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Suspect screening of emerging pollutants and their major transformation products in wastewaters treated with fungi by liquid chromatography coupled to a high resolution mass spectrometry

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ABSTRACT

A new approach for the screening of 33 pharmaceuticals and 113 of their known transformation products in wastewaters was developed. The methodology is based on the analysis of samples by liquid chromatography coupled to high resolution mass spectrometry (HRMS) followed by data processing using specific software and manual confirmation. A home-made library was built with the transformation products reported in literature for the target pharmaceuticals after treatment with various fungi. The method was applied to the search of these contaminants in 67 samples generated along treatment of wastewaters with white-rot fungus *Trametes versicolor*. The screening methodology allowed the detection of different transformation products (TPs) generated from degradation of parent compounds after fungal treatment. This approach can be a useful tool for the rapid screening and tentative detection of emerging contaminants during water treatment in both full and batch-scale studies when pure standards are not available.

1. Introduction

The removal of organic compounds in conventional wastewater treatment plants (WWTPs) is still a challenging task. Therefore, the use of green bioremediation technologies to mitigate the pollution not only in raw wastewater (WW) but also after conventional wastewater treatment (where only incomplete elimination of pollutants is achieved) is of high interest [1]. Bioremediation based on fungi is particularly promising in the decontamination of water streams from trace organic contaminants (*e.g.* endocrine disrupting compounds, drugs or recalcitrant pharmaceuticals) because of the nonspecific nature of the ligninolitic enzymatic system, able to degrade xenobiotic compounds [2]. The main degradation mechanisms of these organisms include: (1) the extracellular oxidation by laccase and peroxidase enzymes and (2) the intracellular degradation involving for example, cytochrome P450 monooxigenases and nitroreductases, which allow either the catabolisation through oxidation and/or reduction or the conjugate formation by transferases (*e.g. O*-glucoside or *O*-glucuronide) [2]. These reactions can lead either to the complete mineralization of the compounds or just to the excretion of metabolites. Therefore, fungi can be considered for the treatment of pollutants that are inefficiently degraded by bacteria. Different authors have tested the efficiency of different fungi in the degradation of a wide range of compounds (Table S1), including recalcitrant organic pollutants [1,3–5] and also to decrease the risk associated with metals, metalloids and radionuclides by chemical modification or by influencing chemical bioavailability [2]. White-rot fungi (WRF), for example, have been used since 1980s [6] due to their capabilities for the removal of a wide range of xenobiotics and their use for bioremediation has increased during the last years [7]. Among this type of fungi, *Trametes versicolor* has been applied with successful results for the degradation of different pharmaceutical compounds [1,3,5,8–10], ibuprofen [3], carbamazepine [11], sulfonamides [12,13], X-ray contrast agent iopromide and the fluoroquinolone antibiotic ofloxacin [8], UV-filters [1,14,15] and polybrominated flame retardants [1].

Removal of many of these pollutants can be monitored along the fungal treatment using liquid chromatography coupled to mass spectrometry in tandem (LC–MS/MS) [1,8,10–15]. During the last years, the use of LC–MS/MS has increased because of its high

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sensitivity as well as selectivity for the measurement of organic pollutants in the environment through the so called target analysis (based on acquisition in selected reaction monitoring mode (SRM) in the mass spectrometer). It implies the search of thousands of known compounds that can be present in a sample and are compared with their pure standard [16–18]. However, from an environmental point of view, it is of high importance the detection of transformation products (TPs) of these pollutants, generated during removal treatments, which can be either more persistent or toxic than the parent compound [19,20]. Nonetheless, the assessment of removal and fate of TPs cannot be based on target analysis in most of the cases because there are no available standards. In this context, different technologies can be applied in order to identify these compounds such as nuclear magnetic resonance (NMR) [17] or high resolution mass spectrometry (HRMS) (*e.g.* time of flight or Fourier Transform mass spectrometers) [8,11–14]. HRMS allows performing the so-called "*suspect screening*", where public and home-made libraries containing a broad set of compounds suspected to be present in the sample are used [17,18,21–24] as a basis for the search methodology. This approach has been used by different authors (Table 1) although it requires several steps including data reduction, MS library search and further processing of MS and chromatographic data [25].

The main aim of this work was to develop an analytical method for the screening of selected emerging contaminants and their major transformation products generated during fungal treatment of different wastewaters. Screening methodology is based on LC coupled to a hybrid ion trap – Fourier Transform Orbitrap mass spectrometer.

2. Materials and methods

2.1. Fungi strains and chemicals

Trametes versicolor (ATCC#42530) information can be seen elsewhere [8,26–28].

All the solvents used during the studies were of the highest purity grade available. Formic acid (98%) was provided by Merck (Darmstadt, Germany). Acetonitrile and water (Optima™ LC–MS) were supplied by Thermo Fisher Scientific.

The calibration mixture used for high resolution mass spectrometry purposes was supplied by Thermo Fisher Scientific (LTQ ESI Positive Ion Calibration Solution and ESI Negative Ion Calibration Solution).

2.2. Water samples

Seven sets of wastewater effluent samples treated with the WRF *Trametes versicolor* were considered within the frame of this work. A total number of 67 samples were analyzed including samples before and along fungal treatment experiments. The samples consisted on: (i) 3 sets of hospital wastewaters (HS, HN1 and HN2) [26]; (ii) 2 sets of wastewaters from a campus university in Barcelona (Catalonia) (US and UN) [28]; and (iii) 2 sets of reverse osmosis concentrate (ROC) obtained from a pilot Reverse Osmosis filtration plant of urban WWTP effluent (RS and RN) [27]. A summary of samples considered is listed in Table 2 and more information can be seen elsewhere [26–28].

The samples were purified and extracted by solid phase extraction according to the methodology described elsewhere [8] before their analysis (see next Section 2.4).

2.3. Library for target chemicals and their major transformation products

A home-made library was built collecting the information reported in several studies about the degradation of selected contaminants and corresponding generation of the TPs during fungi water treatments. The library includes 146 compounds: 33 pollutants and their major transformation products and metabolites (113) (Table 3). The library includes the molecular formula of non-ionized compounds, the molecular formula of ionized compounds and their corresponding exact monoisotopic mass. This list was used to create an acquisition method into the high resolution mass analyser in order to screen for selected analytes, as it is described in detail below (see Section 2.4).

2.4. Suspect screening for selected compounds

The analysis was carried out by a LC system coupled to a hybrid linear ion trap-Fourier Transform Mass Spectrometry Orbitrap analyser (LC-LTQ Orbitrap). Aria TLX-1 chromatographic system (Thermo Fisher Scientific) was used for separation purposes. This system comprised a PAL auto sampler and two mixing quaternary pumps (eluting pump and loading pump). The entire system was controlled *via* Aria software, version 1.6, under the Xcalibur 2.2 software. The system was equipped with a Hypersil GOLD PFP analytical column (100 mm × 2.1 mm; 1.9 μm particle size; Thermo Fisher Scientific, Franklin, MA). This column was used because of its capabilities in the separation of isomeric compounds [29]. 10 μL of sample was injected and the analytes were separated by the LC gradient with water (0.1% formic acid) and acetonitrile for compounds to be analyzed under positive ionization conditions and water (pH 8.5 with a drop of ammonia) and acetonitrile for the ones to be analyzed under negative conditions (Table 4). The total run time for each injection was of 7 min for positive ionization and 10 min for negative ionization.

The LTQ-OrbitrapVelos™ (Thermo Fisher Scientific) was equipped with a diverter valve and an Electrospary Ionization source (ESI). Samples were analyzed in separate runs: one under positive and another under negative ionization polarity. Samples were analyzed in separate runs: one under positive and another under negative ionization polarity. The divert valve was switched in different positions along chromatographic run: under positive mode from 0 to 1.5 min the flow was discharged to the waste, from 1.5 to 6.5 min the valve was switched to injection mode and switched again to the waste from 6.5 to 7 min. In the case of negative ionization, the valve worked from 0 to 1.5 min on waste position, from 1.5 to 7.75 min the valve was switched to injection mode and, finally, from 7.75 to 10 min the flow was discharged again to the waste. Mass calibration was performed prior to every sample run with LTQ ESI Positive or Negative Ion Calibration Solution (Thermo Fisher Scientific) and an optimal mass accuracy was assured, with mass errors within ±2 ppm. The ionization voltage was set at 3.5 kV for positive and 3 kV for negative modes with the sheath gas flow at 40 for positive and 35 for negative (arbitrary units), auxiliary gas flow at 20 for positive and 10 for negative (arbitrary units), S-Lens RF level at 69%, and the capillary temperature and the source heater temperature at 350 °C and 300 °C, respectively, in positive mode, and both of them at 450 °C in negative ionization mode.

The samples were acquired using two different acquisition methods in parallel: (1) the first one through full scan within a mass-to-charge (m/z) range from 100 to 800 m/z at a resolving power of 60,000 (full width at half maximum; FWHM) and (2) by data-dependent analysis at a resolving power of 7500 (FWHM) through the MS^2 fragmentation of the ions from the list built by the authors (Section 2.3). During this last acquisition method the ions were isolated in the ion trap with a width of 2.0 Da, a collision induced dissociation activation type (Q = 0.250 and an activation time of 30 ms) and normalized collision energy (35). All the system was controlled by Xcalibur 2.2 software.