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Removal of total and antibiotic resistant bacteria in advanced wastewater treatment by ozonation in combination with different filtering techniques

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ABSTRACT

Elimination of bacteria by ozonation in combination with charcoal or slow sand filtration for advanced sewage treatment to improve the quality of treated sewage and to reduce the potential risk for human health of receiving surface waters was investigated in pilot scale at the sewage treatment plant Eriskirch, Baden-Wuerttemberg/Germany. To determine the elimination of sewage bacteria, inflowing and leaving wastewater of different treatment processes was analysed in a culture-based approach for its content of *Escherichia coli*, enterococci and staphylococci and their resistance against selected antibiotics over a period of 17 month. For enterococci, single species and their antibiotic resistances were identified. In comparison to the established flocculation filtration at Eriskirch, ozonation plus charcoal or sand filtration (pilot-scale) reduced the concentrations of total and antibiotic resistant *E. coli*, enterococci and staphylococci. However, antibiotic resistant *E. coli* and staphylococci apparently survived ozone treatment better than antibiotic sensitive strains. Neither vancomycin resistant enterococci nor methicillin resistant *Staphylococcus aureus* (MRSA) were detected. The decreased percentage of antibiotic resistant enterococci after ozonation may be explained by a different ozone sensitivity of species: *Enterococcus faecium* and *Enterococcus faecalis*, which determined the resistance-level, seemed to be more sensitive for ozone than other *Enterococcus*-species. Overall, ozonation followed by charcoal or sand filtration led to 0.8–1.1 log-units less total and antibiotic resistant *E. coli*, enterococci and staphylococci, as compared to the respective concentrations in treated sewage by only flocculation filtration. Thus, advanced wastewater treatment by ozonation plus charcoal or sand filtration after common sewage treatment is an effective tool for further elimination of microorganisms from sewage before discharge in surface waters.

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1. Introduction

In Germany so far, no breakpoints exist for the concentration of bacteria or even for antibiotic resistant microorganisms in the effluent of wastewater treatment plants (WWTP), since the focus lies on the elimination of carbon, nitrate and phosphate. Currently, sewage treatment of most plants can be divided into three steps: primary and secondary treatment (mostly activated sludge) combined with nitrification/denitrification, phosphate precipitation and/or filtration as tertiary treatment (Statistisches Bundesamt, 2013). In the last years, the quality of receiving water bodies, which often serve as drinking water reservoir or for recreation, moved more and more into public interest. This is manifested in different guidelines, for instance in the EU Bathing Water Directive (European Parliament and Council, 2006), which defines quality thresholds for bathing water. Among other parameters, maximal numbers of two fecal indicator groups, *Escherichia coli* and enterococci are fixed. These two bacterial groups were chosen as markers for contamination with human feces (Luczkiewicz et al., 2011). In contrary, staphylococci, typical colonizers of skin and mucous membranes of humans and animals, are not classified as fecal indicators. However, some species and subspecies of enterococci and *E. coli* are also relevant human pathogens.

To achieve the goals manifested e.g. in WHO or EU guidelines, improvements of wastewater purification by fourth treatment techniques, such as UV-irradiation, photo Fenton, chlorination or photocatalysis may be useful (e.g. Diao et al., 2004; Rincón and Pulgarin, 2005; Michael et al., 2012). A positive effect of the mentioned techniques is the reduction of living facultative pathogenic bacteria but it is unclear if their establishment will lead to a higher percentage of antibiotic resistant bacteria (e.g. Dodd, 2012).

Ozonation is another of the recently tested advanced oxidation processes: molecular ozone decays in three phases if it is dissolved in water (Gehr et al., 2003). Its decay rates depend on various parameters. If, for instance, alkalinity is low and/or the concentration of organics high, ozone will decay rapidly, forming hydroxyl radicals for non-selective oxidation (Gehr et al., 2003). Hunt and Marinas (1997) found out that molecular ozone primarily inactivated *E. coli*. Up to date, the mode of action and the specific target structures of ozone and its decomposition products in microbial cells are not completely understood: amino acids or proteins, peptidoglycan, lipids in the cell wall and cell membrane, enzymes as well as DNA-molecules may be affected (Dodd, 2012). Furthermore it is still an open question whether antibiotic resistant microorganisms might be less sensitive against oxidative stress that is caused by ozone in aquatic environment.

The effect of different filter materials such as quartz sand or charcoal on the distribution of antibiotic resistance genes is controversially discussed: Grabow et al. (1976) assumed that stony surfaces of biofilters or sand filters are unfavorable for conjugation and could damage sex pili resulting in a stable or even decreasing percentage of antibiotic resistant bacteria. On the other hand, the prolonged hydraulic retention times in the filters may promote horizontal gene transfer. The percentage of antibiotic resistant bacteria may also increase, if temporarily retained microorganisms in a filter would incorporate free DNA fragments of lysed cells after ozonation (Dodd, 2012).

Within the project "SchussenAktiv*plus*" the effect of ozonation followed by sand and/or activated-charcoal filtration per se or in combination was investigated with respect to the removal of total and antibiotic resistant bacteria, *E. coli*, enterococci and staphylococci. The filters were used for post-ozonation treatment to remove e.g. emerging contaminants, estrogenicity or mutagenicity possibly induced by ozone (Magdeburg et al., 2014). For this purpose, part of the sewage entering the wastewater treatment plant of Eriskirch was treated with the mentioned advanced techniques. The concentrations of bacteria as well as the percentage of antibiotic resistant isolates were determined in a culture-based approach and results were compared with those of the effluent of routine tertiary treatment by flocculation filtration.

2. Material and methods

2.1. WWTP characteristics

The study site is a medium sized WWTP in Eriskirch (Germany) with 40,000 inhabitant equivalents located 0.5 km upstream to the Schussen estuary into Lake Constance. The catchment area has a population of 28,000 inhabitants. Standard wastewater treatment processes comprise mechanical retention of large solids, separation of sand and grit in aerated chambers, settling of suspended solids in primary settling tanks, biological removal of nitrogen and phosphorus by activated sludge units (including chemical phosphorus precipitation) and secondary clarifiers. In an additional flocculation filtration step, again phosphorus precipitation takes place, before the treated water is directed to a filtration unit consisting of seven cells with a surface area of 22.1 m^2 each filled with a 15 cm quartz gravel support layer (4–8 mm), a 65 cm quartz sand layer (0.71–1.25 mm) and an 85 cm anthracite layer (1.4–2.5 mm). Back flushing was performed at 6 h intervals from cell to cell, requiring 42 h. Depending on weather conditions this time span may have been shorter. The maximum hydraulic load was 350 l/s. The mean run-out was about 10,296 m^3/d (dry weather minimum 6468 m^3/d, rainy weather maximum 20,318 m^3/d, triennial means 2011–2013) (for details see Triebskorn et al., 2013; www.schussenaktivplus.de).

For the pilot scale study, a partial flow of the effluent (1.5 m^3/h) was piped through an ozonation reactor (contact time 20 min with 0.73 mg O_3/mg DOC), followed by either sand filtration or granulated activated charcoal (GAC) adsorption or a combination of both techniques. The three filters were identically sized (4 m height, 0.3 m diameter, filter area 0.0707 m^2) and were top-down fed and bottom-up back-flushed. The sand filter (back-flush interval 43 h) contained 0.3 m gravel support layer, a 0.6 m sand layer (volume 40 l, grain size 0.71 mm–1.25 mm) and a 0.8 m hydroanthracite layer (volume 55 l, grain size 1.4 mm–2.5 mm). The GAC filter (back-flush interval 5 h) contained 0.3 m gravel support layer