Method Development for Quantification of Nitrogenous Degradation Products after Wet Air Oxidation for Hospital Effluents and Toxicity Evaluation

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1. Introduction

Pharmaceutical compounds are emerging contaminants that can have harmful effects on biota. Indeed, the latter can contribute to an increase in oxidative stress in biota[1] or a decrease in the production of gametes in fish[2]. After ingestion, non-metabolized compounds are excreted through stools or urine, thus ending up in municipal wastewaters. Currently, conventional wastewater treatment plants are not designed to remove pharmaceutical compounds and they are released into the environment. Among the diverse sources of pharmaceuticals in the environment, hospital effluents are one of the most important since they generally contain high loads of pharmaceutical compounds. It is in this perspective that it becomes necessary to study different methods to remove these pharmaceutical compounds from effluents. Multiple advanced oxidation processes (AOP) are commercially available[1] and wet air oxidation (WAO) is of interest since it only uses water in subcritical conditions and air as oxidant. WAO could be applied as a pre-treatment on hospital effluents before they reach wastewater treatment plants. However, a previous study demonstrated that WAO treatment of hospital effluents increases their toxicity towards the crustacean *Daphnia magna*. In that study, the authors observed that median effective concentration (EC_{50}) of untreated effluents was 72 ± 28% and that toxicity increased after 10 minutes of WAO treatment at 290 °C (EC_{50}=39 ± 12%)[3]. A subsequent study demonstrated the presence of four organic acids (acetic acid, formic acid, glycolic acid, and succinic acid) following WAO of these effluents and they represented between 35% to 46% of the residual chemical oxygen demand (COD). Therefore, between 54% to 65% of the residual COD has not been identified yet[4]. In light of these results, it becomes essential to develop a method for quantifying small nitrogenous compounds that may represent a significant portion of the unidentified COD and to measure the toxicity of small organic acids.

The first objective of the present study is to assess the contribution of small organic acids to the toxicity observed in *D. magna* crustaceans. The second objective is to develop a method for the quantification of nitrogenous transformation products following WAO treatment of hospital effluents and assess their toxicity.

2. Materials and Methods

For the toxicity assessment, the *Daphnia magna* acute immobilization bioassay was applied according to the OECD Guideline 202[5] with a few modifications (e.g. daphnids obtained from ephippia were used instead of individuals from a stock). Immobilization assays after 48 hours of exposure to the samples were performed to evaluate the acute toxicity of WAO treated effluents related to detected compounds.

Two methods were developed to quantify nitrogenous transformation products previously identified in the literature as compounds possibly generated during WAO[6-10]: one using gas chromatography quadrupole mass spectrometry (GC-QMS) and the other liquid chromatography-triple quadrupole mass spectrometry (LCQqQMS). Eight small amines (methylamine, ethylamine, dimethylamine, diethylamine, glycine, 2-aminophenol, 4-aminophenol and 4-aminobenzyl alcohol)[6-9] were analyzed by LC-QqQMS using electrospray ionization in the positive mode and dansyl-chloride (DNS-Cl) derivatization prior analysis. 4-aminobenzoic acid[10] was also analyzed by LC-QqQMS but without prior derivatization. An Acquity HSS-T3 column was used, mobile phase A was water and mobile phase B was methanol, both with 0.2% formic acid as an additive. Five volatile or semi-volatile compounds, that can't be derived with DNS-Cl (acetamide, aniline, methenamine, nitrobenzene, nitrosobenzene)[6, 9], were analyzed using GC-QMS using a fused silica

column Agilent HP-5MS Ultra-Inert. The carrier gas was helium and the ion source used was electron ionization.

3. Results and discussion

Test compounds (acetic acid, formic acid, glycolic acid, and succinic acid) were identified and quantified in a previous study (42.8 mgL^{-1} of acetic acid, 16.53 mgL^{-1} of formic acid, 3.71 mgL^{-1} of glycolic acid, and 6.49 mgL^{-1} of succinic acid, after 15 minutes of treatment at 290 °C)[4]. Bioassays with solutions containing a mixture of these organic acids quantified in the WAO treated hospital effluents were carried out. The EC_{50} of this mixture was 22 ± 8%. On the other hand, when the mixture's pH was maintained at 7 (close to the pH of hospital effluents treated by WAO), using a carbonate buffer, no toxicity was observed. These observations make it possible to conclude that transformation products, other than organic acids quantified previously, contribute to the effluents treated by WAO toxicity.

Preliminary results for method development indicate that the LC-QqQMS method detection limits are between 0.04 µg/L and 20 µg/L, depending of analytes. For the GC-QMS method, detection limits are lower than 15.1 mg/L. Method validation results as well as the analysis of spiked wastewater treated by WAO will be presented.

4. Conclusions

The mixture of major organic acids detected in hospital effluents treated by WAO is not toxic *to D. magna*. Therefore, the quantification of nitrogenous transformation products formed after WAO treatment of hospital wastewaters is essential to identify a potential source of toxicity and to evaluate the threat to biota after conventional wastewater treatment. Then, it will be a question of developing a plan to eliminate these compounds during the WAO treatment.

5. References

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