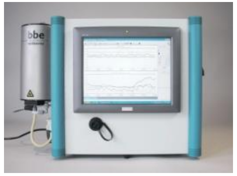




Cyanobacteria Threats – New Approaches of the Assessment of Cyanobacteria Toxin Release

Anna Dahlhaus

03.05.2014

bbe Moldaenke; Preetzer Chaussee 177, 24118 Schwentinental
Tel:+49 (0) 431 380400; Fax:+49 (0) 431 3804010;
bbe@bbe-moldaenke.de

Cyanobacteria

Anchient group of bacteria

Capabele of photosynthesis like higher plants

Can be found in almoust every aquatic habitat

Can appear in blooms

Can build toxins

Table 1. Provisional health-based water guideline values for four cyanotoxins (ppb or µg/L)[1]

Water Use	Anatoxin-a	Cylindrospermopsin	Microcystins	Saxitoxins
Drinking Water	3	1	1	3
Non-drinking and non-cooking uses	20	6	10	100

[1] Cyanotoxins are measured in parts per billion (ppb) or micrograms per liter (µg/L), which are equivalent.

Table 2. Target organs and health effects of cyanotoxins

Cyanotoxins	Target Organ	Health Effects	Onset of Symptoms Following Exposure
Anatoxin-a	Nervous system	• Numbness or tingling in fingers and toes • Dizziness • Convulsions • Paralysis • Death (in some cases)	Immediate up to 24 hours
Cylindrospermopsin	Liver and kidneys	• Nausea • Vomiting • Bloody diarrhea • Abdominal pain • Kidney damage • Protein in urine • Blood in urine • Dehydration • Headache	Up to a week
Microcystins	Liver	• Nausea • Vomiting • Diarrhea • Liver damage • Death (in some cases)	Immediate up to 24 hours
Saxitoxins	Nervous system	• Numbness or tingling around mouth • Numbness spreading to arms and hands • Muscle soreness • Muscle weakness • Paralysis • Difficulty breathing • Death (in some cases)	Immediate up to 24 hours

Cyanotoxins

neurotoxins, hepatotoxins, cytotoxins, and endotoxins

In European water bodies, the most frequently occurring cyanobacteria are the *Microcystis*, *Anabaena* and *Planktothrix* strains (e.g. Chorus & Bartram 1999;TOXIC 2005). Toxins produced by these Cyanobacteria are different forms of microcystins.

Cyanotoxin release

- **Natural toxin release**
  - senescence
  - death
  - lysis
- **Induced toxin release**
  - Mechanical stress
    - Pressure differences
    - Shearing forces
    - Mixing and stirring (coagulants and powder-activated carbon)
  - Oxidative treatment
    - Ozonation
    - chlorine
    - permanganate
  - Physiological stress
    - Osmotic stress, etc.

bbe Moldaenke; Preetzer Chaussee 177, 24118 Schwentinental
Tel:+49 (0) 431 380400; Fax:+49 (0) 431 3804010;
bbe@bbe-moldaenke.de

Light harvesting in Cyanobacteria – Phycobilisoms and Phycocyanin (PC)

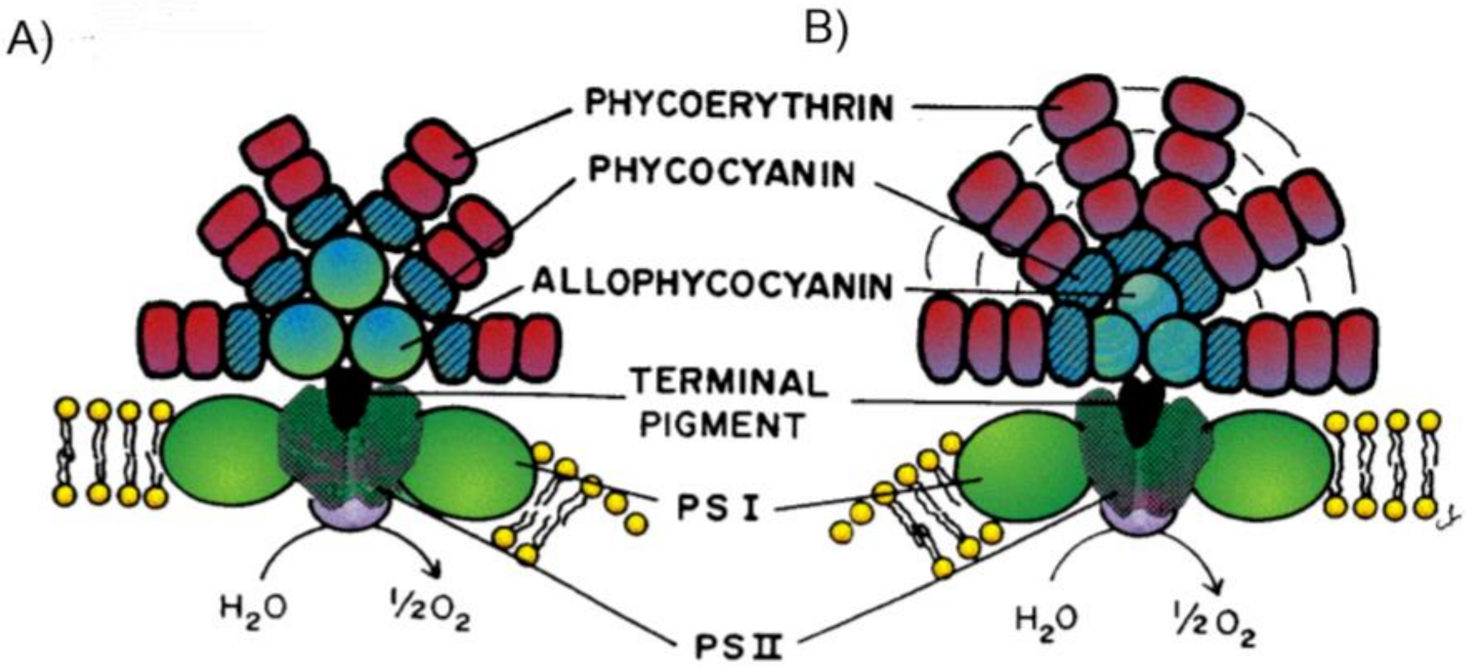


Figure 1.3. Schematic of a (A) hemidiscoidal phycobilisome, (B) and a more complex hemispherical phycobilisome (Gantt (1986))

bbe Moldaenke; Preetzer Chaussee 177, 24118 Schwentinental
Tel:+49 (0) 431 380400; Fax:+49 (0) 431 3804010;
bbe@bbe-moldaenke.de

Correlation between PC and cyanotoxins

W. Schmidt *et al.*, 2009

Table 2 | Ratio of phycoerythrin (PE), phycocyanin (PC) to total microcystin concentration (MC-LR + MC-RR) in native and cultivated *Plankthotrix rubescens*

Total	Phycoerythrine (PE), µg/L	Phycocyanine (PC), µg/L	Sum of MC-LR + MC-RR, µg/L	$[PE]/[MC_{tot}]$	$[PC]/[MC_{tot}]$
Planktothrix rubescens (Waida reservoir)	8	53	2.5	~3	~21
Planktothrix rubescens (culture)	80	320	42	~2	~8

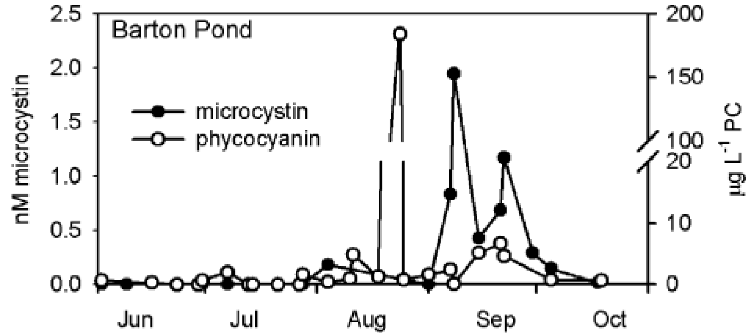
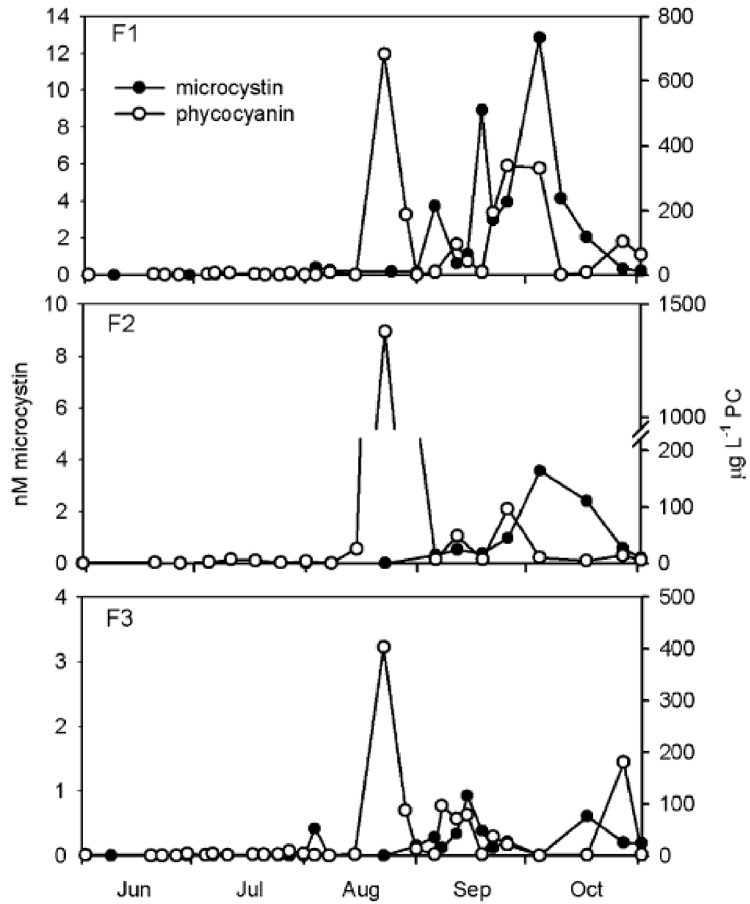


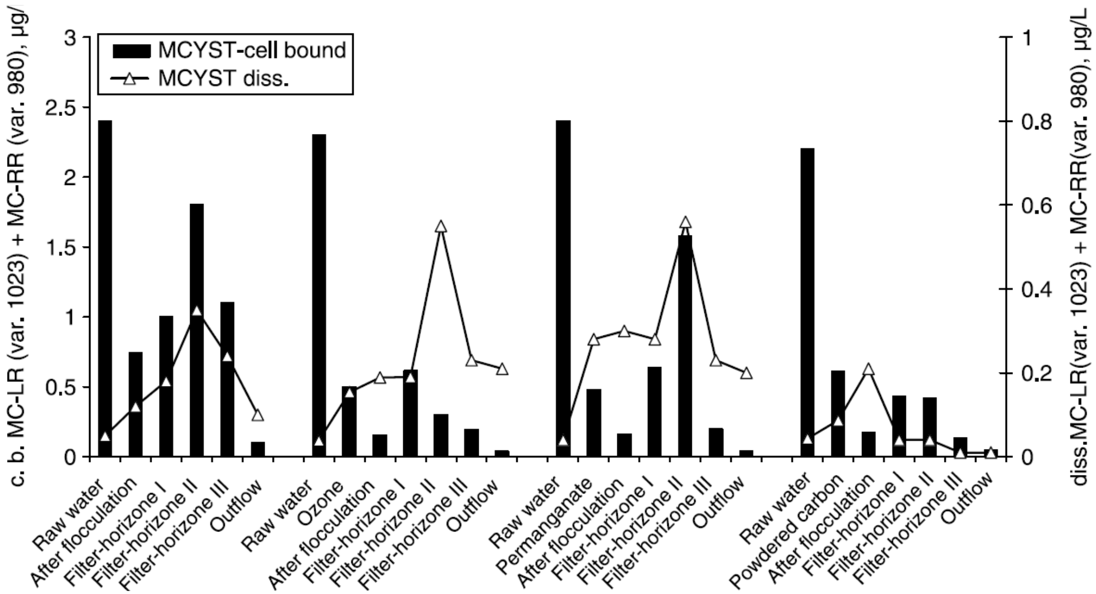
Fig. 2 – Microcystin concentrations (nM) and phycocyanin (PC, µg L^{-1}) of Barton Pond surface water, June to October 2005.

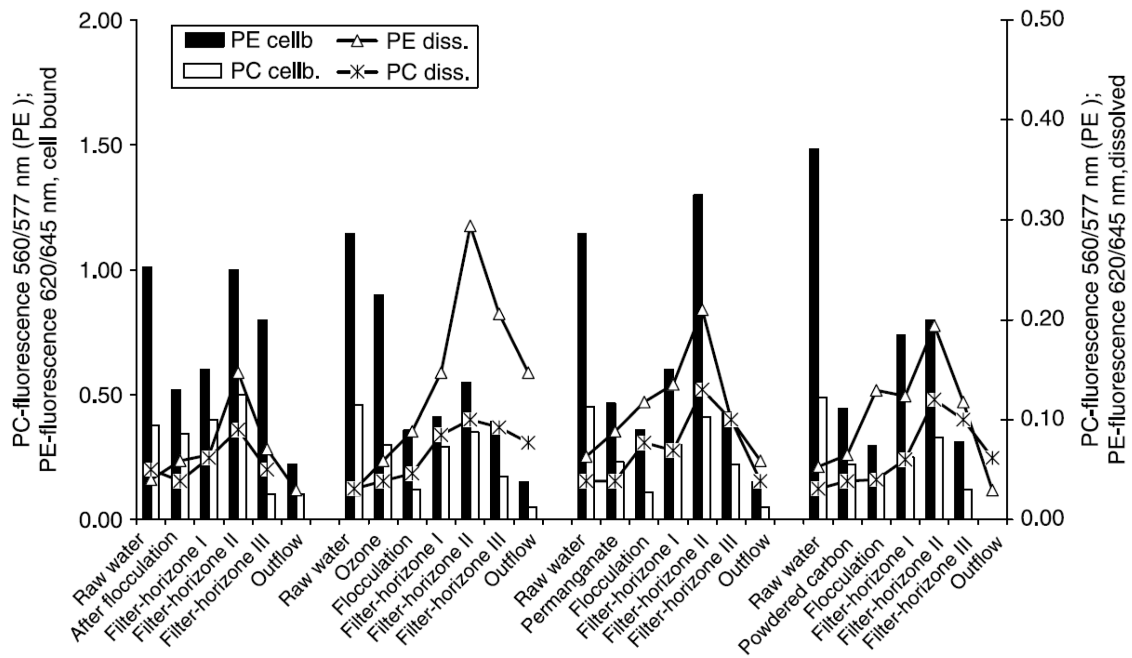


Lehman., 2007

Fig. 3 – As Fig. 2, but for Ford Lake surface water, stations F1, F2, and F3. The phycocyanin maxima on 23 August are attributable to *Aphanizomenon*; subsequent maxima in September and October are *Microcystis*.

Concentration of cell-bound and dissolved microcystin in different drinking water treatment stages; raw water: DOC = 3.8 mg/L; cell count of *Planktothrix rubescens*; 10^6 cells /mL.

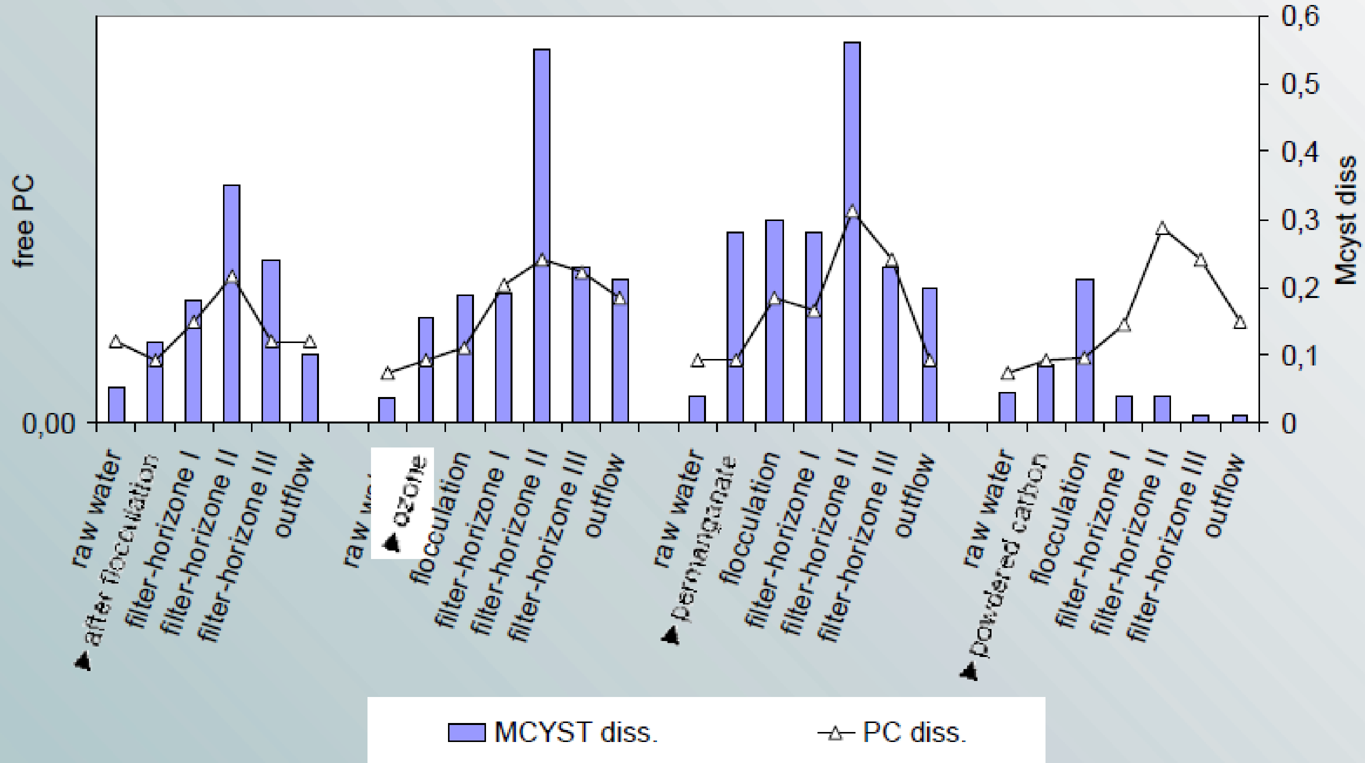




W. Schmidt *et al.,* 2009

Concentration of cell-bound and dissolved phycoerythrin and phycocyanin in different drinking water treatment stages; raw water: DOC = 3.8 mg/L; cell count of *Planktothrix rubescens*; 10^6 cells /mL.

Correlation between free PC and dissolved Microcystins



Measurement of Phycocyanin – fluorescence

- Online instrument
- Two detectors
- 7 LEDs

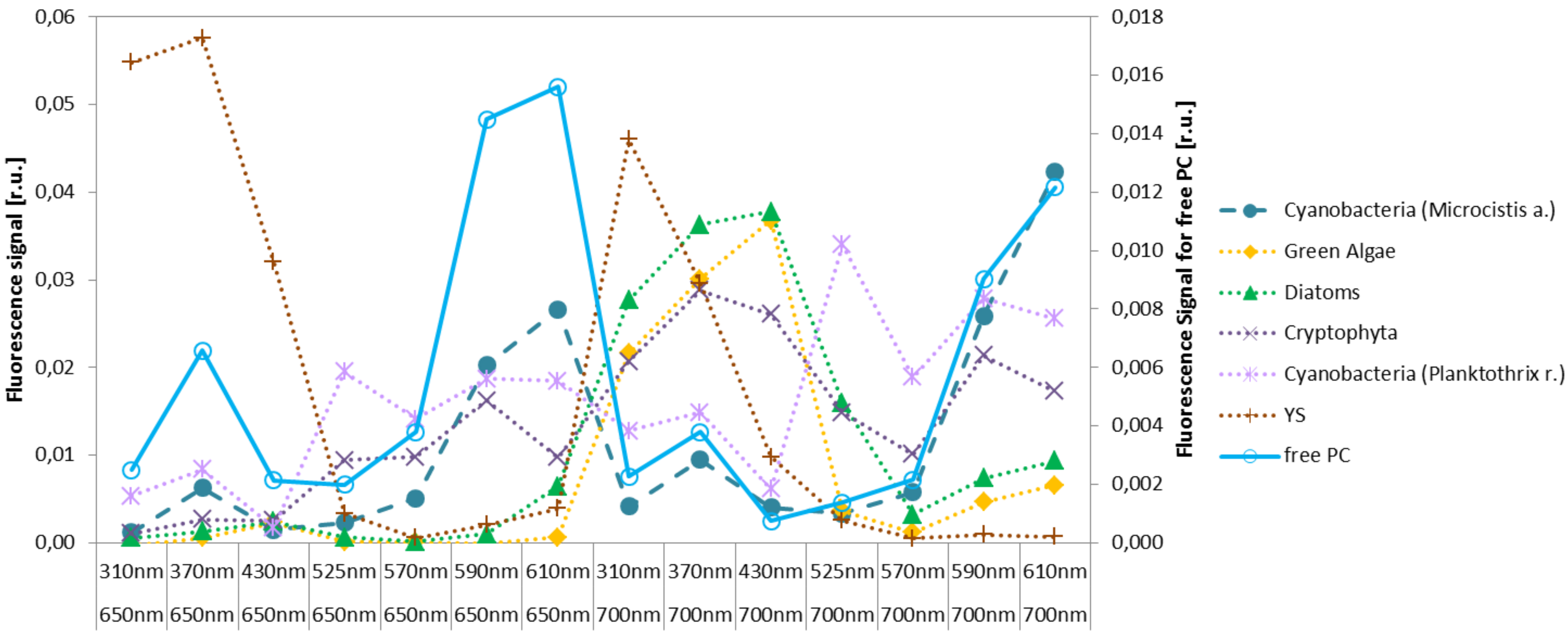
bbe Moldaenke; Preetzer Chaussee 177, 24118 Schwentinental
Tel:+49 (0) 431 380400; Fax:+49 (0) 431 3804010;
bbe@bbe-moldaenke.de

biological · biophysical · engineering

bbe moldaenke

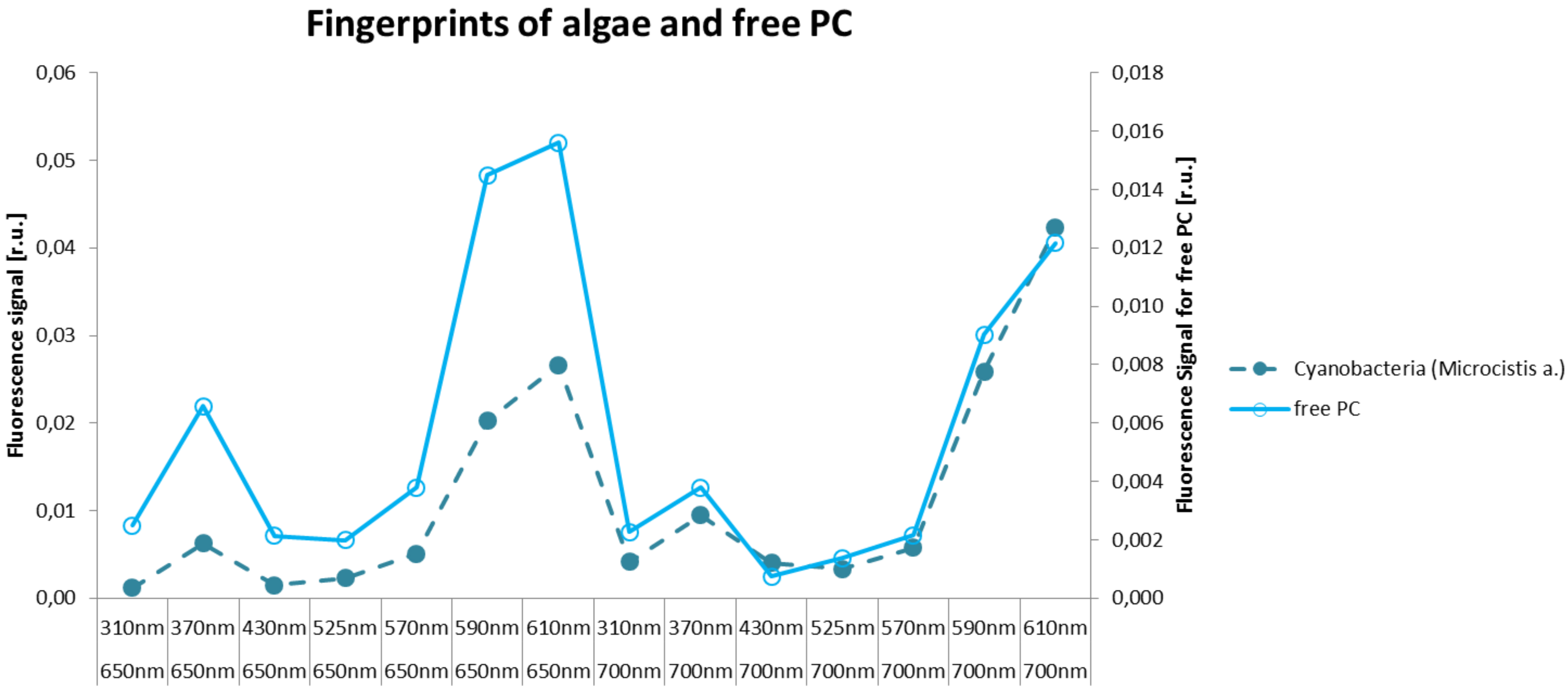
Measurement of Phycocyanin – fluorescence

Fingerprints of algae and free PC

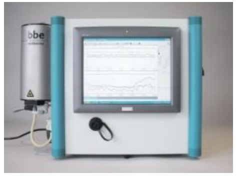


bbe Moldaenke; Preetzer Chaussee 177, 24118 Schwentinental
Tel:+49 (0) 431 380400; Fax:+49 (0) 431 3804010;
bbe@bbe-moldaenke.de

Bound and free PC



bbe Moldaenke; Preetzer Chaussee 177, 24118 Schwentinental
Tel:+49 (0) 431 380400; Fax:+49 (0) 431 3804010;
bbe@bbe-moldaenke.de

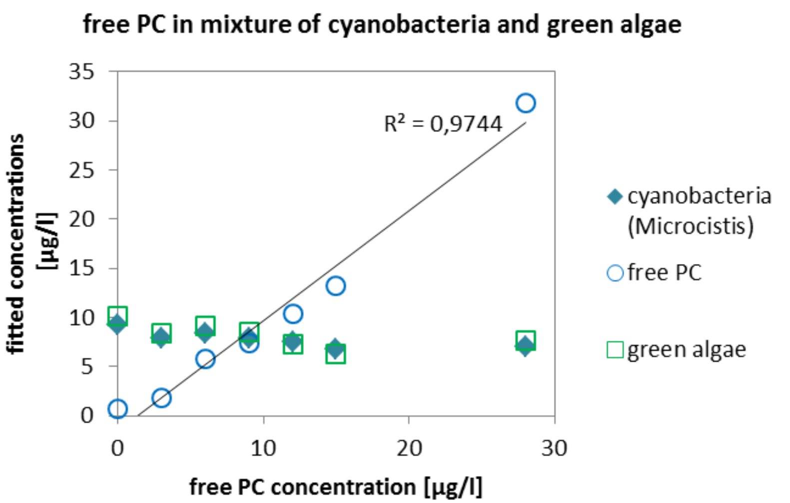


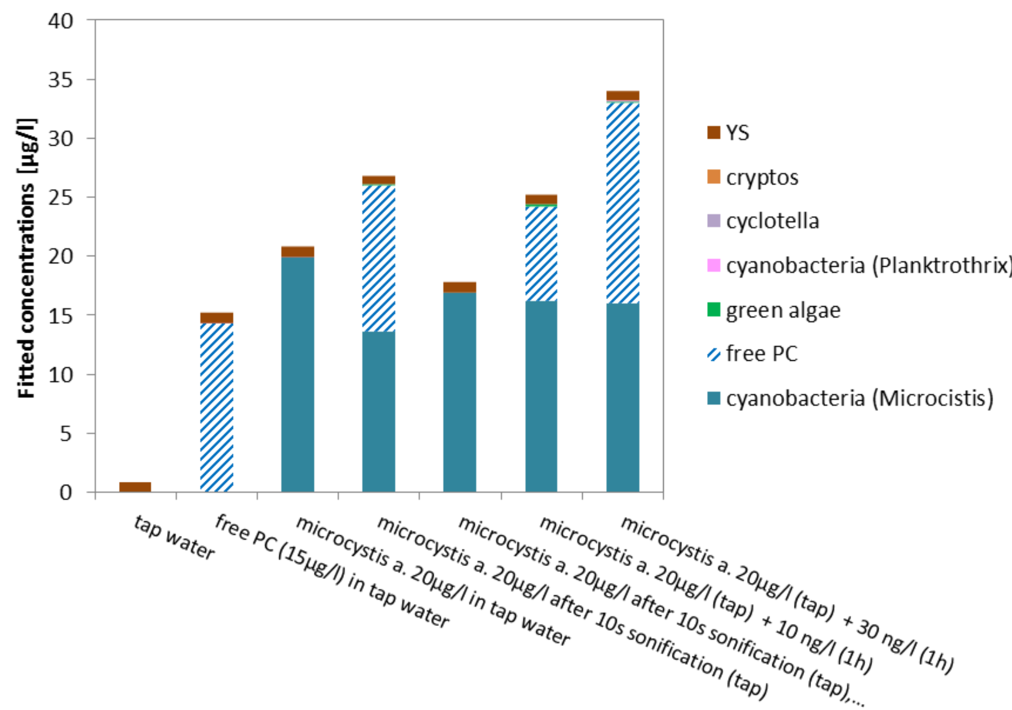


biological · biophysical · engineering

bbe moldaenke

Differentiation between bound and free PC - fluorescence





bbe Moldaenke; Preetzer Chaussee 177, 24118 Schwentinental
Tel:+49 (0) 431 380400; Fax:+49 (0) 431 3804010;
bbe@bbe-moldaenke.de

Table 1 Fluorescence lifetimes of the *Nostoc* cells and the components of *Nostoc* photosynthetic apparatus

Sample	τ_1 (ns)	f_1 (%)	τ_2 (ns)	f_2 (%)	χ^2
Cells					
Growth medium	2.07 ± 0.25	19	0.06 ± 0.015	81	9.43
Water	1.84 ± 0.08	51	0.06 ± 0.02	49	9.66
Supernatant	1.93 ± 0.05	90	0.46 ± 0.09	10	11.95
Thylakoids					
Phosphate buffer	1.81 ± 0.05	58	0.14 ± 0.01	42	3.96
Water	1.82 ± 0.06	88	0.23 ± 0.08	12	15.25

The samples were excited at 590 nm. f_1 and f_2 are the relative contributions of the long- (τ_1) and short-lived (τ_2) components of the emission decay profile

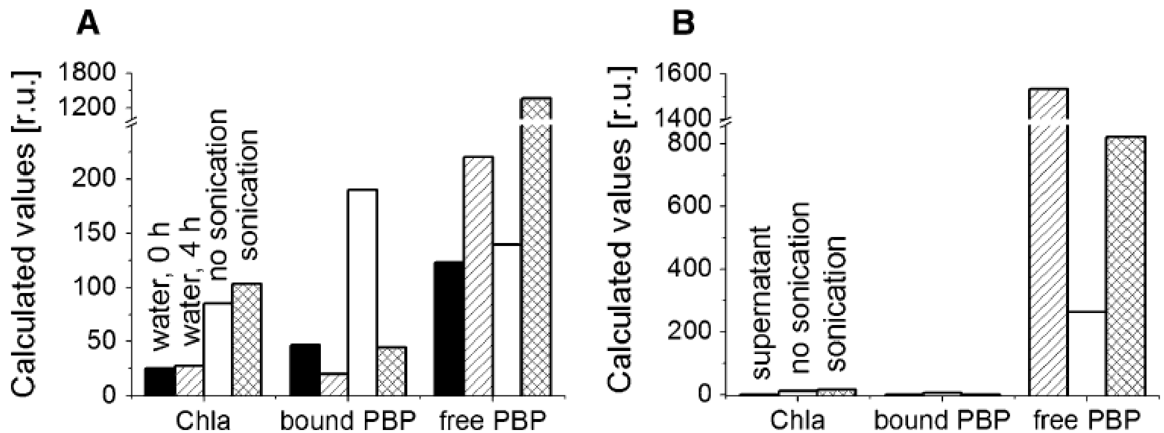
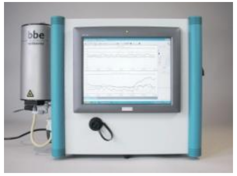


Fig. 4 The results of the fitting of fluorescence profiles of *Nostoc* cells and components of its photosynthetic apparatus, as recorded using a FluoroProbe instrument. The measurements were conducted in a suspension containing the cells (**A**) as well as supernatant obtained after removal of the cells (**B**). The fitting was done applying Eq. (1). See the text for the details of the analysis. "Chl *a*" stands for a total amount of chlorophyll *a* in the thylakoids, "bound PBP" for the amount of the phycobiliproteins energetically coupled to photosystems, and "free PBP" for the amount of the cell-released phycobiliproteins





Conclusions

1. Cyanopigments can be efficient indicators for cyanotoxins and cyanobacteria-originated organic matter.
2. Measurement of free phycocyanin can provide an early warning system agains toxin release.
3. Real-time character of measurement allows to obtain information in a minute, while a other methods for toxin analysis can take hours (ELISA kits) or a day (chromatographic methods).

Table 4 | Assessment of the early warning function of *phycoerythrin* and *phycocyanin* against microcystins in water treatment

	Early warning against dissolved microcystins	Early warning against dissolved cyanobacteria-like organic matter
Chlorine (pre-oxidation, disinfection)	++	++
Chlorine dioxide	–	–
Permanganate (pre-oxidation)	++	++
Ozone (pre- and main-ozonation)	(+)	(+)
Powdered-activated carbon	+	++

++ = efficient early warning; + = limited early warning; (+) = not yet defined; – = no early warning.

W. Schmidt *et al.*, 2009

bbe Moldaenke; Preetzer Chaussee 177, 24118 Schwentinental
Tel:+49 (0) 431 380400; Fax:+49 (0) 431 3804010;
bbe@bbe-moldaenke.de