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Characterization of the microbial community structure and nitrosamine-reducing isolates in drinking water biofilters

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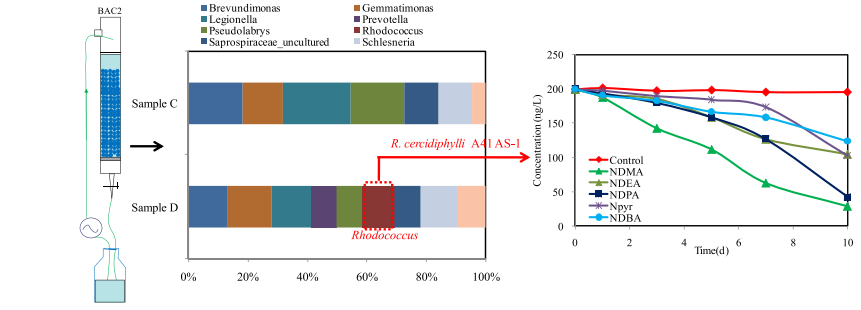
HIGHLIGHTS

- Tracked the changes of microbiome in BAC filters exposed to nitrosamines by 454 pyrosequencing
- Isolated a nitrosamine-reducing bacterium, *R. cercidiphylli* A41 AS-1, from the BAC filters
- Identified the role of nitrosamines as the carbon source in biodegradation by the isolated strain
- The removal ratios of NDMA, NDEA, NDPA, Npyr, and NDBA were 38.1%–85.4% by the isolated strain

GRAPHICAL ABSTRACT



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ABSTRACT

Two biofilters were constructed using biological activated carbon (BAC) and nitrosamine-containing water from two drinking water treatment plants. The microbiome of each biofilter was characterized by 454 high-throughput pyrosequencing, and one nitrosamine-reducing bacterium was isolated. The results showed that nitrosamines changed the relative abundance at both the phylum and class levels, and the new genera were observed in the microbial communities of the two BAC filters after cultivation. As such, the genus *Rhodococcus*, which includes many nitrosamine-reducing strains reported in previous studies, was only detected in the BAC2 filter after cultivation. These findings indicate that nitrosamines can significantly affect the genus level in the microbial communities. Furthermore, the isolated bacterial culture *Rhodococcus cercidiphylli* A41 AS-1 exhibited the ability to reduce five nitrosamines (N-nitrosodimethylamine, N-nitrosodiethylamine, N-nitrosodi-n-propylamine, N-nitrosopyrrolidine, and N-nitrosodi-n-butylamine) with removal ratios that ranged from 38.1% to 85.4%. The isolate exhibited a better biodegradation ability with nitrosamine as the carbon source when compared with nitrosamine as the nitrogen source. This study increases our understanding of the microbial community in drinking water biofilters with trace quantities of nitrosamines, and provides information on the metabolism of nitrosamine-reducing bacteria.

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1. Introduction

Nitrosamines are extremely potent carcinogens, and there is an increasing concern over the risk of nitrosamine-contaminated drinking water. Nitrosamines in drinking water are derived from chloramine found in disinfection by-products (DBPs) and industrial by-products.

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These compounds increase with the distance along the distribution system after disinfection and cannot be effectively removed using conventional drinking water treatment processes. In an effort to reduce nitrosamine levels, reverse osmosis (RO) (Plumlee et al., 2008), ultraviolet radiation and advanced oxidation processes (AOP, i.e., UV/H_2O_2, O_3/H_2O_2) (Jobb et al., 1994; Liang, 2002) have been employed; however, only a partial reduction of nitrosamines can be expected using these methods under practical operating conditions, and their application is limited due to high cost and complexity (Huang et al., 2010; Megan and Martin, 2007; Lee et al., 2005, 2007). Due to the hydrophilic nature of nitrosamines and their negligible sorption by soils and organic matter, the physicochemical properties of these compounds limit possible attenuation mechanisms in water supplies (Gunnison et al., 2000; Yang et al., 2005). Furthermore, pre-oxidation processes such as pre-chlorination, pre-chloramination and pre-ozonation are used widely in drinking water treatment (Susan et al., 2003; Rodgers, 1997; Camel and Bermond, 1998), leading to the formation of nitrosamines before filtration and disinfection processes. In our precious survey, the concentrations of N-nitrosodimethylamine (NDMA) in source water, sedimentation effluent, filtration effluent and finished water in one drinking water treatment plant (DWTP) were 15.1, 28.8, 22.6 and 46.9 ng/L, respectively (Wang et al., 2010); the dominant nitrosamines in source water were NDMA (6.4–13.9 ng/L), N-nitrosodiethylamine (NDEA, 1.9–16.3 ng/L) and N-nitrosodibutylamine (NDBA, 1.0–19.9 ng/L) (Wang et al., 2011). Biological treatment, though slow under environmental conditions, is thought to be an important mechanism for the removal of these nitrosamines and those originating from industrial discharge in DWTPs, and it could also avoid the risks of chemically treating in groundwater (Gunnison et al., 2000; Zhou et al., 2009; Patterson et al., 2012). With the biodegradation of precursors and nitrosamines formed in the pre-oxidation processes, the nitrosamine formation potential might decrease after disinfection (Tezel et al., 2011; Mitch et al., 2003). For the practical operation of DWTPs, the screening and isolation of microbial strains capable of reducing nitrosamine levels is urgently required. However, the isolation of microbes from water treatment systems is a challenge due to the naturally formed indigenous bacterial population. Therefore, an understanding of the structure and biodiversity of the microbial community that forms in the biofilters (biological activated carbon, BAC) used with nitrosamine-contaminated water is a prerequisite for understanding nitrosamine biodegradation and for the isolation of nitrosamine-reducing strains.

Recent work has examined bacteria capable of reducing nitrosamines and other organics in DWTPs (Chung et al., 2008; Liao et al., 2013a, 2013b; Douterelo et al., 2013; Fournier et al., 2009; Paul et al., 2011; Carissa and Jonathan, 2013). Some studies have described a bacterial community structure involved in reducing other organics in the drinking water by filtration processes or in groundwater (Ameet et al., 2012; Joshua et al., 2013; Pranab et al., 2012). However, to the best of our knowledge, there are no studies of the microbial communities in BAC filters exposed to nitrosamines in DWTPs, which could lead to the identification and isolation of nitrosamine-degrading bacteria. Therefore, nitrosamine biodegradation as a water treatment process could potentially be advanced by exploration of the bacterial community structure in BAC filters.

Our understanding of microbial nitrosamine degradation is largely limited to NDMA. Pure culture studies have revealed microbial strains capable of NDMA degradation through the action of monooxygenase enzymes, including a soluble methane monooxygenase (sMMO) in *Methylosinus trichosporium* OB3b (Sharp et al., 2005); propane monooxygenases (PrMOs) in *Mycobacterium vaccae* JOB-5, *Rhodococcus jostii* RHA1, and *Rhodococcus* sp. RR1 (Sharp et al., 2007, 2010); and toluene 4-monooxygenases in *Ralstonia pickettii* PKO129 and *Pseudomonas mendocina* KR1 (Sharp et al., 2005; Fournier et al., 2006). Different aerobic NDMA biodegradation pathways have been reported in *Rhodococcus ruber* ENV425 (Fournier et al., 2009; Hatzinger et al., 2011) and KR1 (Fournier et al., 2006, 2009). However, these studies only focused on NDMA at high concentrations (μM or mM). Carissa and Jonathan (2013) found that the bacterial strain *R. jostii* RHA1 could degrade high concentrations (20–2000 μg/L) of NDMA, NDEA, N-nitrosodi-n-propylamine (NDPA), N-nitrosopyrrolidine (NPyr), and possibly N-nitrosomorpholine (NMor). In these cases, the strains were not isolated from BAC filters. In many DWTPs, nitrosamines are usually present at much lower concentrations (<100 ng/L) in source and finished water (Wang et al., 2011), the degradation rate and attenuation mechanism of nitrosamines at these concentrations is unknown. Further studies on the biodegradation of nitrosamines at relatively low concentrations are therefore of practical significance.

In the present study, to better characterize isolated nitrosamine-reducing bacteria and to clarify the relationship between the bacterial community structure and the nitrosamine-reducing strains in BAC filters in DWTPs, we (1) tracked the changes in the microbiome of BAC filters exposed to nitrosamines after 60 days; (2) clarified the impact of nitrosamines at low concentrations on the microbial communities in BAC filters, (3) isolated nitrosamine-reducing bacteria from the BAC filters, and (4) identified the role of nitrosamines in biodegradation and evaluated reducing behaviors. The findings of the present study provide new insight into the evolution of bacterial communities and will aid in the construction of a BAC filter that more effectively removes nitrosamines from drinking water.

2. Materials and methods

2.1. Chemicals and preparation of nitrosamine-containing water

Standard solutions of 1000 μg/mL NDMA, NDEA, NDPA, NMor, N-nitrosomethylethylamine (NMEA), NPyr, N-nitrosopiperidine (NPip), NDBA and N-nitrosodiphenylamine (NDPhA) were purchased from Supelco (USA), and all other chemicals used in this study were in HPLC grade. One filtered water sample from a DWTP (lake as source water) containing approximately 10 ng/L NDMA, 2.6 mg/L dissolved oxygen (DO), 0.58 mg/L ammonia (NH_3-N), and 0.4 mg/L dissolved organic carbon (DOC) was used as the influent for the bench-scale experiment. Another filtered water sample from a DWTP (surface river as source water) contained 15 ng/L NDMA, 3.0 mg/L DO, 1.23 mg/L NH_3-N, and 0.6 mg/L DOC. Each filtered water sample was spiked with a mixture of nitrosamines (nine nitrosamines at a concentration of 1000 ng/L each).

2.2. Experimental apparatus setup

The BAC samples used for the experiment were obtained during stable operation from two DWTPs employing the same treatment processes but with different water sources. Coal active carbon was used in both BACs. The drinking water treatment processes of DWTPs include pre-chlorination, coagulation and sedimentation, ozonation, BAC filtering, and disinfection (chlorine). Four glass columns (inner diameter = 4.0 cm) with a working volume of 450 mL were filled with the obtained BAC samples, of which two were used as controls by sterilizing the BAC before filling (Fig. 1). Two filtered water samples were circulated using peristaltic pumps (Longer-Pump, YZ1515x Bt00-300 M), and the empty bed contact time (EBCT) was 25 ± 1 min. The filters were maintained at room temperature (25 °C) and backwashed every 10 days, and the experiment was conducted for 60 days.

2.3. DNA extraction and 454 high-throughput pyrosequencing analysis of the microbial community in the biological samples from BAC filters

Four BAC samples – A, B, C, and D – were used for DNA extraction. Samples A and B were collected from the initial bench-scale BAC1 filter, before and after cultivating with the backwashed water for 60 days, respectively. Similarly, samples C and D were from the BAC2 filter. The genomic DNA for each sample was extracted using the Ultra Clean DNA Extraction Kit (Omega Bio-Tek, USA), and then quantified using a

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