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Bacterial growth during the start-up period of pilot-scale biological activated carbon filters: Effects of residual ozone and chlorine and backwash intervals

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ABSTRACT

This study aimed to evaluate the effects of residual ozone and chlorine and the backwash intervals on bacterial activities and density in pilot-scale biological activated carbon (BAC) filters by employing adenosine triphosphate (ATP) measurement and flow cytometry. The BAC filters received water treated by full-scale coagulation–filtration–ozonation processes. The attached bacterial density on the BAC increased rapidly in the first few weeks. The residual chlorine in the influent water caused ca. 1-log reduction of ATP-per-cell in the bacteria attached on the BAC and in the effluent and backwash waters. Extending the backwash interval made the bacteria attached on the BAC more resistant to ozone and chlorine, as suggested by the higher ATP-per-cell of the attached bacteria and the higher percentage of high-nucleic-acid intact bacteria in the backwash water. However, the attached bacterial density was higher for the shorter backwash interval operation than for the longer one during the high-ozone period. Although no significant difference in DOC removal rates was observed between the two BAC filters, fluorescence excitation–emission matrix analysis revealed that high residual ozone decreased the removal of aromatic proteins and soluble microbial product-like compounds.

1. Introduction

Microorganisms can colonize on a granular activated carbon (GAC) surface and establish active biomass, transforming GAC into so-called biological activated carbon (BAC) [1,2]. In general, the GAC in full-scale BAC filters is replaced regularly due to a loss of water treatment capacity, but oxidizing agents, i.e., ozone and chlorine, could hinder the start-up of BAC filters filled with virgin GAC in drinking water treatment plants (DWTPs). In Japan, pre-chlorination is employed in DWTPs for the control of ammonia nitrogen and for the removal of iron and manganese. However, the effect of residual chlorine on the bacterial activity in a BAC filter during the start-up period remains unclear [3]. Residual chlorine may also indirectly affect bacterial proliferation (hereafter referred to as bacterial growth) on the BAC by letting bacteria utilize organic carbon for generating compounds that are required for protection against residual chlorine for their survival, rather than for their growth [4], and by inhibiting bacterial attachment on the BAC [5].

In addition, the backwashing of BAC filters, which is accomplished by applying water and/or air in an up-flow manner through a biofilter, causes a loss of biomass [6,7]. However, studies that closely assess the effect of backwash intervals on the bacterial attachment and growth during the BAC start-up period are scarce.

Adenosine triphosphate (ATP) is an energy-rich metabolic compound that is produced in all active organisms and can therefore be used as a parameter to determine the active microbial biomass in water [8,9]. The biomass ATP in bacteria attached to the BAC was reported to be influenced by water quality, change in growth conditions and vertical stratification of nutrient concentrations [10,11]. In addition, the biomass ATP can be measured in a high dynamic range. Thus, biomass ATP is considered to be a useful parameter for the measurement of microbial activity on the BAC. Flow cytometry (FCM) coupled with nucleic acid-specific fluorescent dyes allows

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for the rapid and accurate enumeration of all cells, including cells that are inactive or unculturable on conventional media. The FCM technique can discriminate between cells having different physiological and/or growth status, including their nucleic acid content [12–14], which can be a better indicator of the fraction of active cells than the total cell counts in aquatic systems. Based on the cellular integrity of the cytoplasmic membrane, bacterial cells are discriminated into intact (both active and inactive or dormant cells) and damaged cells [12,15]. In addition, FCM also has a wide dynamic range of several orders of magnitude, which is required for the analysis of bacterial activities and growth in BAC filters. The combined use of FCM and ATP measurements has previously been shown to hold promise for the understanding of bacterial growth on BAC [10,11]. However, no backwashing was applied to the BAC filters in their studies; therefore, the ATP of attached and suspended bacteria and their nucleic acid content in the backwashed BAC filters has never been reported.

This study aimed to evaluate the effects of the backwash interval and residual ozone and chlorine in the influent water on the activity and growth of bacteria on BAC. A second purpose of the study was to evaluate the removals of dissolved organic matter (DOM) during the start-up period of new BAC filters. Two pilot-scale BAC filters were installed in a full-scale DWTP in the Tone River Basin (Japan), which purifies surface water through pre-chlorination, coagulation and 1st rapid sand filtration (RSF), ozonation, BAC filtration, 2nd RSF and post-chlorination. Ozonated water was taken from the full-scale DWTP as the feed water to the pilot-scale BAC towers. To evaluate the effect of the backwash interval on bacterial activity and growth in BAC filters, two BAC filters were operated at different backwash intervals. Because of the aforementioned advantages, FCM and ATP measurements were employed to quantify bacterial activity using two parameters, i.e., ATP-per-cell and the nucleic acid content. The changes in the counts of intact and damaged cells were also measured by FCM to quantify bacterial growth on the BAC during the start-up period.

2. Materials and methods

2.1. Pilot-scale study

The downflow biofilters (inner diameter 20 cm, GAC bed depth 250 cm on a 15-cm gravel support) received ozonated water from the full-scale DWTP (Fig. S1, suppl. data). Table 1 presents the ozone dose and water quality parameters of the ozonation effluent. The GAC used in the pilot study was identical to that used in the full-scale DWTP and was a coal-based GAC with an average diameter of 1.0 mm and a BET surface area of 1033 m^2/g. The changes in the residual chlorine (0.08–0.32 mg/L in 4–19 weeks) and residual ozone (maximum 0.88 mg/L in 13–19 weeks) levels in the influent water are presented in Fig. 1.

The empty bed contact time (EBCT) of the BAC filters was 18 min at a linear velocity of 8.33 m/h. Over the 26 weeks of operation (October 2012–April 2013), the columns were regularly backwashed at two different intervals: Run 1: 3.5 days (backwashing twice a week); Run 2: 14 days or 7 days (backwashing once in two weeks or once a week). After four weeks of operation, Run 2 experienced high water head loss due to buildup of biomass that resulted in an overflow from the filter inlet; hence, the backwash interval was shortened from 14 days to 7 days. Backwashing was performed with an air flow rate of 80 L/min for 7 min and then with chlorinated tap water (residual between 0.63 and 0.77 mg/L) at a water flow rate of 14.4 L/min (upflow) for 12 min. We took a backwash water sample from the top of the BAC filters when the water level was approaching the top. In addition, we assumed that the effect of the residual chlorine in the backwash water on the activity and

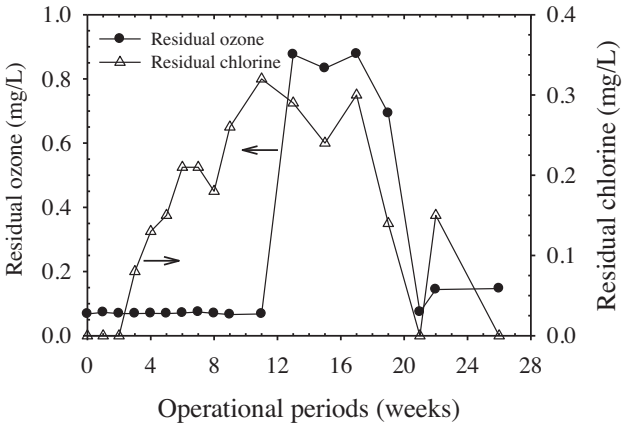

Fig. 1. Residual chlorine and ozone concentrations in the influent of the BAC filters.

Table 1
Water quality parameters of the ozonation effluent.

Parameter	Unit	Arithmetic mean	Standard deviation	Minimum	Maximum
DOC	mg/L	1.027	0.155	0.814	1.421
UVA_{254}	cm^{-1}	0.009	0.002	0.013	0.006
pH	–	7.06	0.22	6.71	7.55
Ozone dose	mg-O_3/L	0.48	0.34	0.11	1.01
Residual ozone	mg-O_3/L	0.25	0.32	0.07	0.88
NH_4-N	mg-N/L	0.10	0.02	0.07	0.15
Bromide	μg/L	71	17	39	96
Temperature	°C	9.8	3.9	4.7	16.8

the numbers of bacteria in the BAC filters was negligible because of the short *Ct* value in the backwashing operation and because later in the filter cycle the biomass recovered to its prebackwashing concentration [16,17].

The influent and effluent waters were collected at 30 min before filter backwashing to monitor the dissolved organic carbon (DOC) and bacterial quality indicators. The backwash water was collected during backwashing by grab sampling. The BAC samples were taken from the top of the BAC bed (30 cm below the bed surface) using a tubular sampler and were analyzed for biomass ATP immediately after the sampling. All samples were transported to the laboratory under cold storage conditions and analyzed for FCM cell concentration within 5 h of sampling.

2.2. ATP analysis

ATP is present in water as cellular and extracellular ATP. Total ATP, which is the sum of cellular and extracellular ATP, was determined with a luciferin-luciferase assay as described in Berney et al. [9] and Hammes et al. [18] using a BacTiter-Glo™ reagent (Promega Corp., Madison, WI, USA) and a luminometer (Sirius L, Berthold Detection Systems GmbH, Germany). The data were collected as relative light units (RLU) and converted to ATP (M) by means of a calibration curve employing a series of rATP standards (P1132, Promega Corp.) ranging from 10^{-7} μM to 1 μM. Extracellular ATP was quantified by measuring ATP after filtering each sample through a 0.22-μm sterile syringe filter (hydrophilic PVDF, Millex-GV, Millipore), and then, cellular ATP was calculated by subtracting extracellular ATP from total ATP. The ATP was measured in triplicate for all samples.

2.3. Flow cytometric analysis

FCM is a method to directly count the number of bacterial cells. FCM was performed with a BD Accuri C6 flow cytometer equipped