Microbial Degradation of a Cyanotoxin in Gravity-Driven Membrane Filtration Biofilms

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Introduction

Gravity-driven membrane (GDM) filtration is a technology for water purification in regions where drinking water is not easily available [1]. However, blooms of harmful cyanobacteria have increasingly become an environmental concern. During the breakdown of these blooms, highly toxic compounds such as microcystins (MCs) may be liberated into freshwaters [2]. Microcystins are hepatotoxic for animals and humans; depending on exposure and concentration they can cause acute poisoning or promote cancer [3]. We investigate the influence of harmful cyanobacteria on bacterial biofilms from GDM filtration systems and the degradation of microcystins by these biofilms using source water from Lake Zurich in different seasons.

Experimental Setup

Two experiments were performed in spring (Exp. A) and autumn (Exp. B) where six biofouling monitors were split into three treatments.
In both experiments, the control directly received water from Lake Zurich; the mc^+ treatment was enriched daily with destroyed cells of the microcystin-producing cyanobacterium *Microcystis aeruginosa PCC7806*; the mc^- treatment (Exp. A) received daily doses of broken cells from a genetically engineered mutant of *M. aeruginosa* that does not produce microcystins and the p^+ treatment (Exp. B) received daily doses of broken cells from the microcystin-producing cyanobacterium *Planktothrix rubescens A7* (Figure 1 & 2).
Both experiments lasted for 20 days. LC-MS was used to determine microcystin concentration, flow cytometry served to analyse microbial regrowth on the filtrates, optical coherence tomography (OCT) was used to determine biofilm thickness and microbial diversity was analysed by Next Generation Sequencing (NGS, Illumina MiSeq) of partial 16S rRNA gene.

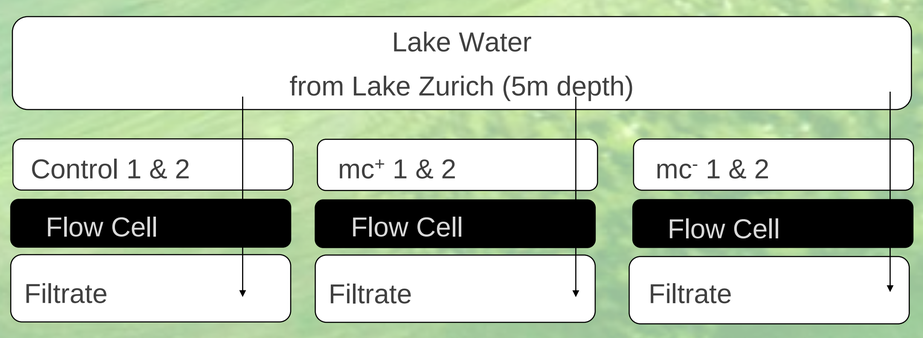

Figure 1- Setup used for experiment A. All three treatments have a biological replicate.

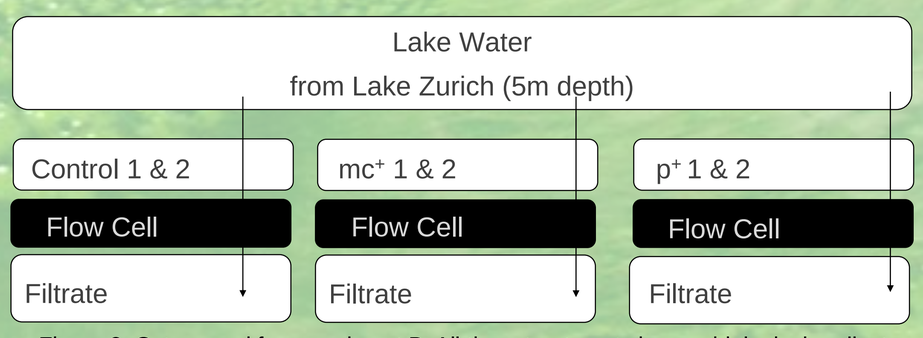

Figure 2- Setup used for experiment B. All three treatments have a biological replicate.

Microcystin removal by GDM biofilms

The permeate flux showed slightly different stabilization periods in experiment A and B (Figure 3). The microcystin removal efficiency represents the difference between the amount of MCs injected and the amount of MCs measured on the respective filtrate after 24h (Figure 4). The regrowth measured in the filtrates of the control exhibited approximately 10% less cells/mL than in the filtrates of treatments where cyanobacterial biomass was added. The mean biofilm thickness point to structural differences between the control and the other treatments. The controls were usually thinner than the treatments with external biomass input in experiment A, whereas in experiment B the control featured a thicker biofilm than the other treatments (Table 1).

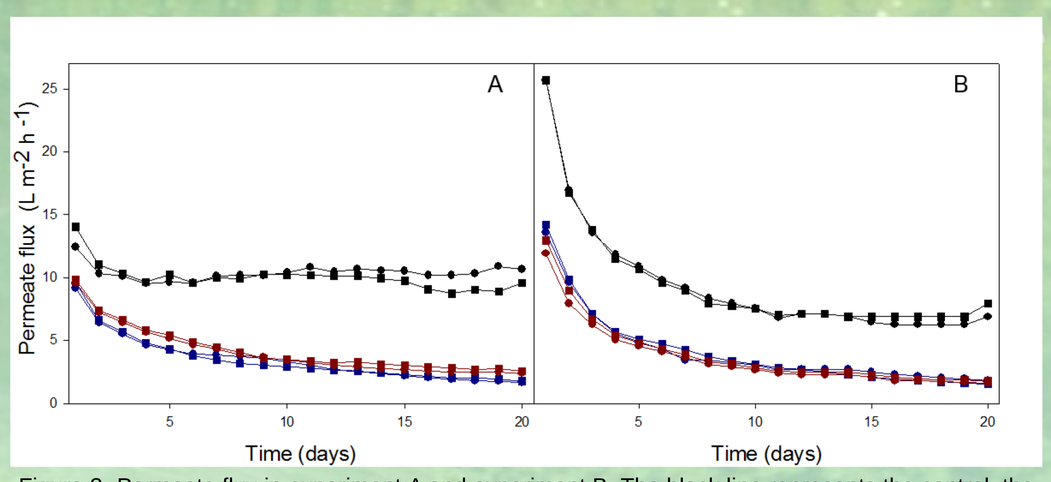

Figure 3- Permeate flux in experiment A and experiment B. The black line represents the control, the blue line the mc^+ treatment and the red line in A represents mc^- and in B the p^+ treatment. Squares and circles in the same colour represent biological replicates.

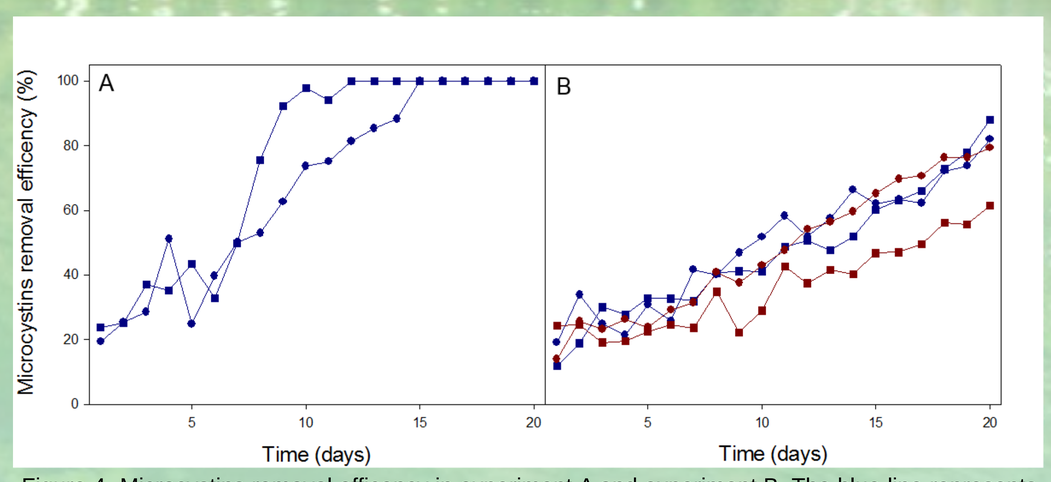

Figure 4- Microcystins removal efficency in experiment A and experiment B. The blue line represents the mc^+ treatment in A and B and the red line in B represents the p^+ treatment. Squares and circles in the same colour represent biological replicates.

Table 1- Mean biofilm thickness from all treatments from experiments A and B.

	Mean biofilm thickness (µm)							
	C1	C2	MC^+1	MC^+2	MC^-1	MC^-2	P^+1	P^+2
Experiment A	99 (±29)	42 (±4)	522 (±38)	MNP	244 (±12)	MNP	NA	NA
Experiment B	647 (±80)	782 (±132)	494 (±80)	326 (±55)	NA	NA	294 (±24)	501 (±45)

MNP- measurement not possible; NA-not applicable

Discussion

The differences on the permeate flux and biofilm thickness between experiments A and B can be explained by seasonal characteristics of Lake Zurich. In autumn when experiment B was performed, *P. rubescens* was present in the lake in very high concentrations. This natural input of external biomass most likely reduced the permeate flux in that experiment. As expected, the biofilm thickness was higher in the treatments with biomass input in experiment A (Table 1). By contrast, the greater thickness of the control biofilms in experiment B was also a consequence of the natural *P. rubescens* population that floated on top of the biofilms and could not be distinguished from it by the OCT measurements (Figure 5). Seasonality did not affect the initial removal efficiency but it possibly influenced biofilm performance over time. Higher microbial regrowth in the filtrates from treatments with higher biomass loading (irrespective of the presence of MCs) suggested that the biofilms did not quantitatively remove the massive external input of substrates and nutrients. Preliminar analysis of NGS data showed that the microbial assemblages in the control treatments were consistently more diverse, which is in line with the concept that more productive conditions may have a negative effect on diversity. In addition, a number of genotypes indicated the presence of anoxic microniches within the biofilms.

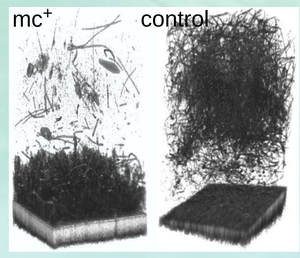

Figure 5- 3D representation of part of the biofilm in the mc^+1 and control1 in experiment B.

Conclusions

Our experiments suggest that the microbial community in water from Lake Zurich, as represented in the biofilms of the GDM systems, is consistently able to remove high additional quota of microcystins from *Microcystis aeruginosa PCC7806* and *Planktothrix rubescens,* and that this capacity is only marginally affected by seasonal phenomena.

References: [1] de Figueiredo, D.R., Azeiteiro, U.M., Esteves, S.M., Gonçalves, F.J.M., Pereira, M.J., 2004. Microcystin-producing blooms--a serious global public health issue. Ecotoxicology and environmental safety, 59, pp.151–163 [2] Peter-Varbanets, M., Zurbrügg, C., Swartz, C., Pronk, W., 2009. Decentralized systems for potable water and the potential of membrane technology. Water research, 43(2), pp.245–65 [3] Rastogi, R.P., Sinha, R.P., Incharoensakdi, A., 2014. The cyanotoxin-microcystins: current overview. Reviews in Environmental Science and Biotechnology, pp.1–35.

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