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Ferrate treatment for inactivation of bacterial community in municipal secondary effluent

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ABSTRACT

This paper demonstrates the effect of ferrate [Fe(VI)-compound], an environmental friendly multi-purpose reagent, in municipal secondary effluent treatment. The purpose was to study the inactivation capability of ferrate and for the first time to compare the effect and efficiency of Fe(VI) with the widely used disinfectant, chlorine gas on the indigenous bacterial community in the case of secondary effluents. The most probable number technique (MPN) was applied for the determination of cultivable heterotrophic bacterial abundance and terminal restriction fragment length polymorphism (T-RFLP) analysis for comparing bacterial communities. The study demonstrated that (i) ferrate and chlorine had different effect on the total bacterial community of secondary effluents, (ii) low ferrate dose [5 mg L^{-1} Fe(VI)] was sufficient for >99.9% reduction of indigenous bacteria, and (iii) a similar dosage was also effective in the inactivation of chlorine-resistant bacteria.

1. Introduction

Environmental protection, regulation and human health interests claim that wastewater must be treated to meet the requirements of given standards before it is discharged to surface waters or reused. Coagulation and oxidation/disinfection are important processes in water and wastewater purification. The most common coagulants are ferric- and aluminum salts, while the most widely used oxidants/disinfectants are chlorine, sodium hypochlorite, chlorine dioxide, ozone, hydrogen peroxide or their combination (Jiang and Lloyd, 2002; Jiang, 2007). Furthermore catalytic, UV assisted processes are also applied for oxidation (Li et al., 2010). However, there are some problems with the application of these methods regarding the formation of potential harmful disinfection by-products, e.g. trihalomethanes and bromates (Rook, 1974; Hagg and Hoigne, 1983; Kumar et al., 2010). Therefore an alternative reagent without any known harmful effects via by-products, ferrate has been introduced (Jiang, 2007; Jiang et al., 2009; Sharma, 2010).

Ferrate can be produced as potassium ferrate (K_2FeO_4) or sodium ferrate (Na_2FeO_4) by several methods, such as dry, electrochemical and wet oxidation processes (Jiang and Lloyd, 2002; Alsheyab et al., 2009). Besides its prominent coagulation, detoxification and deodorization capacity, disinfection properties are also very promising due to the high oxidation–reduction potential in a wide pH range (De Luca et al., 1996; Sharma et al., 2005; Jiang, 2007; Jiang et al., 2009). Many of the pollutants in wastewater can be removed by ferrate-Fe(VI) generating non-hazardous products and a non-toxic, easily separable by-product, Fe(III) precipitate during its reduction (Jiang and Lloyd, 2002; Sharma et al., 2005; Jiang, 2007; Sharma, 2007, 2010; Alsheyab et al., 2009).

The effect of Fe(VI) on microorganisms was demonstrated mainly on coliforms (or on other Gram-negative bacteria), which have special importance in the hygienic properties of drinking water and wastewater (Murmann and Robinson, 1974; Gilbert et al., 1976; Sharma et al., 2005; Cho et al., 2006; Jiang et al., 2006). The inactivation of some more resistant bacteria (such as aerobic spore-formers and anaerobic clostridia; Sharma et al., 2005), viruses (Schink and Waite, 1980) and parasites (Ling et al., 2010) with Fe(VI) was also confirmed. These studies have revealed that Fe(VI) could kill ⩾99.9% of different microorganisms (that corresponds to 3 orders of magnitude reduction of initial concentration) both in laboratory-scale and in pilot-scale experiments with relatively low applied reagent dose. These investigations usually focused on only one or some selected microbial groups and only limited work was performed related to wastewater or secondary effluent samples.

Therefore, the main objective of this study was to assess the effect of Fe(VI) on the total indigenous bacterial community of secondary sewage effluents. Parallel to Fe(VI)-treatment, chlorination

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was also performed on some selected samples to compare the efficiency of Fe(VI) with chlorine gas, a commonly applied oxidant/disinfectant. These investigations were amended with laboratory-scale experiments using pure cultures of chlorine-resistant bacteria.

2. Methods

2.1. Chemical characterization of secondary sewage effluents

Secondary effluents were obtained from the South-Pest Wastewater Treatment Plant (South-Pest WWTP, part of the Budapest Sewage Works Ltd., Hungary). Samples were collected in plastic containers (20 L) and chilled until the laboratory experiments.

The effluents, in terms of pH, specific electric conductivity, turbidity, total and soluble chemical oxygen demand (COD) had been characterized according to Standard Methods (APHA, AWWA, WEF, 2005). The determination of total organic carbon (TOC) and dissolved organic carbon (DOC) was carried out according to the valid international standard (EN ISO 5667-3:1995).

2.2. Treatments with Fe(VI) and chlorine

For the evaluation of Fe(VI) performance in sewage treatment, secondary effluent samples were treated with ferrate and chlorine gas in laboratory. These samples were compared with untreated ones and with samples that were subjected to industrial scale chlorination in the WWTP.

The preparation of sodium ferrate (Na_2FeO_4) was carried out by wet oxidation in laboratory (according to the recipe of Ferrate Treatment Technologies, LLC, Orlando, FL, USA). For determining concentration, the absorbance at its characteristic wavelength (510 nm; Sharma, 2007) was measured with a spectrophotometer (Ocean Optics Inc., Dunedin, FL, USA). Ferrate concentration is expressed as Fe(VI) throughout this paper.

Laboratory-scale treatments were performed using standard jar tests (by flocculator SW1, Stuart Scientific, Redhill, UK) at room temperature with Fe(VI) doses up to 15 mg L^{-1}. A rapid mixing at a speed of 230 rpm for 10 min and then a slow mixing at a speed of 33 rpm for 20 or 50 min were applied (corresponding to 30 and 60 min contact time). Mixing was followed with neutralization using 1 M HCl to adjust the pH to 8.0 ± 0.4 and with a subsequent sedimentation for 20 min.

The chlorination in lab-scale experiments was carried out with chlorine gas where the initial chlorine concentration was 15 mg L^{-1} and the samples were tested after 30 min contact time. The parameters of laboratory scale chlorination were adjusted to mimic the industrial scale treatment. The free chlorine content of the chlorinated samples was controlled by a spectrophotometric method (APHA, AWWA, WEF, 2005).

For comparison of the effect of Fe(VI) and chlorine treatment, four different sample types were compared: secondary effluents without any treatment ('untreated'), treated by chlorine at plant scale ('Cl-ind') and in the laboratory ('Cl-lab') as well as by a determined optimal dose of Fe(VI) in the laboratory ['Fe(VI)-lab'].

Supernatants were decanted and analyzed as described in Section 2.3 and 2.5.

2.3. Determination of heterotrophic bacterial cell count

Viable counts of cultivable heterotrophic bacteria were determined by the MPN (most probable number) technique on microtiter plates in five parallels. The standard serial dilution technique was applied using the liquid growth medium (tryptone 6 g L^{-1}, yeast extract 3 g L^{-1}; pH 7.2 ± 0.2) as diluent. After incubation (22 ± 1 °C, 72 h) growth was detected visually (observed turbidity) in the medium (testing the adequacy of this method is available as online Supplementary material). The MPN values with standard errors of bacterial growth were calculated from the MPN tables described by Garthright and Blodgett (2003) using the Microsoft Excel spreadsheet. Removal efficiency was calculated based on the ratio of cell concentration detected in treated and raw samples.

2.4. Fe(VI)-treatment experiments with selected bacterial strains

Secondary effluent samples were prepared using a sterilized sewage effluent sample (taken on the 17 June 2010, heat sterilization in autoclave at 121 °C for 15 min) inoculated with pure cultures of bacteria. Three selected bacterial strains were applied as model cultures for ferrate treatment experiments: *Bacillus licheniformis* RB1-1B (Felföldi et al., 2010b), *Mycobacterium frederiksbergense* M8-6 and *Mycobacterium setense* M9-4 (Homonnay, Makk, Márialigeti and Tóth, Eötvös Loránd University, Department of Microbiology, unpublished results). Bacterial cell suspensions were prepared in the pre-sterilized sewage effluent and the optical density (measured at 650 nm; OD_{650}) of this suspension was coupled with bacterial cell counts using the above-mentioned MPN technique. Subsequently, OD_{650} of cell suspensions was used as reference to adjust the proper cell abundance values in previously sterilized sewage samples. Bacterial cell concentration was set to 5×10^5 MPN mL^{-1} in the case of separate experiments and to 6×10^5 MPN mL^{-1} in the case of experiments performed with the mixture of the three strains (containing 2×10^5 MPN mL^{-1} from each). Prior and after Fe(VI)-treatment the bacterial cell counts were determined with the method described above.

2.5. Bacterial community analysis with terminal restriction fragment length polymorphism (T-RFLP)

Approximately 100 mL aliquots from secondary effluent samples were filtered by sterile cellulose nitrate filters (0.45 μm, 47 mm diameter; Sartorius, Göttingen, Germany). Filters were cut into small pieces and the total environmental genomic DNA was extracted as described by Felföldi et al. (2010b). T-RFLP analysis was performed as reported earlier in detail (Vajna et al., 2010). Briefly, PCR amplification was conducted using fluorescently labeled 27F (5′-HEX-AGA GTT TGA TCM TGG CTC AG-3′) and 518R primers (5′-ATT ACC GCG GCT GCT GG-3′), which was followed with the enzymatic digestion of PCR products using *Alu*I and *Bsh*1236I restriction endonucleases (Fermentas, Vilnius, Lithuania). Capillary electrophoresis was performed to detect presence and relative abundance of fluorescently labeled terminal fragments having different length. To find corresponding genotypes in different samples, alignment of chromatograms was generated with the T-Align program (Smith et al., 2005). Subsequently, the generated data matrices of the two enzymatic digestions were combined. Principal component analysis was performed with the software PAST (Hammer et al., 2001).

3. Results and discussion

3.1. Inactivation of the indigenous bacterial community by Fe(VI)

The chemical composition of the investigated samples corresponded to an average secondary effluent, having differences within measured parameters among the samples (Table 1). On average, effluents had slightly alkaline pH (7.64 ± 0.31), moderate specific electric conductivity (1340 ± 170 μS cm^{-1}) and low values of turbidity (2.12 ± 1.08 NTU), TOC (16.9 ± 4.6 C mg L^{-1}), DOC