Eutrophication: from nutrients to algae

Problem statement:

Which nutrients are important for algal growth?

Have you ever seen 'foam' on the beach and wondered what it is and where it came from?

Method: Chemical analysis

Skills: Research skills

Target group: Secondary school (14-18 yr.)

Theoretical part:

Foam

In spring time the light increases in duration and intensity so that microscopic particles in seawater - so called phytoplankton – start to develop strongly. For their growth they need **nutrients** such as **nitrates** (NO_3^-) and **phosphates** (PO_4^{3-}). Many of the nutrients enter the sea through our rivers and estuaries and an excess of these nutrients is called **eutrophication** ('eu' = true or well; 'trophy' = food). These nutrients allow algae to multiply very quickly resulting in an algal bloom. The main effects of eutrophication are lack of oxygen, changes in the ecosystem, deterioration of water quality (bad smell and taste of the water) and development of algal species that can produce toxins potentially harmful to humans and animals.

The algal bloom occurring in spring in the North Sea is reflected in the formation of foam on the beach (figure 1). The foam is caused by remnants of decaying and dead algae (scientific name: *Phaeocystis*, figure 2) that are stirred up by the waves and the wind.

Figure 1: "Foam" algae (*Phaeocystis*) on the beach
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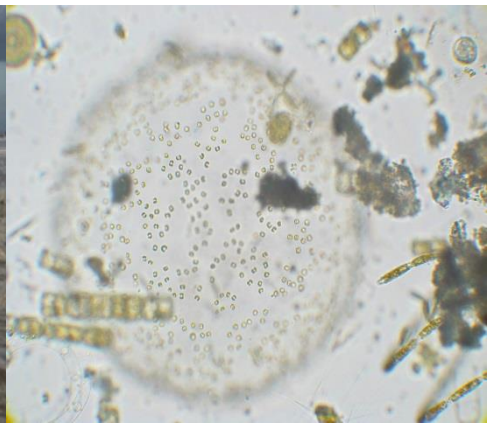

Figure 2: Microscopic image of *Phaeocystis* cells
Source: PAE Ugent

Phaeocystis are unicellular algae species (figure 3) and the size of an individual cell varies from 3 to 7 micrometer in diameter.

Figure 3: Microscopic image of individual *Phaeocystis* cells
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How can such small algaе form so much foam? This is because the cells organize themselves into large spherical **colonies** (figures 4 and 5) when nutrients are abundant. If you fill a bucket with seawater when the sea starts foaming, you can easily see the colonies.

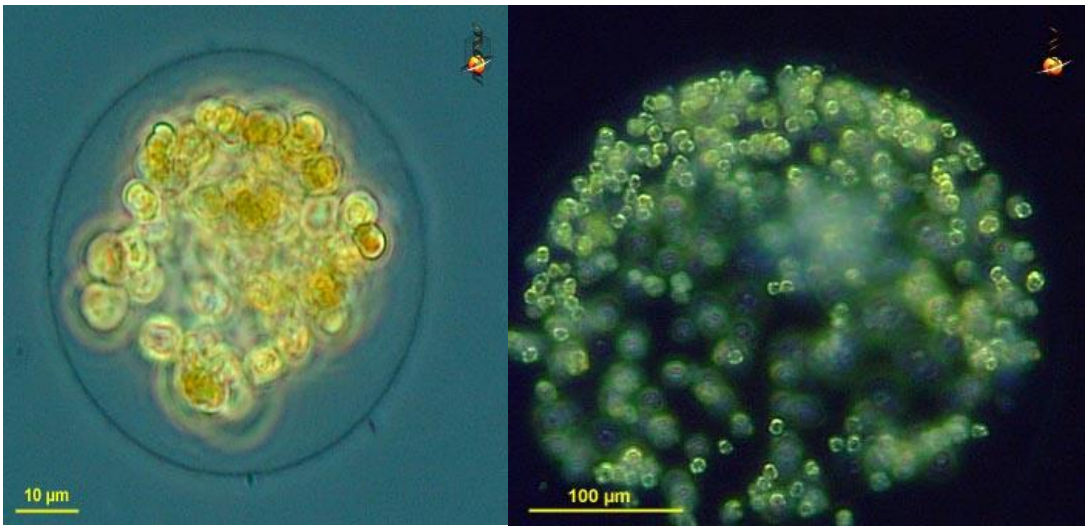

Figure 4 and 5: *Phaeocystis* cells forming gelatinous colonies when nutrients are abundant
©Bob Andersen and D. J. Patterson – Courtesy of NCMA

These colonies can grow very fast, causing large 'blooms'. Most of the cells are actually arranged at the periphery of the colony, which is a mucus-like watery ball (gelatinous). This layer of mucus protects the algae from grazing and is also used as a storage for nutrients so that these algae can continue to grow even at night. Because the colonies are so large, they are hardly eaten by 'grazers' like copepods (small crustaceans) and unicellular grazers ('microzooplankton' – zooplankton 20-200 µm). When they have depleted the nutrients in the water, the colonies deform and start to disintegrate. A part sinks to the bottom and another part is attacked by special viruses, causing the cells to fall apart (lysis). During this decaying phase, the foam is released and when the wind conditions are right, it can form huge quantities on the beach. Although foam is a natural phenomenon and not a direct result of pollution there is still a link between the amount of foam on the beach and eutrophication. These algae can grow far better when more nutrients are present in

the water resulting in more foam. Fortunately the foam is not toxic but fishermen do not like *Phaeocystis* as colonies can clog their nets. As mentioned above, *Phaeocystis* is hardly eaten by copepods, which is preferred food for small fish, which are in themselves good food for larger fish. For this reason *Phaeocystis* does not contribute much to the fisheries industry. In very severe causes they can settle on mussel beds, causing anoxia and killing the mussels.

Materials:

- Microscope
- SERA tests for phosphate and nitrate (you can buy them in any aquarium shop)
- Seawater sample
- Sieve
- (Coffee)filters
- Funnel
- Sampling jars
- Mixer
- Egg
- Copper bowl (for whisking your egg)
- Fertilizer (Roses: 12 % N, 5 % P) and spoon
- Stopwatch

Practical part

1. Collect a sample of seawater and observe with the microscope: can you see some species of phytoplankton and / or zooplankton?
2. Prepare 2 samples:
   - Sample 1: Collect a seawater sample and use a filter to make sure that there is no sand in your sample. You should have about 100 mL filtrate.
   - Sample 2: Collect 200 mL of seawater in a jar and add 4 spoons of fertilizer. Shake a few minutes so that the fertilizer can dissolve. Wait a few minutes. Now pour the content over the (coffee)filter until you have a 100 mL filtrate.
   - Measure the concentrations of nitrate and phosphate with the test kit (see protocol).
   Note: Sometimes it is difficult to determine the exact color of your sample (especially for phosphate, look and compare the difference in color between sample 1 and sample 2).

	Seawater (blanco)	Seawater (+ fertilizer)
NO_3^- (mg/L)		
PO_4^{3-} (mg/L)		
Colour		

Measuring

Phosphate

Protocol SERA test for phosphate

- Rinse the measurement vial several times with the (sea)water to be tested, then fill to the 10 mL mark. Dry the vial on the outside.
- Add **6 drops of reagent 1** into the vial and shake the vial until the liquid is evenly distributed.
- Add **6 drops of reagent 2** into the vial and shake the same manner.
- Add one heaping measurement spoon (white) of **reagent 3**. Close vial with cover and shake. Then, remove the cover.
- Wait **10 minutes.**
- Compare the colors with the color chart (from a position above at natural daylight, avoid direct sunlight).
- If the water is dark blue : repeat the test with a diluted sample (5 mL sample + 5 mL distilled water or 2 mL sample + 8 mL distilled water). Do not forget to read the value in the correct line. If the result does not show any blue color the water is extremely low in phosphate or phosphate-free.
- Clean vial and cover thoroughly with tap water before and after each test.

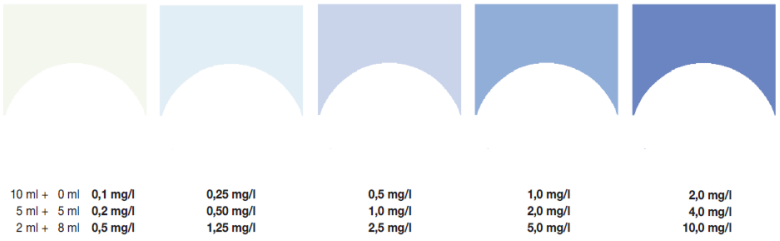

Photo credit: Sera®GmbH

Nitrate

Protocol SERA test for nitrate

- Rinse the measurement vial several times with the (sea)water to be tested, then fill to the 10 mL mark. Dry the vial on the outside.
- Add **6 drops reagent 1** into the vial and shake the vial until the liquid is evenly distributed.
- Add **6 drops reagent 2** into the vial and shake the same manner.
- Add one **measurement spoon** (red) **reagent 3** into the vial.
- Close with the cover and shake vigorously for precisely **15 seconds**.
- Open the vial and add **6 drops reagent 4**. Shake the vial until the liquid is evenly distributed.
- Compare the colors after **10 minutes** with the color chart (from a position above at natural daylight, avoid direct sunlight).
- Clean vial and cover thoroughly with tap water before and after each test.

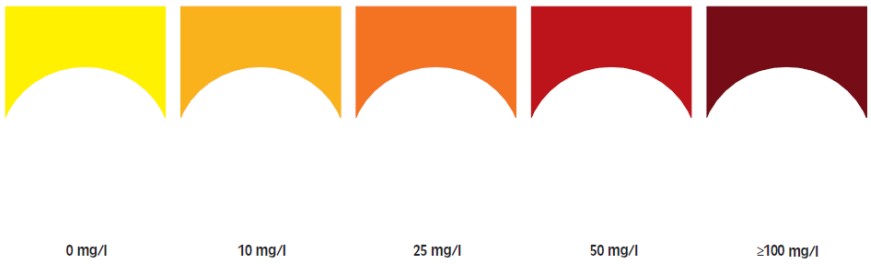

Photo credit: Sera®GmbH

3. It is not always easy to collect natural samples of *Phaeocystis* (due to the seasonal appearance of the foam phenomenon). That is why we will mimic the foam by using an egg. First we have to separate the egg into yolk and the egg white. Add some water to the egg white and whisk. ***Tip:*** If you are able to collect a sample (sea or harbor mouth) during a bloom (usually in spring – May / June – at the Belgian coast) you can have a try and maybe successfully create foam.

Results and comments:

Measurement of nitrates and phosphates

The main causes of eutrophication in the North sea include the use of fertilizers (agriculture) that contain nitrates. Not all parts of the fertilizer are taken up by plants so that the rest is transported via groundwater to the sea and estuaries. An excess of nitrate allows algae to grow faster and better. The use of nitrates in organic and chemical fertilizers for agriculture is a major source of water pollution in Europe. A decline in the use of mineral fertilizers was first recorded in the early nineties. In general agriculture remains responsible for more than 50 % of the total nitrogen emissions into surface water. Phosphates are also present in fertilizers. Phosphorus stimulates the development of plants and an excess of phosphates also leads to an excessive growth of algae. Phosphates are used as water softeners in washing powders (they make the washing more easier by making the water less hard).

The use of phosphates was reduced since the beginning of the 2000s because they are an important contributor to water pollution and eutrophication. Nowadays the most important phosphate source that ends up in our waterways are derived from households, industry and intensive agriculture (fertilizers and animal feed (livestock)). Several companies such as ECOVER are working to create environmentally friendly washing powders free from phosphates by replacing them by alternative softeners. Unfortunately phosphate is also added to some dishwater tablets due to the lower cost as softeners.

Simulating foam

After an intensive and hard work of whisking you finally managed to create the foam phenomenon! We have whipped the proteins into foam just as the waves whip the *Phaeocystis* proteins in the mucus layer. The foam is produced in a mixing process between air (oxygen) and the proteins.

Sources:

- http://www.vliz.be/docs/groterede/GR04_schuim.pdf
- **The life cycle of *Phaeocystis*: state of knowledge and presumptive role in ecology**
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  in: Van Leeuwe, M.A. *et al.* (Ed.) (2007). *Phaeocystis, major link in the biogeochemical cycling of climate-relevant elements. Biogeochemistry,* 83(1-3): pp. 29-47.
- **Current understanding of *Phaeocystis* ecology and biogeochemistry, and perspectives for future research**
  Verity, P.G.; Brussaard, C.P.D.; Nejstgaard, J.C.; Van Leeuwe, M.A.; Lancelot, C.; Medlin, L.K. (2007) *Biogeochemistry 83(1-3)*: 311-330.
- www.iseca.eu
- Sera®GmbH: user manuals Phosphate (PO_4^{3-}) Test and Nitrate (NO_3^-) test http://www.sera.de/en/products/in_category/single-water-test-312/product/sera-po4-test-phosphate-test.html
  http://www.sera.de/en/products/in_category/single-water-test-312/product/sera-nitrate-test.html

This educational tool has been developed for the ISECA project.

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