

Wildrosenweg 3
D - 24119 Kiel-Kronshagen
Telefon +49(0)431/380 400
Telefax +49(0)431/380 4010

e-mail bbe@bbe-moldaenke.de
Internet www.bbe-moldaenke.de

Differentiation of algae by bbe FluoroProbe, bbe Online-Fluorometer, bbe Cuvette-Fluorometer

Due to the fact that algae of the same division contain a similar quantity and quality of photosynthetic pigments their fluorescence excitation (with a fixed emission wavelength at 680nm) spectrum is significant for each division. So it is possible to distinguish divisions of algae by their fluorescence excitation spectrum.

Principle of operation

The bbe FluoroProbe for algae differentiation uses 5 LEDs for fluorescence excitation. The LEDs emit pulsed light at selected wavelengths (450nm, 525nm, 570nm, 590nm and 610nm) Their spectra are shown below. Fluorometric Emission is measured at 680nm by photomultiplier at an angle of 90 degrees to the exciting light source.

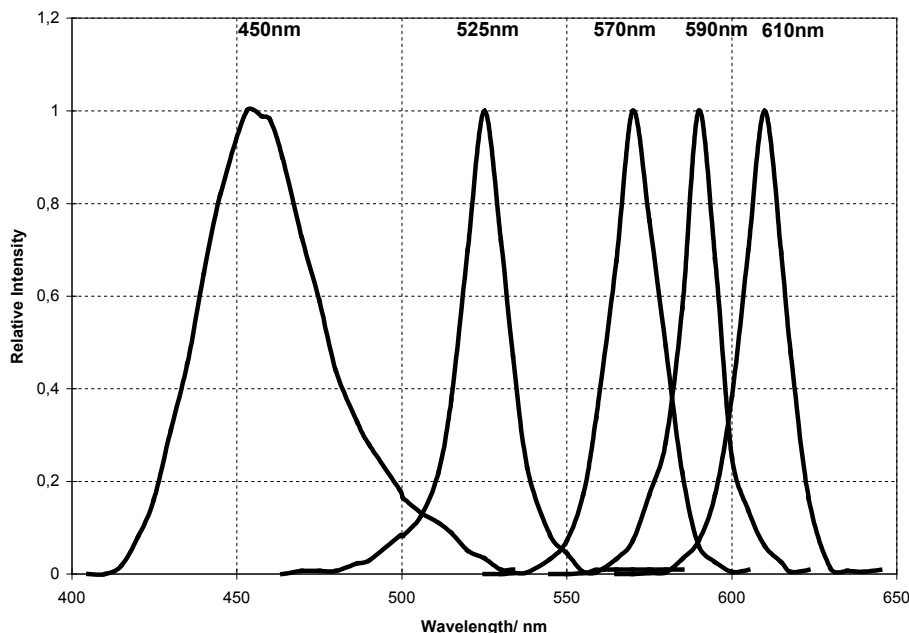


Fig.1: Spectral intensities of used LEDs (intensity is normalised to the maximum of each LED)

Determination of different algae

The division of *chlorophyceae* (- green algae -) shows a broad maximum of fluorescence at the 450nm LED that corresponds to chlorophyll-a and -b excitation. The *cyanophyceae* (- bluegreen algae -) pigments are characterized by maximal excitation at 610nm caused by the photosynthetic antenna pigment phycocyanin. Although *cyanophyceae* contain chlorophyll-a too, spectrum shows low intensity at 450nm. This is due to the masking effect of the phycocyanin. Furthermore, the high peak at the 525nm region for the *bacillariophyceae* originates from the xanthophyll fucoxanthin and from peridinin for the division of *dinophyceae*. The maxima at 450nm are caused by chlorophyll-a and -c. In our last analysed group, *cryptophyceae*, a significant maximum at 570nm that originates from phycoerythrin can be found.

It is obvious from Figure 2 that it is not possible to differentiate *bacillariophyceae* and *dinophyceae* by their „fluorescent fingerprint“ at this level of discrimination. But it can be clearly seen that it is possible to distinguish four groups of algae: *chlorophyceae*, *cyanophyceae*, *dinophyceae* and *bacillariophyceae* (the brown group), *cryptophyceae*.

Additionally, it has to be mentioned that sometimes phycocyanin content per cell in *cyanophyceae* varies leading to increased deviation in chl-determination. Nevertheless, the average fingerprint can be used to differentiate this division as shown below.

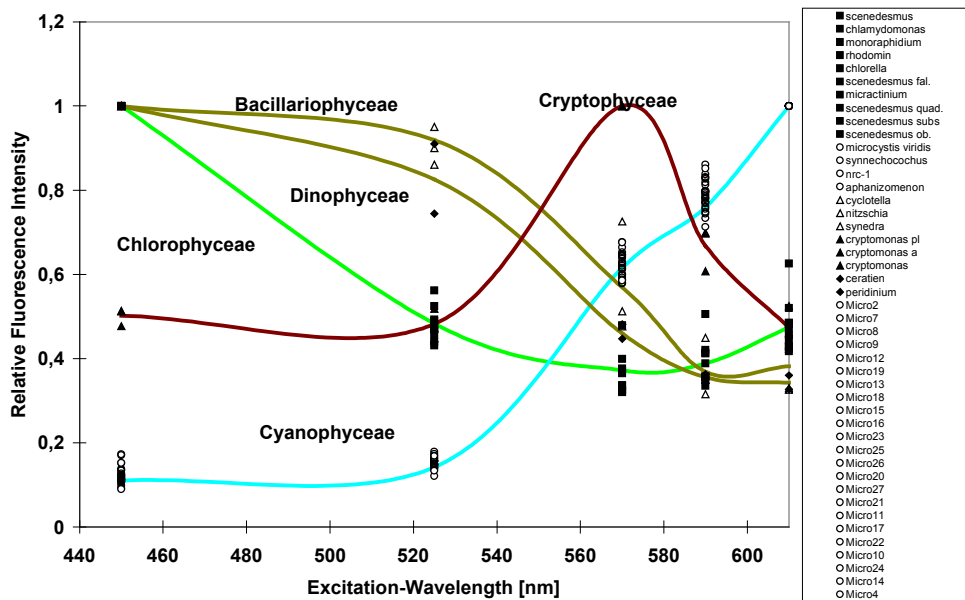


Fig.2: The fluorescence intensities of the 5 divisions multiplied by the intensity of the LED and normalised to the maximum intensity of each division. In this measurement several species of the cyanobacteria myrocystis (abbreviation micro) were tested as well.

Test in laboratory

After recording fluorescence excitation spectra of single cultures we prepared a mixture of *cyanophyceae* (Microcystis), *bacillariophyceae* (Phaeodactylon) and *chlorophyceae* (Scenedesmus). From emission data it was possible to calculate the concentration of each algal division via gaussian fit. This mathematical method was realized by computer: the four spectral fingerprints of tested divisions were compared with the new recorded spectrum and calculated for the concentration for each single division.

During an experiment we added to a mixture of Scenedesmus (concentration 20µg/l chlorophyll-a) and Phaeodactylon (concentration 10µg/l chlorophyll-a) gradually well known amounts of Microcystis (from 5µg/l to 100µg/l). By this means the amount of cyanophyceae increased while the concentration of Scenedesmus and Phaeodactylon was kept constant. In this way we simulated an induced algal bloom.

After each addition of Microcystis the fluorescence spectrum was recorded with the modified "bbe-Cuvette-Fluorometer" and the concentrations of each algal group were calculated.

Figure 3 shows the fluometric response of Microcystis addition together with measured and calculated amount of all three types of algae

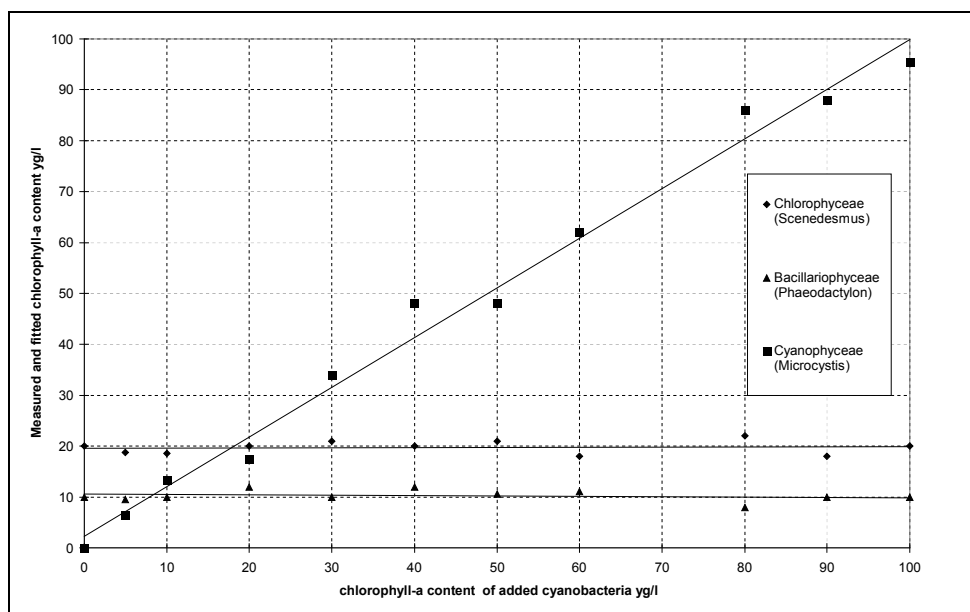


Fig.3: "Induced artificial" Microcystis bloom, as described in text

***In situ* experiment**

Algae differentiation method by fluorometric measurement was tested in a 15-day experiment at Kiel Bay which is part of the Baltic Sea. During this experiment the spectra were recorded once a day at 11:00 am. The results are shown in Figure 4. We can recognize a light bloom of *chlorophyceae* and *cyanophyceae*. Of course we can not decide by fluorometric measurements if the shown concentration of *bacillariophyceae* is caused by *dinophyceae* or caused by *bacillariophyceae*, but we know from microscopic observation that *dinophyceae* were absent in this case.

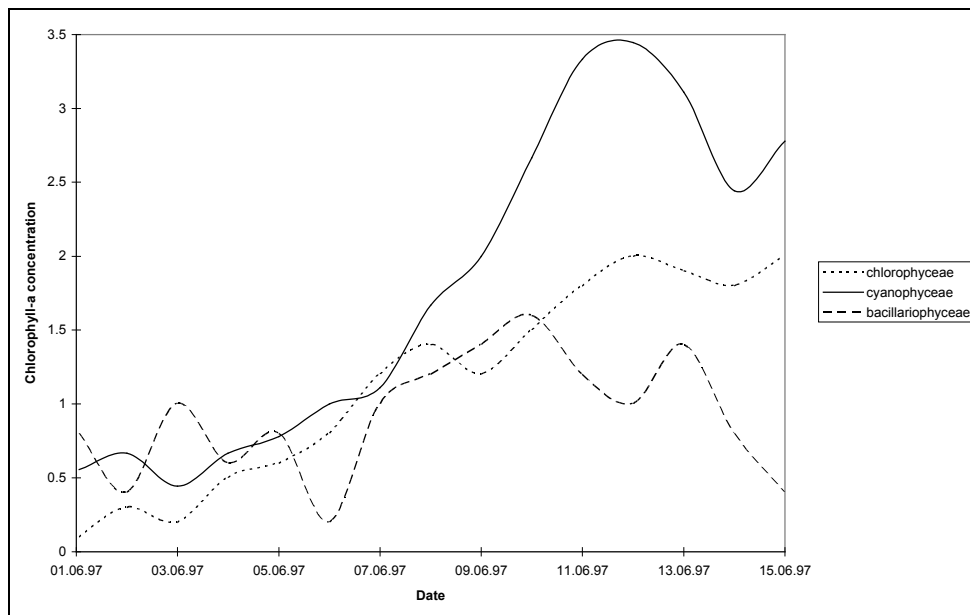


Fig.4: *In situ* measurements at Kiel Bay, Baltic Sea