Q-368

Role of Bacterial Exopolysaccharides in Organic Chloramine Formation in Drinking Water Distribution Systems

Absar Alum, Hyunyoung Jang, Paul Westerhoff, Morteza Abbaszadegan
Arizona State University, Tempe, AZ,

ABSTRACT

Organic chloramines form as inorganic chloramines or aqueous chlorine react with nitrogen-containing organic matter present in surface waters or in biofilms. Determination of disinfectant concentration in distribution system is critical for disinfectant residual compliance by water utilities. Organic chloramines have very poor disinfection capabilities; therefore, accurate measurement of the organic chloramine fraction of the total chlorine is important. The chloramine concentration in water can be determined by different methods. Membrane introduction mass spectrometry (MIMS) is a fast and reliable method to quantify chloramines in water. Scientific evidence suggests that filtration step interfere with the accurate determination of organic chloramines. It was observed that fraction of organic chloramine concentration that can pass through filter, decreases with decrease in the pore size. Our hypothesis was that organic nitrogen from biofilm may be a primary source of organic chloramines formation and that organic chloramines may attach to biomass surface. We compared the organic chloramines detection methods with filtration and without filtration process. In order to support this hypothesis, light and scanning electron microscopic studies were performed. Pure bacterial cultures were used for microscopic studies. *E. coli* was cultured using nutrient broths, and centrifuged at 700 g for 20 min to remove the broth. A small amount of bacteria was placed on a clean slide and spread over the surface of slide and were fixed using heat. The slide was rinsed with PBS and then rinsed with nucleases (DNase and RNase to remove the organic molecules released from cells. Cell membrane of immobilized bacterial was stained with Crystal violet and DPD contained chlorine solution was added to the slide and reaction was visualized under fluorescent microscope. These observations were confirmed by scanning electron microscope observations. The elemental composition analyses of bacterial cell treated with chlorine showed high concentration of chlorine and nitrogen on the surface of *E coli* cells. Microscopic observations and elemental analyses support the conclusion that organic chloramine are formed on the surface of microbial cells.

INTRODUCTION

Organic Chloramines Formation

Organic matter is ubiquitous in the distribution systems. The organic nitrogen-containing materials react with free chlorine (HOCl/OCl-) or inorganic chloramines (NH2Cl, NHCl2) resulting in the formation of organic chloramines (R-NHCl).

$$RNH_2 + HOCl \rightarrow RNHCl + H_2O \text{.....(1)}$$

$$RNH_2 + NH_2Cl \rightarrow RNHCl + NH_3 \text{.....(2)}$$

Relevance to Water Industry

The distribution of organic and inorganic chloramines in water distribution systems is determined by the concentrations and relative chlorination rates of organic nitrogenous compounds and ammonia. Higher levels of organic chloramines are expected to form because the organic nitrogenous compounds readily bind with chlorine.

The EPS are one of the components in biofilm and they play an important role in biofilm formation in distribution systems. The EPS in biofilms contain between roughly 50 and 90% of the total organic matter. In distribution systems, organic nitrogen compounds reacting with chlorine would be more favorable reaction than with ammonia. The excess ammonia can lead to nitrification Unexpected organic chloramines formation would hinder to achieve the target inorganic chloramines residual. Therefore, it is important to understand organic chloramines formation in the drinking water system for proper monitoring of disinfectant residuals.

Acknowledgement: This research was funded by AWWARF and ASU-WQC

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MATERIALS AND METHODS

Preparation of HPC bacteria

HPC bacteria was isolated from a tap water and grown overnight in the nutrient broth at 28°C. Bacteria were concentrated by centrifugation at 1,000 g for 15 minutes and washed three times with phosphate buffer solution (PBS).

Comparison between filtration and non-filtration

The experiments were conducted by exposing HPC to chlorine. , and the after prescribed intervals filter out the HPC biomass by a glass fiber filter (ashed Whatman GF/F].

Measurement of Organic chloramines

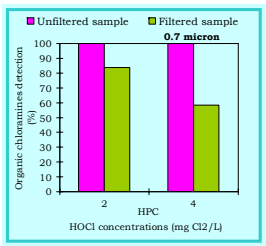
Organic chloramine concentration was calculated as follow:
organic chloramines = total chlorine – free chlorine – monochloramine
Sample was taken and measured free (530 nm) and total chlorines (530 nm) and monochloramine (655 nm) concentrations using a Hach Dr 5000 spectrophotometer. For free and total chlorine, DPD analysis was adapted and monochlorF analysis was used for monochloraimes.

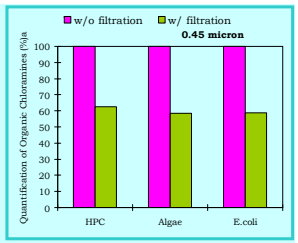
Microscopic Studies

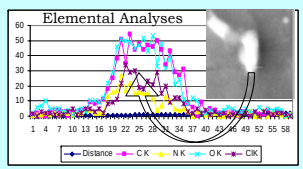
E coli (ATCC 25922) was grown in tryptic soy broth (TSB). Bacterial were concentrated and washed with PBS. Washed cell were exposed to chlorine (0.5mg/L) for 2 minute. Chlorine was quenched using sodium thiosulfate. Chlorinated and non-chlorinated bacteria were visualized under scanning electron microscope and analyzed for elemental composition using EDX.

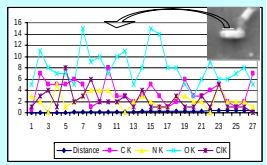
RESULTS AND DISCUSSION

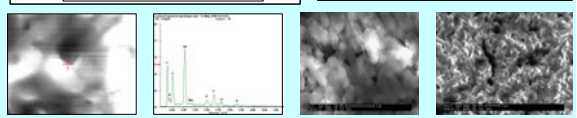
Comparison of organic chloramine concentration in filtered and non-filtered samples show that the concentration of organic chloramine decreased with filtration. This suggests that reactive species are formed on the surface of the cell, they do not solubilize into solution and mostly remain on/within the cell or cellular materials (e.g. EPS). This data also suggest that filtration process should be avoided for proper organic chloramines measurement. The scanning electron microscope data indicate the chlorine and nitrogen molecules are concentrated on the bacterial cell suggesting that bacterial EPS react with chlorine species resulting in the formation of surface bound organic chloramines











Impact of Organic Chloramines Formation

- Formation of organic chloramines can induce nitrification process which can be a serious problem
- Conventional analytical technique can not distinguish the true level of inorganic and organic chloramines
- Organic chloramines give 'false' indication of active disinfectant residuals

CONCLUSIONS

- ✓ Organic chloramines are formed on the surface membrane of microorganisms
- ✓ Filtration should be avoided for accurate measurement of inorganic chloramines and organic chloramines