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Membrane bioreactor followed by heterogeneous TiO₂ and TiO₂-rGO photocatalytic processes or solar photo-Fenton oxidation: bacterial reduction and changes in bacterial community structure

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This study focused on the in-depth investigation of the gaps in research, regarding the disinfection of urban wastewater from selected bacteria including antibiotic-resistant bacteria (ARB) (*Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella* species) the removal of ARGs (*ampC*, *sul1*, *mecA* and *ermB*) and taxon-specific markers (*Enterococcus* rRNA and *Pseudomonas* RNA polymerase factor), and the changes of the microorganism community structure in an urban wastewater effluent after a pilot-scale membrane bioreactor (MBR) followed by selected Advanced Oxidation Processes (AOPs). Namely the solar photo-Fenton oxidation and heterogeneous TiO₂ and TiO₂-rGO composite photocatalytic processes (hydrothermal (HD) or photocatalytic preparation (PH)), at a bench-scale setup were applied.

The MBR unit, located at the University of Cyprus premises has the capacity to treat 10 m³ day⁻¹ of sewage (membrane nominal pore diameter: 0.4 μm). For the AOP experiments, a laboratory-scale solar simulator was used (Newport 91193) (irradiation intensity: 63 W m⁻²). 100 mg L⁻¹ of catalyst (TiO₂, TiO₂-rGO-PH or TiO₂-rGO-HD) were used in the inherently pH-neutral MBR-treated effluent. The established optimum conditions of the solar photo-Fenton oxidation were: [Fe²⁺]₀ = 5 mg L⁻¹, [H₂O₂]₀ = 50 mg L⁻¹, pH = 2.8.

According to TEM analyses, the extent of bacterial disinfection by TiO₂ and TiO₂-rGO particles, depends on their physical proximity of bacteria to the catalyst. Interestingly, total and antibiotic-resistant bacterial repair and re-activation were also observed, due to the produced 'Viable But Not Cultivable' (VBNC) state after treatment. No notable impact on ARGs or taxon-specific markers was observed.

In contrast, solar photo-Fenton was capable of permanently inactivating the examined bacteria (<LOD), without any reactivation phenomena, accompanied by a complete removal of ARGs (<LOD), but not of taxon-specific markers.

According to molecular DGGE analyses which show the guanine-cytosine DNA content, the greatest change in the bacterial community structure was observed in the solar photo-Fenton treated samples. Notably, both TiO₂ and TiO₂-rGO (PH and HD) photocatalysis were not able to drastically change the bacterial community molecular footprint.

Based on 16S rRNA amplicon sequencing, TiO₂ and TiO₂-rGO-PH photocatalysis and the solar photo-Fenton oxidation produced distinct bacterial community structures, different to the ones prior to AOP treatment. However, TiO₂-rGO-HD treatment did not shift the most abundant bacterial genera, compared to the samples before treatment. Remarkably, there was a notably high prevalence of the *Mycobacterium* genus in the photo-Fenton treated effluents (48%), showing the adaptability of this genus to adverse conditions such as the presence of reactive oxygen species, which in combination with simulated solar irradiation, have the capacity to cause damage and mutational alterations on bacteria. Overall, the solar photo-Fenton oxidation was shown superior to heterogeneous photocatalysis for disinfection of wastewater, and at the same time capable of causing a more drastic shift in the bacterial community, a result which needs further investigation.

Overall, further study is needed for the effective disinfection of urban wastewater, whose quality may have implications for safe water reuse and disposal into environmental compartments.

