceeds via a linear pathway, in which methane is first converted to methanol by a (NADH)-dependent mono-oxygenase. The methanol is further oxidized via formaldehyde and formate to carbon dioxide by methanol dehydrogenase, formaldehyde dehydrogenase and formate dehydrogenase. The electrons released in these steps are passed to the electron transport chain for energy conservation and ATP synthesis.

(1) $CH_4 + 2\ O_2 \rightarrow CO_2 + 2H_2O$ $\Delta G° = -773\ kJ.mol_{CH4}^{-1}$

In contrast to aerobic methane oxidation, anaerobic oxidation of methane (AOM) by microbes is less understood and for many years AOM was thought to be impossible. In the seventies of last century, AOM was first discovered by geochemical *in situ* studies in anaerobic marine sediments and waters. Methane diffusing upwards from deeper sediment layers was oxidized before reaching oxic zones. The consumption of methane could be coupled to the consumption of sulfate, diffusing downward from the seawater. Methane oxidation coupled to sulfate reduction occurs according to equation 2.

(2) $CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$ $\Delta G° = -16.6\ kJ.mol_{CH4}^{-1}$

AOM has also been observed in non marine environments, like lakes, ditches and landfills. In most cases AOM was probably coupled to sulfate reduction, but methane oxidation coupled to denitrification was described as well. Microbiological research has indicated that AOM in marine environments likely is a syntrophic process, which proceeds via reversed methanogenesis. It is hypothesized that archaea convert methane to a metabolite, which is the electron donor for sulfate-reducing bacteria. Up to now, attempts to identify the intermediate failed.

Prospectives:

Methane formation and methane oxidation are key processes in marine environments. To understand these processes from a microbiological viewpoint a lot of research is still needed. A profound insight of marine methanogenesis will help to produce biogas from marine biomass. In addition, insight into the process of AOM may result in biotechnological processes with methane as chemical building block.

Recent molecular ecological research has shown that the microbial communities in marine environments are very diverse, but only a small fraction of the marine microorganisms present have been isolated and studied physiologically. It is tempting to assume that in particular marine environments are enormous reservoirs of novel microbial biocatalysts. These novel biocatalysts might be exploited for biotechnological purposes.

Further reading:

Muyzer G and Stams AJM (2008) Ecology and biotechnology of sulphate-reducing bacteria. Nature Rev Microbiol 6: 441-454.

Stams AJM and Plugge CM (2009) Electron transfer in syntrophic communities of anaerobic bacteria and archaea. Nature Rev Microbiol 7: 568-577.