Science of the Total Environment 524–525 (2015) 290–299

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

A multi-parametric approach assessing microbial viability and organic matter characteristics during managed aquifer recharge

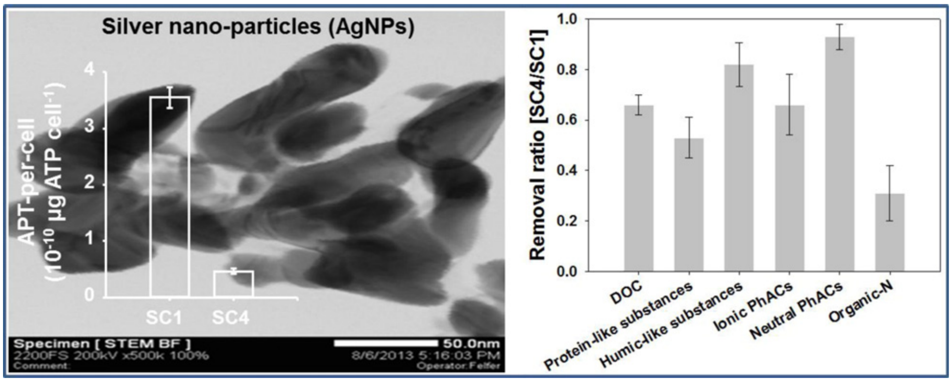
Hyun-Chul Kim [a], Jin Hyung Noh [b], So-Ryong Chae [c], Jaewon Choi [d], Yunho Lee [e], Sung Kyu Maeng [b,]*

[a] Water Resources Research Institute, Sejong University, 98 Gunja-Dong, Gwangjin-Gu, Seoul 143-747, Republic of Korea
[b] Department of Civil and Environmental Engineering, Sejong University, 98 Gunja-Dong, Gwangjin-Gu, Seoul 143-747, Republic of Korea
[c] Department of Biomedical, Chemical, and Environmental Engineering, 701 Engineering Research Center, University of Cincinnati, Cincinnati, OH 45221-0012, USA
[d] K-water Institute, Water Quality Center, Shintanjin-Ro 200, Daeduck-Gu, Daejeon 306-711, Republic of Korea
[e] Department of Environmental Science and Engineering, Gwangju Institute of Science and Technology (GIST), Gwangju 500-712, Republic of Korea

HIGHLIGHTS

- FCM with ATP for quantification as cellular viability was successful.
- AgNPs affected the enzyme activity of viable microbes associated with sand.
- Selected ionic PhACs was strongly associated with biodegradation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 February 2015
Received in revised form 3 April 2015
Accepted 4 April 2015
Available online 18 April 2015

Editor: Adrian Covaci

Keywords:
Managed aquifer recharge
Microbial viability
Silver nanoparticles
Flow cytometry
Pharmaceutically active compounds

ABSTRACT

Soil column (SC) experiments were conducted to investigate the feasibility of using silver nanoparticles (AgNPs) as microbial inhibitors; the microbial viability affecting the degradation of pharmaceutically active compounds (PhACs) and the characteristics of organic matter during managed aquifer recharge were specifically evaluated. Natural surface water samples treated with AgNPs (0, 2.5, 5, and 10 mg L^{-1}) were continually fed into the soil columns for 2 years. The adverse impact of AgNPs on the cell membrane integrity and microbial enzymatic activity was quantitatively determined using flow cytometry and adenosine triphosphate analysis. The increase in AgNP concentration in the feed water (up to 10 mg L^{-1}) resulted in a corresponding deterioration in the performance of the managed aquifer recharge (MAR), with respect to the removal of organic carbon, oxidation of nitrogenous compounds, and PhAC attenuation. The fluorescence excitation–emission matrices of feed water and treated water showed the favorable removal of protein-like substances compared to humic-like substances regardless of the AgNP concentrations; however, the extent of removed fractions decreased noticeably when the microbial viability was lowered *via* AgNP treatment. The biological oxidation of organic nitrogen was almost completely inhibited when 10 mg L^{-1} AgNP was added during soil passage. The attenuation of bezafibrate, ketoprofen, diclofenac, clofibric acid, and gemfibrozil was strongly associated with the significant deterioration in biodegradation as a result of AgNP activity.

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* Corresponding author.
E-mail address: andrew.maeng@gmail.com (S.K. Maeng).

http://dx.doi.org/10.1016/j.scitotenv.2015.04.017
0048-9697/

1. Introduction

Managed aquifer recharge (MAR) systems (*e.g.*, river or lake bank filtration and artificial recharge) have been considered as one of the most environmentally friendly and cost-effective treatment or pre-treatment strategies for the removal of dissolved organic matter (DOM) and pathogens, especially when the goal is to ensure a constant supply of safe drinking water (Abel et al., 2014; Maeng et al., 2010, 2012). The prolonged use of MAR systems in Germany has shown it to be a well-proven treatment step for providing safe drinking water (Eckert and Irmscher, 2006).

MAR has also become part of a multi-barrier concept in treatment schemes for the removal of emerging contaminants of concern, such as pharmaceutically active compounds (PhACs) and endocrine disrupting compounds (EDCs), from drinking water. MAR systems are increasingly being employed to alleviate the burden faced by conventional water treatment facilities to provide potable water in Asia, including Korea. The ever-increasing use of PhACs and EDCs for human and veterinary purposes has contributed to their frequent detection in the aquatic environment and in drinking water sources, especially downstream of municipal wastewater treatment plants, as many of these compounds are not completely degraded by biological treatment processes (Sim et al., 2010; Yoon et al., 2010). Some of these organic micropollutants can cause major problems in aquatic ecosystems (*e.g.*, feminization of fish by estrogens) or for human health (*e.g.*, increasing antibiotic resistance by antibiotics). In addition, various combinations of these compounds might have different, possibly synergistic effects on public health and/or aquatic life, compared to the presence of only a single compound (Kümmerer, 2009).

Several mechanisms are associated with the removal of DOM and PhACs during the infiltration of surface water to a production well through soil passage. Biodegradation has played a key role in the removal of DOM and PhACs during MAR treatment (Maeng et al., 2008, 2011; Shamrukh and Abdel-Wahab, 2008). In particular, the biodegradability of PhACs is an important factor to be considered during risk (and future step) assessment (Cunningham, 2008). Therefore, the inhibition of biodegradation during MAR can deteriorate water treatability, resulting in the need for post-treatments to ensure a supply of safe drinking water for the existing MAR sites.

Sorption is another important removal mechanism involved in the removal of organic micropollutants during MAR. Some attempts have been made to differentiate between the removal efficiency of biodegradation and sorption, by suppressing the microbial activity by chemical inactivation, using sodium azide (NaN_3) and mercury salts. However, these techniques could potentially affect the sorption behavior of compounds by altering the sample water matrix or the solid surface characteristics (Stevens-Garmon et al., 2011). Although the use of non-chemical inactivation methods (*e.g.*, lyophilization and dry-heating technique) has been suggested as an alternative for this purpose (Kerr et al., 2000), the water and soil matrix (including dissolved organic matter) characteristics in the feed water, as well as the soil organic matter content in sand can be also altered.

This study utilized silver nanoparticles (AgNPs), identified as promising bacterial inhibitors by previous studies, which reported the inhibition of sustainable viability of microorganisms in the aquatic environment using AgNPs (Lok et al., 2007; Shoults-Wilson et al., 2011; Sondi and Salopek-Sondi, 2004). AgNPs have previously been implicated as major toxicants, which put aquatic microorganisms in natural waters at risk. Some investigators have suggested that the size and shape of AgNPs play key roles in determining toxicity (Choi and Hu, 2008; Morones et al., 2005; Sondi and Salopek-Sondi, 2004), while others have reported that the dissolution of ionic silver from the nanoparticles is responsible for the derogatory effect on microbial viability (Lok et al., 2007; Navarro et al., 2008). There are some similarities between the mechanisms of toxicity presented by AgNPs and Ag^+ (Fabrega et al., 2011); however, there remains a substantial disagreement regarding their effectiveness in disabling the enzymatic activity of microbes (Lok et al., 2007). The magnitude of the adverse effects depends on the concentration of AgNPs in aquatic environments (Cornelis et al., 2013). However, the effects of AgNPs on natural bacterial communities, *i.e.*, in surface water, are largely unknown.

The flow cytometry-based method employed in this study discriminated intact cells from damaged cells by determining the cell membrane integrity of microbes; therefore, the membrane-impermeable PI cannot bind to deoxyribonucleic acid (DNA) and/or ribonucleic acid (RNA) unless the surface of cells has been ruptured. Co-metabolism may play an important role in the removal of PhACs. Therefore, we also determined the enzymatic activity of intact cells, based on their membrane integrity. Fluorescein diacetate (FDA) was also employed to quantitatively evaluate the number of intact cells with enzymatic activity, by determining the hydrolysis rate of esterase in viable microbes. Determination of the esterase activity is the most common method for the measurement of cellular enzyme activity. The FDA staining procedure has been described in detail in a previous study (Xiao et al., 2011). Therefore, the microbial viability was probed by measuring the cell-bound adenosine triphosphate (ATP), and the number of cells with an intact cell membrane, using a flow cytometer. The intracellular ATP level was divided based on the number of intact cells in the aqueous phase, which is a more reliable method for the quantitative estimation of viable cell activity, compared to conventional methods such as heterotrophic plate counts.

The major goal of this study was to investigate the feasibility of using AgNPs as microbial inhibitors during MAR treatment, in order to discriminate between biodegradation and sorption in the removal of contaminants, especially DOM and PhACs, and evaluate a multi-parametric approach for the estimation of microbial viability affected by AgNPs in the removal of biodegradable fractions *via* biological decomposition and/or assimilation processes. The performance of the MAR treatment with soil columns exposed to microbes of differing viabilities was compared, especially regarding the fate of DOM, oxidation of organic/inorganic nitrogen species, and degradation of PhACs. This study utilized a more reliable method for the quantitative estimation of viable cell activity using flow cytometry, compared to the conventional methods, such as heterotrophic plate counts. We also employed an innovative suite of analytical tools to better elucidate the changes in compositional and functional properties of DOM by MAR treatment.

2. Materials and methods

2.1. Chemicals

Stock solutions of the thirteen PhACs (*i.e.*, gemfibrozil, diclofenac, bezafibrate, ibuprofen, fenoprofen, naproxen, ketoprofen, clofibric acid, carbamazepine, phenacetine, pentoxifylline, acetaminophen, and caffeine) were prepared in methanol. All PhACs were obtained from Sigma-Aldrich (St. Louis, MO). The stock solutions were subsequently diluted with deionized water (Milli-Q Advantage A10; Merck-Millipore, Darmstadt, Germany) that had a minimum resistivity of 18.2 MΩ-cm, in order to prepare 1 mg L^{-1} working solutions; the working solutions were spiked into the feed water. The concentration of PhACs in the influents ranged between 0.5 and 3.5 μg L^{-1}. The physicochemical properties of the selected compounds are listed in Table 1.

2.2. Preparation of AgNP suspensions

AgNPs (<100 nm, 99.5%) were purchased from Sigma-Aldrich, and the energy dispersive X-ray (EDX) spectrum of AgNP is shown in the Supplementary information (SI-1). An aqueous stock suspension of Ag was prepared by adding 1 g pristine Ag powder to 1 L of deionized water; the suspension was sonicated for 9 h using a Cole-Parmer 8890 sonicator (Cole Parmer, Vernon Hills, IL). The resultant mixture was stored in a refrigerator at 4 °C before use.