

Evaluation of innovative water treatments at molecular level based on high resolution mass spectrometry and advanced statistical analysis tools

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Abstract This work aims at integrating the last advances in high resolution mass spectrometry (HRMS) and statistical analysis of data to develop and optimize a smart methodology (workflow) for the assessment of the performance of innovative water treatments using different technological approaches based on advanced oxidation processes with UV-254nm: (i) UV/K₂S₂O₈, where oxidation takes place mainly following the initial formation of sulfate radicals, (ii) UV/KHSO₅, where oxidation begins with the formation of both sulfate and hydroxyl radicals and (iii) UV/H₂O₂, when only the formation of hydroxyl radicals takes place initially.

Experiments were carried out using secondary effluent from a local wastewater treatment plant. The developed workflow allows the evaluation of the treatments in terms of overall oxidation through the careful study of *Van Krevelen* diagrams, where all the masses of the HRMS chromatograms are considered. The potential formation of transformation products with sulfur due to the sulfate radicals was also evaluated using statistical tools based on the isotopic pattern and accurate mass. Finally, the behavior of a large number of micropollutants with a wide range of physicochemical properties was studied using suspect screening strategies.

Keywords: Water treatment, AOPs, HRMS, non-target screening

1. Introduction

The continuous discharge of pollutants from waste water treatment plants (WWTPs) into the environment concerns the scientific and public community, because their potential adverse effects can threaten the ecological status of water bodies as well as human health. In this regard, advances in wastewater treatment technologies are crucial. Appropriate analytical evaluation of novel and alternative treatment options remains one of the main challenges.

Currently, the efficiency of wastewater treatment is routinely evaluated by measurements of chemical and biological oxygen demand and total organic carbon (TOC). Some additional specialized tools such as the measurement of the dissolved halogenated organics, assessment of aromaticity using the specific UV absorbance (SUVA₂₅₄) for the UV active fractions, or specific methods to analyze a small number of hand-picked contaminants, typically using GC-MS or LC-MS, are also available. However, all these approaches either summarize information in agglomerated parameters or neglect hundreds of organic contaminants and little is known about general changes at the molecular level. Therefore, new strategies are needed to assess the efficiency of water treatment. In this sense, a comprehensive characterization of dissolved organic matter (DOM) would give a more comprehensive evaluation of the efficiency and deeper understanding of the treatment processes. This study aims at integrating the last advances in high resolution mass spectrometry (HRMS) and statistical analysis of data to create new tools for the assessment of the efficiency of water treatment at the molecular level. The focus will be put on advanced oxidation processes (AOPs), where the influence of the radicals SO₄^{•-} and OH[•] as initial species, and their corresponding secondary oxidants generated (O^{•-}, CO₃^{•-}, NO₃[•], Cl₂^{•-}, Cl[•], etc) are still unknown in complex natural waters, such as effluents from WWTPs.

2. Material and methods

2.1. Water treatments

Experiments were carried out using secondary effluent from a local WWTP in Quart (Girona, Spain). Treatment consists of a preliminary screening coupled with grit removal, an aeration basin for carbon and nitrogen removal, followed by a secondary clarifier. The AOPs applied are based on radical formation under UV radiation. The radical sources are K₂S₂O₈

(0.4 mM), KHSO_5 (0.4, 0.8 mM), and H_2O_2 (0.4 mM). The experiments were carried out using a quasi-collimated beam UV apparatus equipped with a 130W amalgam Hg UV lamp (Philips TUV 130W XPT SE UNP/20, Netherlands) with near monochromatic emission at $\lambda_{\text{max}} = 253.7$ nm. Illumination was applied to 200 mL of solution in a 10 cm diameter glass reactor, under constant stirring and at 21°C, over a total time of 60 min (Fluence = 1702 mJ/cm^2).

2.2. Instrumental analysis and data treatment

Samples were analyzed in a liquid-chromatography system coupled to a hybrid linear ion trap (LTQ)-Orbitrap mass spectrometer. Data was acquired in data dependent acquisition mode using collision-induced dissociation (CID) and higher-energy collisional dissociation (HCD) fragmentation energies. Peak picking and componentization were carried out following a computational workflow based on open-source R packages (xcms and CAMERA), as previously described by Alygizakis et al. (2019a). In addition, in-house R-based programs were developed and applied for the evaluation and visualization of the results. Suspect screening for $\approx 44,000$ micropollutants were carried out using the NORMAN digital sample freezing platform (Alygizakis et al., 2019b), list “allcompounds”, and the workflow described by Gago-Ferrero et al (2015).

2. Results

3.1. Evaluation of the treatments at the molecular level

Our data analysis approach was applied to evaluate at the molecular level the treatment of a secondary effluent using using different technological approaches based on advanced oxidation processes with UV-254nm: (i) UV/ $\text{K}_2\text{S}_2\text{O}_8$, where oxidation takes place mainly following the initial formation of sulfate radicals, (ii) UV/ KHSO_5 , where oxidation begins with the formation of both sulfate and hydroxyl radicals and (iii) UV/ H_2O_2 , when only the formation of hydroxyl radicals takes place initially.

We identified significant changes in DOM during wastewater treatment. Between 5000 and 7000 peaks corresponding to specific substances were detected in the samples belonging to the different treatments. The average and median mass of the substances that were present decreased in the following order: Initial > UV > UV/ $\text{H}_2\text{O}_2 \approx \text{UV}/\text{K}_2\text{S}_2\text{O}_8 \approx \text{UV}/\text{KHSO}_5(0.4\text{mM}) > \text{UV}/\text{KHSO}_5(0.8\text{mM})$. This is due to a higher degradation capacity of the combination of UV with the different oxidants in relation to the only use of UV. The more degradation, a higher number of substances fall below 100 Da (the lower limit with our instrumental conditions) causing the increase of the median mass. This is in accordance with the fact that the total number of peaks decrease in the opposite direction for the same reason.

Regarding the chemical profile, we calculated molecular formulas for all the features (following strict criteria but also being aware of a certain degree of error) no changes were observed in the average number of hydrogen, carbon or nitrogen atoms in the determined compounds/molecular formulas. On the contrary, we observed a significant increase in the number of oxygen

atoms (and the O/C ratio) in the order: Initial $\approx \text{UV} < \text{UV}/\text{H}_2\text{O}_2 < \text{UV}/\text{K}_2\text{S}_2\text{O}_8 \approx \text{UV}/\text{KHSO}_5(0.4\text{mM}) < \text{UV}/\text{KHSO}_5(0.8\text{mM})$.

By means of methods based on the isotopic pattern, the addition of sulfur atoms during the studied treatments was also evaluated. A significant increase of sulfur atoms was observed in the samples treated with $\text{K}_2\text{S}_2\text{O}_8$ and KHSO_5 , indicating that the presence of sulfate radicals gives rise to additions of this element in formed transformation products. The concentration of these radicals also influences as it is deduced by the significant increase in the number of sulfur atoms obtained with UV/ KHSO_5 at 0.4mM and 0.8mM, respectively. The increase of oxygen that is observed in the processes that involve $\text{SO}_4^{\bullet-}$ is linked to the fact that when a molecule is sulfonated, automatically oxygen and O/C increase.

3.2 Suspect screening of contaminants of emerging concern

Suspect analysis of 40,053 micropollutants was conducted in the initial and the treated samples. Preliminary results showed that hundreds of compounds were tentatively identified in the initial water. For most of these compounds their intensity decreased (or completely disappeared) when AOPs were applied. The decrease in intensity was more pronounced for treatments involving $\text{SO}_4^{\bullet-}$. However, It is still necessary a detailed evaluation for each compound in order to make an assessment from the mechanistic point of view.

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References

- Alygizakis N.A., Gago-Ferrero P., Hollender J., Thomaidis N.S. (2019a). Untargeted time-pattern analysis of LC-HRMS data to detect spills and compounds with high fluctuation in influent wastewater *Journal of Hazardous Materials* **361**: 19-29.
- Gago-Ferrero P., Schymanski E.L., Bletsou A.A., Aalizadeh R., Hollender J., Thomaidis N.S. (2015) Extended suspect and non-target strategies to characterize emerging polar contaminants in raw wastewater with LC-HRMS/MS *Environmental Science and Technology* **49**: 12333-12341.
- Alygizakis N.A., Oswald P., Thomaidis N.S., Schymanski E.L., Aalizadeh R., Schulze T., Oswaldova M., Slobodnik J. (2019b) NORMAN digital sample freezing platform: A European virtual platform to exchange liquid chromatography high resolution-mass spectrometry data and screen suspects in “digitally frozen” environmental samples *Trends in Analytical Chemistry* **115**: 129-137.