Questions - Webinar “Planktothrix” 30/07/2014

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1. Can Planktothrix be measured with a standard Fluorometer?

    a. Yes, but not quantitatively and really not related to the chlorophyll content or biomass. There are sensors available that measure the fluorescence of the phycoerythrin itself but cannot account for the amount of occurring cyanobacteria, In contrast bbe determines the chlorophyll content from the chlorophyll fluorescence including the interaction of all accessory pigments with chlorophyll. This gives a much better estimation of the amount and takes into the calculation the other algae classes.

2. What do you think are the reasons for Planktothrix growing appearance in the last 2 years in Europe?

    a. Summer and sun, together with high amounts of nutrients. *Planktothrix rubescens* is frugal in respect of phosphate and can grow al low P-concentrations. We have now the typical conditions for *Planktothrix rubescens* growth, but also for other harmful algae like *Planktothrix agardhii* and *Microcystis aruginosa*. Daily we receive reports about cyanobacteria warnings from US.

3. Can we get information about toxicity from the fluorometric approach?

    a. Not directly, although there is a high probability that the algae contain harmful cyanotoxins. However there are stains of the same species i.e. *Microcystis aruginosa* which do not express the toxin. In case of an occurrence a chemical analysis for the cyanotoxins is necessary, but this needs precious time before the result is available. The cyanobacteria recognition and estimation of the content with the flow-through AlgaeOnlineAnalyser is done in real time.

4. The algae you used for calibration are from a culture. How can you be sure to have the right species that are comparable to algae in a natural sample?

    a. When we started the development of the advanced algae analysis with the multispectral approach we used a lot of different algae and cyanobacteria from an algae library. The measurements resulted in mean fingerprints for the different algae classes which are still true today and fit in most cases. However from time to time we are faced with algae where the bbe fingerprint is not that well for a superior analysis. We had such a result from a *Phaeocystis* species. Then we recommend to introduce an own fingerprint for this algae. With the new fingerprint the classification will be improved.

5. How often is a calibration needed for an AlgaeOnlineAnalyser?

    a. We recommend every two years. This should be done at the bbe workshop.

6. Although we can have a good estimate of the number of cells of P.rubescens, is there any relation to the toxins they may produce?

    a. There are some figures to give you an idea: 10 µg chlorophyll might be equivalent to the content of 1 µg microcystin, but there is a very high variability. So you should be careful using this relation. Cell number is more difficult because we have small and large cells and colony forming cyanobacteria. Important for toxicity is the status of the toxin. If the cell is damaged the toxin will be released. The intact cell does not release the cyanotoxin.

7. Your validation of algae counts (microscope) with the measurements your equipment, was performed with natural samples, or with pure cultures?

    a. Yes, with natural samples from the place of extraction. We have a lot of results from counting beside fluorometry. Counting show high variability due the specific observer.

8. Cryptophytes, another group of algae, do have PE and are concentrated in deep chl. maximum. Would you be able to detect differences between Planktothrix and Cryptophytes?

    a. It is difficult because of the partial overlapping excitation spectra. We recommend to skip the cryptophytes detection when you want to measure the Planktothrix. So they are similar but not identical. Of course afterwards the data are still available to calculate for any other algae class or combination. Deep chlorophyll maximum let me assume that you will find a spatial distance between the Cryptophytes and the *P. rubescens*.

9. Is there a risk that the cells will burst (> gas vesicles) before the measurements and will burst cells give a reasonable detection limit?

    a. Of course there is a risk of bursts. We know that damaged cells are subjected to rapid decay of chlorophyll. Microcystin lifetime is longer and may persist after all chlorophyll is dissipated. This fact emphasizes the necessity to recognize the occurrence of potential harmful algae in an early stage.

10. Can we measurement all algae with this machine? How about calibration for quantitative measurement?

    a. Not all, the technique is limited to microphytoplankton but includes also pico- and nanoplankton. Pico- and Nanoplankton aren´t visible with the light microscope. Macrophytes cannot be measured. For benthic algae a special instrument – the BenthoTorch – is available. The calibration is

in accordance to pigment analysis and quantification by use of HPLC pigment separation and absorbance measurement. We perform the comparison periodically to ensure consistent measurement results. The bbe workshop provides a “calibration”-fluorometer only for calibration purposes of our customer´s instruments. Algae cultures are kept under constant and ideal conditions in a logarithmic growth phase with high photosynthetic activity. We understand this as an advantage over secondary standards. However we also use a dye with our calibration – fluorometer to check the performance and reproducibility.

Again, thank you very much for your attendance and the shown interest in our bbe webinars.

We are looking forward to meet you at our next webinar!
Best regards,

Your bbe-team

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