Understanding the impact of water distribution system conditions on biodegradation of HAAs and detection of dehalogenase genes

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Presentation Outline

- Background
- Purpose of the study
- Materials and Methods
- Experimental results
- Conclusions
- Ongoing Study
- Acknowledgement
Background

Conventional water treatment processes generally yield lower molecular weight NOM

NOM enters water distribution system
Background

Disinfection by products (DBPs)

- Products of reaction between organic matter and disinfection agents (e.g. chlorine) in water treatment

DBPs formation in water distribution systems

- Chlorinated DBPs are carcinogenic compounds, causing adverse birth outcomes and birth defects, etc
- Brominated DBPs exhibits higher relative toxicity

DBPs adverse health effects

Biofilm

Natural Organic Matter

Disinfection

C-DBPs (Carbonaceous) & N-DBPs (Nitrogenous)
Background

DBP Regulations

- United States Environmental Protection Agency (USEPA) started regulating DBPs in tap water under stage (I) disinfectants and disinfection byproduct rule.
- Maximum contaminant levels (MCLs) for trihalomethans (THMs) and haloacetic acids (HAAs) are 80 µg/l and 60 µg/l, respectively.

DBP degradation in water distribution systems

- Some water utilities and research groups have reported decreases in HAA concentration with increased water residence time in the distribution system.
- The observed loss of HAAs is usually attributed to microbial degradation.

HAA biodegradation mechanisms

- HAAs are biodegraded aerobically via a hydrolysis-oxidation pathway.
- This pathway involves a major substitutive dehalogenation step in which the halogen atom is replaced by a hydroxyl group.
- This step is catalyzed by enzymes called halocarboxylic acid dehalogenases.
Background

HAAs biodegradation in water distribution system (continue)

Genes encoding dehalogenases enzymes are categorized into two phylogenetically unrelated groups, called *dehI* and *dehII*. Dehalogenases from group II proceed via a nucleophilic attack resulting in a covalent ester-enzyme link between an aspartate residue and the dechlorinated substrate. Dehalogenases from group I do not form any ester bond with the substrate.
Purpose of this study

- Investigating biodegradation of five HAAs (mono-, di- and trichloroacetic acids and mono- and dibromoacetic acids) using collected biomass from local water utilities

- Evaluating the impact of water distribution system conditions \([pH, \text{residual disinfectant (chlorine), total organic carbon (TOC), and phosphorous}]\) on biodegradation of HAAs

- Understanding HAA biodegradation mechanisms: detecting \(deh\) gene groups
Materials and Methods

Bacterial enrichment

- 4 batch master culture reactors containing mineral buffer were inoculated with mixed species bacteria collected from local water utilities (500 ml volume)
- Reactors were fed with 5 HAAs (1mM total concentration) as the sole source of carbon for 3 months
- Half of each reactor volume was replaced with fresh mineral buffer and HAA solution twice a week
- Bacterial quantity were monitored in the reactors using Heterotrophic Plate counting (HPC) method
- Upon reaching steady state conditions in the reactors, harvested biomass was collected and preserved in 20% glycerol at -80 Celsius
- 2 reactors did not show any HAA degradation and were shut down after 45 days of maintenance (their biomass was stored in freezer for further experiments)
Materials and Methods

Experimental design

- City of Toledo tap water was filtered using granular activated carbon (GAC) column to remove residual chlorine and organic contents (TOC/TN)
- Frozen culture stock was thawed, washed, and resuspended for degradation tests
- Considered water distribution system conditions and their respective levels are:
  1. pH (9.3 and 7)
  2. Residual chlorine (0 and 0.5 mg/l)
  3. Total organic carbon (0 and 3 mg/l)
  4. Phosphorous (tap water level ≈ 0.5 mg/l and 3 mg/l)
- All experiments were conducted in 50 ml vials (vials were prepared with granular activated carbon filtered tap water containing 200 µg/l HAAs and 10⁴ CFU/ml bacteria)
- All vials were placed in a rotator at speed of 40 rpm
- Sampling time (0, 12, 24, 48 and 72 hrs)
- A set of control samples for checking abiotic degradation of HAAs and potential formation of HAAs in the presence of chlorine were also prepared
Materials and Methods
HAA analysis method (EPA method 552.2 with small modifications)

Gas chromatograph (GC) (Shimadzu, Japan, GC-2010 Plus)
Materials and Methods

DNA extraction protocol (2 ml biomass sample)

- Centrifuging the sample and decanting the supernatant
- Resuspending pellet in 467µl of TE buffer +30µl 10% SDS + 3µl of proteinase k
- Addition of equal volume phenol/chloroform and centrifuging at 12000 for 2 times
- Addition of 1/10 volume sodium acetate and 0.6 volume isopropanol
- Washing precipitated DNA with 1 ml of 70% ethanol
- Resuspending DNA in 200µl TE buffer and storage in the refrigerator

DNA extraction verification

- O.D. measurement using Spectrophotometer at wavelength of 260 nm
- Gel electrophoresis (%0.8 agarose gel)
Materials and Methods

PCR protocol using universal degenerate primers to amplify *deh* genes

- Degenerate primers were obtained from Integrated DNA Technologies (based on the work of Hill et al, 1999)
- For *dehI* group one forward primer and two reverse primers were tested (*dehIFor*, *dehIRevI*, *dehIRevII*)
- For *dehII* group one forward primer and two reverse primers were tested (*dehIIFor*, *dehIIRev*)

PCR reaction mix

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SYBR Green reaction mix</td>
<td>12.5</td>
</tr>
<tr>
<td>Forward primer (10 µM)</td>
<td>2.5</td>
</tr>
<tr>
<td>Reverse primer (10 µM)</td>
<td>2.5</td>
</tr>
<tr>
<td>Sterile distilled water</td>
<td>6.5</td>
</tr>
<tr>
<td>DNA (add sterile DI water for NTC)</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
</tr>
</tbody>
</table>
Materials and Methods

Amplification program for *dehI* group

- 94°C for 2 min, 92°C for 20 s, 70°C (-1 degree per cycle) for 30 s cycled 20 times, the adjoining reaction includes 92°C for 20 s, 51°C for 30 s, 75°C for 30 s; cycled 20 times, with an extension of 75°C for 7 min

Amplification program for *dehII* group

- 94°C for 10 min, 36 cycles of (94°C for 45 s, 55°C for 1 min, 75°C for 45 s) and finally 75°C for 7 min

Gel electrophoresis (%1.5 agarose gel) was run for checking quality of PCR products

Thermo cycler (BIO-RAD, USA, MyiQ single color Real-Time PCR Detection System) was used for running PCR.
Materials and Methods

Isolation of HAA-degrading bacterial strains

HAA-degrading bacteria isolates were obtained from HAAs harvested biomass using HAA-amended agar plates

HAA degrading bacteria isolation steps:
- Preparing of 25 mmol/l phosphate buffer solution
- Adding pH indicator (5 mg/l bromocresol purple) to the buffer (purple at pH>6.8 and yellow at pH<5.7)
- Autoclaving the mixed agar-buffer-pH indicator solutions
- Adding the HAA mixture (10 mmol/l) to the basal medium
- Preparing agar plates
- Spreading the adequate amount of mixed culture on the agar plates
- Incubation of plates in appropriate temperature
Results

Biodegradation of HAAs under different DWDS conditions

Effect of pH
Results

Biodegradation of HAAs under different DWDS conditions

Effect of residual chlorine
Results

Biodegradation of HAAs under different DWDS conditions

Effect of total organic carbon (TOC)
Results

Biodegradation of HAAs under different DWDS conditions

Effect of phosphorous
Results

Biodegradation of HAAs under different DWDS conditions

HPC counting

![Graph showing biodegradation of HAAs under different conditions]
Results
Biodegradation of HAAs under different DWDS conditions

Key Results

- HAA degradation at neutral pH value was higher than basic pH value
- Chlorine even at low quantities (<0.5 mg/l) stops HAA degradation
- High TOC concentration improves biodegradation of all HAAs
- The effect of phosphorous on biodegradation was not considerable
- No HAA formation observed in control samples in the presence of low concentrations of chlorine
Results
DNA extraction

DNA was extracted successfully from collected biomass samples from 3 master culture reactors
Results

PCR amplification products obtained from collected samples from master culture reactors using degenerate *dehI* and *dehII* primers

- Lanes 1, 7, 13 are DNA marker
- Lanes 2, 8, 14 are negative control
Results

PCR amplification key findings

- The primer pairs *dehI* (For, Rev<sub>1</sub>) amplified a single product between 200-300 bp for samples from 2 master culture reactors
- The primer pairs *dehI* (For, Rev<sub>II</sub>) did not amplify any product
- The primer pairs *dehII* (For, Rev) amplified a single product between 400-500 bp for samples from 2 master culture reactors

It has been reported by other researchers that *dehI* genes should be amplified at 230 bp and *dehII* genes should be amplified at 420 bp

Since there is only one band for the amplified products, it can be concluded that these degenerate universal primers are specific for *deh* genes and can be used for q-PCR
Results

Isolation of HAA degrading bacteria

- HAA degrading bacteria grew on HAA amended agar plates (bacteria from reactors that did not show degradation did not grow on HAA-agar plates)

- Since degenerate primers are specific for deh genes, isolated strains will not be used to design a new pair of specific primers
Conclusions

- Drinking water distribution system conditions (pH, TOC concentration and residual disinfectant concentration) are influential on biodegradation of HAAs

- $dehI$ and $dehII$ dehalogenase genes were existed in 2 out of 4 different biomass sources (HAA were degraded by hydrolysis-oxidation mechanism)

- Degenerate $dehI$ and $dehII$ PCR primers were found to be specific for gene expression
Ongoing Study

- Identification of HAA degrading bacteria using isolated strains

- Verifying the impact of water distribution system conditions on HAA biodegradation by measuring the expression of dehalogenase genes

- Microbial community structure analysis under different water distribution system conditions

- Investigating biodegradation of HAA in simulated water distribution system with different pipe materials
Acknowledgement

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Thank you