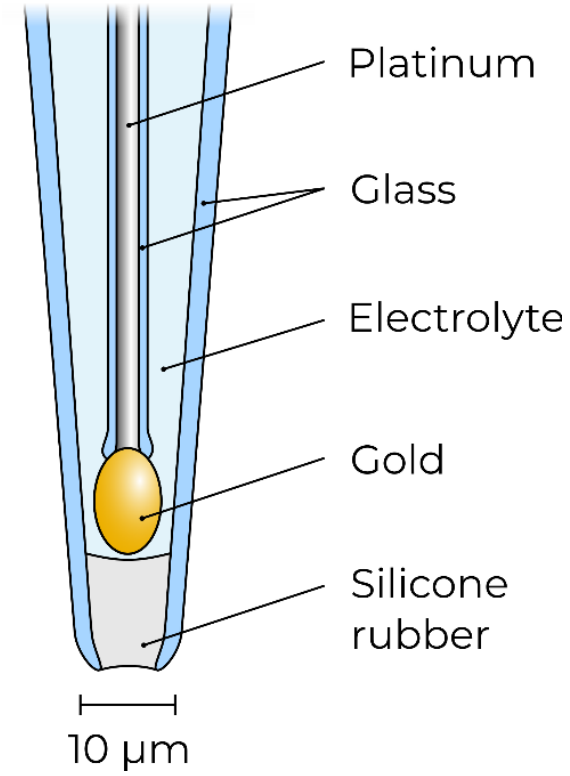
Microsensors: A look into the microbial environment

Miss: we can see microbes with a microscope
but how can we see what they are doing?



Left: Tip of oxygen microsensor. Tips can be made with diameters down to less than 2 µm. Right: Oxygen and hydrogen sulfide microsensors inserted into a photosynthetic biofilm. The microsensors are inserted by use of a **micromanipulator**. Graphics by Fabian Steininger, photo by Niels Peter Revsbech.

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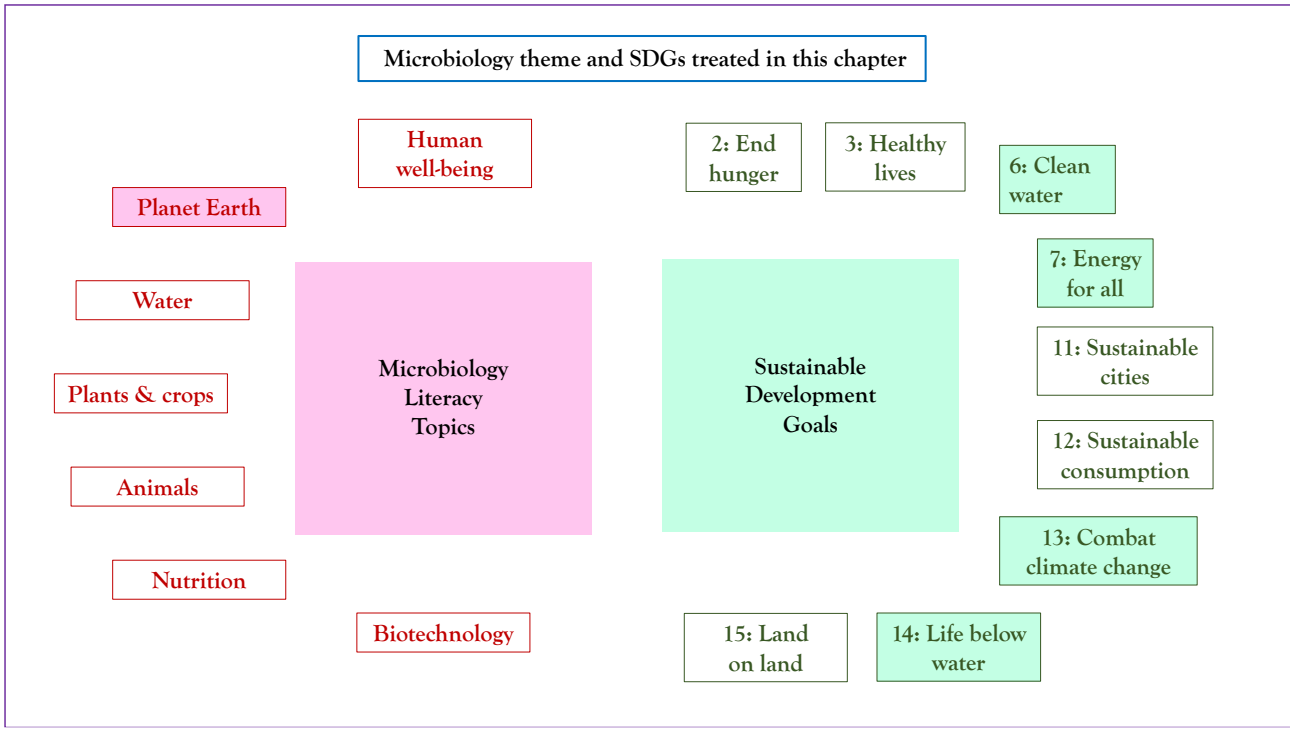
Storyline

The environment is heterogeneous: when we look at fields of crops, one will be growing wheat to feed us, one corn to feed cattle and chickens, and yet another hay to feed horses. In each field, we can observe the different plant populations growing and producing different things for us. But microbes cannot be seen, so to figure out which ones are present in a given environment and what they are doing, we need to replace our eyes with special instruments. A powerful one is microsensors, which enable us to measure what is going on at the microscopic level.

The Microbiology and Societal Context

The microbiology: Environmental microbiology; microsensors; micromanipulation; biofilms; cable bacteria; greenhouse gases. *Sustainability issues:* monitoring of greenhouse gases and environmental toxicants.



Microsensors: the Microbiology

1. ***The need to describe the environment of microbes.*** When we want to see bacteria and other microbes we need to use a microscope. The first one to do so and to realize the existence and diversity of microbes was Antonie van Leeuwenhoek around 1675. He could do this because he was able to make simple but functional microscopes. But how can we know what they are doing, what they are eating, and how well they feel under the conditions in which they live? For this, we need to be able to describe the immediate environment of microbes – its chemical and biological components – and especially how it changes in real time, and in response to environmental changes, natural and experimentally imposed.

2. ***Homogeneous versus heterogeneous systems.*** If you place a drop of dye in a bowl of water, it quicky disperses throughout the water, giving a uniform colour. The colour is said to be homogeneous, or evenly distributed. If you would measure its concentrations at different parts of the bowl, they would always be the same. Now think of a school playground: when children are out to play, they gather in groups to chat or they chase a ball. Their distribution in the playground is not homogeneous; it is heterogeneous! If one person has a birthday and brings in a bag of sweets, everyone rushes to that person, making the distribution even more heterogeneous.

3. ***Microbial environments are heterogeneous and vary enormously at the microscopic level.*** Like children in a playground attracted to someone with a bag of sweets, because microbes can move towards sources of food or away from environmental conditions they do not like, they distribute themselves heterogeneously in their habitats. And, because microbes metabolise the chemicals in their immediate environment, they change it. So: the greater the heterogeneity of the distribution of microbes (e.g. a high cell density in a particular part of the habitat due to the availability of a lot of an especially appetizing food and/or energy source), the greater the heterogeneity of the environmental conditions. Therefore, a measurement in one part of the habitat says nothing about any other part of the habitat. We have to take measurements precisely where the microbes are doing what interests us. And, since microbes are very small, we need to make measurements over very small distances, micro-distances, in real time, in order to describe the microenvironments of microbes.

4. ***Microenvironment measurements became possible with the invention of microsensors.*** It was only as recent as about 1980 that it become possible to observe the chemical microenvironment within dense microbial communities, such as the microbial slimes (plaques, **biofilms)** coating our teeth. The inventions making this possible were microsensors for chemicals like oxygen (O_2) and hydrogen sulfide (H_2S), and physico-chemical parameters like acidity (pH), etc. At present, microsensors have been developed for a large number of environmentally relevant chemical species, and we are therefore able to observe in real time the microbial consumption and production, i.e. biologically-mediated transformations, of many chemicals in biofilms, sediments, soils, etc. Such a transformation could for example be the formation of oxygen due to photosynthesis, or consumption of oxygen due to respiration.

5. ***What does a microsensor look like?*** An example of a microsensor is shown above. Notice how thin the tip is. A typical oxygen microsenor has a tip diameter of 10 µm (1 mm = 1000 µm). A human hair has a diameter of about 70 µm, and is thus 7 times thicker than the tip of a typical oxygen microsensor. And it is possible to make some types of microsensors with a diameter down to about 1 µm, that is the same diameter as a typical bacterium.

6. ***And how does a microsensor function?*** Different microsensors work in different ways but, in the case of an oxygen microsensor, we measure the electrical current produced by the reduction of O_2. The reduction of oxygen is basically the same reaction that happens within the human body when we respire with oxygen. We respire with oxygen because it is a very oxidizing and reactive chemical that readily reacts with many types of reductants to produce a lot energy that we need to grow and do all the things we enjoy. Reductants are chemicals that want to get rid of electrons and thereby get oxidized. In the oxygen microsensor, we do not have a

chemical reductant that can donate electrons, but we have a gold surface that is negatively charged with electrons from a battery, and this gold surface is thereby able to reduce oxygen:

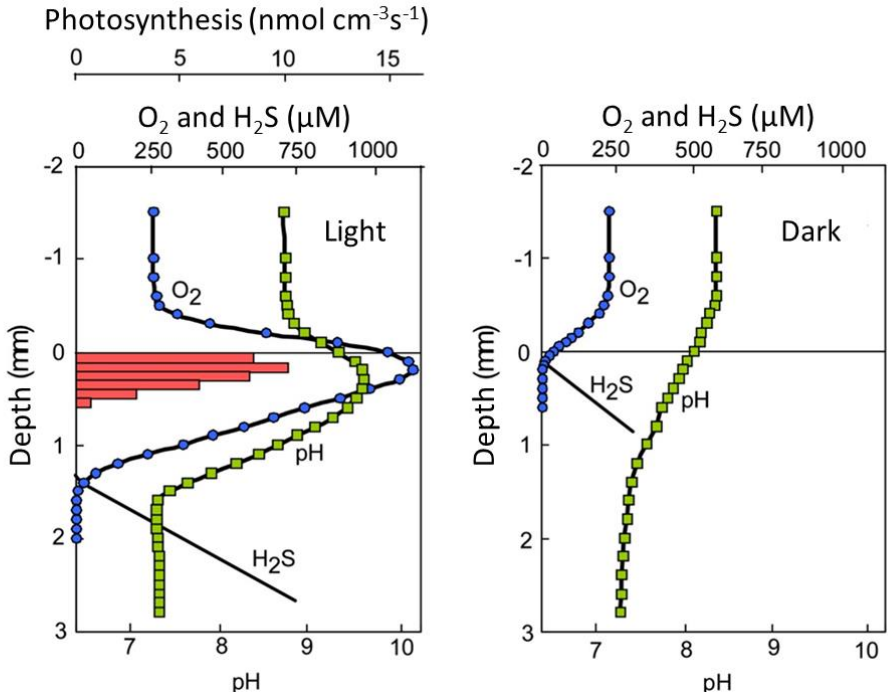
$$O_2 + 4e^- + 2H_2O \rightarrow 4OH^-$$

When the gold surface (the **cathode**) donates electrons by the process of oxygen reduction, these electrons have to be replenished by the battery, and we get an electrical current in a circuit between the gold cathode and a silver **anode** (connected to the positive pole of the battery) inside the sensor (the silver anode is not shown in the figure). The current in this circuit is proportional to the concentration of oxygen outside the oxygen-permeable silicone membrane in the tip. The silicone membrane ensures a stable chemical environment in the liquid (electrolyte) inside the microsensor and prevents the entry of chemical species that could disrupt microsensor functioning. The basic design of similar macroscale sensors was invented as early as 1954 by a British physiologist, L.C. Clark, and such sensors are therefore named Clark sensors or Clark electrodes.

7. ***Microsensors need to be operated with precision by a micromanipulator.*** To examine the variation in levels of a parameter like oxygen within a given microenvironment, it is necessary to introduce the microsensor into the substrate in a stepwise and very controlled manner. First of all, microsensors are very delicate and we do not want to break them, but accuracy is also necessary as, for example, the concentration of oxygen may change very much over distances of much less than a millimeter. Microsensors are thus usually mounted onto a device called a **micromanipulator**, whereby the microsensor can be moved incrementally in steps of down to 1 µm in a sample or habitat. This allows us to "scan" the habitat by moving the sensor and taking readings at each position, and thereby to "map" changes in oxygen levels in space and over time.

The insertion of two microsensors held by such a micromanipulator while being introduced into a photosynthetic microbial biofilm is shown in the figure above. For such environmental analysis it is normal to introduce the sensors at steps of 0.1 mm (100 µm) and then wait for a few seconds at each depth to get a stable reading.

8. ***What kinds of things can we do with microsensors? Example: investigate a photosynthetic biofilm.*** As indicated above, microbiologists want to know what the microbes are doing under certain conditions, such as when they are producing or consuming **greenhouse gases.** A set of data for oxygen (O_2), hydrogen sulfide (H_2S) and acidity (pH) measured in a photosynthetic biofilm is shown in the figure below. Depth zero marks the surface of the biofilm. You can see that when the biofilm was kept in darkness, oxygen in the overlying water decreases the closer the microsensor gets to the biofilm, becoming zero 0.2 mm into the biofilm. However, when the biofilm was illuminated, oxygen concentration increases as the microsensor approaches the biofilm, reaching a peak 0.2 mm into the biofilm that is 5x the concentration of the air-saturated overlying water, and becoming zero at a depth of only 1.5 mm. This is because microbes in the biofilm carried out photosynthesis when illuminated, and produced oxygen. The rates of photosynthesis at various depths can be calculated from oxygen microsensor data and are shown as bars in the figure. Photosynthesis could be detected down to 0.6 mm depth, below which the light intensity became too low. Light intensity inside such substrates can be measured by various types of fiber optic microsensors.



Depth distribution of oxygen (O_2), hydrogen sulfide (H_2S) and acidity (pH) in a photosynthetic biofilm during illumination (left) and darkness (right). Redrawn after original of Bo Barker Jørgensen.

As can also be seen, the acidity (pH) rose and fell more or less in step with oxygen. During illumination, carbon dioxide (CO_2, in water partially found as carbonic acid, H_2CO_3) was consumed by photosynthesis, so the pH was higher in the photosynthetic layer. During darkness, CO_2 was produced by microbial respiration and pH decreased.

Hydrogen sulfide and O_2 only co-existed within a very narrow zone of the biofilm. This overlap zone is often less than 0.1 mm thick, and specialized bacteria live in it and get their energy by mediating the oxidation of H_2S. H_2S moves up into the oxic layer by a process called diffusion, and oxygen also moves into the O_2/H_2S overlap zone by diffusion. Diffusion is the process of random movement of individual molecules/ions in liquids and gases; it results in the net transport of molecules/ions from regions of high concentration to regions of lower concentration. This diffusional transport of chemicals is comparable to heat transport from layers with high temperature to layers with low temperature: if you dip a metal teaspoon into hot tea you will soon feel the handle warming up.

9. ***The discovery of cable bacteria.*** A very important discovery was made in 2012 when microsensor data showed that O_2 and H_2S profiles in a marine sediment did not overlap, in fact that they were well separated. How on earth could H_2S be oxidized if no oxygen was available? It turned out that long filaments of bacteria, consisting of more than 10,000 cells joined end-to-end, connected the surface oxic and deeper sulfidic layers that were separated by a distance of about 1 cm. And, amazingly, these filaments transported electrons from H_2S to O_2, creating an electric current in the sediment. The interaction of H_2S and O_2 that results in oxidation of H_2S and the reduction of O_2 involved physically-separated reactions! At the time of writing, it has not yet been clarified what the electrical wires consist of. The electrically conducting microbial

filaments is just one example of how use of microsensors has changed our understanding of how nature works.

10. ***There exist microsensors for measurement of many physico-chemical parameters.*** Oxygen, hydrogen sulfide and acidity are just examples of physico-chemical parameters that can be measured with microsensors. Other biologically important chemical species that can be measured with microsensors are the plant and microbial nutrients nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+), and the key future energy carrier hydrogen (H_2).

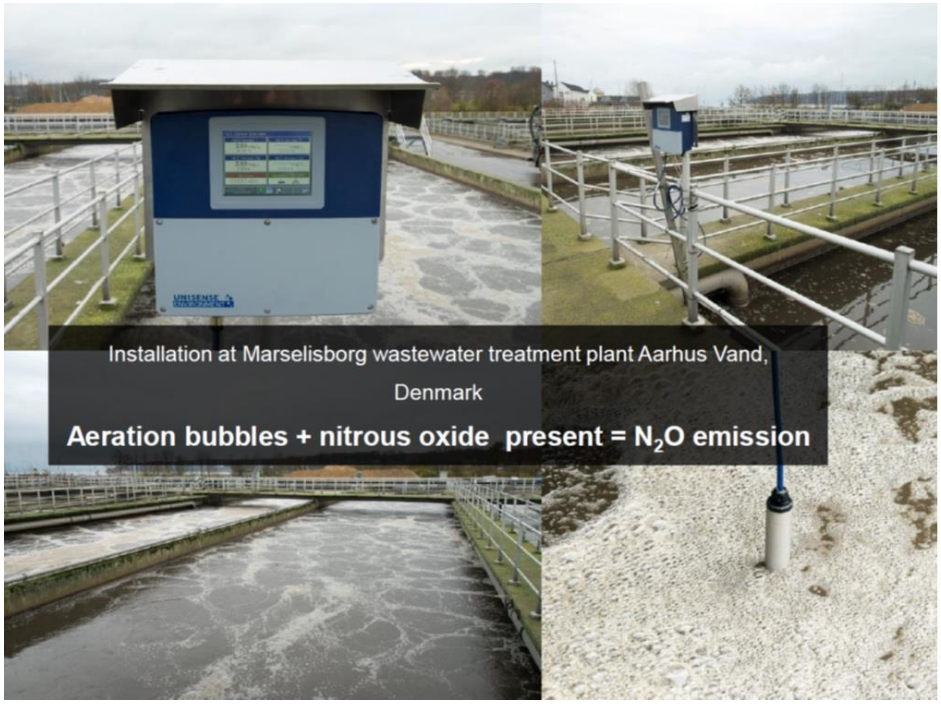
By construction at a microscale, it has been possible to make microsensors where no macroscale version exists. The main reason for the success of microscale construction is that the transport of molecules and ions over the short distances inside the sensor is very rapid. Reactants for -and waste products from - reactions are thus efficiently transported to or away from the sensor tip by diffusion. An example of this is the microsensor for nitrous oxide (N_2O). It is not possible to measure N_2O with an electrode if O_2 is present, but the O_2 can be removed by alkaline ascorbic acid. New ascorbic acid is supplied to the tip of the microsensor by diffusion and the consumed, oxidized ascorbic acid is taken away by the same process.

11. ***Microsensors can inform about the production and consumption of greenhouse gases.*** Microsensors exist for the greenhouse gases carbon dioxide (CO_2), nitrous oxide (laughing gas, N_2O), and methane (CH_4), and so represent key tools to investigate the production and consumption of these agents of global warming. As a greenhouse gas, nitrous oxide is almost 300 times more potent than carbon dioxide, so its emissions from all kinds of sources need to be minimized.

Wastewater treatment plants are a significant source of N_2O. The reason is that wastewater contains large amounts of nitrogen in the form of cellular compounds like proteins which, in the plant become converted to amino acids, then ammonia, then nitrogen oxides (the process of nitrification), and finally nitrogen gas (the process of denitrification) which escapes to the atmosphere. However, not all nitrogen in wastewater is converted to nitrogen gas, and some ends up as N_2O, which is also released to the atmosphere as a greenhouse gas. How much N_2O is formed is determined by the operating conditions of the plant which in turn influence the microbial activities involved in N_2O production. Prompt adaptation of these operating conditions to minimize N_2O production requires accurate, continuous, real-time measurement of N_2O production levels. The nitrous oxide microsensor is an example a sensor where no macroscale analogue has been made and is now widely used to monitor and control emission of nitrous oxide from wastewater treatment plants, as shown at the figure below.

12. ***Microsensors are being deployed in many different settings.*** Chemical microsensors have expanded our knowledge in many areas. They have for example been used extensively to explore the seabed at water depths down to 11,000 m. It is often stated that we know less about the deep ocean that we know about the moon, but robotic instruments ("landers") equipped with microsensors for measurement in the sediment have supplied valuable knowledge. The picture below shows such an instrument before deployment in the deep Pacific Ocean. A bank of microsensors is mounted on the pressure-proof electronics cylinder in the lower center of the frame. The instrument can ascend to the surface by release of ballast when the measurements are completed.

Microsensors have also been used extensively for exploration of animal and plant physiology.



Microsensor meaurement of N_2O in the tanks of a wastewater treatment plant where bacteria mediate the degradation of wastewater constituents. The signal from the electronics box is transmitted to the control room of the plant. The foam at the water surface is created by vigorous bubbling with air. Illustration by Unisense A/S, with permission.

Deployment of a deep sea "lander"in the Pacific Ocean. The lander is equipped with microsensors for analysis of the deep sea sediments. The lander was constructed by R.N. Glud, University of Southern Denmark. Photo by Niels Peter Revsbech.

Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 6. Ensure availability and sustainable management of water and sanitation for all**. Sewers are degraded by corrosion caused by hydrogen sulfide, causing enormous economic losses every year. Macrosensors that are microsensors in thick casing are used to help mitigate build-up of hydrogen sulfide (https://sulfilogger.com/wastewater/).Wastewater treatment may be controlled by use of microscale **biosensors** for nitrate and nitrite, but further research is needed to obtain the required long-term sensor stability.
- **Goal 7. Ensure access to affordable, reliable, sustainable and modern energy for all.** We expect a future "hydrogen economy", and research in hydrogen transformations in for example biogas plants is facilitated by H_2 microsensors.
- **Goal 13. Take urgent action to combat climate change and its impacts** Microsensors for greenhouse gases enable not only measurement of their production and consumption by biological systems, and their monitoring in engineered systems like wastewater treatment plants, but also insights into their mechanisms of production. All of these will be relevant to controlling and reducing their production and release to the atmosphere.
- **Goal 14. Conserve and sustainably use the oceans, seas and marine resources for sustainable development**. Microsensors have been used to elucidate transformations of algal nutrients in oceans and seas. Microsensors may also be used to prevent toxic build-up of H_2S in aquaculture.

The Evidence Base, Further Reading and Teaching Aids

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Glossary

Anode: A positively charged electrode
Biofilm: A matrix composed of microorganisms and the slimy compounds they excrete. Dental plaque is a good example.
Biosensor: A sensor where a biological component such as enzymes or living cells is creating the signal
Cathode: A negatively charged electrode
Greenhouse gases: Gasses that absorbs and emits heat radiation and thereby lower heat emission from our globe to space.
Micromanipulator: A device used to position for example the tip of a needle-shaped microsensor at high accuracy. A micromanipulator can be manually operated, or by computer control of small electric motors moving in one, two or three dimensions.