Schedule for “Measuring photosynthesis and respiration in a microbial mat” 8/3-2006

Note! – it is a good idea to have looked into the articles that are relevant for the exercise (Kühl et al. 1996, and Revsbech&Jørgensen 1983)

9-10.30 Intro lecture on “Measuring microbial photosynthesis” and introduction to microsensor setup and measurements.
11-17 Experimental work with microsensor measurements in a microbial mat. You manage lunch breaks and other breaks yourself. See also a more detailed exercise plan below.

PRACTICAL WORK:

Familiarize yourself with the setup. Refresh how a micromanipulator works. What are the strip-chart recorder settings etc.

Measure the intensity of the incident light for different setting of the lamp (position 1-5) by using the quantum irradiance meter. NB! Carefully slide the experimental chamber to one side. Watch out not to break the microsensor tip! We have only one light meter available. Not to loose time, one team may thus continue with the procedures outlined below and then measure the light intensities at the end.

Measure the temperature and salinity of the water with an electronic thermometer and a refractometer, respectively – the bulk water can be regarded air-saturated.

Position the chamber with the microbial mat sample below the microsensor setup. Position the oxygen microsensor into the water above the mat. Wait for a stable signal and note on the strip-chart that the signal corresponds to the oxygen content in air-saturated water at the determined temperature and salinity.

Measure 2-3 oxygen profiles with the mat kept in darkness (lamp off and dim light in the classroom). Note the recorder output for “zero” oxygen on the strip-chart..

Position the microsensor tip on the mat surface and wait for a stable signal in darkness. Turn on the lamp (setting 2 or 3) and mark the time on the recorder paper. Do also note the recorder speed (it should be about 1 cm per min.). The oxygen concentration on the mat surface is now followed onto it stabilizes at a new and higher level – this takes 10-15 min.

When the oxygen concentration reaches a steady-state in light, i.e. a stable oxygen signal over time, try out the light-dark-shift method for measuring gross photosynthesis. This may involve at least 2 persons:

- One person is in charge of the micromanipulator and darkening. A small black plate is quickly introduced into the light path and another person marks on the recorder paper when this happens.
- It is only necessary to darken for 2-4 seconds – enough to record a clear slope on the chart paper. Remove the plate and wait for the oxygen signal to get back to the steady state level. Then the measurement can be repeated – in the same depth or after positioning the microsensor 100 µm deeper.
• NB! In order to obtain a clear slope on the chart paper, it may be necessary to increase the chart paper speed. But this is only necessary to do just before and during the light-dark-shift. NOTE DOWN THE CHART SPEED USED DURING THE LIGHT-DARK SHIFT. As soon as the mat is illuminated again you can decrease the chart speed to save paper.
• It is important to put a mark next to the recorder pen exactly when the light-dark-shift is done, in order to identify a lag in oxygen sensor response (see e.g. in Revsbech & Jørgensen 1983 what such a delayed response may indicate)

When you are familiar with the light-dark-shift method, you are ready to measure depth profiles of oxygen concentration and gross photosynthesis:
• Slowly retract the oxygen microsensor from the mat surface until it gives a constant reading. The microsensor tip is now in the turbulent bulk water above the diffusive boundary layer.
• Now go down in steps of 100 µm (remember to mark each step clearly on the recorder paper). In the diffusive boundary layer, the sensor signal may be a bit fluctuating over time, but when the signal fluctuations are dampened you are close to the mat surface. At this position you make the first light-dark-shift measurement.
• After getting back to steady state, you go 100 µm deeper into the mat, record the steady-state level at this depth and then make a light-dark-shift measurement. This procedure is repeated at increasing depths until there no longer is a clear response to a light-dark-shift.
• Continue to measure the oxygen profile until you reach zero oxygen.

Based on profile and light-dark-shift measurements, you can now investigate how the photosynthesis in the mat is regulated by e.g. increasing light intensity or e.g. flow.

As a minimum, ALL TEAMS should measure a set of oxygen profiles and gross photosynthesis profiles at different light intensities (darkness, low, moderate and high light).

If time does not allow to measure gross photosynthesis profiles differen light intensities, try at least to measure oxygen profiles at 3-4 different light levels.

When you change the light level, it is a good idea to have the microsensor tip positioned at the mat surface. This allows you to follow how the oxygen level responds and reaches a new steady-state level after the light conditions have been changed – this will typically take 15-30 min.

You can also try to record complete light-dark cycles with the microsensor tip positioned on the mat surface (or e.g. in the most active region of the mat) recording the change from steady state dark to steady state light and back to steady state dark conditions.
Or you may want to modify the flow velocity keeping the light condition constant.
QUESTIONS, DATA ANALYSIS etc.:

Note again! – it is a good idea to have read Kühl et al. (1996) and Revsbech&Jørgensen (1983), where calculations are described in detail.

During the next long day (15/3 2006) there is time allocated for the groups to finish data analysis and to prepare answers to some questions (given below), which will be discussed later on the same day.

ALL GROUPS should bring their results as data and graphs into a spreadsheet for presentation.

Tables for i) the oxygen solubility in air saturated water and ii) the oxygen diffusion coefficient at various temperature and salinity have been handed out.

Basic data treatment of data on stripchart:

Calculate all oxygen levels in units of $\mu$mol O$_2$ liter$^{-1}$ ($1 \mu$mol O$_2$ l$^{-1} = 1$ nmol O$_2$ cm$^{-3}$)

Calculate fluxes of oxygen with Fick’s first law in units of nmol O$_2$ cm$^{-2}$ s$^{-1}$.

On oxygen profiles measured in light, try to determine both the oxygen flux up into the water and down into the aphotic zone of the mat.

Calculate the gross photosynthesis rate in each of the measuring points, $P(z)$, in units of nmol O$_2$ cm$^{-3}$ s$^{-1}$.

Find the total areal gross photosynthesis, $P(G)$ (in units of nmol O$_2$ cm$^{-2}$ s$^{-1}$), by depth integration of the measured rates:

$$P(G) = \text{porosity} \cdot \sum P(z) \cdot (\text{depth interval between measurements}).$$

The diffusion coefficient for oxygen in water at experimental temperature and salinity, $D_0$, can be found in the tables. The diffusivity of oxygen in the mat is assumed to be: $\phi D_s = 0.7 \times D_0$. The porosity of the mat is assumed to be 0.95.

Graphs:

Plot the oxygen concentration and the gross photosynthesis rate as a function of the depth in the mat for each of the light intensities used in the experiments.

Plot the net photosynthesis and the total gross photosynthesis as a function of the light intensity.

Plot the oxygen concentration and the gross photosynthesis rate measured about 0.1-0.2 mm below the mat surface as a function of the light intensity,
Questions:

Describe and explain your oxygen profiles measured in dark and light conditions. Explain the shape and why they look as they do (how thick is the diffusive boundary layer? Where is there a net production of oxygen – and where a net consumption? What is the oxygen penetration? etc.)

Describe the depth distribution of gross photosynthesis measured at different light intensities. What is the maximum thickness of the photic zone? What limits the thickness of the photic zone?

How fast does it take the mat to reach a new steady-state level after the light has been manipulated? What is determining the time it takes?

Explain how the net photosynthesis and the total gross photosynthesis vary with light intensity. What do the curves show?

Compare with a curve of the gross photosynthesis in 0.1-0.2 mm of the mat as a function of light intensity – explain similarities and differences.

Calculate and compare the oxygen consumption of the mat in darkness and in light. Is the oxygen consumption the same? Why/why not?

How will increasing light intensity affect other chemical parameters and biological processes in the mat? What other factors than light affect photosynthesis and oxygen consumption in the mat – and how?

Mention and discuss some methodological problems with microsensor-based measurements of photosynthesis.