



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

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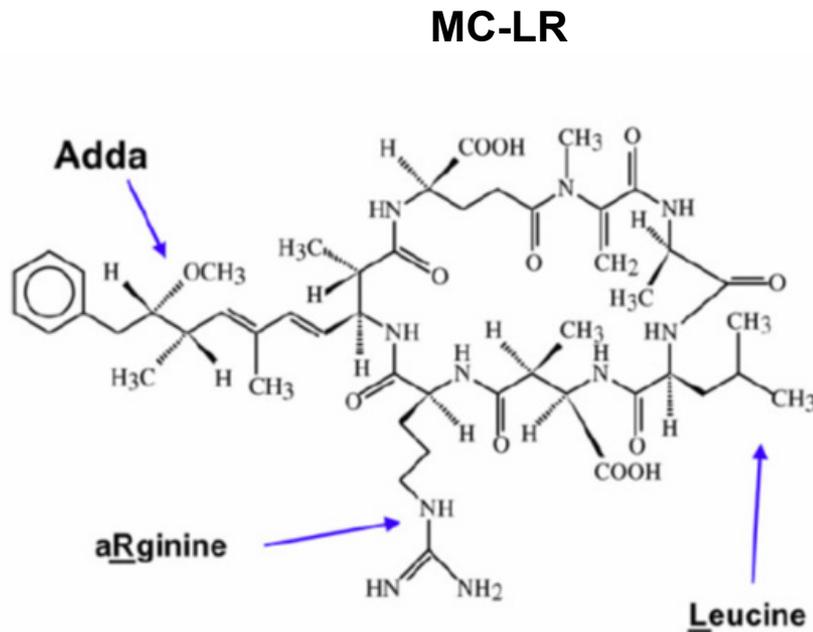
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Hepatotoxin Microcystin-LR

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



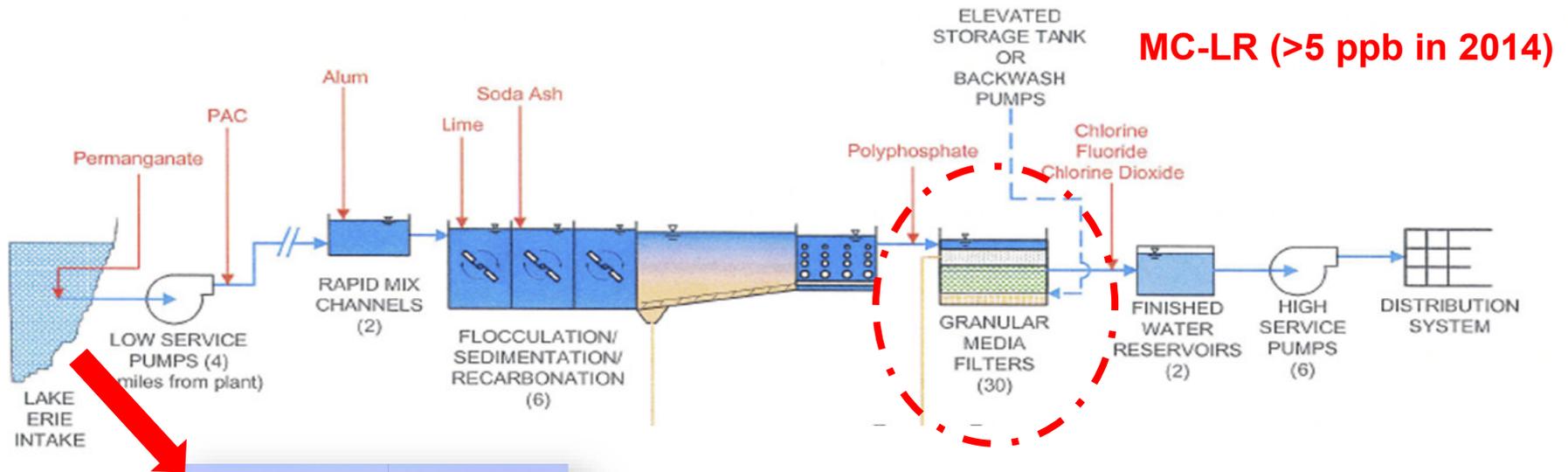
Lake Erie Water Intake



From Toledo Blade

Toledo Water Treatment Plant

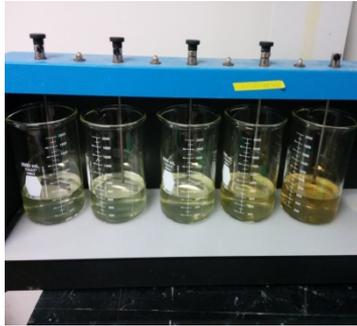
- ❑ At high levels AOM inhibits the efficiency by increasing the negative charges of particles
- ❑ Some proteins are likely to form complexes with coagulants, resulting in the reduction of available coagulant doses for destabilization.
 - ➔ Coagulation/Flocculation processes are not able to completely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



Schematic of Toledo Drinking Water Treatment Plant



Controlling Methods for Cyanotoxins



Pretreatment using KMnO_4



Ozonation



UV/ H_2O_2



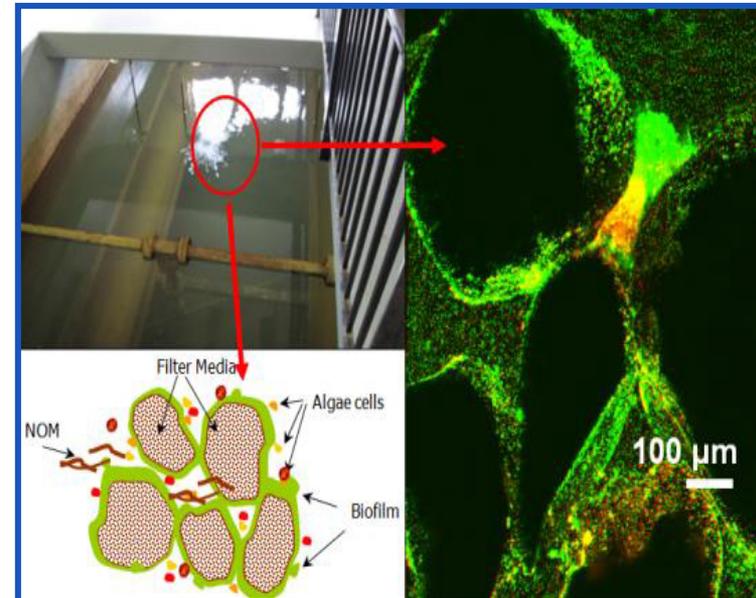
Membrane Filtration



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, anthracit, or granlar-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

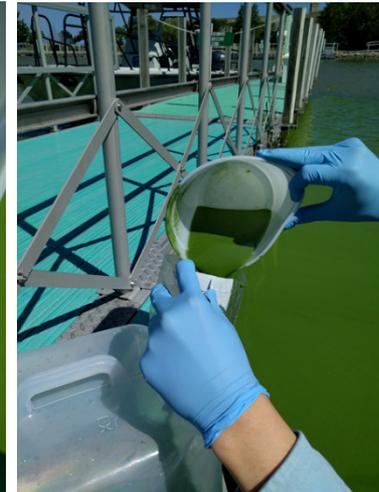
Preparation of source water and solution of AOM

- ❑ 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

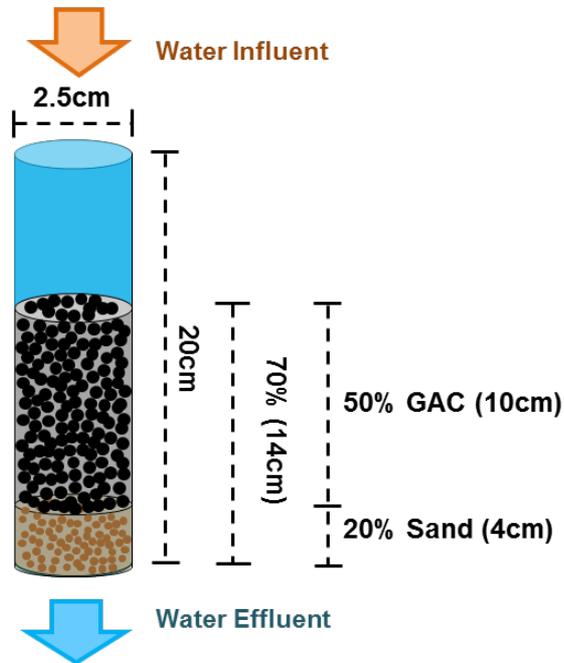


Intracellular algal compounds

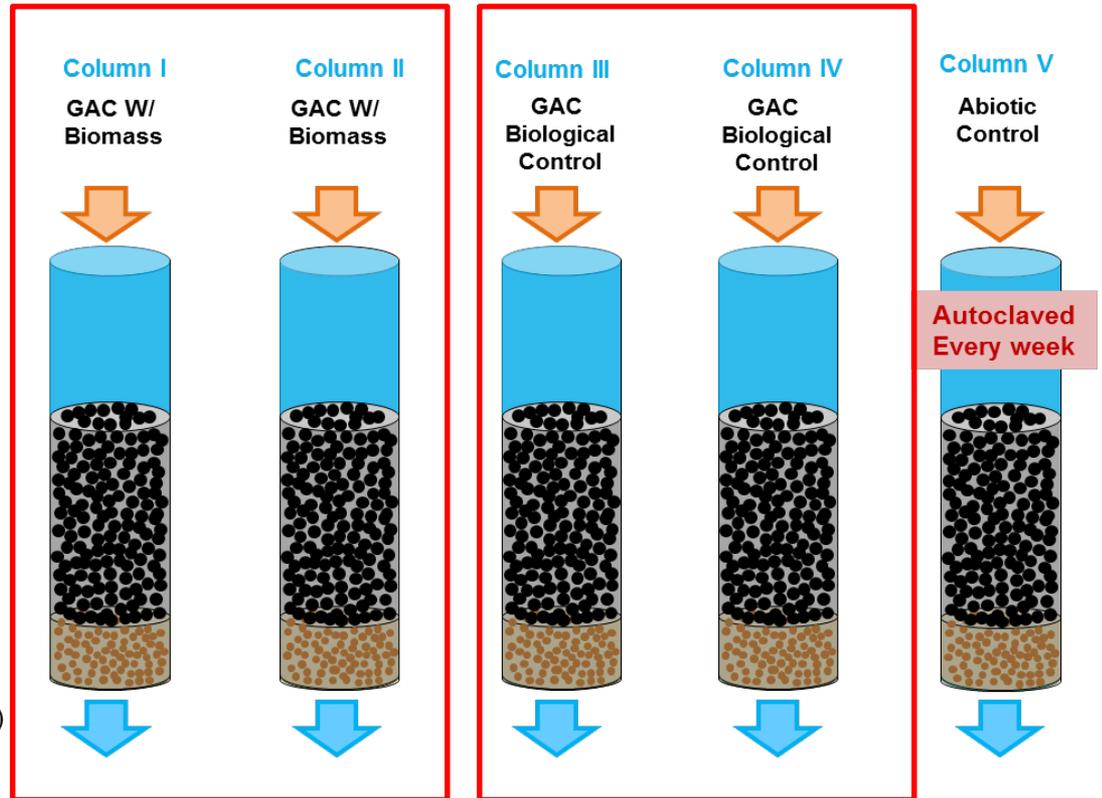


Materials & Methods

Biological Filtration Column



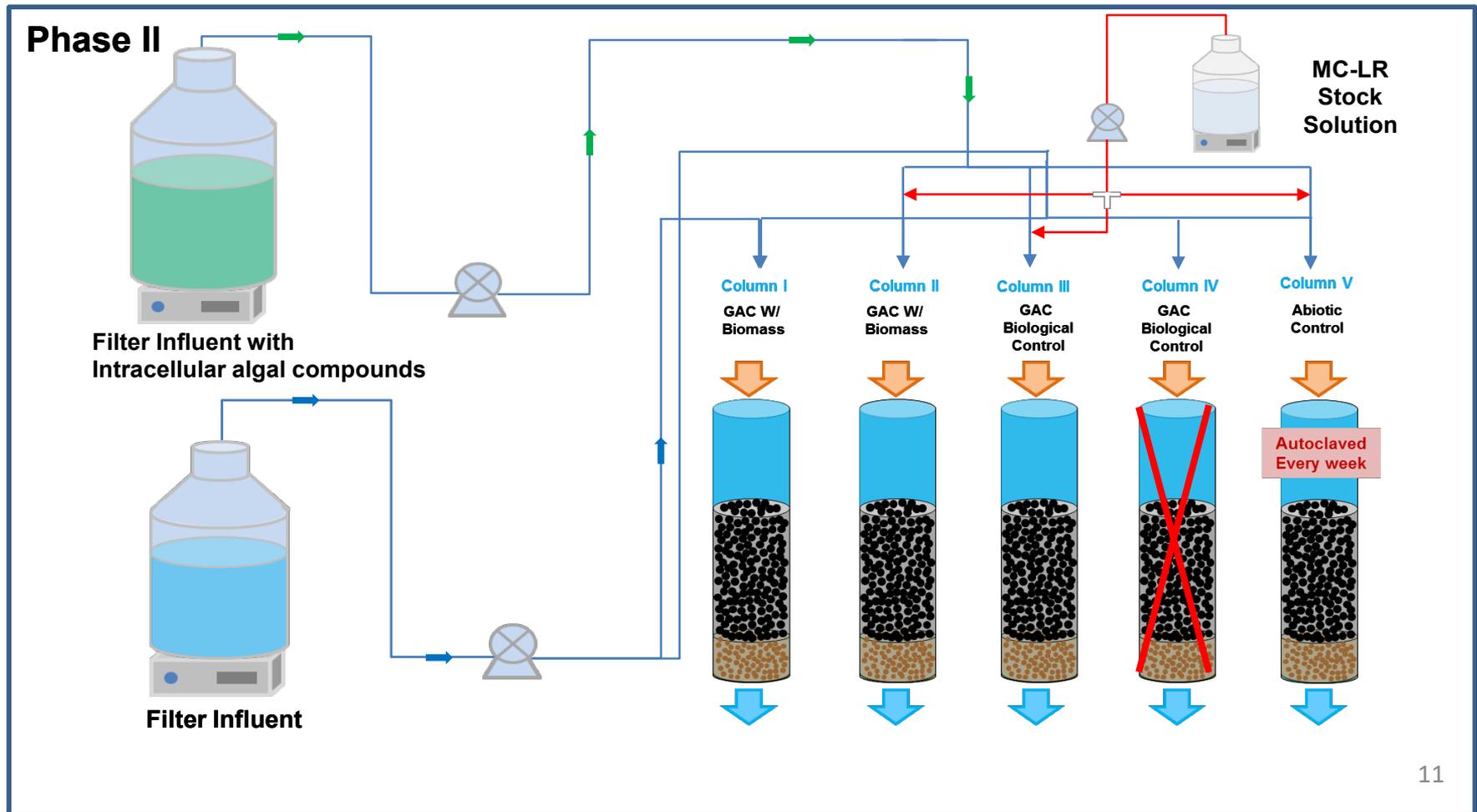
- **Column Dimensions**
: Autoclavable glass column, 2.5 cm (D) × 20 cm (L)
- **Flow Rate**
: 2.54 ml/min
- **Empty Bed Contact Time (EBCT)**
: 20 min



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)

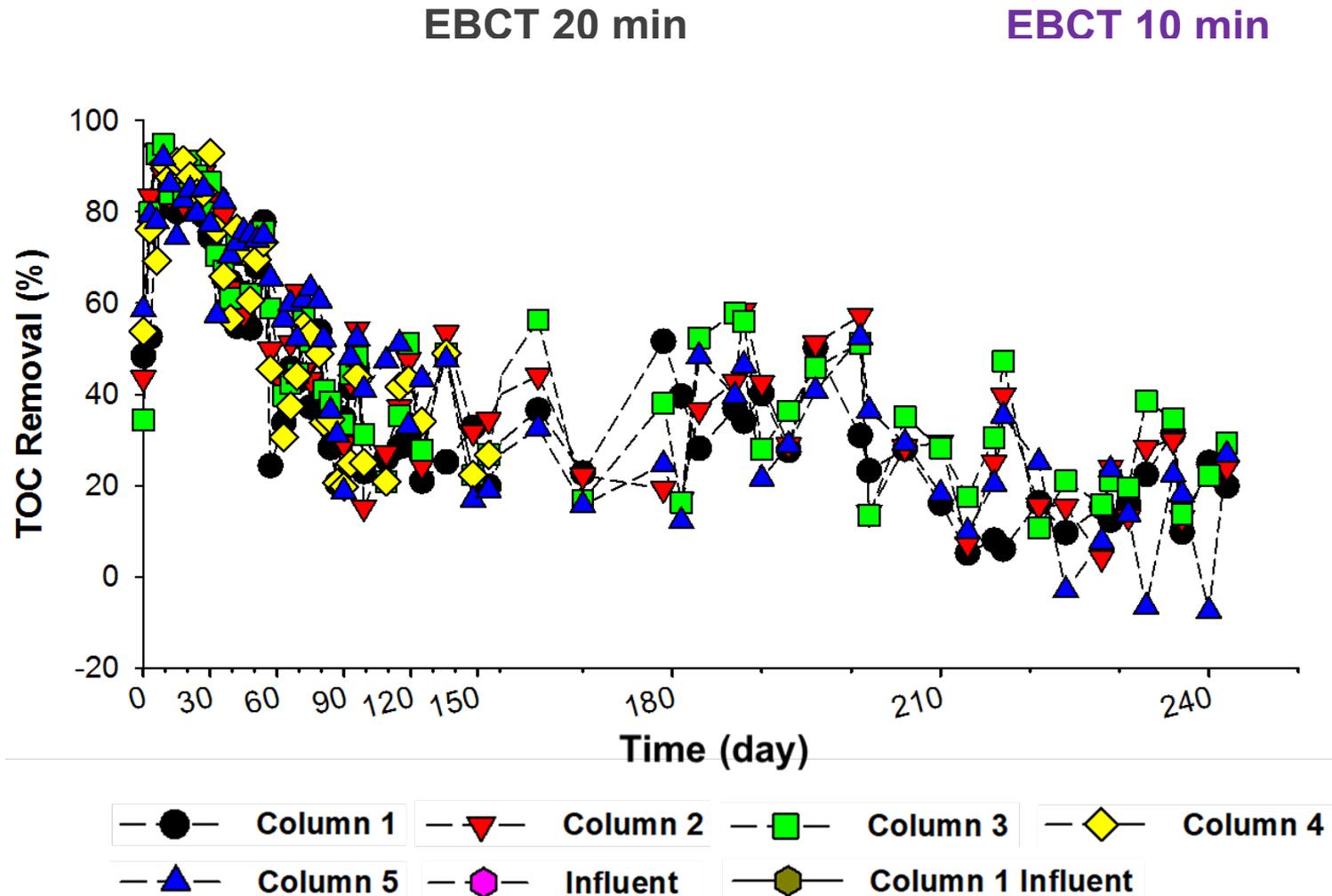
Microbial Community Structure Analysis

- ❑ 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

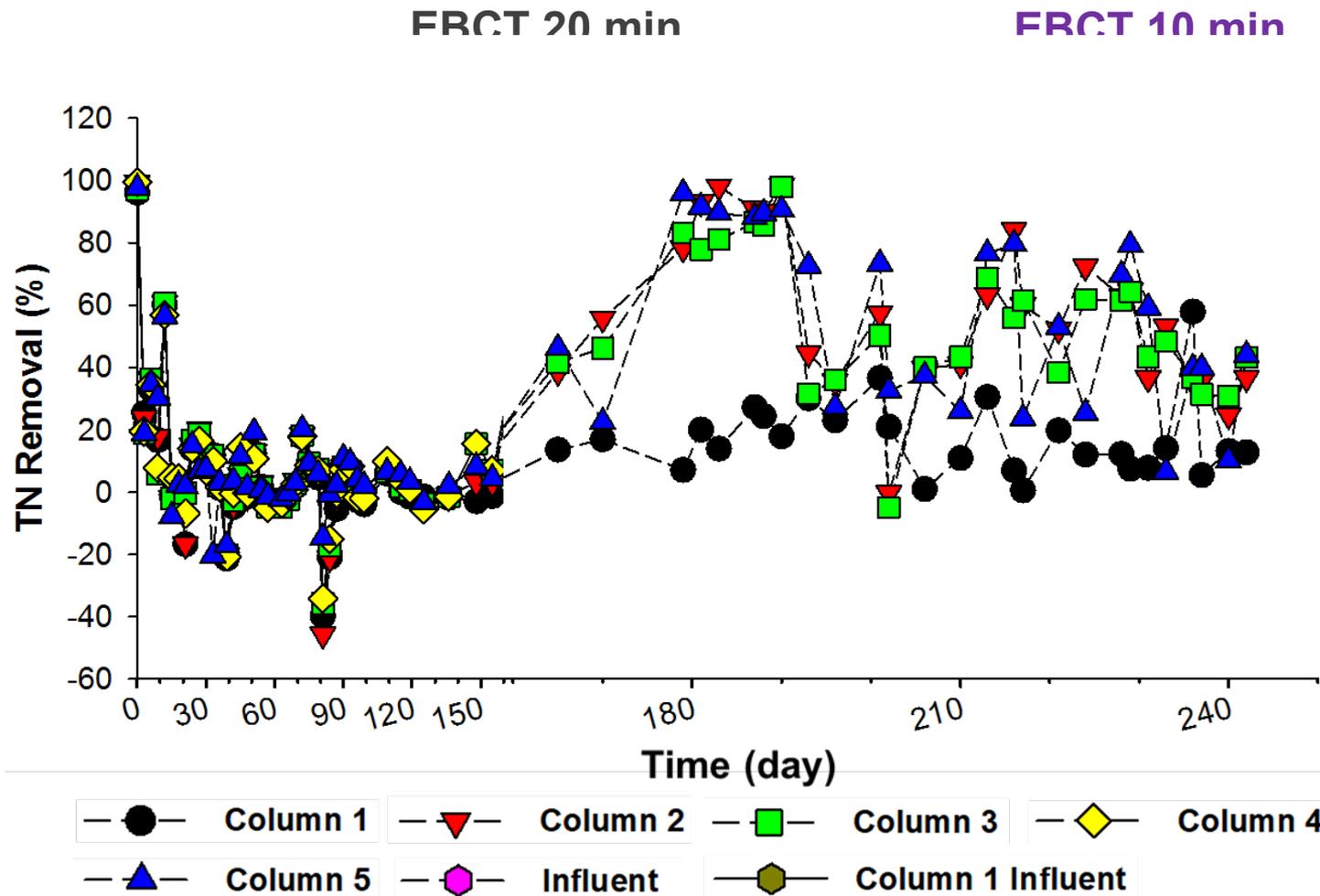
- Fresh GAC was slowly exhausted and TOC removal efficiency decreased over time



Results

Effect of filter media conditions on the removal of TN

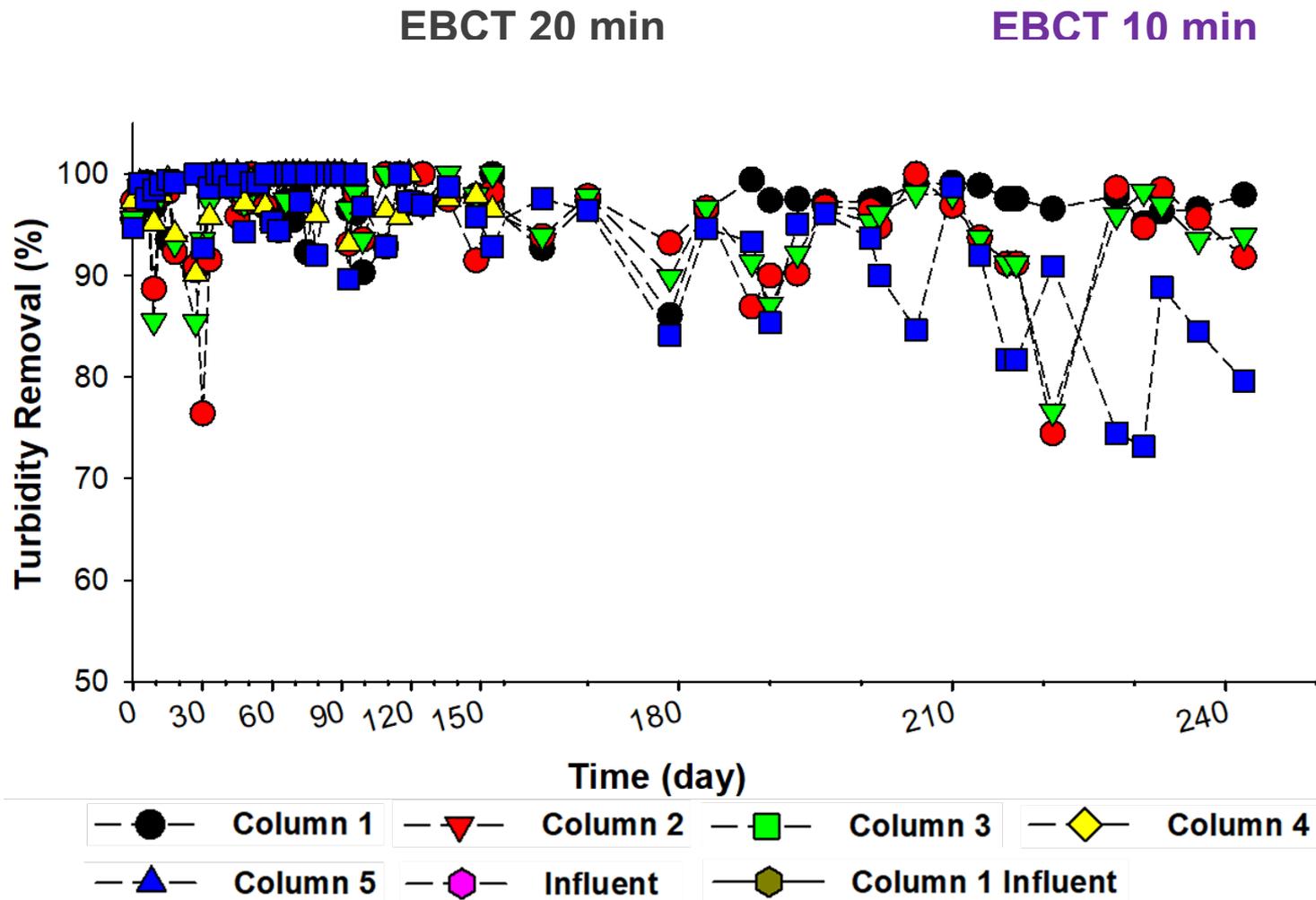
- Addition of AOM increased the removal efficiency of TN



Results

Effect of filter media conditions on the removal of turbidity

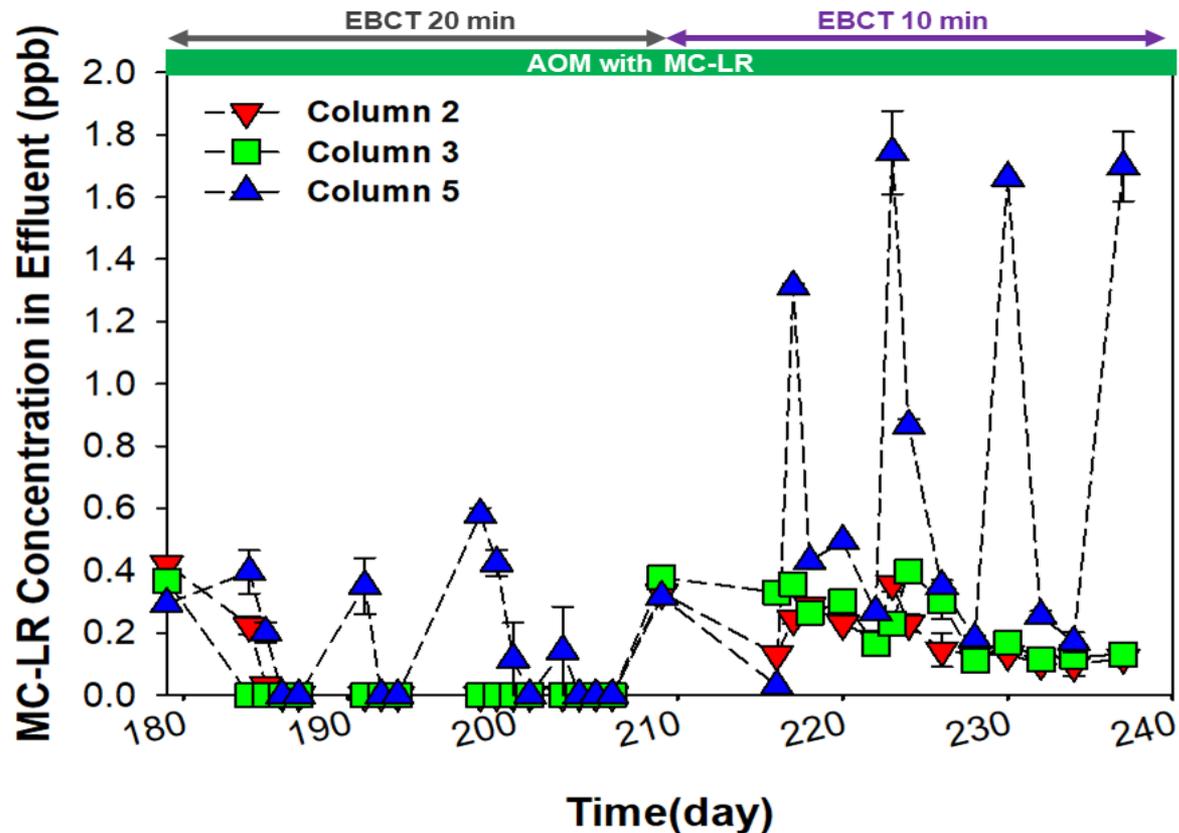
- Particle removal efficiency decreased by the attrition of AOM
- Column 5 at the EBCT of 10 min without active biofilm showed the lowest removal efficiency of particles



Results

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

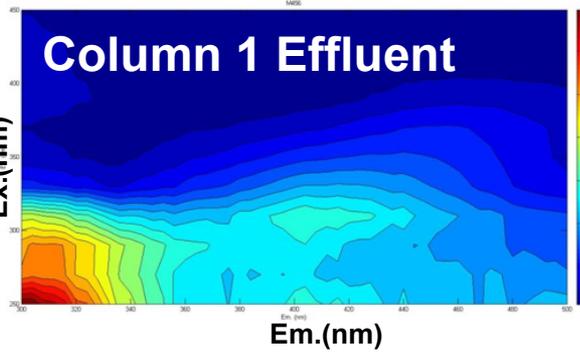
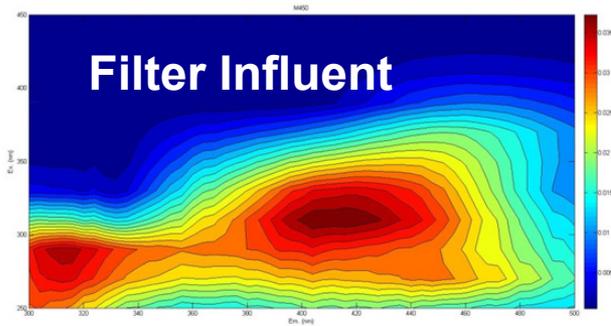
- A short EBCT (from 20 min to 10 min) and deactivation of biofilms significantly affected MC-LR removal by decreasing the removal efficiency



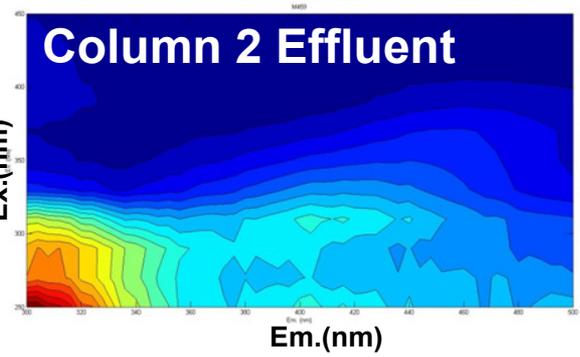
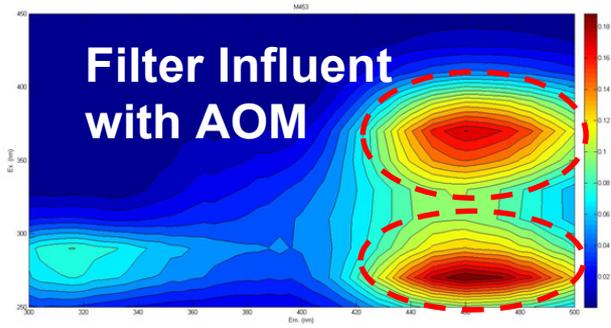


Results

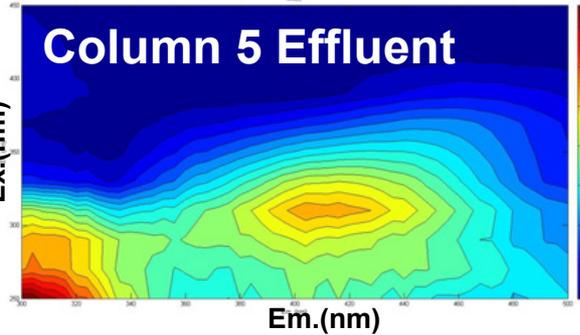
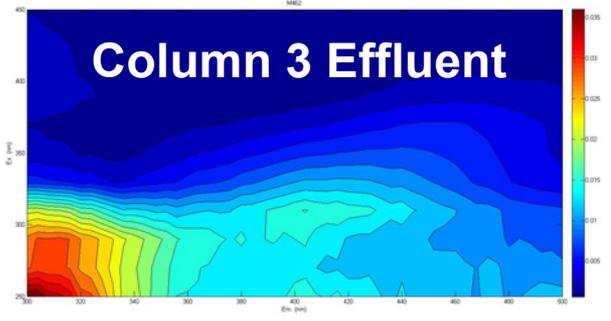
Fluorescence excitation-emission matrices (EEM) Analysis



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



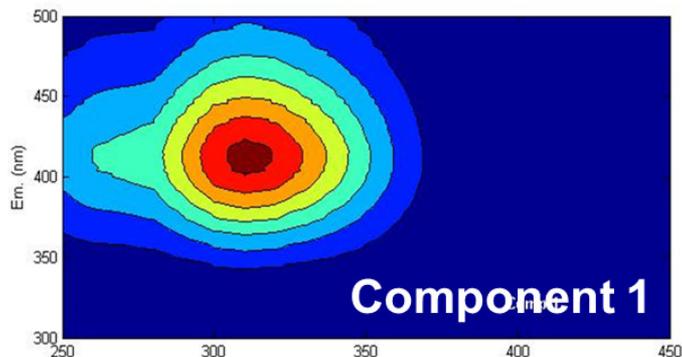
■ EEM results showed intense peaks became weak after filtration, especially intense peaks by algal compounds got almost disappeared



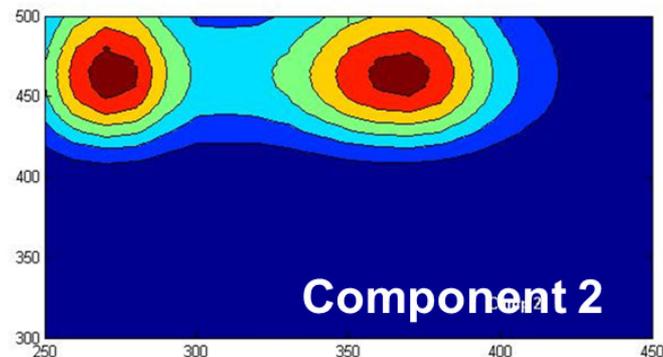
Results

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

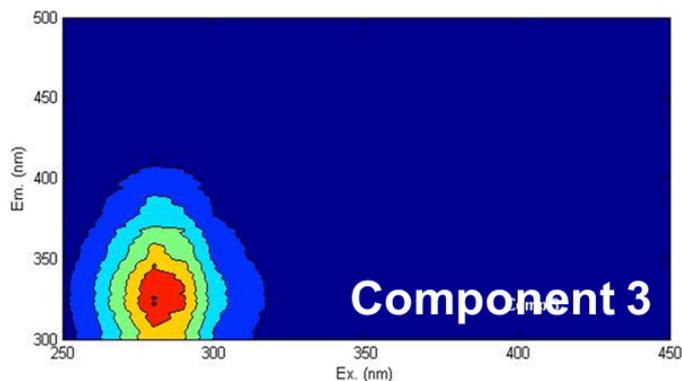
- A total ninety six EEM samples from filter effluents were used to obtain a PARAFAC model
- The model suggested 4 different components



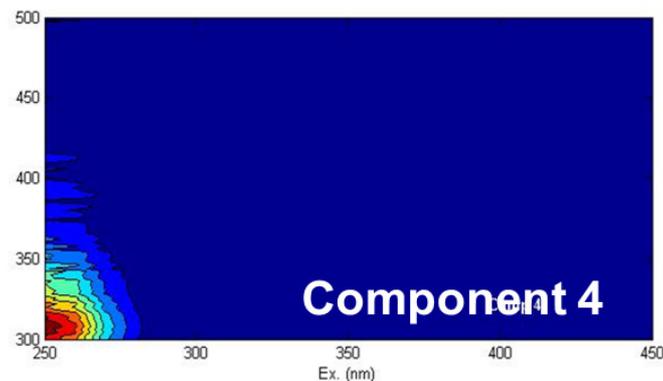
Marine Humic-like Substances



Terrestrial Humic-like Substances



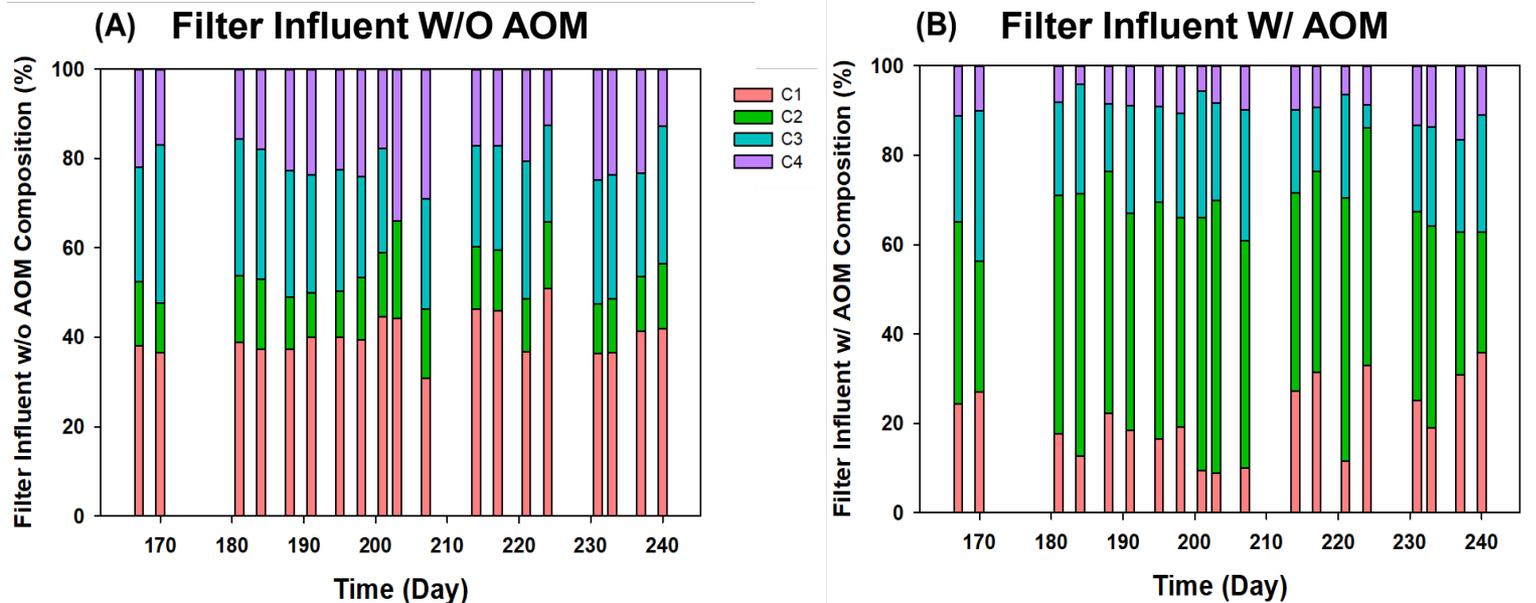
Protein, Tyrosine-like Substances



Protein, Tyrosine-like Substances

Results

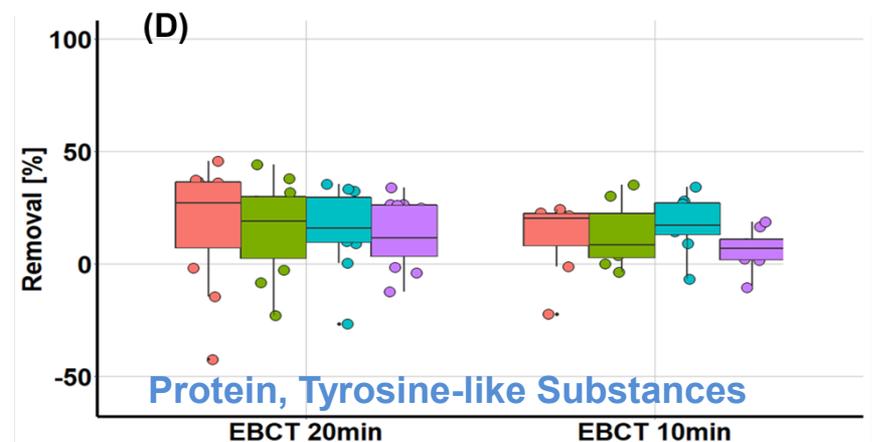
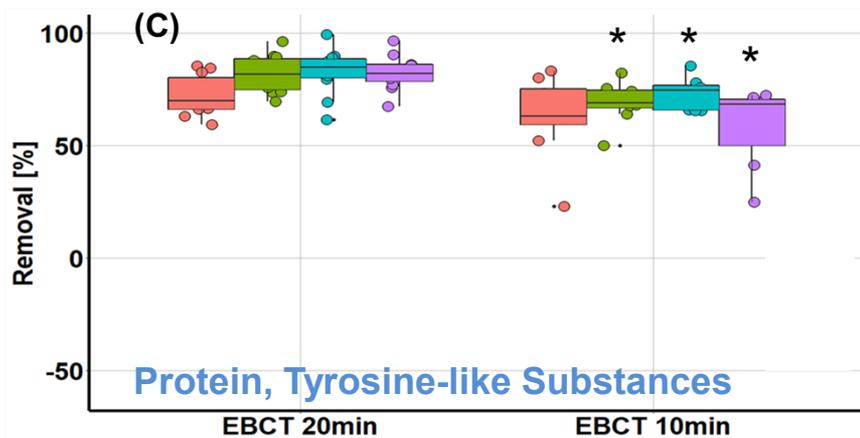
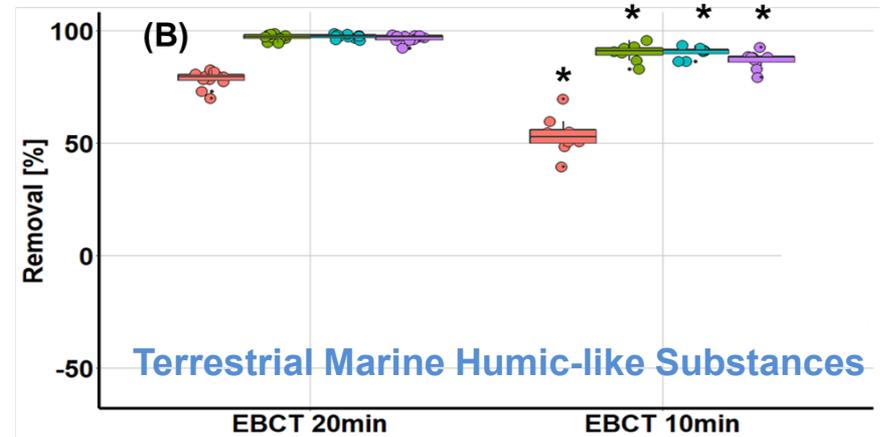
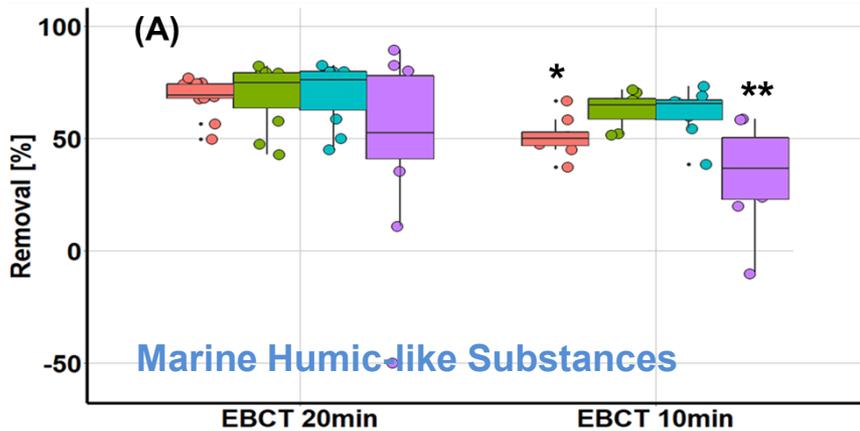
- The addition of AOM in filter influent augmented Component 2 and Component 3 proportions



Results

Percentage reduction of the components in GAC filter

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



Column 1 Column 2 Column 3 Column 5

Results

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

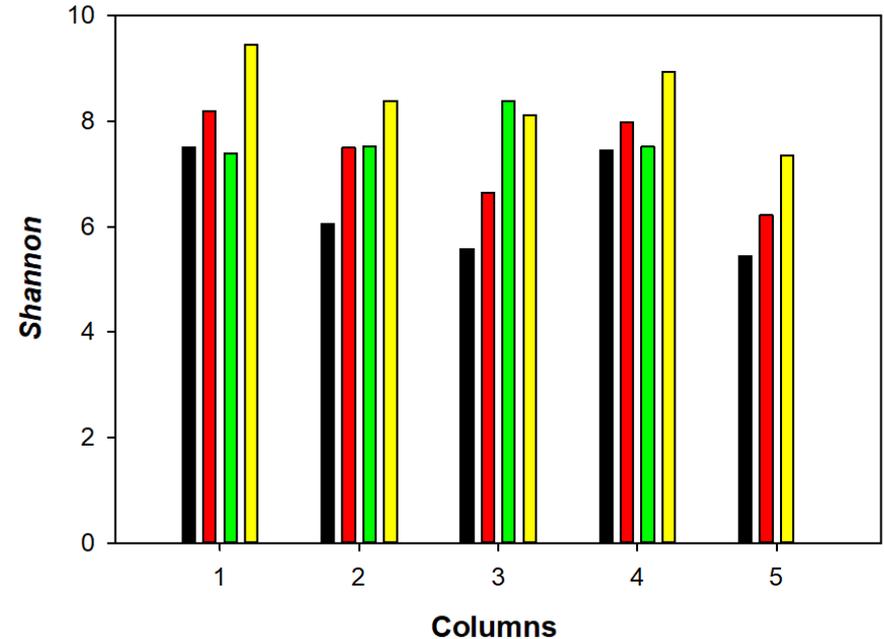
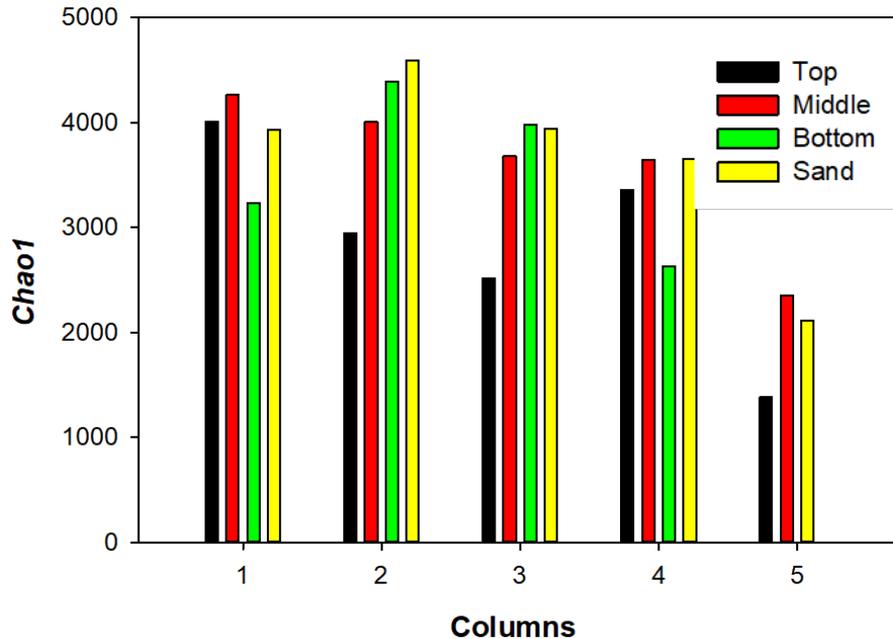
Spearman's rank correlation coefficients between PARAFAC component removal and MC-LR (* Correlations are significant, $p < 0.05$, based on two tailed test)

	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

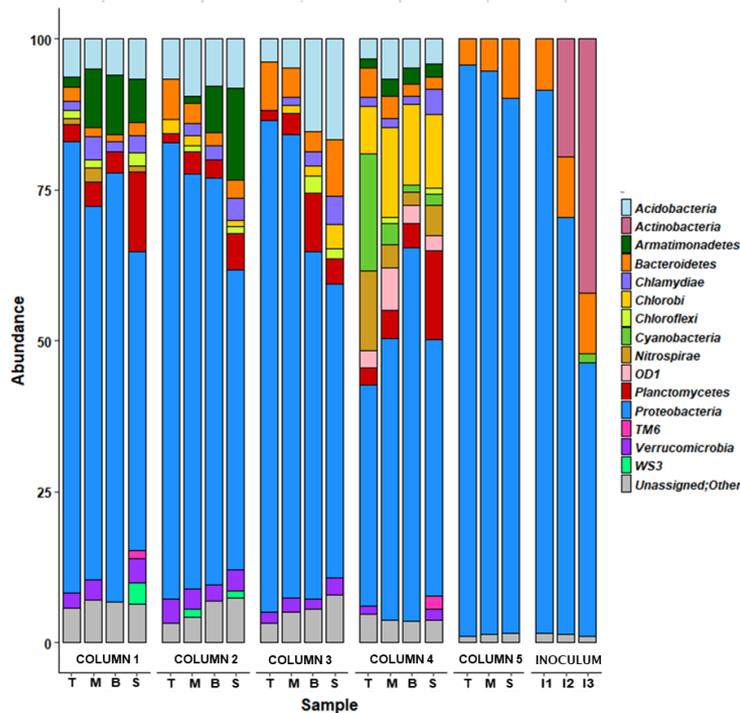


Results

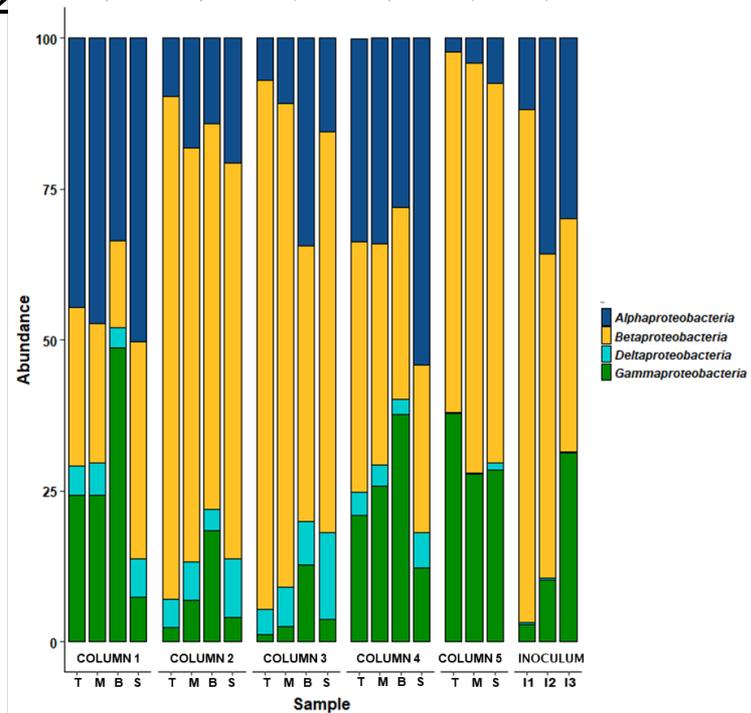
Phylum and Class Level Composition of Bacterial Sequences (>1% Abundance Level)

- 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

con



2



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

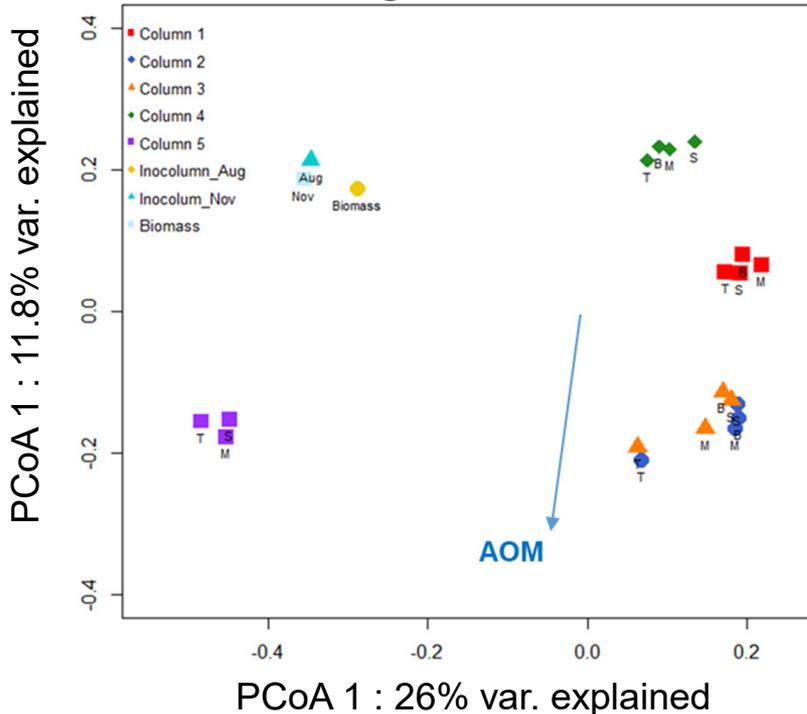
** T:Top, M:Middle, B: Bottom, S: Sand

Results

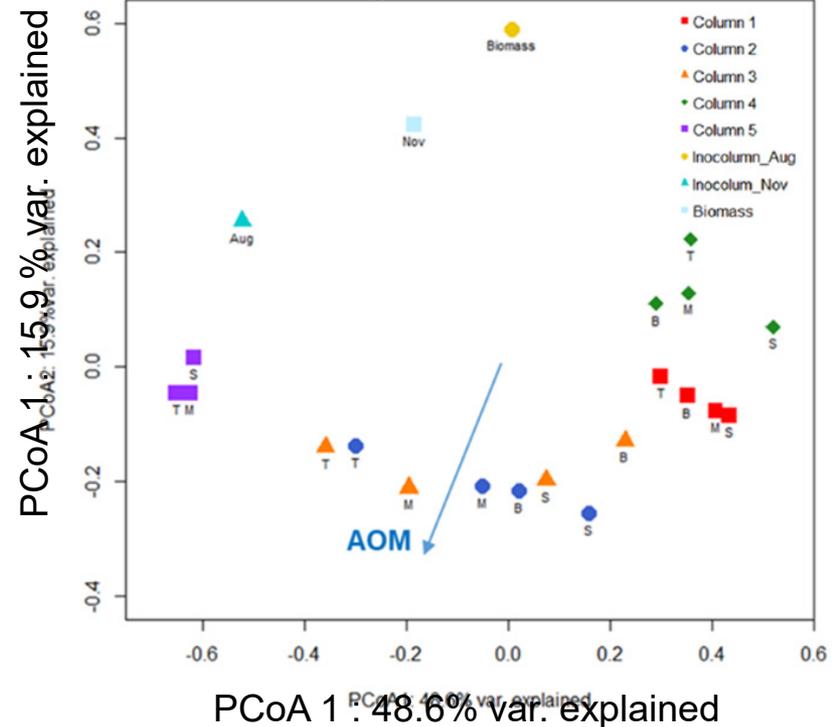
PCoA of Unweighted and Weighted Unifrac Distances

- 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



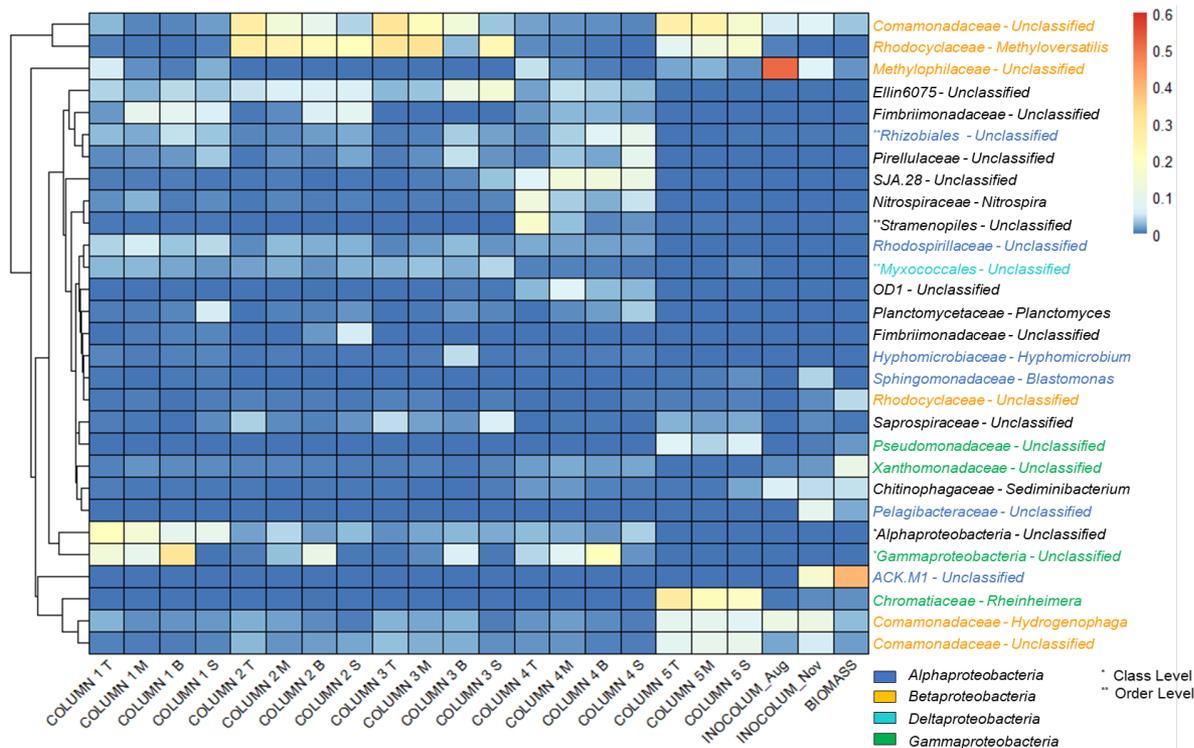
Weighted Unifrac



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

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Questions? /Comments?



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