MONITORING OF NITRIFICATION IN CHLORAMINATED DRINKING WATER DISTRIBUTION SYSTEMS WITH MICROBIOME BIOINDICATORS USING SUPERVISED MACHINE LEARNING

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SUPPLEMENTAL MATERIAL

SUPPLEMENTARY MATERIALS and METHODS

Drinking water distribution system (DWDS) simulator

A semi-closed pipe-loop DWDS simulator (**Gomez-Alvarez et al., 2016**) was operated through successive operational schemes (see schematic). Pipe-loop feed water was created from a free chlorine municipal drinking water (DW) source. The DW source was amended as necessary with ammonium sulfate and sodium hypochlorite to generate a target 3 mg L^{-1} monochloramine (Stable [SS; SI and SII] and Failure [SF]) or free chlorine (Restore [SR]) residual concentration. For monochloramine formation, a 4.5:1 chlorine to ammonia-nitrogen mass ratio (Cl_2:N) was used. The operational scheme sequence was designed to parallel a practical scenario where a chloraminated drinking water system progresses from normal operation where a chloramine residual is maintained in the pipe-loop (SS) to a failure period where no chloramine residual is maintained as a result of nitrification (SF). Subsequently, the drinking water system operation is modified by switching disinfectants from chloramine to free chlorine to eliminate nitrification and maintain a disinfectant residual (SR). After a period, the drinking water utility then switches back to chloramine and resumes normal operation (SS). To accelerate the development of nitrification and disinfectant residual loss, the temperature of the pipe-loop water was increased (from 18°C to 24°C) for the Failure operational scheme.

Water quality

Bulk water (BW) concentrations were analyzed from a composite of two samples taken from two different ports in the pipe-loop. Temperature, pH, turbidity, and ORP measurements were taken from online sensors. Nitrate and phosphate analyses were performed with a discrete colorimetric SmartChem 200 autoanalyzer (Westco Scientific, Danbury CT) using the EPA methods 353.2 (**USEPA 1993a**) and 365.1 (**USEPA 1993b**). Chemical concentrations for free chlorine (HACH Method 8021), monochloramine and free ammonia (HACH Method 10200), total chlorine

(HACH Method 8167), and nitrite (HACH Method 8507) were determined with a HACH DR/2400 Portable Spectrophotometer (HACH, Loveland, CO, USA).

Sample Collection

Duplicate BW samples (3 L) were collected from two ports in the pipe-loop DWDS simulator using sterile polypropylene bottles (Nalgene, Rochester, NY). Samples were individually filtered on-site through polycarbonate membranes (47 mm diameter, 0.22 μm pore size). Membranes were individually overlaid with 100 μmol L^{-1} Propidium Monoazide (PMA) and incubated in the dark for 20 min, followed by a 15 min exposure to 460 nm light (**Hellein et al., 2012**). PMA can inhibit PCR amplification of DNA from membrane-compromised cells and effectively discriminates between live and dead bacteria (**Nocker et al., 2010**). Membranes were stored and transported on ice for DNA processing.

DNA Extraction and Sequencing

Total DNA was extracted using the MoBio PowerWater® DNA Isolation Kit. DNA extractions followed the manufacturer's instructions (MoBio Laboratories, Solana Beach, CA). DNA concentrations were measured using the Qubit® Fluorometer (Life Technologies, Carlsbad, CA) and stored at -80°C. The V4 region of the 16S rRNA sequence was amplified using the bacterial primer set 515F and 806R (**Caporaso et al., 2012**). Paired-end 125 bp libraries were prepared using the Illumina MiSeq® Reagent v2 (500-cycles) kit on the MiSeq platform (Illumina Inc., San Diego, CA).

16S rRNA sequence analysis

Reads were analyzed using the software MOTHUR v1.37.6 (**Schloss et al., 2009**) and were screened following the procedure described in **Gomez-Alvarez et al. (2016)**. Briefly, fastq files with forward and reverse reads were used to form contigs. Reads were screened and removed if they (i) had a length less than 292 bp, (ii) contained ambiguous bases (N's), (iii) contained homopolymers greater than 7 bases, (iv) were identified as chimera, or (v) were classified as unknown, Chloroplasts, or Mitochondria. Reads were aligned against the SILVA SEED release 123 reference dataset and grouped with 97% sequence identity as the cut-off point for each Operational Taxonomic Unit (OTU). Taxonomic classification was obtained using the Ribosomal Database Project (RDP v16) reference database. The sequences and taxonomic outlines for the RDP hierarchies were downloaded from the MOTHUR website (https://www.mothur.org). Prior to community analysis, samples were rarefied to the smallest dataset (5 000 reads).

Biomass quantification

Total ATP of bacterial cells was used as a surrogate for biomass present in BW samples. The amount of ATP was determined by ATP-bioluminescence quantification using the Promega BacTiter-Glo™ Microbial Cell Viability Assay kit (Promega, Madison, WI), following the protocol by **Berney et al. (2008)**. Briefly, the BacTiter-Glo™ Buffer was mixed with the lyophilized BacTiter-Glo™ Substrate and stored over night at room temperature. 100 mL of bulk water (BW) sample and an equal volume of BacTiter-Glo™ reagent (stored on ice) were warmed separately for 2 min in a 30°C water bath. BW and reagent were mixed, and the luminescence of the sample was immediately measured with a GloMax® 96 Microplate Luminometer (Promega, Madison, WI). All samples were analyzed in triplicate and free ATP (in BW) was also measured. A calibration curve was prepared with dilutions of pure ATP (Promega, Madison, WI).

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