



Assessing the Impact of Algal Organic Matter on the Performance of Biological Filtration Systems

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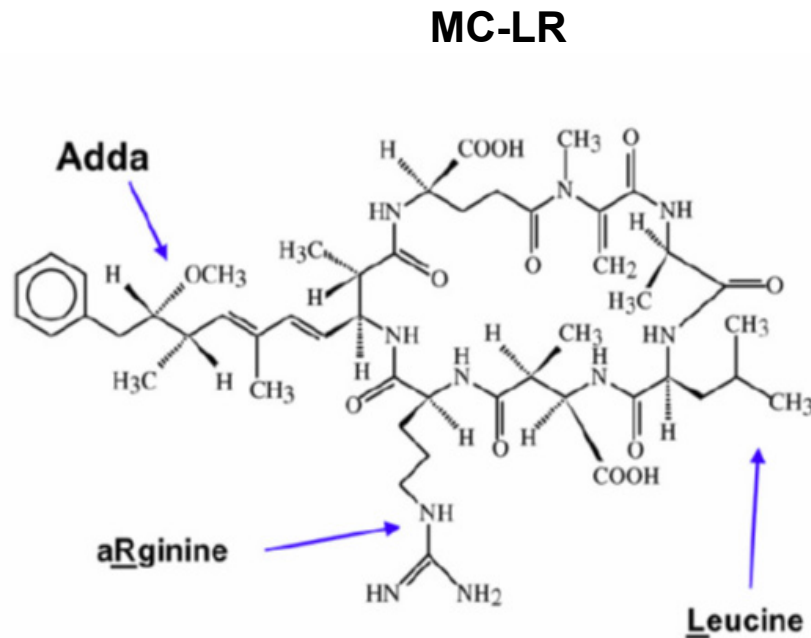
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Hepatotoxin Microcystin-LR

Microcystin-LR (MC-LR)

- ❑ Produced as secondary metabolites by cyanobacteria
- ❑ Stable in water and resistant to Hydrolysis, Oxidation and High Temperature



- ❑ Hepatotoxin (**Liver damage, Liver tumor promoter**)
- ❑ World Health Organization (WHO): **1 µg/L** for microcystin variants in drinking water

Toxic Harmful Algae Bloom (HAB)

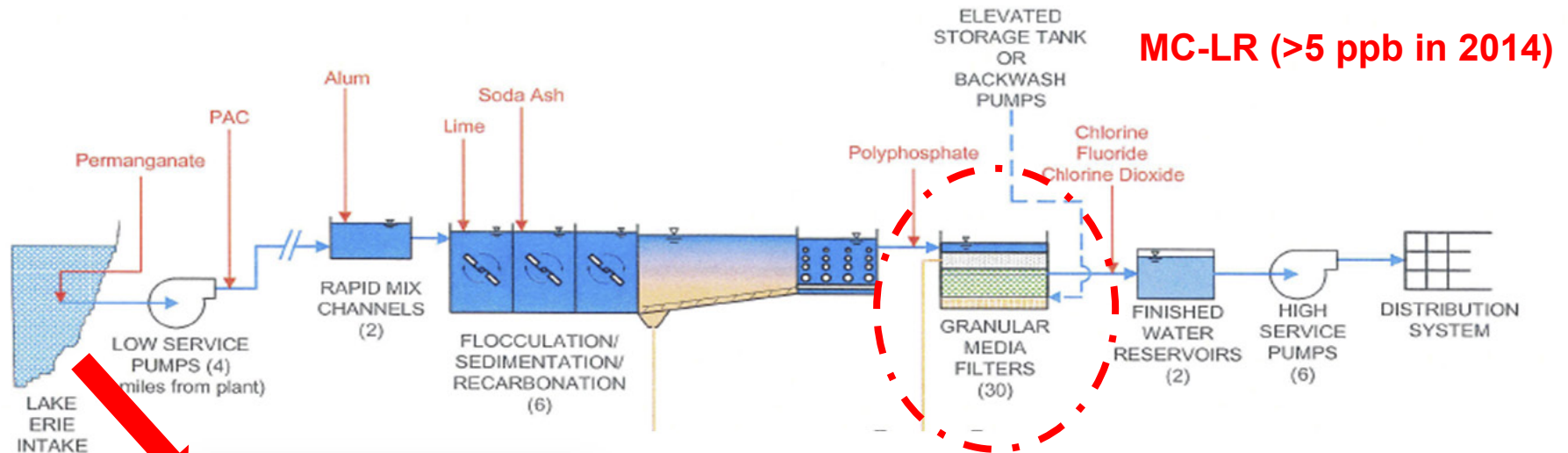
- ❑ In Aug. 2014, over 500,000 residents in Toledo and neighboring communities experienced a water crisis due to HAB
- ❑ Microcystin-LR from *Microcystis* species is a toxic cyclic heptapeptide and has become prominent in Lake Erie



From Toledo Blade

Toledo Water Treatment Plant

- ❑ At high levels AOM inhibits the efficiency by increasing the negative charges of particles
- ❑ Some proteins are likely to form complexes with coagulants, resulting in the reduction of available coagulant doses for destabilization.
 - ➔ Coagulation/Flocculation processes are not able to completely remove extracellular MC-LR

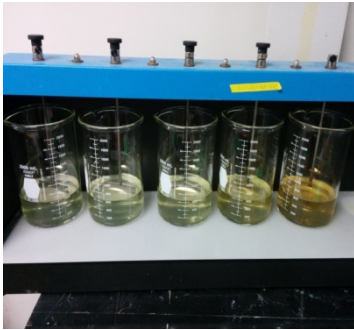


Schematic of Toledo Drinking Water Treatment Plant



HARAZ N. GHANBARI / AP

Controlling Methods for Cyanotoxins



Pretreatment using KMnO_4



Ozonation



UV/H₂O₂



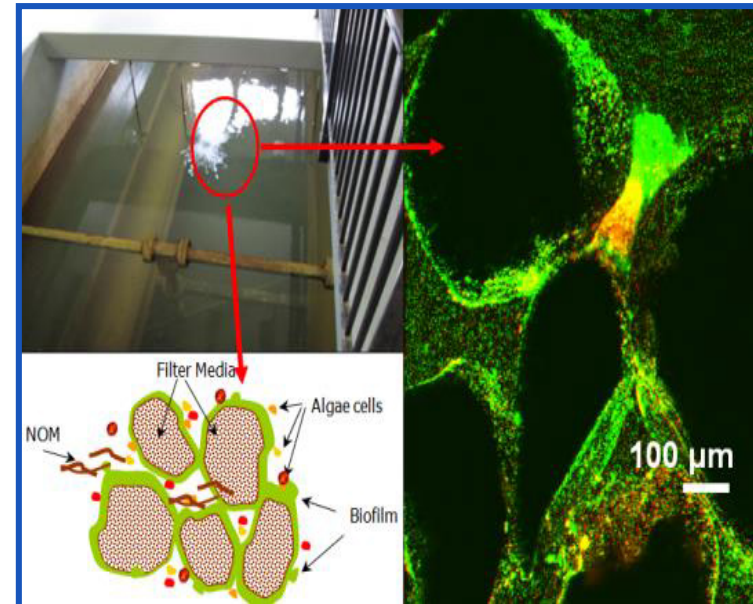
Membrane Filtration



Biological Filtration

Biological Filtration Systems (BFSs)

- ❑ One of the oldest and basic form of water treatment
- ❑ Do not require major systems improvements
 - ➔ Use the granular filter media comprised of
 - Sand, anthracite, or granular-activated carbons (GAC)
- ❑ Can remove various contaminants with low maintenance
- ❑ Biofilms are crucial to remove various pollutants including cyanobacterial toxins
- ❑ No operational protocols and monitoring methods for cyanobacteria toxin removal are currently available



Purpose of Study

STEP I

BFS Monitoring (Field Study)

- ❑ Toledo WTP
- ❑ Monitoring Period:
Jan 2016 ~ Jan 2017
- ❑ Investigating the
performance of two
different filter media
(GAC vs Anthracite)

STEP II

Lab-Scale Column Study

- ❑ Impacts of **algal organic matter (AOM)** on the performance of conventional GAC filtration and the removal of MC-LR
- ❑ Changes in bacterial community structure in response to AOM

STEP III

Bioaugmentation Using MC-Degrading Bacteria

- ❑ Testing biodegradation of MC-LR in GAC and anthracite filters using MC-degraders

** STEP II were discussed in this presentation*

Materials & Methods

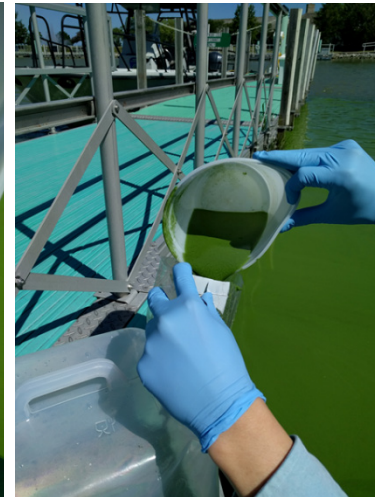
Preparation of source water and solution of AOM

- ❑ Filter influent from Toledo WTP was continuously fed through the columns
- ❑ Algal water samples were collected near Maumee Bay State Park
- ❑ GAC (particle size: 1.0-1.2 mm) and virgin sands were used to fill columns

Toledo WTP filter influent

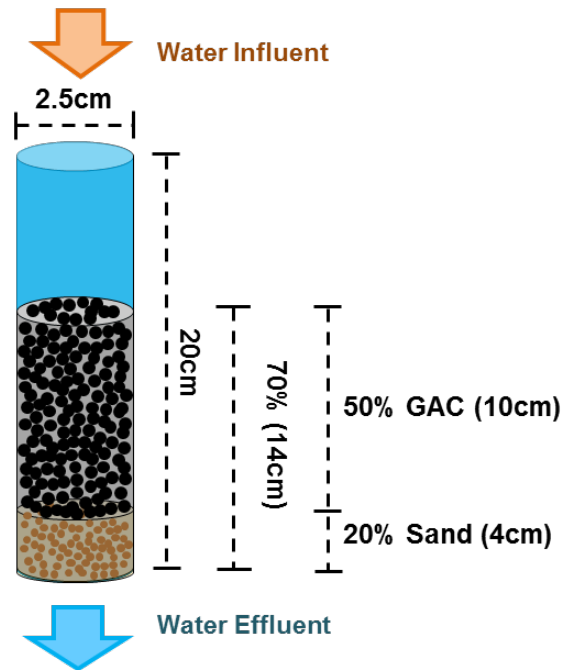


Intracellular algal compounds

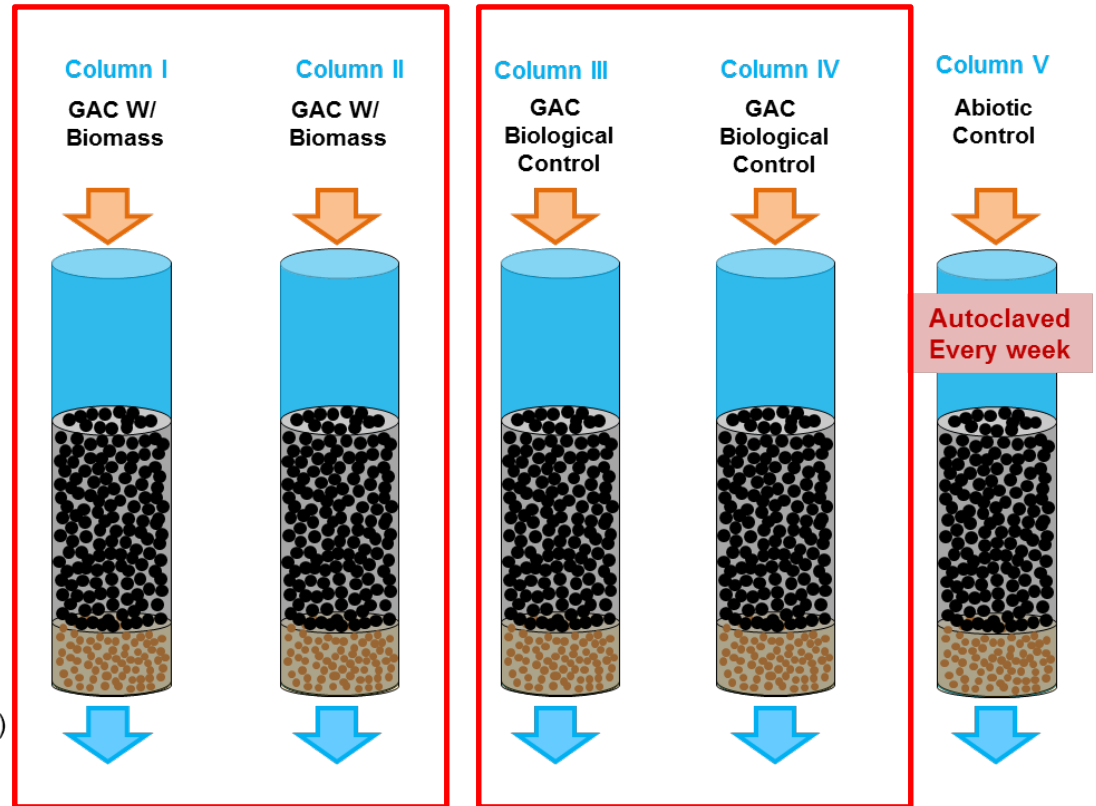


Materials & Methods

Biological Filtration Column



- **Column Dimensions**
: Autoclavable glass column, 2.5 cm (D) × 20 cm (L)
- **Flow Rate**
: 2.54 ml/min
- **Empty Bed Contact Time (EBCT)**
: 20 min

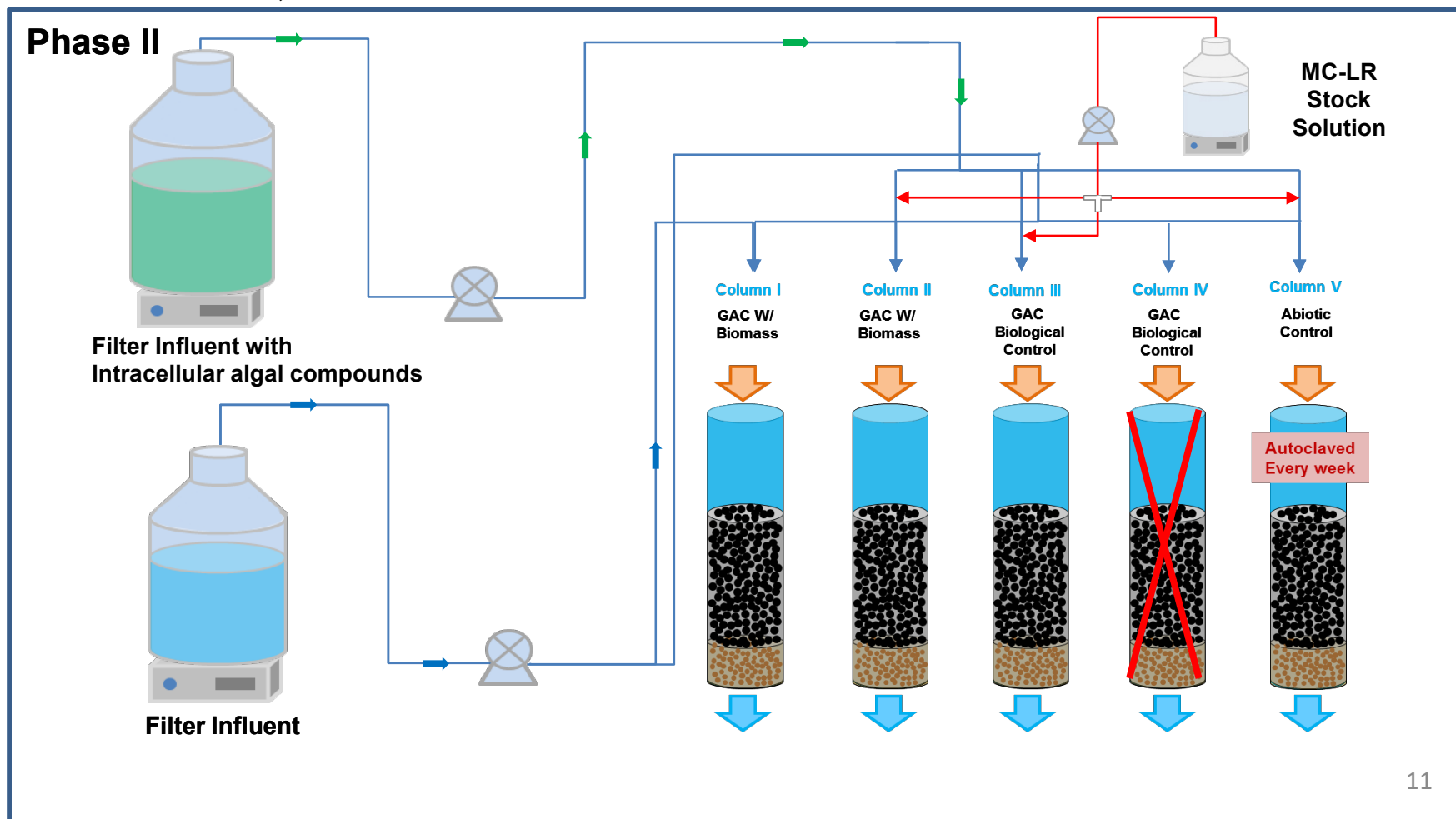




Materials & Methods

□ Column Set-up and Operation

- ✓ Biological filter biomass was injected into the column 1 and column 2
- ✓ Phase I: Operated using Toledo WTP filter influent (all columns)
- ✓ Column 4 was stopped and filter media samples were collected
- ✓ Phase II: Intracellular algal compounds (**TOC:0.5 ~1 ppm, TN: <0.2 ppm**) were additional injected to column 2,3 and 5



Materials & Methods

Monitoring Water Quality Parameters

- ❑ Total organic carbon (TOC)/ Total Nitrogen (TN) : TOC-VCSH (Shimadzu, JP)
- ❑ Adenotriphosphate (ATP): PhotonMaster Luminometer using Deposit & Surface Analysis (DSA TM) kit
- ❑ Turbidity (HACH, USA)
- ❑ MC-LR (≈ 5 ppb) using HPLC
- ❑ Fluorescence excitation-emission matrices (EEMs): Fluoro Spectrophotometer (Shimadzu, Japan)

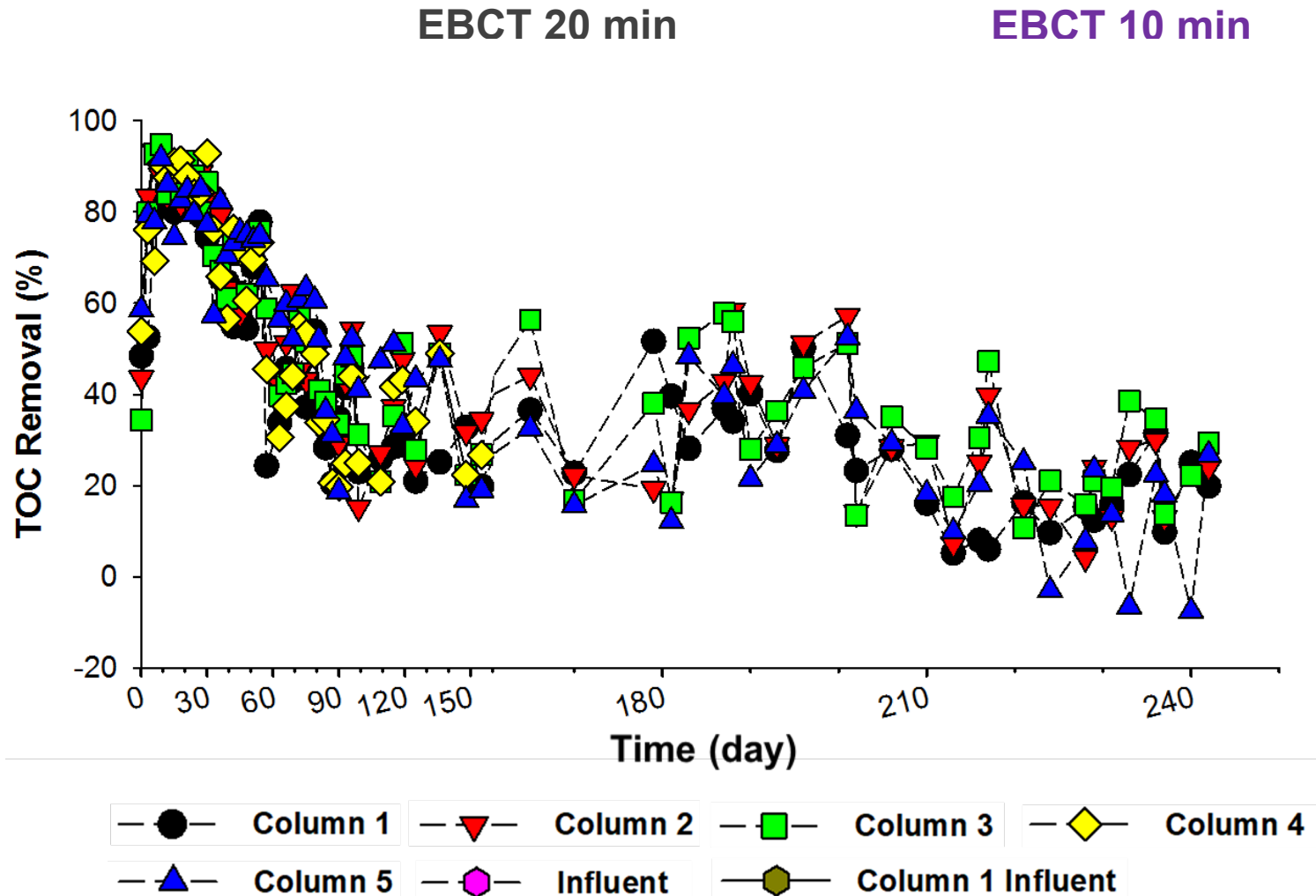
Microbial Community Structure Analysis

- ❑ DNA extraction of filtered water and filter media samples (Dneasy PowerSoil Kit, Qiagen)
- ❑ High-throughput amplicon sequencing targeting 16s rRNA V4 Region (i.e., 515F and 806R) using an Illumina MiSeq PE250 sequencing kit.
- ❑ Downstream analysis (Qiime and R)

Results

Effect of filter media conditions on the removal of TOC

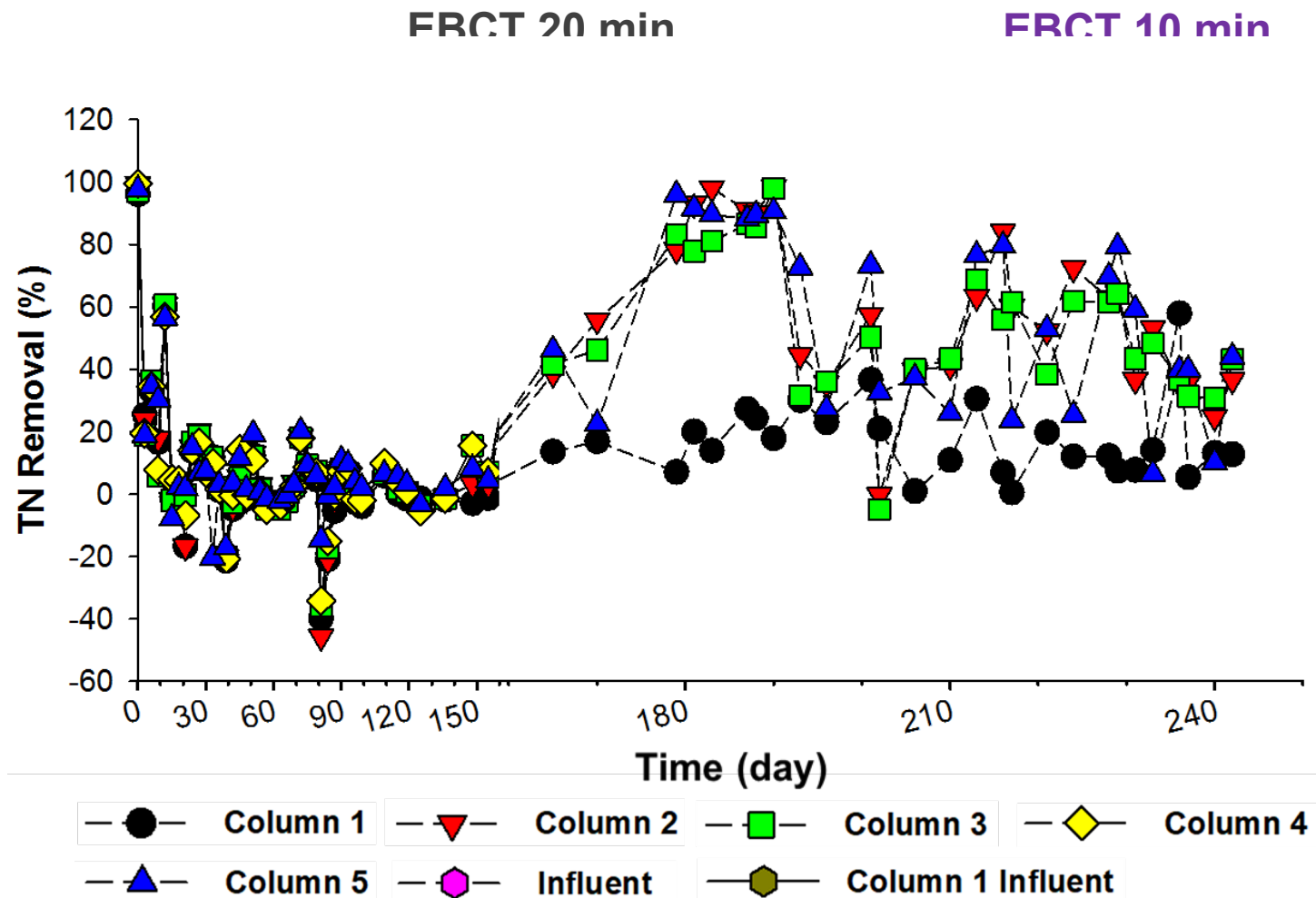
- Fresh GAC was slowly exhausted and TOC removal efficiency decreased over time



Results

Effect of filter media conditions on the removal of TN

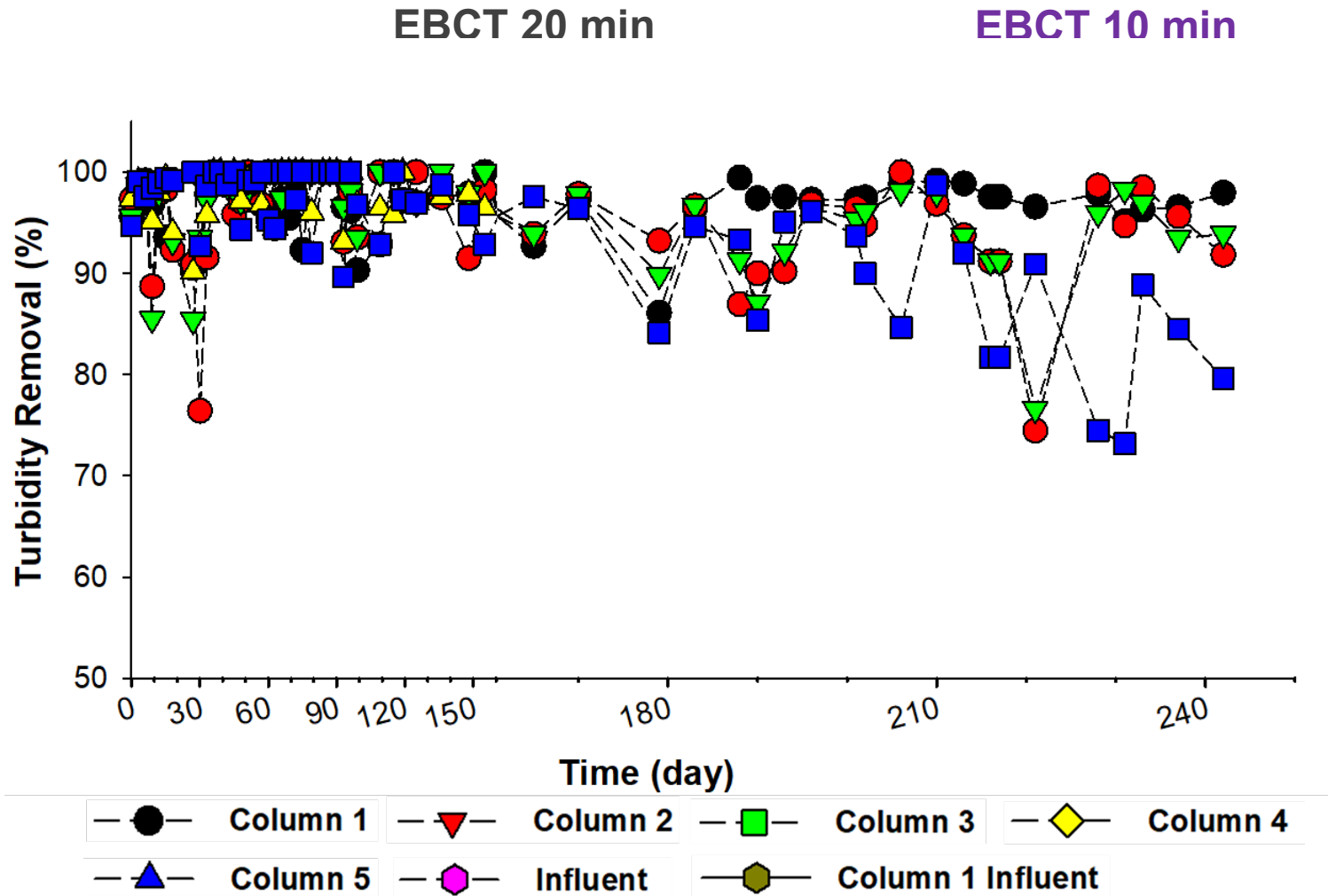
- Addition of AOM increased the removal efficiency of TN



Results

Effect of filter media conditions on the removal of turbidity

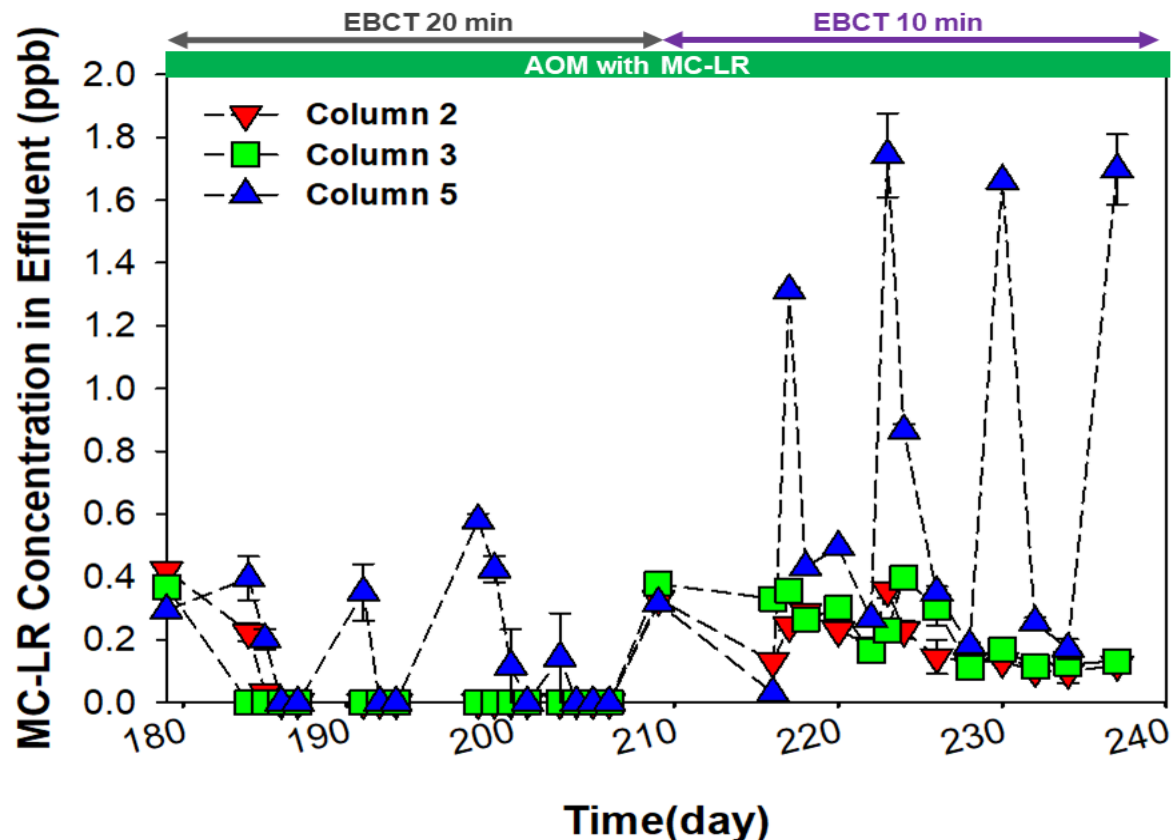
- Particle removal efficiency decreased by the attrition of AOM
- Column 5 at the EBCT of 10 min without active biofilm showed the lowest removal efficiency of particles



Results

Removal of MC-LR through GAC filtration under the presence of AOM

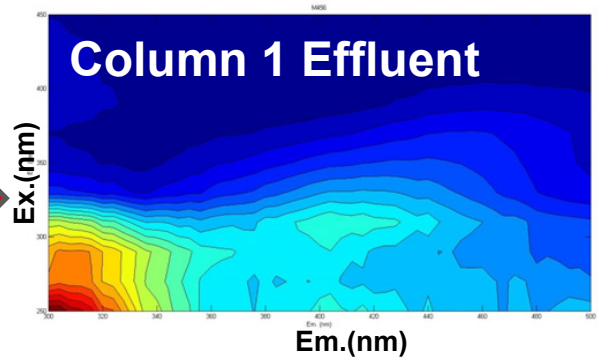
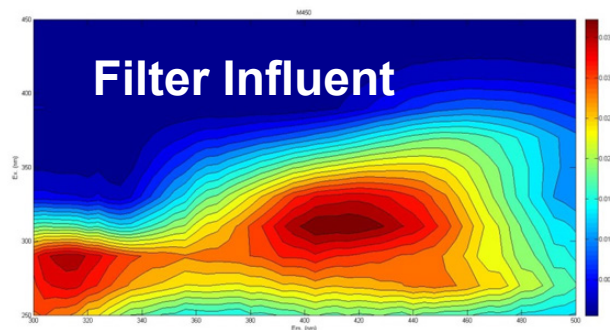
- A short EBCT (from 20 min to 10 min) and deactivation of biofilms significantly affected MC-LR removal by decreasing the removal efficiency



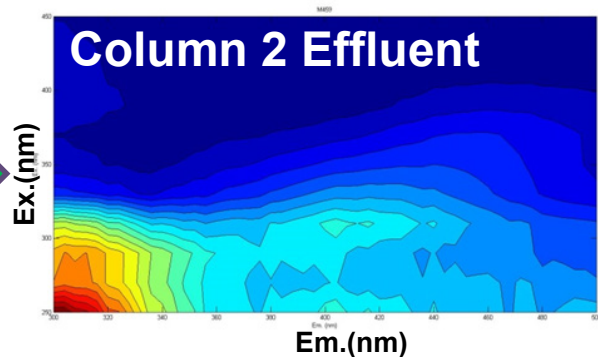
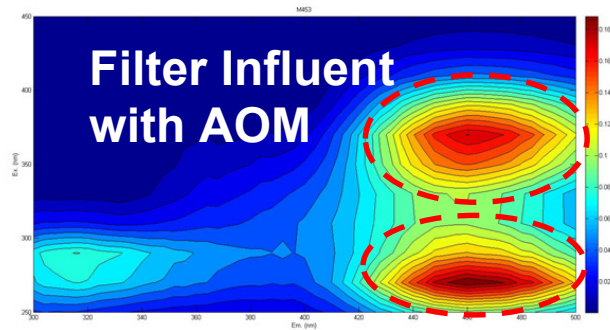


Results

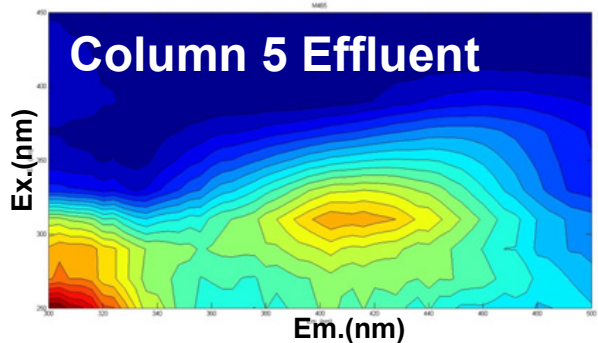
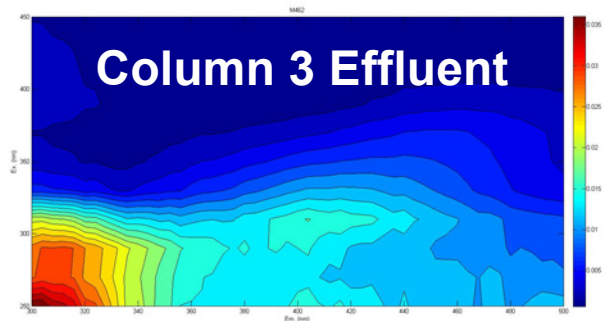
Fluorescence excitation-emission matrices (EEM) Analysis



- Injected algal compounds increased peaks intensity at 352/441(Ex./Em.) and 282/353 (Ex./Em.)



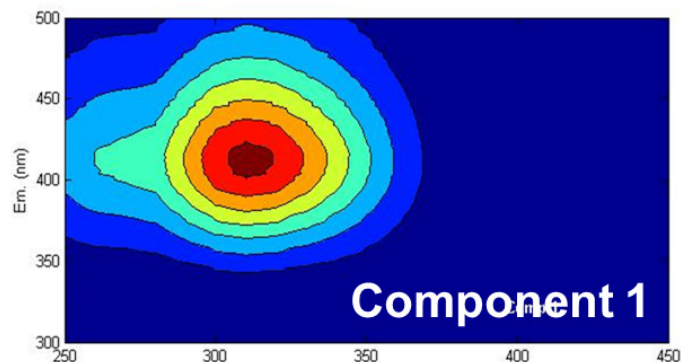
- EEM results showed intense peaks became weak after filtration, especially intense peaks by algal compounds got almost disappeared



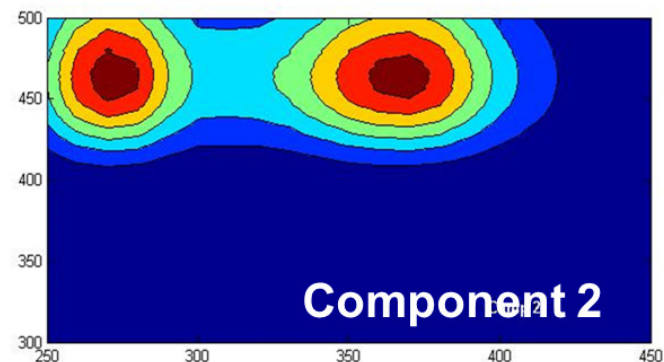
Results

FEEM contour plots of the 4 model components (C1, C2, C3, and C4) obtained by using PARAFAC

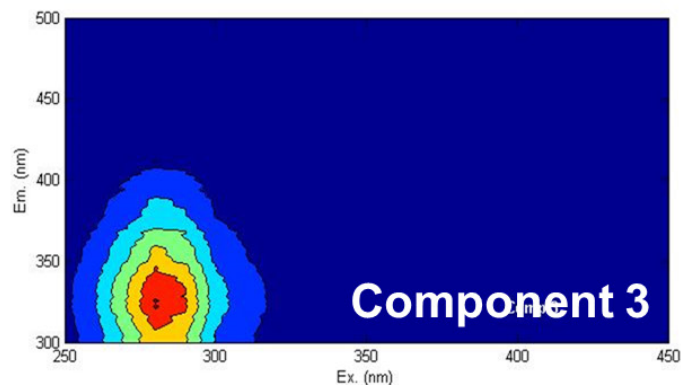
- A total ninety six EEM samples from filter effluents were used to obtain a PARAFAC model
- The model suggested 4 different components



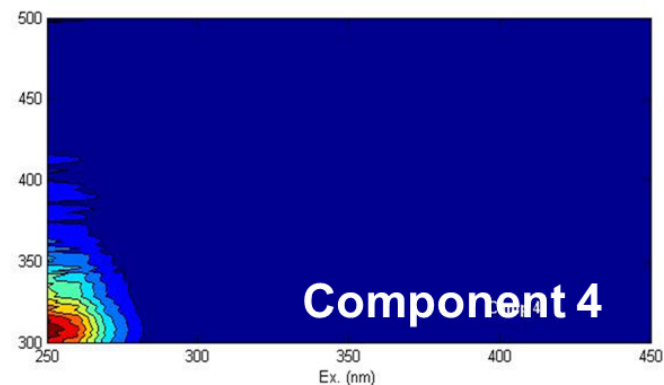
Marine Humic-like Substances



Terrestrial Humic-like Substances



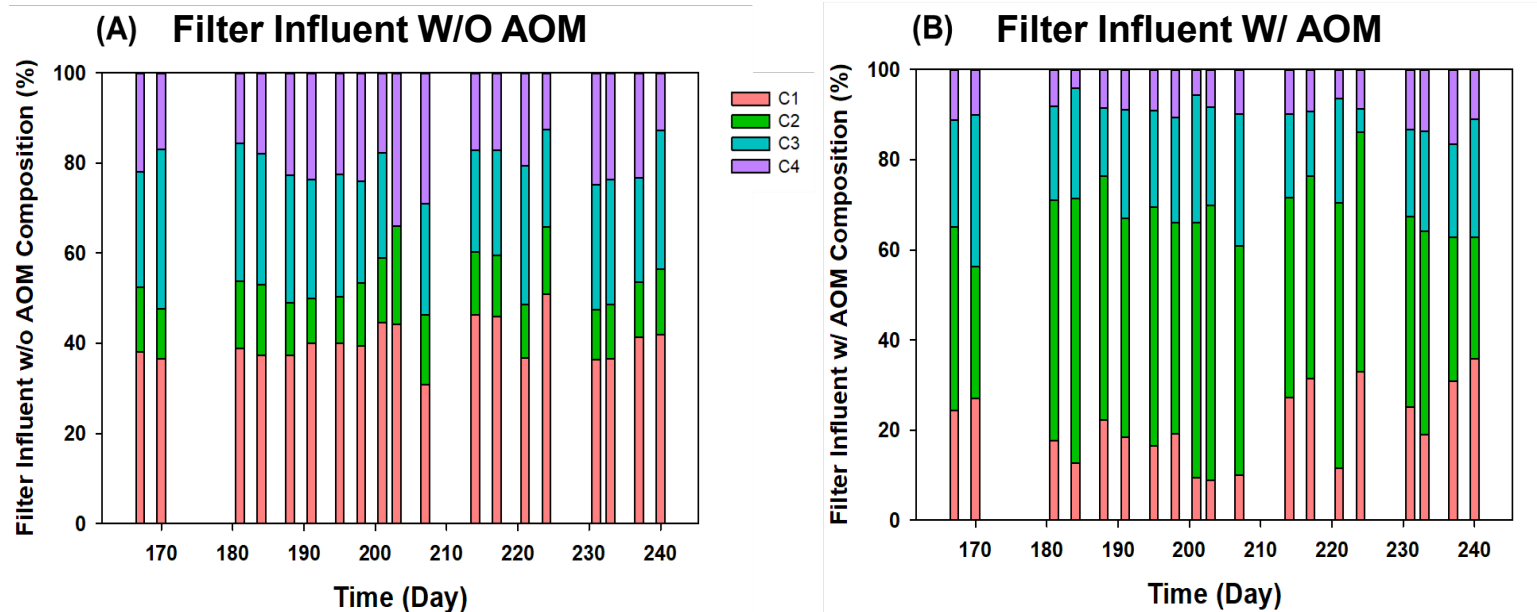
Protein, Tyrosine-like Substances



Protein, Tyrosine-like Substances

Results

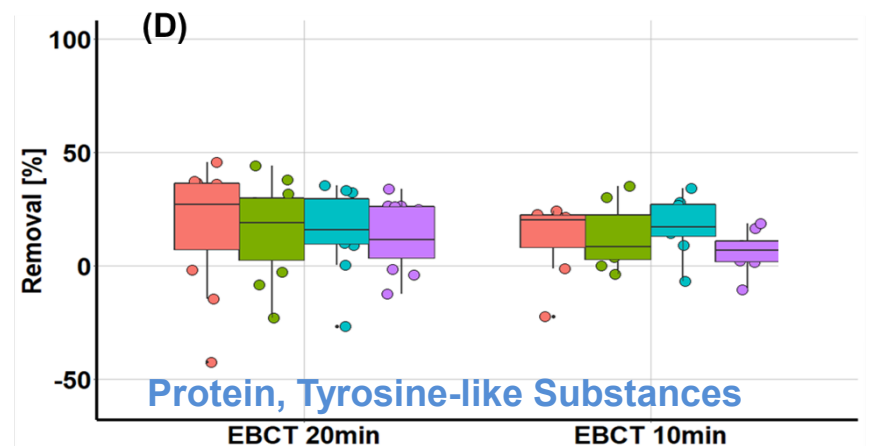
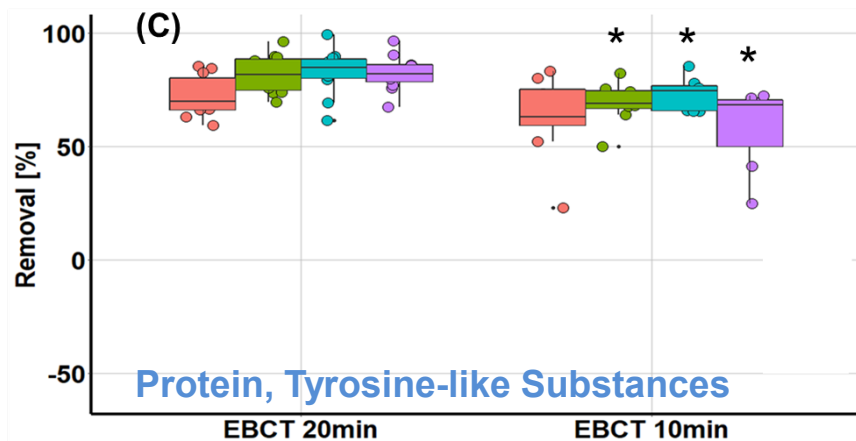
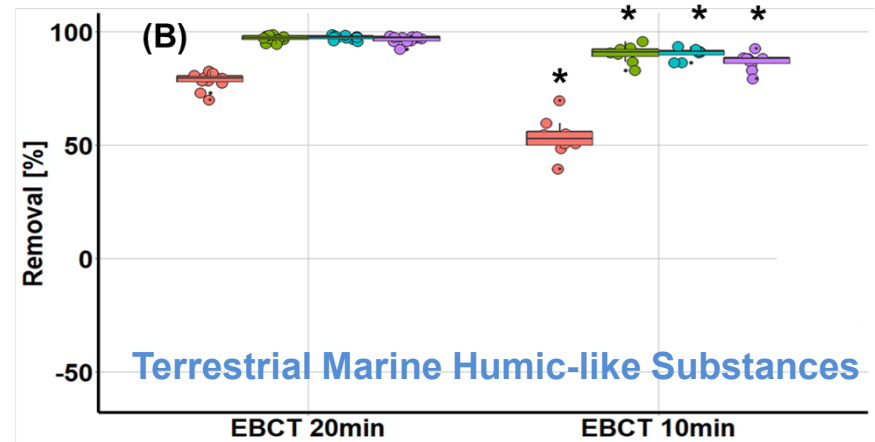
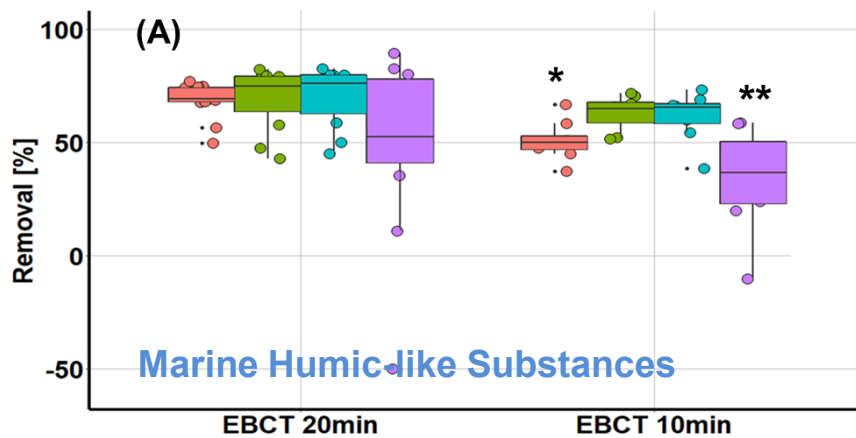
- The addition of AOM in filter influent augmented Component 2 and Component 3 proportions



Results

Percentage reduction of the components in GAC filter

- GAC showed better removal efficiencies for humic-like substances than protein, tyrosine-like substances



Results

- The removal of Component 2 was closely correlated with the removal of MC-LR

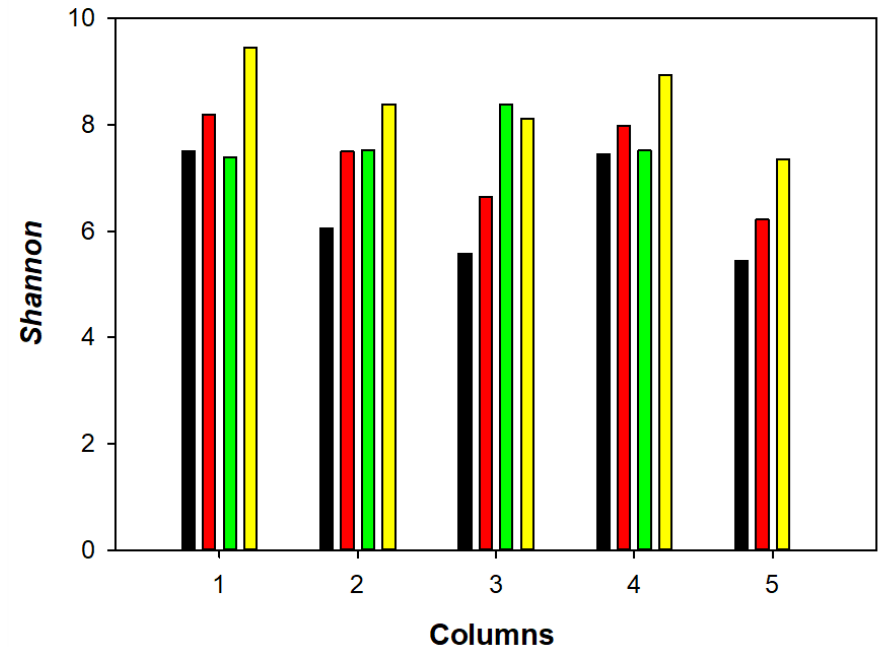
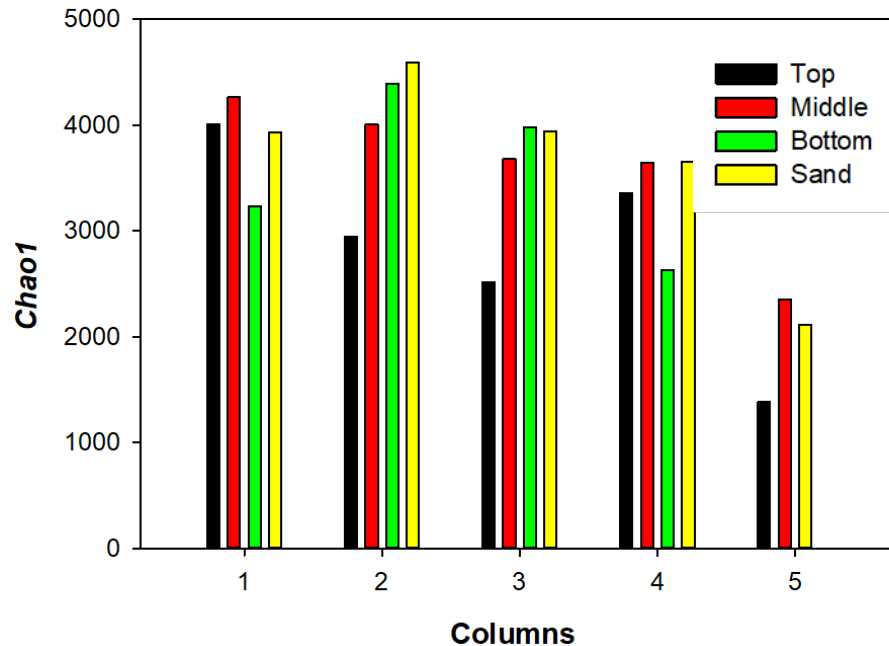
Spearman's rank correlation coefficients between PARAFAC component removal and MC-LR (* Correlations are significant, $p < 0.05$, based on two tailed test)

	MC-LR removal		
	Column 2	Column 3	Column 5
Component 1 removal	0.19	0.24	0.41
Component 2 removal	0.54*	0.60*	0.66*
Component 3 removal	0.47	0.26	0.14
Component 4 removal	0.18	0.22	0.29
Component 1/ Component 2	0.55*	0.75*	0.65*
Component 1/ Component 3	0.36	0.40	0.46
Component 1/ Component 4	0.19	-0.01	0.02

Results

Alpha Diversity Indices

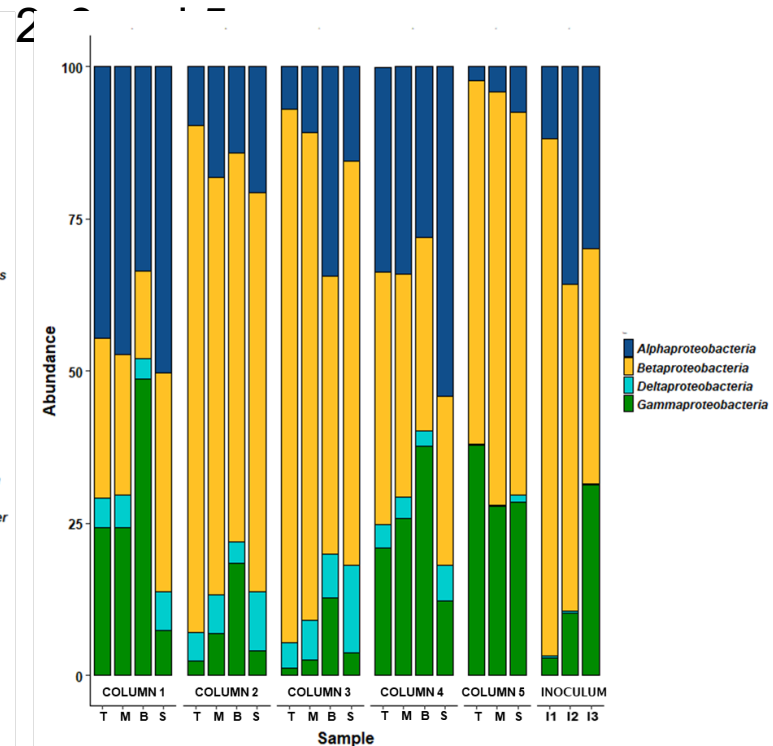
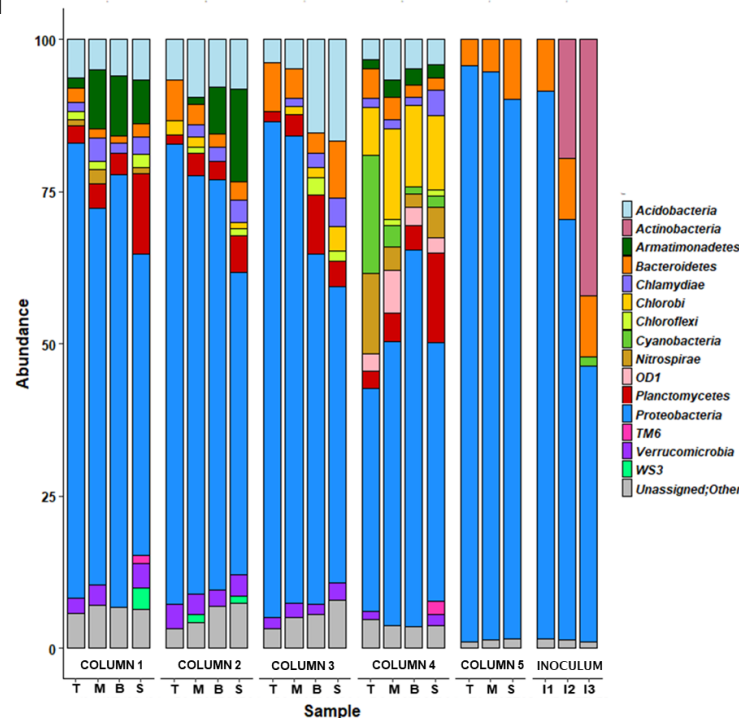
- Sand samples showed higher alpha diversity indices than those of the GAC samples from the bottom layer
- AOM decreased richness and diversity of bacterial communities in column 2 and 3



Results

Phylum and Class Level Composition of Bacterial Sequences (>1% Abundance Level)

- At phylum level the group of *Proteobacteria* comprised major proportion of the microbial community
- The *Betaproteobacteria* overwhelmingly dominated both column 2 (63-83%) and column 3 (45 -87%)
- The *Bacteroidetes* accounted for the second highest abundance of the community



* I1: Influent in August, I2: Influent in November, I3: Injected biomass to Column 2

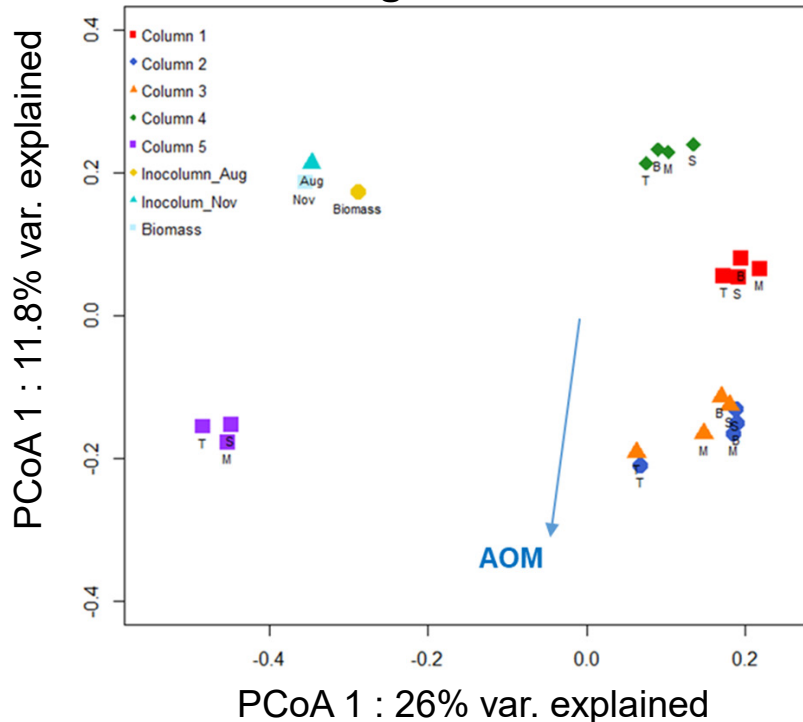
** T: Top, M: Middle, B: Bottom, S: Sand

Results

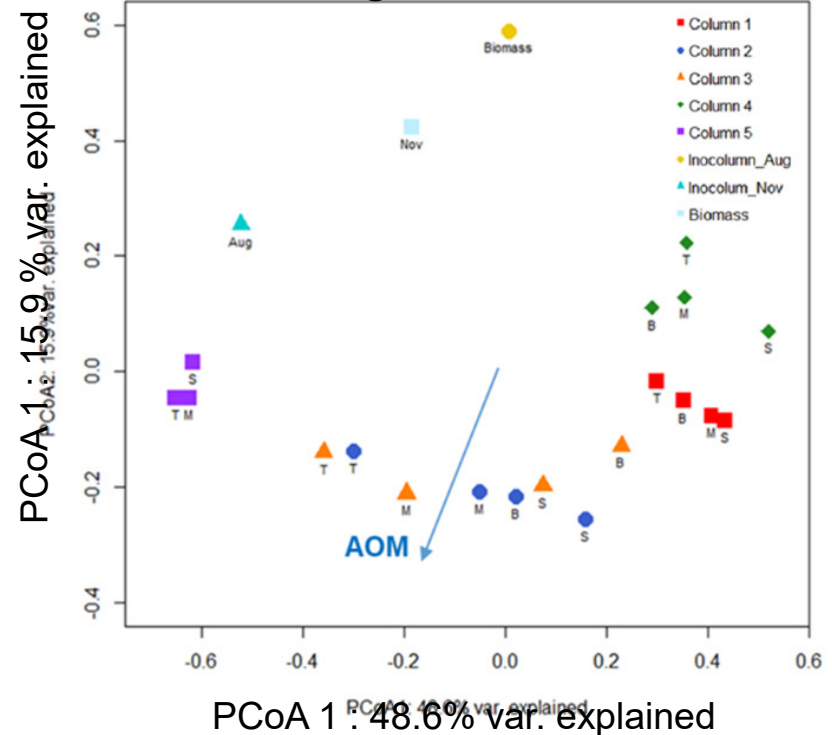
PCoA of Unweighted and Weighted Unifrac Distances

- Column 1, column 4, and column 5 were three distinct groups far separating from the inoculum, while column 2 and 3 were aggregated together in Unweighted Unifrac PCoA
- In Weighted Unifrac PCoA, a similar pattern was observed but Column 2 and column 3 samples were more horizontally scattered between the clusters of column 1 and column 5

Unweighted Unifrac



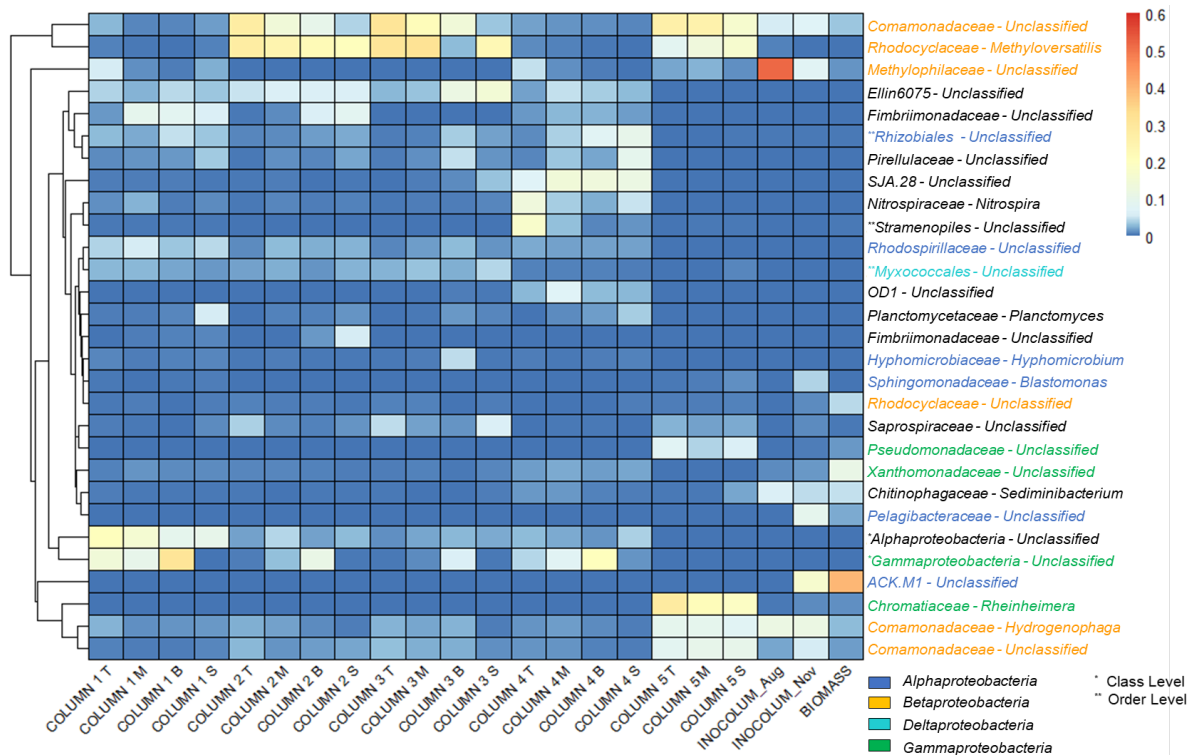
Weighted Unifrac



Results

Comparison of Column Samples at the Genus Level (4%>Abundance)

- *Rhodocyclaceae* and *Comamonadaceae* belong to *Betaproteobacteria* group were dominant in column 2, 3, and 5
- Those two groups are capable of oxidizing $\text{NH}_4\text{-N}$ under aerobic condition and reducing nitrate and nitrite to N_2 under anoxic condition (AOM is rich in organic-nitrogen)



Conclusions

- ❑ AOM induced biofilm formation on GAC and decreased the efficiency of particle removals
- ❑ The reduced EBCT and deactivation of biofilms significantly affected the filter performances including MC-LR removal
- ❑ Humic-like substances showed higher affinity than protein, tyrosine-like substances against GAC filter media
- ❑ A comprehensive 16S rRNA-based amplicon sequencing analysis revealed that AOM affected the bacterial community composition largely at the class level by shifting the most abundant fraction from *Alphaproteobacteria* to *Betaproteobacteria*
- ❑ Dominant taxa within the *Betaproteobacteria* were *Rhodocyclaceae* and *Comamonadaceae*. Their prevalence in column 2,3, and 5 is presumably related to the utilization of AOM-related components

Acknowledgements

- ❑ This research was supported by NSF GOALI (1605185), the Ohio Department of Higher Education (R/SDW-2-BOR), and the Ohio Water Development Authority (7174)



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EPA Disclaimer

The U.S. Environmental Protection Agency, through its Office of Research and Development, partially funded and collaborated in the research described herein. It has been subjected to the Agency's peer and administrative review and has been approved for external presentation. Any opinions expressed in this presentation are those of the authors and do not necessarily reflect the views of the Agency, therefore, no official endorsement should be inferred. Any mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Thank You !

Questions? /Comments?



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