2018 Joint Call
Mid-Term Progress Report
Closing the water cycle gap - Sustainable management of water resources

Supporting tools for the integrated management of drinking water reservoirs contaminated by Cyanobacteria and cyanotoxins – BlooWater

MILESTONE SUMMERY & DELIVERABLES

Milestone 1.1 Identification of the study areas

Lake Erken and lake Mälaren are two of the very few lakes in Northern Europe that have a long history of monitoring, including both manual and high-frequency automatic measurements of lake and stream stations. The monitoring programme includes measurements of physical and chemical parameters as well as plankton composition.

Lake Erken is a moderately eutrophic lake located in east-central Sweden near the Baltic coast (59.8 N 18.6 E). The lake has a surface area of 24 km^2, an average depth of 9 m, a maximum depth of 21 m and a water residence time of approximately 7 years. The Lake has been the site of Uppsala University's limnological field station for nearly 80 years, where a large variety of research covering all aspects of limnology have been carried out. The field station was first established in 1944, and later followed by the addition of a research Laboratory on the lake shore, and a small meteorological station on an island offshore (Fig. 1).

Figure 1 - Lake Erken and its watershed. The bottom shows the location of the automated monitoring systems that are operational on the lake.

This meteorological station on Malma Island, completed in 1958, was one of the first developed to support limnological studies. In addition to standard meteorological measurements of wind solar radiation and air temperature, a stilling well was installed to allow continuous monitoring of lake level, and underwater thermistors were installed to automatically monitor water temperature. Originally, all data were collected on paper charts and these were transcribed to produce daily summary statistics. Between 1987 - 1988 the meteorological station measurements were converted to record data digitally, and measurements made at 1 min frequency were summarized and saved at hourly and daily time intervals. During this time a water temperature monitoring system was also established at the eastern end of the main basin of the lake at a depth of 15 m. Measurements at 0.5 m intervals made at this station allow detailed analysis of the lake thermal structure and supplementary measurements of wind speed close to the water surface. Water discharge is measured entering the lake from the largest input at Kristineholm, and the lake outflow discharge is measured at Stensta. At Kristineholm the inflow concentrations of dissolved organic carbon (DOC) are estimated by monitoring the fluorescence associated with DOC in the inflowing stream water. In 2014 the Erken laboratory joined the SITES (https://www.fieldsites.se/en-GB) research network and as result of increased funding and support was able to further increase the automated monitoring program to include a YSI profiling system that collects hourly profiles of dissolved oxygen, turbidity, pH and fluorescence measurements of phytoplankton, chlorophyll, cyanobacteria phycocyanin and DOC . Automated lake monitoring from

Malma Island and other sites (Fig. 1) continues to this day (http://130.238.87.115:8080/Erken4/index.html). Lake Erken joined the GLEON lake monitoring network (http://www.gleon.org/) in 2007 and has one of the longest data records of the GLEON sites.

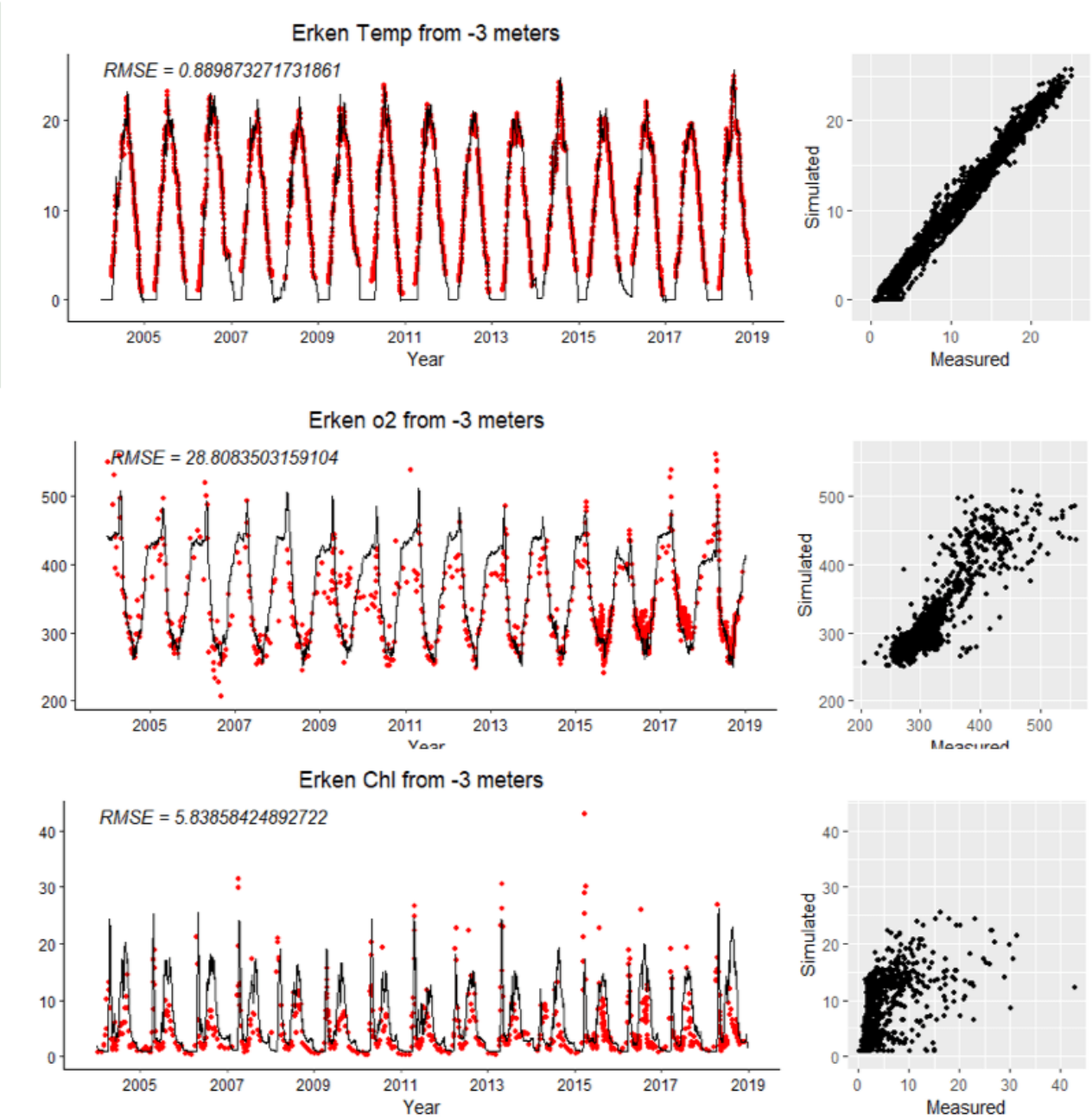
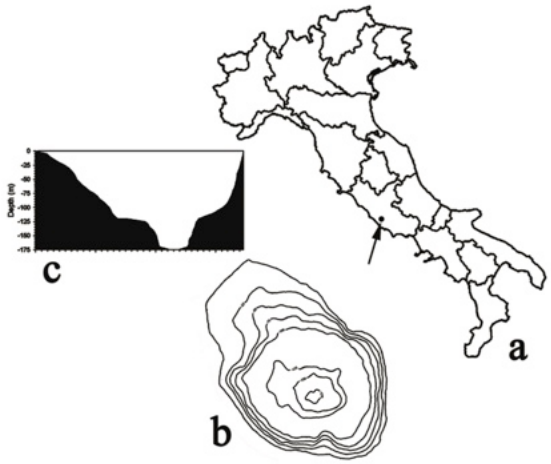


Figure 2. Model simulations of Lake Erken water temperature (top) dissolved oxygen (middle) and chlorophyll concentration (bottom). Red points are data from Erken's monitoring programs. Black line is the simulated values.

Lake Albano is a closed crater basin located on the west side of the Colli Albani volcano in Latium (central Italy), nestled between small hills and a few municipalities of the area

known as Castelli Romani, 25 km south-east of the city of Rome. On its coasts there are important prehistoric and Roman archaeological remains, such as the Village of Macine, the artificial emissary and the Doric and Bergantino nymphaeums, along with a part of the complex of the Villa Albana of Domitian.



Geographical position of Lake Albano within the Italian peninsular (a), bathymetric map (b) and a cross sectional profile of the lake from NW to SE of the lake (c). (source Ellwood et al., 2009)

The lake is located at 293 m above sea level and has an area of 5.2 km2, a perimeter of 10 km and a depth of ~ 175 m. Unlike most volcanic lakes, Albano is elliptical in shape, stretched in the NW-SE direction, filling two craters that represent the last known eruptive products of the volcano. For the frequent seismic activity in the volcanic system, the local uplift, the gaseous emissions (mostly CO2, H2S, HF) and the hydrothermal activity the area is still deemed geologically hazardous.

According to a hydrogeological study (Capelli et al. 2000), the lake is fed mainly by groundwater as it intercepts water from two aquifer systems, a regional one and a perched one.

Since 1960s the lake water level has dramatically lowered by more than 4 meters, mainly due to ongoing anthropogenic processes such as urbanization and over-exploitation of lake water and groundwater, that is not balanced by the meteoric intake, which has also decreased.

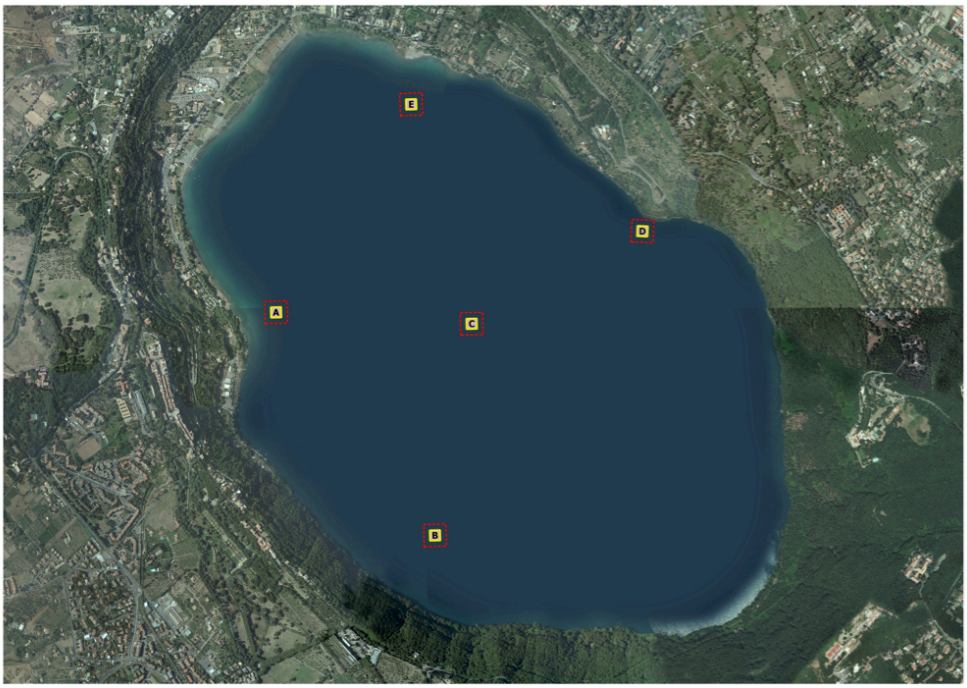
In addition, a 47 years-water renewal time combined with increase pollutants, mainly from sewage effluents and to a lesser extent from surface run-off, results in elevated levels of nitrogen and phosphorus that affect the water quality and increase the anthropogenic lake eutrophication.

Massive algal blooms are a well-known consequence of the excess of nutrient loading and storage to water bodies with low hydro dynamism, and lake Albano is also affected by algal blooms.

Following the large toxic algal bloom detected in February 2001, indeed, a study carried out by Istituto Superiore di Sanità (2001-2003) showed the presence of two steady populations of the toxic Cyanophycea *Planktothrix rubescens*. The toxins from these cyanobacteria, when produced in sufficient quantities can cause death of animals and pose risk to human health.

The high Nitrogenous/Phosporous ratio presents in Lake Albano is particularly favourable to algal bloom of the toxic *P. rubescens.*

For these reasons, bathing and lake fruition are in general forbidden to the population during the period of algal blooms (winter-early spring).



Bloowater-Flight areas and water sampling points

Sources

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Sylvain Merel, David Walker, Ruth Chicana, Shane Snyder, Estelle Baurès, Olivier Thomas, *State of knowledge and concerns on cyanobacterial blooms and cyanotoxins,* Environment International, Volume 59, 2013, Pages 303-327, ISSN 0160-4120, https://doi.org/10.1016/j.envint.2013.06.013.

Istituto Superiore di Sanità *Toxic algal species dynamic in the lakes of Albano and Nemi.* Milena Bruno, Valentina Messineo, Daniela Mattei, Serena Melchiorre 2004, 55 p. Rapporti ISTISAN 04/32 (in Italian)

Lake Castreccioni is a lake created in the 1980s when a dam was placed across the Musone River near Monte San Vicino, at about 70 km far from the coast. The biggest artificial lagoon in the Marche region (Central Italy), Lake Castreccioni covers about 2.4 km^2 and reaches depths of 55 feet. The dam (67 m high and 280 m long) is situated on a homogeneous calcareous-majolica formation (Upper Jurassic-Lower Cretaceous) belonging to the prevalently limestone unit of the Umbro-Marche sequence. The volume of the lake at the maximum altitude is approximately 50 million cubic meters.

The potabilization plant (Acquambiente Marche) is located near the district of Castreccioni in the municipality of Cingoli and is responsible for drinking water for the member municipalities: Cingoli, Filottrano, Numana and Sirolo. Furthermore, through the Castreccioni pipeline, the treated water also supplies the Municipalities of Osimo and Castelfidardo.

Acquambiente Marche has the purpose of treating and drinking water from the reservoir of the Castreccioni dam from which it is fed by gravity through the intake present in the dam body. The plant represents the center of the activities of Acquambiente Marche both for its strategic importance, supplies water for a total of about 65,000 inhabitants in the winter and about 95,000 in the summer, using technologically advanced and equipped of an advanced ozone disinfection system.

The drinking water plant was started in 2000 and over the years has treated about 140 million cubic meters of water. The maximum capacity is 500 l/sec divided on two equal lines of 250 l/sec. The flow of treated water is a function of the needs of the distribution network and is accounted for both influent and effluent. The process steps are: Pre-disinfection with ozone (pre-ozonation), clariflocculation, sand filtration, disinfection, ozonation, activated carbon filtration, accumulation tanks and final disinfection with chlorine dioxide. There is also accessory equipment thanks to which the chemical and physical parameters such as temperature, turbidity, pH, conductivity, dissolved oxygen for raw water or pH, nitrite conductivity and active free chlorine for the treated water and aluminium and ozone in intermediate samples are continuously determined. The variability of the organoleptic and chemical characteristics of the water coming from the Castreccioni reservoir obliges the Company to continuously and dynamically manage to promptly intervene in all cases in which

unfavourable interventions occur under the conditions of the drinking water process also in case of cyanobacteria concentrations in the influent.

Based on the monitoring campaign by ARPAM conducted since 2014, it is concluded that:

- Lake Castreccioni is characterized by relatively low nutrient values (oligo-mesotrophic lake); also identified as "phosphorus as the limiting element for algal productivity" (typical for most Italian lakes).
- The genus *Planktothrix* constitutes the prevalent part of the phytoplankton (except for the summer period, in which there are more algal species). The same is typical of the initial stages of cyanobacterial contamination, so that once they reach the oligotrophic reservoir, they proliferate in the absence, or scarce co-presence of other algal species. Over the years, as a rule, there should be an increasingly articulated structure of the phytoplanktonic populations, a structure that should guarantee "a greater ecological balance between the species, also determining containment of the intensity of the algal blooms".

P. rubescens can produce numerous types of toxins (microcystins) with a hepatotoxic, gastroenteric and carcinogenic value. Since one of the possible means of the migration of these toxins is drinking water, Acquambiente monitor the concentrations of these substances in the distributed water through periodic analyses.

Fig. 1 - Lake Castreccioni, Cingoli (Marche Region, Italy)

Fig. 2 - Basin of the municipalities through the Castreccioni pipeline.

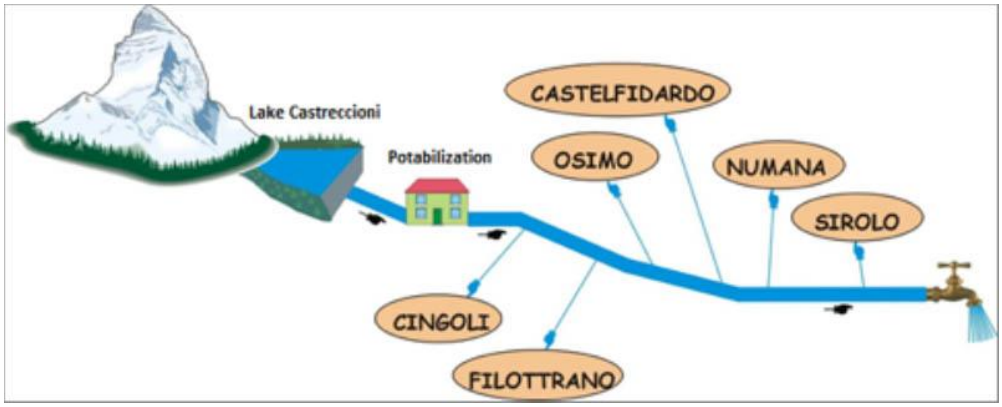


Fig. 3 - Potable water supply of Lake Castreccioni (obtained and modified from the Acquambiente website)

BLOOWATER Mid-Term Review Work Package 2

Don Pierson – Uppsala University Sweden
Francois Clayer - Norwegian Institute for Water Research

The purpose of this work package is to test two different, but at the same time complimentary, methods for simulating the occurrence of cyanobacteria blooms:

- Process based (PB) modeling
- Machine learning (ML) based methods.

During the first half of BLOOWATER the goals of WP 2 were to 1) develop the data sets needed to test either of these approaches; and 2) set up PB models on the lakes chosen as test sites by the BLOOWATER Project. And, un the PB models using historical data to produce hind cast simulations that can be used to evaluate model efficacy for use in in bloom forecasting in the second half of the project. There are no specific milestones or deliverables related to ML in the first half of the project, but clearly there was a need to begin evaluating these methods.

While we have not completely fulfilled the milestones and goals associated with the project mid-term (Table 1), work has progressed on all of the tasks that were to be completed in this work package

Table 1

Milestone	Due Date	Description	Status
M2.1	Month 3	Final decision on case study sites made. Data needed for model forcing and calibration collected and collated.	All case study sites have been choosen including one addtional site studied by the Norwegian Partner. Complete data sets available for the Swedish and Norwegian sites. Data collection continues for the Italian lakes
M2.2	Month 9	GOTM hydrothermal model setup and calibrated for all case study sites.	Completed in Sweden and Norway. Cannot be completed in Italy until full data set is compiled
M2.3	Month 18	SELMA water quality model setup and calibrated for all case study sites.	Completed in Sweden and Norway. Cannot be completed in Italy until full data set is compiled
Deliverable			
D2.1	Month 6	Publicly available data archive of all data used to force and calibrate lake water quality models	Preliminary database has been created and populated with available data. Presently it is publicly accessible, but not finally published.

Data Collection

The status of data collection for the WP2 is shown in Table 2. Complete or nearly complete data sets are available for all sites in Sweden and Norway, while some data

from the two Italian lakes that will be used in this study have been collected, and data collection continues. Deliverable 2.1 specified that a publicly available data archive of all data used for BLOOWATER model testing and development would be placed in a public archive. An initial version of this archive is now available at http://www.hydroshare.org/resource/10e1281196d34550b42501f611e268f9. Final publication will occur once a complete data record from all sites can be uploaded.

Table 2

Site	Country	Meterological Forcing Data	Inflows and nutrient inputs Reservoir opperations	Calibration Hydrothermal lake Water Temperature	Calibration lake water quality. Nutrients Chlorophyll Phytoplankton
Erken	Sweden	1961-2018	2004-2018	1989-2018	2004-2018
Mälaren/Ekoln	Sweden	1979-2016	1979-2016	1998-2005	1998-2005
Vansjö	Norway	1980-2018	1984-2018	2005-2018	2005-2018
Albano	Italy			2015-2019	2015-2019
Castereccioni	Italy		2014-2019 (reservoir level)		2013-2019 Phytoplankton counts

Process Based Modelling

The modeling methods that will be developed by BLOOWATER have the goal of forecasting the probability of cyanobacteria blooms using PB and/or ML models that accept a combination of near real-time (NRT) lake monitoring data, and weather forecasts as input. In the first half of the project we have focused on the PB modeling approach, setting up PB models and developing methods to calibrate these models and access their accuracy. Such calibrated lake models capable of simulating lake conditions and cyanobacteria will become the basis for the forecasts produced in the second half of the project.

The hydrodynamic model GOTM (Burchard, 2002) is the foundation for all the PB modeling done in BLOOWATER and is coupled to the SELMA (Simple Ecological Model for Aquatic Systems) using the Framework for Aquatic Biogeochemical Models FABM (Bruggeman and Bolding, 2014) SELMA which was originally named ERGOM (Neumann et al., 2002) was extensively modified in Water JPI PROGNOS project and as a consequence renamed. It is capable of simulating lake chemistry (nutrients and oxygen), as well as the abundance of three generic groups of phytoplankton: diatoms, flagellates, and cyanobacteria. It is our hope that changing concentrations of cyanobacteria simulated by SELMA can be used as a predictor of conditions favoring cyanobacteria blooms.

The PARSAC (https://bolding-bruggeman.com/portfolio/parsac/) calibration program developed for use with FABM was used for the demanding task of calibrating the coupled GOTM SELMA models. These calibrations require optimizing many model

parameters, which greatly increases the number of model simulations needed to find an optimal parameter set. In BLOOWATER we have continued to refine the calibration methodologies originally developed and tested in PROGNOS, which calibrated groups of parameters in regards to different simulated state variables using an iterative model workflow.

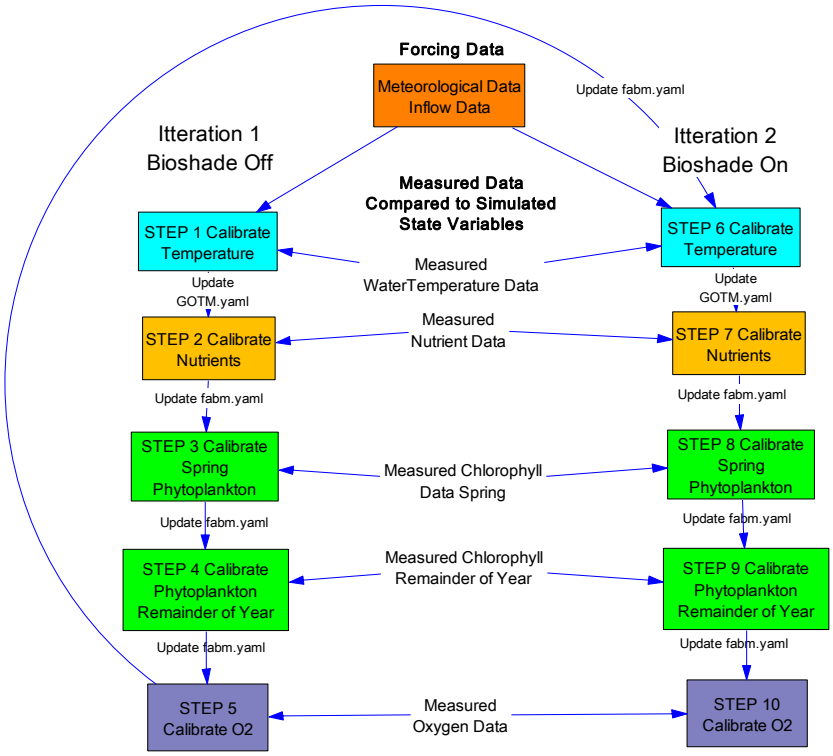
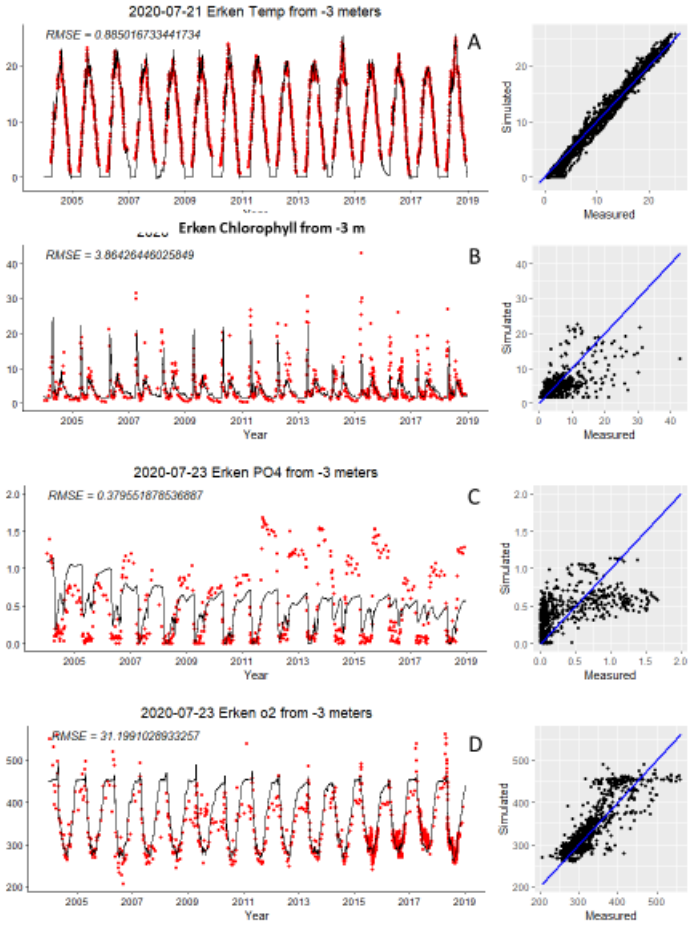


Figure 1. Example of the sequential steps of model calibration used to calibrate the GOTM – SELMA model at Lake Erken. The simulated model state variables associated with each step are compared the measured data in the middle of the figure. Following each step the model parameter files (gotm.yaml and fabm.yaml) are set to the optimum value determined from the calibration. Calibrated parameters from one step continue to be calibrated in the remaining steps however the range of possible values is restricted, and the objective function used to evaluate the calibration is only based on data values related to the present step.

The calibration workflow used for Lake Erken is illustrated by (Fig 1). The lake hydrothermal model is first calibrated by comparison with measured lake water temperature data. Following this the biogeochemical and phytoplankton model parameters affecting lake nutrient concentration and lake chlorophyll concentrations are calibrated, and finally the model parameters affecting dissolved oxygen concentrations are calibrated. For each calibration step simulated values of key state variables are compared to measured values of the same variable. Following each step, the range over which the model parameters can be further adjusted is restricted to +/- a percentage of the determined optimal values. In each step the parameters from all previous steps are also calibrated, but within this more restricted range, and using an objective calibration function that is based on the comparison of simulated and measured data in the present step. For example, in step 3 (Fig 1) the all the parameters associated with steps 1-3 are calibrated, but range of possible parameter values is restricted for the parameters previously

calibrated in steps 1-2, and the calibration is based only on the comparison of simulated diatom chlorophyll with measured chlorophyll during the spring bloom period. Following the first iteration of five calibration steps, the process is repeated after turning on feedback between the simulated water quality and the light extinction coefficient, which can be expected to have some effect on the calibration of the subsequent steps. In the latest calibrations developed for BLOOWATER a final iteration of five additional steps has been added to ensure the model parameters reach stable values. For Lake Vansjø, a simpler calibration workflow is being evaluated. As for Lake Erken, the hydrothermal model is first calibrated. Then, only a second and final calibration step is performed where the model parameters affecting dissolved oxygen, chlorophyll and cyanobacteria concentrations are calibrated. The simulated values for dissolved oxygen, chlorophyll and cyanobacteria concentrations are compared to measured values of the same variable to estimate the skill of the



model.

Figure 2 Model calibrations results for lake Erken as a time series of simulated (solid line and measured (red dots) data from 3 m depth The scatter plots show the measured data vs the simulated data at the time of measurement.

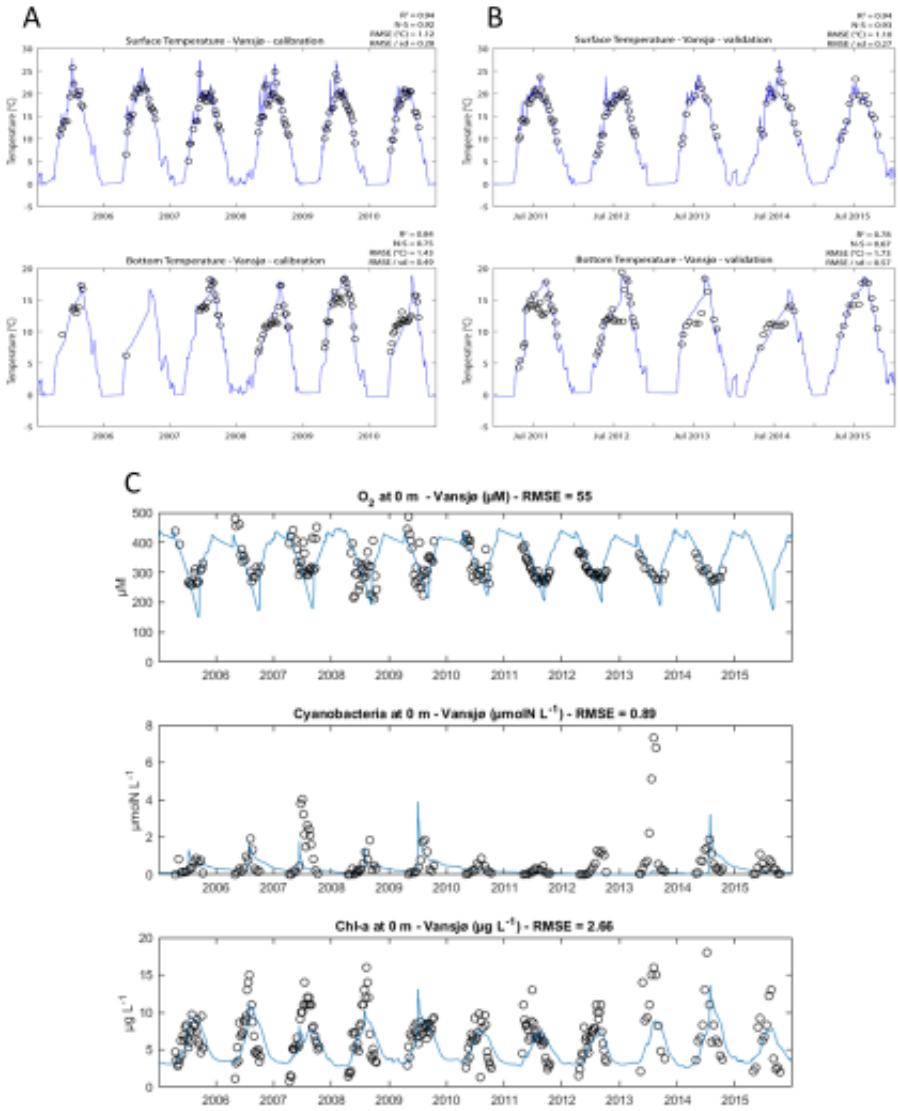
Some results comparing the simulated and measured water quality data for the three meter depth of Lake Erken are shown in Fig. 2., and for Lake Vanjjö in Figs 3-4. The

GOTM hydrothermal model simulations of water temperature are most accurate (Fig. 2A, and Fig. 3A-B), as is always the case, since the hydrothermal algorithms are based on well described physical processes. The hydrothermal model (water temperature and mixing depth) forms a strong foundation for the SELMA biogeochemical simulations, and these results are also very encouraging since biological state variables that are always more difficult to simulate, are predicted with a good degree of accuracy. (Fig. 2B-D, and Fig. 3C). For Lake Erken, both the magnitude and timing of simulated chlorophyll concentrations, a measure of the total phytoplankton, match well with the measured lake concentrations, In particular the model is able to simulate the correct timing of the spring and late-summer phytoplankton blooms that occur in Lake Erken every year. The factors controlling the phytoplankton (phosphate concentration) and affected by the phytoplankton (dissolved oxygen) are also simulated with good accuracy. The calibrated PB model for Lake Vansjø also predicted water quality variables (O2, Chl-a and cyanobacteria) within realistic concentration ranges, displaying well-defined seasonal patterns. However, the magnitude and timing of the simulated chlorophyll and cyanobacteria concentrations are predicted with a lower degree of accuracy in Lake Vansjø compared to Lake Erken. The relative lower skills for the Lake Vansjø model application compared to that of Lake Erken might be attributed to the simpler calibration workflow used in the former case. The comparison of the two calibration workflows highlighted the added-value of the more complex multi-step calibration.

To evaluate the ability of SELMA to forecast the onset of a cyanobacteria bloom we also compared the simulated chlorophyll of the generic cyanobacteria group in SELMA with proportion of chlorophyll associated with cyanobacteria based on the microscopic cell counts (Fig 4). Here model performance was less satisfactory, but still may be of value for predicting the timing if not the absolute magnitude of the blooms. In the first example (Fig 4A) The timing of the initial increase in cyanobacteria is well simulated, but the simulated concentrations are apparently overestimated during the following four sampling dates and then more correctly estimated during the initial decline of the bloom. In the second example (Fig 4B) the timing of the onset of the bloom is well simulated but the magnitude is less than the measured data. The third example (Fig 4C) is one where the model predicts a bloom of cyanobacteria that apparently did not occur. While there are clearly greater levels of uncertainty in the prediction of cyanobacteria, some of this is also related to the measured data by which the model is being judged. The concentrations of cyanobacteria can be particularly difficult to quantify due to difficulties in obtaining a truly representative sample of the lake on any occasion. At Lake Erken a vertically integrated sample is collected at a single point, but it is often the case that there are large horizontal variations in cyanobacteria in the presence of surface blooms (Ostlund et al., 2001) Day to day variations in wind, mixing, and horizontal gradients in surface concentration can lead to large variations in cyanobacteria concentration that can make the single point sample unrepresentative of the conditions for the lake as a whole.

Calibrations such as these are a necessary first step in developing a local model applications that can be used as the basis for a forecasting system on any lake. The development of automated methods to produce these calibrations in a consistent, non-biased and repeatable manner, is an important first step in developing PB model

forecasts for the BLOOWATER project. We continue to evaluate the calibration methods, particularly in regards to the simulations of the cyanobacteria that are key

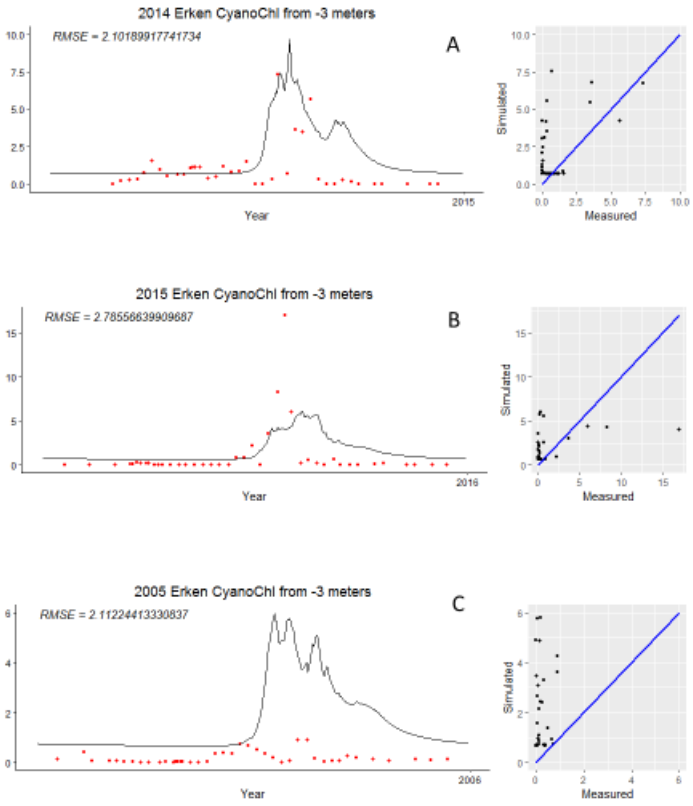


to the use of these models for forecasting purposes.

Figure3 Model calibrations results for lake Vansjö as a time series of simulated (solid line and measured (dots) data. A) surface and bottom water temperatures during model calibration, and B) surface and bottome temperatures during model validation. C shows the simulated concentrations of oxygen cyanobacteria and chlorophyll at the lake's surface.

Machine Learning methods

In the event of failure of accurately predicting cyanobacteria blooms with the PB models, the hydrothermal predictions, because of their extremely high accuracy, can add significant knowledge into Machine Learning algorithms. Given our initial success with the PB models and the potential for ML models to account for complex processes not explicitly described by the PB based models we will make developing hybrid PB-ML models a priority in the second part to the project. The post doc hired



to work in BLOOWATER by Uppsala University will have this work as a central task.

Figure4 Detailed evaluation of the SELMA model to simulate the occurance of cyanobacteria in lake Erken. Solid line is the simulated chlorophyll attributed to cyanobacteria. Red dot are measured values. These are based on the total measured chlorophyll concentration which is portioned into SELMA phytoplankton groups based on biomass estimates from microscopic phytoplankton counts.

Reason for Delays

There were a number of factors that delayed this work package. First delays in the funding of some project partners caused some initial delays in starting up and this was then compounded by additional delays related to the COVID 19 pandemic that made it much more difficult to communicate and collaborate between partners. To date it still has not been possible to have an in person meeting, and our experience shows that model development and applications aimed at specific project goals

greatly benefit from in person modelling workshops, and also direct contact with stakeholders. Both a late start and long delayed physical meetings has led to some of the delays in data collection and model development discussed above. A hiring of post doc by Uppsala University was delayed, in order to allow them to be in place when the project was more fully underway, and also due to the issues of trying to hire someone who would potentially need to move to Sweden during the pandemic. This position has now been advertised and we hope to fill it by the end of November.

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Deliverable D3.1 in the BLOOWATER Project

Waterworks as barriers to cyanobacteria and their toxins

An assessment of removal efficiencies and economic aspects associated with conventional treatment technologies

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Preface

This report is the deliverable D3.1 in the BLOOWATER project under the Water JPI initiative “Closing the Water Cycle Gap” and funded by the national research foundations of Italy, Sweden and Norway (MIUR, FORMAS and RCN). The original working title of this deliverable was “D3.1 Practical report on data performances and economical assessment of conventional technologies for cyanobacteria reduction (M8)”. This report has been a joint effort between the Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA), Polytechnic University of Marche (UNIVPM) and the Norwegian Institute for Water Research (NIVA).

Oslo, 16.10.2020

Christian Vogelsang

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1 Background

Toxin-producing cyanobacterial blooms and measures to provide efficient barriers towards these in drinking water systems have been studied extensively for decades (Chorus and Bartram, 1999; Newcombe et al., 2010). However, despite the large variability in the group of toxin-producing cyanobacteria, most of the in-depth studies that have been conducted on the removal of cyanotoxins have been focusing on microcystins and particularly microcystin-LR, the most toxic of the microcystins. Due to their size, microcystins are typically found intracellularly. Since cyanobacteria are relatively easy to remove by conventional treatment methods (e.g. chemical coagulation/flocculation and filtration), it has for good reasons been strongly advised to avoid actions that could release the toxins during or after treatment. However, during the course of a cyanobacterial bloom many microcystin-producing cells die off and release their toxin. Furthermore, many of the other cyanotoxins are smaller and are found extracellularly to a much larger extent. The different toxins do also have other properties that differ from microcystin-LR and that strongly influences their expected removal by treatment properties.

In this report, we are first looking at the dynamics and variability of cyanobacterial blooms and their cyanotoxins before we discuss the expected removal of cyanobacteria and cyanotoxins by conventional treatment applied at waterworks utilising surface waters as raw water sources for their drinking water production. After that, we focus on a hybrid system that combines two conventional treatment processes in the so-called polymer-enhanced ultrafiltration (PEUF) process that is more extensively studied in the BLOOWATER project. Finally, we look at economic aspects comparing the PEUF process with conventional treatment processes.

2 Cyanobacterial blooms

Cyanobacteria are unicellular, but while some exist as individual cells, others exist as colonies which can form filaments, sheets or hollow balls (**Figure 1**). Cyanobacteria occur in almost all Norwegian lakes, but the amount and species composition varies. About 20 species of toxin-producing cyanobacteria have been recorded and Anabaena is the most common toxin producing genus found in Norwegian lakes (Skulberg, 1980; Berg et al., 1986; Skulberg et al., 1994) (**Table 1**). Microcystis, Anabaena and Planktothrix are among the most common toxin-producing genera in the Northern Hemisphere (Sivonen and Jones, 1999).

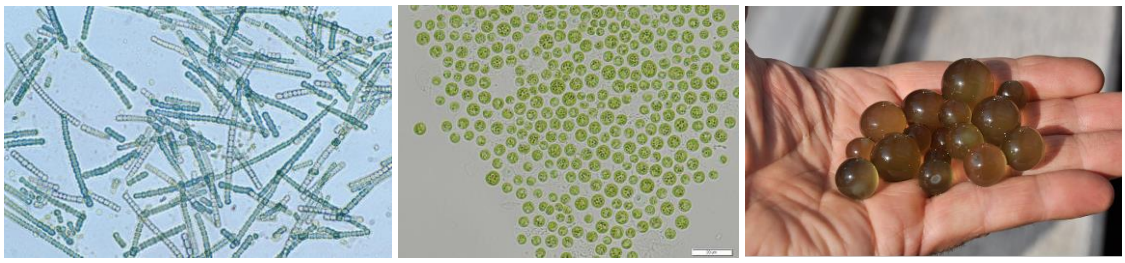

Figure 1. *Selected growth forms of selected cyanobacteria. Left: Filamentous Cylindrospermum sp (Photo: CSIRO); Middle: Colony with Microcystis aeruginosa (Photo:CyanoRO); Right: Nostoc pruniforme (Mare's eggs) (Photo: CC BY_NC)*

Table 1. Toxigenic cyanophytes found in Norwegian inland waters (Skulberg, 1996)

Order	Cyanobacteria	Toxin
Chroococcales	*Microcystis aeruginosa* Kütz.	Microcystins
	Microcystis botrys Teil.	Microcystins
	Microcystis Lemmermann sp.	Microcystins
	[1)]*Snowella lacustris* (Chod.) Kom. et Hind.	Microcystins
	[2)]*Woronichinia naegeliana* (Unger) Elenk.	Microcystins
Nostocales	*Anabaena circinalis* Rabenh.	Unknown
	Anabaena flos-aquae (Lynb.) Bréb.	Microcystins
	Anabaena lemmermani P. Richt.	Microcystins Anatoxin
	Anabaena mendotae Trel.	Microcystins
	Anabaena solitaria Kleb.	Unknown
	Anabaena spiroides Kleb.	Unknown
	Aphanizomenon flos-aqua (L.) Ralfs	Unknown
	Nostoc Vaucher spp.	Unknown
Oscillatoriales	[3)]*Planktothrix agardhii* (Gom.) Anagn. et Kom.	Microcystins Anatoxin
	Planktothrix mougeotii (Bory ex Gom.) Anagn. et Kom.	Microcystins
	Planktothrix rubescens (DC. ex Gom.) Anagn. et Kom.	Microcystins
	Planktothrix prolifica (Gom.) Anagn. et Kom.	Microcystins
	[4)]*Phormidium formosum* (Bory ex Gom.) Anagn. et Kom.	Homoanatoxin Microcystins
	Phormidium Kützing spp.	Unknown
	Trichodesmium lacustre Kleb.	Unknown

Synonyms:

1) *Gomphosphaeria lacustris* Chod.
2) *Gomphosphaeria naegeliana* (Unger) Lemm.
3) *Oscillatoria agardhii/rubescens* group
4) *Oscillatoria Formosa* Bory ex Gom.

The filamentous species of the genus Planktothrix, adapted to low light conditions, and especially the red variants containing the pigment phycoerythrin often dominate blooms in the metalimnion (see **Figure 2**), the boundary layer formed by the thermocline. The accumulation in the metalimnion during summer is attributable to the presence of very strong gas vesicles which lighten the cells while polysaccharide accumulation acts as ballast (Utkilen et al., 1985; Walsby, 1994). This is of particular concern as blooms occurring at these depths are not readily visible from above. And moreover, many waterworks have their water intake at this depth.

Heavy cyanobacterial blooms are associated with lakes receiving extensive amounts of nutrients, but they are able to survive periods with low supply by accumulating reserve compounds (e.g. carbohydrates) while relevant nutrients are still available (Whitton and Potts, 2000). Concentrations of phosphorus less than 0.01 mg/L as filterable reactive phosphorus (FRP) are considered to be growth limiting (Vollenweider and Kerekes, 1980) and 0.1 mg/L soluble inorganic nitrogen is considered the minimum concentration to maintain growth during the growing season (Reynolds, 1992). Higher concentrations support rapid growth and higher biomass. However, cyanobacteria are not growing as

fast as diatoms and many single-cell green algae, therefore cyanobacteria do not bloom in water with short retention times (Reynolds 1997).

Note that Planktothrix compete well in late autumn and winter when low insolation and deep circulation combine to decrease available light, thereby maintaining a population all year round in contrast to phytoplankton species that sink to the bottom in the autumn (Micheletti et al., 1998; Davis et al., 2003).

Note that cyanobacteria prefer basic conditions (pH 8-10) and do not grow under acidic conditions (<pH 7), but they can manipulate their micro-environment by taking up CO_2 and carbonates providing alkaline conditions.

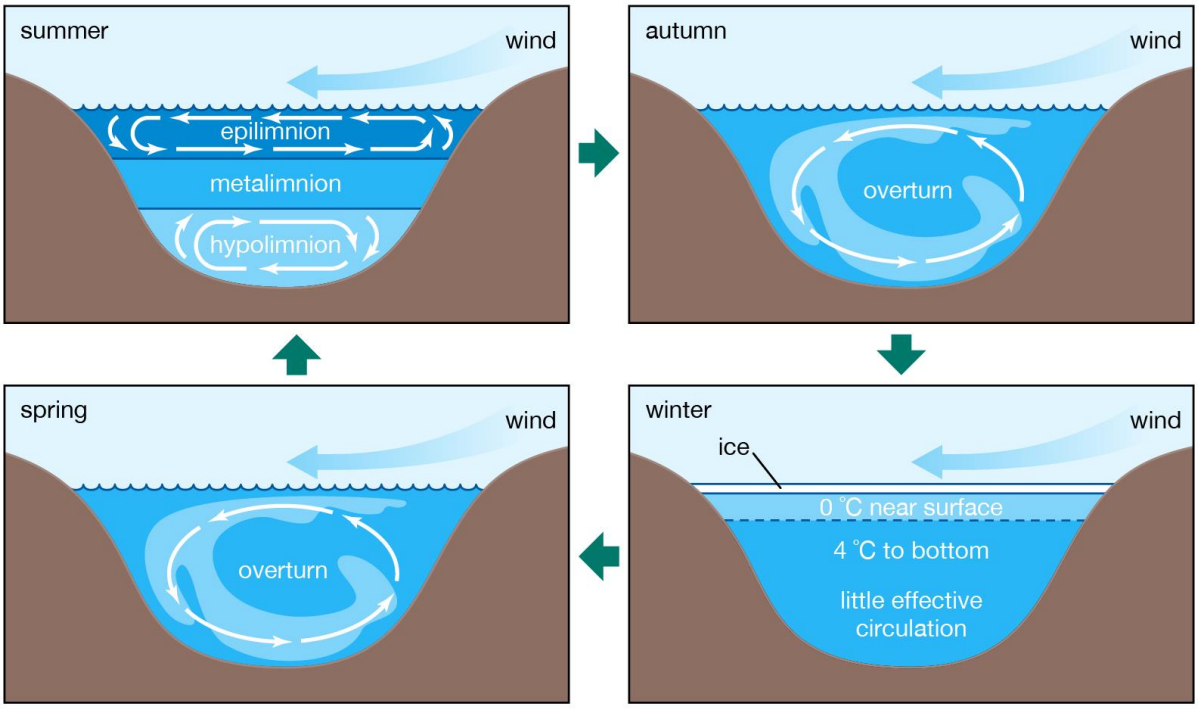

Figure 2. *Seasonal changes in a dimictic lake with stratification during summer (due to warm and less dense water on the surface) and winter (due to ice cover and higher density 4°C water below).*

3 Cyanotoxins

3.1 Structure and properties

A large variety of cyanotoxins have been identified, typically divided in two groups, oligopeptides and alkaloids, as summarised in **Table 4**. As also shown in **Appendix A**, some of the toxins are relatively large and cyclic compounds (microcystin-LR, anabaenopeptin B, cyanopeptolin 954, microviridin B and aplysiatoxin), while others are smaller, more simplistic and linear compounds (aeruginosin B, glycyclamide and anatoxin-a). The others could be regarded as something in between. Microcystin-LR has been very much studied and are well characterised (see **Box 1**).

Table 2 and **Table 3** show some general characteristics and properties of some selected cyanotoxins that are of interest when predicting their removal by different types of treatment processes (see **Section** Error! Reference source not found.). A cyanobacterial bloom may constitute several different cyanobacterial strains and each cyanobacterium may produce several different cyanotoxins. Though all cyanobacteria have toxic lipopolysaccharides (LPS) embedded in their cell wall, not all cyanobacteria produce toxins.

Box 1. *Microcystin-LR*

Microcystins are cyclic heptapeptides, i.e. contain seven amino acids in a ring structure (see **Figure 3**). One of the invariant amino acids is a unique β-amino acid called Adda. Microcystins contain two variable residues, which make the differentiation between variants of microcystins. These two variable elements are always standard L-amino acids. In microcystin-LR these are leucine and arginine. About 80 microcystins have been identified, representing differences in the two variable residues and some modifications in the other amino acids. The different microcystins have different toxicity profiles, with microcystin-LR found to be the most toxic.

The microcystins are larger than most of the other cyanotoxins (their estimated hydrodynamic size is about 1-2 nm). They are primarily hydrophilic due to the many charged groups, but they contain also hydrophobic sections.

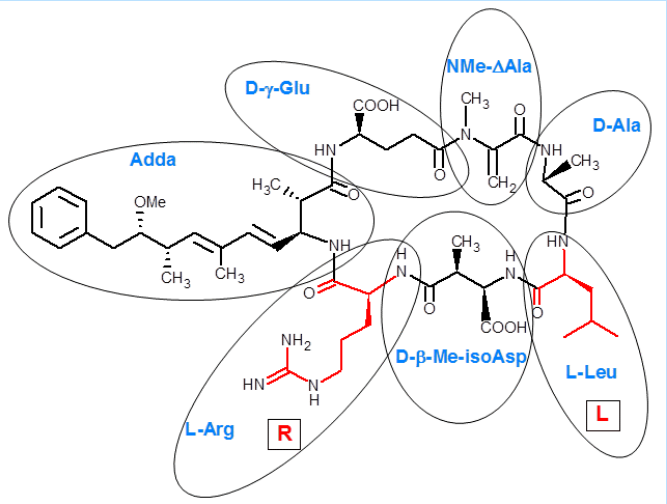

Figure 3. *Microcystins are cyclic heptapeptides contain seven amino acids in a ring structure, here exemplified by microcystin-LR. [DrJohn1100[1]]*

[1] Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=34824812

Table 2. *General characteristics and properties of selected cyanotoxins. The water solubilities at pH 7 and log K_{ow} values are predicted values using OPERA models and made available through the Comptox database. Numbers in brackets should be regarded as highly questionable.*

Toxin	Formula	Mw (g/mol)	Pred. water sol. (mg/L)	Pred. log K_{ow}
Oligopeptides				
Microcystin-LR	$C_{49}H_{74}N_{10}O_{12}$	995,19	(7 500)	-1.44
Aeruginosin B	$C_{14}H_{11}N_3O_5S$	333,32	8 700	(9.43)
Anabaenopeptin B	$C_{41}H_{60}N_{10}O_9$	836,99	(-13 000)	(1.74)
Cyanopeptolin 954	$C_{46}H_{63}ClN_8O_{12}$	955,50	(-520)	(2.09)
Glycyclamide	$C_{14}H_{20}N_2O_3S$	296,39	18	2.90
Microginin 527	$C_{25}H_{41}N_3O_7S$	527,68	26 000	-0.0892
Microviridin B	$C_{84}H_{106}N_{16}O_{24}$	1723,86	-	-
Alkaloids				
Anatoxin-a	$C_{10}H_{15}NO$	165,24	36 000	1,07
Aplysiatoxin	$C_{32}H_{47}BrO_{10}$	671,62	35	(2.25)
Cylindrospermopsin	$C_{15}H_{21}N_5O_7S$	415,42	11 300	(-1.72)
Lyngbyatoxin-a	$C_{27}H_{39}N_3O_2$	437,63	16	4.17
Saxitoxin	$C_{10}H_{17}N_7O_4$	299,29	(2 200)	(9.56)

Table 3. *Minimum and maximum projected diameter and projected area based on … using the geometrical descriptors in the model software MarvinView. Graphics are provided in* ***Appendix A.*** *Predicted molecular charge at pH 4-8 using the isoelectric point … in MarvinView. More detailed figures are provided in* ***Appendix B****.*

Toxin	Projection diameter (Å)		Projection area (Å²)		van der Waals volume (Å³)	Charge				
	min	max	min	max		pH 4	pH 5	pH 6	pH 7	pH 8
Oligopeptides										
Microcystin-LR	16.1	26	119	940	937	-0.5	-1.0	-1.0	-1.0	-1.0
Aeruginosin B	10.6	14.8	39	97	261	-0.7	-1.0	-1.0	-1.0	-1.0
Anabaenopeptin B	15.4	21.8	109	173	777	0.5	0.0	0.0	0.0	0.0
Cyanopeptolin 954	15.4	21.4	114	199	865	0.0	0.0	0.0	-0.2	-0.6
Glycyclamide	10.0	14.3	48	84	268	-0.3	-0.8	-0.98	-1.0	-1.0
Microginin 527	16.5	19.9	93	143	502	0.3	0.0	0.0	0.0	-0.3
Microviridin B	21.3	26.7	225	301	1528	-1.0	-1.0	-1.0	-1.0	-1.0
Alkaloids										
Anatoxin-a	7.6	9.9	34	53	165	1.0	1.0	1.0	1.0	0.98
Aplysiatoxin	13.0	15.1	102	127	587	0.0	0.0	0.0	0.0	-0.2
Cylindrospermopsin	10.6	15.9	53	100	332	0.0	0.0	0.0	0.0	-0.2
Lyngbyatoxin-a	13.5	13.9	80	106	442	0.0	0.0	0.0	0.0	0.0
Saxitoxin	9.5	13.2	48	69	243	2.0	2.0	2.0	2.0	1.75

Table 4. Cyanotoxins

Toxin group	Toxin	Structure	#	Observed in Norway	Cyanobacterial genera
Oligopeptides	Microcystins, nodularins	Cyclic with Adda	89	Yes	*Anabaena, Hapalosiphon, Microcystis, Nodularia, Nostoc, Planktothrix*
	Aeruginosins	Linear	27	Yes	*Microcystis, Planktothrix, Nodularia*
	Anabaenopeptins	Cyclic	32	Yes	*Anabaena, Aphanizomenon, Microcystis, Planktothrix, Plectonema, Nodularia, Schizothrix*
	Cyanopeptolins	Cyclic	82	Yes	*Anabaena, Dolabella, Lyngbya, Microcystis, Planktothrix, Scytonema, Symploca*
	Cyclamides	Multicyclic	21	?	*Lyngbya, Microcystis, Nostoc, Planktothrix, Stigonema, Westelliopsis*
	Microginins	Linear	38	Yes	*Microcystis, Planktothrix, Nostoc*
	Microviridins	Cyclic	10	?	*Microcystis, Planktothrix, Nostoc*
Alkaloids	Anatoxin-a	Linear	?	Yes	*Anabaena, Planktothrix (Oscillatoria), Aphanizomenon, Cylindrospermopsis*
	Anatoxin-a(S)	Linear	?	No	*Anabaena*
	Aplysiatoxins	Cyclic	?	No	*Lyngbya, Schizothrix, Planktothrix (Oscillatoria)*
	Cylindrospermopsins	Linear	?	No	*Cylindrospermopsis, Aphanizomenon, Umezakia, Raphidiopsis, Anabaena*
	Lyngbyatoxin-a	Cyclic	?	No	*Lyngbya*
	Saxitoxins	Linear	?	No	*Anabaena*[2]*, some Aphanizomenon, Cylindrospermopsis, Lyngbya, Planktothrix.*

[2] Anabaena circinalis has recently been renamed to Dolichospermum circinalis (Wacklin et al. 2009).

Microcystins are produced intracellularly by several cyanobacterial species (*Microcystis*, *Planktothrix*, *Anabaena*, *Oscillatoria* and *Nostoc*) with microcystin-LR particularly associated with *Microcystis aeruginosa*. Microcystins (and all other oligopeptides?) are mostly retained in the living cyanobacterial cells. Light intensity and temperature seems to be the most important environmental factors regulating the production of microcystins (Wicks and Theil 1990). After senescence (the cells get old and weary) and cell lysis they are released into the surrounding water (see **Table 5**). Note that in a natural bloom there will probably be cells in all stages of growth. Due to their chemical stability microcystins can persist in the water for several days or weeks after the breakdown of the cyanobacterial bloom.

Table 5. *Distribution of microcystins during laboratory culture of Microcystis aeruginosa (Source: National River Authority, 1990).*

Age of culture	Distribution of toxins (%)	
	Cells	Water
Young		
Slowly growing cells	100	0
Rapidly growing cells	75-90	10-25
Old		
Slowly growing intact cells	70-80	20-30
Decaying ells (leaking cell contents)	30-40	60-70

The smaller and linear cyanotoxins such as cylindrospermopsins, anatoxin-a and saxitoxins are typically found extracellularly to a much higher degree than microcystins.

3.2 Natural degradation of cyanotoxins

3.2.1 Microcystins

Microcystins are very chemically stable and can persist for months or years in natural waters in the dark. Slow hydrolysis has been observed at high temperatures (40°C) and at very low pH (90% breakdown over 10 weeks at pH 1) or relatively high pH (90% breakdown over 12 weeks at pH 9) (Harada et al., 1996). However, in full sunlight, intracellular microcystins undergo slow photochemical breakdown in a couple of weeks, enhanced by the presence of water-soluble cell pigments (Tsuji et al., 1993). Microcystins are, however, susceptible to breakdown by aquatic bacteria found naturally in rivers and reservoirs. There is usually a lag period that may last from days to several weeks before the biodegradation process commences and 90% removal of microcystin is typically complete within 2-10 days (Jones et al., 1994; Lahti et al., 1997).

3.2.2 Anatoxin-a

Under normal day and night light conditions at pH 8 or pH 10, and at low initial concentrations (10 µg/L), the half-life for anatoxin-a breakdown was found to be approximately 14 days (Smith and Sutton, 1993).

3.2.3 Saxitoxins

In the dark at room temperature, saxitoxins undergo a series of slow chemical hydrolysis reactions. The C-toxins lose the N-sulphocarbamoyl group to form the much more toxic (by a factor of 10-100)

dicarbamoyl gonyautoxins (dc-GTXs), hence the toxicity can actually increase over a period of up to three weeks during a bloom before toxicity begins to abate during the succeeding 2-3 months (Jones and Negri, 1997).

3.2.4 Cylindrospermopsin

Cylindrospermopsin is relatively stable in the dark, but breakdown in sunlight and in the presence of cell pigments occurs quite rapidly being more than 90 per cent complete within 2-3 days (Chiswell et al., 1999). Biodegradation occurs in natural waters.

3.3 Expected cyanotoxin concentrations during cyanobacterial blooms

The concentrations of cyanotoxins during a bloom will vary considerably, also the ratio between intra- and extracellular toxins. **Figure 4** shows typical concentrations of intracellular microcystins found in Germany during blooms with different types of dominating cyanobacteria. The concentrations of extracellular cyanotoxins will depend on the type of toxin and the stage of the bloom (as discussed above), as well as on the intensity of the bloom. Reported concentrations of microcystins are typically in the range of 0.1-1 µg/L, but as high concentrations as 1800 µg/L has been reported when a bloom was treated with algicide resulting in release of intracellular microcystin. Nevertheless, this indicates that much higher naturally occurring extracellular concentrations can be expected of smaller cyanotoxins such as cylindrospermopsins, anatoxin-a and saxitoxins during blooms High regional, seasonal, spatial, temporal and diel variations of toxin concentrations have been reported (WHO, 1999).

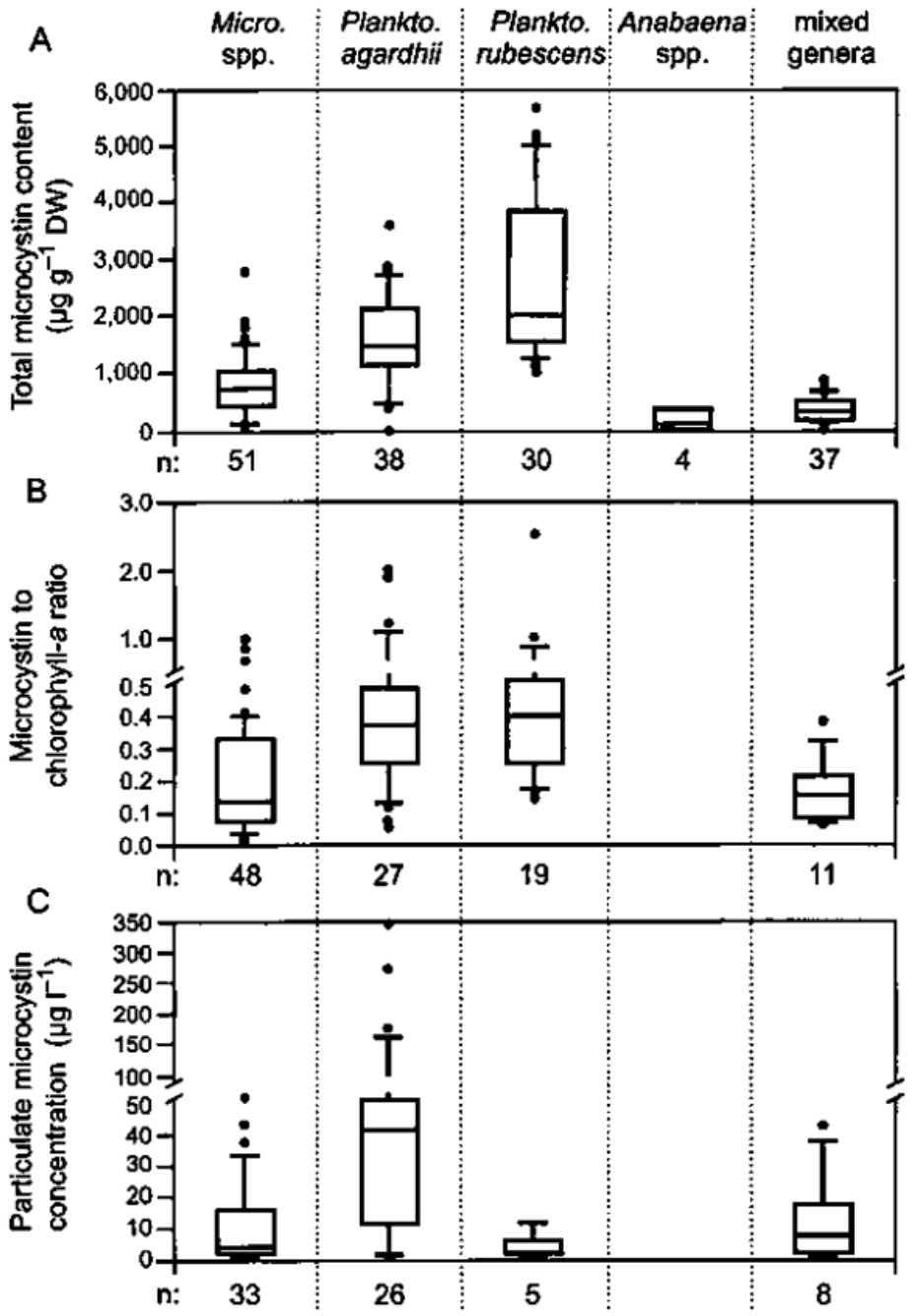

Figure 4. *Cell-bound total microcystin content (measured by HPLC) of samples taken in Germany between 1995 and 1996 dominated by different cyanobacteria; on a dry weight basis (**A**), on a chlorophyll a basis (**B**) and on a volume basis (**C**). Micro. spp. = Microcystis spp.; Plankto. agardii = Planktothrix agardii; Plankto. rubescens = Planktothrix rubescens. Boxes show median values and the values within the 50 percentile range; bars indicate the 10th and 90th percentile; n = number of samples. Modified from Fastner et al. (1998) by WHO (1999).*

3.4 Target concentrations in drinking water

The World Health Organisation (WHO) has develop a provisional guideline value for microcystin-LR in drinking water of 1 µg/L (Cupta, 1998). It was considered provisional because the available data were insufficient to develop values for other microcystin congeners. Many countries have accepted this guideline value, with some exceptions. A summary of national guideline values for different cyanotoxins is given in **Table 6**.

Table 6. *National guideline values for different cyanotoxins in drinking water. Based on de la Cruz et al. (2020).*

Cyanotoxin	Value	Country
Microcystins	1.5 µg/L	Canada
	1.0 µg/L	Brazil, France, Spain, Ohio (USA), Oregon (USA)
	1.6 µg/L	US EPA (people in general)
	0.3 µg/L	US EPA (≤5 years)
	0.1 µg/L	Minnesota (USA)
Microcystin-LR	1.5 µg/L	Canada
	1.0 µg/L	Many countries*
Nodularin	1 µg/L	New Zealand
Saxitoxins	3 µg/L	Brazil, New Zealand, Oregon (USA)
	0.2 µg/L	Ohio (USA)
	1.6 µg/L	Oregon (USA; people in general)
	0.3 µg/L	Oregon (USA; ≤5 years)
Cylindrospermopsins	15 µg/L	Brazil
	1.0 µg/L	New Zealand, Ohio (USA), Oregon (USA)
	3.0 µg/L	US EPA (people in general)
	0.7 µg/L	US EPA (≤5 years)
Anatoxin-a	6.0 µg/L	New Zealand
	3.7 µg/L	Canada
	3.0 µg/L	Oregon (USA; people in general)
	0.7 µg/L	Oregon (USA; ≤5 years)
Homo-anatoxin-a	2.0 µg/L	New Zealand
Anatoxin-a(s)	1.0 µg/L	New Zealand

*) Czech Republic, New Zealand, Finland, Singapore (in both free and cell-bound forms), South Africa (target range 0-0.8 µg/L), Uruguay, Japan, Korea, Norway and China.

4 Removal of cyanobacteria and their toxins by conventional drinking water treatment processes

In order to select the appropriate treatment process, a drinking water manager will need to consider the types of cyanotoxins that may be present (see **Table 4**) and whether they are contained within the cyanobacteria cells (intracellular) or dissolved in the water (extracellular). In a natural bloom there will probably be cells in all stages of growth. Intracellular toxins can be eliminated by removing the cyanobacteria cells. Extracellular toxins are dissolved in the water and are generally more challenging to remove. Hence, the following two-step strategy for the removal of cyanotoxins is preferred:

Step 1: Removal of cyanobacterial cells using processes that will not release any intracellular toxins. Making sure that the applied sludge management also prevent any reintroduction of any released cyanotoxins to the water.

Step 2: Removal of any extracellular toxins using treatment process(es) adequate to the cyanotoxins that are present.

4.1 Step 1: Removal of intracellular cyanotoxins

Error! Reference source not found. summarises the expected treatment performances of different conventional treatment processes applied at waterworks that are expected to remove cyanobacteria and therefore also their associated intracellular cyanotoxins. Comments to each treatment process is given in the following sub-sections.

Table 7. *Summary of expected water treatment performance for the removal of cyanobacteria with intracellular cyanotoxins*

Treatment process	Expected removal of intracellular cyanotoxins
Coagulation – sedimentation-filtration	++/+++
Coagulation – dissolved air flotation	++/+++
Slow sand filtration	+++
Microfiltration	0/+/++
Ultrafiltration	+++

4.1.1 Chemical coagulation followed by sedimentation and/or depth filtration

The standard conventional treatment train and key line of defence for many waterworks is coagulation, flocculation, and sedimentation, followed by depth filtration. At Norwegian waterworks coagulation and direct filtration (i.e. omitting the sedimentation step) is very common. A well-optimized coagulation and sedimentation-filtration step is critical to cyanobacterial cell removal during treatment. The typical removal achieved by coagulation/flocculation is in range of 30% to >90%, with factors impacting removal such as: type and dose of coagulant, pH, presence of NOM, type of

cyanobacteria (morphological differences, stage of growth, size and shape, zeta potential) (Hiskia et al. 2020). The size and shape of cyanobacterial cells influence the removal efficiency. It has been reported that smaller cyanobacterial cells are not as efficiently removed through coagulation as the larger ones. On the other hand, spherical cells display better removal than ellipsoidal or elongated cells (Ma et al., 2007). Since efficient coagulation is dependent on charge neutralisation, the pH-dependent surface charge of the specific cyanobacteria in each bloom should be considered (by e.g. measuring its zeta potential at different pHs). Hence, jar testing for site-specific conditions to optimize coagulant dose, pH, and settling time are critical to setting full-scale operational parameters to meet expected blooms (AWWA 2016). Both alum and ferric chloride coagulation have been shown effective at removing intact *Microcystis* and *Anabaena* cells in addition to other algal and cyanobacterial cells (Knappe et al. 2004, Chow et al. 1999, Chow et al. 1998), however, release of toxins have been observed when using ferric or aluminium coagulants for direct coagulation in natural water reservoirs (Pietsch et al., 2002; Han et al. 2012; Han et al. 2013). Whether there is some damage to the cells during the process appears to be dependent on the health of the cells, and the stage of growth of the bloom.

The depth filters may consist of a single medium, such as sand or anthracite, dual media, a combination of these two or multimedia including materials such as garnet with effective sizes of 0.1–1.0 mm. The depth filters are run as rapid sand filters with flow rates of 2-20 m/h and filter runs of typically 20-60 hours before backflushing. During a bloom situation where some cells are carried through to the filters, backwash frequency will probably increase. This is desirable to reduce the risk of dissolved algal metabolites released into the filtered water.

4.1.2 Chemical coagulation followed by dissolved air flotation (DAF)

DAF is a clarification process that can replace the sedimentation step after the chemical coagulation step described above, or it may be used a pre-treatment before membrane filtration (see below). In DAF, fine air microbubbles (10-100 µm) are introduced in the liquid phase, the microbubbles attach to and become enmeshed in solid particles, producing bubble-solid aggregates less dense than water, and these aggregates are transported to the water surface. Hence, DAF is particularly effective for light cyanobacterial cells and species containing gas vesicles, which typically form surface scums (Sklenar et al., 2014; U.S. EPA, 2014a; Tokodi et al., 2012, Markham et al., 1997; Mouchet and Bonnélye, 1998). The efficiency of DAF depends more on the particle destabilization than on the floc size. Cyanobacteria removals in the range of 71-99% have been reported (Hiskia et al. 2020).

4.1.3 Slow sand filtration (SSF)

The slow sand filters are typically operated with flow rates of 0.1-5.0 m/day with runs taking 2-6 months. A key feature of slow sand filters is the formation of the “dirt blanket” on the upper surface of the filter. This is a biologically active layer of biofilm which serves both as a physical filter, removing many pathogens, and an active bioremediation filter eliminating many organic pollutants including algal toxins [108]. Whilst SSF can be highly efficient for removal of cyanobacterial cells and toxins, successful operation is dependent on reasonable quality of feed water, hence, some sort of pre-sieving (e.g. microstraining) is often needed to prevent blockage and reduced run lengths. Moreover, cyanobacteria may accumulate on the filter surface, acting as a source of dissolved cyanotoxins after cyanobacteria are no longer present in the water body. The top layer of sand should therefore be removed on a regular basis. Removals in the range of >85-94% have been reported for microcystin, but the removal efficiency can be drastically reduced at lower temperatures (Hiskia et al. 2020).

4.1.4 Low-pressure membrane filtration (MF and UF)

Membrane filtration is a physical separation process that utilizes a semi-permeable membrane to divide an influent water stream into two fractions: a permeate that contains any material passing through the membrane and a retentate (or concentrate) that contains the materials that have been separated out. Membranes used for microfiltration (MF) typically have a pore size of 0.1-10 µm and a molecular weight cut-off (MWCO) of 800-2000 kD, while membranes used for ultrafiltration (UF) typically have a pore size of 0.01-0.1 µm and a molecular weight cut-off (MWCO) of 1-500 kD.

The pore sizes of the low-pressure membranes are in theory too large to remove extracellular toxins, but cyanobacteria and therefore the intracellular cyanotoxins may be well retained.

- Cross-flow and dead-end MF and UF: more than 98% of the *M. aeruginosa* cells were removed; at 200 kPa some damaged cells were found in the backwash water; but no release of microcystin into the permeate.
- Mouchet and Bonneley (1998) observed that 40-70% of the cyanobacterial species tested were removed using a MF with pore size of 25-35 µm, but noted that the smaller species were not removed (<10%).
- Sorlini et al. (2013) evaluated the removal of algae, 17 cyanobacterial species and intracellular microcystin-LR from lake water using a pilot-plant with a 200 kDa PVDF hollow fiber MF membrane. MF achieved removal rates higher than 98% for a large number of species of cyanobacteria and the removal efficiency of total algae content was about 98–99%. Moreover, there was no observable release of microcystin-LR into the permeate water as a result of the low mechanical stress from the system.
- UF was reported to be a very efficient treatment technology to remove intracellular cyanotoxins with removals higher than 98% (Gijsbertsen-Abrahamse et al., 2006).
- During experiments with cyanobacteria cells, a 100 kDa hydrophilic cellulose acetate (CA) UF membrane provided complete removal of single cells of *Microcystis aeruginosa*, producing chlorophyll-a free permeate (Campinas and Rose, 2010). In the experiments with extracellular cyanotoxins, the study also looked into the removal and cell lysis of microcystin. The microcystin removal was below 20% due to cell damage and subsequent release of microcystins. However, despite this cell lysis, the permeate quality during the microcystin experiments was not deteriorated and its microcystin concentration was always identical or lower than the dissolved concentration in the feed water. The hydrophilic UF membrane presented low adsorption of microcystins.

Despite the relatively large pore sizes of UF membranes, some microcystin removal have been reported:

- A 100 kDa hydrophilic PVDF UF membrane totally rejected *Microcystis* cells with their intracellular toxins. But it also provided 10-55% rejection of extracellular microcystin-LR from the simulated surface water (Liu et al., 2017). The rejection rates decreased from the initial 30-55% to as low as 10% before slightly increasing and stabilizing, indicating exhaustion of the membrane adsorption capacity. However, gradually improved rejection of microcystin-LR was observed during the final filtration periods with removal rates varying from 20% to 45% as the transmembrane pressure (TMP) increased from 50 kPa to 250 kPa. Cake layer filtration was regarded as the dominating mechanism for improved toxin rejection during the later filtration periods. The cell breakage during filtration was less than 5% and mainly occurred in the cake layer due to hydraulic shear, but the breakage did not substantially vary with increasing TMP.
- UF membranes with MWCO of 4 kDa, 2 kDa and 1 kDa provided 35%, 55%, and 70% removal of microcystin-LR, respectively. After reaching steady-state conditions, the removal was mainly attributed to size exclusion and the factors like membrane characteristics, feed concentration,

water recovery and operating pressure impacted the removal rates (Lee and Walker, 2008). Hydrophobic polysulfone membranes adsorbed the microcystin significantly, as opposed to hydrophilic cellulose acetate membranes that did not. Since the negatively charged UF membranes significantly sorb microcystin-LR they should be cleaned by backwashing or chemical cleaning after blooms of cyanotoxins, to minimize the risk of toxin release to the permeate.

- The removal rate of microcystin-RR and microcystin-LR by 50 kDa PVC UF membrane decreased over time from original 26% and 42% to 2% and 10 %, respectively (Li et al., 2009). Low removal could be due to the hydrophilic nature of the membrane and the MWCO being far greater than these microcystins. By increasing the permeate flux the removal of microcystin-RR and microcystin-LR increased markedly, possibly due to greater adsorption of microcystins onto the membrane.

Cyanobacteria may be concentrated at the membrane surface. The extent of damage to the cells will depend on the flux through the membranes, pressure and the time period between backwashes [195].

However, direct membrane application to raw water with high algae concentration brings several challenges such as fouling due to algal cells and algae-derived organic matters (Qu et al., 2012; Wei et al., 2016) and/or cell rejection that cause an increase of extracellular toxins in the permeate due to cell breakage during filtration (Liu et al., 2017). For instance, Sorlini and co-authors obtained high removals of cyanobacteria (98%) and total algae (99%) using a pilot-scale MF, and further reported severe fouling that caused a rapid increase in the transmembrane pressure. At this point, the use of integrated membrane systems with enhanced coagulation (see **Section 4.3**) has high potential to remove both intra- and extracellular cyanotoxins, to reduce fouling on membranes and to produce high quality water (Dixon et al., 2011; Yan et al., 2017).

4.1.5 Treatment processes that may release intracellular cyanotoxins

4.1.5.1 Pre-oxidation

Oxidants are often added at the intake to address one or several concerns: 1) reduce taste and odour compounds; 2) discourage bio fouling (zebra mussels, biofilm, and algae) of the intake pipe; 3) reduce the production of disinfection by-products; 4) assist with coagulation; and 5) remove dissolved metals, such as iron and manganese. However, the addition of an oxidant at the intake may provoke cell lysis and thereby strongly contribute to the release of intracellular cyanotoxins from the exposed cyanobacteria. If oxidation is required to meet other treatment objectives, consider using lower doses of an oxidant less likely to lyse cells (e.g. potassium permanganate). If oxidation at higher doses must be used, sufficiently high doses should be used to not only lyse cells but also destroy total toxins present.

4.1.5.2 Sludge management

Once confined in sludge of any type, cyanobacteria may lose viability, die, and release dissolved algal metabolites into the surrounding water (Drikas et al., 2001). This can occur within one day of treatment for some cyanobacteria and could potentially result in very high dissolved concentrations of algal metabolites. Similarly, algal cells carried onto sand filters, in flocs or individually, could rapidly lose viability. Where cyanobacteria are potentially toxic, all sludge and sludge supernatant should be isolated from the plant until the toxins have degraded sufficiently, wherever this is possible.

Microcystins are readily biodegradable (Bourne et al. 1996) so this process should take 1-4 weeks. Cylindrospermopsin appears to be slower to degrade (Senogles et al., 2002) and the biological degradation of saxitoxins has not yet been studied. However, the latter are known to be stable for prolonged periods in source water, so caution is recommended.

4.2 Step 2: Removal of extracellular cyanotoxins

Due their much smaller sizes and relatively high water solubility (see **Table 2**), extracellular cyanotoxins are usually not efficiently removed by the treatment processes described for the removal of cyanobacteria in Step 1. Treatment processes that have been shown to be effective in the removal of at least some cyanotoxins are summarised in **Table 9** and described in more detail in the following.

4.2.1 Adsorption by activated carbon

Activated carbons are the primary materials used as adsorbents in waterworks. The activated carbons are characterized by a high specific surface (500-2500 m^2/g), highly reactive surface, and differ in the distribution and size of the pores (micropore, mesopore, or macropore structure) according to the original raw material (Westrick et al., 2010). Powdered activated carbon (PAC) is usually employed as a temporary treatment for transient contaminants and is fed at the front of the treatment process at a point that will provide sufficient contact time before the particle removal processes. A PAC dose of >20 mg/L is usually necessary but needs to be established together with the contact time in each case [Chorus and Bartram, 1999; Song et al. 2005). Granulated activated carbon (GAC) is used in fixed beds to reduce NOM, taste and odour compounds, and synthetic organic compounds from source waters contaminated by anthropogenic pollutants (Westrick et al., 2010). The main removal mechanisms for extracellular cyanotoxins by both PAC and GAC are adsorption and/and ion exchange with functional groups on the surface of the activated carbon. Though particulate matter (e.g. cyanobacteria) may be trapped in the GAC filter, the filtration action itself will not increase the removal of (dissolved) extracellular cyanotoxins. When used as a post-filter adsorbent, GAC can be a highly effective barrier for microcystin but must be replaced or regenerated with sufficient frequency to minimize breakthrough (AWWA, 1999). An active biofilm is often developing on the surface of the GAC, forming the so-called biological activated carbon (BAC), providing potential biodegradation of cyanotoxins as well as an increased bed life (Huang et al. 2007). However, when applied as a BAC with several years of service, the adsorption of cyanotoxins may be minimal (AWWA, 1999). The biodegradation rate is significantly impacted by previous exposures to the same cyanotoxin(s) (positive correlation) and by the water temperature (negative correlation).

Activated carbon adsorption removals of microcystins of up to 99% are possible, however different activated carbon variants have different adsorption efficiencies. The removal by PAC is reportedly in range of 65-98% depending on the dose, whereas GAC removes typically 90% of extracellular cyanotoxins and 60% of intracellular cyanotoxins (Hiskia et al. 2020). The BAC is also effective, but the removal may drop to 40% (MC-LA) and 70% (MC-LR) after app. 6-mth operation.

There is a relationship between the adsorption-efficient pore size range of the activated carbon and the molecular size of the cyanotoxin:

- Activated carbon with microporous structure have been shown to have the highest adsorption capacity for saxitoxins (Ho et al. 2009).
- Activated carbon with mesopore structure (2-50 nm) have been shown to have the highest adsorption capacity for microcystins and cylindrospermopsin (Newcombe, 2002; Ho et al. 2008). The molecular weight of cylindrospermopsin (415 g/mol) indicates that it would be

removed by activated carbons similar to those recommended for saxitoxins. However, laboratory results have shown that carbons possessing higher volumes of larger pores are the most effective, suggesting the molecule has a larger hydrodynamic diameter than indicated by its molecular weight (Huang et al. 2007).

For the removal of anatoxins, more studies are needed to determine which type of activated carbon, dosage and contact time are appropriate (Westrick et al., 2010).

Natural organic matter (e.g. humic and fulvic acids) will compete with the cyanotoxins and reduce the adsorption capacity [Donati et al. 1994; Svrcek and Smith, 2004; Campinas et al. 2013). Note also that GAC or PAC that is selected for control of typical water contaminants for a utility may not be the optimal carbon for cyanotoxin control (AWWA, 1999).

4.2.2 High-pressure membrane filtration (NF and RO)

Membranes used for nanofiltration (NF) typically have a MWCO of 0.2-2.0 kD and provide effective removal of various extracellular cyanotoxins typically in the range of 70-99%, but the removal may also be as low as 40% for some membranes or toxins (Hiskia et al. 2020). The reverse osmosis (RO) membranes provide removals of microcystin greater than 95% (Neumann and Weckesser, 1998), but site-specific tests are recommended as removal efficiency may vary as well. The rejection percentage of a cyanotoxin by high-pressure membranes is affected by various properties of the membrane, including its molecular weight cut-off (MWCO), desalting degree, porosity, morphology and hydrophobicity, properties of the cyanotoxin such as their MW, molecular size, charge and hydrophobicity, as well as the feedwater chemistry. Due to the higher pressure applied it is expected that cell lysis is highly likely (EPA 2014). Below is a list of studies looking at the removal of cyanotoxins using NF membranes:

- Fawell et al. (1993): complete rejection of MC-LR and no toxins accumulation on the membrane surface of a 200 Da NF membrane.
- Smith et al. (2002) & Simpson and McLeod (2002): 8 commercially available NF membranes with MWCO of 60-300 Da. The rejection of microcystins were greater than 80%. For 7/8, microcystin in permeate <1 µg/L (WHO guideline). Membranes of 225-300 Da outperformed membranes with lower MWCO. GSM removal > 70% for 6/8 NF membranes.
- Mody (2004): 4 commercial NF, NF90, NF270 and LFC1 (100-300 Da) removed MC-LR below 1 µg/L. NTR7450 (600-800 Da) removed up to 40%.
- Texeira and Rosa (2005): NFT50 (NF/RO) removed MC-LR, MC-LY and MC-LF below detection level. Rejections mainly related to size exclusion effects, and on the microcystin net charge (negative but weakly charged). Presence of CaCl2 and NOM had no influence on the microcystin rejection by the membrane under experimental conditions tested.
- Gijsbertsen-Abrahamse et al. (2006): Anatoxin-a and MC-RR rejection of 96% and 99% using Trisep TS80 4040 NF.
- Alt et al. (2005): 7 NF, recovery 80%, typically 85% MIB and GSM removal, up to 97-99% for NF200 and ESNA1-LF, except Trisep XN45-TSF, Hydranautics Hydracore, and Koch 4720-SR-2.
- Reiss et al (2006): 99% removal of MIB and GSM using PA NF compared to 35-50% CA NF
- Dixon et al (2012): 6 commercial NF, for 4 NF with 100 Da 91-97%, for 1x300 Da 82-89%, for 1x800 Da 50-57% of MIB and GSM. 90-100% removal of cylindrospermopsin (CYN) with low MWCO (NF, NF90, DK, SWRO, BWRO). MC-LR removal dropped from 95-100% (6 hr) to 50-80% after 24 hr for NF270 and DK. Special attention to MWCO, membrane material and solution pH (can affect hydrophilicity of MC-LR which increase between pH of 6-9).

Table 8. Expected (Exp.) and observed (Obs.) average removal of selected cyanotoxins by different types of NF membranes over a 220 hours period. The MWCO of each membrane is given as well if it is hydrophobic (HFO) or hydrophilicity (HFI). Observed values are based on Dixon et al. (2012) and are given as average values (min-max) interpreted from figures. The expected removal is indicated by +++ (very good; ">95%"), ++ (good; "80-95%"), + (moderate; "20-80%"), or 0 (negligible (<20%). The MWCO is interpreted as 90% of molecules of this size will be retained by the membrane.

Cyanotoxin	Properties		100 Da, HFO[1)]		100 Da, HFI[2)]		300 Da, HFI[3)]		600-800 Da, HFO[4)]	
	Mw (g/mol)	Pred. log K_{ow}	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
Microcystin-LR	995	-1.44	+++	85-100%	+++	50-100%	+++	75-100%	++	75-100%
Aeruginosin B	333	(9.43)	+++		++		++		0	
Anabaenopeptin B	837	(1.74)	+++		+++		+++		+	
Cyanopeptolin 954	956	(2.09)	+++		+++		+++		++	
Glycyclamide	296	2.90	+++		+++		++		0	
Microginin 527	528	-0.089	+++		+++		+++		+	
Microviridin B	1724	-	+++		+++		+++		+++	
Anatoxin-a	165	1,07	++		++		0		0	
Aplysiatoxin	672	(2.25)	+++		+++		+++		+	
Cylindrospermopsin	415	(-1.72)	+++	90-100%	+++	95%	+++	80-90%	0	15-85%
Lyngbyatoxin-a	438	4.17	+++		+++		+++		0	
Saxitoxin	299	(9.56)	+++		+++		++		0	

1) NF90, SWRO and BWRO; 2) DK; 3) NF270; 4) NTR7450

Significant research gaps

Most of the research on membranes have been conducted on microcystins, and particularly microcystin-LR. This literature study has revealed a number of significant gaps that need to be studied further. The main topics are listed here, while more details are given in **Appendix C**.

- Effects from shear on the integrity of the cyanobacteria and concomitant release of intracellular cyanotoxins
- Characterize removal performance for other cyanotoxins than microcystins
- Membrane configurations
- Develop more hydrophilic membranes
- Factors controlling fouling
- Investigate removal effectiveness over a longer filtration runs
- Experiments with realistic environmental water matrices
- …

4.2.3 Oxidation by ozonation

Ozonation is effective for all dissolved toxins except the saxitoxins. A residual of at least 0.3 mg/L for 5 minutes will be sufficient. Doses will depend on water quality (e.g. ozone demand caused by NOM) (Newcombe et al., 2010).

4.2.4 Oxidation by chlorination

Chlorination is not effective with doses typically used for drinking water treatment. However, it is effective when the free chlorine is >0.5 mg/L after >30 min at pH <8 and when the NOM concentration is low: The effect is negligible when the dose is low or when pH >8. Microcystin-LA and saxitoxins may require a higher residual (Newcombe et al., 2010).

4.2.5 Potassium permanganate

Potassium permanganate readily oxidise microcystin, but there is limited data for other toxins (Newcombe et al., 2010).

Table 9. *Summary of expected water treatment performance for the removal of selected extracellular cyanotoxins.*

Treatment process	Expected removal based on observed values			Comments
	Microcystins	Anatoxin-a	Cylindrospermopsin	
Slow sand filtration	++/+			
Nanofiltration	+++	(0/++)	++/+++	
Adsorption on activated carbon	++/+++	(+++)	+++	Adsorption varies by carbon type and type of cyanotoxin; competition with NOM
Ozonation (post clarification)	+++	+++	+++	
Free chlorine (post filtration)	+			
Potassium permanganate	++	++	0	Effective on soluble toxin, but only in absence of whole cells

4.3 Polymer-enhanced ultrafiltration (PEUF)

Polymer enhanced ultrafiltration (PEUF) is a promising, effective and sustainable alternative for the removal of cyanobacteria and cyanotoxins, where the flocculation process helps to remove/immobilise membrane foulants prior to filtration. In comparison to other membrane technologies, PEUF has the capability of separating low molecular weight compounds combined with a high permeability for solvent transport at relatively low transmembrane pressures, which is desirable from an energy and capital cost perspective (Huang and Feng, 2019). At this point, the determination of proper coagulant dosage is an important factor controlling membrane fouling in membrane process of algal rich water as highlighted by (Park et al., 2019) that tested the combination of PACl-coagulation and MF. In the combined coagulation-membrane filtration of *Chlorella vulgaris*, a dosage of 50-500 mg/L PACl (Al_2O_3) increased the filtration flux of a 0.9 μm MF membrane by 3–7 times relative to suspension without coagulant (Lee et al., 2012b).

The PEUF using moringa seeds as a natural coagulant provided 20-91% removal of Microcystis sp. cells in the coagulation/flocculation process depending on the coagulant concentration and feed water

turbidity (Nishi et al. 2012). After filtration step using 50 kDa polyether sulfone UF membrane, microcystis sp. cells were not detected in the permeate.
There are many studies that focused on the removal of various cyanobacteria species and/or cyanotoxins via coagulation using chitosan (Pei et al., 2014; Xu et al., 2013) as well as other coagulants (Sun et al., 2013). In the study of (Jin et al., 2017), chitosan quaternary ammonium salt (HTTC) was used to coagulate *Microcystis aeruginosa* cells in different coagulation conditions including HTTC dose, mixing speed and time. The authors found complete removal of algal cells at the HTCC dosage of 1.5 mg/L, rapid mixing for 0.5 min at 5.04 g and slow mixing for 30 min at 0.20 g. On the contrary, chitosan was found to be effective on the removal of *Microcystis* spp. but not *Cylindrospermopsis* spp. (Miranda et al., 2017). This finding points out the importance of algal species and the selection of relevant coagulant. Another critical point in chitosan-aided coagulation processes is that, elevated pH and high alkalinity have been reported to hamper cyanobacteria removal efficiency (Lürling et al., 2017). Moreover, Al species distribution affects flocs characteristics, which is important for the membrane flux decline. Zhang et al. (2017) found that the larger and stronger (high resistance to breakage) flocs formed by the Al_{13}-coagulant showed lowest flux decline. The fouling process and concomitant flux decline in the PEUF hybrid process was mainly determined by the floc size and the content of the residual soluble microbial products (high MW organics). Liang et al. (2008) emphasised the importance of an initial sedimentation step prior to the membrane filtration step to maintain a high flux and produce high water quality when treating algae-rich waters based on their experience with a pilot-plant PEUF using an aluminium-based coagulant and a 100 kDa polyacrylonitrile UF membrane.

The large bulk of research has focused on the understanding and alleviating membrane fouling caused by algal organic matter during MF/UF processes (Lee et al., 2012a: Zhang et al., 2014; Discart et al., 2015; Xu et al., 2018; Du et al. 2020), which is outside of the scope of this deliverable and thus is not reviewed here.

In the literature, some examples exist regarding the hybrid treatment systems with coagulation/flocculation using various coagulants and UF units to remove cyanobacteria and/or cyanotoxins. For instance, PAC-aided coagulation and UF was investigated (Dixon et al., 2011) with 90% of intracellular saxitoxin and 92% intracellular microcystinremoval from two naturally occurring blooms. The authors highlighted the importance of PAC-coagulation on the cell removal and improving the flux of the UF. Similarly in another study (Şengül et al., 2018), up to 94% of microcystin was effectively removed when the coagulation/flocculation and PAC systems were combined with UF membranes, while coagulation/flocculation alone could not lead to an appreciable removal of microcystin. Eventually, the adaptation and optimization of such hybrid systems are crucial since their performance is governed by multiple mechanisms. Furthermore, a multi-barrier approach is therefore highly applicable to minimize the effects of cyanotoxins in water bodies that includes the minimization of the release of toxins within the DWTP, optimizing process operations and developing monitoring system to facilitate effective treatment and provide an early warning (He et al., 2016; Recknagel et al., 2017).

5 Economic assessment

Membrane processes are increasingly being considered as an alternative to conventional water treatment methods in anticipation of future demands for high standards and reduced environmental impact. In the last years, membranes become more feasible in water treatment due to their high potential in solids separation, and relatively low operational cost. A pilot test was conducted (Lermontov et al., 2014) to make a comparative cost assessment of conventional water treatment (with coagulation, flocculation, settling, filtration steps followed by chlorine for disinfection) and UF unit. An economical evaluation was made considering the following factors: use of chemical products, plant operator's expenditure, amount of sludge produced, and the energy consumed. The data was used to create larger case scenarios, 20 and 100 L/s of water intake, as given in **Table 10**.

Table 10. *Overall financial comparison of UF with conventional treatment (Lermontov et al., 2014).*

	20 L/s		100 L/s	
OPEX	**UF (US$/y)**	**Conventional (US$/y)**	**UF (US$/y)**	**Conventional (US$/y)**
Energy	33,102.96	40,669.35	165,323.44	203,111.65
Sludge	2,514.96	15,341.29	12,560.29	76,617.75
Chemical products	3,452.23	17,734.88	17,804.32	88,651.23
Operator	-	54,466.20	-	54,466.20
OPEX	39,070.15	128,211.71	195,688.04	422,846.83
CAPEX	488,153.32	204,248.25	1,062,090.90	453,885.00
Net present value	-794,585.95	-928,673.02	-2,596,899.44	-2,843,063.89

The initial investment in ultrafiltration is higher compared to conventional plants. However, it also brings a thorough consistency in the water quality, reduction of failures in the system and drop of occasional non-conformities with the law, which can lead to fines. Both UF scale plants show indeed a very interesting solution. Even though it comprises of a larger initial investment, its net present value shows a lower cost than the conventional option. When considering the land required and its cost for each solution, the UF solution indicates an equivalent or even more financially feasible option, once the UF-DWTP occupies eight times less land than a conventional DWTP. However, the cost of membrane exchange or personnel, along the operational period were not considered. In another study, life cycle assessments were conducted of a UF system and a conventional water treatment system. The results showed comparable impacts for the two treatment systems; high impacts of energy (80%); minor impacts of construction (<15%); negligible impacts of membranes, chemical transport, decommissioning (<1%) (Friedrich, 2002). Studies have also been done on the techno-economic feasibility of PEUF based integrated processes for the treatment of heavy metal polluted effluents (Llanos et al., 2011), which PEUF is shown to be advantageous. Currently, two major approaches are used: 1) addition of strong ligands or acids to elute the bonded target contaminants from the water-soluble polymers and 2) electrolysis of the retentate from PEUF to result in electrodeposition of target contaminants while retaining the water-soluble polymers in the stream (Huang and Feng, 2019). Further progresses in the development of efficient techniques to recover the polymer agents are required to make PEUF process technically and economically competitive with the existing conventional treatment configurations.

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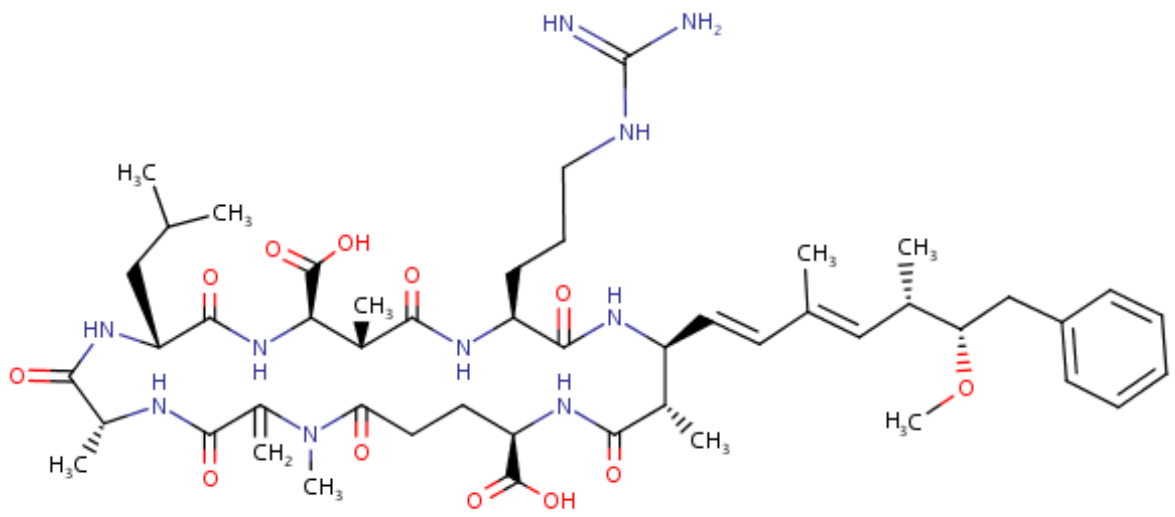
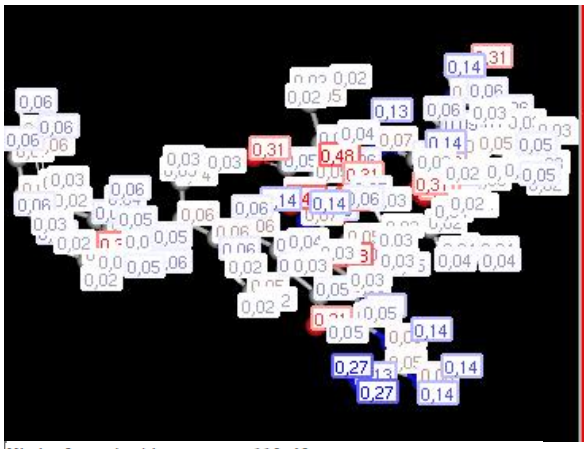
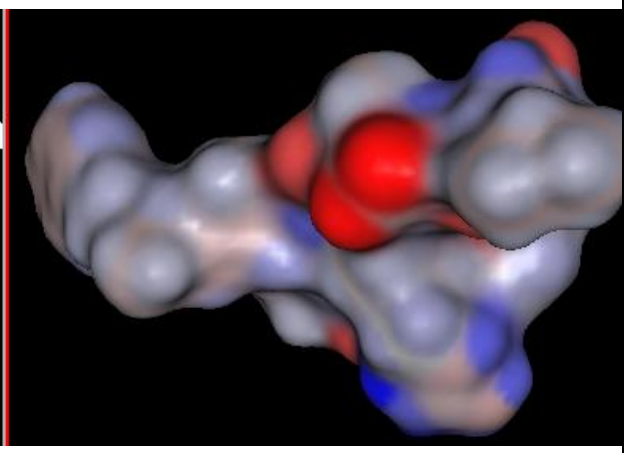
Appendix A. Structures of selected cyanotoxins

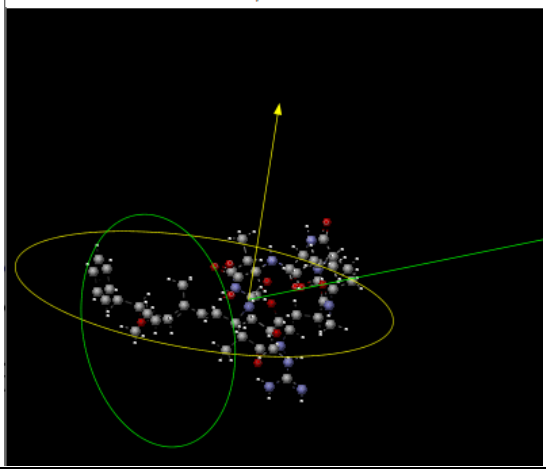
Microcystin-LR



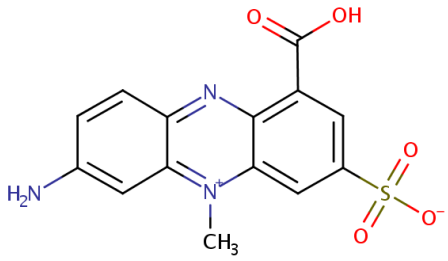


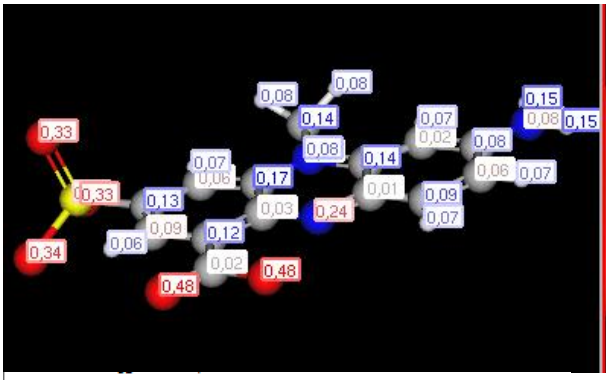


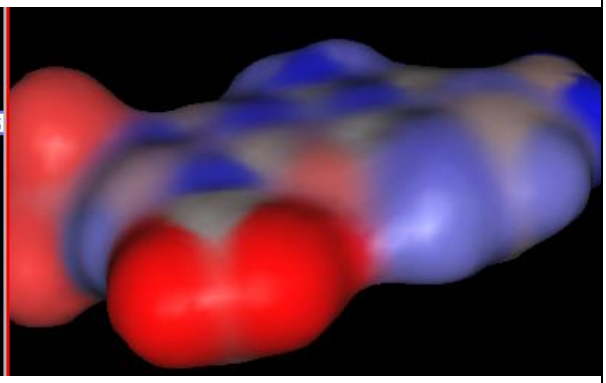
Minimal projection area = 119,42
Maximal projection area = 939,83
Minimal projection radius = 8,04
Maximal projection radius = 12,77
Length perpendicular to the max area = 13,51
Length perpendicular to the min area = 25,82
van der Waals volume = 936,93



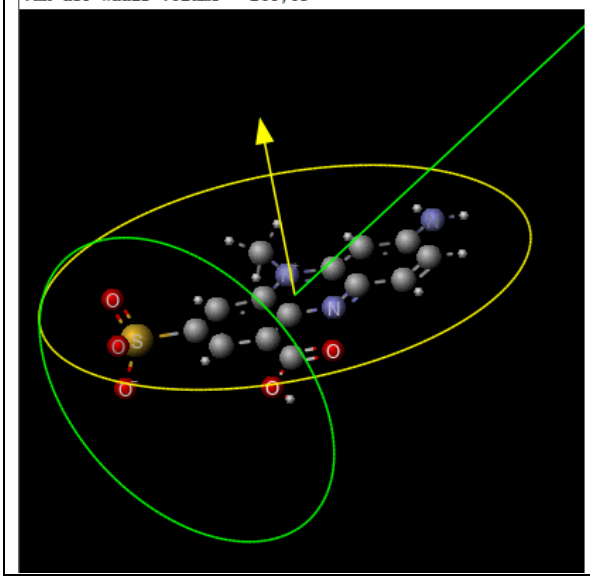
Aeruginosin B



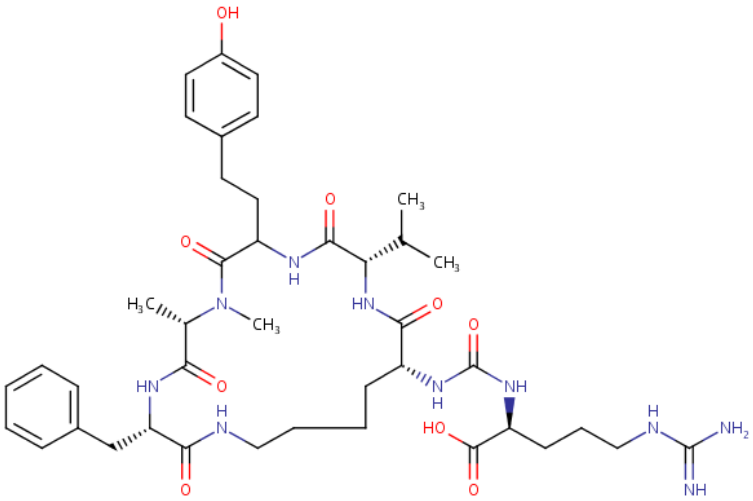


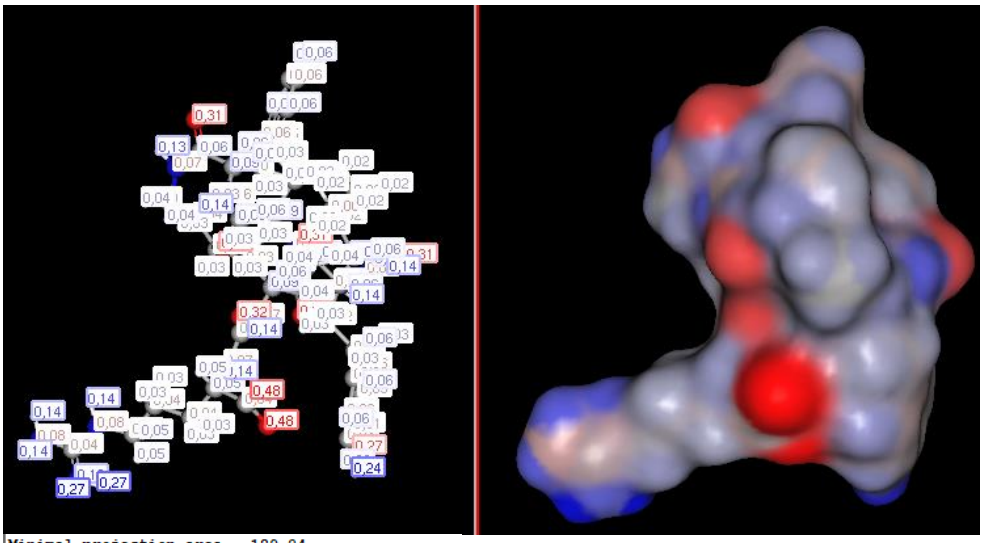


Minimal projection area = 38,75
Maximal projection area = 97,37
Minimal projection radius = 5,31
Maximal projection radius = 7,42
Length perpendicular to the max area = 5,40
Length perpendicular to the min area = 13,94
van der Waals volume = 260,65

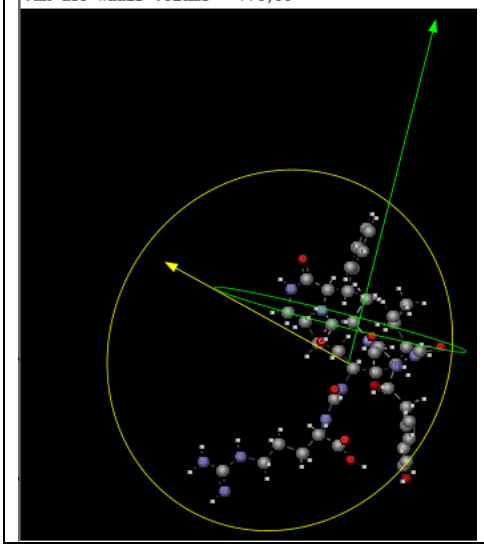


Anabaenopeptin B

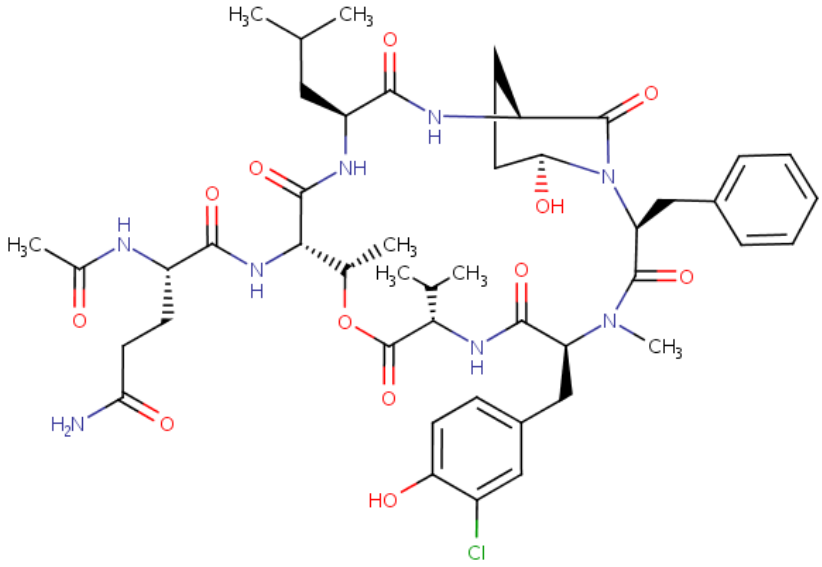


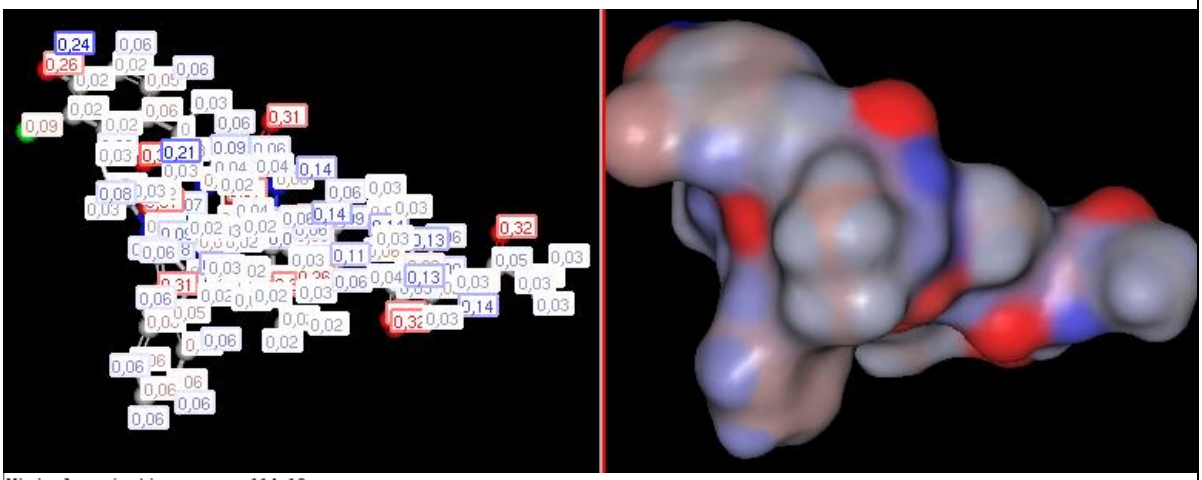


Minimal projection area = 109,24
Maximal projection area = 172,81
Minimal projection radius = 7,72
Maximal projection radius = 10,92
Length perpendicular to the max area = 12,50
Length perpendicular to the min area = 21,12
van der Waals volume = 776,99



Cyanopeptolin 954





Minimal projection area = 114,18
Maximal projection area = 199,12
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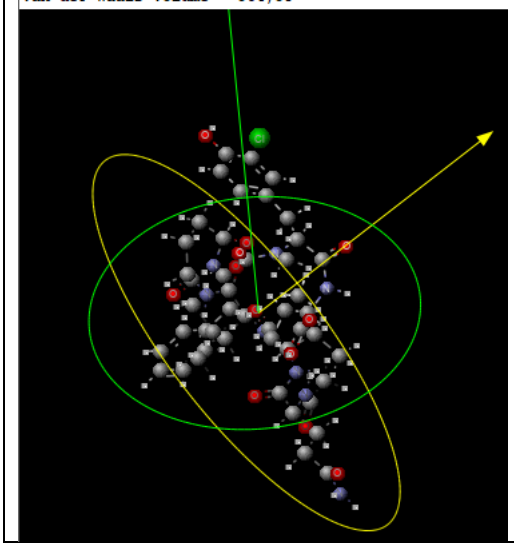
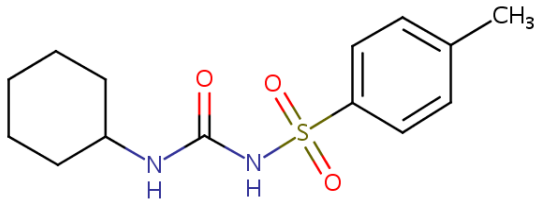
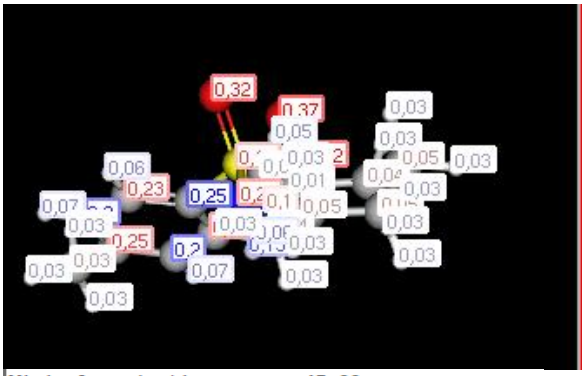
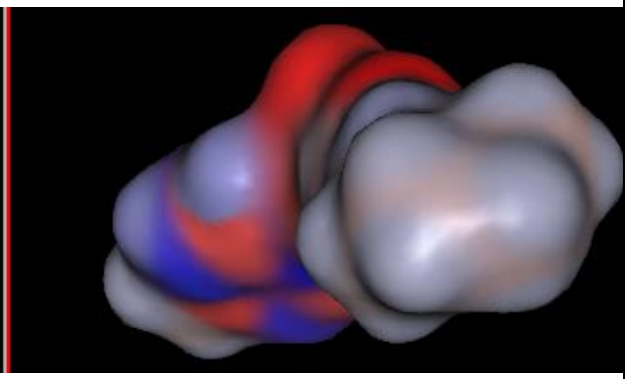
Maximal projection radius = 10,70
Length perpendicular to the max area = 13,81
Length perpendicular to the min area = 18,95
van der Waals volume = 864,80



Glycyclamide







```
Minimal projection area = 47,88
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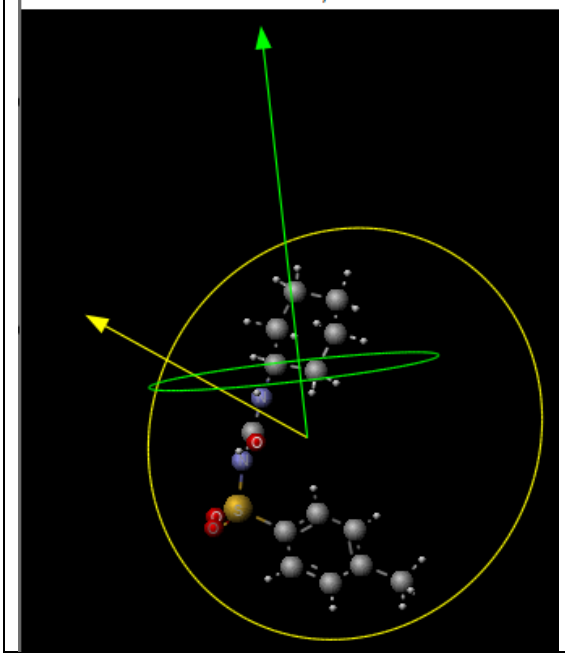
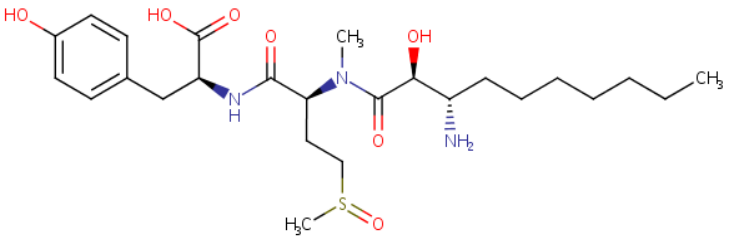
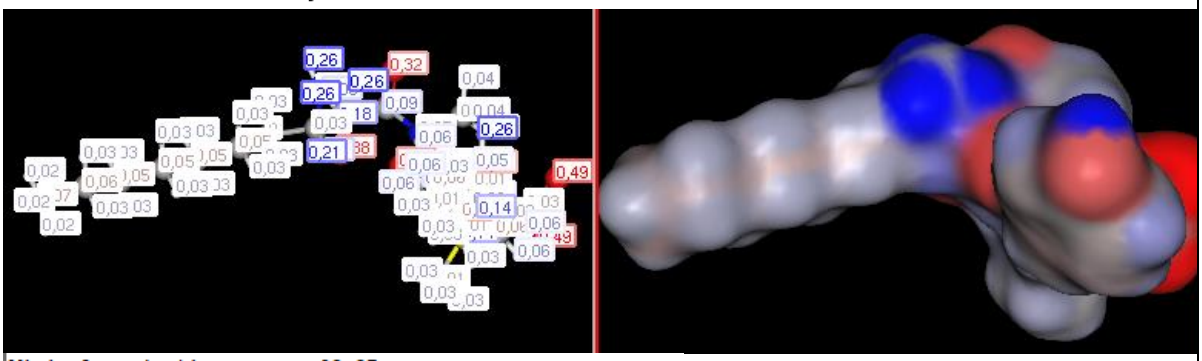
Maximal projection radius = 7,16
Length perpendicular to the max area = 8,66
Length perpendicular to the min area = 14,15
van der Waals volume = 267,75
```



Microginin 527





```
Minimal projection area = 93,37
Maximal projection area = 143,42
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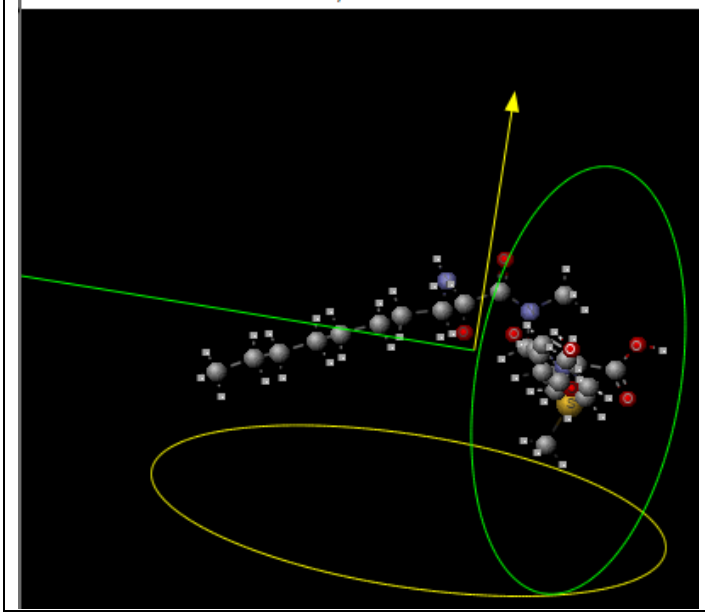
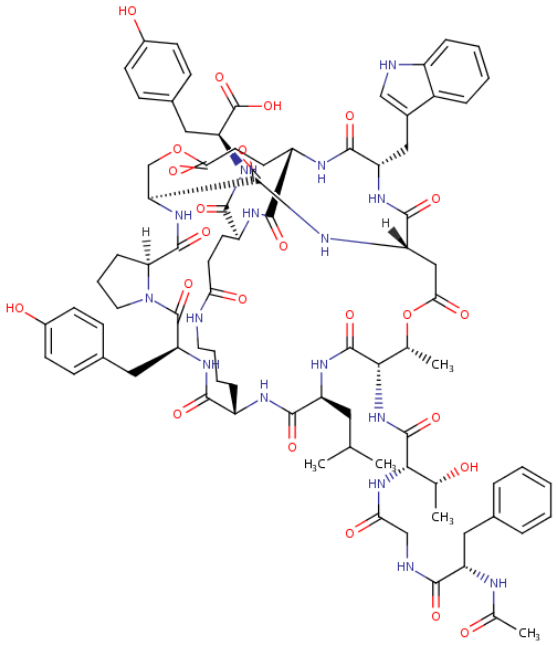
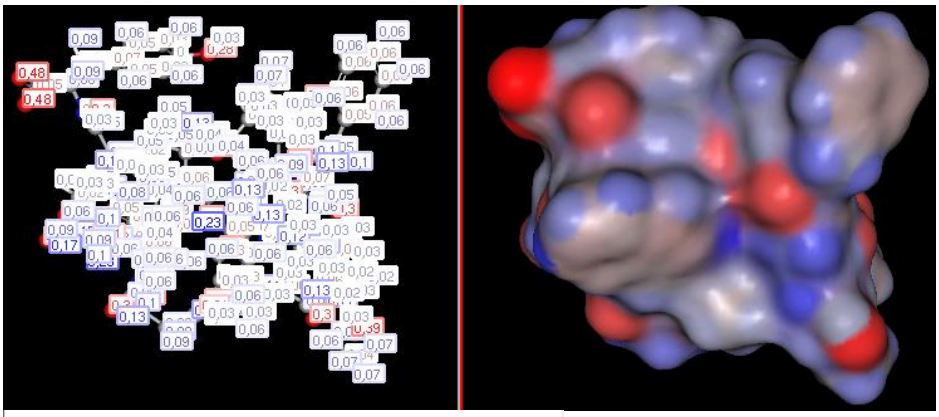
Length perpendicular to the max area = 10,04
Length perpendicular to the min area = 18,89
van der Waals volume = 501,86
```



Microviridin B





Minimal projection area = 224,63
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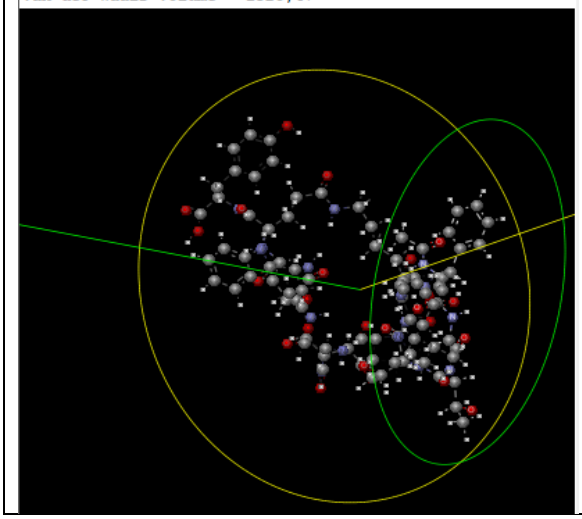
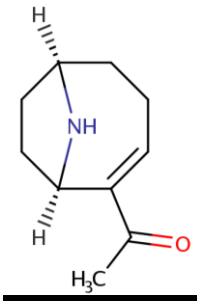
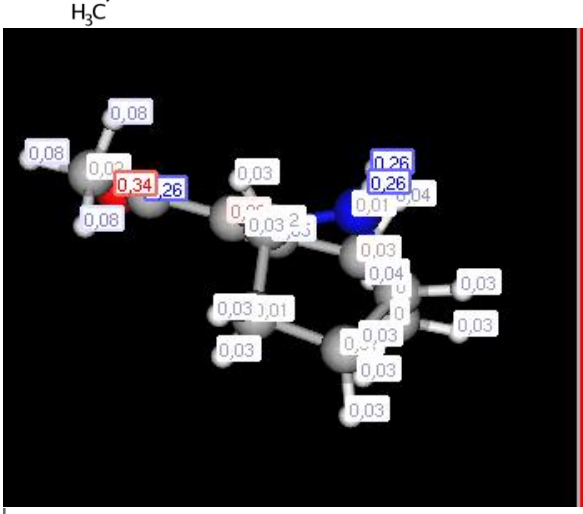
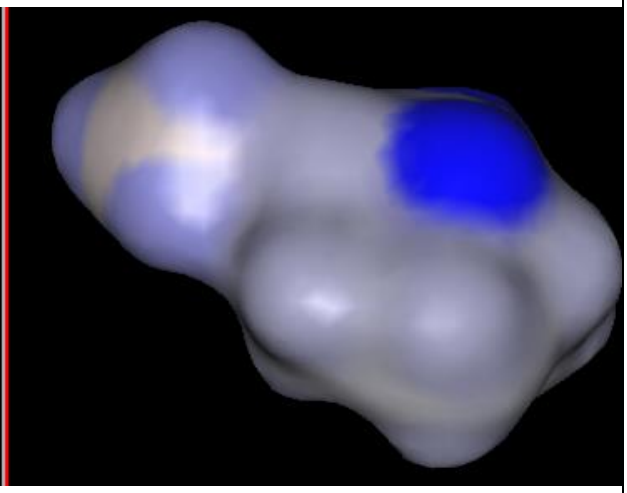
Maximal projection radius = 13,35
Length perpendicular to the max area = 20,11
Length perpendicular to the min area = 23,95
van der Waals volume = 1528,47



Anatoxin-a







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Maximal projection radius = 4,95
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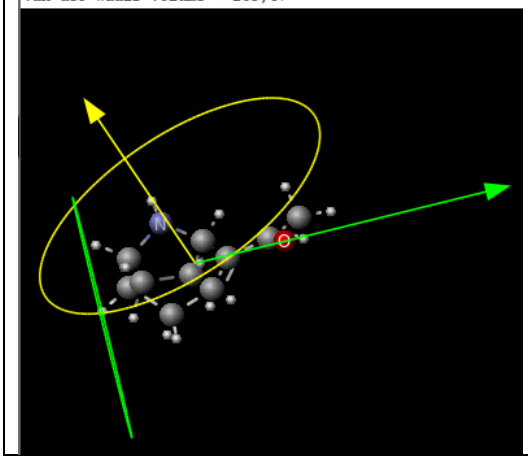
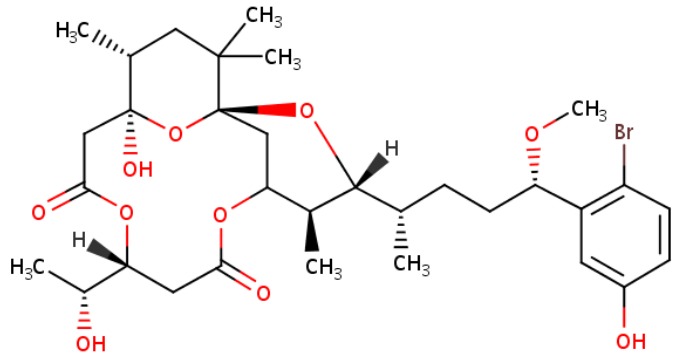
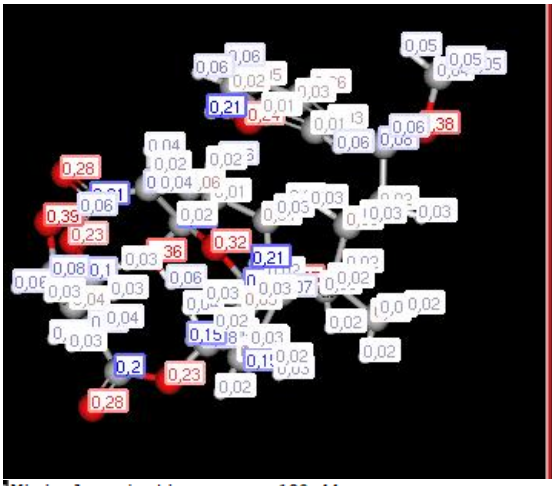
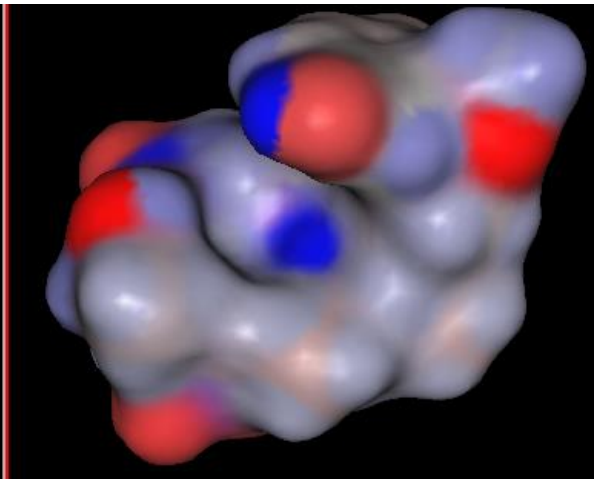
Length perpendicular to the min area = 9,92
van der Waals volume = 165,47



Aplysiatoxin







```
Minimal projection area = 102,44
Maximal projection area = 126,54
Minimal projection radius = 6,52
Maximal projection radius = 7,56
Length perpendicular to the max area = 12,30
Length perpendicular to the min area = 15,24
van der Waals volume = 587,36
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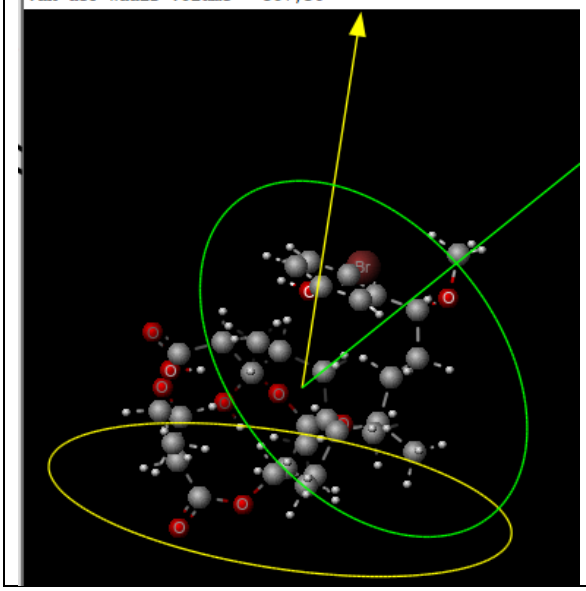
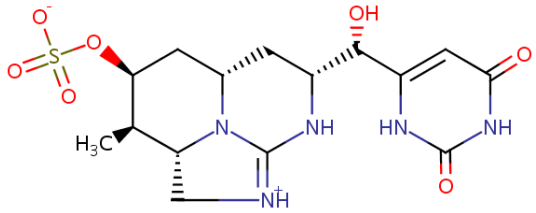
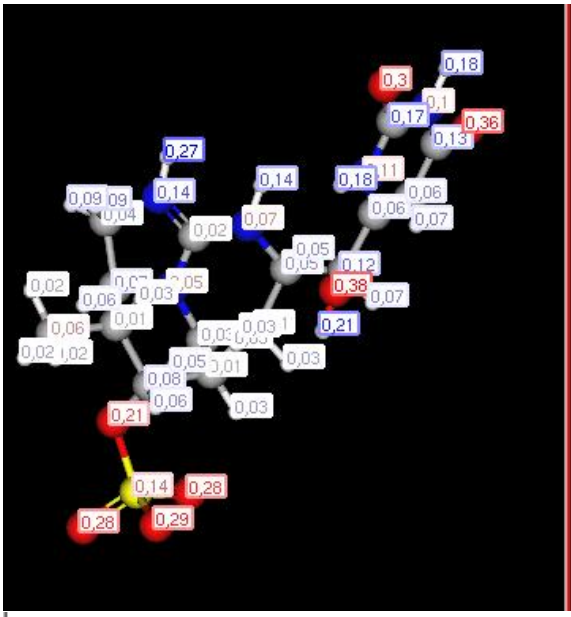
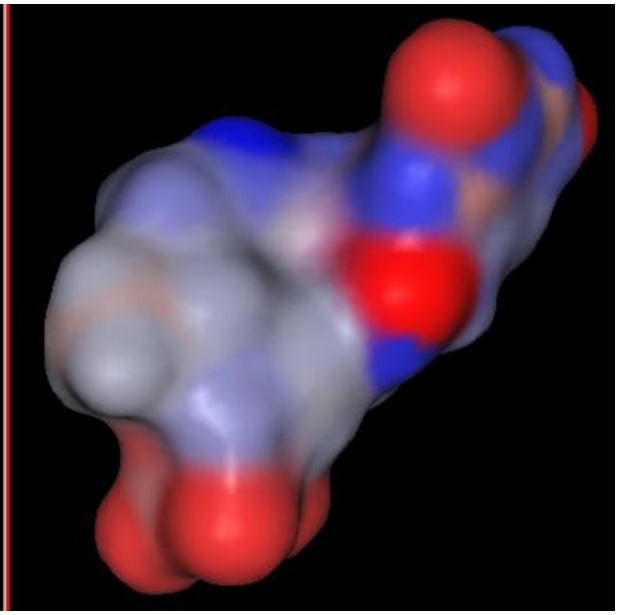


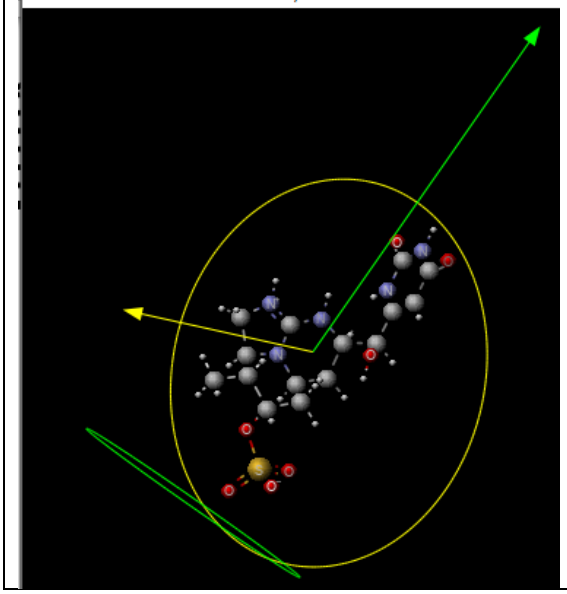
Cylindrospermopsin



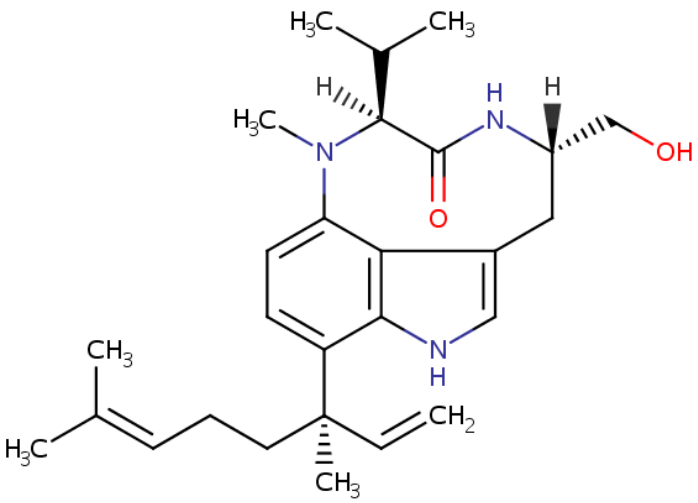


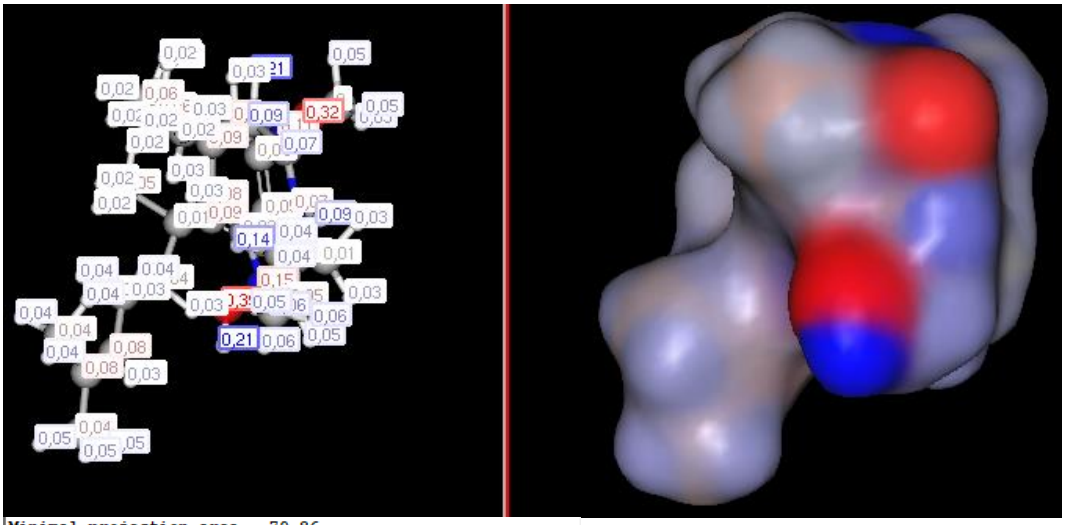


```
Minimal projection area = 53,15
Maximal projection area = 99,87
Minimal projection radius = 5,30
Maximal projection radius = 7,96
Length perpendicular to the max area = 7,93
Length perpendicular to the min area = 16,17
van der Waals volume = 332,34
```

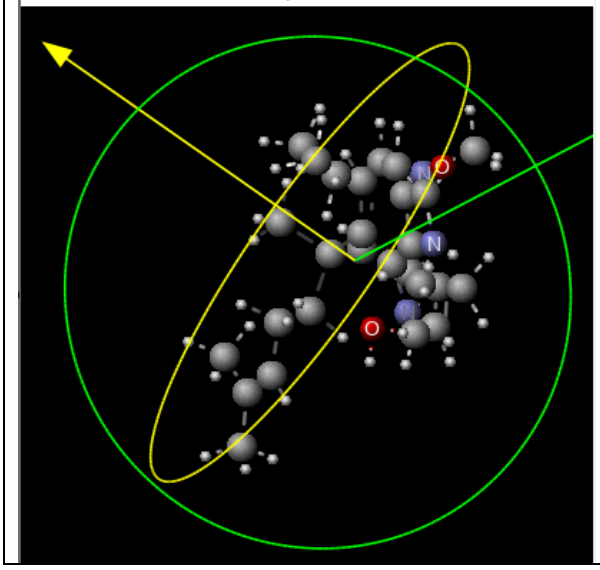


Lyngbyatoxin-a

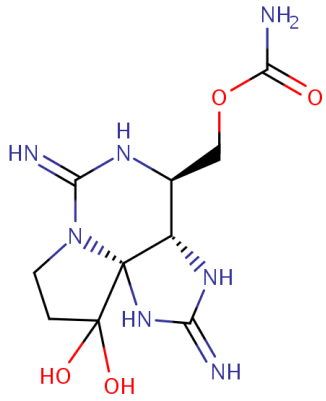


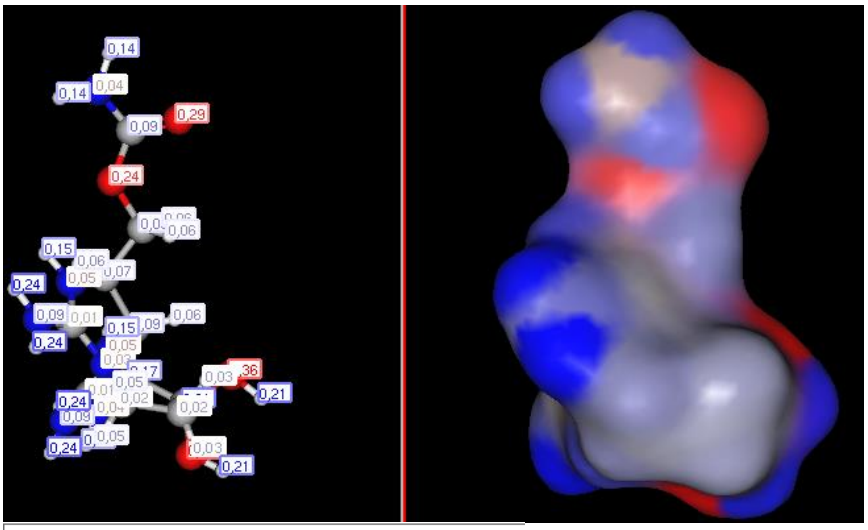


```
Minimal projection area = 79,86
Maximal projection area = 105,92
Minimal projection radius = 6,75
Maximal projection radius = 6,93
Length perpendicular to the max area = 10,04
Length perpendicular to the min area = 13,25
van der Waals volume = 441,99
```

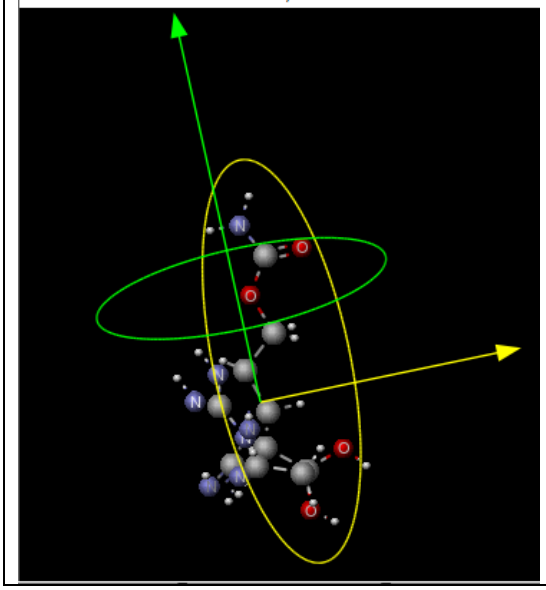


Saxitoxin

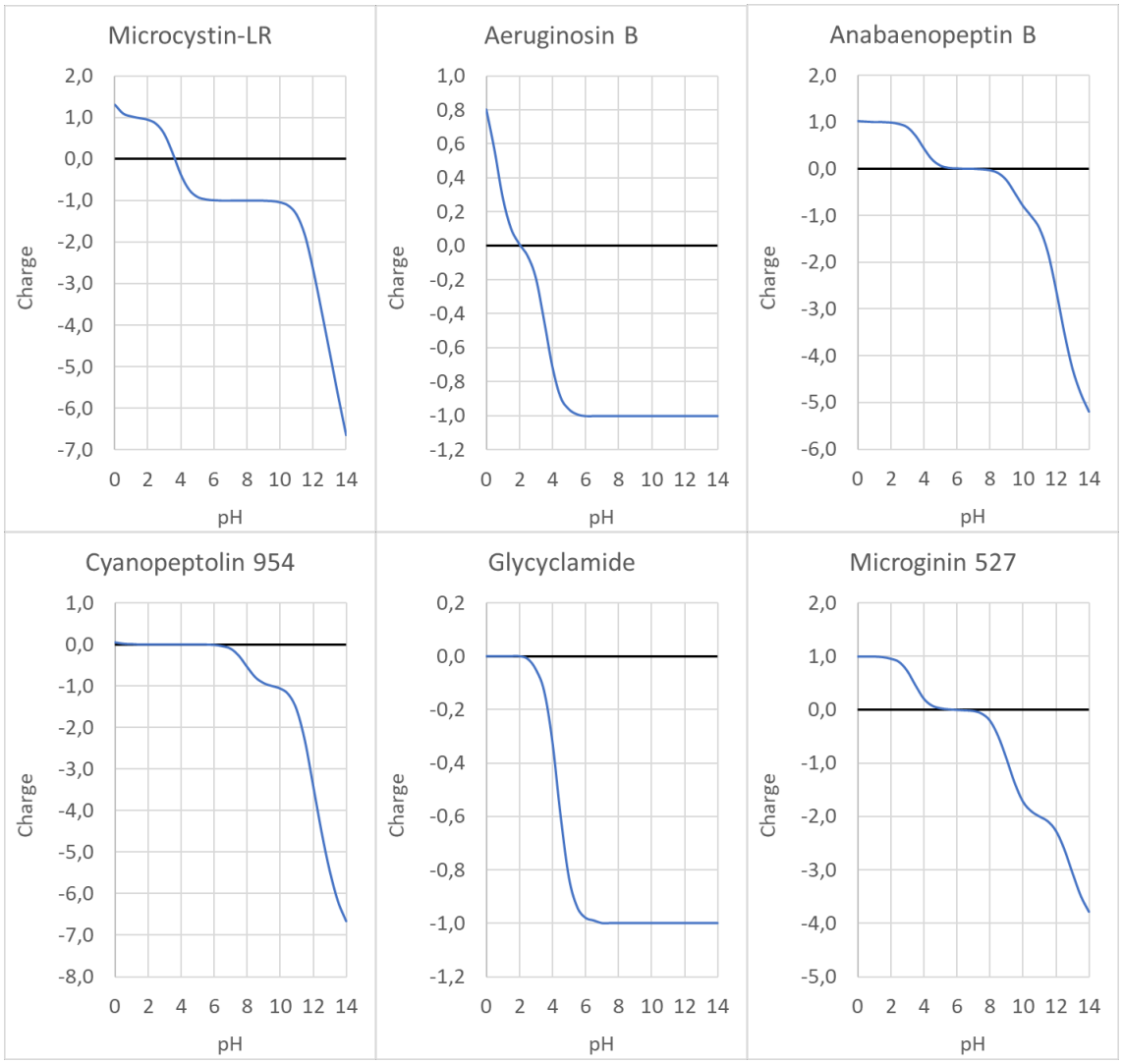


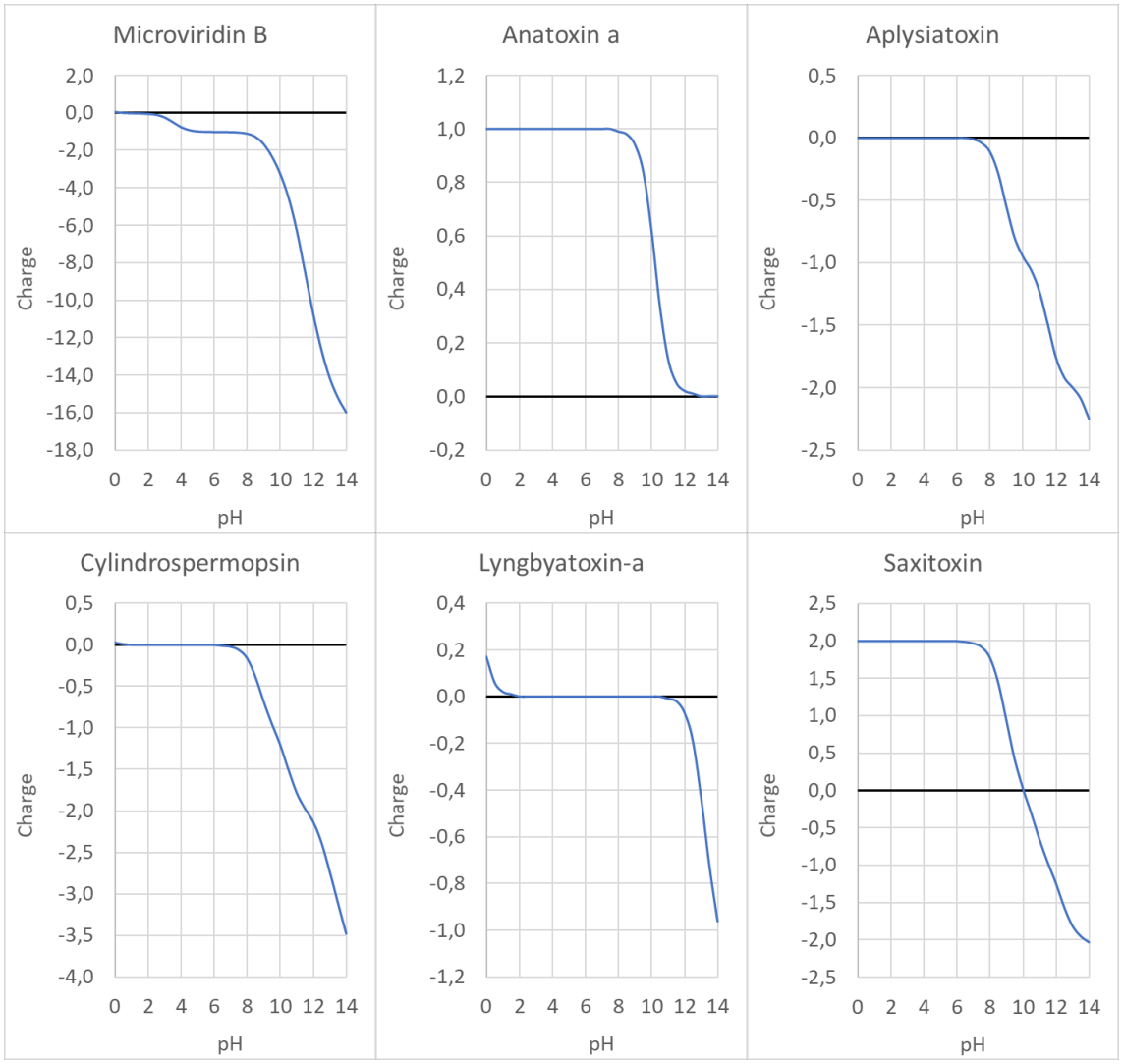


```
Dreiding energy = 51,73 kcal/mol
MMFF94 energy = -221,02 kcal/mol
Minimal projection area = 48,03
Maximal projection area = 69,03
Minimal projection radius = 4,76
Maximal projection radius = 6,62
Length perpendicular to the max area = 8,58
Length perpendicular to the min area = 12,86
van der Waals volume = 242,67
```



Appendix B. Calculated charge of selected cyanotoxins in the pH region 0-14.




Microviridin B
Charge
2,0
0,0
-2,0
-4,0
-6,0
-8,0
-10,0
-12,0
-14,0
-16,0
-18,0
0 2 4 6 8 10 12 14
pH
Anatoxin a
Charge
1,2
1,0
0,8
0,6
0,4
0,2
0,0
-0,2
0 2 4 6 8 10 12 14
pH
Aplysiatoxin
Charge
0,5
0,0
-0,5
-1,0
-1,5
-2,0
-2,5
0 2 4 6 8 10 12 14
pH
Cylindrospermopsin
Charge
0,5
0,0
-0,5
-1,0
-1,5
-2,0
-2,5
-3,0
-3,5
-4,0
0 2 4 6 8 10 12 14
pH
Lyngbyatoxin-a
Charge
0,4
0,2
0,0
-0,2
-0,4
-0,6
-0,8
-1,0
-1,2
0 2 4 6 8 10 12 14
pH
Saxitoxin
Charge
2,5
2,0
1,5
1,0
0,5
0,0
-0,5
-1,0
-1,5
-2,0
-2,5
0 2 4 6 8 10 12 14
pH

Appendix C. Research gaps related to the removal of cyanobacteria and cyanotoxins by membranes

Ladner (2009)

- Study **effects of shear on algae**. The full-scale treatment train should be evaluated to determine the magnitude of shear in the intake systems, pumps, valves, and membrane modules. These studies will help to determine how much cell breakup—and thus release of highly-fouling organic matter—is occurring at full scale. During such tests, fluorescence measurements can be used as an effective indicator of shear.
- Because algae rapidly foul low-pressure membranes, alternative removal strategies should be sought. **Coagulation before filtration** may be an effective strategy since large flocs should have lower fouling potential. Dissolved air floatation is another possibility, since cells do not settle well. Whatever the case, an algal-removal strategy should be designed to minimize shear and cell breakup.

Merel et al. (2013)

- no treatment process has been proven to simultaneously remove or transform all the cyanotoxins. Consequently, the elimination of cyanotoxins during drinking water treatment must be based on a **multibarrier** approach. Therefore, future studies should focus on the **removal of a mixture of toxins (including less common** ones such as **ANTX-a(s) or BMAA** for which no data are currently available) **through several treatment processes in series**.

Discart et al. (2015)

- Coagulant assisted filtration results suggest that the optimum coagulant dosing is also a function of the membranes, which suggests that the **coagulant and dosing optimization could be customized for every membrane**. A more detailed study is required to unravel this finding, which possibly is related to membrane charges or polarity.
- The clear difference in the behavior of the two applied coagulation/flocculation agents in this study suggests that a unique **interaction behavior** is expected for other **coagulation/flocculation agents**, which opens possibilities to investigate them in the future.

Liao et al. (2018)

- Further research should be conducted on **membrane configurations** (types of membrane geometry (hollow fiber vs. flat sheet), membrane module design) to improve the efficiency and simplicity of fouling control.
- EPS and SMPs are controlling factors of membrane fouling in ARMP; therefore, in-depth investigation of **EPS and SMPs properties** (quantity, composition, hydrophobicity, surface charge) and their **interactions** with the process and environmental conditions and microalgal species, is necessary. This helps to understand the role of EPS and SMPs from different microalgal species in membrane fouling and its control.
- Developing **new membranes** with a low binding affinity toward hydrophobic macromolecules using different hydrophilic antifouling co-polymers or surface grafting is required for efficient

microalgal culture filtration. Very few systematic studies have been accomplished in optimizing the properties of the hydrophilic membranes for the microalgal biorefinery.

- **Optimal** MPBR, AnMBR and MBR configurations in terms of the **shear stress and filtration mode** are indeed required because each membrane configuration may have different hydrodynamic regimes where shear and filtration mode could be problematic.
- Developing effective fouling characterization techniques and reliable membrane autopsy tools are particularly important for achieving a **better understanding of fouling** in algal-related membranes.
- As microalgal biofilm has much higher density and larger sizes, compared with suspended microalgal flocs, an integration of the advantages of microalgal biofilm and membrane separation will lead to the development of **advanced membrane microalgal biofilm reactor**, a new type of bioreactor for ARMP.

Xu et al. (2018)

- Interaction of algal organic matter [extracellular organic matter (EOM) (derived from algae metabolites in its growth process) and intracellular organic matter (IOM) (released into water once algae is dead and damaged)] during membrane filtration.

Hiskia et al. (2020) Water Treatment for Purification from Cyanobacteria and Cyanotoxins

- **UF experiments** run for a **longer timeframe** (than 3 hr by Chow et al. [2]) to allow any adsorption capacity of the membrane to become exhausted.
- **shear sensitivity and toxin release** still need to be tested with further cyanobacterial species and strains by small scale UF experiments as this is necessary to optimize the UF performance in direct surface water treatment.
- **Bench and pilot testing** results are important as the recovery and flux can be varied to the optimum values required to meet water quality goals, including concentrations of cyanobacterial metabolites.

Kong et al. (2020)

- Experiments with realistic environmental water matrices
- Occurrence and transport of AOM and toxins in membrane processes
- impacts of membrane permeability and selectivity on the transport of AOM and toxins in membrane separation,
- novel methods for management of the membrane concentrate in drinking water treatment
- integrated/composite membranes for algal separation
- emerging pollutants and the impacts on concentrate management

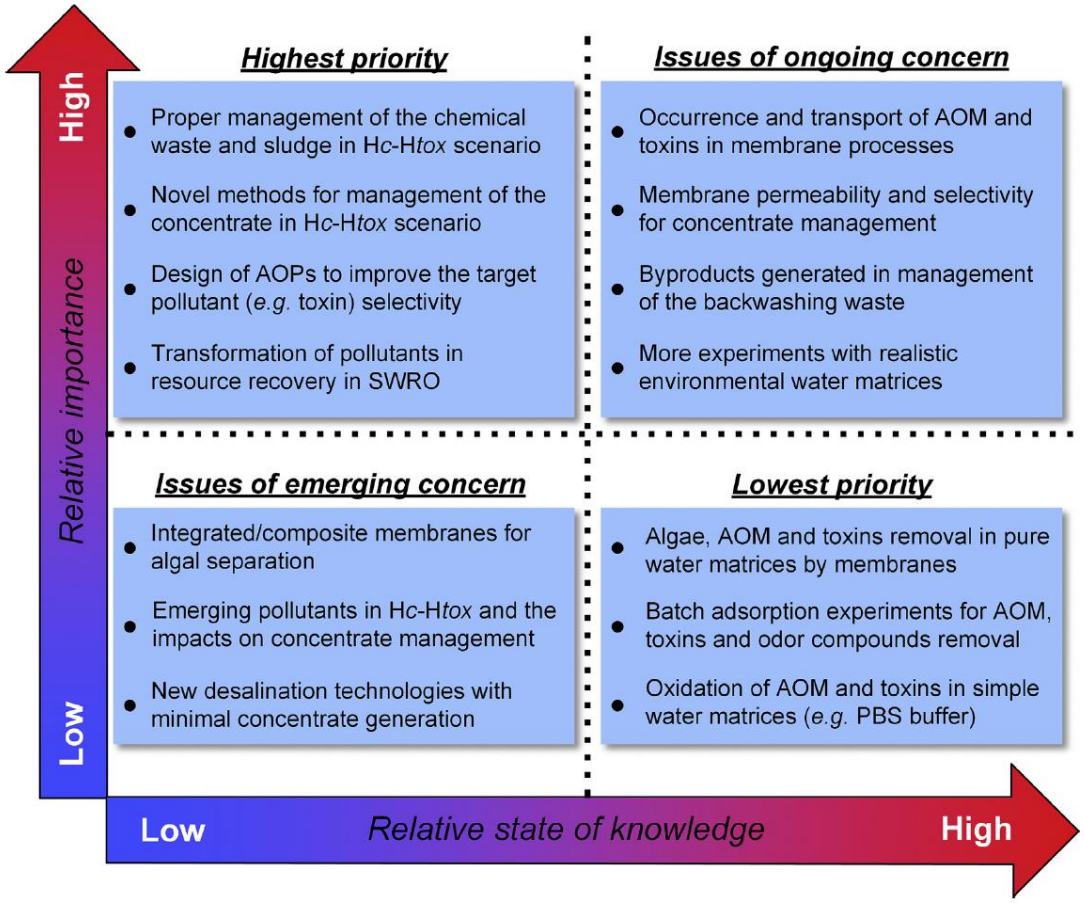


Fig. 5. Eisenhower framework of research perspectives in management of the concentrate and waste streams for membrane-based algal separation.

Research gaps on coagulation-oxidation-low pressure membrane filtration:

UV/Fe(II)/PMS: The **dose of PMS** should be carefully optimized to avoid excess, because excess PMS is highly oxidative and would cause environmental issues. To solve the problem of residual sulfate in the water, subsequent advanced treatment by high pressure membrane filtration, such as nanofiltration or reverse osmosis can be further utilized for algae-laden water treatment.

Fe(II)/PS(persulfate): Work was performed on a lab scale using only Microcystis aeruginosa and two types of oxidants. Hence, more extensive research is still warranted on **real applications of Fe(II)/persulfate-UF** for algae-laden water treatment.

Ferrate: I think that it's better to **focus on sedimentation or inline coagulation prior to membrane** treatment. **Combine ferrate with MnO2** by adding a small dose of KMnO4 in the supernatant of ferrate (i.e. prior to membrane system). The intermediate of permanganate, hydrous manganese dioxide (MnO2) could enhance the sedimentation process by adsorbing on algal cells to increase the specific gravity and settling velocity or improve membrane filterability by promoting the formation of a loose cake layer on the membrane. Alternatively, switch to Inline mode (without any sedimentation or DAF step) and optimize the dose of chemicals and the pH of the solution according to membrane fouling enhancement. Most studies on ferrate reported focused on ferrate's oxidation efficiency in surface- and waste-water treatment, and only few studies investigated the potential **disinfectant capabilities of ferrate**.

Regarding the removal and degradation of algae, their organic materials (AOM) and particularly toxins (ex:MC-LR) by **oxidation or coagulation-oxidation coupled with low pressure membranes**, the number of studies is very limited, and many combinations need to be investigated. Moreover, In the present review, we can find only two works on this combination for the **treatment of toxin**.
In the absence of guideline values for HAB toxins internationally, the best method for ensuring that toxins do not enter the drinking water distribution system is by employing the **multiple barrier** approach to **treatment** which means in general that further research is needed on that topic.

Bench Scale Testing of PEUF

Site and plant description

Lake Castreccioni is in the municipality of Cingoli in Central Marche Region, Italy. It was created in the 1980s when a dam was placed across the Musone River near Monte San Vicino, at about 70 km far from the coast. The biggest artificial lagoon in the Marche region, Lake Castreccioni covers about 2.4 km^2 and reaches depths of 55 feet. The dam (67 m high and 280 m long) is situated on a homogeneous calcareous-majolica formation belonging to the prevalently limestone unit of the Umbro-Marche sequence (Bartolelli et al. 2005). The volume of the lake at the maximum altitude is approximately 50 million cubic meters. Lake Castreccioni is characterized by relatively low nutrient values (oligo-mesotrophic lake), which is typical for most Italian lakes (Rogora et al. 2018).

The drinking water treatment plant (managed by Acquambiente Marche S.r.l.) is located in the district of Castreccioni and responsible for drinking water for the member municipalities: Cingoli, Filottrano, Numana and Sirolo. Furthermore, the treated water is also distributed to the Municipalities of Osimo and Castelfidardo through the Castreccioni pipeline. The plant supplies water for a total of about 65,000 inhabitants in the winter and about 95,000 in the summer, using an advanced ozone disinfection system. The maximum capacity of the plant is 500 l/sec divided on two equal lines of 250 l/sec. The flow of treated water is a function of the needs of the distribution network and accounted for both influent and effluent. The process steps include pre-disinfection with ozone (pre-ozonation), clariflocculation, sand filtration, disinfection, ozonation, activated carbon filtration, accumulation tanks and final disinfection with chlorine dioxide.

The water quality data of the intake of the Castreccioni DWTP was obtained from Acquambiente Marche S.r.l over six bloom seasons from January 2014 to November 2019. The climatic parameters for the monitoring period was obtained from digital data base of Cingoli Province, Marche Region.

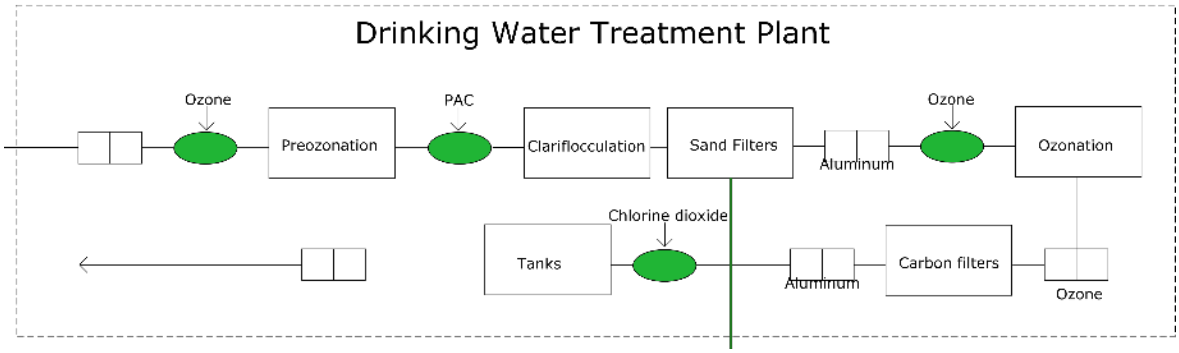


Fig XX. Flow scheme of the Castreccioni DWTP.

Sampling and characterization for the bench-scale tests

Raw water was taken from the water intake of the DWTP that supplies water from 314 m depth of the Lake Castreccioni. Based on the information gathered from the water utility, the current treatment configuration is highly effective to remove cyanobacteria and cyanotoxins in the final effluent. However, sand filter backwash water (SFBW) was reported to be rich in cyanobacteria and microcystin by the operators of the plant, which is usually being discharged into a river. From this point of view, SFWB was the main problematic flow in the treatment scheme in terms of cyanobacteria and cyanotoxin occurrence. Hence, SFWB was also sampled and characterized together with the raw lake water for further studies. Physical and chemical characterization of the water intake and SFBW are reported in **Table XX**. All analyses were done according to the Standard Methods (APHA 2012).

The sample taken from the water inlet of the plant for the experimental tests contained 0.099 ng/mL microcystin since the sampling was done in a non-bloom period. In the meantime, SFBW contained 12.32 ng microcystin/mL, which highlighted that the sand filter unit is the main accumulation point for cyanobacteria and cyanotoxins, in particular *P. rubescens* and microcystin. On the other hand, the microcystin concentration in the effluent of the sand filter was measured as 0.115 ng/mL, which indicated that there was no re-release of microcystin in the effluent. In fact, considering the following treatment units in the plant, such as ozonation, carbon filtration and chlorination,

microcystin concentration in the final effluent is always much lower than the limits. Consequently, we considered the SFBW as the most critical flow in the plant in terms of microcystin release into the environment, as well as in any other similar DWTPs, since these (waste)waters are discharged in water bodies without any treatment. At this point, further lab-scale PEUF experiments were conducted on the enriched *P. rubescens* culture and SFBW.

Table XX. Characteristics of the water intake and sand filter backwash water (SFBW) in the Castreccioni DWTP during the sampling for PEUF tests.

Parameter	Unit	Lake water	SFBW
pH	-	7.3	7.8
EC	µs/cm^2	367	485
Alkalinity	mg$CaCO_3$/L	184	185
DO	mg/L	7.6	7.3
Chemical oxygen demand	mg/L	73	124
Total suspended solids	mg/L	5	60
Total dissolved solids	mg/L	680	520
Total phosphorus	mg/L	<0.1	0.3
Nitrate	mg/L	2.89	<0.1
Sulphate	mg/L	57.8	55.4
Chloride	mg/L	18.4	20.7
Sodium	mg/L	15.6	16.5
Potassium	mg/L	2.4	2.6
Magnesium	mg/L	14.3	14.6
Calcium	mg/L	96.8	99.2
Iron	mg/L	0.044	0.169
Copper	mg/L	0.044	0.123
Manganese	mg/L	0.005	0.034
Aluminium	mg/L	0.044	0.202
Zinc	mg/L	0.113	0.558
Barium	mg/L	0.124	0.069
Lead	mg/L	<0.0086	<0.0086
Chromium	mg/L	<0.001	<0.001
Nickel	mg/L	<0.001	<0.001
Cadmium	mg/L	<0.0004	<0.0004
Microcystin	ng/mL	0.099	12.32

Algal isolation and cultivation

Isolation of *Planktothrix rubescens* filaments was made from a sample of raw water taken from the water intake of the drinking water treatment plant, following the capillary pipette method (Hoshaw and Rosowski 1973) under an inverted microscope (Zeiss Axiovert 135) equipped with phase

contrast, at 200x magnification. After an initial growth in microplates, cells were cultured at 21 ± 0.1 °C under a 12:12 h L:D photoperiod and an irradiance di 90-100 μmol $m^{-2}s^{-1}$, in BG11 medium. For the molecular identification, genomic DNA was extracted from the cells collected by centrifugation at 4,000×g for 20 min from 30 mL monoclonal culture of *P. rubescens* in logarithmic growth phase using the CTAB (N-cetyl-N,N,N-trimethylammoniumbromide) method (Richards et al. 2001). Extracted DNA was amplified by Polymerase Chain Reaction (PCR) technique, carried on with a SimpliAmpTM Thermal Cycler, as described in (Savichtcheva et al. 2011). PCR products were visualized and quantified in 1.5% agarose gels stained with GelRedTM (Biotium, Hayward, CA, USA) using Low DNA Mass Ladder (Invitrogen, Carlsbad, CA, USA) as reference, and visualized under UV light. PCR products with expected lengths and yields were purified and directly Sanger-sequenced by Macrogen Europe (Amsterdam, The Netherlands). Sequences were compared to the NCBI database by BLAST search with default settings (https://blast.ncbi.nlm.nih.gov/Blast.cgi).The cultures were harvested at the final cell yield up to 10^5 cells/mL and diluted to 10^4 cells/mL with the lake water of Castreccioni to simulate dense algae period according to the historical lake data. The lake water was filtered through 0.45 μm fiber membrane to remove any background algae prior to dilution.

Polymer-enhanced Ultrafiltration (PEUF)

Optimization tests

Chitosan powder from crab shells was obtained from Sigma–Aldrich Company Ltd. and used as the coagulant in the coagulation experiments. A chitosan solution of 1 mg/mL was prepared according to the protocol described elsewhere (Divakaran and Pillai 2002). The operating parameters (i.e. coagulant dose, rapid mixing velocity and time) for the PEUF experiments were initially optimized in 28 experimental systems (Table XX).

Briefly, chitosan was added to 500 mL of cyanobacterial culture (10^4 cells/mL) at the concentrations of 1, 2, 3, 4, 5, 10 and 20 mg/L, and different rapid mixing conditions were tested for each concentration as follows: 100 rpm at 3 min, 150 rpm at 2 min, 200 rpm at 1.5 min and 250

rpm at 1.2 min. Then, the suspensions were slowly mixed at the velocity of 40 rpm for 30 min and finally left for sedimentation for 30 min. Each test was run in duplicates.

Table XX. Optimization of chitosan dosage and contact time in preliminary coagulation/flocculation.

Test (in duplicates)	Chitosan dose (mg/L)	Rapid mixing		Slow mixing		Sedimentation
		Velocity (rpm)	Time (min)	Velocity (rpm)	Time (min)	Time (min)
1	1	100	3	40	30	30
2	1	150	2	40	30	30
3	1	200	1.5	40	30	30
4	1	250	1.2	40	30	30
5	2	100	3	40	30	30
6	2	150	2	40	30	30
7	2	200	1.5	40	30	30
8	2	250	1.2	40	30	30
9	3	100	3	40	30	30
10	3	150	2	40	30	30
11	3	200	1.5	40	30	30
12	3	250	1.2	40	30	30
13	4	100	3	40	30	30
14	4	150	2	40	30	30
15	4	200	1.5	40	30	30
16	4	250	1.2	40	30	30
17	5	100	3	40	30	30
18	5	150	2	40	30	30
19	5	200	1.5	40	30	30
20	5	250	1.2	40	30	30
21	10	100	3	40	30	30
22	10	150	2	40	30	30
23	10	200	1.5	40	30	30
24	10	250	1.2	40	30	30
25	20	100	3	40	30	30
26	20	150	2	40	30	30
27	20	200	1.5	40	30	30
28	20	250	1.2	40	30	30

The supernatants were carefully collected and subjected to UF (Merck Milipore Amicon^{TM} stirred cells - 0.013 μm) separately. The clarification efficiency (CE) of the supernatants after coagulation and UF were further determined via the measurement of the optical density at 750 nm (OD_{750}) with a ONDA UV-20 spectrophotometer. The CE was then calculated by CE = (1 – ODs/ODf) x 100% (Xu et al. 2013), where ODf is the OD of the feed sample, ODs is the OD of the supernatant after coagulation-flocculation-sedimentation and UF. Based on the clarification efficiencies (Fig. XX),

samples were selected (tests including 1, 2, 4 and 20 mg chitosan/L) for further microcystin analysis.

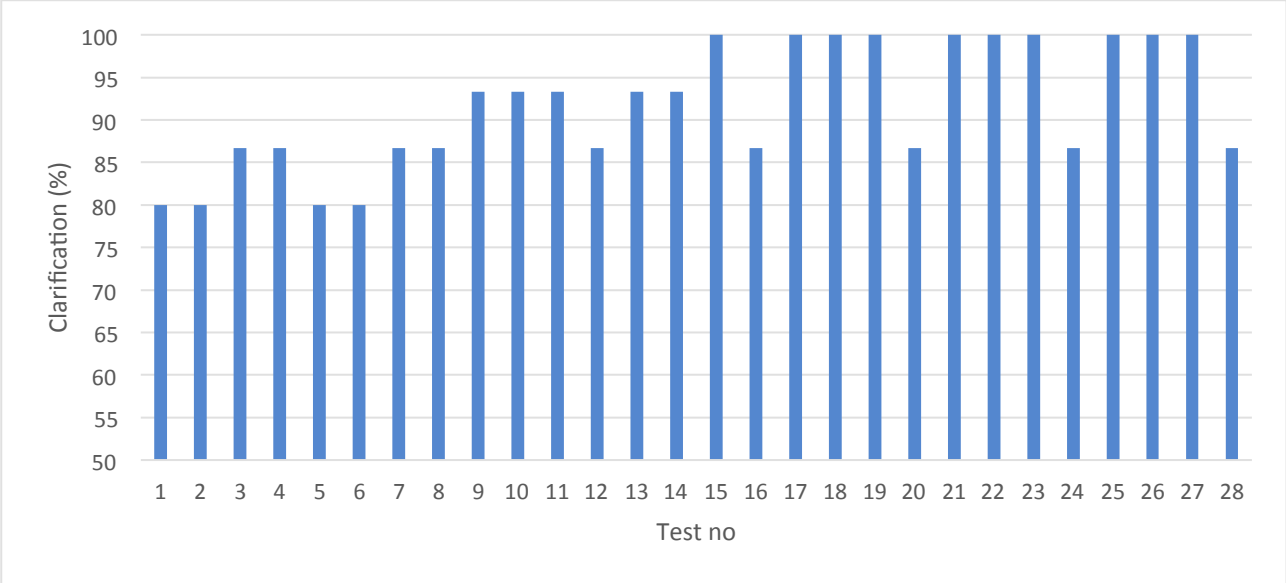


Fig. XX. Clarification efficiency in the preliminary batch tests.

Indirect Competitive ELISA Assay

Water samples of 10 mL were conditioned at room temperature, mixed by vortex for 60 s and subjected to ultra-sonication (180 W, 40 kHz DU-65, Argolab, Carpi, Italy) for half hour for cell lysing. The Enzyme-Linked-Immunosorbent-Assay (ELISA), was performed by the commercial Microcystins-ADDA-Enzyme-Linked Immunosorbent Assay (Abraxis, Product No. 520011, Lot.No. 19GO113) following the manufacturer's instructions. All reagents, such as, microtiter plate (12 x 8 strips) coated with an analogue of Microcystins conjugated to protein, ready-made Standards solutions at 0, 0.15, 0.40, 1.0, 2.0, 5.0 , ppb (µg/L), Control at 0.75 ± 0.185 ppb, Sample Diluent, Antibody solution, Anti-sheep-HRP conjugate solution, Wash buffer (5x) concentrate, substrate solution (color solution), and Stop solution, were provided by manufacturer. The absorbances were read at 450 nm in an Multiskan FC, Labsystems microtiter plate reader (Thermofisher). Concentrations of Microcystins in water samples were calculated by interpolation using the standard curve constructed by plotting the %B/B0 for each standard versus the

corresponding Microcystins concentration (0, 0.15, 0.40, 1.0, 2.0, 5.0 ppb). The detection limit (LOD) for this assay, based on MC-LR, was 0,10 ppb (µg/L). Coefficient of variations (CVs) for standards were always < 10%; for samples always < 15%. Recovery for control standard (QCS, 0.75± 0.185 ppb), were always between 75 and 125%. A final estimate for microcystins content was obtained and calculated as the mean of at least two subsamples (i.e., two replicates per subsample). Microcystin concentrations in the enriched cyanobacteria culture and selected preliminary PEUF tests (i.e. 1, 2, 4 and 20 mg/L chitosan-dose) are given in Fig. XX. The *P. rubescens* culture had an initial microcystin concentration of 1.236 ng/mL, and it decreased to 1.133, 0.923, 0.945 and 1.055 ng/mL after the coagulation/flocculation at 1, 2, 4 and 20 mg chitosan/L, respectively. After the UF, overall removal efficiency of the PEUF was in the range of 87-92% in all tests. Considering the clarification efficiency and microcystin removal, further PEUF tests were conducted in the following optimal conditions: the chitosan dose of 4 mg/L and the rapid mixing velocity at 200 rpm for 1.5 min.

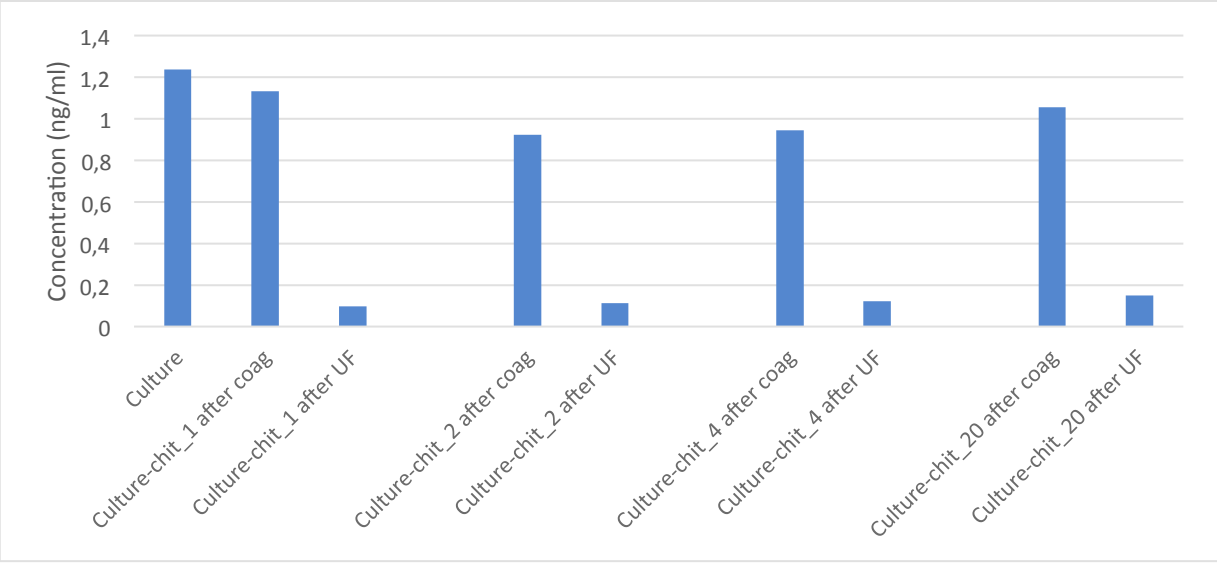


Fig. XX. Microcystin concentrations in the preliminary batch tests.

PEUF tests in batch operation

Further PEUF experiments were conducted at the pre-determined optimal conditions using two different flows: cyanobacterial culture (10^4 cells/mL) and SFBW. In addition to the chitosan dose of

4 mg/L, a high concentration of 20 mg/L were also tested to evaluate any possible enhanced removals of algae and microcystin. In parallel, the effectiveness of the PEUF was experimentally compared to that of conventionally used UF, where the *P. rubescens* culture and SFBW were directly filtered without the prior coagulation. After each test, the samples were collected for microcystin analysis. The samples after UF were also checked to ensure any microbiological contamination in the final effluents using membrane filtration method. All experiments were carried out in duplicate and mean values are presented.

Fig. XXA and XXB show the results obtained from pure culture (PC) of *P. rubescens* and SFWB with respect to microcystin concentration, and removal rates are given in **Fig. XX**C. PC had an initial microcystin concentration of 1.236 ng/mL and decreased by 54.3% to 0.562 after the coagulation-flocculation at 4 mg/L of chitosan. After the UF, the effluent has 0.122 ng/mL of microcystin, corresponding to an overall 90.1% removal. Differently, 20 mg/L chitosan contributed to 94.1% removal after the coagulation-flocculation with 0.073 ng/mL of microcystin. Following the UF, the microcystin concentration in the effluent were detected as 0.13 ng/mL (overall 89.5% removal). Meanwhile, direct UF of the PC (without prior coagulation step) resulted in 89.9% overall microcystin removal rates with the remaining concentration of 0.125 ng/mL. The initial concentration of microcystin in the SFBW was measured as 12.32 ng/mL, which is much greater than the PC. With the aid of 4 mg/L of chitosan, there was only 14.1% of microcystin removal, and the effluent after coagulation-flocculation has still 10.583 ng/mL of microcystin. On the other hand, further UF application ended up with overall 99.1% removal in the final effluent that corresponded to 0.106 ng/mL, respectively. There was a significant contribution of the addition of 20 mg/L of chitosan compared to 4 mg/L, where 80.2% reduction was achieved with remaining 2.44 ng/mL of microcystin. After the UF, there was 0.057 ng/mL microcystin in the final effluent, where the highest removal efficiencies were achieved as 99.5%. At the same time, the direct UF of SFBW resulted in 0.119 ng/mL of microcystin with 99% removal efficiency.

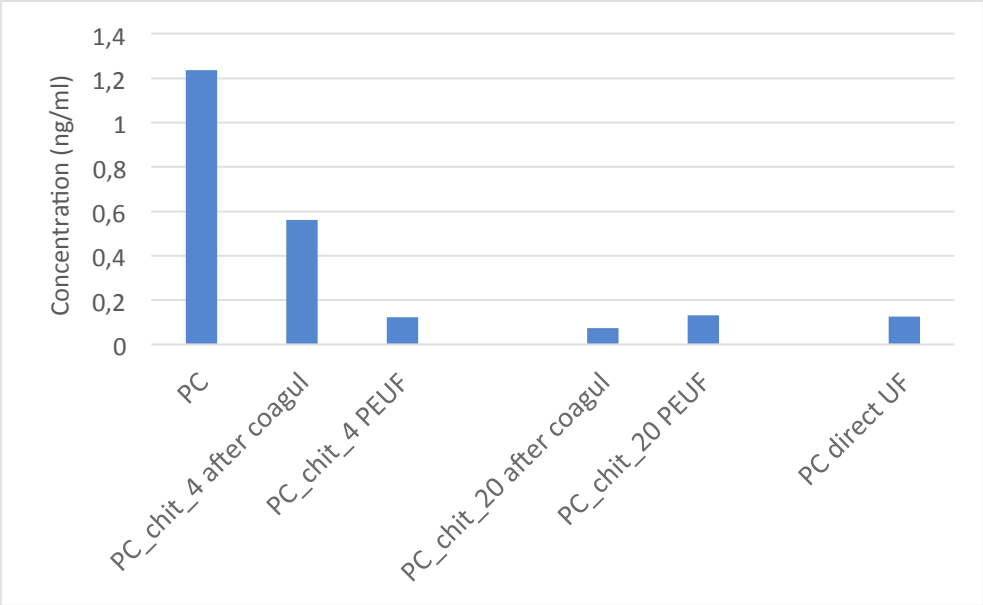


Fig. XXA. Microcystin concentrations in pure culture (PC) after chitosan-enhanced coagulation/flocculation and PEUF with the addition of 4 and 20 mg/L chitosan.

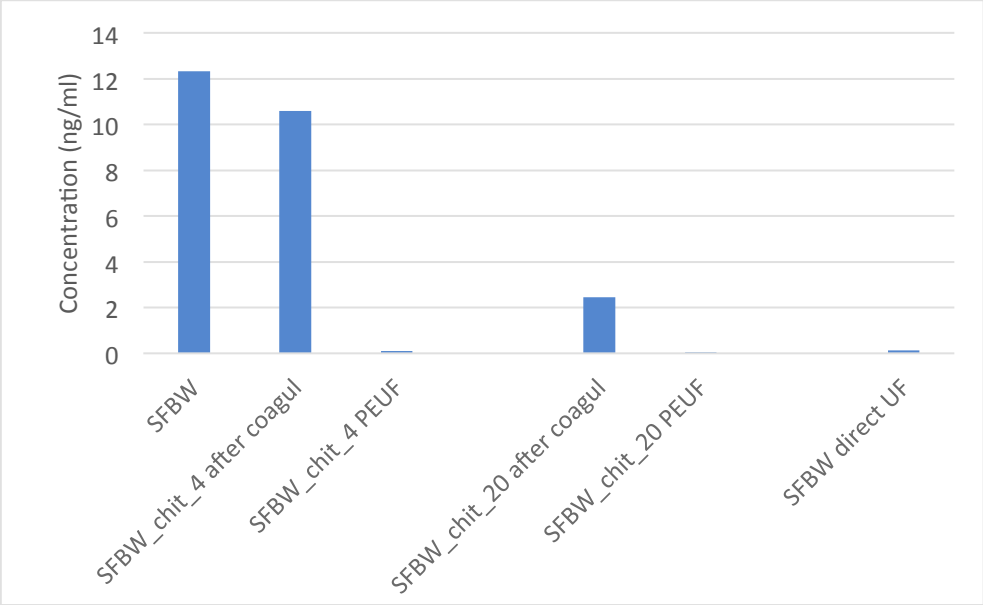


Fig. XXB. Microcystin concentrations in sand filter backwash water (SFBW) after chitosan-enhanced coagulation/flocculation and PEUF with the addition of 4 and 20 mg/L chitosan.

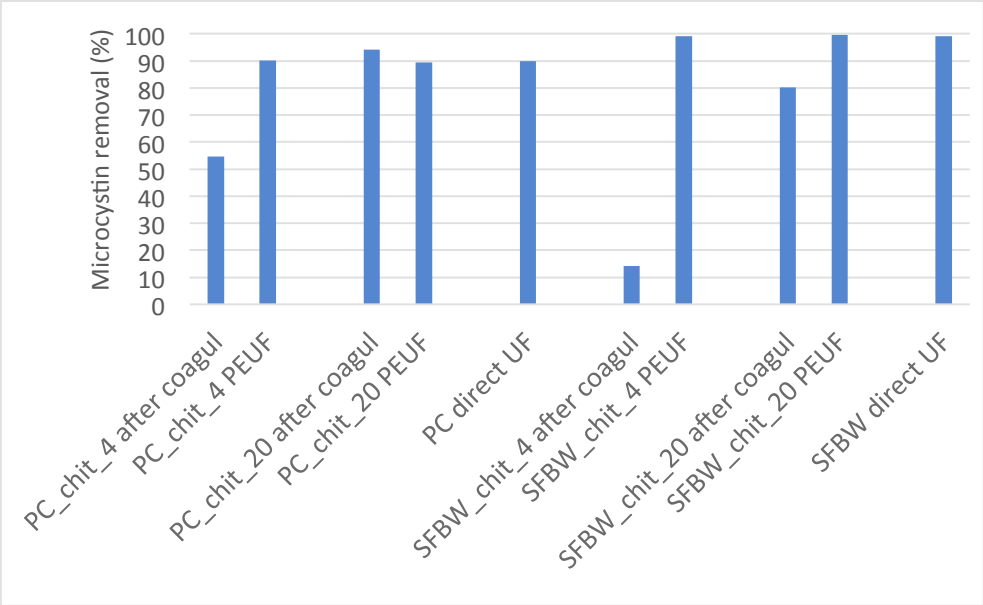


Fig. XXC. Overall microcystin removal efficiencies of PC and SFBW samples.

The clarification efficiencies of the PEUF tests, as shown in **Fig. XXD**, confirmed the microcystin removals in the experiments. Chitosan-aided coagulation-flocculation had approximately 13.3% clarification efficiency in PC samples at both concentrations, it increased to 60% after PEUF for the concentrations of 4 and 20 mg/L. Similarly, there was not a difference in the clarification efficiency between 4 and 20 mg/L of chitosan in the SFBW samples after the coagulation-flocculation process, calculated as 72.9% and 71.8%, respectively. After applying UF to the effluents of SFBW samples after the coagulation-flocculation, the maximum clarification efficiency of the PEUF was obtained that was calculated 92.9%.

In all conditions, the microcystin concentration in the final effluents were < 0.15 ng/mL. In fact, a slight increase in microcystin concentration was even observed in the final PEUF effluents in PC samples. This can be attributed to high transmembrane pressure that may have caused a slightly release of microcystin. Meanwhile, direct UF application without prior coagulation resulted in poorer removal efficiencies. In addition to this, fouling is a serious problem in direct membrane applications.

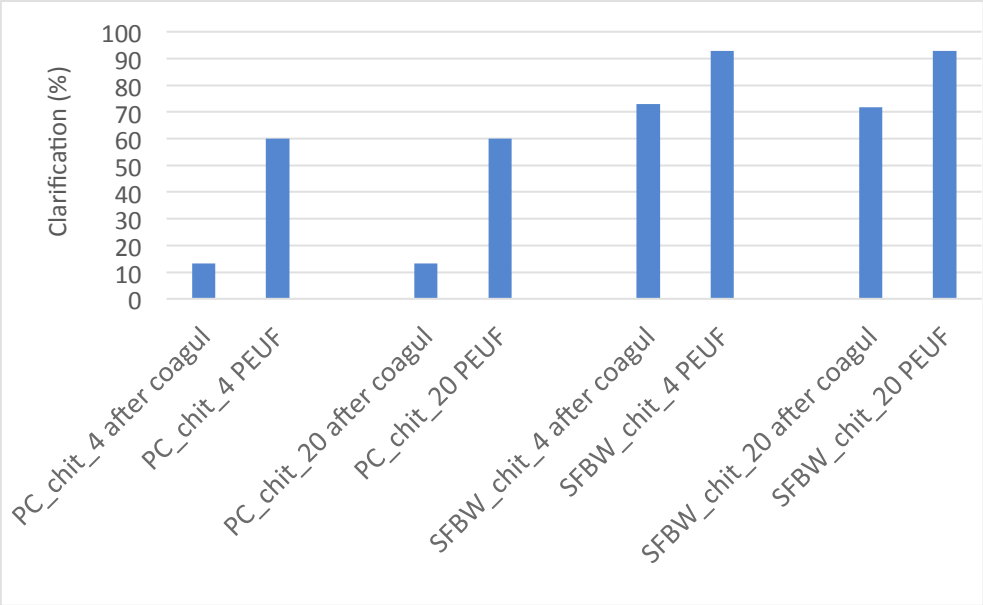


Fig. XXD. Clarification efficiencies of PC and SFBW samples.

Further tests will be conducted in the following months using small-scale PEUF units in continuous operation and to understand the relationship between cyanobacterial removal and possible re-release of cyanotoxins as well as the fouling mechanism of membranes.

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WP4- DECISION SUPPORT SYSTEM DEVELOPMENT

D 4.1 Country data acquisition on the water cycle management with special focus on the Country needs for the pilot action and special mapping of relevant stakeholder.

Work Package 4 regards the development of an integrated, well known and applied in many fields, DSS-GIS approach for the water cycle management. The first action of this work package was the definition of a comprehensive set of data that needs to be collected concerning the priority area identified for the participating countries. In parallel, an identification of the stakeholders (social, political, technological etc.) that operate in the water management at local, national, and international level will commence.

It is important to underline that the developing DSS can be improved in order to manage the whole water cycle at different level from local to national.

Taking the above into consideration, an excel spread sheet is developing to help partners with the data collection. The data that needs to be collected till now is categorized as follows:

1. Statutory Legislation;
2. Bibliography/projects concerning, in particular, the cyanotoxin problem and the applicable technologies;
3. Phenology and bacteria species;
3. Stakeholders;
4. Water related ancillary data;
5. Ancillary data miscellanea;
6. Water quality and characteristics;
7. Drinkable water treatment plants;
8. Cartography of the pilot action site;
9. GIS

The DSS

Due to the complexity of a technical process and the scarcity of well-trained operators, intelligent decision support systems (DSS) which can recommend what correcting actions should be taken to manage the process to its desired state after an individual (or multiple) parameter disturbance would be extremely useful for both abnormal as well as normal operating conditions subjected to day to day disturbances.

Every real-time control system management using computational intelligence comprises three components:

- The real-time database which is the repository for real-time data acquired from the process;
- The knowledge base which contains the knowledge and experience about the process and how it must be managed;
- The answers to the questions (a sort of the *if-then* rules) and the inference engine (algorithm) that computes the actions that must be applied to the process to achieve the desired objective.

Following completion of data acquisition (a combination of on-line and off-line measurements and visual observations) the inference engine is executed and searches the knowledge base exhaustively to determine the degree to which every rule contributes to the final decision. If the result is unique then this action is proposed. When more than one rule contributes to a decision, the inference engine must weigh the influence of every rule before arriving at a crisp action to propose. Here the challenge is one of developing a simple and robust decision support system whose decisions, which will lead to a performance commensurate to that of an expert plant manager. It must be emphasized that the DSS cannot be expected to be better than the human whose decision-making process it emulates, but it can be expected to be more consistent.

The drinkable water management requirement
Regarding the drinkable water management, using only data acquired at irregular intervals from infrequent, unreliable and erratic measurements of the parameters concerning the cyanobacteria growing in order to manage the Drinkable Water Treatment Plants (DWTP), the task of the plant manager is to maintain performance variables within prescribed limits. The manager specifies the set-points of the desired performance variables over a future time horizon and the process is allowed to coast until the next measurements are acquired. It is hoped that nothing unforeseen will occur in the meantime. Generally the DWTP is sensitive to changes in the influent parameters caused by the presence of pollutants and in particular to the presence of the seasonal cyanobacteria.
Due to the complexity of the process and the scarcity of well-trained operators, DSS which can recommend what correcting actions should be taken to restore the process to its desired state after a disturbance would be extremely useful for both abnormal as well as normal operating conditions subjected to disturbances.
An extended (to the whole area, territory, cities, etc) DSS can be useful also for the stakeholder in order to obtain a significant and urgent challenge if a decision/problem must be resolved quickly especially in large urban areas or in important environment such as a protected area.

Due to the COVID emergence, data collection and acquisition it is not yet complete. Till now only a draft of the list and the partitioning of the data it is compiled. Moreover it is developing a list of:

- the stakeholder, at local (regional) level, has been identify (public health, consumers and environmental associations, administrations, plant management and water supply companies, waste management companies, engineering companies, etc);
- the list of the data concerning the characteristics of the pilot action area (water surface, surface of the phenomenon, evolution time, bacteria species, cyanotoxins concentration evolution, nutrient contents, pH, TSS, etc);
- the list of the process parameters (water inflow, pH, temperature, abatement kinetic, polymer concentration, etc);
- the water supply needs;
- legislation (local, national and international) about the drinkable water management and characteristic.

Legislation
In this category, partners must address all the statutory legislation on water protection / water management that currently exist in their countries.
If currently exists, the parameters that need to be examined under this category are the following:

1) *H_2O Management Legislation for*:
   - Urban Wastewater (alternative resource for irrigation);
   - Use of drinkable water in the industries;
   - Drinking Water;
   - Drain/Sewer.

2) *H_2O Protection Regulations*
   - provide necessary strategic and technical tools for the decision makers to make informed decisions on the use of non-conventional water resources based on economic, environmental and social justification;
   - provide the necessary rules and regulations for the protection of surface water resources and reduce health exposure and hazards;

- establishing institutional and consultation mechanisms for the management of these resources in a transparent, and accountable manner.

Bibliography/projects
This category records all the previous projects performed and the bibliography concerning the drinkable water and the relative technologies applied for in order to emphasize the previous experiences.
The information that needs to be collected for each project is as follows:

- Name of the project and data such as partners involved, main objectives, level of interest (local, national, internatiomnal), identification of the stakeholders of each project, impact/relevance, sponsor, amount of founding, the site, the year, etc;
- Bibliography: list the references of the past projects
- Website / Link Hyperlink: list the website (if any) of project/ equipments/ technical records.

Phenology and bacteria species
A great effort on these data will be devoted. Firstly data obtained from the scientific literature has been collected but, naturally, the data of the pilot sites will be of primary importance.

Stakeholders
Stakeholders are those who may be affected by or have an effect on the project's efforts, they can be divided into three very broad groups:

Primary: the people or groups that stand to be directly affected, either positively or negatively, by an effort or the actions of the project;

Secondary: the intermediaries in the aid delivery process;

Key Stakeholders: who might belong to either or neither of the first two groups, are those who can significantly influence, or are important to the success of the project;

The effectiveness and sustainability of the project, depend practically, in part, on the commitment of interested parties (stakeholders), thus participation is a central element in achieving aid objectives.
The purpose of this category is to record all the stakeholders that will contribute for the successful implementation of the project. It is important to provide information of each stakeholder emphasizing their experience.
Information that needs to be included in this section is the following:

- Name of the stakeholder: It may be a person, group or organization with an interest in a project;
- Legal status: Public/Governmental, Private, NGO, National, Local etc.
- Reference administration/interested geographic area (when local stakeholder): location
- Contact Person: List the name of the person who the partner can contact to get in touch with the stakeholder
- e-mail Address: This is for the above person

Water Related Ancillary Data
The Water Related Ancillary Data section is about collecting data regarding the water usage, the water discharges and the water distribution network, in order to estimate the quantity of water that

is used (and to treat) per day. This collection data can be improved to increase the DSS application to the whole water cycle management.
The parameters that are needed to be taken into consideration for the collection of data, are the following:

- Water use - identification of the amount and sources of water usage for:
- Residential area (water consumption per capita);
- Industrial / Commercial/ Institutional;
- Agricultural;
- Municipal;
- Tourism.

Untreated water:
- If a drinkable water treatment plant exist then data will be filled in in the specific excel worksheet (named WATER TREATMENT PLANT CHARACTERISTICS);
- If a water treatment plant doesn't exist please give a characterization for each inflow water in terms of the most important parameters, such as:
  - ✓ Flow rate: It depends upon population density, water consumption, and the extent of the commercial or industrial activity in the community.
  - ✓ Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD);
  - ✓ Principal pollutants concentration: The principal pollutants that can be found in a water network such as suspended solids, nutrients (Nitrogen and phosphorus), heavy metals;
  - ✓ Cyanobacteria.

Water distribution network, this is to measure the amount of water that distributed by the following water networks:

- Drinking water distribution network;
- Sewage network;
- Reservoirs;
- Wells;
- Rivers.

Ancillary Data Miscellanea
In this category the partner has to estimate the quantity of water used from each interested area (local and regional). Population estimates are one of the greatest data challenges for water supply planners. It is critical to know how many people a utility serves and to project how many may be served in the future to ensure adequate water supply. Accurate and consistent estimates of population are a necessary component of calculating metrics such as gallons per capita per day.

The Parameters that the partner needs to take into consideration are the following:
Population: Estimate the seasonal equivalent inhabitants (or population) living in each study area in order to be able to calculate the amount of water used per person.

Industrial plant: water use for the process purposes (i.e. textile, agroindustry, etc). The characteristics of the to wastewater treatment plant (if any) should be recorded.

H_20 Quality
As water quality depends on all the substances that compose the hydric solute system therefore for water management purposes it is important to examine the water quality of each study area, in order

to be able to suggest ways of improvements related with the water cycle management. Partners have to take into consideration the parameters according to the legislation limits and, of course, relating to the cyanobacteria presence. Moreover the monitoring systems (if any) should be recorded.

Drinkable Water Treatment Plant

It is important to collect information about any existing Drinkable Water Treatment Plant in order to optimize the process allowing the removal of cyanotoxins.

The information that needs to be collected is the following: adopted technology, the year the water treatment plant was constructed, maintenance records of the plant, water flow rate, water characteristics, seasonality, produced waste, water use after treatment, etc.

Cartography

The data that will be collected in this category will be combined also with the GIS section. The data needed is as follows:

- Landuse, a landuse map illustrates types and intensities of different land uses in a particular area;
- Hydrographical Network, hydrography is the mapping of water features;
- Water distribution network, a map of water distribution illustrates the routes that the water networks follow ending up into different water bodies;
- Wastewater network, a map of wastewater network illustrates the routes that the wastewater follows ending up into different water bodies;
- Temperature, a temperature map could present the past, current and future temperature of the area of interest;
- Precipitation/rainfall, precipitation/ rainfall map presents the rate of precipitation in areas of interest;
- Administrative boundary, subdivisions of areas/territories/jurisdictions recognized by governments or other organizations for administrative purposes;
- Terrain morphology, for example, to generate elevation, area, and volume curves for a set of selected drainage areas;
- Geology, a geology map is a special-purpose map made to show geological features;
- Protected areas, a map could be prepared illustrating if there are any protected areas in specific locations of interest (i.e. NATURA 2000 areas, National Parks etc.);
- Water Treatment plant, all the existing industrial plants for water treatment, locating in specific areas of interest, could be presented in a map;
- Reservoirs, locations of water reservoirs.

D 4.3 Geographic Information Systems

A Geographic Information System (GIS) lets us visualize, question, analyze, and interpret data to understand relationships, patterns, and trends. GIS software is designed to capture, manage, analyze, and display all forms of geographically referenced information.

GIS allows us to view, understand, question, interpret, and visualize our world in ways that reveal relationships, patterns, and trends in the form of maps, globes, reports, and charts.

GIS software helps you answer questions and solve problems by looking at your data in a way that is quickly understood and easily shared—on a map.

This first data collection system will be upgraded at national level to ensure the future sustainability of the project. The software requirement to realize the DSS is under cinsuderation.