Science of the Total Environment xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Antibiotic resistance genes fate and removal by a technological treatment solution for water reuse in agriculture

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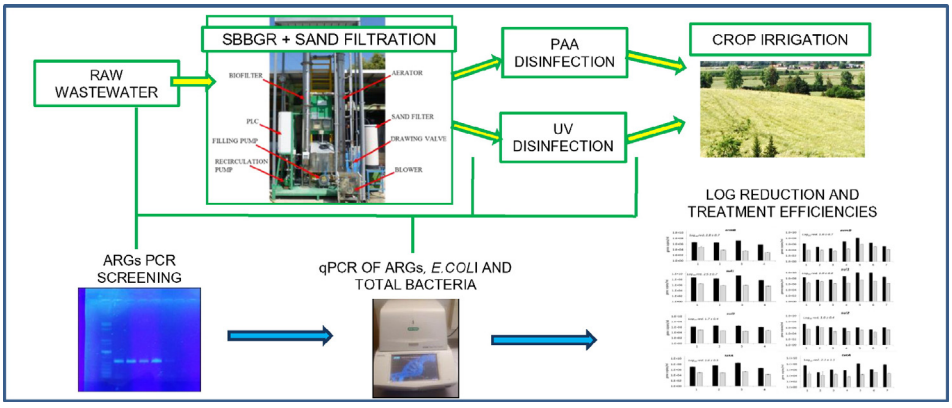
HIGHLIGHTS

- ARGs reduction by a technological treatment solution for water reuse was assessed
- SBBGR and sand filtration showed high efficiency in terms of ARGs reduction
- ARGs are removed together with the total bacteria and not specifically eliminated
- Disinfection treatments by UV and peracetic acid did not affected ARGs level
- ARGs behaved differently under treatments implying diverse ecologies

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 May 2016
Received in revised form 6 July 2016
Accepted 7 July 2016
Available online xxxx

Editor: D. Barcelo

Keywords:
Antibiotic resistance genes
Sequencing Batch Biofilter Granular Reactor
Water reuse
Disinfection
Wastewater treatment
E.coli

ABSTRACT

In order to mitigate the potential effects on the human health which are associated to the use of treated wastewater in agriculture, antibiotic resistance genes (ARGs) are required to be carefully monitored in wastewater reuse processes and their spread should be prevented by the development of efficient treatment technologies. Objective of this study was the assessment of ARGs reduction efficiencies of a novel technological treatment solution for agricultural reuse of municipal wastewaters. The proposed solution comprises an advanced biological treatment (Sequencing Batch Biofilter Granular Reactor, SBBGR), analysed both al laboratory and pilot scale, followed by sand filtration and two different disinfection final stages: ultraviolet light (UV) radiation and peracetic acid (PAA) treatments. By Polymerase Chain Reaction (PCR), the presence of 9 ARGs (*ampC, mecA, ermB, sul1, sul2, tetA, tetO, tetW, vanA*) were analysed and by quantitative PCR (qPCR) their removal was determined. The obtained results were compared to the reduction of total bacteria (16S rDNA gene) and of a faecal contamination indicator (*Escherichia coli uidA* gene). Only four of the analysed genes (*ermB, sul1, sul2, tetA*) were detected in raw wastewater and their abundance was estimated to be $3.4 \pm 0.7 \times 10^4$ - $9.6 \pm 0.5 \times 10^9$ and $1.0 \pm 0.3 \times 10^3$ to $3.0 \pm 0.1 \times 10^7$ gene copies/mL in raw and treated wastewaters, respectively. The results show that SBBGR technology is promising for the reduction of ARGs, achieving stable removal performance ranging from 1.0 ± 0.4 to 2.8 ± 0.7 log units, which is comparable to or higher than that reported for conventional activated sludge treatments. No reduction of the ARGs amount normalized to the total bacteria content (16S rDNA), was

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http://dx.doi.org/10.1016/j.scitotenv.2016.07.055
0048-9697/

instead obtained, indicating that these genes are removed together with total bacteria and not specifically eliminated. Enhanced ARGs removal was obtained by sand filtration, while no reduction was achieved by both UV and PAA disinfection treatments tested in our study.

1. Introduction

In an effort to alleviate water scarcity, treated wastewater reuse in agriculture is increasingly being applied to achieve sustainable water management in arid regions, especially those of the Mediterranean basin. The benefits of reusing water in agriculture are mainly related to nutrient recovery possibilities, more rational allocation and conservation of freshwater resources, control of pollution and increase in food production in water-scarce areas (Asano et al., 2007; Fatta-Kassinos et al., 2011). Innovative and sustainable systems need to be developed and tested as an alternative to conventional systems to reclaim wastewaters for agricultural reuse, especially in small and medium communities where low maintenance and easy operation are essential.

The Sequencing Batch Biofilter Granular Reactor (SBBGR) is a system developed during the last decade, which can perform in a single stage the entire wastewater treatment train (i.e., primary and secondary treatment) carried out at the conventional plants (Di Iaconi et al., 2008). The high biomass retention capacity, due to its particular structure, and the dynamic operating conditions, typical of periodic systems, are the main strength points of this system. From these, several advantages follow, like good robustness, high removal efficiencies and low excess sludge production (Di Iaconi et al., 2010). Moreover, when a high effluent quality is needed for agriculture reuse, SBBGR treatment can be also implemented by post- chemical (e.g. chlorine, ozone, PAA) or physical (e.g. UV radiation, filtration) disinfection treatments. Furthermore, due to the operational features of this system, these chemical/physical processes can be activated/deactivated on the basis of water demand. Recently, the efficiency of a lab scale SBBGR and its integration with different disinfection strategies (UV radiation, peracetic acid) to produce an effluent suitable for agricultural use were investigated by De Sanctis et al. (2016). In particular, the SBBGR hygienization performances were higher than those reported for conventional activated sludge plants comprising primary treatments (Carducci et al., 2008; Koivunen et al., 2003; Wen et al., 2009): SBBGR was able to remove approximately 2.5 log units of total coliforms and 2.8 log units of *E.coli*, producing an effluent that met the limit fixed by Italian regulation for discharge into water bodies (5000 CFU/100 mL). Consistently, SBBGR treatment was more efficient than conventional processes in removing pathogenic protozoa, such as *C. parvum* and *G.lamblia*, with removal values of 2.0 log units and 3.8 log units, respectively. Finally, disinfection treatments of the secondary effluent at mild conditions with UV (40 mJ/cm^2) or peracetic acid (PAA 1 mg/L) reduced *E. coli* content in the effluent below the restrictive Italian limit for agricultural reuse (<10 CFU/100 mL), (De Sanctis et al., 2016).

Beside the potential presence of pathogens, the spread of clinically relevant antibiotic resistance genes (ARGs) and antibiotic resistance bacteria that are released from anthropogenic sources, is currently considered to be a serious environmental problem of global dimensions (Berendonk et al., 2015) eventually hindering the use of this renovated resource for irrigation purpose (Fatta-Kassinos et al., 2011).

The intensive use of antibiotics for human, veterinary and agricultural purposes is a worldwide practice providing both desirable and undesirable effects. Links have been found between the massive use of antibiotics and the increased dissemination of antibiotic resistance to known human commensal and pathogen bacteria (Allen et al., 2010; Bouki et al., 2013; T. Zhang et al., 2009; X.X. Zhang et al., 2009). In contrast to many chemical contaminants, ARGs, which provide bacteria with the ability to survive after antibiotic exposure, are capable to persist in the environment and even to spread through it (Berendonk et al., 2015). The ARGs carried by mobile genetic elements (namely plasmids, transposons, integrons) can multiply in their hosts, be transferred to other bacteria and be subjected to further evolution (Lupo et al., 2012). Since a huge variety of ARGs (more than 20,000 potential genes) confers resistance to antibiotics in bacterial communities (Davies and Davies, 2010), the identification of ARGs present in the target environment is necessary to define suitable monitoring parameters. Many different ARGs, encompassing resistance for all the classes of antibiotics, have been detected in wastewaters and wastewater treatment plants (WWTPs) (Auerbach et al., 2007; Chen and Zhang, 2013; Du et al., 2014; Munir et al., 2011; Yang et al., 2014). Recently, in an exhaustive review by Berendonk et al. (2015) an array of some genetic determinants has been selected and proposed as possible indicators to assess the antibiotic resistance level in environmental settings, focusing mainly on genes spread in the environment and commonly associated to mobile elements and thus more prone to horizontal transfer.

WWTPs have been specifically individuated not only as the main sources of antibiotics, but also as platforms for the spread of both antibiotic resistant bacteria and ARGs through the environment (Rizzo et al., 2013). Favourable conditions in WWTPs, such as high nutrient loadings, dense microbial populations and persistent antibiotics, are supposed to facilitate horizontal transfer of ARGs among bacteria, potentially contributing to further antibiotic resistance proliferation (Schlüter et al., 2007; Tennstedt et al., 2003). Of particular concern is the possible co-occurrence of antibiotic resistant bacteria and enteric pathogens, in wastewaters and WWTPs, which might favour the transfer of resistance from non-pathogenic to pathogenic microorganisms. For this reason, a careful examination of the antibiotic resistance level and removal in any kind of process directed to reclaim water, needs to be investigated in order to reduce the health risk at an acceptable level. In parallel, the definition of technological solutions that can reduce the environmental contamination by ARGs is also a priority. While the effectiveness of the SBBGR system in terms of pathogen removal has already been investigated and demonstrated (De Sanctis et al., 2016), information about ARGs occurrence and removal in this system is still missing.

The objective of this study was therefore to investigate the fate and removal of ARGs in a technological solution, based on the SBBGR system, for the treatment and reuse in agriculture of wastewaters produced by small communities. SBBGR performances were evaluated at both laboratory and pilot scale. Moreover, the effect of a tertiary disinfection step on ARGs removal, based on sand filtration followed by UV radiation or peracetic acid treatment, was also assessed. Occurrence and removal of ARGs by SBBGR and tertiary treatments were evaluated using culture-independent methods, in comparison to the removal of total bacteria (16S rDNA) and of a bacterial indicator of faecal contamination (i.e. *Escherichia coli*). In details, ARGs present in the monitored environment were initially identified by PCR screening of 9 ARGs, commonly found in raw and treated wastewaters and representative of resistance to 5 of the main classes of antibiotics: beta-lactam (*ampC*, *mecA*), glycopeptide (*vanA*), macrolide-lincosamide-streptogramin B (*ermB*), sulfonamide (*sul1,sul2*), tetracycline (*tetA, tetO, tetW*) (Auerbach et al., 2007; Böckelmann et al., 2009; Czekalski et al., 2012; Chen and Zhang., 2013; Fahrenfeld et al., 2013; Munir et al., 2011; T. Zhang et al., 2009; X.X. Zhang et al., 2009). Successively, by qPCR, the levels of the detected ARGs, *Escherichia coli (uidA* gene) and total bacteria (16SrDNA gene) were determined in raw and treated wastewater, in order to assess the absolute removal performances in the applied treatments.

Please cite this article as: Luprano, M.L., et al., Antibiotic resistance genes fate and removal by a technological treatment solution for water reuse in agriculture, Sci Total Environ (2016), http://dx.doi.org/10.1016/j.scitotenv.2016.07.055