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Microbial activities and chemical gradients in the chemocline of a meromictic lake in relation to the precision of the sampling procedure

Knut Yngve Børsheim [a], J. Gijs Kuenen [b], Jan Gottschal [c]* and Ian Dundas [a]

[a] *University of Bergen, Department of Microbiology and Plant Physiology, Allegt. 70, 5000 Bergen, Norway,* [b] *Delft University of Technology, Laboratory of Microbiology, Julianalaan 67a, Delft, The Netherlands, and* [c] *Rijksuniversiteit Groningen, Biologisch Centrum, Laboratorium voor Microbiologie, Kerklaan 30, 9751 NN Haren, The Netherlands*

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1. SUMMARY

The dark CO_2 fixation rate, and sulfide and oxygen concentrations, were measured in the chemocline of the eutrophic, meromictic lake Sælenvannet in Western Norway. Sulfide and oxygen coexisted at a depth of 4–5 m in a narrow layer, only 2.5–10 cm wide. Coexistence of oxygen and sulfide coincided with an increase in the rate of dark CO_2 fixation. Maximal potential for light-dependent CO_2 fixation was found 2.5 cm below the sulfide and oxygen coexistence region. Our results demonstrate that a number of conventional sampling techniques are unsuitable for the study of such interfaces, and that very precise sampling techniques are needed to measure the chemical gradients and biological processes taking place in the chemocline of shallow meromictic lakes.

2. INTRODUCTION

Meromictic lakes and meromictic marine basins almost invariably contain sulfide in their stagnant bottom waters [1]. The anaerobic water bodies serve as sources of nutrients for the oxic layers in the meta- and epilimnion. However, the sulfide in particular may also represent a threat to the aerobic life. The microbial community in the interface may act as a biological lid on the bottom layer, consuming both energy-rich degradation products and inorganic nutrients, including sulfide [2–4]. The latter is detoxified by its oxidation to sulfate, by either aerobic or denitrifying chemolithotrophs, or by phototrophic bacteria living at the interface. A detailed knowledge of the processes occuring at the interface is thus the basis for full understanding of the ecosystems of meromictic lakes, and of the interactions between anaerobic and aerobic communities in these systems.

In large marine basins, such as the Black Sea, the width of the interface (here defined as the layer where sulfide and oxygen coexist) is 30–50 m, and samples can be taken with the usual type of sampling devices [3,5]. In shallow meromictic lakes, however, the interface regions are extremely narrow. In a study of 3 meromictic lakes, Sorokin [1] sampled with a closing cylinder (Susljaev bottle), which did not allow an estimate of the width of the interface. Better precision can be obtained by siphoning or pumping [6,7]. Jørgensen et al. [7]

* To whom correspondence should be addressed.

designed a pumping-type sampler for their investigations of a shallow (5 m) highly saline pond in Sinai. They found that the width of the interface was between 0 and 10 cm, and that its exact profile and location depended on the diurnal cycle. Another refinement in their study was the simultaneous and independent measurement of oxygen and sulfide in the same samples.

In the course of a study on several aspects of primary production and the mineralization in a meromictic lake in Norway, Sælenvannet, attention was particularly focussed on the interface of this lake [8,9]. Using a Ruttner sampler, Indrebø et al. [8] were unable to accurately analyze the interface between the aerobic and the anaerobic part of the lake. Therefore it was decided to work out a more accurate sampling method to study the chemical and biological gradients in this interface. Using a pumping-type sampler, as used by Jørgensen et al. [7] we succeeded in measuring oxygen and sulfide gradients and in obtaining data on the rate of microbial production within the very narrow interface region.

3. MATERIALS AND METHODS

Sælenvannet is a meromictic lake on the west coast of Norway (60°20'N, 5°17'E). The lake is connected to a landlocked fjord through a shallow channel, and occasionally receives an input of brackish water with a salinity of approx. 20‰. The surface area of Sælenvannet is 0.6 km^2, and the maximum depth is 24 m. The stagnant bottom water is anoxic and sulfide-containing below a depth of 4–5 m [9]. Just below the interface there is a bloom of photosynthetic sulfur bacteria. The bloom is most pronounced during the autumn and winter. The major portion of the photosynthetic bacteria consists of *Chlorobium phaeovibrioides* [8]. In subsequent studies, a minor population of *Chromatium* cells was detected by phase contrast microscopy.

Sampling was carried out at the deepest part of the lake. The boat was secured by 4 steel wires, anchored about 50 m apart and drawn tight. We used a pumping-type sampler [7]. The sampling inlet consisted of two polyvinylchloride cones with their bases spaced 1 cm apart, hung by a steel chain at the depth sampled. The upper cone had a central hole connected to a tube, through which the samples were pumped to the surface with a peristaltic pump. The pumping rate was 500 ml · min^{-1}.

Dissolved oxygen and sulfide were measured in the same samples [10].

CO_2 fixation was measured in 60-ml glass-stoppered bottles after the addition of 20 μCi carrier-free $^{14}CO_2$ and incubated for 2 h. The high radioactivity was needed to ensure sufficient sensitivity for the detection of the dark CO_2 fixation. Dark CO_2-fixation rates were linear for 3 h.

As shown by the light measurements, there was an extremely steep gradient of light intensity from 495–505 cm in the chemocline, so in situ incubation of the light CO_2 fixation bottles was not attempted. Instead, light CO_2 fixation bottles were incubated 1 m above the interface, ensuring light-saturating conditions. Dark CO_2 fixation bottles were incubated at the depth sampled. Samples fixed with 2% formaldehyde before adding the radioactivity served as blanks. After incubation, the samples were fixed with formaldehyde and filtered within 2 h through Whatman GF/F filters. This fixation procedure caused a slight loss (less than 10%) of radioactivity in the particulate material.

The filters were fumed with HCl, dried at room temperature, and Instagel scintillation cocktail (Packard) was then added. The radioactivity was counted in a Packard Tri-Carb Scintillation Counter. Samples fixed at zero time served as blanks for the dark bottles. Net light CO_2 fixation was calculated by subtracting the values from the dark bottles from the values from the corresponding transparent bottles.

In one experiment, DCMU (3-(3,4-dichlorophenyl-1)-1-dichloromethyl-urea) was added to a parallel series of light bottles (final concentration 10 μM), CO_2 was measured with an infrared analyzer from The Analytical Co LTD, type 225 HK2 [9]. Light intensity was measured with a submersible lux-meter (Dr. Lange, Gmbh, Standard-Beleuchtungsmesser 1).

Protein in particulate matter was measured with the method of Lowry et al. [11] after concentration

by filtration and washing with 100% ethanol to remove sulfur and sulfides.

Pigments were measured in whole cells, concentrated by centrifugation and resuspended in 2 M sucrose solution. Absorbance was measured in a spectrophotometer (Shimadzu MPS-50L). The distinct peaks at 720 nm and 675 nm, very close to the absorbance maxima reported for algal and bacterial chlorophylls, respectively [12], were extrapolated to a symmetrical shape (deconvoluted) and integrated.

4. RESULTS

Fig. 1 shows the dark CO_2 fixation rate and sulfide and oxygen gradients as measured during an experiment conducted before sunrise. A very steep gradient of sulfide and oxygen concentration was observed, at a depth of approx. 5 m, with the sulfide concentration increasing by 20 $\mu M \cdot cm^{-1}$ just below the chemocline. A peak in the dark CO_2 fixation rate was detected in the chemocline region.

To rule out the possibility that the increase in dark CO_2 fixation was a consequence of increased biomass in the chemocline, specific CO_2 fixation was calculated (rate of CO_2 fixation per mg protein). The protein concentration in the samples varied from 2–3 mg protein $\cdot l^{-1}$ above the chemocline, to approx. 8 mg protein $\cdot l^{-1}$ at 5.00 m. The peak in dark CO_2 fixation was still pronounced when the specific CO_2 fixation was calculated. Fig. 2 shows the results of an experiment conducted at noon, comparing sulfide and oxygen profiles with specific dark CO_2 fixation. At noon, the peak was less pronounced, and the coexistence region was narrower than before sunrise.

The light intensity approached the detection limit of our instrument (1 lux) coinciding with the region of H_2S/O_2 coexistence. Since the gradient of light intensity was very steep, and photosynthesis was probably light-limited, no attempt was made to measure in situ light CO_2 fixation. Fig. 3 shows the potential light CO_2 fixation, as measured in samples incubated one meter above the chemocline. The maximum potential for light CO_2 fixation was found in the sample just below the

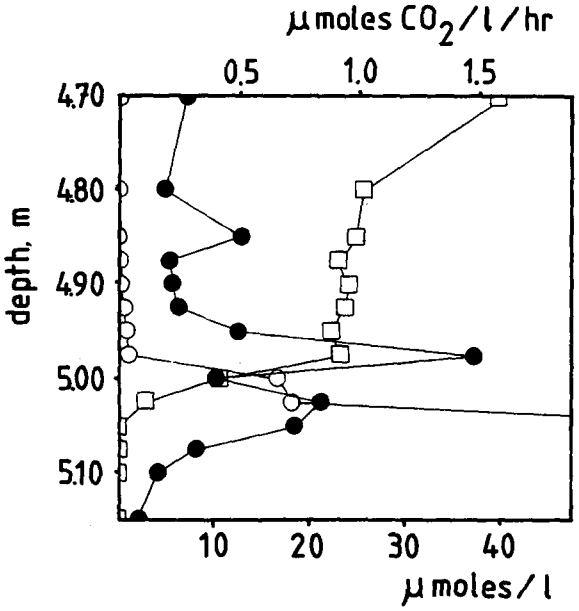

Fig. 1. Rate of dark CO_2 fixation (●———●), oxygen concentration (□———□) and sulfide concentration (○———○) as a function of depth, before sunrise (5–7 a.m.).

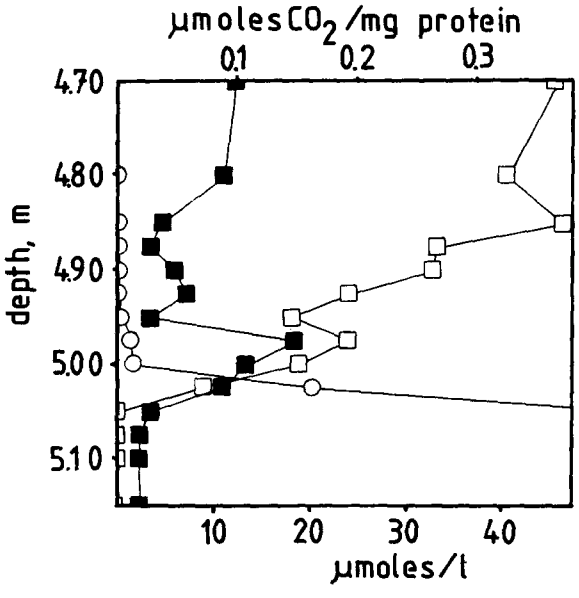

Fig. 2. Specific rate of dark CO_2 fixation (■———■), oxygen concentration (□———□) and sulfide concentration (○———○) as a function of depth, at noon (1–3 p.m.).

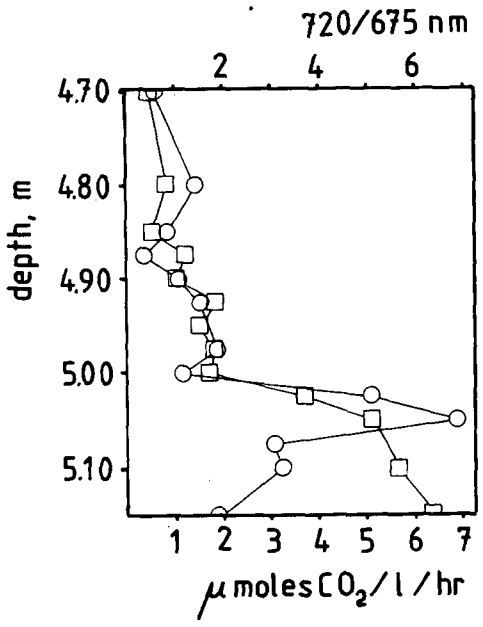

Fig. 3. Net light-dependent CO_2 fixation in samples incubated at a depth of 4 m at midday (1–3 p.m.) (○——○) and the ratio between absorption by pigments at 720 nm (phototrophic bacteria) and 675 nm (algae) (□——□).

chemocline. This maximum potential coincided with an increase in *Chlorobium* pigments (Fig. 3). The *Chlorobium* pigment concentration continued to increase below the interface, but the light CO_2-fixation potential decreased from 5 cm below the interface.

When DCMU was added to a series of samples taken at depths of 4.70–4.97 m, light-dependent CO_2-fixation was lowered by 66–74% compared to a parallel series of samples incubated in the absence of DCMU. Using samples from 5.00–5.15 m (including the depths where maximum light-dependent CO_2 fixation was observed), a similar experiment yielded differences in the CO_2 fixation rates of $< 14\%$.

5. DISCUSSION

Interfaces between aerobic and anaerobic, sulfide-containing layers in meromictic lakes have been shown to represent highly dynamic systems [1,7]. Biological and chemical oxidation/reduction reactions take place simultaneously or alternately. Lake Sælenvannet does not seem to be an exception to this rule.

Even sampling intervals of 2.5 cm may not be precise enough to estimate the exact width of the chemocline. We could show that the total width of the interface in Sælenvannet extended over only 5–10 cm, and that its exact width varied during the diurnal cycle. Before sunrise, when the activity of the photosynthetic bacteria was minimal, the chemocline was wider and the dark CO_2-fixation was higher than in the middle of the day. The anaerobic phototrophic sulfur bacteria, and the aerobic sulfur oxidizing chemolithotrophs influence each other in a rather complex way. Both are dependent on the availability of reduced sulfur compounds. The aerobic sulfur bacteria can only use what is left over by the *Chlorobium* population. However, *Chlorobium* is a strict anaerobe, and is dependent on a population of oxygen consumers to remove the oxygen from their immediate environment. The interaction between the two is thence rather more mutualistic than competitive.

The presence of both sulfide and oxygen coincided with an increase in the specific rate of dark CO_2 fixation. This suggests that the increase was caused by chemolithoautotrophic activity. Moreover, since the protein measurement gives an estimation of total biomass, including algal and detrital protein, the estimated specific activities must be considered as minimal values for the bacterial chemotrophic activity in the interface.

In spite of the demonstrated chemosynthetic activity, attempts to isolate thiobacilli from the chemocline samples were not successful (Gottschal and Kuenen, unpublished). These findings parallel earlier results from interfaces in other marine meromictic basins [13,14]. There is little reason to exclude the possibility of a bacterial group being present just because attempts to isolate members of the group have failed. It is also possible that the increased dark CO_2-fixation is caused by mixotrophic bacteria or heterotrophic bacteria stimulated by the availability of reduced sulfur compounds [15,16]. The strict anaerobic nature of the *Chlorobium* rules out the possibility that the high dark CO_2 fixation was due to these phototrophs. The

Chromatium species detected in the samples may have been responsible for some of the dark fixation, as it has been shown that some of these bacteria can grow not only as photolithotrophs under anaerobic conditions, but also as aerobic chemolithoautotrophic sulfide-oxidizers in the dark [17,18].

From our results it is clear that the sampling technique is crucial in the study of interfaces of lakes such as Sælenvannet, where different populations of bacteria are separated in narrow layers, and the chemical gradients are very steep. Had the Ruttner sampler been used [8], the different layers would inevitably have been mixed, resulting in a dilution of the locally high population densities. This would have made the detection of the elevated dark CO_2-fixation in the interface very difficult or even impossible.

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