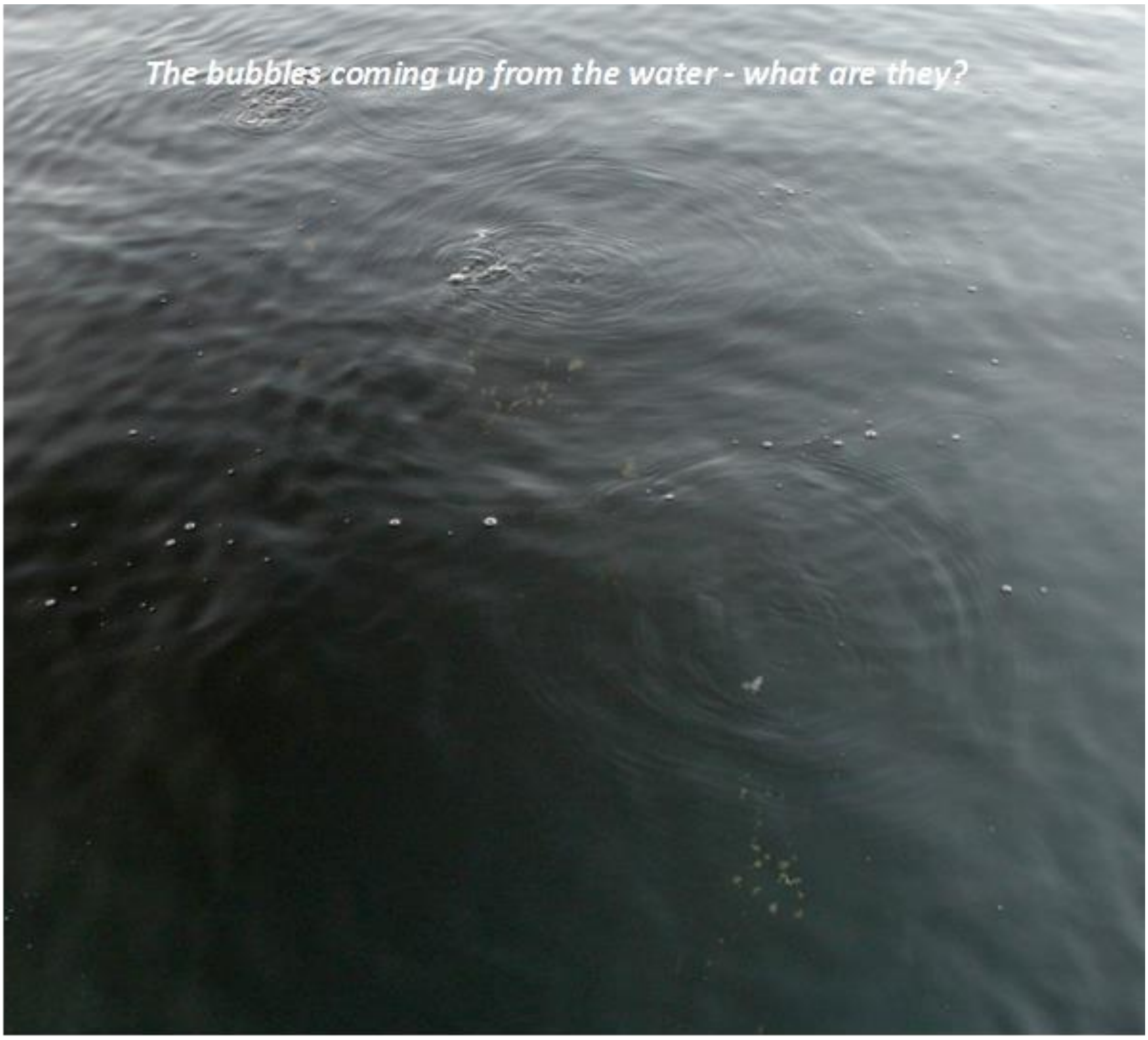
Microbes and the Greenhouse Gas Methane



Odd Gunnar Brakstad, Ida Beate Øverjordet and Bjørn Henrik Hansen

SINTEF Ocean, Trondheim, Norway

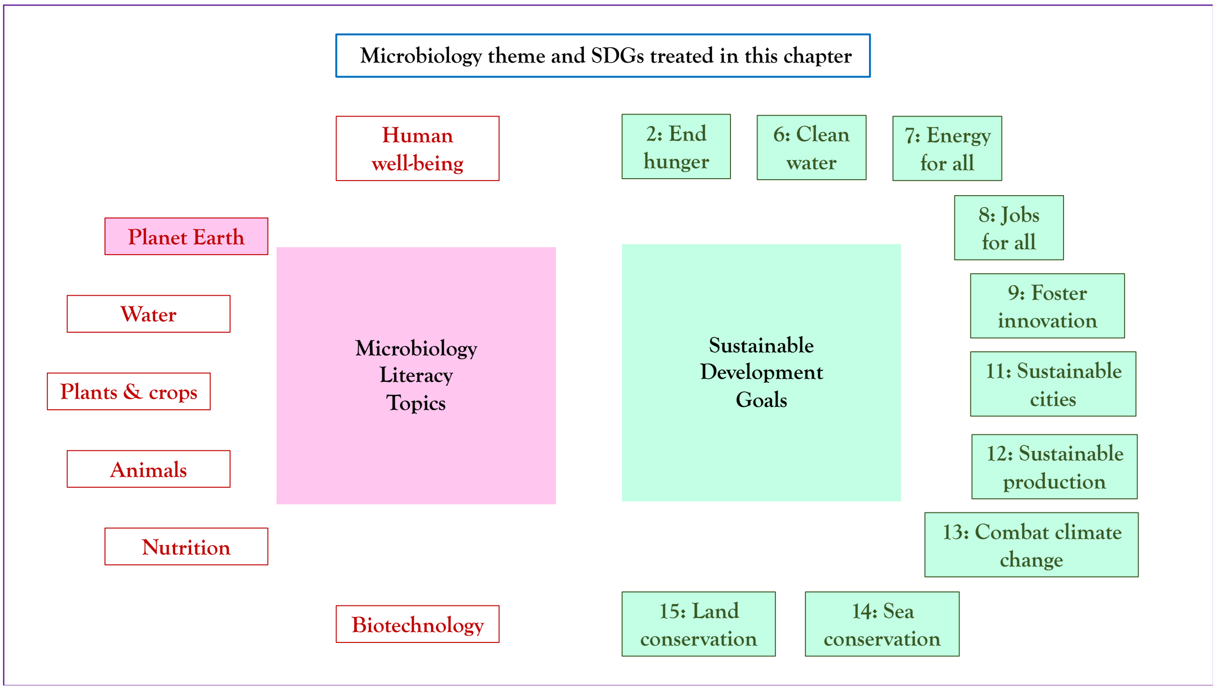
Microbes and Methane

Storyline

Methane is a greenhouse gas which is associated with global warming. The gas can be formed in the deeper parts of the earth, or by microorganisms – the *anaerobic methanogens* – in oxygen-free environments like lake or seawater sediments, landfills, and peatlands, by decay of organic materials. Methane produced in sediments can be seen as bubbles in the water which rise to the surface. A major concern is the formation of methane in thawing permafrost triggered by temperature increases in the Arctic and Antarctic regions. On the other hand, methane can be captured and transformed (oxidized) to carbon dioxide (CO_2) by *methanotrophic microbes*, which thereby reduce the escape of this greenhouse gas to the atmosphere. The methanotrophic microbes can therefore be considered as a 'biofilter'. Scientists are working with developments of such 'biofilters' as *bioremediation* tools to reduce methane emissions from landfills or for restoration of peatlands. The microbiology of methane is therefore associated with several Sustainable Development goals.

The Microbiology and Societal Context

The microbiology: carbon cycle; microbial formation and oxidation of methane; greenhouse gas production; pollution; gas production; enrichment of methane-oxidizing microbes; natural attenuation; bioremediation. *Sustainability issues:* clean water and clean energy; sustainable economic growth, industrialization, and cities/communities; responsible consumption and production; climate action; life below water and on land.



Microbes and Methane: the Microbiology

1. ***Methane is a colourless gas, so how do we detect it?*** If you have visited a pond, swamp or a peatland area, you may have observed bubbles that rise up through the water. And even in waters covered by clear ice in the winter, you may have observed white spots in the ice. These bubbles are trapped in the ice since the ice will not let them through.

So what are these bubbles? They may consist of the greenhouse gas (GHG) *methane*, which you have heard of as a cause of *global warming*.

Methane is also an important energy source and the most important part of natural gas used in householdings and industry.

2. ***Methane as a greenhouse gas (GHG).*** We are aware of the risk from global warming and the emissions of GHGs as important contributors to global warming. Methane (CH_4) is one of several GHGs, which also include carbon dioxide (CO_2) and laughing gas (N_2O). Although global warming is associated with increased concentrations of CO_2 in the atmosphere, both methane and laughing gas are more *potent* GHGs than CO_2. According to the Intergovernmental Panel on Climate Change (IPCC), the global warming potential of methane is 25 CO_2-equivalents, and for laughing gas 298 CO_2-equivalents. For methane this means that in a perspective of one hundred years, 1 tonne of methane released into the atmosphere will create the same warming in the atmosphere as 25 tonnes of CO_2.

One main source (and concern) of methane as a GHG is that the temperature is increasing in the Arctic, and this results in thawing of stored organic materials in the permanently frozen lands (*permafrost*).

Methane is produced in a variety of environments and is the second most abundant carbon compound in the atmosphere (after carbon dioxide). Methane is also present in sediments in seawater and lakes, in oxygen-free water ('rotten water'), and rice-fields, in landfills and waste, in the gut contents and faeces of man and animals, and in many other environments.

3. ***Measurement of methane.*** There are several ways of measuring methane released from water to the atmosphere. The released methane gas can be trapped in simple closed floating chambers, and the gas content in these chambers will increase over time. By taking samples at specific times, and analysing the methane concentrations in the chambers, the rate of release of this gas to the atmosphere may be determined, for instance as mg methane per m^2 water surface per day.

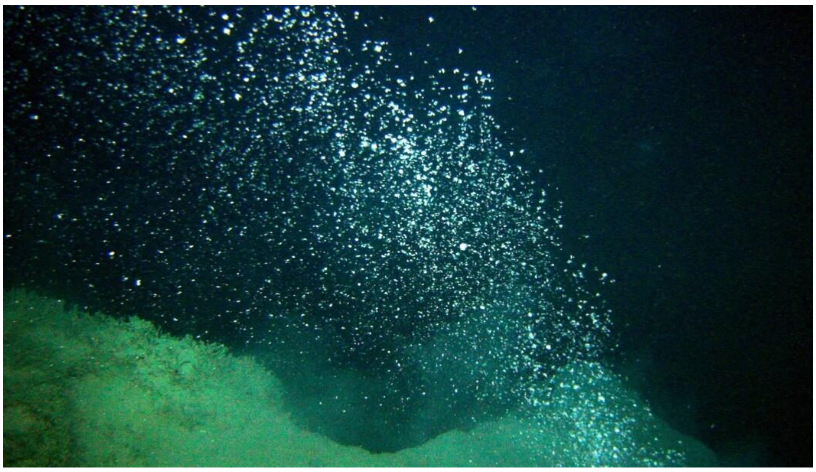
Systems for sampling gases released from lake surfaces (photo, SINTEF Ocean)

Some determinations require that collected samples are taken to a laboratory for analyses. However, special greenhouse gas analysers have also been developed which may be directly used in field analyses.

A system of inverted plastic cases with bicycle tubes as floating elements and small tubes leading the released gases directly to a greenhouse gas analyser (photo, SINTEF Ocean).

4. ***Microbial and non-biological production of methane.*** Methane can be produced by biological or non-biological processes. Methane produced by biological is *biogenic methane*, while methane produced by non-biological processes is often called *thermogenic methane*. Biogenic methane is caused by decay of organic material from plants and animals, and these processes occur at temperatures where biological processes can take place. However, thermogenic methane is formed in deeper parts in earth at high temperatures where biological processes cannot take place (often more than 150°C). Both biogenic and thermogenic methane may be produced as part of natural gas, together with other gases like ethane, propane and butane, but methane is the predominant gas. While thermogenic methane seeps up from deep and hot layers below the marine seabed, biogenic methane is formed in more shallow and cooler sediments.

Methane will sieve through the sediments and will reach the sediment surface on the bottom of the sea or a lake, often as bubbles. In shallow waters, these bubbles will rise quickly to the water surface, and the methane will then be released to the atmosphere as a GHG.



Bubbles of methane released from the seabed in the Arctic. This picture is taken from a methane seep in the Barents Sea (Source, Alfred Wegener Institute, Germany).

In cold seawater, methane sieving up from deep layers and reaching the seabed with low water temperatures, may be trapped in *gas hydrates*. Gas hydrates look like ice and consist of natural gas (mainly methane). If brought the surface, methane may be released from the hydrates, and the hydrates may become flammable.

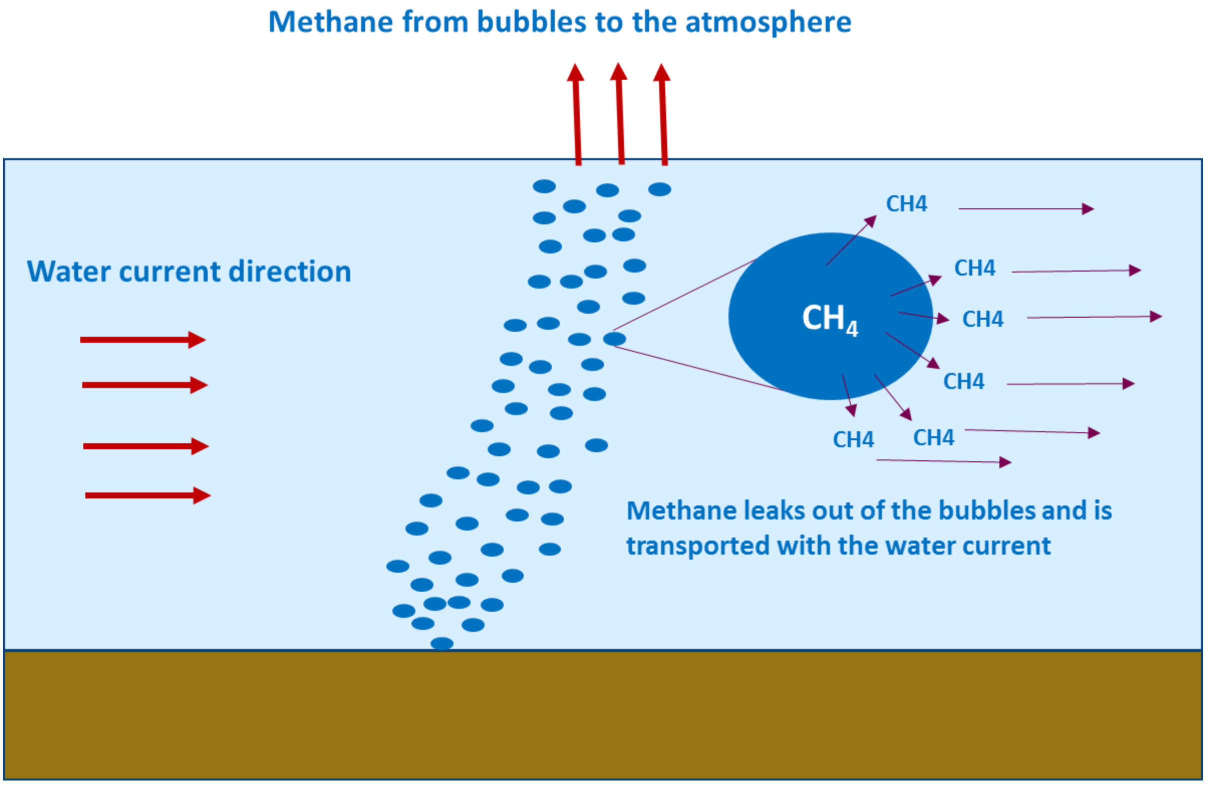
A flammable gas hydrate (U.S. Department of Energy, Office of Fossil Energy and Carbon Management).

5. ***The microbes of methane formation.*** Biogenic formation of methane is carried out by microbes in the total absence of oxygen. These microbes are therefore classified as strict anaerobic microbes, since they do not thrive in the presence of oxygen. They are therefore living in natural oxygen-free environment, like lakes, marine sediments, peatlands, and in various soil environments. These microbes may also be present in many oxygen-free environments created by man, and with high content of organic materials, for instance in landfills, rice fields, animal husbandry (although large emissions of methane also come from coal mining and for oil and gas production, these emissions are usually not the results of microbial activities). They typically have names starting with *Methano-*, like *Methanosaeta* (e.g. see MicroStars Portrait Gallery), and are also called methanogens. The methanogens digest organic material from plants which has ended up in the sediments. The same process also occurs when the permafrost thaws, and previously frozen organic material becomes available for the methanogenic microbes to decay.

While people usually relate microorganisms to the kingdom Bacteria, most methanogens belong to another kingdom, called Archaea. The Archaea have different cell wall structures than Bacteria, and they are sometimes considered as relicts from the period of extreme environments on the earth. Archaea are typically inhabitants of what we consider to be very 'unfriendly' environments, characterised for example by high temperatures, a lack of oxygen, high salt concentrations, or high acidity.

Methanogens also have the ability to grow at different temperatures. Some cold-loving methanogens may grow and form methane at low temperatures (down towards 0°C), while other heat-loving methanogens may produce methane at temperatures up to nearly 100°C. However, most methanogens prefer temperatures of 15-25°C.

The methanogens living in the oxygen-free parts of the sediments produce methane which sieves through the sediments to reach the upper surface of the sediment on the floor of the sea or a lake. The water they then encounter usually contains oxygen. The gas reaching the water as bubbles rises through the water to the surface. However, during the journey from the bottom of the lake/sea to the top, the methane in the bubbles may dissolve in the water. If there is a current in the water, as there is in the sea, the dissolved methane will be transported in the current direction.



If methane is released from sediments to the water as bubbles, these will rise to the surface. However, methane will then be dissolved from the bubbles and may follow the water current.

6. ***Microbes also destroy methane.*** When methane dissolves in water which contains oxygen, it can be transformed to carbon dioxide. This is of advantage for the atmosphere. since carbon dioxide is regarded to be a much less dangerous GHG than methane which is considered have a global warming potential of 25 times higher than carbon dioxide.

Methane oxidation is also a process performed by microbes, called *methane-oxidizing microbes* or *methanotrophs*. Most of these microbes are Bacteria (not Archaea), although some Archaea and fungi are also able to transform methane to carbon dioxide. Some of the Archaea may even transform methane in the absence of oxygen in the sediments on the seafloor.

7. ***The microbes of methane oxidation.*** While the microbes involved in methane formation, the *methanogens*, usually start their name with had names starting the '*Methano-*', the microbes causing methane oxidation, the *methanotrophs*, often have names starting with '*Methylo-*', like *Methylococcus*,. Other microbes with names starting with '*Methylo-*' are able to oxidize for instance methanol (CH_3OH), which also contains only one carbon. The names of these microbes may therefore tell us something about the main functions of the microbes in nature, that they are able to oxidize molecules with one carbon, usually to carbon dioxide (CO_2).

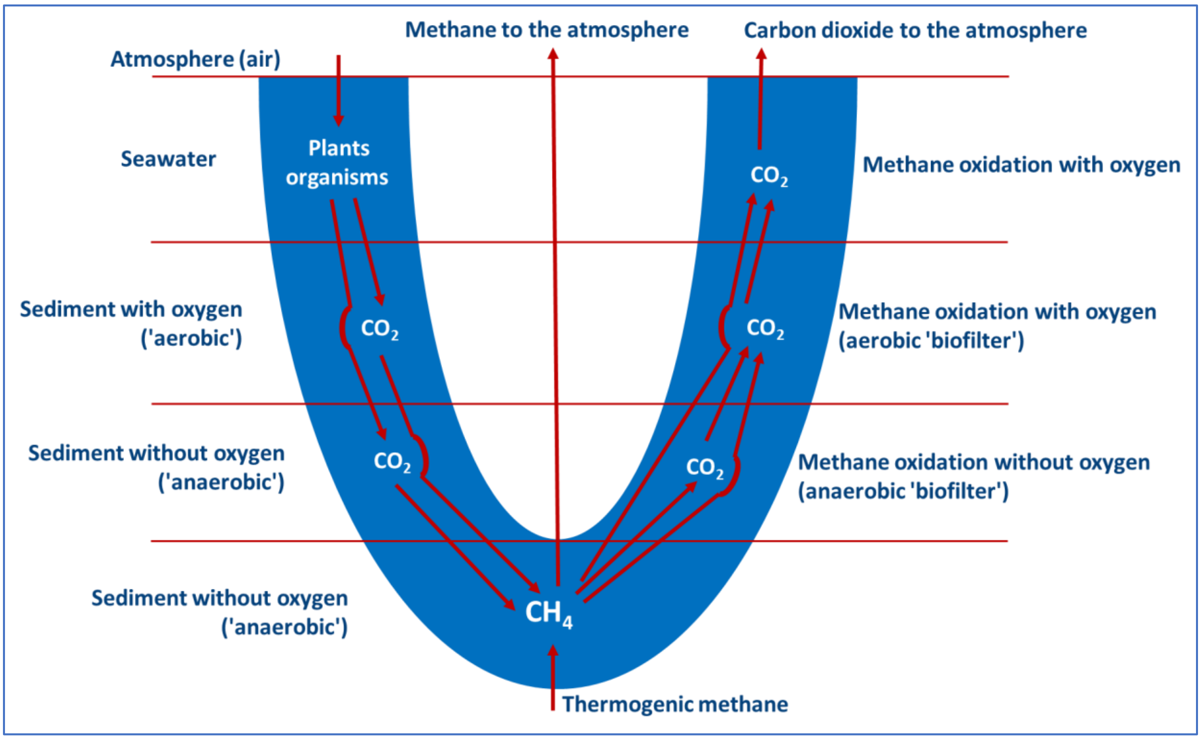
The oxidation of methane is possible because these microbes possess an enzyme called *methane monooxygenase*, which combines methane and oxygen to form carbon dioxide. As with the methane-forming microbes (methanogens), these methane-oxidizing microbes can live in a variety of environments and, as a general rule, they are present in most environments containing methane and oxygen (although also sometimes in the absence of oxygen), both natural and man-made environments.

8. ***Methane-oxidizing microbes as 'biofilters'.*** Methane oxidation may take place in most environments where methane is formed by biological processes, and the process may become effective if the gas reaches an environment with oxygen. These microbes then become something we may call a '*biofilter*'. This biofilters may occur in natural systems without any human intervention, or they may be stimulated by microbiologists to increase the 'rates' of methane oxidation.

And methane oxidizers often work in collaboration with other microbes, for instance so-called sulphate-reducing bacteria (bacteria known to produce smell of rotten eggs). This cooperation is also called *syntrophy* – one organism caries out one part of a process, and another completes it.

As methane percolates up through the sediments, it meets these '*biofilter*' microbes which convert some of it to carbon dioxide. Methane oxidation can therefore occur both in the oxygen-free and oxygen-containing parts of sediments, and in the water above the sediments. However, methane in gas bubbles rising rapidly to the water surface may escape these processes, since the microbes can only get access to the gas that becomes dissolved in the water.

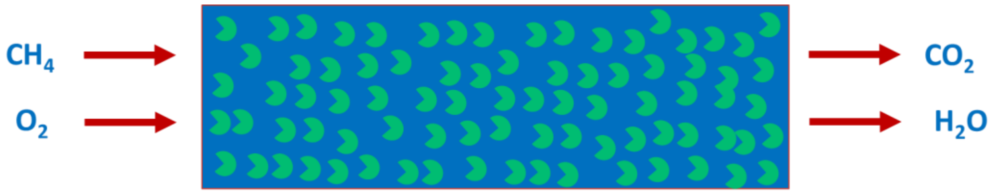
These processes can be considered as the ‘natural biofilters’, and they occur by themselves, meaning that they do not need to any help from man-made operations. The presence of methane itself is enough to stimulate these processes.



A simplified illustration of the process in the marine environment, where organic material in the form of plants or organism are biodegraded to carbon dioxide (CO2), and carbon dioxide is further biodegraded to methane. Methane is then further oxidized by methanogenic microbes to carbon dioxide in the sediments either without or with oxygen, and in the water above the sediments. Carbon dioxide can then be released to the atmosphere. However, methane can also be released to the atmosphere, either as gas bubbles, or as dissolved methane not degraded in the environment.

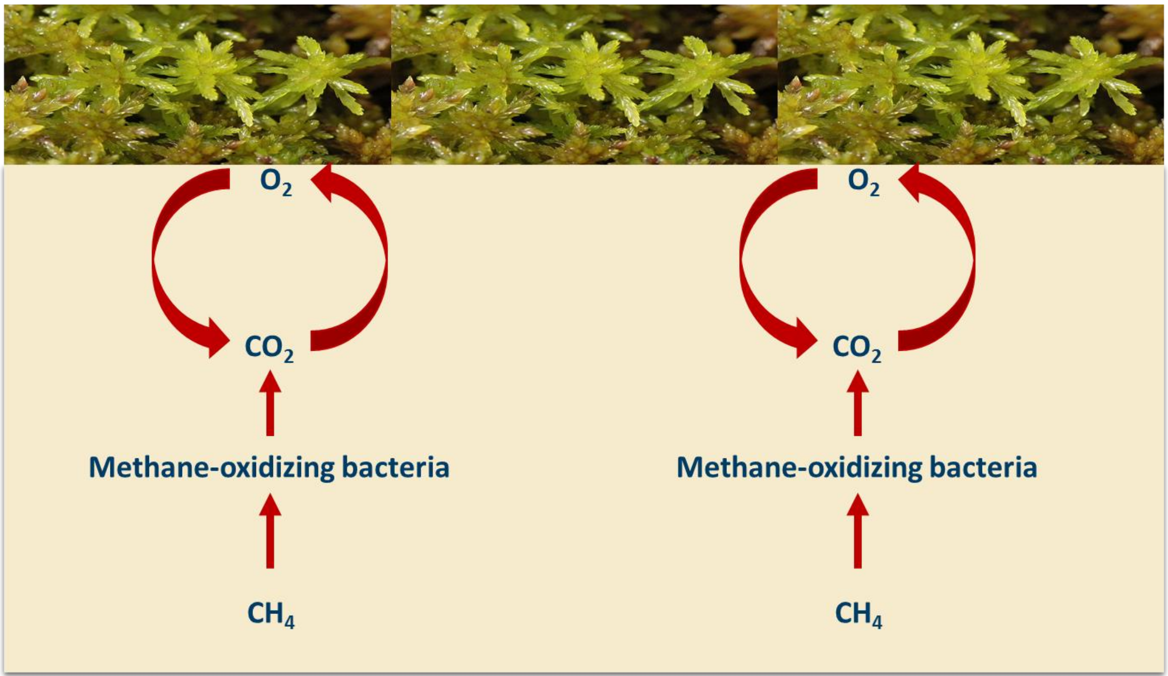
9. ***Biotechnological application of methano-/methylotrophs.*** However, scientists have also developed methods to help (or stimulate) methane oxidation. While the 'natural biofilters' work through a process of *natural attenuation*, we often refer to such man-made stimulations as

bioremediation. Bioremediation has been used for biological treatments of for instance oil spills, but it may also have possibilities for removing methane or reducing the concentrations of methane. One example is to stimulate methane-oxidation in restricted environments with high methane production, for instance in compost and landfills.



Cartoon of a column packed with methane-oxidizing bacteria. The bacteria form biofilms (layers of bacteria) growing on solid surfaces, for instance small beads. This creates large surface areas of bacteria. Methane (CH_4) from an environment is then pumped into one end of the column together with air (here shown as O_2). Ideally, the products coming out of the other end should be carbon dioxide (CO_2) and water (H_2O).

Scientists also try to take advantage of methane-oxidizing microbes for restoration of peatlands. These environments contain a lot of organic matter. During restoration, oxygen-free conditions may occur, which may cause methane to be formed. However, mixtures of mosses and methane-oxidizing microbes may be used to reduce methane releases.



This figure shows the collaboration between mosses and methane-oxidizing bacteria for restoration of peatlands. Consumption of organic material may result in oxygen-free soil, which generates methane by anaerobic microbes. This is not favourable for the mosses of this environment. However, by introducing more mosses, which harbour methane-oxidizing bacteria, during restoration of the peatlands, the bacteria will transform methane to carbon dioxide. This is favourable for the vegetation, which also generate oxygen which is of benefit for the bacteria.

The mosses (*Sphagnum*) harbour a lot of methane-oxidizing bacteria which transform methane to carbon dioxide, which is used by the plants. These plants then produce oxygen through photosynthesis, which again is beneficial for the bacteria. This is an example of a *symbiotic* process. The bioremediation action here is therefore to introduce more mosses, to increase the process of methane biofiltration.

10. ***Can the methane-oxidizing microbes solve the climate crisis?*** Let's go back to the bubbles in the water. Methane bubbles coming from environments all over the world may cause large amounts of methane to be released to the atmosphere. One particular worry is the large permafrost areas in the Arctic, which are thawing as the result of increased temperatures in the Arctic. The methane-oxidizing microbes can definitely help in reducing the emissions of methane, particularly the methane which becomes dissolved in water bodies like ponds, lakes and dams. These microbes are stimulated by the presence of methane, and they will therefore increase in numbers in the presence of the dissolved gas.

During the Deepwater Horizon oil spill in the Gulf of Mexico in 2010, scientists estimated that these microbes were quite effective in removing methane from the marine waters. Most focus was of course on the oil pollution, but methane was probably the most abundant hydrocarbon compound during the spill. Concentrations of methane-oxidizing bacteria increased during the spill period, and it was suggested that these bacteria removed nearly all the released methane. It was further estimated that the capacities of the methane-oxidizing microbes to oxidize methane increased more than 100 times during the spill period.

If these processes of methane oxidation also occur during other incidents of increased methane production, the methane-oxidizing bacteria may be of great importance as 'natural biofilters' in nature's contribution to reducing emissions of this GHG. In addition, future development of man-made technologies for stimulating these microbes in 'hot-spot' areas of methane emissions may also be of help in reducing methane emissions.

Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 2. End hunger, achieve food security and improved nutrition and promote sustainable agriculture**. Sustainable agriculture will reduce methane formation, if driven towards plant-based food rather than meat production, which increases methane formation. However, rice paddies may also be sources of methane formation, and substitution of rice production with other plant-based products may be beneficial to reduce methane formation and global warming.
- **Goal 6. Ensure availability and sustainable management of water and sanitation for all** (*assure safe drinking water, improve water quality, reduce pollution, protect water-related ecosystems, improve water and sanitation management)*. Ensuring sustainable water quality will reduce the risk of methane formation. Organic pollution and eutrophication (increased levels of N and P nutrients in the water) will promote microbial activities and may result in low oxygen concentrations (hypoxia) in some water environments, which eventually may be favourable for methanogens. Water monitoring, wastewater treatment and strict boundaries between waters and pollutant sources (industrial and agricultural) are therefore important to reduce the risk of methane formation.
- **Goal 7. Ensure access to affordable, reliable, sustainable and modern energy for all** (*ensure access to clean, renewable and sustainable energy, and increase energy use efficiency)*. A change to renewable sources like hydropower, wind and sunlight is important for reducing atmospheric emissions of greenhouse gases. Nuclear power is also a clean energy source, but the devastating risk associated with nuclear emissions should be considered in this context. Hydropower may also contribute to microbial methane formation when new dams are established by flooding of large land areas.

- **Goal 8. Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all** (*promote economic growth, productivity and innovation, enterprise and employment creation)*. Sustainable economic growth with reduced industrial emissions to air and water will also reduce greenhouse gas formation. Air emissions can be reduced by for instance carbon capture technologies, while reduced discharges of pollutants and N/P nutrients (e.g by the use of fertilizers) to lakes and rivers will be a contribution to maintenance of good water quality and avoiding the risk of hypoxia and anaerobic microbial activities.
- **Goal 9. Industry, innovation and infrastructure** (*build resilient infrastructure, promote sustainable industrialization and foster innovation*). Sustainable industry development and infrastructure will reduce pollution and are important water and air quality contributors. Reduced air and water pollution will also reduce the emissions of greenhouse gases directly to the air or from microbial production in water sources. Innovation is important to create environmental technologies to reduce and mitigate pollution. Environmental technologies may include the use of both physical and biological tools for greenhouse gas reductions. Circular economy and industrial re-use of waste are important tools in pollution reduction.
- **Goal 11. Sustainable cities and communities** (*make cities inclusive, safe, resilient and sustainable*). Sustainable cities and communities are crucial for reductions of pollution and greenhouse gases. Sustainable cities and communities have well-established control to avoid or reduce air and water pollution and should perform monitoring programs of air and water quality. They should also be able to handle pollution incidents by established preparedness systems.
- **Goal 12. Ensure sustainable consumption and production patterns** *(achieve sustainable production and use/consumption practices, reduce waste production/pollutant release into the environment, attain zero waste lifecycles, inform people about sustainable development practices)*. Reductions of waste production and pollutant release into the environment will improve air and water quality, Pollution control will reduce formation of hypoxic water environments and anaerobic activities, including methanogenesis. Systems for utilization of waste to avoid emissions to the environment are crucial for pollution control.
- **Goal 13. Take urgent action to combat climate change and its impacts** *(reduce greenhouse gas emissions, mitigate consequences of global warming, develop early warning systems for global warming consequences, improve education about greenhouse gas production and global warming)*. Since control of emissions of greenhouse gases like methane is the main action to reduce global warming, this goal covers all aspects of systems and methods to control and reduce methane formation and to develop and establish efficient methods to combat emissions. Prevention of deforestation is of particular importance. Carbon capture, storage and utilization of industrial emissions is also considered very important. In addition, efficient combat methods will include the development of biofilters and other remediation methods in hotspot areas like peatlands, rice paddies, and other environments of particular concern
- **Goal 14. Conserve and sustainably use the oceans, seas and marine resources for sustainable development** *(reduce pollution of marine systems by toxic chemicals/agricultural nutrients/wastes like plastics, develop mitigation measures for acidification, enhance sustainable use of oceans and their resources)*. Large amounts of greenhouse gases like methane are released to the oceans by seepages from the subsurface, and the oceans contain large amounts of methane captured in gas hydrates. Ocean warming may result in releases of methane from these hydrates to the water column and to the atmosphere. Increased concentrations of dissolved methane in

the seawater will also stimulate methanotrophic activities, while gas bubbles may rapidly rise to the water surface and release methane to the atmosphere. In coastal seawater, organic and inorganic input from land (including a variety of pollutants), may affects microbial methane cycles, promoting both methane formation and oxidation. In areas with large methane reserves, like Arctic/Antarctic areas with large gas hydrate deposits, releases of large methane concentrations may trigger methanogenic activities to such levels that seawater acidification may occur with reduced pH-levels, potentially harming organisms with calcium carbonate shells and skeletons, like diatoms, colars (e.g. cold-water corals) and coral organisms.

- **Goal 15. Life on land** (*Sustainably manage forests, combat desertification, halt and reverse land degradation, halt biodiversity loss*). As a sink of greenhouse gases, forests are important in the combat of global warming. In particular tropical rainforests are important for sequestering carbon dioxide. Global warming will also result in thawing of permafrost in polar and alpine areas, releasing large carbon sources available for the activities of methanogenic microbes. Melting permafrost will result in formations of small lakes which may become hotspots for methane formation and release.

Pupil Participation

1. Class discussion of the issues associated with microbial methane production and consumption

2. Pupil stakeholder awareness

a. How can you personally help to decrease emissions of the GHG methane (think about the food you eat)?

b. How can you personally help to decrease emissions of the GHG carbon dioxide (think about anything you do that consumes energy)?

c. Peat has historically been used to produce soil and compost used for growing seedlings and plants, but this practice is now discouraged in many countries in order to protect peatlands. Why are peatlands so important for global warming?

3. Exercises

a. Obtain maps of the world showing its peatlands. Calculate the proportion of total landmass that peatlands represent. Draw a graph showing how these proportions have changed over the years for which you have maps.

b. Which are the regions of the world most affected by the changes?

The Evidence Base, Further Reading and Teaching Aids

Cadena S., Cervantes S.J., Falcon, L.I., García-Maldonado, J.Q. The Role of Microorganisms in the Methane Cycle. Frontiers for young minds, December 4, 2019 (https://kids.frontiersin.org/articles/10.3389/frym.2019.00133)

Kanna, K. How Methanogenic Archaea Contribute to Climate Change. American Society for Microbiology, May 6, 2022 (https://asm.org/Articles/2022/May/How-Methanogenic-Archaea-Contribute-to-Climate-Cha).

American Society for Microbiology. Microbes and Climate Change – Science, People & Impacts. Report on an American Academy of Microbiology Virtual Colloquium held on November 5, 2021 (https://asm.org/ASM/media/Academy/Academy%20Reports/Microbes-Climate-Change-Science,-People-Impacts-Report.pdf).

Video: Methanogenesis. (https://www.youtube.com/watch?v=3yW4xB6HVFU).
Video: Let's Learn About Methanotrophs | Future Energy Systems. https://www.youtube.com/watch?v=clUiBQNdvWQ).

Glossary

Archaea: These microorganisms are single-cell microbes. Archaea possess genes and several metabolic pathways with differ from Bacteria, the other major *domain* of single-cell microbes. Many Archaea live in extreme environments and are therefore called *Extremophiles* (e.g. at high temperature, acidic or alkaline conditions, or at high salinity).
Anaerobic microbes: These are microorganisms living under anaerobic conditions are growing in the absence of oxygen. These organisms may either be obligate or facultative anaerobic. Obligate anaerobes will grow only in oxygen-free environment, and oxygen may be poisonous to them. Facultative anaerobic can grow both in the presence and absence of oxygen, using different metabolic pathways in the presence or absence of oxygen.
Biofilter: A biofilter consists of living organisms which capture and degrade pollutants. This type of technology is commonly used in wastewater treatment, but can also be used for treatment of dissolved gases.
Biogenic: Produced by living organisms. Biogenic gases are usually formed at temperatures <50 °C, at temperatures enabling the organisms to execute their metabolism.
Bioremediation: This process refers to biological processes which make pollutants less harmful. In the same way as with microbial *natural attenuation* (see below), this typically refers to biodegradation of environmentally harmful organic pollutants to carbon dioxide (mineralization). As a pollution clean-up technology, bioremediation can be performed as a) stimulating the natural population in the polluted environment by adding nutrients and aeration as in landfarming (biostimulation), or b) adding additional microbes specialized in degradation of the pollutant (bioaugmentation).
Gas hydrate: A gas hydrate is an ice-like form of water and gases like methane, ethane, and carbon dioxide. Gas hydrates occur in some cold marine sediments and in association with permafrost.
Greenhouse gas: When the sun heats the surface of the earth, some of the irradiation becomes heat, while some is reflected and escape into the atmosphere. A greenhouse gas will allow sunlight radiation to pass through the atmosphere and heat the planet. However, these gases will absorb heat emitted from the earth and send it back to the earth (re-radiation). Additional heat energy will then be trapped on the earth instead of escaping into the atmosphere. The most common greenhouse gases are water vapour, carbon dioxide, methane, nitrous oxide and ozone. Methane is considered to have an impact on global warming 100 times higher than carbon dioxide, but because of shorter atmospheric lifetime that than carbon dioxide, the global warming potential of methane over a 100-year perspective is considered to 27-30 times higher than for carbon dioxide, according to the Intergovernmental Panel on Climate Change.
Methanogens: These are microbes that produces methane. Methane may typically be formed in the absence of oxygen by reducing carbon dioxide in the presence of hydrogen to methane and water ($CO_2 + H_2 \rightarrow CH_4 + H_2O$).
Methanotrophs: These are microbes that use methane as their source of carbon and chemical energy. In the presence of oxygen, the carbon from methane can either be incorporated into organic compounds or be released as carbon dioxide. Methane can also be metabolized in the absence of oxygen.
Natural attenuation: This term refers to natural processes which make pollutants less harmful. In microbiology, this typically refers to biodegradation of environmentally harmful organic

pollutants to carbon dioxide (mineralization) by members of the naturally bacterial population in the polluted environment.
Permafrost: Permafrost is sediments constantly frozen, meaning that the temperature is below 0 °C all the year around. This is typically for Polar regions and for high mountain areas. Biological activities are very low or negligible in the permafrost.
Symbiosis: Interactions between two different organisms which are mutually beneficial for both of them.
Syntrophy: This is the process of one species feeding on the metabolic product of another species.
Thermogenic: Produced at high temperature. Thermogenic gases are formed at temperature well above the boiling point of water (usually >150 °C).