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Wastewater disinfection by AOPs: effect on antibiotic resistance and contaminants of emerging concern

Urban wastewater treatment is called for facing different challenges worldwide, such as energy saving, resource recovery and reuse. Conventional wastewater treatments that are the state of art in most world regions, are not designed to remove the so-called contaminants of emerging concern (CECs) whose concentrations range from high ng L-1 to low μg L-1. Among CECs antibiotics play an important role because they may promote the selection and the diffusion of antibiotic resistance patterns into the environment, such as the development of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs). Due to the incomplete removal by conventional treatments, CECs, ARB and ARGs occur in treated wastewater and may exert chronic toxic effects in aquatic and terrestrial organisms as well as they may pose environmental and public health risks. This scenario raises major concern in the light of wastewater reuse that is regarded as an interesting option for augmenting available water supplies in many Countries. Advanced oxidation processes (AOPs) have been deeply investigating in the removal of such micropollutants accordingly. The main aim of this PhD thesis work was to investigate solar driven AOPs as wastewater disinfection methods to control or minimise the diffusion of antibiotic resistance into the environment especially in the light of reuse of treated wastewater for agricultural purposes. In particular, this thesis was focused on the following objectives: (i) the evaluation of different solar driven AOPs (H2O2/sunlight, H2O2/TiO2/sunlight, TiO2/sunlight, photo-Fenton) on the inactivation of ARB from urban wastewater; (ii) the investigation of the effect of UV/H2O2 process on the potential of antibiotic resistance transfer by means of molecular biology methods; (iii) the simultaneous removal of target ARB and CECs by H2O2/sunlight process and (iv) the subsequent assessment of chemical and microbial cross contamination on crops that have been irrigated with treated wastewater. PhD thesis work was finalized with a multidisciplinary approach including environmental engineering, microbiology, molecular biology and analytical chemistry. xi

Cultivation methods were used to address the first relevant issue and an antibiotic resistant E. coli strain was selected from urban wastewater. When implemented at laboratory scale, H2O2/TiO2/sunlight process allowed to achieve the detection limit (DL) with a cumulative energy per unit of volume (QUV) ranging between 3.3 and 4.2 kJ L-1, depending on H2O2/TiO2 ratio. Good performances were also obtained with photo-Fenton with Fe2+/H2O2 ratio as low as 5/10 mg L-1. Therefore, the energy was observed to be higher (QUV=14.9 kJ L-1) than that required by

H2O2/sunlight process (QUV=8.7 kJ L-1, H2O2 dose of 50 mg L-1). Subsequently disinfection experiments were carried out at pilot scale in compound parabolic collectors (CPCs) photo-reactors. The best disinfection efficiency was found for photo-Fenton at pH 4.0 (Fe2+/H2O2 ratio of 5/10 mg L-1), in terms of treatment time (20 min to reach DL) and required energy (QUV=0.98 kJ L-1). All processes resulted in a complete inactivation (5 Log decrease) of antibiotic resistant E. coli strain until DL but, due to the expected higher costs related to both solar TiO2 photocatalysis and solar photo-Fenton, H2O2/sunlight process at low hydrogen peroxide doses, namely 20 and 50 mg L-1, may be regarded as the most feasible and cost-effective in small communities. Since none of the implemented solar driven AOPs affected the antibiotic resistance of survived antibiotic resistant E. coli colonies, quantitative polymerase chain reaction (qPCR) based methods were investigated. blaTEM, qnrS and tetW were selected as target ARGs and were quantified by qPCR in the survived colonies as well as in the whole suspension (total DNA). In spite of a bacterial inactivation both in tests on distilled water and on urban wastewater, UV/H2O2 process did not affect blaTEM gene copies number per mL in total DNA. The presence of qnrS and tetW genes was not unequivocally proven in tests on distilled water, while no difference ($p>0.05$) was found for qnrS gene in urban wastewater between the initial (5.1×104 copies mL-1) and the final (4.3×104 copies mL-1) sample and a decrease ($p<0.05$) was observed for tetW gene. According to these results, H2O2/UV process may not be an effective tool to limit or minimise the potential spread of antibiotic resistance into the environment. H2O2/sunlight process was also implemented as disinfection/oxidation treatment for urban wastewater to evaluate the simultaneous removal of ARB and CECs and the subsequent assessment of chemical and microbial cross contamination on crops irrigated with treated wastewater.

When the final bacterial density was below the DL in the treated wastewater, no microbial contamination of pathogens, such as antibiotic resistant E. coli and antibiotic resistant E. faecalis, was observed in lettuce leaves and soil samples. When H2O2/sunlight process resulted in a residual bacterial density, such as 5.6×102 CFU mL-1 for AR E. faecalis, the complete absence of microbial contamination in crops samples could not be guaranteed. Carbamazepine (CBZ) and thiabendazole (TBZ) were partially degraded (36.9% and 68.2%, respectively), while flumequine (FLU) was totally removed (99.9%), after 300 min of solar exposure with the addition of 20 mg L-1 of H2O2. This led to a chemical contamination in lettuce and soil irrigated with treated urban wastewater. In fact after irrigation tests with 300 min treated wastewater, 48.0 ng g-1 of CBZ and 10.8 ng g-1 of TBZ were detected in lettuce samples, as well as 374.0 ng g-1 of CBZ and 120.8 ng g-1 of TBZ were measured in soil samples. On the base of the obtained results, solar driven AOPs may be successful in the inactivation of ARB. H2O2/sunlight process may be used as advanced treatment of urban wastewater in small communities as a cost-effective technology but it must be properly designed and applied to effectively inactivate pathogens, such as ARB, as well as to maximise CECs degradation in order to reduce their subsequent accumulation/deposition/transfer/uptake on crops irrigated with the treated wastewater. However, this process may not entail the control of the potential spread of antibiotic resistance into the environment, at least at the applied oxidant dose and with respect to the investigated ARGs. Further research is then needed to deeply investigate how ARB and ARGs transfer antibiotic resistance, in order to minimise the spread of antibiotic resistance into the environment. An environmental and health risk assessment may be also a useful tool to evaluate other aspects in this research field.