



Performance comparison of secondary and tertiary treatment systems for treating antibiotic resistance



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ARTICLE INFO

Article history:

Received 15 June 2017

Received in revised form

10 October 2017

Accepted 11 October 2017

Available online 12 October 2017

Keywords:

Antibiotic resistance

Treatment

Modified trickling filter

Ozone

UV

ABSTRACT

Rapid emergence of antibiotic resistance (AR) in developing countries is posing a greater health risk and increasing the global disease burden. AR proliferation mediated by treated/untreated discharges from sewage treatment plants (STPs) is a prime public health concern. Efficient sewage treatment is among our key defenses against the dissemination of infectious diseases. The present study aims to estimate the efficiency of aerobic [activated sludge process (ASP) and modified trickling filter (MTF)] and anaerobic reactors (anaerobic flow-through reactor) along with the three disinfection techniques (UV, ozone and chlorination) in reducing ARB and ARGs present in the domestic sewage. The three treatment systems were operated at different HRTs for 1 year and their performances in terms of treatment of conventional and emerging pollutants (ARB and ARGs) were assessed. The results indicated higher removal of ARB and ARGs in aerobic reactors compared to anaerobic reactor. Treatment studies in various bioreactors showed that the use of MTF along with UV/Ozone was superior to ASP and anaerobic flow-through reactor in reducing both the conventional and emerging pollutants. However, higher reduction of the pollutants was observed at higher HRTs. Though complete removal of coliforms and ARB was observed by treating the wastewater using MTF followed by UV or ozone but substantial levels of ARGs were observed in the effluent. Therefore, different advanced and effective treatment technologies such as filtration (RO), use of zero valent iron, TiO₂ photocatalysis and other strong oxidizing agents which can ensure complete removal of ARGs along with ARB need to be evaluated. Though addition of these units will increase the treatment cost, but the increased cost would be negligible compared to the present disease burden of AR.

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1. Introduction

Extensive use of antibiotics for human and veterinary purposes results in the development of antibiotic resistant bacteria (ARB) in the guts of humans and animals (Canto and Baquero, 2008), which are subsequently released into the natural environments through the faecal matter (sewage). The antibiotics consumed by the humans and animals are not metabolized completely and are also discharged with the fecal matter (Hendricks and Pool, 2012). The presence of these residual antibiotics in the environment creates selection pressure for the proliferation of ARB (Canto and Baquero, 2008). Antibiotic resistant genes (ARGs) present in the ARB are mostly associated with integrons and transposons present in the plasmid (Mazel, 2004; Thong et al., 2009). These mobile genetic elements fosters the transfer of ARGs from one organism to another

by horizontal gene transfer (Davies and Davies, 2010).

The residual antibiotics, ARB and ARGs present in the sewage reach the sewage treatment plants (STPs) along with other household organic pollutants for treatment before getting discharged into the surface water bodies. The conventional STPs (mostly based upon biological processes) are designed for treating the organic pollutants (C, N and P) present in the sewage but are inefficient for treating the emerging pollutants such as residual antibiotics, ARB and ARGs (Kümmerer, 2003; Miao et al., 2004; Zhang and Li, 2011; Lamba and Ahammad, 2017). Indeed, STPs are considered as the reservoir for the proliferation of antibiotic resistance (AR). High nutrient content and huge microbial biomass creates a suitable habitat for the proliferation and transfer of antibiotic resistance in the STPs and also leads to the development of multi-drug resistant microbes (Lapara et al., 2011; Schlüter et al., 2007).

Recently, studies have examined the removal of ARB and ARGs in the STPs. Studies have reported that the removal of ARB and

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ARGs in the STPs depends upon the treatment technology used and the operating conditions (Christgen et al., 2015; Kim et al., 2007; Manaia et al., 2010). A recent study reported that aerobic reactors as well as combination of aerobic-anaerobic reactors (anaerobic system followed by aerobic system) are superior to anaerobic reactors in removing the ARGs from the domestic sewage (Christgen et al., 2015). Conversely, aerobic systems have been shown to select for greater levels of multidrug resistance microbes compared to anaerobic systems (Christgen et al., 2015; Lapara et al., 2011). Earlier studies have reported that longer hydraulic residence time (HRT) might improve the microbial removal rates as observed by the higher removal in activated sludge process (ASP) operating at 8hr compared to trickling filter operating at 0.5 h HRT (Manaia et al., 2010). However, longer residence time has also been reported to favour the ARGs exchange and thus promoting multidrug resistance (Manaia et al., 2010). Organic loading rate and growth rate have also been reported to affect the abundance of AR in the STPs as shown by the increased tetracycline resistance abundance in the effluent with increasing organic loading rate and growth rates (Kim et al., 2007).

The chemical and biological processes alone cannot efficiently remove ARB and ARGs from the wastewater. This highlights the need for using disinfection strategies along with the present biological treatment options for combating the problem of AR by destroying both the ARB and ARGs. Destruction of ARGs along with the ARB is very crucial because even after destroying the ARB, DNA containing ARGs are released into the environment and may get transferred to other cells through the process of horizontal gene transfer, thus causing proliferation of AR (Crecchio et al., 2005). UV, ozone and chlorination treatments are commonly used as disinfection processes in wastewater treatment plants (WWTP). UV and ozone are of particular interest because UV and ozone have the ability to cause DNA damage and thus have the potential to cause ARG damage compared to chlorination (Macauley et al., 2006; Öncü et al., 2011; Rastogi et al., 2010). Few efforts have been made in previous studies to estimate the effectiveness of these disinfection techniques regarding ARB/ARGs, and conflicting results exist. For instance, previous studies have reported the efficiency of UV disinfection for treating the ARB (Macauley et al., 2006; Mckinney and Pruden, 2012). 4–5 log reduction of methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecium*, *E. coli* SM-3-5, and *Pseudomonas aeruginosa* 01 has been reported at a UV dose of 10–20 mJ/cm² (Mckinney and Pruden, 2012). Another study reported that a fluence of 220 mJ/cm² resulted in 3.4–4.2 log reduction of ARB (Macauley et al., 2006). Conversely, other studies indicated that UV disinfection is inefficient in treating ARB and ARGs (Munir et al., 2011; Rusin and Gerba, 2001). UV disinfection led to no significant change in the abundance of ARB and ARGs in the Michigan WWTPs (Munir et al., 2011). Reports have also highlighted that UV exposure can lead to the variations in the characteristics of ARB. The authors highlighted that this discrepancy in the results is probably because of different doses used for disinfection and the susceptibility of the organisms. Studies have also highlighted the efficiency of chlorination disinfection in reducing the abundance of ARB (Huang et al., 2011; Macauley et al., 2006). Contrary to this, other similar studies have reported that no significant reduction in the abundance of ARB and ARGs was observed after chlorination disinfection (Munir et al., 2011; Rusin and Gerba, 2001). The conflicting results on the effect of chlorination on AR removal have been partly explained by the earlier studies which reported that the effect of chlorine on AR varied with chlorine dosage, the chlorination conditions and concentration of suspended solids present in the wastewater (Bouki et al., 2013; Macauley et al., 2006). Similarly, studies have shown contrasting results for ozone disinfection of ARB and ARGs as well (Alexander

et al., 2016; Macauley et al., 2006; Zhuang et al., 2015). For example, Macauley et al. revealed that 100 ppm dose of ozone could lead to 3.3–3.9 log reduction of ARB (Macauley et al., 2006). The author also said that low ozone dose was ineffective in reducing the microbial load. A recent study indicated that only a high ozone dose of 177 ppm resulted in 2 log reduction of ARGs whereas lower doses were ineffective in removing ARGs (Zhuang et al., 2015). Alexander et al. reported no substantial change in the abundance of *Pseudomonas aeruginosa* and an increase in the abundance of *vanA* and *bla_{VIM}* genes after ozone treatment (Alexander et al., 2016). Reports have highlighted that the efficiency of ozone treatment depends upon the concentration of radicals, contact time and the susceptibility of the target organism.

However, the current information regarding the potential of secondary and tertiary treatment technologies for the removal of ARB and ARGs is very limited. So, further study is required to understand the effect of these treatment techniques on antibiotic resistance. The aim of this study was to evaluate the efficiency of commonly used aerobic (activated sludge process and modified trickling filter) and anaerobic reactors (anaerobic flow-through reactor) along with the three different disinfection techniques (UV, ozone and chlorination) to eliminate ARB and ARGs from the treatment plant effluent and thus ensure public health security. The presence of ARB and their resistant determinants in the treated wastewater is of primary concern since this is the principle route for the entry of these contaminants in the water bodies, thus implementing suitable treatment technologies to treat these reservoirs will result in substantial benefits.

2. Material and methods

2.1. Reactor design and operation

2.1.1. Activated sludge process

The reactor setup consists of an aeration basin followed by a clarifier. The volume of the lab scale aeration tank is 4 L (working volume 3.5 L) and that of the clarifier is 5 L. The residual dissolved oxygen (DO) in the aeration basin was 1.5 mg/L and. The solid retention time of 11 days was maintained in the aeration tank. The aeration tank was initially inoculated with aerobic sludge collected from the return activated sludge line (RAS) from local STP (Mehrauli sewage treatment plant, Delhi). The reactor was run in the batch reactor for one week after which it was run in continuous mode. The reactor was fed with the wastewater collected after the grit chamber from the same STP (Mehrauli sewage treatment plant).

2.1.2. Modified trickling filter (MTF)

The reactor is designed to have two zones: aerobic (upper) and anaerobic (lower) zones. Both the zones have porous media layers to retain the microbes. In the upper zone, instead of using a single porous media, various porous media units were used to have a larger surface area for the attachment of the microbes and the subsequent removal of the contaminants. All the sponge units were enclosed in a cylindrical ring made up of plastic material. The reactor was operated in a downflow mode. The bottom region has two porous media units. The volume of the upper zone is 15 L, and that of the lower zone is 3.3 L. The reactor was fed with sludge and wastewater collected from Mehrauli wastewater treatment plant for biofilm formation. The reactor was stabilized after one month after which it was run in a continuous mode. The effluent from the reactor was recirculated at a rate three times the rate of the influent flow rate for better removal of organics.

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