Influence of Algal Organic Matter on Biofilm Development and Disinfection By-product Formation in Simulated Water Distribution Systems

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1. Introduction

2. Experiment Methods

3. Results & Discussion

4. Conclusion
Introduction
Natural Organic Matter (NOM) in Source Water

- **Humic substance (HS)**
  - Allochthonous source NOM
    - Aromatic carbon
    - Phenolic
    - Conjugate double bonds
  - Autochthonous source NOM
    - Nitrogenous contents
    - Hydrophilic faction
    - Small molecules
Water Treatment Process

Conventional Water Treatment

Limitation
Treatment capacity
The presence of NOM might cause several issues in drinking water distribution system (DWDS):

- Color problem
- Odor problem
- Supporting the growth of microorganism

Biofilm in DWDS:

- 90% of microorganism can be found in matrix enclosed microbial colonies.
- Biofilm survives even in the presence of disinfectant residue.
- Biofilm affects the taste, odor, and color as well as harboring opportunistic pathogens which may later released them into water flow.

Overlook problems:

- Forms disinfection-by products.
Study Overview

Goal:
1. Examine the biomolecular composition of biofilm changes in response to AOM or HS.
2. Understand the influence of biofilm on subsequently DBP formation.

Mushroom structure like Biofilm on pipe

Toxic???
Experiment Method
Experiment Preparation

Jar test for humic substance (Sigma-Aldrich)

Freeze and thaw 5 cycles

Jar test for algal organic matter
Operating a simulated DWDS using biofilm reactor

- **Biofilm reactor**
  - Influent
  - Effluent
  - Polypropylene coupon holders
  - PVC coupons
  - Magnetic stir bar

- **Humic substance** prepared from commercial product (Sigma - Aldrich)
- **Algal organic matter** collected from Lake Erie water sample during algal bloom season
- **Bacterial inoculum** prepared from local drinking water treatment plant

Continuous operating 6 month under ~ 1.5mg/L TOC influent condition for each reactor. Coupons were taken out from reactor every three weeks to conduct related tests.

- **Coupon number**: 24
- **Flow rate**: 0.5mL/min
- **Hydraulic retention time**: 12 hours
Experimental Methods

Biofilm structure analysis

- Confocal laser scanning microscopy: cell (green), protein (blue), polysaccharide (red)
**Experiment Methods**

**DBP formation potential test (DBPFP)**
- 5 days DBPFP test followed the standard method
- **DBP extraction**: Modified EPA method 551.1 and 552.2
- **Analytical instrument**: gas chromatograph (GC) (Shimadzu, Japan, GC-2010 plus)
- **DBP speciation**: THM$_4$, HAA$_9$, HK$_2$, HAN, HNM

### Analyzed DBP speciation table

<table>
<thead>
<tr>
<th>DBP categories</th>
<th>DBPs class</th>
<th>Analyte name</th>
<th>DBPs abbreviations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regulated</td>
<td>Haloaetic acids (HAA$_9$)</td>
<td>Monochloroacetic acid</td>
<td>MCAA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dichloroacetic acid</td>
<td>DCAA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monobromoacetic acid</td>
<td>MBAA</td>
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<tr>
<td></td>
<td></td>
<td>Bromochloroacetic acid</td>
<td>BCAA</td>
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<tr>
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<td></td>
<td>Dibromoacetic acid</td>
<td>DBAA</td>
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<td></td>
<td></td>
<td>Trichloroacetic acid</td>
<td>TCAA</td>
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<td></td>
<td></td>
<td>Bromodichloroacetic acid</td>
<td>BDCAA</td>
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<tr>
<td></td>
<td></td>
<td>Tribromoacetic acid</td>
<td>TBAA</td>
</tr>
<tr>
<td></td>
<td>Trihalomethanes (THMs)</td>
<td>Chloroform</td>
<td>TCM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bromodichloromethane</td>
<td>BDCM</td>
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<td>Dibromochloromethane</td>
<td>DBCM</td>
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<tr>
<td></td>
<td></td>
<td>Bromoform</td>
<td>TBM</td>
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<tr>
<td>Unregulated</td>
<td>Haloaetonitrile (HANs)</td>
<td>Dichloroacetonitrile</td>
<td>DCAN</td>
</tr>
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<td></td>
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<td>Trichloroacetonitrile</td>
<td>TCAN</td>
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<td>Bromochloroacetonitrile</td>
<td>BCAN</td>
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<tr>
<td></td>
<td></td>
<td>Dibromoacetonitrile</td>
<td>DBAN</td>
</tr>
<tr>
<td>Nitrogenous DBP (N-DBP)</td>
<td>halonitromethane (HNM)</td>
<td>trichloronitromethanes</td>
<td>TCNM</td>
</tr>
<tr>
<td>Carbonaceous DBP (C-DBP)</td>
<td>haloketones (HKS)</td>
<td>1,1-dichloro-2-propanone</td>
<td>1,1-DP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1,1,1-trichloro-2-propanone</td>
<td>1,1,1-TCP</td>
</tr>
</tbody>
</table>
Experiment Methods

Biomolecular characterization of biofilm:
- Excitation emission matrix fluorescence with parallel factor analysis (EEM-PARAFAC): Quantify DOM composition in biofilm matrix
- Analytical instrument: Spectrofluorophotometer (Shimadzu, Japan, RF-6000)
Result & Discussion
Biofilm Structure Image

3D CLSM image of biofilm structure in each reactor

Week 9

Week 15

Week 21

- Cells  - Protein  - Polysaccharides
EEM-PARAFAC Component in Biofilm

EEM raw spectra data of the samples (Removed background)

<table>
<thead>
<tr>
<th>Component</th>
<th>Exmax(nm)</th>
<th>Emmax(nm)</th>
<th>Fluorophore type</th>
<th>Description from previous study</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>275</td>
<td>314</td>
<td>Tyrosine-like</td>
<td>Amino acid, may indicate more degraded peptide material</td>
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<tr>
<td>C2</td>
<td>265/285</td>
<td>288</td>
<td>Microbial degradation product</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>255/280</td>
<td>326</td>
<td>Tryptophan-like</td>
<td>Amino acid, may indicate intact proteins or less degraded peptide material</td>
</tr>
<tr>
<td>C4</td>
<td>300</td>
<td>420</td>
<td>humic-like</td>
<td>Low molecular weight humic-like substances, associated with biological activity</td>
</tr>
<tr>
<td>C5</td>
<td>275/320</td>
<td>462</td>
<td>humic-like</td>
<td>High molecular weight humic-like substances, widespread</td>
</tr>
</tbody>
</table>

EEM fingerprint and spectra of 5 identified components

Fit the PARAFAC model
EEM-PARAFAC Components in Biofilm from AOM Reactor

Dynamics changes of relative abundance of EEM components in biofilm matrix

PCA biplot of EEM components changes in biofilm matrix
EEM-PARAFAC Component in Biofilm from R2A Reactor

Dynamics changes of relative abundance of EEM components in biofilm matrix

Biplot of EEM components changes in biofilm matrix
The bimolecular composition of biofilm shifted from high molecular weight humic-like substance and tryptophan like materials to SMP, tyrosine, and low molecular weight humic-like substance except the biofilm in the humic reactor.
C-DBP Formation Potential from Biofilm _Normalized by Carbon Concentration

- Compared to the biofilm from HS and R2A reactor, the biofilm from AOM reactor produced relative higher amount of C-DBP based on per carbon, especially at the beginning phase (3 weeks and 6 weeks incubation).

- DBP speciation:
  - HAA: Monochloroacetic acid (MCAA), Trichloroacetic acid (TCAA)
  - THM: Chloroform
  - HK: 1,1,1-trichloro-2-propanone
• For HAN, did not see obvious trend.
• Halonitromethane (TCNM) formation from biofilm in AOM and HS reactor have decreasing trend and became stable.
• DBP speciation
  ✓ HAN: Dichloroacetonitrile (DCAN)
Either C-DBP or N-DBP formation form biofilm in different reactor did not show any significant difference.

Showed different patterns compared to the DBPFP from biofilm normalized by carbon.
Conclusion

- The biomolecular composition of biofilm matrix were impacted by the substrate.

- Different substrate render different fraction of biofilm biomolecular composition and subsequently might affect the DBP formation potential.

In this study we have observed relative more C-DBP and N-DBP formation from biofilm in AOM reactor based on per carbon. But the result of the DBPFP calculated from coupon area did not showed significant difference for three reactors.
On Going Study _ The Correlation of DBPFP and Other Parameters

- In progress
  - Correlation analysis
  - Method: spearman correlation analysis
  - Goal: based on the spearman coefficient, determined the correlation of DBFP and EEM component
Microbial community analysis
  - Illumina Miseq
    - Target at 16S rRNA gene paired end sequencing (V4 region)

Sequence data analysis
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Questions?

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