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Seasonal occurrence of picoplanktonic cyanobacteria in water treatment plant Milence and their application in nanobiotechnology

Bachelor thesis

Kateřina Sochorová

Supervisor: RNDr. Jitka Jezberová, Ph.D.

Consultant: Prof. Ing. Ivo Šafařík, Ph.D., DSc.

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Annotation

This bachelor thesis deals with freshwater picocyanobacteria. The first part of it is focused on the occurrence of picocyanobacteria in the water reservoir Nýrsko and their penetration into the individual stages of technological treatment of drinking water in the water treatment plant Milence. The filter separation efficiency of picocyanobacteria in dependency on filter operational time and subsequently, the possible variants of optimalization or addition to the technological process for separation of picocyanobacteria from treated water are presented. The second part of this bachelor thesis deals with the use of picocyanobacteria in nanobiotechnology. It consists of pilot experiments with application of picocyanobacteria in addition with microwave synthesized magnetite for the nanobiotechnological application – sorption of the industrial dyes from water. For the preliminary tests, cultures of picocyanobacteria from the collections of the Hydrobiological institute of the Biology Centre of the Czech Academy of Sciences were tested. The continuous experiments were performed using a picocyanobacterial culture from the water reservoir Nýrsko. As the model pollutants the industrial dyes, Malachite green, Methylene blue, Rhodamine B, Crystal violet, were utilized.

Keywords: picocyanobacteria, water treatment, drinking water, magnetite, biosorption, dye, Malachite green, Methylene blue

I declare that I am the author of this qualification thesis and that in writing it I have used the sources and literature displayed in the list of used sources only.

Student's signature

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List of abbreviations

2-MIB – 2-methylisoborneol
AA – after accumulation
AG – aggregation
AOC – assimilated organic carbon
a.s. – public limited company
AU – absorbance unit
BA – before accumulation
BG-11 – blue-green medium
BMAA – β-N-methylamino-L-alanine
Cat – catalogue
COD_{Mn} – chemical oxygen demand by the permanganate
col% – percentage of picocyanobacterial colonies in total amount
coll. – collection of laws
ct – concentration of free dye
ČR – Czech Republic
CV – Crystal violet
ČSN – Czech technical standard
ČSN EN ISO – Czech version of the European Standard
ČSVTS – Český svaz vědeckotechnických společností z.s. (Czech Union of Scientific and Technical Societies z.s.)
DAF – dissolved air flotation
DAPI – 4′,6-diamidino-2-phenylindole
DNA – deoxyribonucleic acid
EN – European norm
FEMS – Federation of European Microbiological Societies
ind% – percentage of individual picocyanobacteria in total amount
ISO – International Organization for Standardization
IUPAC – International Union of Pure and Applied Chemistry
IWA – International water association
LC-MS – liquid chromatography – mass spectrometry
LPS – lipopolysaccharide layer
MASL – metres above sea level
MB – Methylene blue
MG – Malachite green
mil. – million
MW Mag – microwave synthesized magnetite particles
NA – numerical aperture
NCBI – National Center for Biotechnology Information
No. - number
p.a. – per analysis
PAX 18 – polyaluminum chloride
PCTE – polycarbonate
PCR – polymerase chain reaction
Pcy – picocyanobacteria
PS – polystyrene
PVL – Povodí Vltavy
qt – amount of adsorbed dye on specific amount of sorbent
RCF – relative centrifugation force

RS – reservoir surface
rpm – revolutions per minute
RNA – ribonucleic acid
rRNA – ribosomal RNA
RW – raw water
SEM – scanning electron microscope
SOVAK – Sdružení oboru vodovodů a kanalizací (Water Supply and Sewerage Association)
sp. – species
s.p. – state enterprise
s.r.o. – private limited company
TEP – transparent exopolymeric particles
UB VŠCHT – Department of Biotechnology, University of Chemistry and Technology, Prague
UF – ultrafiltration
UV – ultraviolet
ÚV – úpravna vody (water treatment plant)
VIS – visible light
VŠCHT – University of Chemistry and Technology, Prague
WC – Wright Chu
WHO – World Health Organization
WTJ – water reservoir Janovice
WTP – water treatment plant

1 Introduction ... 1
1.1 Picoplanktonic organisms ... 3
1.2 Picoplanktonic cyanobacteria ... 3
1.2.1 Taxonomy ... 4
1.2.2 Morphological and physiological characteristics ... 6
1.2.3 Occurrence of picocyanobacteria ... 10
1.2.4 Ecological and environmental importance of picocyanobacteria ... 11
1.2.5 Toxicity of picocyanobacteria ... 12
1.2.6 The influence of picocyanobacteria on water treatment technology ... 14
1.3 Elimination methods of hydrobiological contamination of water ... 17
1.3.1 Enhanced coagulation ... 17
1.3.2 Electrocoagulation ... 17
1.3.3 Dissolved air flotation ... 18
1.3.4 Membrane separation methods ... 19
1.4 WTP Milence ... 21
1.4.1 Water reservoir Nýrsko ... 21
1.4.2 Chemical quality of raw water ... 22
1.4.3 Hydrobiological conditions of raw water ... 22
1.4.4 Technology of water treatment ... 23
1.5 Magnetic modification of cells ... 25
1.6 Biosorption and biosorbents ... 27
1.6.1 Biosorbents of dyes ... 28
1.7 Dyes ... 29
1.7.1 Malachite green ... 29
1.7.2 Methylene blue ... 30
1.7.3 Rhodamine B ... 30
1.7.4 Crystal violet ... 31
2 Work objectives ... 33
3 Methods ... 34
3.1 Water sampling ... 34
3.2 Counting of picocyanobacteria ... 34
3.2.1 Preparation of the sample ... 34
3.2.2 Counting of picocyanobacteria ... 36
3.3 Cultivation and harvesting of picocyanobacteria ... 37
3.4 Magnetic modification of the picocyanobacterial cells ... 39

3.4.1 Preparation of non-stoichiometric magnetite 39
3.4.2 Magnetization of the cells 39
3.4.3 Adsorption experiments 40
3.4.4 Evaluation of the measurements 43
3.4.5 Scanning electron microscope (SEM) 45
4 Results 46
4.1 Development of the picocyanobacteria in reservoir Nýrsko and WTP Milence 46
4.2 Penetration through the filters 51
4.3 Preliminary tests for nanobiotechnological experiments 51
4.4 Biosorption of Malachite green dye by magnetically modified picocyanobacteria 54
4.4.1 Malachite green equilibrium adsorption isotherm 56
4.5 Biosorption of the dyes using picocyanobacterial culture from water reservoir Nýrsko 57
4.6 Extended biosorption experiments using Nýrsko picocyanobacterial culture and Methylene blue dye 61
4.6.1 Adsorption of Methylene blue dye using non-dried culture of magnetized picocyanobacteria 61
4.6.2 Adsorption of Methylene blue dye using lyophilizated magnetized picocyanobacteria 65
5 Discussion 69
5.1 Picocyanobacteria´s occurrence in water reservoir Nýrsko and WTP Milence 69
5.2 Biosorption of the dyes by magnetized picocyanobacteria 71
6 Conclusion 75
7 Bibliography 76
8 Supplemental materials 93

1 Introduction

Improvement of the drinking water quality via development of new technologies and monitoring of new parameters is one of the current ways and aims of water-related sciences. Requirements for water quality intended for the human consumption are rapidly evolving depending on newly gained knowledge in order to achieve the best possible drinking water quality without harmful or problematic substances. Currently, in addition to the classical pollutants (e.g. nitrates, heavy metals, natural organic substances), specific organic anthropogenic contaminants (e.g. pesticides and their metabolites, residues of industrial origin) have also begun to be discussed. Attention is also paid to the natural components occurring in water. Continuously, with new findings about hydrobiological composition of raw water, the requirements for biological aspects of treated water are revised. Among them, the excessive occurrence of picocyanobacteria observed, which is the subject of this study.

In the first part of the thesis, the occurrence of picocyanobacteria during the vegetation season at the water reservoir Nýrsko, which serves as the raw water source for the water treatment plant Milence (further referred as WTP), was analysed. In recent years, the site has suffered from hydrobiological contamination caused by picocyanobacteria, which subsequently partially permeated the water treatment technology. In sampling campaigns, the quantification of the picocyanobacterial cells was carried out in various sampling profiles from the source of raw water to the point of the distribution and the penetration into the technological line was investigated. The filter separation efficiency for picocyanobacteria in dependence on the filtration operation time was analysed. Simultaneously, the methods for removing picocyanobacteria and other hydrobiological pollution from water by means of optimised processes or by incorporation of additional separation stage into an existing water treatment technology, were discussed.

The second part of the bachelor thesis, which was simultaneously worked on, was focused on the laboratory experiments with picocyanobacterial biomass as the potential biosorbent for the pollutant removal, specifically dyes. The aim is not primarily an application in field of drinking water treatment (as it is currently not envisaged to use such polluted water as raw water for the treatment), but for example for the treatment of industrial wastewater so that it can be safely released into the environment or recycled, which has been an ongoing and highly discussed trend in water management in recent years. In preliminary tests, picocyanobacterial strains grown in a collection of phototrophic organisms at the Hydrobiological institute of the Biology

Centre of the Czech Academy of Sciences were experimented with. These cultures were together with microwave synthesized magnetite (used for separation purposes of the picocyanobacteria from the water/dye solution) utilized for the biosorption of dye, specifically Malachite green, from water. In the final stage, the picocyanobacterial culture, isolated from the water sample obtained from the reservoir Nýrsko, was modified by the methodology adjusted according to the results of the preliminary test, and the biosorption of the Methylene blue, and three other common industry-used dyes, was analysed.

The common factor of both parts of the bachelor thesis are picocyanobacteria found in the water reservoir Nýrsko. While in the treatment of reservoir water for drinking purposes, these organisms are considered a negative component and an effort is made to entirely eliminate them from the treatment process, in the case of using these organisms as a source for nanobiotechnology, their ability to biosorb dyes is beneficial and potentially useful for reducing environmental contamination from anthropogenic activities. Hence, the knowledge gained in the second part of the study can serve as a basis for the follow-up applied research.

1.1 Picoplanktonic organisms

Picophytoplanktonic organisms (photoautotrophic, prokaryotic, and eukaryotic) can be defined by their specific size from 0.2 to 2 μm. Because of their small dimensions, these species were overlooked in studies and research in the past. This fact was also caused due to the widely used sedimentation technique (presented by Utermöhl (1958)), which were applied for quantitative determination of phytoplankton in general, including the discussed organisms. This approach, with applied sedimentation chambers and inverted microscopy as defined by EN 15204 (Guidance for the enumeration of phytoplankton using inverted microscopy; rules for phytoplankton analyses in the water treatment plant laboratory) is unsuitable for the detection and subsequent quantification of picophytoplankton – the settling of particles of such small size is slow and, furthermore, contains undistinguishable particles (Guillou et al., 1999; Ning et al., 2021; Worden et al., 2004)

Picophytoplankton is an essential component of the aquatic environment that contributes to primary productivity, especially in less trophically loaded reservoirs. Due to the lower phosphorus and nitrogen content, the picophytoplankton structure is essentially stable and quite rich in terms of species diversity. Moreover, the physiological structure of picophytoplankton has some competitive advantages in the microbial community. Analyses of the size structure of phytoplankton are certainly necessary and desirable to understand and at the same time to control its dynamics in water bodies. Significantly, this is also related to the approach to better water management of different quality and from different aquatic ecosystems. These organism groups are complexly tied to nutrient availability, nitrogen and phosphorus forms and light sources, temperature, or salinity (CHEN et al., 2003; Husiman & Weissing, 1995; Ning et al., 2021).

1.2 Picoplanktonic cyanobacteria

Picoplanktonic cyanobacteria (further referred as picocyanobacteria or Pcy) are autotrophic prokaryotes belonging to the picoplanktonic organisms, more specifically to the subgroup of "algae of bacterial size" (Callieri, 2008; Raven, 1998). Picocyanobacteria are viewed as the ubiquitous organism and can be found in every biotope and water environment – freshwater, marine and brackish (Häggqvist et al., 2016; Sandoval Valencia et al., 2022). Until the 1970s, picoplanktonic organisms were not mentioned or considered in the scientific literature and research (Callieri, 2008; Raven, 1998). The neglect of these small-sized specimens was caused

by the insufficient methods and approaches (containing common bottle sampling and basic light microscopy) applied for the sampling and subsequent recognition (Caroppo, 2015).

The term “picocyanobacteria” was originally assigned to individual single cells which were present in the water environment. Throughout the time, the expression was extended also to colonies aggregating more than 50 individual cells. The intermediate structure is called microcolony and is defined as a colony with number of cells counting in range from 2–49 (Callieri, 2008; Raven, 1998).

Picocyanobacteria are observed in the nature in all three structures – individual cells, microcolonies, and colonies. Within the colony and microcolony, the cells are attached together with the extracellular mucilaginous slime, which is produced by the individual community members (Harper, 2005; Huber et al., 2017). The distribution of picocyanobacteria structures in water column is as follows: Individual cells are usually represented in the deeper layers of the water columns, in contrast to the colonies of picocyanobacteria, which can be mostly found on the surface (Psohlavec, 2022). This ability, otherwise known as phenotypic plasticity, is crucial also in the food chain, where the changing in size can eliminate some of the predators (Huber et al., 2017).

1.2.1 Taxonomy

Originally, the picocyanobacteria were classified as one of the two following specimen groups – *Synechocystis* (spherical cells) or *Synechococcus* (elongated or oval cells). Through the years, these genera were observed in many various environments (such as water or soil). Furthermore, the researchers were able to identify subclasses and other genera, specifically containing only the cyanobacteria species (Komárek et al., 2020).

As mentioned in Chapter 1.2 about the general characteristic of the picocyanobacteria, these organisms can be found in two different structures – as unicellular or in colonies of different sizes. Both of these forms have multiple genera of which these structures are typical. The unicellular specimens are stated to be *Prochlorococcus* and *Synechococcus* as representatives of marine ecosystems accompanied with the specimen of freshwater ecosystems – *Synechococcus*, *Synechocystis*, *Cyanothece*, *Cyanobacterium* and *Cyanobium*. These previously stated genera are the main subgroups considered to be in picocyanobacteria (Callieri et al., 2013; Jasser & Callieri, 2016;). The most common freshwater genera of colonial picocyanobacteria are *Aphanocapsa, Aphanothece, Chroococcus, Coelosphaerium, Anathece,*

Cyanodictyon, Merismopedia, Romeria, Snowella, and *Tetracercus* (Callieri et al., 2013; Jasser & Callieri, 2016; Komárek et al., 2011).

The correct taxonomy of picocyanobacteria is not only essential for classification of individual species into the same taxonomic groups and genera and thus combining characters into larger subunits, but also for predicting and confirming hypotheses concerning the ecology of individual species and their contribution to global biogeochemical cycles and processes (Farrant et al., 2016). However, the correct taxonomy classification of the picocyanobacteria is complicated and arduous – not only because of complications regarding the genera system, but also of the fact that conventional taxonomical system does not correspond with the physiological interpretation of the organisms' characteristics (Rippka et al., 1979; Walter et al., 2017).

The main issue of the taxonomy of picocyanobacterial genera lies in which system, whether botanical or microbiological, should these organisms be categorized into and with which taxonomic methods. Botanists suggested the initial 'definition' and characterization of the picocyanobacteria as they are green and belong to the phytoplankton. This classification is justified by the morphological and ecological approach using microscopy techniques. However, phylogenetically, these organisms are more related to bacteria, where the taxonomy is genetically based (Lefler et al., 2023; Švecová, 2022). According to these two systems, we can arrange the picocyanobacteria into both *Cyanobacteria* (in accordance with International Code of Nomenclature of Prokaryote (Oren et al., 2023)) and to the *Cyanophyta* (conforming to International Code of Nomenclature for algae, fungi, and plants (Turland et al., 2018)).

Nowadays, the previously mentioned genera are obsolete and from some points of view also incorrect and not sufficient for the exact classification. The genera system has to be united and redesigned so that the two systems are linked and sufficient for the legitimate classification. Nevertheless, the taxonomy system of the cyanobacteria and picocyanobacteria is still a developing field (Jezberová, Jitka, thesis supervisor, [oral communication], 2024; Komárek et al., 2014).

For the factual classification of picocyanobacteria and its individual species, the multidiscipline approach – so called polyphasic – has to be adopted and put into practice. In the approach several techniques and experiments are performed and combined: observation of morphological traits not only with low-magnification techniques but also with higher magnifications (ultrastructure examination), electron microscopy analysis, consideration of ecological aspects,

monitoring of biochemical features, and genetics experiments (predominantly 16S rRNA gene sequence). The gene research in the framework of cyanobacteria taxonomy groups reveals the high diversity of these specimen (Komárek et al., 2020; Komárek et al., 2014; Lefler et al., 2023; Nabout et al., 2013).

1.2.2 Morphological and physiological characteristics

The shape of picocyanobacteria can be various (diagram of different shapes and sizes can be seen in Figure 1) and can alter from spheres through ovoid structure to rods (Harper, 2005). To distinguish the shape, literature suggests the use of epifluorescence methods, which can analyse not only the shape but also the colour of the auto-fluorescence of picoplanktonic cyanobacteria (Komárková, 2002; Švecová, 2019).

Genus (Number of species)	Shape of cells	Type of cell division	Thylakoid pattern	Type of nucleoids	Type of binary fission	Involution cells	Type of S-layer	GC (% mol)	Cell dimensions (µm)
Synechocystis (21)	Spherical					-	P6	30-48	0.7-15 diameter
Cyanobium (13)	Oval to short rod-like	Symmetric		Band like	Pinching	Irregular	(p4?)	(36) 49.1-71	0.4-4.5x0.2-3
Synechococcus (24)	Cylindrical		Parietal			Filamentous	P4	47-56	1.2-28 x 1-6
Cyanobacterium (8)	Cylindrical to widely oval	Symmetric or asymmetric	Length wise	Irreg. Granular to net like	Cleavage	Irregular to short filamentous		39-41	3.4-12x2-12
Cyanothece (6)	Cylindrical to widely oval	Symmetric	Radial infrath. spaces*	Net like		Irregular	P2	41	7-70x7-52

N.B Radial infrath. spaces = radial position of thylakoids with tendancy to form intrathlylakoidal spaces.

Figure 1. Overview of the possible picocyanobacteria´s cell shapes (Harper, 2005)

Cell wall

Regarding the picocyanobacterial cell wall and its structure, the experiments show the presence of elements for both Gram staining categories. Nevertheless, the picocyanobacteria are considered to be part of the Gram-negative bacteria. Despite the inclusion of picocyanobacteria in the group of Gram-negative organisms, there are still several differences in the assembly of the cell wall compared to the mentioned category. Due to its larger thickness, the picocyanobacteria´s wall is acting as unquestionably efficient barrier for large molecules. To maintain the transport of the compounds into and out of the cell, picocyanobacteria have evolved sophisticated transport systems compared to other bacteria. These mechanisms can be differentiated according to the required-transfer energy, according to the categories of substances that are transferred across the membrane, or according to the complexity of the

individual transporting processes and mechanisms (Hoiczyk & Hansel, 2000; Jasser & Callieri, 2016).

The cell wall structure and properties of the individual and complex groups located on the wall (such as carboxylic, ester, or amino functional groups) are crucial part for the understanding of the processes, which are able to be carried out by the picocyanobacteria, such as calcium precipitation or biosorption capacity (see Chapter 1.6). In general, the picocyanobacterial cell walls exhibit the deprotonation property, i.e. a negatively charged surface at the neutral pH (enabling to attract the positively charged particles such as metals ions) (Dittrich & Sibler, 2005). The picocyanobacterial cell wall is displayed in Figure 2.

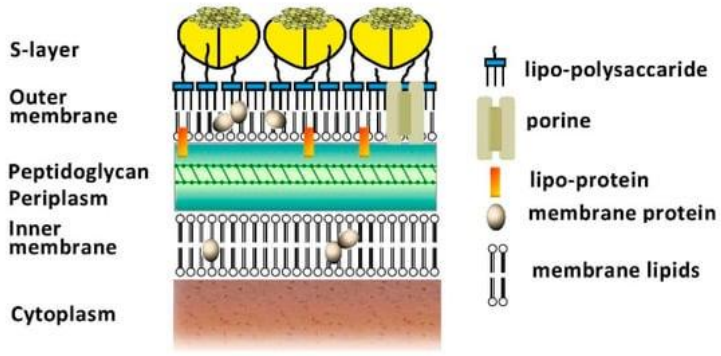

Figure 2. Scheme of the picocyanobacterial cell (Retrieved from Callieri et al. (2022))

To be able to form larger structures (also known as colonies), the picocyanobacterial cells are able to secrete the adhesive exopolymer substance. The secretion mostly consists of the acid polysaccharides (causing the characteristic stickiness and tendency of negatively charged particle bind) with high ratio of covalently linked sulphates and carboxylate groups. Subsequenlty, this structure forms the polymer structures containing the transparent exopolymer particles causing the gel feature. The production of the mucus is influenced also by the physiological modification or growth stage (showing the excess of secretion in late stages of organism's life) of the picocyanobacteria, which means connection to environment, such as nutrient limitations. The mucus secretion was observed to be essential in various processes, such as fixation of dissolved organic carbon (Deng et al., 2016; Kiørboe & Hansen, 1993; Passow, 2002; Pereira et al., 2009)

Part of this wall structure is peptidoglycans, and, in many cases, there is an S-layer (surface layer) on the surface that contains a group of substances called glycoproteins in crystalline monomolecular form (Rachel et al., 1997). The S-layer can be described as a symmetrical continuous protein chain encircling the entire cell (Schultze-Lam & Beveridge, 1994).

However, not all of the species, which were experimented with, showed the presence of the discussed layer. The absence of the above-mentioned phenomenon could be caused by the lack of the functional genes responsible for the development of this structure or by experiment failure in research by Rachel et al. (1997). The crystalline form of the layer varies through the individual species. The information whether two different species belong to a close subgroup is based on the assumption that the specimen close to each other in the taxonomic tree cannot have a completely different crystalline structure of the S-layer (Rachel et al., 1997).

The function of this cell wall component is to defend the prokaryote against the protozoa, maintaining the cell integrity, as the molecular sieves, traps for different ion species or, as shown with the specific line of *Synechococcus* sp. strain GL24, as the crystallization nuclei for calcium in oversaturated conditions. Overall, it is assumed that this layer has a vital function for the organisms (Jasser & Callieri, 2016; Rachel et al., 1997; Sleytr, 1999; Šmarda et al., 2002). Nevertheless, it participates in the allergic reaction caused to the human in the case of the cyanobacterial blooms (Rachel et al., 1997).

Not only that the cyanobacteria´s wall contains the peptidoglycans, with crossing characteristics typical for Gram-positive bacteria, lipopolysaccharides, which indicates the gram-negative bacteria´s cell wall distinctive feature, but also part of the composition is cyanobacterial antigen O ensuring the possibility of the adsorption of the cyanophage or aquatic endotoxicity (Hoiczyk & Hansel, 2000; Jürgens et al., 1983). The lipopolysaccharide layer (LPS) consists of three main parts – lipid A, antigen O, and oligosaccharides' core with polysaccharide chain. First-mentioned component – lipid A – is having the structure of the biphosphorylated lipid used by the cell to attach the LPS to the cellular membrane and is considered as the protective part against antibiotic and the own destruction of the cell through the cytotoxicity (complement-mediated lysis) (Caroff et al., 2002; Gemma et al., 2016; Raetz et al., 2007). The core of the LPS contains 10 to 12 saccharide units divided into two segments – outer and inner core, also called proximal and distal core, with the binding site for the above-mentioned antigen O (Gemma et al., 2016). The lipid A and the polysaccharide core is connected together by the 3-deoxy-D-manno-oct-2-ulopyranosonic acid (as the most often acidic saccharide as used by these organisms for this purpose) (Caroff et al., 2002). Furthermore, talking about polysaccharide antigen O, this component is crucial for not only the previously-mentioned absorption processes and endotoxicity, but also in pathogenesis or the fact that the picocyanobacteria can be found in most of the water environments. In other words, they are ubiquitous (Reeves, 1995).

Genetic information

The genetic information of picocyanobacteria is carried by a complexly coiled circular DNA molecule.

The morphological characteristics of picocyanobacteria are not that explicit and obvious as it is for other species of phytoplankton (Psohlavec, 2022). There are only few of typical features from which the picocyanobacteria can be determined (colour and shape). Due to this fact, the classification to the taxonomic groups is done by specific molecular methods. The 16S rRNA gene analysis is applicable and widely used technique (Ohki et al., 2012). By application of this procedure to the picocyanobacterial conserve gene, Crosbie et al. (2003) observed that the distinctions among the freshwater picocyanobacteria could be more complex then was initially expected – the difference among the picocyanobacteria species was according to this around 5% pairwise. Nowadays to differentiate the species of the picocyanobacteria, the methods based on the DNA analysis and also with eco-genomics approaches (containing metagenome and genome sequences-based techniques or phycobilisomes analyses embedded in an ecological context) are preferred and more effective (Becker et al., 2002; Cabello-Yeves et al., 2022; Psohlavec, 2022).

Thylakoids and pigments

The main photosynthesis centrum – thylakoids – are parietally arranged in the cell and contain photosynthesis pigments, which are essential for the right and sufficient photosynthesis process. The thylakoid membrane contains chlorophyll *a* (green pigment). On the surface of the thylakoid there are phycobilisomes, which contain specific pigments. The pigments, specifically phycocyanin (blue-green pigment, absorbed light: 620–630 nm), phycoerythrin (red pigment, absorbed light: 490–550 nm), phycoerythrocyanin, and allophycocyanin, belong to the structure of phycobilisomes representing group of phycobiliprotein hexamers. These pigments fulfil the function of a light-harvesting antenna. The main function of these antennae is to capture the energy and transfer it to the membrane photosystems containing the green photosynthetic pigment chlorophyll *a*. These light-collecting antennas facilitate the use of wavelengths that chlorophyll cannot absorb under normal conditions (Grébert et al., 2018; Haverkamp, 2008; Six et al., 2007; Stomp et al., 2004).

According to the predominant photosynthetic pigment, the picocyanobacterial cells can vary in colour – red, green, or blue-green. The exact composition of the pigments varies, and it can be connected to the ecology distribution of the individual species, to be more specific, the variety

helps with the coexistence of the different picocyanobacteria specimens through the absorption of different light wavelengths (Grébert et al., 2018; Stomp et al., 2007). Light-harvesting system consists of the core, which includes a different number (2, 3, or 5) of protein allophycocyanin cylinders. This structure is then bound to the other substructure of the other photosynthetic pigments in the shape of peripheral rods (Grébert et al., 2018; Haverkamp, 2008; Watanabe & Ikeuchi, 2013). Thylakoids and their composition of pigments work with the energy flow as follows: one of the three pigments catches the light which is subsequently transferred through the rods to the core of the phycobilisomes, from which, continuously, is excitation energy relocated to the thylakoids, more specifically, to the photosystem II, continuing to take part in the whole photosynthesis process (Ducret et al., 1996; Mullineaux, 2008; Watanabe & Ikeuchi, 2013).

1.2.3 Occurrence of picocyanobacteria

Picocyanobacteria survive in most water conditions – they can be found in oligotrophic, mesotrophic, eutrophic, or even in hypertrophic types of waters. Due to their body structure and other features, these organisms are able to survive in conditions with low nutrient concentrations and under reduced light. Their small body size and ratio of surface to volume give them an advantage in catching and absorption of the nutrients present in water (Agawin et al., 2000; Raven, 1998). Additionally, the body size helps to reduce the minimum energy needed for the processes within the cell (Callieri & Stockner, 2002). These ones and other qualities such as smart and adaptive usage of nutrients help picocyanobacteria with the ability to grow and survive in deeper section of the water column (Jakubowska & Szeląg-Wasielewska, 2015).

Photosynthesis, photosynthetic pigments, and phycobilisomes are crucial part of the ubiquitous character of picocyanobacteria. The picocyanobacterial plasticity potential is able to modify the characteristic ratio of the pigments, allowing adaptation to the depth of the water column and with that to survive under various conditions (Śliwińska-Wilczewska et al., 2020; Stockner, 1988).

Picocyanobacteria have two peaks of their maximum growth number in every year vegetative season, the first at the turn of spring/summer, and the second one at the turn of summer and early autumn. This type of development is called bimodal. However, this pattern is not always applicable due to the few exceptions which are occurring in several cases (Jasser & Callieri, 2016; Psohlavec, 2022; Stockner et al., 2002).

1.2.4 Ecological and environmental importance of picocyanobacteria

Picocyanobacterial organisms are known for their significant phenotypic plasticity and further capability to survive various conditions. The discussed ability is crucial in terms of ecological status and environmental conditions in which the picocyanobacteria are able to live and survive. This ability is also demonstrated in case of food chain surveillance – the picocyanobacteria are able to modify their size and thus eliminate some of the predators (Huber et al., 2017; Śliwińska-Wilczewska et al., 2020). The growth of picocyanobacterial cells and constant physiological modifications are influenced by three main conditions, temperature, light, and nutrient quantity (Malinsky-Rushansky, 2002). Due to the ability mentioned above, picocyanobacteria could be differentiated into various characteristics groups (the groups are further broken down into subclusters) depending on the photosynthetic pigments, prerequisites for nitrogen conditions, motility, or requirements in terms of water salinity (Ahlgren & Rocap, 2012; Śliwińska-Wilczewska et al., 2018; Stomp et al., 2007).

Throughout the climate change and changes in pressure values of carbon dioxide in the atmosphere, these organisms must also modify to achieve the ability of the prosperity in the water with increasing CO_2 concentration. The research by Lu et al. (2006) is concerned with the mentioned problematics and physiological changes of marine *Synechococcus* sp. due to the CO_2 increase issue. The analysis showed the increase in RNA/DNA ratio growth rate, however, the photosynthetic system was also modified in the direction of uncoupling of this needed process from phycoerythrin structures causing the decrease of metabolism´s rate (Lu et al., 2006).

Furthermore, picocyanobacteria, as well as the whole group of phytoplankton, are a key component of primary production and play a major role in the global carbon cycle and in other biogeochemical cycles (Jürgens & Matz, 2002). Not only that the picocyanobacteria are remarkable part of the picoplankton population but also the numbers showed that 50% of the cyanobacterial biomass present in the world's ocean comes from the picocyanobacteria organisms. Furthermore, 80-90% of total carbon mass is attributed to these creatures. Considering the global warming and its effects, it is expected that their involvement in the above-mentioned phenomena will expand further (Dittrich et al., 2010; Farrant et al., 2016; Śliwińska-Wilczewska et al., 2018).

Not only that these organisms are fundamental part of the primary production, but also their ability to respond to fluctuations and disruptions in the environment can be used in fields such

as signage in water quality or in ecological research about water habitats. In addition, picocyanobacteria, along with other species from the picoplankton, are crucial in many other processes being part of the microbial loop, engaging in the energy and matter transfers, in relocation between the biotic and abiotic areas or in adjusting the tropic levels (Azam et al., 1983; Caroppo, 2015; Di Poi et al., 2013; Magazzù & Decembrini, 1995).

Picocyanobacteria are essential part of natural processes also including the fact of being a prey (mainly talking about protozooplankton and metazooplankton) for the predators in the natural food chain, or the role of picocyanobacteria in development of eukaryotic photosynthesis (Callieri et al., 2022). Furthermore, these organisms can be also beneficial in the biogenic precipitation, such as in precipitation of calcite in different water environments, as shown in the research paper by Dittrich et al. (2010) or Schultze-Lam & Beveridge (1994). This ability, also known as whiting event (Schultze-Lam et al., 1997), is acquired through their surface area, which are significant for the adsorption processes.

1.2.5 Toxicity of picocyanobacteria

Unlike larger cyanobacteria, the toxicity caused by picocyanobacteria is reported rarely. However, as already mentioned, the sampling methods and techniques for experiments were not adapted for this type and size of organism. Nevertheless, in several cases, the production of bioactive compounds and secondary metabolites was indicated by several studies. The mostly observed ones are microcystins (belonging to the groups of hepatotoxins), neurotoxins and lipopolysaccharides (as a class of dermatoxins). Nonetheless, there are more exposed biologically active compounds which are generated by the picocyanobacteria like various types of peptides: oligopeptides, post-translationally modified peptides synthesized through ribosomal pathway (so called cyanobactins), 2-methylisoborneol, 1,2,7,7-tetramethyl-2-norborneol (otherwise known as geosmin). Not all of the mentioned compounds and groups of substances are toxic or harmful to the human health (Błaszczyk et al., 2021; Hitzfeld et al., 2000; Jakubowska & Szeląg-Wasielewska, 2015; Jasser & Callieri, 2016; Pagliara et al., 2021).

Throughout the years, various specimen of cyanobacteria, including picocyanobacterial species – *Prochlorococcus, Synechococcus* – were indicated to produce numerous types of bioactive substances – cyanobactins – through their biochemical ribosomal pathways and continuously transformed in post-translational modification. The previously mentioned group of biochemical compounds contains, from a chemical structure point of view, heterocyclized amino acids and

different isoprenoid derivates of amino acids (Jakubowska & Szeląg-Wasielewska, 2015; Torres-Hernandez et al., 2024).

Cyanotoxins produced by the cyanobacteria in algal blooms are well known. The release of these substance by picocyanobacteria is however reported infrequently. Nonetheless, at present, the production of these harmful compounds was observed in some picocyanobacterial specimens. For example, the strains of *Synechococcus* sp. are able to generate the neurotoxic compound β-N-methylamino-L-alanine (BMAA) and microcystin (Bláha & Maršálek, 1999; Gin et al., 2021). The BMAA is one of the factors that can cause neurodegenerative diseases, such as amyotrophic lateral sclerosis, Parkinson's disease, or Alzheimer´s disease (Lobner et al., 2007; Réveillon et al., 2014).

One of the most abundant toxins, which is generated by the cyanobacteria, is toxin from the family of microcystins belonging to the group of hepatotoxins (Jasser & Callieri, 2016; Yang et al., 2016). Its production by picocyanobacteria was detected by Bláha and Maršálek (1999), however, the present concentrations were much lower than the ones in the cyanobacterial blooms. The production of microcystin was then confirmed by other studies like Domingos et al. (1999) or Vareli et al. (2013). Furthermore, Bakr (2022) investigated the toxicity of picocyanobacteria species *Merismopedia minima* and showed the release of the discussed toxic substances and subsequently insufficiency of the Egyptian water treatment in order to remove these harmful compounds. The exposure to these harmful substances causes liver-related disorders and haemorrhages leading, in acute toxicity cases, to death. In mild cases, the health is affected by the nausea, intestinal issues, lungs damage or kidney issues (Van Hassel et al., 2022; Yang et al., 2016). Furthermore, the carcinogenic character is assumed by the inhibition of phosphatases protein and subsequent hyperphosphorylation of cellular proteins. In the last few years, the genotoxicity character is also considered (Lone et al., 2015).

Cox et al. (2005) claim that different groups of cyanobacteria, as well as picocyanobacteria, are also able to produce harmful neurotoxins. The results of the study were supported, among others, by Cervantes Cianca et al. (2012). Not only neurotoxins but also endotoxins, like lipopolysaccharides, can be found in the picocyanobacterial species (Schmidt et al., 1980). Other cases of toxins produced by the picocyanobacteria, which were described in the recent years, are delineated in a few studies such as Leland et al. (2023) or Gin et al. (2021).

1.2.6 The influence of picocyanobacteria on water treatment technology

For the best drinking water quality, the monitoring of the various aspects is essential in order to cover all possible issues. Besides the physical-chemical properties examination, the hydrobiological evaluation is done to prevent complications through technological process in individual water treatment stages and further to reduce the number of organisms and various contaminants passing through the treated water. Organisms influence the quality of water not only with their presence, but also with the possible metabolite production (Ambrožová & Říha, 1998; Říhová Ambrožová, Jana, Hydrobiology specialist, VŠCHT, [oral communication], Prague 2. 4. 2024).

Although picocyanobacteria may be the dominant type of hydrobiological contaminants in several water reservoirs, they are often overlooked and, unlike in marine picocyanobacteria case, the ecology and eventual toxicity is poorly researched. One of the reasons is small percentage of representation in overall phytoplankton composition. The second, substantial reason, is unsuitable methodology of their detection in water industry (Janák & Říhová Ambrožová, 2023).

The Czech laboratories (in accredited and non-accredited mode) perform a microscopic image determination (qualitative and quantitative representation of bioseston) according to ČSN 75 7712 (“Water quality - Biological analysis - Determination of bioseston”), also mentioned in the decree of the Ministry of Health No. 252/2004 Coll (“Decree laying down the sanitary requirements for drinking and hot water and the frequency and scope of drinking water inspections”). Although the methodology of sample preparation by centrifugation followed by microscopic analysis and examination of the concentrated sample on the counting chamber grid is nationally accepted and preferably used to detect the presence of microorganisms, it is completely unsuitable for the detection of phytoplankton cells (picocyanobacteria and picoalgae). The insufficiency of the method lies in completely and fundamentally underestimated results of microscopic analyses (Říhová Ambrožová, Jana, Hydrobiology specialist, VŠCHT, [oral communication], Prague 2. 4. 2024).

Since microscopic analyses are routinely carried out according to ČSN 75 7712, there are no relevant data available in the Czech Republic indicating the actual abundance of pico-sized cells in water sources of different trophic types. Thus, the Czech legislation does not reflect the fundamental influence of these small representatives on the quality of treated and drinking water and the potential contribution to the occurrence of organoleptic water defects

(trihalomethanes, chloroform, geosmin, 2-MIB, etc.) or biological instability (biofilms). The risk of penetration of pico-sized cells and their extracellular polymers through the water technology is comprehensively underestimated. In addition to a suitable method for the detection of picoplankton, it is desirable to reflect this issue in legislation with regard to the importance of risk analysis and safe drinking water according to the previously mentioned Decree No. 252/2004 Coll. (Říhová Ambrožová, Jana, Hydrobiology specialist, VŠCHT, [oral communication], Prague 2. 4. 2024).

According to Janák & Říhová Ambrožová (2023), the most effective technique of picophytoplankton detection is method of membrane filtration and subsequent evaluation of the sample using a light microscope with a fluorescence extension, where this method detects up to four times the amount of picoplankton compared to the currently used technique of quantifying bioseston in waters according to ČSN 75 7712 (concentration by centrifugation).

Since a detailed determination of the type and species of picoplanktonic organism is not possible without molecular biology methods and their toxic potential is understudied, the occurrence of picophytoplanktonic organisms in drinking water must be viewed as a potential threat (Jakubowska & Szeląg-Wasielewska, 2015).

In particular, due to their small cell size, picocyanobacteria are able to penetrate from the raw water source into water treated by conventional technologies and pass through the entire treatment process to the treated water and into the distribution network. Several risks can occur after the cells have penetrated from raw water to treated water. The impact can be twofold – the influence on the operational parameters of the treatment and on the quality of the water.

The first category includes, for example, the risk of shortened filter cycles due to filter clogging (Janák & Říhová Ambrožová, 2024). In the second mentioned case, picocyanobacteria can break through the filters and affect the quality of the treated water in the parameters of colour, turbidity, odour or even taste (Jakubowska & Szeląg-Wasielewska, 2015; WHO, 2015). The cause may be the decomposition of biological material, presence of geosmin or the metabolic activity of the organisms. The issue of the risk of internal harmful substances produced by picocyanobacteria is addressed in Chapter 1.2.5 (Toxicity of picocyanobacteria).

In addition, bioseston can contribute to the formation of chlorinated organic derivatives of the group halogenated methanes that are formed in treated water as secondary products of water disinfection (Janák & Říhová Ambrožová, 2024). Water treatment technology with the use of disinfectant oxidising agents such as chlorine dioxide, cause the formation of assimilated

organic carbon (AOC) by cell breakdown, which can then be used by living organisms such as bacteria, which can potentially develop microbial growth in the distribution system and subsequently generate biofilms in the distribution network (Müller et al., 2003).

According to Janák & Říhová Ambrožová (2024), some picoplankton organisms are capable of withstanding the exposure to disinfectants, thus resulting in further problems in the distribution network. With their development under appropriate conditions (no food competition, no predation, and appropriate temperature), the potential for the decomposition of the distribution network material and its objects increases, as well as the existence of general degradation of water quality in chemical and physical parameters can exist.

Last but not least, the inefficiency of the technology with regard to the separation of picocyanobacteria water may cause incompliance with legislation of drinking water quality – exceeding the hydrobiological requirements for drinking water quality, which are set at 50 dead individuals per 1 mL and 0 live organisms per 1 mL in the microscopic view. These limits are set for the Czech Republic in the Decree of the Ministry of Health No. 252/2004 Coll. entitled "Decree establishing the sanitary requirements for drinking and hot water and the frequency and extent of drinking water inspections" (Česká republika, 2004), including the limitation on quantification of picoplanktonic organisms addressed in the first paragraphs of this chapter.

1.3 Elimination methods of hydrobiological contamination of water

In practice, two different picocyanobacteria elimination principles from the treated water can be encountered. The first group of methods is based in the removal of unbroken full picocyanobacterial cells, while the second one works on the principle of destruction of the cells to form residues that are needed to be dealt with further in the treatment process (Aktas et al., 2013).

This chapter focuses only on selected methods in the group of whole cell elimination, in order to minimise the risk of residues resulting from the cell integrity damage. Four techniques of previous-mentioned full cell elimination of picocyanobacteria are discussed – enhanced coagulation, electrocoagulation, dissolved air flotation (DAF) and membrane methods.

The time period of the technique development also varies. The coagulation process is applied in the drinking water treatment techniques as early as the 18th century, however, the electrocoagulation was introduced and put into operation in the mid-20th century (IWA Publishing, 2024; Vik et al., 1984). A significant development of the DAF application for the water treatment of eutrophic or humic waters occurred in the 1990s also with the ultrafiltration, which has been intensively used for the removal of hydrobiological contamination from raw water for approximately the same period (Haarhoff, 2008; Laîné et al., 2000).

1.3.1 Enhanced coagulation

The conventional coagulation technique, continuously followed by the separation step, can be optimized for efficient removal of picocyanobacteria under fitting conditions (e.g. pH, type, and the dose of coagulant) – this phenomenon is discussed for example in a study by Aktas et al. (2013). In the mentioned study, the removal rate of *Synechococcus* sp. was determined to be in range from 24 to 36% depending on the type and dose of coagulant. However, in the research by Aktas et al. (2012), a removal rate of 56% was achieved.

1.3.2 Electrocoagulation

Another possible separation method is electrocoagulation, which differs from the conventional coagulation by inserting the potential into a reactor with the treated water (Krusinova et al., 2023; Magnisali et al., 2022;). The electrocoagulation process is shown in Figure 3.

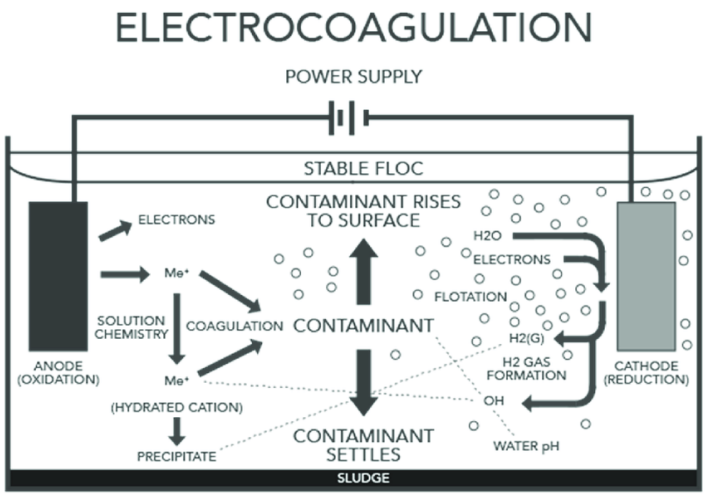

Figure 3. The scheme of the electrocoagulation process (Posavčić et al., 2019)

The application of electrocoagulation, mainly in water treatment, is wide (Jing et al., 2021). Moreover, the discussed technique shows sufficient efficiency in removal of biomass including algae or, if the electrocoagulation is added as the supplementary measure to the treated water technology, also picocyanobacteria. The possibilities of removing picocyanobacterial cells by electrocoagulation technique are currently under investigation by various scientific groups (Aktas et al., 2012; Krusinova et al., 2023).

1.3.3 Dissolved air flotation

Dissolved air flotation (DAF) is nowadays a method frequently placed into the water treatment process to remove various contaminant, including variety of colloids, precipitates, ions, but also microorganisms, proteins, or oils from water (Rodrigues & Rubio, 2007). The first step of the DAF is coagulation. The water containing products of coagulation are linked to the air-bubbles continuing up to the water surface, whereas the treated water is separated from the deeper parts (scheme shown in Figure 4) (Crossley & Valade, 2006). The separation efficiency for hydrobiological contamination in drinking water treatment is as high as 98.5% for DAF (Dolejš, 2006).

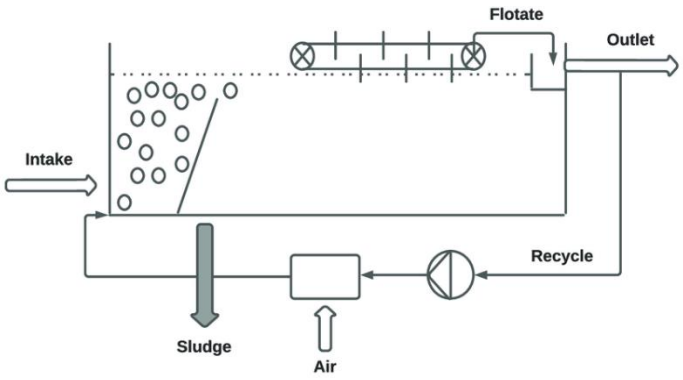

Figure 4. Scheme of the dissolved air flotation system (Philipp et al., 2021)

1.3.4 Membrane separation methods

Membrane separation methods are classified as a physico-chemical process. The technique is based on a semi-permeable membrane – a barrier with the ability to let through only a definite group of particles based on the membrane pore size (Honzajková et al., 2010; Peters, 2010).

The membrane processes are further subdivided according to the pore sizes on microfiltration, ultrafiltration, nanofiltration, and reverse osmosis. However, to this day, the division is not very precise and varies in different sources (Honzajková et al., 2010; Peters, 2010). In Figure 5, categories of membrane separation processes in water treatment and their efficiency are shown.

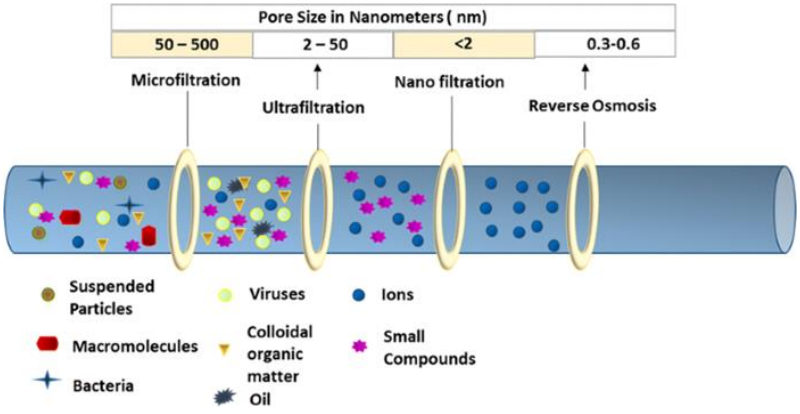

Figure 5. Categories of membrane separation methods and their efficiency of molecules (Rezakazemi et al., 2018)

Ultrafiltration (UF) is a membrane filtration process that is used to separate bacteria, viruses, or parasites and molecules of size in the interval 10-100 nm, however, likewise the division of individual methods of the membrane separation, the interval size of the UF varies (which is observable also in the difference between the Figure 4 and other citated literature). The principle is based on the size exclusion – molecules larger than the membrane pore size remain in front

of the membrane, while, smaller molecules pass through. Compared to e.g. reverse osmosis, minerals are still present in water even after this step of the water treatment (Nakatsuka et al., 1996; Woodard, 2022; Xia et al., 2004).

1.4 WTP Milence

The water treatment plant (hereinafter referred to as WTP), located in the village of Milence about 20 km far from the town of Klatovy, began its operation in 1988. WTP Milence produced water intended for human consumption for the towns on the route of the group water supply system – Klatovy, Domažlice and other towns and municipalities, which amounts to approximately 60 thousand consumers. Originally, the treatment plant was dimensioned for 440 L/s, after reconstruction and modernization in 2005, an average of 110 L/s of drinking water is produced (Gubric, 2008).

1.4.1 Water reservoir Nýrsko

The source of raw water for the WTP Milence is the water reservoir Nýrsko (Figure 6), which is located on the upper stream of the Úhlava river. This reservoir is placed in the 3rd zone of the National Park Šumava (Marval et al., 2017).

Figure 6. Water reservoir Nýrsko (Znachor, 2023)

The flooded area is 148 ha and the reservoir itself receives a total volume of 20.75 mil. m^3. The catchment area collects water from 80.9 km^2. The crest length is 320 m (Figure 7), and the height above the terrain 36.2 m. The drainage and communication tunnels are stretched along the left bank. The circular tower was built at one end of the communication tunnel. It contains raw water intake points for WTP, a shaft overflow, and two lower outlets. The raw water comes from the Nýrsko water reservoir into the WTP Milence from the water intake point

at 506.5 MASL at depth of 17.75 m from the water level in the full reservoir (Marval et al., 2017; Středa et al., 2017).

Figure 7. The dam of the water reservoir Nýrsko (author´s photo)

1.4.2 Chemical quality of raw water

The chemical quality indicators of the raw water from the water reservoir Nýrsko are very good and stable. The content of organic substances (including humic substances) expressed as COD_{Mn} is low (COD_{Mn} 1.2-2.0 mg/L), water reaction value pH above 6, very low nitrate concentrations. The water is slightly mineralized and therefore very soft (Rutová & Balejová, 2023).

1.4.3 Hydrobiological conditions of raw water

The first note of the higher number of organisms[1] in the reservoir water was detected in 2014, the found organisms were determined as nonspecified picocyanobacteria (Sochor, 2020). In Figure 8, the picocyanobacteria from the water reservoir Nýrsko can be seen. They were determined by Associate Professor Jan Kaštovský (Department of Botany, Faculty of Science University of South Bohemia in České Budějovice) as *Synechococcus* sp.

[1] The definition of the terms "organism" and "picocyanobacteria" are defined by the standard ČSN 75 7712 ("Kvalita vod - Biologický rozbor - Stanovení biosestonu(Water quality - Biological analysis - Determination of bioseston)) and ČSN 75 7717 ("Kvalita vod - Stanovení planktonních sinic (Water quality - Determination of planktonic cyanobacteria)) – as "organism" is described as every living creature inside of the sample, as "picocyanobacteria" are defined these creatures fulfilling the characterisation of this group of organisms (see Chapter 2.1).

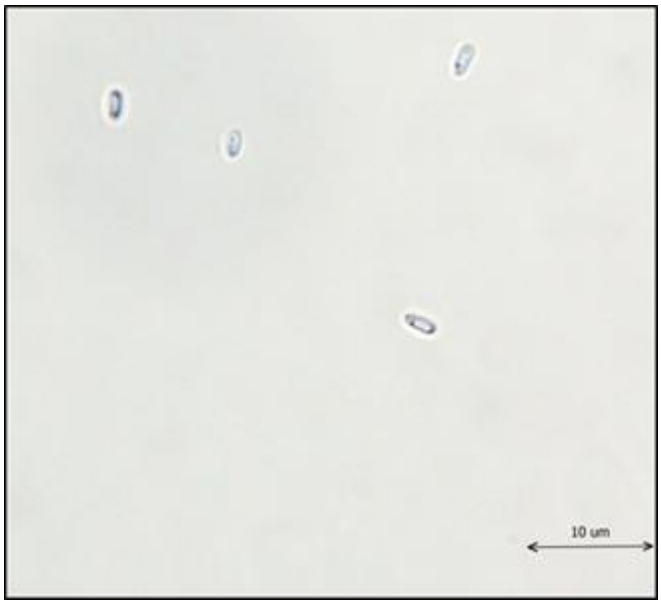

Figure 8. Synechococcus sp. from the reservoir Nýrsko (Source: Povodí Vltavy, Dr. Hess, 2016)

Further analyses confirmed a higher number of picocyanobacteria in the water, especially in the summer season – more than 10^3 picocyanobacteria in 1 mL of water. The largest detected amount was in the sample from 5. 8. 2015, namely 27.6×10^3 org./mL of picocyanobacteria (Sochor, 2020).

1.4.4 Technology of water treatment

Raw water uses coagulation filtration coagulated with prepolymerized coagulant PAX 18 (Pivokonský et al., 2020). The pre-alkalization is not used. The aggregation of flakes is observed in the aggregation reservoir. Then the water with the products of aggregation – commonly called flakes – is filtrated using 4 sand-type filters with filtration cycles of 96 hours (system: 3 in operation, 1 in cleaning), which contain the Leopold drainage system. The following chemicals are added to the filtered water: lime water ($Ca(OH)_2$), carbon dioxide (CO_2), and chlorine dioxide (ClO_2), whereby the water is stabilized to a calcium carbonate balance and sanitized. The treated water is collected in the water tank (2 x 2,500 m^3) and is pumped in the direction towards Domažlice. In the direction of Nýrsko, Janovice nad Úhlavou and Klatovy, water is forced to flow by the gravitation (Sochor, 2020).

The scheme of current technology of water treatment at WTP Milence is shown in Figure 9.

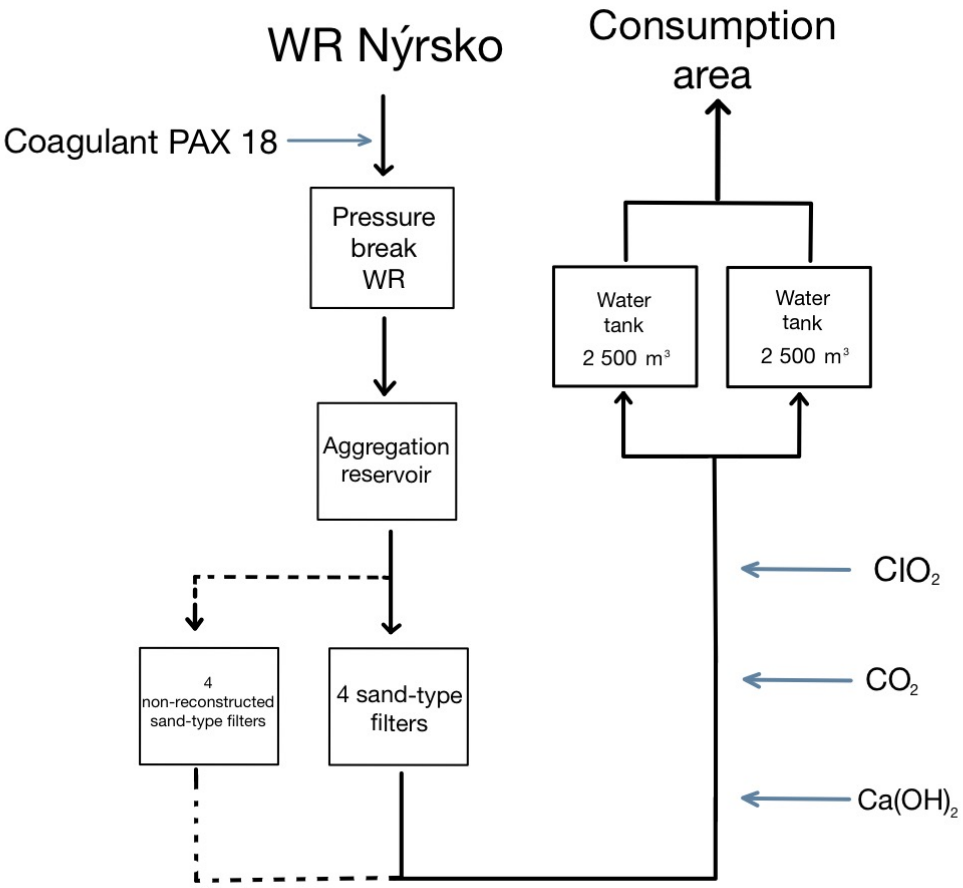

Figure 9. The scheme of the WTP Milence (author´s scheme)

1.5 Magnetic modification of cells

The water companies, technologists and scientists try to remove not only hydrobiological material but also harmful substances coming from the anthropologic sources such as industrial dyes or agricultural pesticides. This thesis discusses and experiments with the idea of the application of the magnetized picocyanobacteria as a sorbent of pollutants, in this particular case, dyes.

For the majority of cells, the diamagnetic behaviour occurs; the only group of magnetically responsive cells are magnetotactic bacteria (Faivre & Schüler, 2008; Wang et al., 2020). The main property of these magnetically modified cells is interaction with external magnetic fields (Safarik et al., 2014; Safarik et al., 2016). Magnetic conversion is usually achieved by the attachment of the (nano)particles exhibiting ferro- or ferrimagnetic properties. This process is called "magnetic labelling", or, when talking explicitly about the labelling of the cells, the process is termed as "magnetic decoration." The term "decorated cells" is assigned to cells with magnetic micro- or nanoparticles present on the surface of the cell (Safarik et al., 2014).

The iron oxides are applied as these magnetic labels – particularly two compounds with formulas Fe_3O_4 and γ-Fe_2O_3 called as magnetite and maghemite. The most frequent method of preparing these magnetic compounds from their precursors is precipitation in alkalic conditions ($pH \approx 12$) of ferrous and ferric salts (Safarik et al., 2016). However, many micro- and nano-particles are also available commercially (Safarik et al., 2012). The increased utilization of these particles is nowadays caused by high biocompatibility, easy regeneration and manipulation using basic tools and non-damaging magnetic field (Prochazkova et al., 2013).

Many of the cells or other biological materials can be subjected to magnetic modifications and are object of many research and trials. Experiments with yeast were performed and presented by Safarik et al. (2014), with cells of microalgae described by Safarik et al. (2016) or macroalgae studied by Mullerova et al. (2019) or with peanut husks as sorbent of heavy meals as reported in article by Rozumová et al. (2016).

Furthermore, many micro- and nano- particles (magnetized or not) are investigated also in the biological and chemical research in various contexts containing the topics of diverse kinds of sensing, drug delivering, different types of imaging (such as magnetic resonance), as polymer fillers or using the ability to affect UV protection or different types of dyes (De et al., 2008; Stark et al., 2015). Currently, the magnetic particles are a highly attractive tool in the field of biomedical research. These particles can be used for specific biomolecules tagging or tagging

of possibly or realistically harmful organisms for the human body – such as viruses or variety of cells. Not only they can be used as the tags but also as the hyperthermia agents, in chemotherapy or tools for malignant tumour treatment (Giustini et al., 2012; Xu et al., 2019).

1.6 Biosorption and biosorbents

The definition of biosorption is generally described as the removal of target substances from solutions by biological materials. Biosorption is a physical-chemical process that is independent of metabolism and involves different mechanisms. These can contain absorption – the substance is taken up (= is absorbed) by another substance, molecule or a living organism (such as absorption of dyes by fish scales (Kabir et al., 2019)), adsorption – the process when the molecules are sticked to the sorbent forming a thin film on the outside (which is the case of the experiments performed in this bachelor thesis), and precipitation – the mechanism when the substance is precipitating on the surface of the sorbent – this process is often used for the sorption of heavy metals (such as biosorption of heavy metals by the exopolysaccharide produced by *Paenibacillus jamilae* (Morillo Pérez et al., 2008)), but also the ion exchange – the process of swapping of ions present in any carrier (as the example, the Pb(II) ion exchange adsorption presented in the study by Ngah & Fatinathan (2010) can be used) or surface complexity meaning the sorption of some molecule or atom to the surface forming complex – this mechanism can be used in processes such as removal of copper ions by hyacinth roots (Zheng et al., 2009). The mechanisms behind the applications are based on interactions of the target molecules with specific functional groups (Safarik et al., 2021). All different types of biomaterials – such as bacteria and algae but also biological industrial waste, have generally demonstrated strong biosorption abilities for all different kinds of metal ions (Vijayaraghavan & Yun, 2008). The rate and quality of biosorption depends on lots of factors – such as temperature, pH, salt concentration (salinity) and/or concentration of the target substance (Kalita & Baruah, 2023).

After a biosorbent is used for the initial purpose, it can be continuously recycled for other purposes. In this matter, the circulation and recycling of the yet used biomass can be essential part for the world economy. With a smart approach to recycling of this biomass, waste can be significantly reduced down to the so-called “end of the waste” scenario (Ciani & Adessi, 2023). In these actions of the recycling, remediation, and recovery of the biosorbents are essential for the appropriate and sufficient base for the circular economy (Madeła & Skuza, 2021). According to Hassan et al. (2020), few laboratory experiments observed the possible recycling of the magnetic biosorbent using various types of acids and bases or organic solvents for the removal of the pollutant. Nevertheless, this approach, used for example in wastewater treatment, could lead to another risk, which is the observation of the secondary waste, such as mixtures of inorganic and organic solvent with the pollutants. That can potentially introduce

hazard for the environment in terms of proper waste handling (Hassan et al., 2020). In a review by Yadav et al. (2021), the author stated that cyanobacteria used for the removal of heavy metals in wastewater treatment process can be further applicable for the production of the biofuel. Continuously, these products further help to reduce greenhouse gases by replacing diesel fuel. In the cases of uptake of ions beneficial to the soil, Robalds et al. (2015) suggests using the biosorbents, specifically the peat biomass, in agriculture and environment revitalization to improve the quality of the soil conditions, in terms of higher amounts of phosphorus, organic carbon or ability to hold water content.

1.6.1 Biosorbents of dyes

Currently, wastes containing synthetic dyes cause serious water pollution, especially in industrial wastewater. It has become a significant environmental issue (Diaz-Uribe et al., 2021). Numerous industries are especially implicated in water pollution as a result of various municipal, agricultural, and industrial activities. These represent significant threats to both the environment and human health at the same time (Elgarahy et al., 2021). These pollutants are also chemically, biologically, and photolytically stable and tend to be carcinogenic and toxic (Nidheesh et al., 2018).

In recent years, research has been published on the applications of various organisms in the matter of pollution absorption. Yeast, fungal, bacterial and algae biomass, or spent coffee grounds have been part of research as biosorbents to remove various pollutants (Safarik et al., 2021). Specifically, for the removal of dyes the magnetized cells of yeast (Safarik et al., 2007) or *Chlorella vulgaris* (Khorasani & Shojaosadati, 2019) were used.

1.7 Dyes

Variety of inorganic or organic pollutants is transferred into the environment from the wastewaters coming from a wide range of industries. Especially dyes are the second largest class of pollutants regarding the water contamination (Teo et al., 2022). These substances can continuously cause different medical issues and complications to the human organism. Not only that these dyes obstruct the light to penetrate into the deeper water column parts and subsequently limit the organism but can also cause serious health problems to humans with their carcinogenic or mutagenic properties (Ismail et al., 2019). The complications which can be caused by the presence of these polluters in water are vomiting, shocks or heart disorders (everything depends on the type of the dye and its quantity). This thesis experiments with five dyes widely present in the wastewater. All belonging to a group of organic dyes and confirmed by various institutions as hazardous and harmful for humans.

1.7.1 Malachite green

Malachite green (continuously as MG) is a green-coloured organic compound with the formula $C_{23}H_{25}ClN_2$ (Figure 10) belonging to triarylmethane dyes (NCBI, 2023).

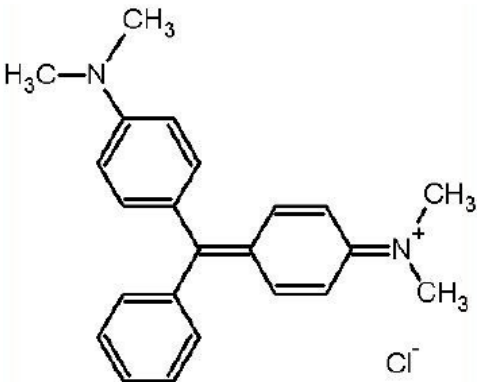

Figure 10. Structure of Malachite green (Prabakaran & Arivoli, 2012)

MG itself is used for various purposes. This substance is used in aquaculture as a biocide, especially due to its ability to eliminate fungi, different types of parasites, and protozoans. This dye is also used in the textile industry for colouring different types of materials, such as cotton, leather, wool, or silk (Safarik & Safarikova, 2002; Srivastava et al., 2004).

Concerns regarding Malachite green are mentioned in many literary sources. The cytotoxicity of mammalians and its effect as an enhancement factor and ability to cause defects in a developing fetus has been proven for this particular compound. At present, MG is banned or

regulated in countries of the European Union due to the toxic properties of this dye (Li et al., 2019; Safarik & Safarikova, 2002; Srivastava et al., 2004).

1.7.2 Methylene blue

The Methylene blue (further referred to as MB) is a water-soluble, basic, blue to dark green dye with the heterocyclic structure having the molecular formula of $C_{16}H_{18}N_3ClS$ and IUPAC name 3,7-bis(dimethylamino)-phenothiazine chloridetetramethylthionine chloride (structure displayed in Figure 11) (Khan et al., 2022).

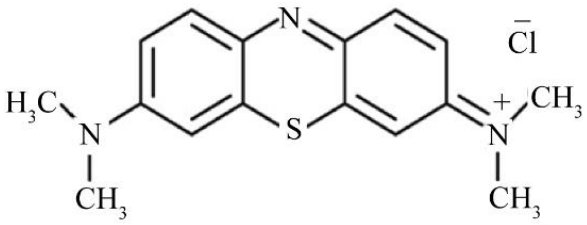

Figure 11. Structure of Methylene blue (Elmorsi, 2011)

The applications of this dyes are various, especially in medicine and biotechnologies. The diluted solutions are applied in treatment of anaemia, cancer (as a part of the chemotherapy treatment), or used as an antiseptic reagent, as an agent against the RNA viruses (causing the inactivation through the photo sensitivity of the dye to activate the drug) (Khan et al., 2022; Oz et al., 2009;). Toxicity of the discussed dye was proven to be in many factors as teratogenicity, embryotoxicity, or carcinogenicity. Furthermore, due to poor biodegradability, mainly of the aromatic ring, the intoxication with MB is accompanied by the following symptoms: issue in gastrointestinal system, respiratory complications, cardiovascular problems, impact on central nervous system or dermatological influence (Li et al., 2023; Oladoye et al., 2022).

1.7.3 Rhodamine B

Rhodamine B is classified into the group of xanthine dyes with basic and cationic properties. Its sum formula is $C_{28}H_{31}ClN_2O_3$ with structural molecular formula [9-(2-carboxyphenyl)-6-diethylamino-3-xanthenylidene]-diethylammonium chloride (the overall structure can be seen in Figure 12) (Saigl, 2021; Xu & Ma, 2021).

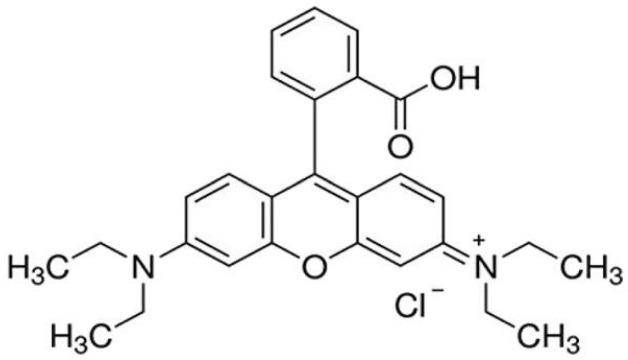

Figure 12. Molecular structure of Rhodamine B dye (Amaterz et al., 2020)

The discussed dye has a wide range of applications, mainly in textile industry, but also in food or paper colouring, and as a label agent for water tracking. However, this organic dye has also application in scientific research mostly for the microscopy purposes (Hakami et al., 2021; Saigl, 2021; Tran et al., 2021). The Rhodamine B is considered to be an exceptionally toxic dye not only for humans but also for aquatic organisms in general (Tran et al., 2021). It is proven that this organic dye has carcinogenic, neurotoxic, and teratogenic properties and can cause issues and complications in different parts of human body including gastrointestinal tract or respiratory system. Nevertheless, the difficulties may arise also in terms of various skin or eye irritations, nausea, pain in miscellaneous parts of the human anatomy. It can cause tumour or issues with reproduction. In terms of aquatic environment hazards, the Rhodamine B dye causes the reduction of the photosynthesis rate due to redundant catch of light instead of the photosynthetic organism. The lethal dose concerning the vertebra, more specifically fish, was stated to be 83.9 mg/L (Imam & Babamale, 2020; Jain et al., 2007; Kornbrust & Barfknecht, 1985).

1.7.4 Crystal violet

Crystal violet, further referred to as CV, is a dye belonging to the group of triaryl methane dyes with blue to violet colour with a formula $C_{25}N_3H_{30}Cl$ and IUPAC name 4,4′,4″-tris(dimethyl-amino)triphenylmethyl chloride (the structure can be seen in Figure 13) (Chakraborty et al., 2011; Chen et al., 2008; Hakami et al., 2021).

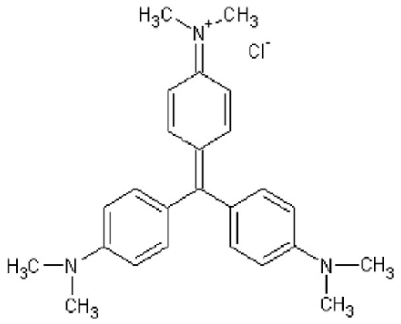

Figure 13. Structure of Crystal violet dye (Sudha et al., 2018)

The applications of CV are various, however, the application in the textile industry, as the colouring agent for cotton and wool materials, is mentioned as one of the most essential one. In small concentrations, this dye can be also used for biomedical or forensic purposes – as agents against viruses, bacteria, or fungi, for initial burns treatment, in forensic analysis, the dye may be applicable for the detection and enhancement of blood fingerprints (Hakami et al., 2021; Maley & Arbiser, 2013).

The toxicity of CV lies mainly in its non-biodegradable carcinogenic characteristics. However, this compound can also cause several types of irritation and if the amount of the substance is sufficient, the failure of various organs including kidneys or lungs or, when in contact with eyes, permanent blindness can appear (Chakraborty et al., 2011; Cheruiyot et al., 2019).

2 Work objectives

The aim of the bachelor thesis was to study picocyanobacteria in terms of their occurrence in raw water source and their removal during the treatment of drinking water. The second aspect of the work was to find out whether it is possible to use picocyanobacterial cells to remove undesirable substances from water. The aims were divided into two interconnected parts, containing the following objectives:

Part A:

- Observation of the amount and development of picoplanktonic cyanobacteria in water reservoir Nýrsko in the season 2022 and individual technological stages of WTP Milence.
- Determination of how deep the picoplanktonic cyanobacteria penetrate into the water treatment technological line.
- Determination of dependence of separation efficiency of the filters on the filtration time. Analysis of elimination options of the picoplanktonic organisms from treated water.

Part B:

- Testing of magnetically modified picocyanobacterial cells for use as a biosorbent of dyes.

3 Methods

3.1 Water sampling

The water samples for the investigation were collected according to "ČSN EN ISO 5667 (75 7051) Kvalita vod – odběr vzorků" method by 2 instruments – a sampler and a tap. The list of samples and their sampling method are shown in Table 1 (the images of the individual sampling profiles are attached in supplements Figure S1 (a-i).

Table 1. Sampling profiles and their sampling method

Sampling profile	Collection method
Water Reservoir Surface	Sampler
WTP Raw Water	Tap
WTP Aggregation	Sampler
WTP Filters (3x)	Tap
WTP Before Accumulation	Tap
WTP After Accumulation	Tap
Water Tank Janovice	Tap

Due to the limited access to merging of all three water streams that come from the filters, the sampling point "WTP Filter" is placed after each of the operating filters. The subsequent sampling profile is "Before Accumulation" after the disinfection and other quality-adjusting chemicals were administrated.

The sampling campaign took place from May to October in 2021 in the water reservoir Nýrsko (49.2530003N, 13.1526539E) and the WTP Milence (49.2667014N, 13.1546392E). The water samples were collected into plastic bottles marked with the location and date of collection. In the case of samples coming out from the filters – the filtration time was specified for further examination. The volume of the sample was equal to 60 mL. 3 mL of formaldehyde (36-38%, p.a.; PENTA) were added to the water sample causing the preservation of the organisms present. The fixed water samples were stored in the fridge in the dark to conserve all present organisms and to protect the photosynthetic pigments autofluorescence from degradation.

3.2 Counting of picocyanobacteria

3.2.1 Preparation of the sample

For the staining of the cells (to focus and review the background of picocyanobacterial data), the DAPI staining was used. DAPI is a dye with the chemical formula 4′,6-diamidino-2-

phenylindole. This substance is used to stain DNA by binding to A-T clusters in DNA (Porter & Feig, 1980).

First, the staining apparatus was prepared in the fume hood (Figure S10). The polycarbonate (PCTE) membrane black filter was placed (with the shiny part up) on the column covered with a glass cylinder and secured with a clamp. The stated water volume depends on the expected number of picocyanobacteria – 1 mL in the summer samples, 5 mL in the autumn ones, 20 mL in case of the very low number of the picocyanobacteria in the sample (cases of samples "Before Accumulation", "After Accumulation", "Water Tank Janovice ") – was poured into the staining apparatus. Concerning samples with higher water volume, an appropriate volume of liquid was let through the apparatus until 1 cm above the filter was left. Then the 60 µL of DAPI stain was pipetted into the sample and allowed to sit for 60 seconds. Subsequently, the suction filtration was performed. The glass slide was labelled, and the filter was transferred on the glass slide. The immersion oil droplet was added on both, the top of the filter, that was placed on the marked slide, and on the top of the cover slide, which covered the filter, to enable clear microscopy using high magnification.

The apparatus used for DAPI staining is shown in Figure 14.

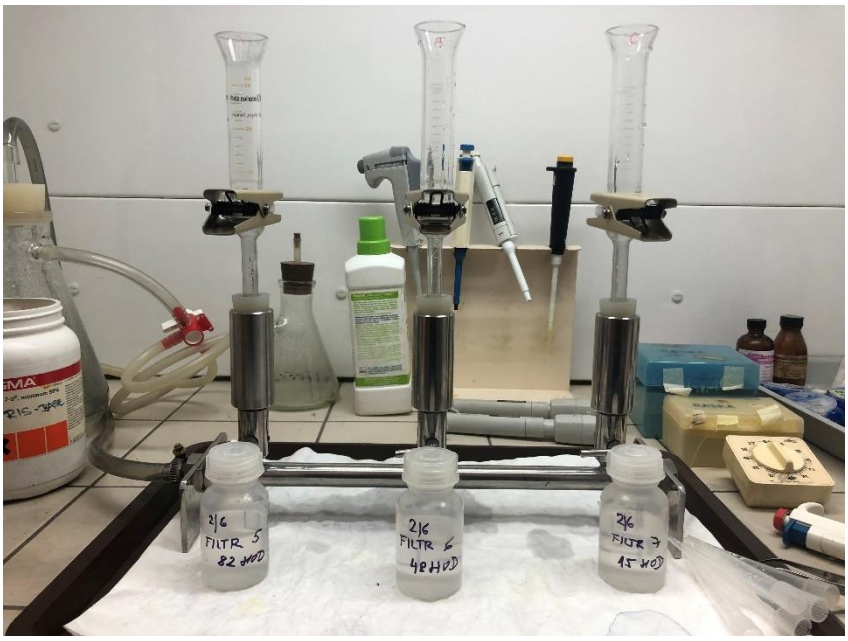

Figure 14. The apparatus used for the purpose of DAPI staining of picocyanobacterial cells and counting filter preparation (author´s photo)

3.2.2 Counting of picocyanobacteria

The fluorescence microscope (shown in Figure 15) used for the purposes of counting picocyanobacteria was Olympus BX60 with ocular Olympus WH10X/22.

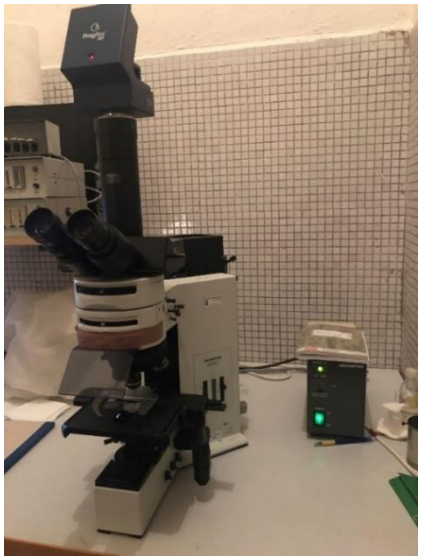

Figure 15. Fluorescence microscope Olympus BX60 used for the purpose of the thesis (author´s photo)

The procedure was as follows: Firstly, cells were focused with the fluorescent microscope. The focused cells are shown in Figure 16. Excel-based programme for cell counting was used. The setting for the program was following: First, the experimental name was entered. Subsequently, the name of the sample (location and date) was defined. Then, the sample volume (used while preparation), dilution factor (1), fit. area (314), counting grid (0.1965), and grid factor (1) were entered into the programme. After that, using fluorescence microscopy and keyboard, picocyanobacteria in individual samples were counted. The entire filter surface was examined step by step counting all shining picocyanobacterial cells on the whole visible field. The enumeration was finished after reaching 500 cells (by screening the whole filter surface) or 50 fields (in case of low number of the picocyanobacterial cells). The data were then copied to the prepared Excel spreadsheet (Microsoft 365 Office, version 2403) where they were further processed.

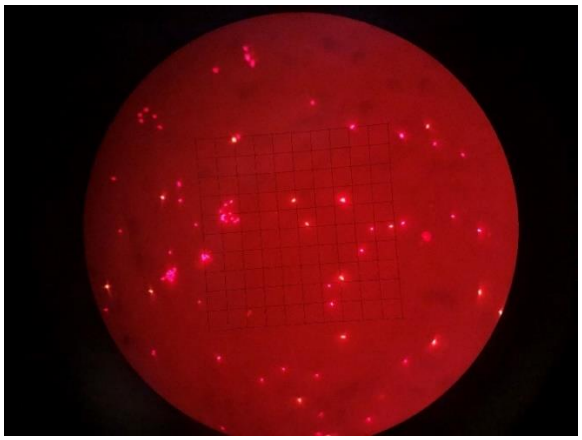

Figure 16. Image of the counted picocyanobacteria sample (prepared by DAPI staining) in the fluorescence microscope (author´s photo).

To determine the final amount of picocyanobacteria in the individual samples the counted numbers have to be recalculated as in Equation 1:

$$Amount\ of\ Pcy = \frac{\bar{x} \cdot \mathrm{FltA}}{V \cdot \mathrm{FOV\ A}}, \quad (1)$$

where $\bar{x}$ is an average number of picocyanobacteria in the sample calculated by the programme, *FltA* is fit. area (314), *V* is the volume of the sample and *FOV A* is the factor for the used visual field (0.1965). Furthermore, the ind% (percentage of individual free cells) and col% (percentage of the colonies in total amount) were calculated by the division of the respective number by total number of picocyanobacteria. The re-calculated results were transferred to the other Tables S1-S9 (see supplements).

The graphical representation of the results was performed in the software GraphPad Prism (version 10).

3.3 Cultivation and harvesting of picocyanobacteria

Cultivation of 6 various picocyanobacterial strains from the collections of the Hydrobiological institute of the Biology Centre of the Czech Academy of Sciences (RD51, Vír, RDKV18, STRS29, D1, Nýrsko picocyanobacteria strain) was performed for the nanobiotechnology experiments. The entire inoculation process was carried out in a sterile environment (a sterile clean bench, Figure S2) to avoid contamination with other microorganisms from the environment.

Foremost, the Erlenmeyer flasks were labelled with the type of the culture medium – WC (Guillard & Lorenzen, 1972), or BG-11 (Stanier et al., 1971), the name of the culture and the date of inoculation (Figure 17). The medium was previously prepared and autoclaved by the

laboratory technician. Before the usage of the WC medium, 1 mL of a sterile vitamin mixture – Cobalamin (B12), Thiamine (B1) a Biotin (B7, H) – was added to 1 L of the sterile and cooled solution using a micropipette in the sterile clean bench. Then, the sterile Erlenmeyer flasks and the bottles with the prepared medium were transferred to sterilised conditions in the clean bench. The neck of the flask and of the medium bottle were burnt over the flame to remove external microorganisms from the surface. The cotton plug was removed, 200 mL of medium was poured into the flask, and 1 mL of the pre-cultured picocyanobacteria were pipetted inside. Subsequently, the Erlenmeyer flask was closed with the sterile cotton plug quickly sterilized above the burner.

Cultures of picocyanobacteria were grown for 6 weeks in a culture room with a constant temperature of 19°C and a light cycle of 16h/8h (light and dark ratio). Light intensity was on average 22.23 µmol photons/(m^2s).

Figure 17. Erlenmeyer flasks inoculated with picocyanobacterial strands RD51 and Vír (two of the cultures used for the biosorption experiments) (author´s photo)

The picocyanobacterial strains were harvested in 50 mL falcon tubes in the centrifuge Avanti JXN-26 (Beckman Coulter), spinned for 20 min at 4000 rpm. During the experiments mentioned in Chapter 4.6.2, the picocyanobacterial biomass was harvested and lyophilizated. Lyophilization was performed in the Lyofilizator L10-55 (GREGOR Instruments s.r.o.) for 48 hours and -26°C.

3.4 Magnetic modification of the picocyanobacterial cells

3.4.1 Preparation of non-stoichiometric magnetite

In one-litre beaker 1 g of iron (II) sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$; p.a., Lach:Ner, catalogue number: 30193-APO, batch number: 2013/09403) was dissolved in 250 mL of distilled water. While mixing continuously, 1M solution of sodium hydroxide (NaOH; index number: 011-002-006, batch number: 1704190412, PENTA) was added until the complete precipitation of magnetite and pH of 12 controlled by universal pH paper. The reason for the non-use of a pH meter is the fact that the synthesis of the microwave synthesised iron oxides can be performed in the pH range from the 10-13 – this statement is also supported by a variation of experiments mentioned in the bachelor thesis written at the Department of Experimental Physics at Palacký University Olomouc by Ochmann (2015). After this step, the entire volume was placed in the home microwave oven for 10 minutes at the maximum output of 800 W. After heating, the magnetite was separated using a NdFeB magnet and washed three times with deionized water to neutral pH, which was checked by the pH paper (Figure 18). Subsequently, the supernatant was decanted until the ratio of magnetite to supernatant was 1:4.

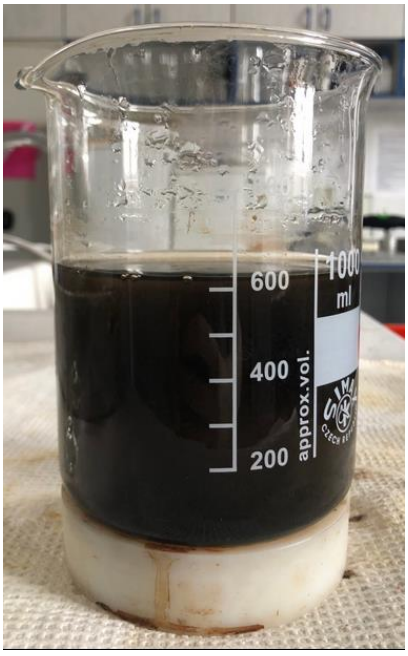

Figure 18. Microwave synthesized magnetite on the NdFeB magnet (author´s photo)

3.4.2 Magnetization of the cells

For the experiments with magnetised cells, dense picocyanobacterial cultures inoculated for specific time period were used. The inoculation and growth of picocyanobacteria are described in Chapter 3.3. Before performing the experiments, 150 mL of the picocyanobacteria

culture was centrifuged for 30 minutes with the rate of 4000 rpm, after that the supernatant was discarded. 9 mL of the cultivated strain of picocyanobacteria was transferred to the labelled container and 1 mL of the magnetite suspension (microwave synthesized magnetite, 1:4 (magnetite to supernatant)) was added. The solution was left to mix using the rotator (Stuart) for 20 hours at 20 rpm at the laboratory conditions (20°C, standard atmospheric pressure). After this period, the magnetised cells were fixed with formaldehyde in a ratio 1: 20.

3.4.3 Adsorption experiments

Based on the previous experiments and research with other biomass types, the volume of dye and picocyanobacterial biomass with microwave synthesized magnetite volume were selected. The selection was based on the literature for Malachite green – "Magnetically modified spent coffee grounds for dyes removal" (Safarik et al., 2012) and "Dyes adsorption on magnetically modified *Chlorella vulgaris* cells" (Safarikova et al., 2008). Volumes of the picocyanobacteria and microwave synthesized magnetite were advised from the previous experiences by supervisors prof. Ing. Ivo Šafařík, Ph.D., DSc. and RNDr. Jitka Procházková, Ph.D. and scientific papers such as Baldikova et al. (2016) or Safarik et al. (2016). The volume of magnetized picocyanobacteria prepared as in the Chapter 3.4.2 (V = 1 mL) was pipetted into the resealable tube and 5 mL of the dye with known concentration (depending on the experiment in range of 10 to 5,000 mg/L) was added.

To prove that the sorption is predominately done by the picocyanobacterial biomass, the control tubes, analogically with the same volumes, only with microwave synthesized magnetite and pure picocyanobacterial mass were set with the same conditions (5 mL of 100 mg/L of MG dye).

This mixture was allowed to rotate for 1 hour (Chapter 4.6.1 and Chapter 4.6.2) or 24 hours at 20 rpm and at laboratory temperature and standard atmospheric pressure (Figure 19).

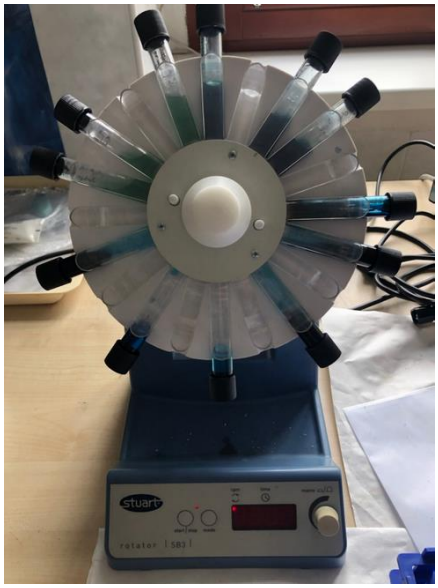

Figure 19. Solution of magnetised picocyanobacteria and dye left for the incubation on the rotator (author´s photo)

After reaction time the suspension of picocyanobacteria and dye was measured using UV spectroscopy. To obtain clear solution without any interfering particles, the magnetic suspension was placed on the magnet to separate the magnetised picocyanobacteria with the absorbed dye from the residue of the unabsorbed dye. In the case of the non-magnetized picocyanobacterial cells, the centrifugation was used. For the absorbance measurement of the individual sample, the volume of 1 mL of solution was pipetted into the plastic cuvette (1.5 mL semi-micro, PS, Cat. No. 7590 15, ISO 9001-14001, BRAND).

If the absorbance of the initial concentration was greater than 1.500 AU, the solution was respectively diluted. As a blank, distilled water was used.

To enhance the performed experiments in order to find out the best conditions for the picocyanobacterial adsorption ability analysis and to generalize and prove the good ability of the picocyanobacterial cells adsorb dye pollutants, the experiments were continuously modified to reach the best adsorption results and incubation conditions. The upgraded methodology was performed with the picocyanobacterial genera from the water reservoir Nýrsko were successfully cultivated and tested for the nanobiotechnology experiments and applications. The basic conditions of the experiment remained constant – the volume of used picocyanobacteria (V = 1 mL), the volume of the dye (V = 5 mL), time of cultivation of the picocyanobacteria with

microwave synthesized magnetite (24 hours), evaluation method (UV spectroscopy). The only parameters which were updated were used dyes, to achieve, if possible, no side effects of the potentially released chlorophyl and the best differences between initial dye absorbance and the absorbance of the dye solution after adsorption. For the experiments in Chapter 4.5, the following dyes were used – Malachite green (taken from the previous experiments as the one with the best adsorption on the picocyanobacteria, possible risk of chlorophyl interference), Malachite blue (one of the measured samples can be seen in supplements Figure S5), Rhodamine B and Crystal violet.

For further analysis of the picocyanobacterial biomass and its properties in terms of pollutant adsorption, the two picocyanobacterial life stages – in exponential phase ("Log phase", the phase with the highest picocyanobacterial growth rate, 5 weeks old) and in the end of their life cycle ("Old Pcy", 12 weeks old), were tested in the same experiment with the same conditions (Chapter 4.5). To be able to see the difference between the two samples (initial and after adsorption) the concentration of the dyes was also adjusted to two cases of higher concentrations – 10 mg/L and 50 mg/L.

Due to the fact that most of the biosorbents (biomass used for the pollutant sorption) are stored and subsequently used in the lyophilized form, the experiments in Chapter 4.6.2 were performed with the freeze-dried biomass of the equivalent genera and amount of picocyanobacteria as in the above-mentioned previous experiment. The volume of picocyanobacteria dried for the experiments was 1 mL (to maintain the quantitative parameters of the previous experiments) with the final mass of the average of 1.3 mg of dried biomass. Subsequently, the Methylene blue was chosen from the previous experiments as the best exemplary sample (in the case of adsorption parameters, visibility, and non-interference with released chlorophyl). The concentrations of dye used for conducting the experiment were 5 – 500 mg/L to find the maximum adsorption ability of the picocyanobacteria (for more information see Chapter 4.4).

The experiments were performed in triplicates for the most precise measurement. For the purpose of this thesis, the Spectrophotometer V-1200 VIS SING (VWR, Belgium) was used. To compare the ability to get attached on the microwave synthesized magnetite, the magnetized cultivated picocyanobacterial biomass from water reservoir Nýrsko were analysed using fluorescence microscopy.

3.4.4 Evaluation of the measurements

The absorbances of individual triplications were used in the calculation of the *ct* concentration of free dye and the *qt* value for the amount of dye bound per unit of sorbent. The formulas for the *ct* an *qt* were derived using following assumptions.

In photometry, the Lambert-Beer law (Equation 2) is applicable:

$$A = \varepsilon \cdot c \cdot d, \tag{2}$$

where ε is molar absorption coefficient in $dm^2/(mol \cdot cm)$, c is concentration in mol/L, and d is path length in solution in cm (in this case, this value is 1 cm and thus it´s not included in other calculations).

In Equation 2, ε can be considered as a slope due to the fact that dye concentrations in this case are not molar but in mg/L (ε is the derivative of the original coefficient in the calibration graph. The respective calibration curves were plotted in GraphPad Prism (version 10) software as well as the performance of the linear regression analysis). The "ε" has the function of the slope "k" in Equation 3.

$$y = k \cdot x \tag{3}$$

Overall equation for the *ct* (t – time dependence) is seen in Equation 4:

$$ct = \frac{A}{k}, \tag{4}$$

where *A* is the absorbance (with subtracted blank) in absorbance unit (AU) and *k* is the slope calculated from the calibration curve.

The *qt* (Equation 5) was based on the formula of mass which is equal to concentration multiplied by volume. Consequently, the amount of dye bound to the sorbent is equal to the difference between the amount of dye entering in time 0 and the amount of free dye remaining in time t. The total amount of dye bound is expressed per unit weight of sorbent - in this case grams.

$$qt = \frac{(0.005 \cdot c_0 - 0.005 \cdot ct)}{m} \tag{5}$$

In Equation 5, the c_0 is the initial concentration of the dye in mg/L, *ct* is the concentration of the free dye in the solution in mg/L and *m* is the mass of the magnetically modified

picocyanobacteria in the solution (weight of the picocyanobacterial mass of centrifuged 1 mL of solution), 0.005 represents the 5 mL of the dye used.

The calculations in the Microsoft Excel software ((Microsoft 365 Office, version 2403) were performed as follows: Firstly, the absorbance in AU was measured using the UV spectroscopy instrument, the triplication was arithmetically averaged and subsequently multiplied by dilution factor resulting in relative absorbance *A*. Then, the blank was subtracted. To get the *ct*, the absorbance was divided with the slope of the calibration curve (Equation 4). Afterwards, to get the amount of the absorbed dye from the initial 5 mL, the *ct* value was subtracted from the initial dye concentration and multiplied by the 0.005 (the volume of the dye in the experiment, in L) to get the final value. Subsequently, the *qt* value was calculated using Equation 5, where the unit of mass was obtained from the weight of the picocyanobacteria cells in 1 mL, resulting in the value of mg of adsorbed dye on the gram of the picocyanobacterial mass. Furthermore, from the two characteristic aspects, *ct* (Equation 4) and *qt* (Equation 5), the graphs were constructed.

The statistical analyses were performed in the Microsoft Excel software (Microsoft 365 Office, version 2403). Averages throughout this work were calculated as arithmetic (AVERAGE function), Outlier elimination was not performed due to the nature and amount of obtained data. Uncertainties in the statistical evaluation were determined using sample standard deviations (STDEV.S function).

For the plotting of the equilibrium adsorption isotherms, the SigmaPlot software (version 15) was used. The software evaluated the experimental data using a simple Equation 6:

$$y = \frac{ax}{(b+x)}, \tag{6}$$

where *a* represents the limit of the equation (analogue of maximum adsorption capacity) and *b* represents equilibrium liquid-phase concentration of the free dye, where 50% of dye is adsorbed. All constants were collected from the SigmaPlot software (version 15) and its calculations. The equilibrium solid-phase concentration of the absorbed dye was calculated using Equation 7:

$$Q_e = \frac{V(C_0 - C_e)}{m}, \tag{7}$$

where V is the volume of the dye in L, C_0 is the initial concentration of the dye in mg/L, C_e represents the equilibrium liquid-phase concentration of the unabsorbed dye in mg/L and m is the mass of the adsorbent in g (Mullerova et al., 2019).

In case of Chapter 4.5, the loss of the dye absorbance was calculated with Equation 8 as follows:

$$Loss\ [\%] = \frac{A_{initial} - A_{adsorbed}}{A_{initial}} \cdot 100 \quad (8)$$

where $A_{initial}$ is relative absorbance of initial dye in AU and $A_{adsorbed}$ is relative absorbance of the dye after adsorption in AU.

3.4.5 Scanning electron microscope (SEM)

The SEM microscopy was used for the visualization of aggregates of picocyanobacteria with magnetite, verification of cell binding to magnetite and their size differences. The SEM photos were taken at the Palacký University Olomouc by the following procedure: the samples of magnetised picocyanobacterial were drop-casted in lacey-carbon grids, subsequently, left to dry at a room temperature and continuously examined under Jeol-7900K SEM microscope, with accelerating voltage of 1.5 kV.

4 Results

The further-discussed chapters contain the results of both parts of the bachelor thesis. Chapters 4.1 and 4.2 present the data and their conclusions of Part A. The remaining chapters show the results of the biosorption experiments of the individual experiments and discuss their positive and negative outcomes (Part B).

4.1 Development of the picocyanobacteria in reservoir Nýrsko and WTP Milence

The following graphs shown in Figure 20 present the trend of picocyanobacteria at the sampling points in the individual sampling dates of the vegetative season from May to October 2022. The tables summarizing the data of the individual sampling dates can be found in the supplement (Table S1 – S9).

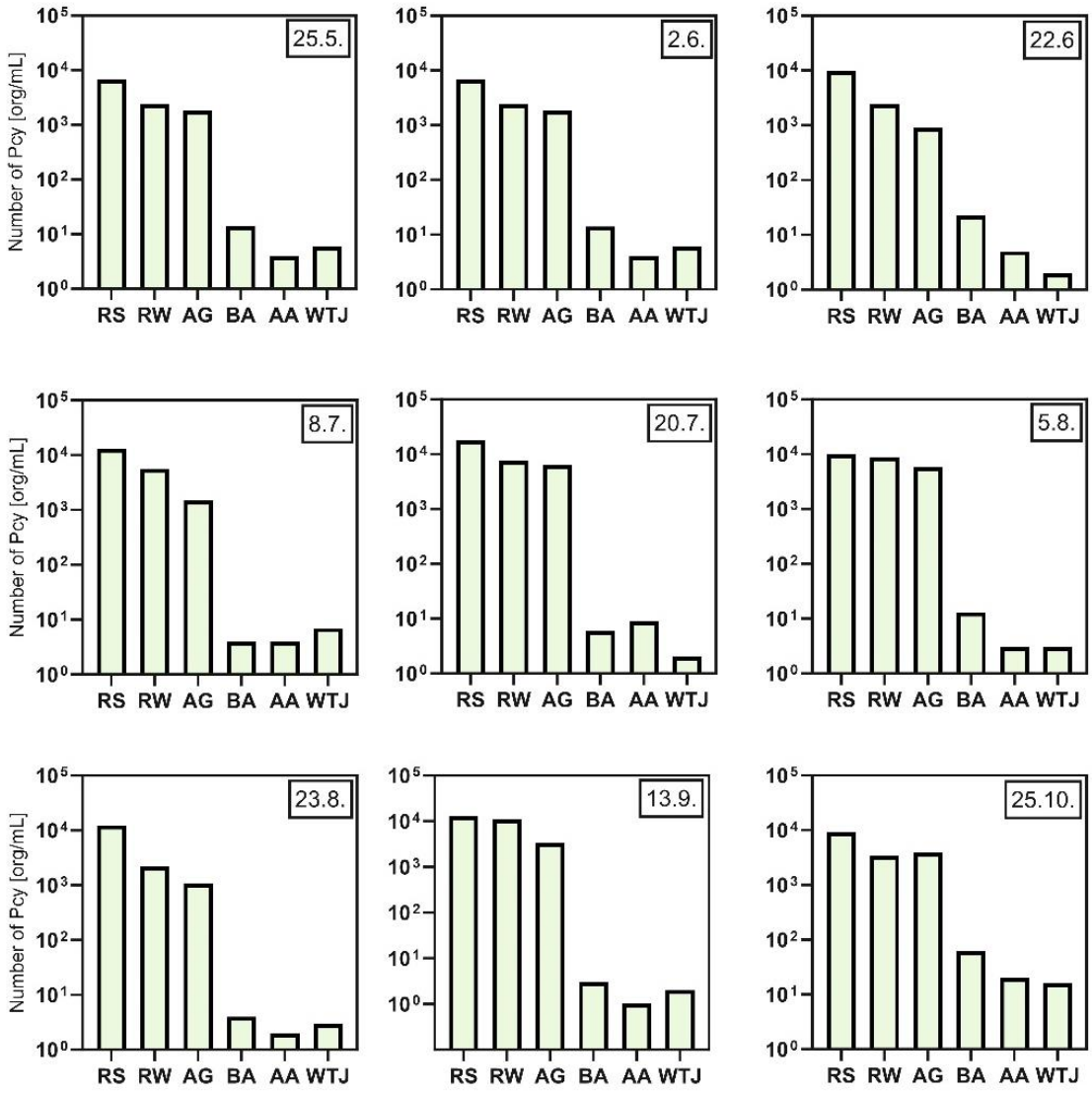

Figure 20. Development of picocyanobacteria through the individual sampling points in the sampling period May to October 2022 (logarithmic y-axis). RS — Reservoir Surface, RW – Raw Water, AG – Aggregation, BA – Before Accumulation, AA – After Accumulation, WTJ – Water Tank Janovice

The highest abundance of picocyanobacteria on all sampling days can be seen in the samples from the water reservoir Nýrsko, slightly lower numbers of picocyanobacteria are detected in the "Raw Water" sampling profile followed by the aggregation step. While most of the picocyanobacteria are eliminated in the technological stage of filtration and then with addition of the batch treatment chemicals. However, all organisms were not removed.

The trend of the picocyanobacteria in reservoir Nýrsko indicates the peak of the vegetative season on 20. 7. 2022 – 1.79×10^4 org/mL, the minimal amount was determined on 25. 5. 2022 – 3.3×10^3 org/mL. The data of picocyanobacteria at the sampling point "Raw Water" showed

the maximum picocyanobacteria number at 13. 9. 2022 – 1.1 × 10^4 org/mL, the minimum was also indicated at 25. 5. 2022 – 606 org/mL.

In Figure 21, the development of picocyanobacteria and picocyanobacterial forms throughout the sampling period at the individual sampling points is shown.

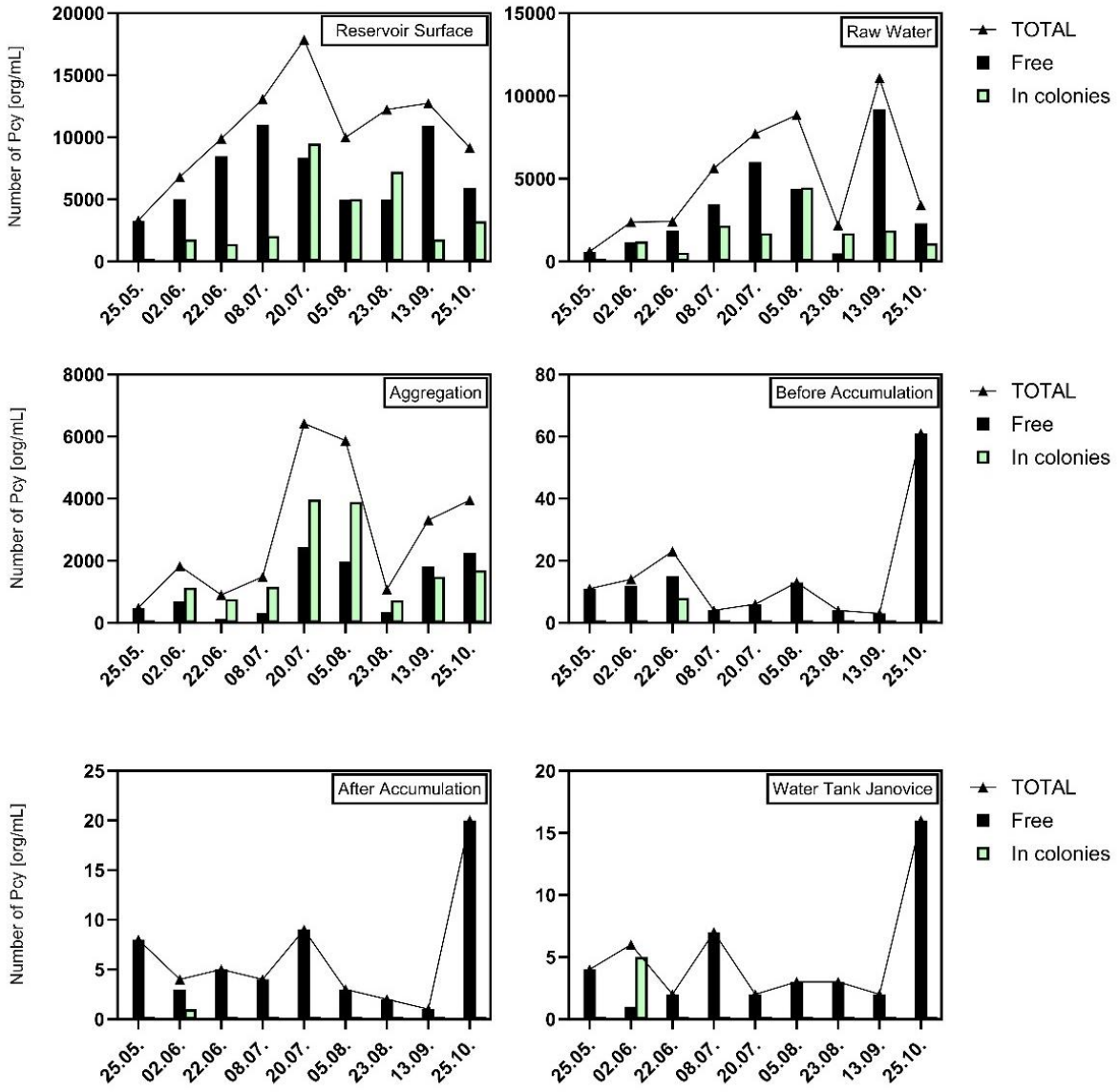

Figure 21. Development of picocyanobacteria and their forms (free living or colonial) in the individual sampling points in the sampling period May to October 2022

In Figure 21, the largest amount of picocyanobacteria can be detected in the "Water Reservoir", "Raw Water" and in "Aggregation" samples. Through the individual technological stages, the number of picocyanobacteria is gradually decreasing. In the "Water Tank Janovice", cells in the range 0 – 10 per millilitre are observed. One exception can be seen on the sampling date 25. 10., where the picocyanobacteria are in the number of 16 org/mL.

The “ind(%)”, showing the percentage of picocyanobacteria as individual cells, and the “col(%)”, the percentage of picocyanobacteria in colonies, are calculated and shown for the individual sampling points in the tables attached in the supplement (Table S1-S9). The ratio of the colonies and individual picocyanobacteria in “Reservoir Surface” and “Raw Water” varies. At the beginning of the vegetative season, individual cells were mostly observed. In the sample “Reservoir Surface”, the predominance of colonies occurred during three samples all in the summer period - 20. 7., 5. 8. and 23. 8. Sample “Raw Water” showed a predominance of colonies occurring in the samples from dates – 2. 6., 5. 8., 23. 8. The dates 2. 6. (“After Accumulation” – 33% of colonies; “Water Tank Janovice” – 83% of colonies), 22. 6. (“Before Accumulation” – 35% of colonies) and 13. 9. (“F6” – 83% of colonies) showed the presence of colonies also behind the filter steps.

Figure 22 shows the picocyanobacterial colony from the Nýrsko water reservoir captured by the fluorescence microscope and DAPI staining.

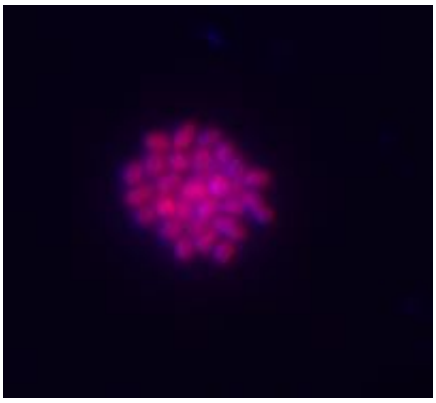

Figure 22. Colony of picocyanobacteria of Cyanobium sp. from reservoir Nýrsko captured by fluorescence microscope (with objective lens´ magnification 100x; multichannel photo) using DAPI staining. Size of the cell: 2 µm (author´s photo)

From the graphic representation of the individual results can be seen that the most diverse ratios of the individual cells and colonies are in the first three technological stages – samples “Reservoir Surface”, “Raw Water” and “Aggregation”. The other stages show mostly only individual cells in the samples. The amounts of picocyanobacteria determined through the sampling points reveal a continuously decreasing cell number. The reduction during water treatment is shown in Figure 23.

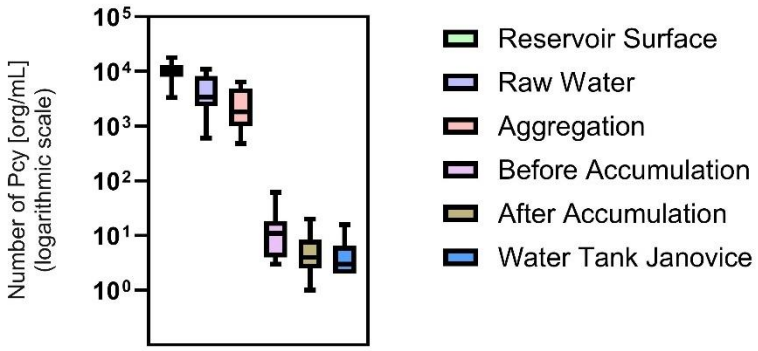

Figure 23. Summary of all data from the sampling period May-October 2022 (logarithmic y-axis)

The data of the average efficiency of the technological line parts can be seen in Table 2.

Table 2. Average efficiencies of the technological line parts

Data of the average efficiency of the technological line parts (%)		
Step specification	Efficiency	Std. Deviation
Raw Water - Aggregation	37.2	29.6
Raw Water - Before Accumulation	99.4	0.7

The calculated data showed that the average efficiency of the technological stages between samples "Raw Water" and "After Accumulation" obtained from the individual sampling dates is equal to 99.7% (Table 3)

Table 3. Separation efficiency of the complete technological process

Overall average efficiency (%)		
Step specification	Efficiency	Std. Deviation
Raw Water - After Accumulation	99.7	0.4

To statistically prove that there are significant differences between stages, a one-way ANOVA was chosen to be performed based on the literature by Ostertagova & Ostertag (2013). The summary of this test can be seen in Table 4. The conclusion of this test is that there are significant differences between the individual technological stages concluded from p-value and evidence that the technology of WTP is sufficient to meet hydrobiological requirements.

Table 4. Results of the statistical test – one-way ANOVA, showing significant differences between individual technological stages of WTP Milence

ANOVA Summary	
F	27.31
P value	<0.0001
Significant diff. among means (P < 0.05)	Yes
R squared	0.7399

4.2 Penetration through the filters

A complete table with the whole data set for the filters is placed in supplements (Table S10).

To determine the dependency of two variables – filtration time and separation efficiency, the Spearman correlation statistical test was selected and resulted in the value of -0.2501. The interpretation based on the work by Schober et al. (2018), suggests that there is a very weak correlation between the separation efficiency and time filtration (Figure 24).

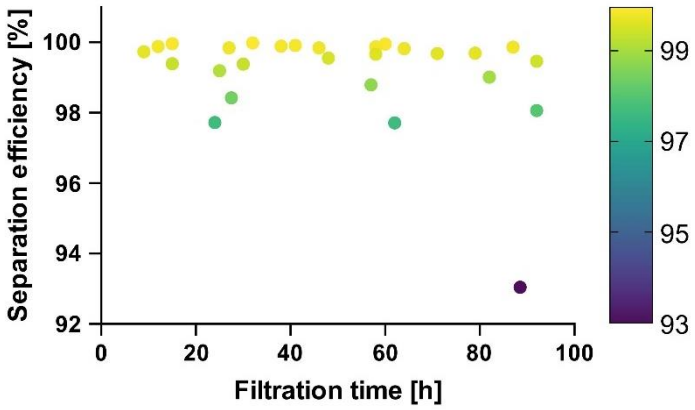

Figure 24. Graphical representation of filters´ separation efficiency dependence on filtration time

4.3 Preliminary tests for nanobiotechnological experiments

First of all, five cultures of picocyanobacteria were tested for magnetization to prove that picocyanobacteria are able to bind with the microwave synthesized magnetite and can be used for the purpose of pollutant adsorption continuously. Cultures were chosen in cooperation with previous unpublished research by RNDr. Jitka Jezberová, Ph.D. and prof. Ing. Ivo Šafařík, Ph.D., DSc. coming from the collection of the phototrophic organisms at Hydrobiological institute of Biology Centre of the Czech Academy of Sciences. The chosen picocyanobacterial cultures can be seen in Table 5 together with the results of the pre-test, which picocyanobacterial culture were able to bind the microwave synthesized magnetite in the best possible rate. The test tubes with the magnetized picocyanobacteria are attached in the supplements (Figure S3).

Table 5. Chosen picocyanobacteria cultures for magnetisation pre-test with numbers and percentage of magnetised cultures (XXX MAG – cultures with magnetite particles)

Culture	Initial Pcy amount in 100 µL	non-magnetised cells in 100 µL	% not magnetized	% magnetized
RDKV18	6.96E+07		97.77	2.23
RDKV18 MAG		6.80E+07		
Vir	6.88E+07		76.65	23.35
Vir MAG		1.48E+07		
RD51	6.88E+07		13.75	86.25
RD51MAG		9.46E+06		
STRS29	4.28E+07		89.14	10.86
STRS29 MAG		3.82E+07		
D1	6.90E+08		97.19	2.81
D1 MAG		6.71E+08		

The best competence for the following experiments showed culture RD51, which is shown with microwave synthesized magnetite particles in Figure 25a (taken by a light microscope) and Figure 25b (taken by a fluorescence microscope). For further characterization, the cells of initial culture RD51 viewed by scanning electron microscope (SEM) are shown in Figure 25c and the cells of RD51 with attached particles of microwave synthesized magnetite are displayed in the Figure 25d. The figures were provided by prof. Ing. Ivo Šafařík, Ph.D., DSc. (the images were taken by Eirini Ioanno from the Regional Centre of Advanced Technologies and Materials, Czech Advanced Technology and Research Institute (CATRIN), Palacky University, Olomouc).

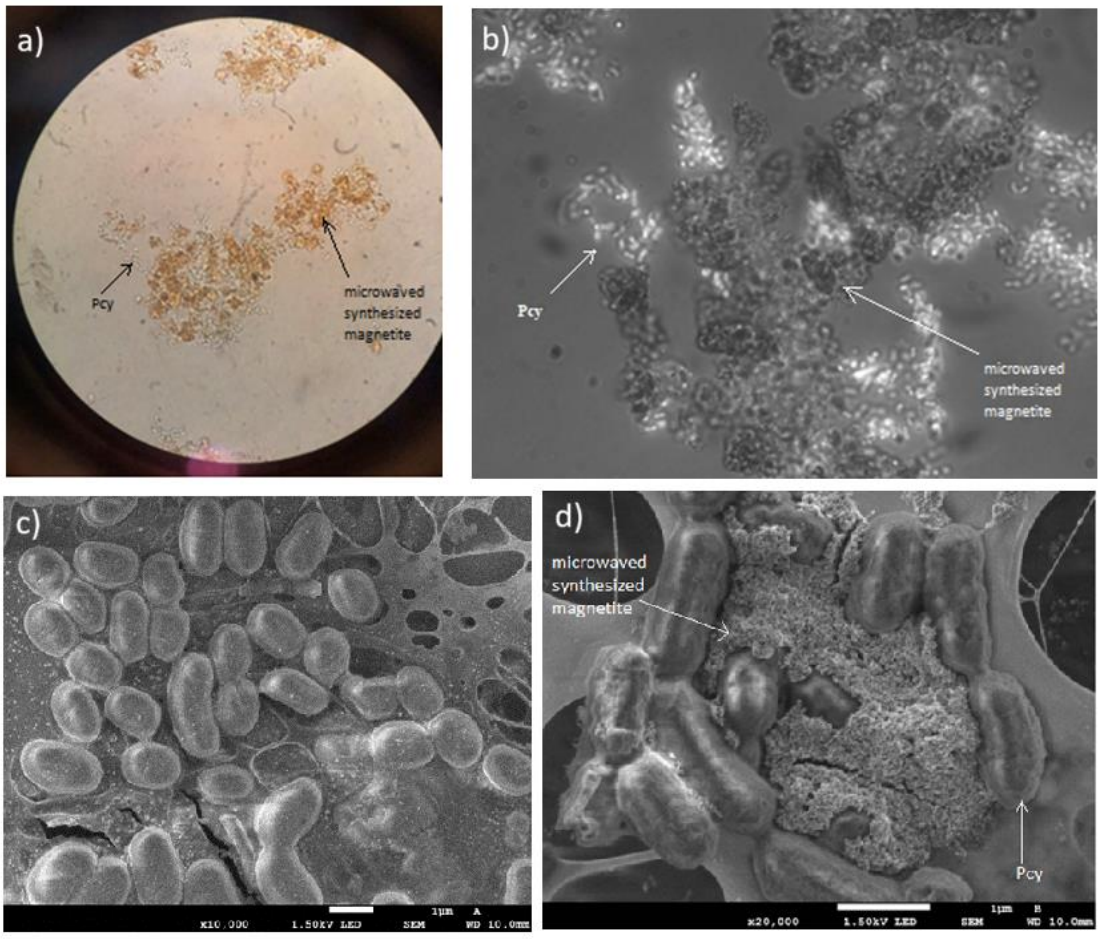

Figure 25. a) Light microscope image of the picocyanobacterial culture RD51 with particles of microwave synthesized magnetite (author´s photo); b) Fluorescence microscope image of the picocyanobacterial culture RD51 with particles of microwave synthesized magnetite (author´s photo); c) Cells of the picocyanobacterial culture RD51 imaged by the scanning electron microscope (received from prof. Ing. Ivo Šafařík, Ph.D., DSc.); Cells of the picocyanobacterial culture RD51 combined with microwave synthesized magnetite imaged by the scanning electron microscope (received from prof. Ing. Ivo Šafařík, Ph.D., DSc.).

After the confirmation that picocyanobacterial cells are attached to the microwave synthesized magnetite, the experiment for adsorption ability detection of picocyanobacteria/microwave synthesized magnetite was performed. The further mentioned data were used for the verification of the fact that picocyanobacteria are the main adsorption factor. The data of absorbance measurement of the pure picocyanobacterial mass and pure magnetite particles are displayed in Table 6 (dilution in the case of 10 μL microwave synthesised magnetite and initial dye exceptionally not performed).

Table 6. The measured values for the pure microwave synthesized magnetite and pure picocyanobacterial mass (A – absorbance of initial dye)

Malachite green (λ = 618 nm)			
A = 1.520			
Picocyanobacterial mass		Microwave synthesized magnetite	
Volume [µL]	Absorbance [AU]	Volume [µL]	Absorbance [AU]
100	1.139	10	1.520
500	1.122	50	1.370
1000	1.169	100	0.950

The decrease of the absorbances of the dye before and after the adsorption process is higher in the case of pure picocyanobacterial biomass than in the samples with pure microwave synthesized magnetite resulting in following observation: The performed experiments (the test´s image of the MG is attached in supplements Figure S4) show that the sorption of the dye analysed in the following experiments happened mainly due to picocyanobacterial cells, however, the partial absorption on the microwave synthesized magnetite cannot be neglected.

4.4 Biosorption of Malachite green dye by magnetically modified picocyanobacteria

The calibration curve for the Malachite green was prepared with the use of linear regression using and its graphical representation can be seen in Figure 26. Table S11 in supplements shows the measured data for the calibration curve.

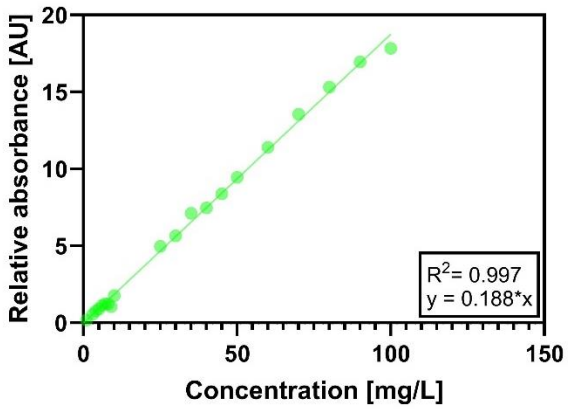

Figure 26. Calibration curve of Malachite green dye

The measured data and obtained results from the calculations of the adsorption experiment of the MG can be seen in Table 7. The blank (water) used in the calculation was 0.202 AU and the mass of picocyanobacterial culture in the experiment was determined to be 0.720 g.

Table 7. Measured data and calculation of the individual concentration experiments with the Malachite green dye (Blank – water, A_{blank} = 0.202 AU; m_{Pcy} = 0.720 g).

Dye concentration [mg/L]	Relative absorbance [AU]	Dilution
10	0.280	1
100	4.94	10
1000	57.5	100
2500	454	1000
5000	741	1000
ct concetration of free dye [mg/L]	Amount of absorbed dye from 5 mL [mg/L]	*qt* (mg adsorbed dye/ g sorbent)
0.418	0.048	0.067
25.3	0.37	0.52
306	3.47	4.82
2420	0.41	0.57
3950	5.23	7.26

These graphical representations can be seen in Figures 27 and 28.

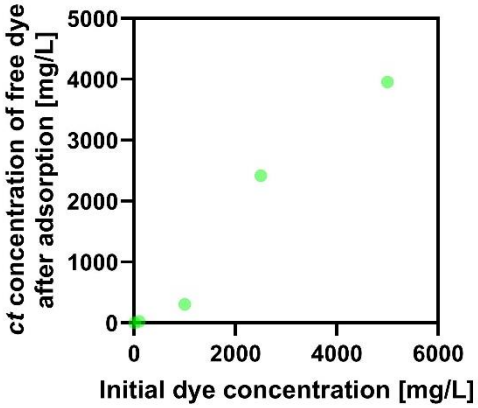

Figure 27. ct concentration of Malachite green dye after the magnetized picocyanobacteria´s biosorption

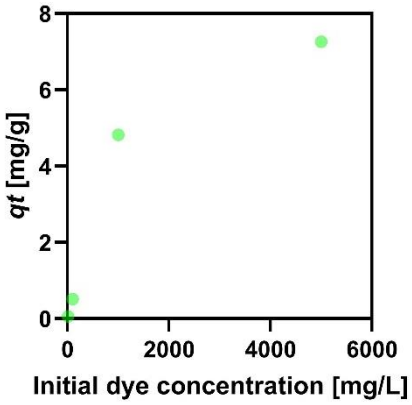

Figure 28. Graphical representation of qt dependency on the initial Malachite green concentration

In Figure 27 and 28, the graphical representations of the *ct* and *qt* dependency on the initial dye concentration can be seen. The data for the 2,500 mg/L were not included to the graphical representation of *qt* due to the error, which occurred during the experiment, and thus the number would give rise to the subsequent error in the calculation of the isotherm and in the assumptions.

The graphs (Figure 27, Figure 28) suggested that the picocyanobacteria have a good adsorption quality due to the right development of the curve, which is parabolic, and the increasing trend can still be observed. This indicates that picocyanobacteria can still remove larger amounts of dyes with acceptable amounts of adsorbed dye from the solution. This presumption is also displayed in Figure 29 (see Chapter 4.4.1) showing the adsorption isotherm calculated and constructed from the discussed experiment. However, the adsorption ability of the biomass is close to reach its limit, which is also observed from the curve development.

The statistical data for the absorbances in experiments for the MG are displayed in Table 8.

Table 8. Calculated statistical values by for the absorbance triplication of the experiments with the Malachite green

Concentration [mg/L]	Average A [AU]	Dilution	Standard deviation	Relative standard deviation
10	0.280	1	0.082	0.294
100	0.494	10	0.033	0.067
1000	0.575	100	0.060	0.104
2500	0.454	1000	0.101	0.222
5000	0.742	1000	0.060	0.081

4.4.1 Malachite green equilibrium adsorption isotherm

The equilibrium adsorption isotherm for Malachite green using picocyanobacteria biomass treated with magnetic iron oxide particles is presented in Figure 29. Neither Langmuir nor Freundlich isotherms could describe the adsorption process with sufficient precision, using specialized software. The graphical representation shows the visibility of the reach to the adsorption process limit, which can be equivalent to the maximum adsorption capacity obtained from the Langmuir isotherm.

The corresponding model constants and the coefficient of determination (R^2) are a = 7.68 mg/g, b = 198 mg/L and R^2 = 0.997 (Table 9).

Table 9. Data of the Malachite green equilibrium adsorption isotherm

Initial MG concentration [mg/L]	Dilution	Equilibrium MG concentration [mg/L]	Equilibrium solid-phase concentration of adsorbed MG [mg/g]
0	-	0	0
10	1	0.418	0.066
100	10	25.28	0.52
1000	100	305.8	4.8
5000	1000	3955	7

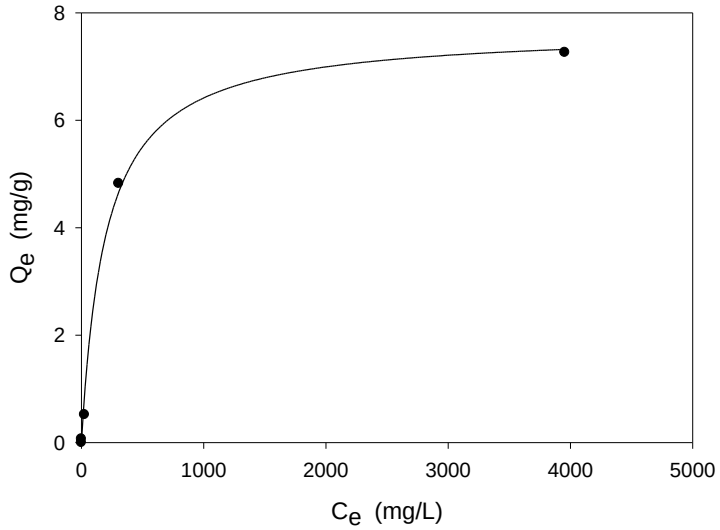

Figure 29. Equilibrium adsorption isotherm of Malachite green using the biomass of picocyanobacteria treated with magnetic iron oxide particles. C_e – equilibrium liquid-phase concentration of the unabsorbed dye (mg/L); Q_e – equilibrium solid-phase concentration of MG

In Figure 29, the equilibrium adsorption isotherm of Malachite green using the biomass of picocyanobacteria treated with magnetic iron oxide particles is displayed. This isotherm was calculated using Equation 7. From the graph, the assumptions that the magnetized picocyanobacterial biomass can adsorb larger concentrations (which means higher amounts) can be drawn due to the fact that the curve still continues. In other words, the curve didn´t reach the constant state. The data for the 2,500 mg/L were not included to the adsorption isotherm due to the error, which occurred during the experiment, and thus the number would give rise to the subsequent error in the calculation of the isotherm and in the assumptions.

4.5 Biosorption of the dyes using picocyanobacterial culture from water reservoir Nýrsko

After preliminary experiments with Malachite green dye, the sorption of dyes on the picocyanobacterial biomass cultivated from the water reservoir Nýrsko samples was investigated. Modifications in methodology were carried out and addition of other problematic dyes was done. For further analysis of the picocyanobacterial cells and its properties in terms of biosorption, two life stages – in exponential phase ("Log phase", the phase with the highest picocyanobacterial growth rate, 5 weeks old) and in the end of their life cycle ("Old Pcy", 12 weeks old), were tested in the same experiment with the same conditions.

Figure 30 shows the picocyanobacterial cells from water reservoir Nýrsko together with attached microwave synthesised magnetite.

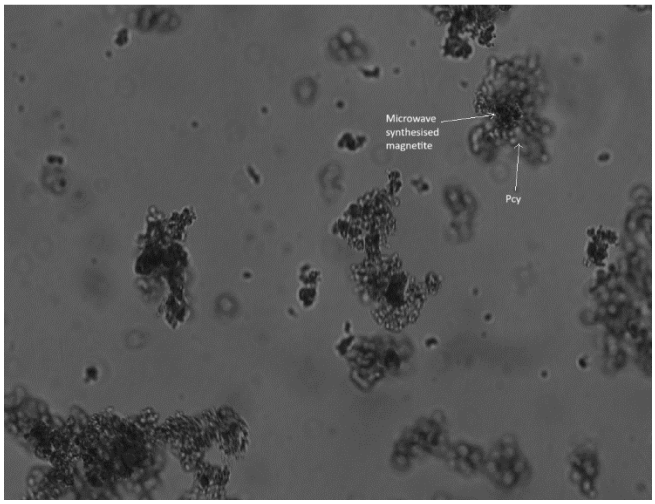

Figure 30. Fluorescence microscope visualisation of picocyanobacterial biomass from water reservoir Nýrsko and microwave synthesised magnetite (Size of the cell: ~2 µm (author´s photo))

The results of the adsorption loss of the individual dyes, concentration and picocyanobacterial culture are displayed in Tables 10-13.

Table 10. The measured data of the Rhodamine B adsorption on the different growth phases of picocyanobacteria in comparison with non-magnetised picocyanobacteria and non-bound magnetite without biomass

Rhodamin B – 50 mg/L (λ = 554 nm)					Rhodamin B – 10 mg/L (λ = 554 nm)				
A = 8.316					A = 1.505				
Sample	Addition of:	Relative absorbance [AU]	Dilution	Loss [%]	Sample	Addition of:	Relative absorbance [AU]	Dilution	Loss [%]
9	Old Pcy	7.516	5	10	25	Old Pcy	1.455	1	3
10	Old Mag Pcy	8.217	5	1	26	Old Mag Pcy	1.494	1	1
11	Log phase Pcy	7.500	5	10	27	Log phase Pcy	1.429	1	5
12	Log phase Mag Pcy	7.922	5	5	28	Log phase Mag Pcy	1.419	1	6
41	Only Magnetite	8.152	5	2	45	Only Magnetite	1.462	1	3

Table 11. The measured data of the Malachite green adsorption on the different growth phases of picocyanobacteria in comparison with non-magnetised picocyanobacteria and non-bound magnetite without biomass

Malachite green – 50 mg/L (λ = 616 nm)					Malachite green – 10 mg/L (λ = 616 nm)				
A = 6.249					A = 0.821				
Sample	Addition of:	Relative absorbance [AU]	Dilution	Loss [%]	Sample	Addition of:	Relative absorbance [AU]	Dilution	Loss [%]
13	Old Pcy	3.898	5	38	29	Old Pcy	0.114	1	86
14	Old Mag Pcy	2.360	5	62	30	Old Mag Pcy	0.141	1	83
15	Log phase Pcy	5.103	5	18	31	Log phase Pcy	0.108	1	87
16	Log phase Mag Pcy	2.314	5	63	32	Log phase Mag Pcy	0.118	1	86
42	Only Magnetite	3.802	5	39	46	Only Magnetite	0.114	1	86

Table 12. The measured data of the Methylene blue adsorption on the different growth phases of picocyanobacteria in comparison with non-magnetised picocyanobacteria and non-bound magnetite without biomass

Methylene blue – 50 mg/L (λ = 664 nm)					Methylene blue – 10 mg/L (λ = 664 nm)				
A = 4.735					A = 1.021				
Sample	Addition of:	Relative absorbance [AU]	Dilution	Loss [%]	Sample	Addition of:	Relative absorbance [AU]	Dilution	Loss [%]
17	Old Pcy	2.987	5	37	33	Old Pcy	0.583	1	43
18	Old Mag Pcy	3.983	5	16	34	Old Mag Pcy	0.865	1	15
19	Log phase Pcy	4.352	5	8	35	Log phase Pcy	0.750	1	26
20	Log phase Mag Pcy	3.796	5	20	36	Log phase Mag Pcy	0.874	1	14
43	Only Magnetite	4.367	5	8	47	Only Magnetite	1.006	1	1

Table 13. The measured data of the Crystal violet adsorption on the different growth phases of picocyanobacteria in comparison with non-magnetised picocyanobacteria and non-bound magnetite without biomass

Crystal violet – 50 mg/L (λ = 590 nm)					Crystal violet – 10 mg/L (λ = 590 nm)				
A = 4.982					A = 1.479				
Sample	Addition of:	Relative absorbance [AU]	Dilution	Loss [%]	Sample	Addition of:	Relative absorbance [AU]	Dilution	Loss [%]
21	Old Pcy	2.201	10	56	37	Old Pcy	1.455	1	2
22	Old Mag Pcy	3.439	10	31	38	Old Mag Pcy	0.276	1	81
23	Log phase Pcy	2.892	10	42	39	Log phase Pcy	1.125	1	24
24	Log phase Mag Pcy	3.320	10	33	40	Log phase Mag Pcy	1.089	1	26
44	Only Magnetite	3.542	10	29	48	Only Magnetite	1.465	1	1

Tables 10-13 display the proofs of picocyanobacterial sufficient adsorption relating to the dye pollution. The best adsorption capability was observed in Malachite green and Methylene blue dyes. Regarding the most suitable growth phase investigation, it was proved that the cells in the exponential phase ("Log phase") are capable of the preferred rate of pollutant sorption. Moreover, the picocyanobacteria in the end phase of their life also showed sufficient adsorption for the examined pollutants.

The graphical representation of the individual dyes and their absorbances after the adsorption can be seen in Figure 31.

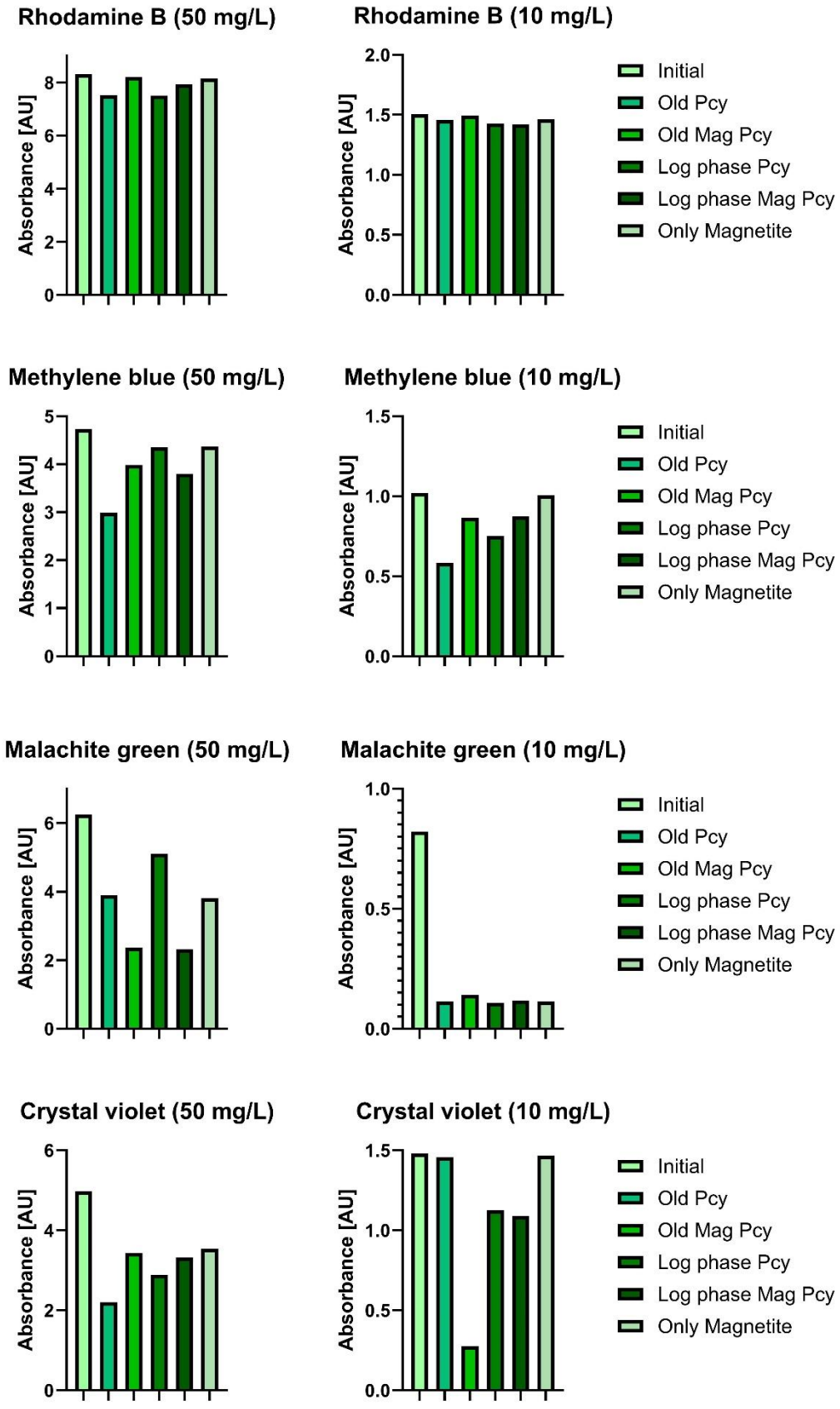

Figure 31. Graphical representation of the biosorption of the dyes in two different concentrations using picocyanobacterial culture from water reservoir Nýrsko ("Old Pcy" - 12 weeks old; "Log phase Pcy"- 5 weeks old)

The performed experiment included a blank sample of microwave synthesized magnetite solution containing an equal volume of dye. The control samples showed the inactivity of the magnetite particles on the sorption. This statement holds the exception in three cases – Malachite green (50 mg/L, 10 mg/L) and Crystal violet (50 mg/L), where the adsorption of pure magnetite synthesized by microwave exceeded the value of 28%, in case of 50 mg/L concentration of MG this value was 86%.

4.6 Extended biosorption experiments using Nýrsko picocyanobacterial culture and Methylene blue dye

Further-mentioned experiments were performed with the application of the lyophilizated picocyanobacterial mass due to the fact that freeze-dried biomasses are widely used in the cases of biosorption. The Methylene blue dye has been chosen by the criteria of the most significant and visible dye loss through the adsorption process and its advantage of non-interference with the chlorophyll wavelength.

The volume of picocyanobacteria dried for the experiments was 1 mL (to maintain the quantitative parameters of the previous experiments) with the final mass of the average of 1.3 mg of dried biomass.

Figure 32 shows the graphical representation of measured data of calibration curve (the relevant data can be found in the supplements, Table S12).

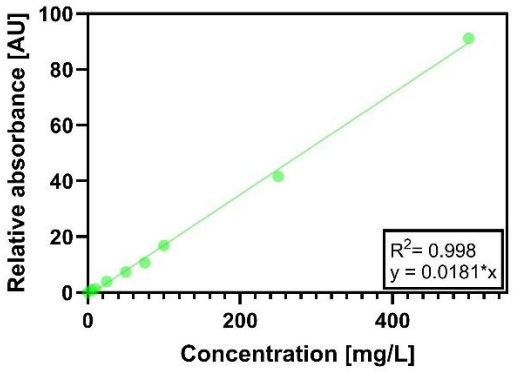

Figure 32. Calibration curve of Methylene blue dye

4.6.1 Adsorption of Methylene blue dye using non-dried culture of magnetized picocyanobacteria

The calculated values for *ct* and *qt* along with the absorbance of the sample and its dilution through the absorbance measurement can be found in Table 14 (1 hour adsorption time) and in

Table 15 (24 hours adsorption time). The description of the calculation can be found in Chapter 3.4.4.

Table 14. Measured data and calculated variables ct and qt of the individual concentration experiments with the Methylene blue (adsorption time: 1 hour)

Dye concentration [mg/L]	Relative absorbance [AU]	Dilution	*ct* [mg/L]	*qt* (mg of adsorbed dye/ g of sorbent)
0	0	-	0.000	0.000
5	0.642	1	3.472	5.877
10	0.735	1	3.993	23.104
25	2.351	2	13.079	45.852
50	5.299	8	29.646	78.285
75	9.960	10	55.85	73.66
100	14.140	15	79.34	79.45
250	37.132	41	208.59	159.28
500	76.923	81	432.26	260.55

Table 15. Measured data and calculated variables ct and qt of the individual concentration experiments with the Methylene blue (adsorption time: 24 hours)

Dye concentration [mg/L]	Relative absorbance [AU]	Dilution	*ct* [mg/L]	*qt* (mg of adsorbed dye/ g of sorbent)
0	0	-	0.000	0.000
5	0.532	1	2.850	8.270
10	0.970	1	5.312	18.031
25	2.455	2	13.659	43.618
50	7.099	8	39.764	39.370
75	10.570	10	59.28	60.47
100	14.560	15	81.71	70.37
250	37.419	41	210.20	153.08
500	78.084	81	438.78	235.45

Figure 33 shows graphical representations of the *ct* and *qt* depending on the initial concentration of dye for the two sets of biosorption samples of Methylene blue dye and picocyanobacterial biomass with two different adsorption cultivation times (1 hour and 24 hours).

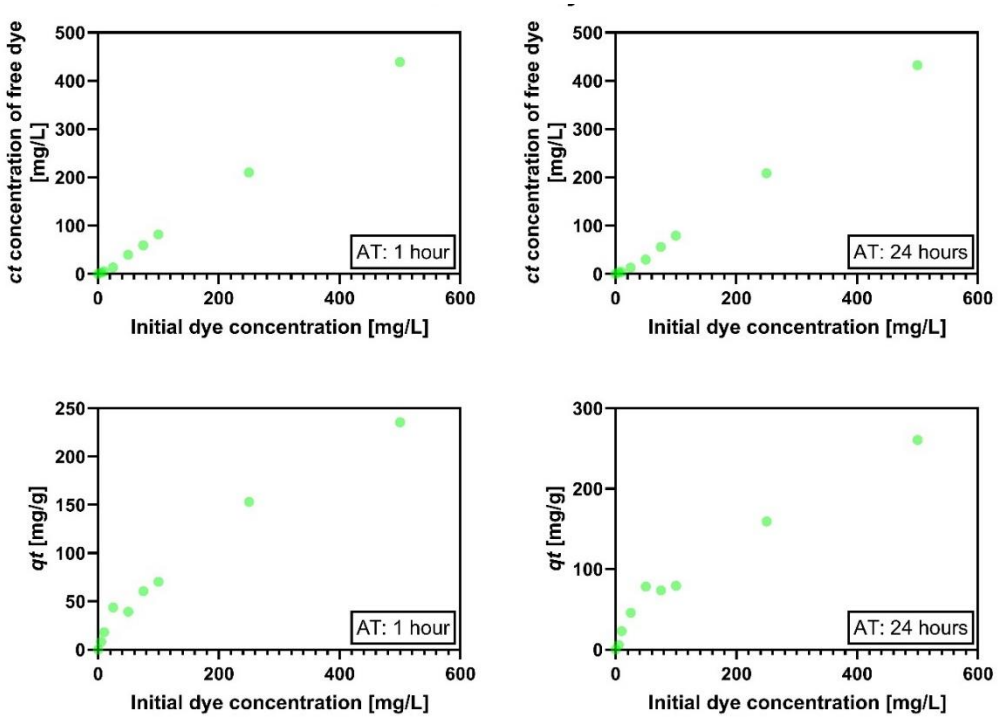

Figure 33. The graphical representations of ct and qt variables for the biosorption of Methylene blue dye by picocyanobacterial biomass from water reservoir Nýrsko (AT = Adsorption time)

The curve observed in the graphical representations of the two variables indicates a high adsorption capacity of the picocyanobacterial cells in terms of dye pollution. Nonetheless, the adsorption capacity of picocyanobacteria cells was not fully utilised. This observation was made based on the non-constant nature of the curve at higher dye concentrations. The capacity of the picocyanobacteria is fulfilled at the moment when the values and curves of *ct* and/or *qt* show a constant trend and non-increasing character. This predisposition is not attained.

The adsorption isotherm for biosorption of Methylene blue by picocyanobacterial biomass treated with microwave synthesized magnetite particles is presented in Figure 34 (adsorption time: 1 hour) and Figure 35 (adsorption time: 24 hours). The procedure was conducted using the same principle as described in Chapter 3.4.4. Tables 16 and 17 show the characteristics coefficient and statistical values for the discussed adsorption isotherms.

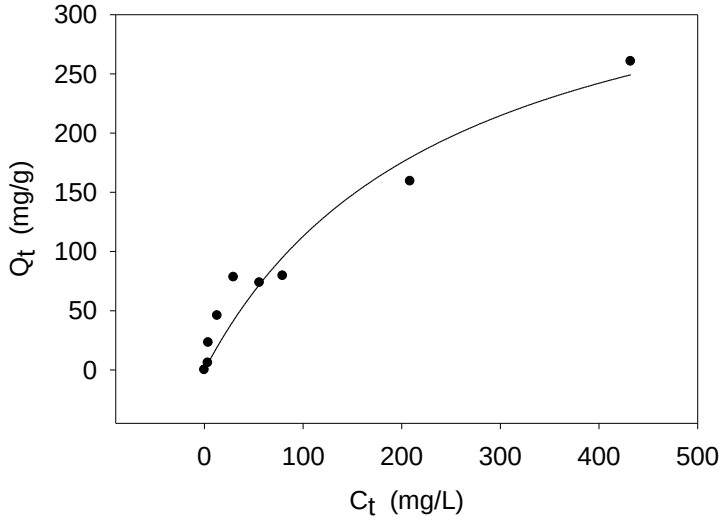

Figure 34. Adsorption isotherm of Methylene blue using the biomass of picocyanobacteria treated with magnetic iron oxide particles (adsorption time: 1 hours). Ct – concentration of the unabsorbed dye (mg/L); Qt – concentration of bound dye on the gram of sorbent

Table 16. The adsorption isotherm specific values of the Methylene blue adsorption by picocyanobacterial solution biomass with adsorption time of 1 hour. P – p-value, t – t-value

Adsorption time: 1 hour				
	Coefficient	Std. Deviation	t	P
a	392	80	4.92	0.0017
b	247	99	2.49	0.0418
$R^2 = 0.945$				

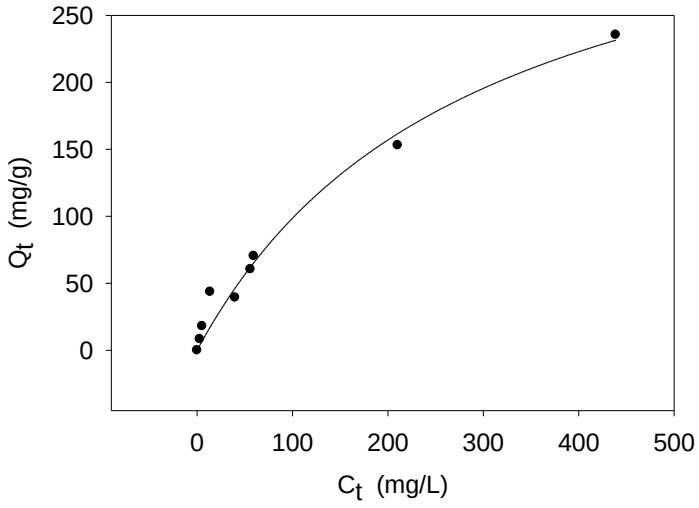

Figure 35. Adsorption isotherm of Methylene blue using the biomass of picocyanobacteria treated with magnetic iron oxide particles (adsorption time: 24 hours). ct – concentration of the unabsorbed dye (mg/L); qt – concentration of bound dye on the gram of sorbent

Table 17. The isotherm specific values of the Methylene blue adsorption by picocyanobacterial solution biomass with adsorption time of 24 hours. P – p-value, t – t-value

Adsorption time: 24 hours				
	Coefficient	Std. Deviation	t	P
a	384	53	7.19	0.0002
b	289	77	3.77	0.007
$R^2 = 0.979$				

Figures 33 (adsorption time: 1 hour) and 34 (adsorption time: 24 hours) displays the adsorption isotherms of performed experiments with picocyanobacteria in solution form. The curves of the graphical representations present higher pollutant adsorption capacity potential of these organisms.

4.6.2 Adsorption of Methylene blue dye using lyophilizated magnetized picocyanobacteria

The results with calculated *ct* and *qt* can be found in Table 18 (1 hour adsorption time) and Table 19 (24 hours adsorption time). The description of calculations can be found in Chapter 3.4.4.

Table 18. The measured data and calculated variables ct and qt for the adsorption of Methylene blue on magnetized picocyanobacterial lyophilizate (adsorption time: 1 hour)

Dye concentration [mg/L]	Relative absorbance [AU]	Dilution	*ct* [mg/L]	*qt* (mg of adsorbed dye/ g of sorbent)
0	0	-	0	0
5	0.511	1	2.102	11.145
10	0.679	1	3.047	26.744
25	0.965	1	4.654	78.253
50	3.030	10	16.26	129.76
75	6.690	10	36.84	146.79
100	8.325	15	46.03	207.59
250	33.128	41	185.45	248.28
500	85.212	81	478.22	83.78

Table 19. The measured data and calculated variables ct and qt for the adsorption of Methylene blue on magnetized picocyanobacterial lyophilizate (adsorption time: 24 hours)

Dye concentration [mg/L]	Relative absorbance [AU]	Dilution	*ct* [mg/L]	*qt* (mg of adsorbed dye/ g of sorbent)
0	0	-	0	0
5	0.431	1	1.973	11.642
10	0.671	1	3.322	25.684
25	0.944	1	4.857	77.474
50	2.79	10	15.23	133.72
75	7.07	10	39.29	137.34
100	8.25	15	45.92	207.98
250	32.759	41	183.69	255.03
500	87.642	81	492.20	30.01

Figure 36 shows a graphical representation of two calculated variables demonstrating and describing the adsorption capacity of the freeze-dried magnetized picocyanobacteria - *ct* and *qt*. The values at concentration 500 mg/L were in both cases of *qt* identified as the outliners and were removed from the graphical representation of this variable.

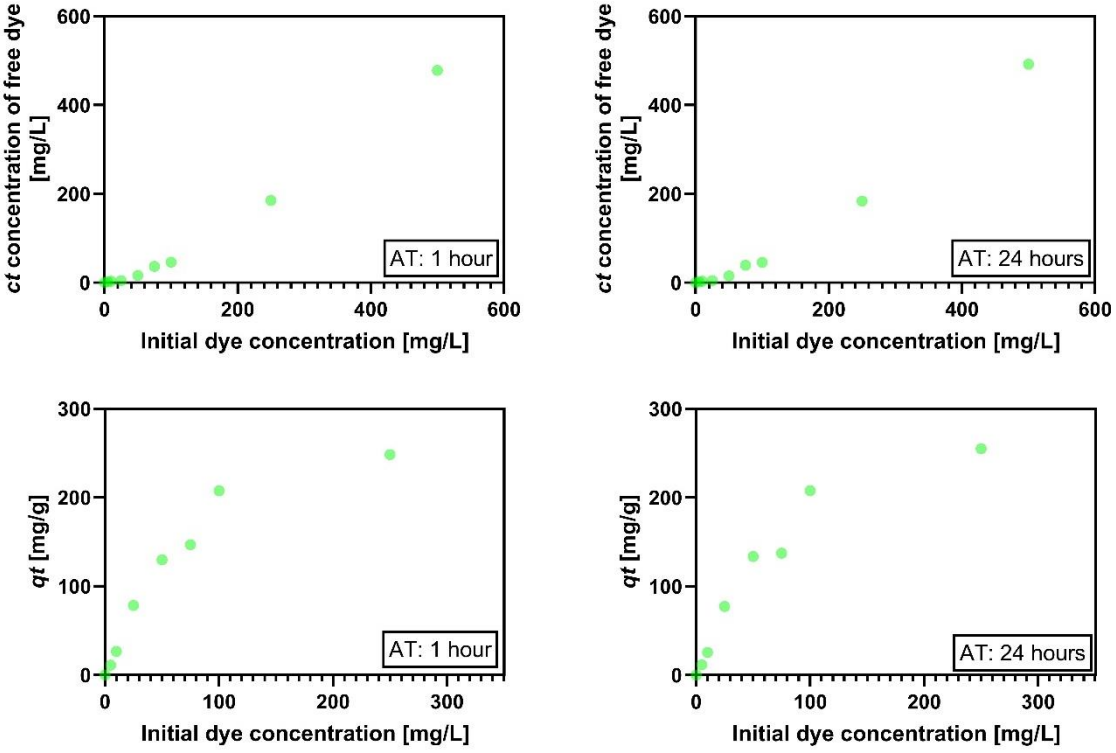

Figure 36. The graphical representations of ct and qt variables for the biosorption of Methylene blue dye by freeze-dried picocyanobacterial biomass from water reservoir Nýrsko (AT = Adsorption time)

In Figure 36 the graphical representations of the *ct* and *qt* for the two experiments are displayed. From the graph, the observation of unreached adsorption capacity for the magnetized lyophilized picocyanobacterial cells can be made. Nevertheless, the diagrams suggested that the culture has a sufficient adsorption ability indicated by the correct parabolic development of the curve and observation of the increasing trend in the adsorption range. Furthermore, the magnetized picocyanobacteria may still be exposed to higher concentrations and still exhibit good adsorption capabilities.

The adsorption isotherm for biosorption of Methylene blue by lyophilized picocyanobacterial biomass treated with microwave synthesized magnetite particles is presented in Figure 37

(adsorption time: 1 hour) and Figure 38 (adsorption time: 24 hours). The calculations were proceeded with the same principle as described in Chapter 3.4.4. Tables 20 and 21 show the characteristics coefficient and statistical values for the discussed adsorption isotherms.

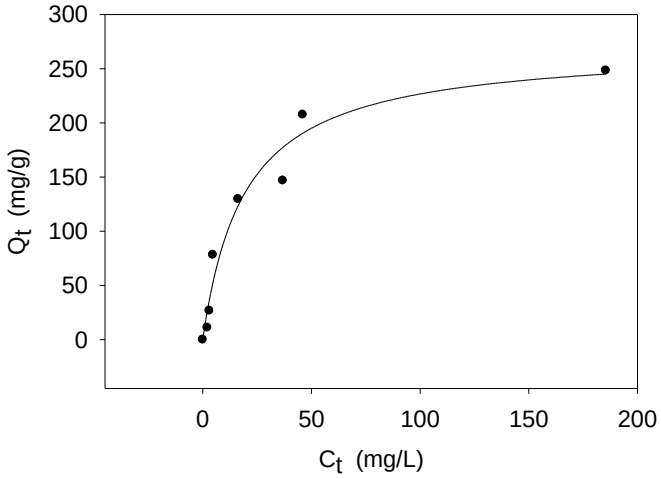

Figure 37. Adsorption isotherm of Methylene blue using the freeze-dried picocyanobacterial biomass treated with magnetic iron oxide particles (adsorption time: 1 hour). Ct – concentration of the unabsorbed dye (mg/L); Qt – concentration of bound dye on the gram of sorbent

Table 20. The adsorption isotherm specific values of the Methylene blue adsorption by picocyanobacterial freeze-dried biomass with adsorption time of 1 hour. P – p-value, t – t-value

Adsorption time: 1 hour				
	Coefficient	Std. Deviation	t	P
a	271	25	11.0	<0.0001
b	19.4	5.5	3.53	0.0123
R^2 = 0.962				

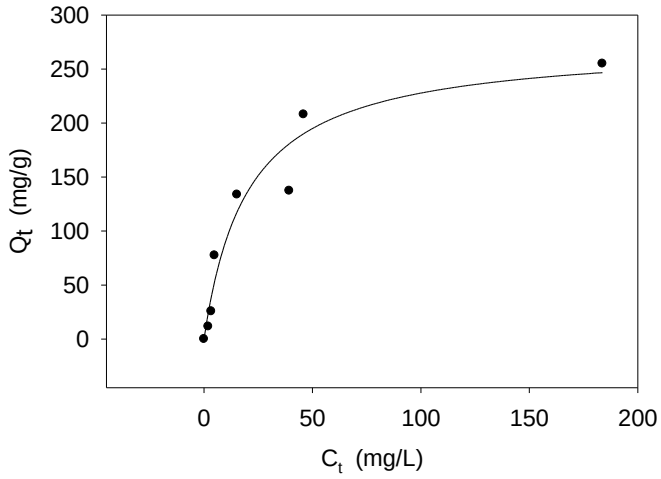

Figure 38. Adsorption isotherm of Methylene blue using the freeze-dried picocyanobacterial biomass treated with magnetic iron oxide particles (adsorption time: 24 hours). Ct – concentration of the unabsorbed dye (mg/L); Qt – concentration of bound dye on the gram of sorbent

Table 21. The adsorption isotherm specific values of the Methylene blue adsorption by picocyanobacterial freeze-dried biomass with adsorption time of 24 hours. P – p-value, t – t-value

Adsorption time: 24 hours				
	Coefficient	Std. Deviation	t	P
a	274	31	8.90	0.0001
b	20.2	7.0	2.90	0.0272
$R^2 = 0.943$				

Figures 37 (1 hour) and 38 (24 hours) shows the adsorption isotherms of the two performed experiments with different adsorption time for the freeze-dried picocyanobacteria. The curves exhibit increasing tendency proposing possible higher adsorption competence of these organisms. However, the cells are reaching almost its maximum capacity. Furthermore, the experiments display the reach of the biosorption ability already after 1 hour of adsorption time.

5 Discussion

5.1 Picocyanobacteria´s occurrence in water reservoir Nýrsko and WTP Milence

The objective of the first part of the introduced bachelor thesis was to validate and quantify the presence of picocyanobacteria in water coming from the water reservoir Nýrsko and individual technological stages of WTP Milence. Furthermore, the dependency of the picocyanobacteria filtration separation efficiency on the filtration operational time and the identification of the possible elimination options of the picoplanktonic organism from treated water were analysed.

It has been verified that there is a hydrobiological contamination in the water reservoir by picocyanobacteria in accordance with (Sochor, 2020). During the sampling campaign in vegetative season (May-October 2022), the maximum number of picocyanobacteria was observed in sample "Reservoir Surface" from the date 20. 7. 2022 with the value of 1.79×10^4 org/mL. This output complied with the research by Somogyi et al. (2020). The author stated the occurrence of picocyanobacteria in oligotrophic water reservoir from spring to autumn with predominance of microcolonies in the middle of summer period.

In the sampling profile "Raw Water", the observed maximum was determined on 13. 9. 2022 with the value of 1.1×10^4 org/mL. The lower value, found relative to the "Reservoir Surface" profile, as well as the different date of the detected maximum, is related to the fact that raw water for the treatment plant is not drawn from the water reservoir surface. According to Janák & Říhová Ambrožová (2023), the maximum occurrence of picocyanobacteria is indicated in the illuminated euphotic layer and towards the deeper layers of the reservoir the amount of picocyanobacteria decreases due to low light intensity, which was validated in the thesis.

Another outcome of the thesis was the confirmation of the picocyanobacterial occurrence in the complete technological line of the water treatment plant Milence. The finding that the picocyanobacteria are penetrating into the stages of the water treatment corresponds with the source (Jasser & Callieri, 2016). The penetration of the picocyanobacteria through the sand filters has been already confirmed by Ambrožová (1999). It was observed that in most cases only single picocyanobacterial cells are found in the water samples after passing through the filter technological stage – the colonies are separated from the remaining treated water by the mentioned technology. This fact is expectable, as the single cells are small, measuring approx. 1 μm in diameter, whereas the colonies are larger with a bigger chance to be separated from the

drinking water. The exception (i.e. the presence of the colonies in three samples) was observed on the following dates – 2. 6. ("After Accumulation" – 33% of colonies; "Water Tank Janovice" – 83% of colonies), 22. 6. ("Before Accumulation" – 35% of colonies) and 13. 9. ("F6" sample – 83% of colonies). The reason, why this was observed is hard to explain. Apparently the picocyanobacterial cells are so small even in colonies and so resistant, that they manage to pass so far in the water system.

Although the problematic separation of these small organisms (picocyanobacteria) is pointed out by Ambrožová & Říha (1998), the overall separation from the process stage "Raw Water" to the process stage "After Accumulation" was found to be 99.7%. This means that the efficiency of WTP Milence was found to be sufficient in removing picocyanobacteria from raw water within the range of legislative requirements. The insignificant separation efficiency between the raw water and aggregation stages and the large scatter of values may be due to the fluctuating aggregation effect of picocyanobacterial cells into the coagulant flakes. The influence on the water quality can be varied also because the variation of raw water quality during the year such as the pH value of the water (Aktas, 2012).

The effect of dosed chemicals, particularly the impact of chlorine dioxide disinfection/oxidation process, may contribute to the reduction of picocyanobacterial contamination downstream of the filtration stage. The significant decrease weren´t observed in the investigated samples. Due to the constellation of the WTP, it was not possible to sample the water directly from the filters causing the sampling from the Before Accumulation profile influenced by the chemical dosing. The strong oxidizing agent can cause cell burst which is one of the reasons for using ClO_2 for disinfection in water treatment plant technologies (Al-bayaty et al., 2010).

Excessive occurrence of picocyanobacteria (Figure 21) into further technological stages on 25. 10. from the profile "Before Accumulation" was observed. This can be caused by several reasons. Apparently, there was a nonstandard operational situation when the excess amount of picocyanobacteria may have passed through the filtration systems as a result of the enormous amount of impurities trapped in the filters, generation of short-circuit currents in the filter medium or caused by technical defect that may have occurred.

The dependence of the filter´s separation efficiency on the filtration cycle, that is, the time of operation of the individual filters, was not found. To confirm the findings, repetition of the experiment in a longer period with more frequent sampling dates is required.

In the case of a significant presence of picocyanobacteria in a source of water for drinking water, it is necessary to act on the precautionary principle. The impact of the presence of picocyanobacterial cells in the water treatment process and their risks to drinking water quality and WTP operations are discussed in detail in Chapter 1.2.6. The previously mentioned problematics was also confirmed by the work by Bláha & Maršálek (1999) who stated that the issues in different water treatment areas due to the occurrence of picoplanktonic cyanobacteria can occur. Within the bachelor thesis, existing or newly installed technologies for the separation of the whole picocyanobacterial cells during the water treatment process, such as enhanced coagulation, electrocoagulation, DAF, and membrane methods (in particular ultrafiltration), were discussed. From the point of view of maximum elimination of picocyanobacteria, the most advantageous method of the method mentioned above is ultrafiltration with removal of all water components larger than the size of the membrane. This arrangement will ensure that all safety requirements are met using a multibarrier water treatment approach as a total barrier (Lopes et al., 2013). The multibarrier path with the different methods is described as an efficient option as reported in the article by He et al. (2016). The inclusion of ultrafiltration technology prior to water disinfection process would also reduce the risk of an increase in AOCs in the distribution network and associated risks.

The sampling campaign was carried out during the vegetative season of 2022. As the occurrence of picocyanobacteria is significantly dependent, apart from e.g. nutrient availability, on water temperature (maximum growth in freshwater systems below 14 °C) according to Callieri et al. (2022), it is advisable to extend the study to years with different seasonal temperature patterns to validate the data.

This part of the bachelor thesis dealt with a so far little accentuated problem of water supply and managed to verify that the picocyanobacterial transition from raw water to the treatment technology is real even in our conditions and should be paid attention in the future to ensure good quality of drinking water.

5.2 Biosorption of the dyes by magnetized picocyanobacteria

The second part of the bachelor thesis was focused on experiments with magnetically modified picocyanobacterial cells for the use as a biosorbent of dyes. The interaction of dyes and magnetite particles has already been observed by Panda et al. (2021) and the utilisation of various types of biomasses for adsorption has also been investigated for some time (such as cyanobacteria *Gloeocapsa* sp. by Pokrovsky et al. (2008) (also showing its application in

wastewater treatment plants) or by different types of fungi and *Saccharomyces cerevisiae* (yeast) according to research by Kumari & Abraham (2007). In contrast, biosorption by magnetically modified picocyanobacteria is a new specific direction for biomass applications in nanobiotechnology and water treatment science.

Before the start of the experiments, a selection of picocyanobacterial cultures from the Hydrobiological institute of the Biology Centre of the Czech Academy of Sciences´ phototrophic organism collections with the best potential for magnetic modification was carried out. Of the five cultures tested, the RD51 culture was chosen, which achieved the level of 86.2% magnetisation. Furthermore, based on the basis of the literature, such as Safarikova et al. (2008) or Safarik et al. (2012), MG dye was selected for use in preliminary tests because it shows sufficient difference in absorbance after magnetized picocyanobacteria adsorption from the initial absorbance value of the dye.

In order to prove that the adsorption is predominantly performed by picocyanobacteria, an experiment was performed with pure picocyanobacterial biomass and pure microwave synthesised magnetite. The results are presented in Table 6 and show that adsorption is mainly carried out by picocyanobacteria, not by microwave synthesized magnetite particles; however, microwave synthesized magnetite can also be involved in the adsorption process, although its primary function is to ensure easy separation of the biosorbent from the water. The possibility of the adsorption process happening on the wall of the glass tube has to be also mentioned.

The involvement of pure magnetite in adsorption can be observed in the experiment in Chapter 4.5 showing high values of sorption by microwave synthesized magnetite – 86% loss of dye concentration in the case of MG with the initial dye concentration of 10 mg/L and 29% loss of dye concentration in the case of CV with initial dye concentration of 50 mg/L. The phenomenon of dye adsorption by magnetite was observed in several studies, such as in research by Panda et al. (2021).

The actual result of the preliminary experiment containing the Malachite green dye and the culture RD51 was the construction of the equilibrium adsorption isotherm (Figure 29). From the shape of the graph curve, one can assume a practically fulfilled capacity of MG picocyanobacterial adsorption. The correctness of the results may be influenced by the bursting of the picocyanobacterial cells, subsequently releasing chlorophyll causing interference with the green wavelength spectrum, causing increase in absorbance. Another error, occurring during

the absorbance measurement, was brought by the dilutions of the samples to fit under the 1.500 AU. This arrangement was taken to obtain the most accurate measurement possible with the instrument used.

Using fluorescence microscopy, it was verified that the biomass cultured from picocyanobacteria from the Nýrsko reservoir shows a similar capability to microwave synthesized magnetite compared to the original RD51 culture. It was further verified that this biosorbent exhibits dye adsorption ability. The best results of the adsorption capacity were found for the MG with high adsorption percentage - 62% loss of dye concentration in the case of MG at initial concentration of 50 mg/l dye ("Old mag Pcy") and 63% loss of dye concentration at the initial concentration of 50 mg/l dye ("Log phase mag Pcy"). Even better results were obtained when MG was adsorbed at an initial concentration of 10 mg/l, but here the efficiency of magnetite alone was measured to be practically the same compared to magnetised picocyanobacteria. These data contradict the results of the adsorption capacity for Table 6 and therefore it is advisable to repeat these experiments and verify the results.

However, the picocyanobacteria showed insufficient adsorption ability for the Rhodamine B, showing the incompatibility of picocyanobacterial cell adsorption with the mentioned dye. There is no adsorbent which is able to adsorb any pollutant or substance given. The problematics of the nonadsorption of the dyes on various microbial cells is further discussed in the research from Šafaříková et al. (2005). To achieve a better adsorption, a few of the parameters can be changed, such as pH of the solution, temperature, or time of the sorption incubation.

Furthermore, the picocyanobacterial cultures in the exponential phase ("Log Phase") showed a better adsorption quality, however, the differences between the exponential phase and phase at the end of the life weren´t significantly different.

The final phase of the experiments was the measurement of data for the construction of MB adsorption isotherms and the comparison of adsorption times for biomass cultured from picocyanobacterial culture from the water reservoir Nýrsko. It was found that picocyanobacteria reached almost full adsorption capacity in 1 hour of reaction with the dye, therefore no significant differences were found between the relative absorbances of samples reacting with the dye for 1 and 24 hours. From the curve of the adsorption isotherms, it can be deduced that,

for the amounts of dyes used, the depletion of the picocyanobacterial adsorption capacity was not reached for the duration of the experiment.

Because most of the biosorbents are used and stored in the dried form, the subsequent test took the picocyanobacterial biomass, freeze-dried them, and then applied them in the adsorption experiments. Lyophilised picocyanobacterial cells showed analogous sufficiency in the adsorption of the dyes as the picocyanobacterial suspensions. However, the freeze-dried picocyanobacterial cells exhibit almost full adsorption capacity. Overall, this result demonstrates the potential of using this form of picocyanobacteria-based biosorbents.

From the experiments, the potential of picocyanobacterial organisms to be used as biosorbents for the dye pollution can be observed. The differences in absorbances between the initial dye and the solution of dye with the picocyanobacteria were significant enough to prove that these organisms can be used and modified for the purpose of dye removal. Nevertheless, the microwave synthesized magnetite was determined as an efficient tool for the separation of the picocyanobacteria with adsorbed dye with a sufficient ability to get the cells of picocyanobacteria attached so it enables the separation from the solution. This claim was in accordance with the research of magnetite and its effectivity by Procházková et al. (2012). Overall, the picocyanobacteria have shown sufficient biosorption capability to conduct further experiments with them and the potential of putting the mechanism into practice in industrial wastewater treatment plants.

For the further research, the experiments should be replicated with other alarming dyes present in the water. Not only dyes are pollutants with which experiments should be performed. The picocyanobacteria can be also investigated for biosorption of heavy metals, such as antimony, which has been already investigated in the field of cyanobacteria biosorption in the study by Sun et al. (2011). Other substances which were already investigated but not with the cyanobacteria are for example pesticides (Hussein et al., 2017), which also represent large environmental danger, or radioactive uranium (Smječanin et al., 2023). In context of magnetic modification, not only cells modified with microwave synthesized magnetite can be investigated, but also the experiments focused on the modification of cells by ferrofluids (magnetic fluids) and their subsequent application are prospering research field. As the example, the application of ferrofluid modified yeast cells as potential biocatalysts (Safarikova et al. (2009)) or biosorbents (Safarik et al. (2007)) has been described.

6 Conclusion

The common factor of both parts of the present work is the picocyanobacteria from the water reservoir Nýrsko, which were investigated on two levels. The first part of the thesis dealt with the presence of picocyanobacteria in the water reservoir Nýrsko and in individual technological stages of the water treatment plant Milence. Significant findings were that picocyanobacteria, determined to be present in the water reservoir Nýrsko, were detected in each of the technological stages located in the WTP Milence. According to the findings, the picocyanobacteria go through the entire technological line and get all the way to the distribution network, only with the number decrease. Despite these findings, the treated water met the hydrobiological requirements for drinking water in the Czech Republic. However, the risks associated with the presence of picocyanobacterial cells in water are not eliminated. The dependency of the separation efficiency on the filter operational time was disproved. Since the presence of picocyanobacteria in the treatment process and drinking water carries risks (both operational and water quality), technologies suitable for the elimination of picocyanobacteria cells from water were sought. By assessing them, the membrane technology, more specifically ultrafiltration, was determined as the most suitable one (see Chapter 5.1). The mentioned technique is capable of ensuring the total barrier against penetration of picocyanobacterial cells into treated water. This system will ensure that all safety requirements are met using a multibarrier water treatment approach.

In the second part, the suitability of picocyanobacteria, retrieved from various locations such as Nýrsko, for biosorption of dyes was verified. The nanobiotechnological analysis of picocyanobacteria revealed the satisfactory biosorption capacity of harmful dyes from water enhanced with the good biosorbent removal from the solution thanks to the attached microwave synthesized magnetite. The picocyanobacterial cells were effective in both forms of the usable biomass – in solution not-dried naturally cultivated and in lyophilized form, which is more often used for the experimental procedures and practical usage in technologies. The performed tests showed the potential of industrial dye biosorption by picocyanobacteria, which can be applied especially in the treatment of wastewater from industrial production.

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8 Supplemental materials

Table S1. Results of counted picocyanobacteria at the sampling date 25. 5. 2022

Date	Sampling point	Free	In colonies	TOTAL	ind(%)	col(%)
25.05.2022	WR Surface	3276	57	3333	98.30	1.70
25.05.2022	Raw Water	574	32	606	94.64	5.36
25.05.2022	Aggregation	476	8	484	98.32	1.68
25.05.2022	F5 (24 hrs)	14	0	14	100.00	0.00
25.05.2022	F7 (88.5 hrs)	42	0	42	100.00	0.00
25.05.2022	F8 (57 hrs)	7	0	7	100.00	0.00
25.05.2022	Before Accumulation	11	0	11	100.00	0.00
25.05.2022	After Accumulation	8	0	8	100.00	0.00
25.05.2022	WT Janovice	4	0	4	100.00	0.00

Table S2. Results of counted picocyanobacteria at the sampling date 2. 6. 2022

Date	Sampling point	Free	In colonies	TOTAL	ind(%)	col(%)
02.06.2022	WR Surface	5021	1786	6807	73.77	26.23
02.06.2022	Raw Water	1166	1217	2383	48.92	51.08
02.06.2022	Aggregation	695	1136	1832	37.96	62.04
02.06.2022	F5 (82 hrs)	24	0	24	100.00	0.00
02.06.2022	F6 (48 hrs)	11	0	11	100.00	0.00
02.06.2022	F7 (15 hrs)	14	0	14	100.00	0.00
02.06.2022	Before Accumulation	14	0	14	100.00	0.00
02.06.2022	After Accumulation	4	2	6	66.67	33.33
02.06.2022	WT Janovice	1	5	6	16.67	83.33

Table S3. Results of counted picocyanobacteria at the sampling date 22. 6. 2022

Date	Sampling point	Free	In colonies	TOTAL	ind(%)	col(%)
22.06.2022	WR Surface	8479	1407	9886	85.77	14.23
22.06.2022	Raw Water	1869	546	2416	77.38	22.62
22.06.2022	Aggregation	134	771	905	14.77	85.23
22.06.2022	F5 (25 hrs)	19	0	19	100.00	0.00
22.06.2022	F7 (92 hrs)	13	0	13	100.00	0.00
22.06.2022	F8 (58 hrs)	8	0	8	100.00	0.00
22.06.2022	Before Accumulation	15	8	23	65.48	34.52
22.06.2022	After Accumulation	5	0	5	100.00	0.00
22.06.2022	WT Janovice	2	0	2	100.00	0.00

Table S4. Results of counted picocyanobacteria at the sampling date 8. 7. 2022

Date	Sampling point	Free	In colonies	TOTAL	ind(%)	col(%)
08.07.2022	WR Surface	11030	2059	13089	84.27	15.73
08.07.2022	Raw Water	3463	2170	5633	61.48	38.52
08.07.2022	Aggregation	317	1163	1481	21.43	78.57
08.07.2022	F5 (9 hrs)	11	4	15	75.00	25.00
08.07.2022	F6 (58 hrs)	7	0	7	100.00	0.00
08.07.2022	F8 (41 hrs)	5	0	5	100.00	0.00
08.07.2022	Before Accumulation	4	0	4	100.00	0.00
08.07.2022	After Accumulation	4	0	4	100.00	0.00
08.07.2022	WT Janovice	7	0	7	100.00	0.00

Table S5. Results of counted picocyanobacteria at the sampling date 20. 7. 2022

Date	Sampling point	Free	In colonies	TOTAL	ind(%)	col(%)
20.07.2022	WR Surface	8368	9496	17864	46.84	53.16
20.07.2022	Raw Water	6009	1704	7713	77.90	22.10
20.07.2022	Aggregation	2448	3977	6425	38.11	61.89
20.07.2022	F5 (30 hrs)	48	0	48	100.00	0.00
20.07.2022	F6 (64 hrs)	14	0	14	100.00	0.00
20.07.2022	F7 (87 hrs)	11	0	11	100.00	0.00
20.07.2022	Before Accumulation	6	0	6	100.00	0.00
20.07.2022	After Accumulation	9	0	9	100.00	0.00
20.07.2022	WT Janovice	2	0	2	100.00	0.00

Table S6. Results of counted picocyanobacteria at the sampling date 5. 8. 2022

Date	Sampling point	Free	In colonies	TOTAL	ind(%)	col(%)
05.08.2022	WR Surface	4956	5032	9988	49.62	50.38
05.08.2022	Raw Water	4380	4464	8844	49.53	50.47
05.08.2022	Aggregation	1978	3896	5873	33.67	66.33
05.08.2022	F5 (27 hrs)	14	0	14	100.00	0.00
05.08.2022	F6 (15 hrs)	4	0	4	100.00	0.00
05.08.2022	F7 (60 hrs)	5	0	5	100.00	0.00
05.08.2022	Before Accumulation	13	0	13	100.00	0.00
05.08.2022	After Accumulation	3	0	3	100.00	0.00
05.08.2022	WT Janovice	3	0	3	100.00	0.00

Table S7. Results of counted picocyanobacteria at the sampling date 23. 8. 2022

Date	Sampling point	Free	In colonies	TOTAL	ind(%)	col(%)
23.08.2022	WR Surface	5010	7223	12234	40.96	59.04
23.08.2022	Raw Water	495	1704	2199	22.51	77.49
23.08.2022	Aggregation	346	730	1077	32.16	67.84
23.08.2022	F5 (79 hrs)	7	0	7	100.00	0.00
23.08.2022	F6 (46 hrs)	4	0	4	100.00	0.00
23.08.2022	F7 (12 hrs)	3	0	3	100.00	0.00
23.08.2022	Before Accumulation	4	0	4	100.00	0.00
23.08.2022	After Accumulation	2	0	2	100.00	0.00
23.08.2022	WT Janovice	3	0	3	100.00	0.00

Table S8. Results of counted picocyanobacteria at the sampling date 13. 9. 2022

Date	Sampling point	Free	In colonies	TOTAL	ind(%)	col(%)
13.09.2022	WR Surface	10957	1786	12742	85.99	14.01
13.09.2022	Raw Water	9188	1883	11071	82.99	17.01
13.09.2022	Aggregation	1818	1493	3311	54.90	45.10
13.09.2022	F5 (38 hrs)	13	0	13	100.00	0.00
13.09.2022	F6 (71 hrs)	3	16	19	16.67	83.33
13.09.2022	F8 (32 hrs)	2	0	2	100.00	0.00
13.09.2022	Before Accumulation	3	0	3	100.00	0.00
13.09.2022	After Accumulation	1	0	1	100.00	0.00
13.09.2022	WT Janovice	2	0	2	100.00	0.00

Table S9. Results of counted picocyanobacteria at the sampling date 25. 10. 2022

Date	Sampling point	Free	In colonies	TOTAL	ind(%)	col(%)
25.10.2022	WR Surface	5909	3246	9155	64.54	35.46
25.10.2022	Raw Water	2305	1104	3409	67.62	32.38
25.10.2022	Aggregation	2256	1696	3953	57.08	42.92
25.10.2022	F5 (92 hrs)	45	0	45	100.00	0.00
25.10.2022	F7 (27.5 hrs)	37	0	37	100.00	0.00
25.10.2022	F8 (62 hrs)	53	0	53	100.00	0.00
25.10.2022	Before Accumulation	61	0	61	100.00	0.00
25.10.2022	After Accumulation	20	0	20	100.00	0.00
25.10.2022	WT Janovice	16	0	16	100.00	0.00

Table S10. Filters and their filtration times at the individual sampling dates with calculated separation efficiencies

Date	Filter	Filtration time [hrs]	Total	Pcy Raw Total	Separation efficiency	% of going through Pcy	Max of the day	Min of the day
25.05.2022	F5	24	14	606	97.72	2.28		
25.05.2022	F7	88.5	42	606	93.04	6.96	6.96	1.21
25.05.2022	F8	57	7	606	98.79	1.21		
02.06.2022	F5	82	24	2383	99.01	0.99		
02.06.2022	F6	48	11	2383	99.55	0.45	0.99	0.45
02.06.2022	F7	15	14	2383	99.39	0.61		
22.06.2022	F5	25	19	2416	99.19	0.81		
22.06.2022	F7	92	13	2416	99.46	0.54	0.81	0.34
22.06.2022	F8	58	8	2416	99.66	0.34		
08.07.2022	F5	9	15	5633	99.73	0.27		
08.07.2022	F6	58	7	5633	99.87	0.13	0.27	0.09
08.07.2022	F8	41	5	5633	99.91	0.09		
20.07.2022	F5	30	48	7713	99.38	0.62		
20.07.2022	F6	64	14	7713	99.82	0.18	0.62	0.14
20.07.2022	F7	87	11	7713	99.86	0.14		
05.08.2022	F5	27	14	8844	99.84	0.16		
05.08.2022	F6	15	4	8844	99.96	0.04	0.16	0.04
05.08.2022	F7	60	5	8844	99.95	0.05		
23.08.2022	F5	79	7	2199	99.69	0.31		
23.08.2022	F6	46	4	2199	99.84	0.16	0.31	0.12
23.08.2022	F7	12	3	2199	99.88	0.12		
13.09.2022	F5	38	13	11071	99.89	0.11		
13.09.2022	F6	71	36	11071	99.68	0.32	0.32	0.02
13.09.2022	F8	32	2	11071	99.98	0.02		
25.10.2022	F5	92	45	2305	98.06	1.94		
25.10.2022	F7	27.5	37	2305	98.42	1.58	2.29	1.58
25.10.2022	F8	62	53	2305	97.71	2.29		

Table S11. Calibration curve data for the Malachite green dye

Concentration [mg/L]	Relative absorbance [AU]
0	0
1	0.086
25	1.798
50	3.98
75	6.50
100	9.08
150	24.5
200	30.1
250	30.8
500	61.2
1000	103
2500	280
5000	558

Table S12. The Calibration curve data for Methylene blue dye

Dye concentration [mg/L]	Relative absorbance [AU]
5	0.835
10	1.407
25	3.98
50	7.36
75	10.68
100	16.9
250	42
500	91

Figure S1. Individual sampling points (author´s photo) a) "Reservoir Surface" b) "Raw Water" c) "Aggregation" d) "Filter 5" e) "Filter 6" f) "Filter 7" g) "Filter 8" h) "Before Accumulation" i) "After Accumulation"

Figure S2. Sterile flow-box used for the inoculation of picocyanobacteria cultures (author´s photo)

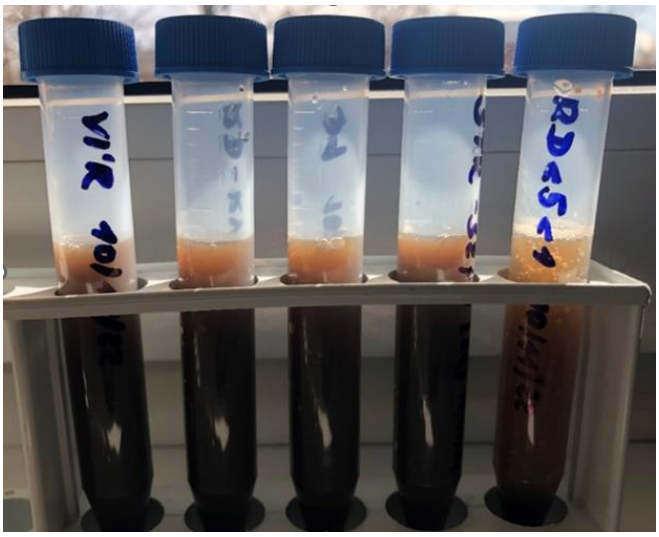

Figure S3. Pretest solutions of picocyanobacteria with microwave synthesized magnetite (author´s photo)

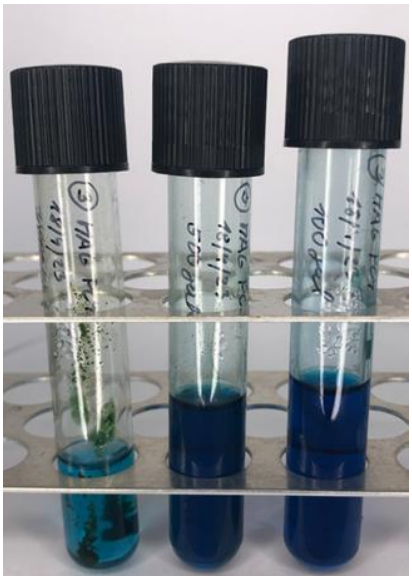

Figure S4. Three samples of three different concentrations of the magnetized picocyanobacteria with 5 mL of 0.1 mg/L Malachite green after 24 hours of incubation at the laboratory temperature (from the left 1000 µL, 500 µL and 100 µL) (author´s photo)

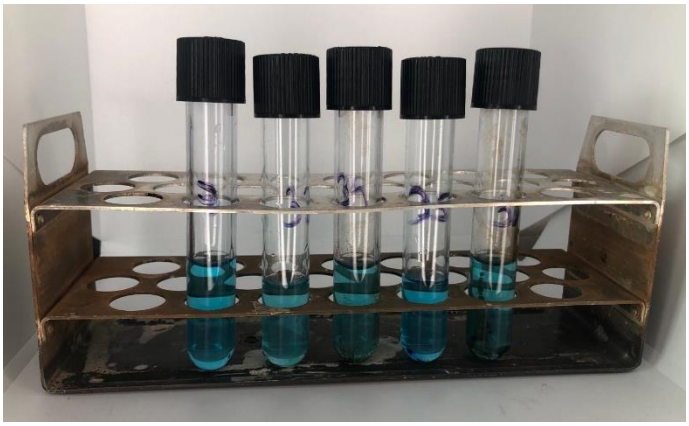

Figure S5. The samples of the Methylene blue biosorption on the picocyanobacterial biomass form water reservoir Nýrsko (from the left – 10 mg/L MB dye without any addition, Methylene blue with old picocyanobacteria culture, Methylene blue with old magnetically modified picocyanobacterial culture, Methylene blue with log phase (author´s photo)