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Application of a multivariate analysis method for non-target screening detection of persistent transformation products during the cork boiling wastewater treatment

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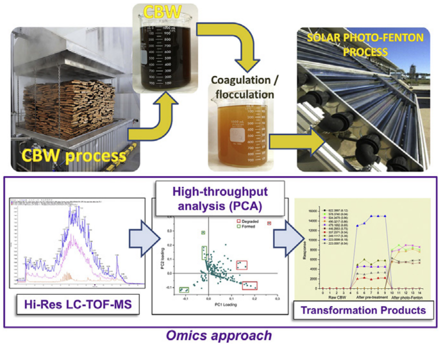
HIGHLIGHTS

- The complex mixture of CBW was fractionated in various groups with different polarities.
- A non-Target screening methodology was used to detect persistent substances during the treatment line of a complex mixture.
- 48 masses were classified as transformation products using the described methodology.

GRAPHICAL ABSTRACT



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ABSTRACT

Cork boiling wastewater is a very complex mixture of naturally occurring compounds leached and partially oxidized during the boiling cycles. The effluent generated is recalcitrant and could cause a significant environmental impact. Moreover, if this untreated industrial wastewater enters a municipal wastewater treatment plant it could hamper or reduce the efficiency of most activated sludge degradation processes. Despite the efforts to treat the cork boiling wastewater for reusing purposes, is still not well-known how safe these compounds (original compounds and oxidation by-products) will be. The purpose of this work was to apply an HPLC-high resolution mass spectrometry method and subsequent non-target screening using a multivariate analysis method (PCA), to explore relationships between samples (treatments) and spectral features (masses or compounds) that could indicate changes in formation, degradation or polarity, during coagulation/flocculation (C/F) and photo-Fenton (PhF). Although, most of the signal intensities were reduced after the treatment line, 16 and 4 new peaks were detected to be formed after C/F and PhF processes respectively. The use of this non-target approach showed to be an effective strategy to explore, classify and detect transformation products during the treatment of an unknown complex mixture.

1. Introduction

Industrial effluents pose a serious risk to environment and human health and their remediation is impeded by the presence of toxic and/

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or bio-recalcitrant organic compounds. Selection of the best treatment option is a complicated task, even when the compounds to be treated are known. However, if the initial compounds are unknown or in a complex matrix the difficulty grows exponentially. Thus, we are left with the basic water treatment parameters and a paramount analytical challenge. The treatment and reuse of a complex industrial wastewater, such as the one generated by cork processing, is a good example of this problem. Cork boiling wastewater (CBW) contains undesirable corkwood extracts, phenolic acids, 2,4,6-trichloroanisol, pentachlorophenol, and so forth, as well as a high organic load not easily removed by conventional treatments (De Torres-Socías et al., 2013). Despite their characteristics, these effluents are usually discharged into the environment without any previous treatment or directly into municipal wastewater treatment plants (MWTPs), lowering the efficiency of active sludge, and thereby causing a real environmental impact on surface and underground aquatic/soil systems (Beltrán de Heredia et al., 2004). Since conventional biological processes cannot satisfactorily deal with cork boiling wastewater, one feasible option is the use of Advanced Oxidation Processes (AOPs). Recently, specific AOP treatment lines have been described that reduce the mostly organic load (De Torres-Socías et al., 2014; Pintor et al., 2011; Vilar et al., 2009; Ponce-Robles et al., 2017). However, since CBW is a very complex mixture, nothing is known about the substances that could be generated during these treatments of the by-products.

Alternative strategies for determining non-target (unknown) compounds and degradation products in complex samples have been developing recently. Advances in high resolution mass spectrometry (HRMS) and ultra-HRMS, such as triple quadrupole coupled with time-of-flight-MS, orbitrap-MS, Fourier transform-MS and others, have enabled the accurate detection of unknown compounds in different matrices with enormous sensitivity and resolution (Ross et al., 2012). Nevertheless, a major issue is how to manage and process the massive amount of information that these methods produce per sample. To solve this high-throughput data analysis and subsequent multivariate analysis needs to be applied to process all the chromatographic and accurate mass information, transform into a lower-dimension data set from which conclusions could be extracted. Such non-targeted approaches with small molecules are common in metabolomics, and are useful exploration tools (Sleighter et al., 2010; Ross et al., 2012; Chen et al., 2015; Neumann and Böcker, 2010; Theodoridis et al., 2008).

Metabolomics is defined as the study of the overall metabolite profiles in a biological system under specific conditions. Translating this into other fields with the omics label, it can be defined as an analysis that attempts to understand all or the majority of small-molecules in a global system while exposed to a specific set of conditions (Worley and Powers, 2013; Hassani et al., 2010). The corner stone of every "-omics" application is the high-throughput data analysis using different types of multivariate analysis, such as Principal Component Analysis (PCA), which is an orthogonal transformation of multivariate data used for exploratory analysis by extracting and displaying systematic variations. PCA is used to find abundance levels across runs to determine the principle axes of abundance variation. As a result compounds from various experiments can be viewed in two-dimensional spaces in such a way that compounds that change in a similar way or under the same conditions appear together on the PCA loading plot. Thus, this can be used to determine which compounds are the most important to distinguish a particular sample group. PCA represents a useful analysis for display, exploration and classification purposes as it provides a low-dimension projection of the data by reducing the dimensions of a dataset (Hassani et al., 2010; Antignac et al., 2011; Masiá et al., 2014). This approach and its combination with other analysis has been described for the treatment of different waters and wastewaters in recently published works (Kiss et al., 2018; Parry and Young, 2016; Soulier et al., 2016; Prasse et al., 2015).

This paper describes the use of a metabolomics exploration approach for the high-throughput screening of LC-HRMS data acquired before and after CBW treatment, for a broader understanding of the treatment, the fate of unknown compounds in a complex mixture, and the impact caused by each process in the treatment line.

2. Materials and methods

2.1. Cork boiling wastewater and reagents

Real CBW was collected in 200-L barrels at a cork-processing plant located in San Vicente de Alcantara (Extremadura, Spain). Barrels were shipped to the Plataforma Solar de Almeria (PSA) (Tabernas, Spain), and stored upon receipt in a cold room (4 °C) until use.

For flocculation/coagulation and solar photo-Fenton processes, anhydrous ferric chloride ($FeCl_3$) and reagent-grade hydrogen peroxide (30% w/v) were supplied by Panreac. The following reagents were used in sample preparation and LC-MS analysis: reagent-grade dichloromethane (99.8%) and hexane (97%) purchased from Sigma-Aldrich (Steinheim, Germany), HPLC-grade methanol (99.9%), acetonitrile (AcN) and formic acid (98%) acquired from Fluka (Buchs, Germany).

Cartridges used for the SPE and SSPE steps were octadecyl silica (C18) sorbent (500 mg, 6 mL) from Sartorius Stedim Biotech GmBH, Goettingen (Germany) and a styrene-divinylbenzene sorbent Lichrolut® EN (200 mg, 6 mL) from the Millipore Corp, Darmstadt (Germany).

2.2. Cork boiling wastewater treatment

CBW was treated with a combination of an optimized physicochemical pretreatment using 0.5 g/L of anhydrous $FeCl_3$ followed by solar photo-Fenton oxidation. Details of this treatment line are reported elsewhere (Ponce-Robles et al., 2017).

A physicochemical pretreatment is usually applied to eliminate suspended solids and decrease turbidity to increase the following photochemical oxidation treatment efficiency. Selected raw wastewater flocculation/coagulation conditions developed at lab scale were therefore scaled up in a flocculation/filtration pilot plant. After that, solar photo-Fenton experiments were performed in a compound parabolic collector (CPC) solar pilot plant with a temperature control system designed for solar photocatalytic applications. (Malato et al., 2009). Samples were taken during the CBW treatment line for LC-HRMS analysis and studied from a metabolomics approach.

2.3. Sample treatment

The complex nature of CBW (as well as the partially treated samples) makes it necessary to apply a signal cleanup and enhancement step. Thus, in order to fractionate the analytes into different polarity groups and eliminate possible matrix effects, a modified sequential solid phase extraction (SSPE) method was applied based on one previously described for analysis and characterization of complex industrial wastewater (Castillo et al., 1999. Prior to extraction samples were filtered through a 0.22-μm cellulose nitrate membrane filter. Then 200-mL aliquotes were extracted using an octadecyl silica (C18) cartridge (500 mg, 6 mL, Sartorius stedim Biotech GmBH, Goettingen, Germany) for nonionic organic compounds in series with a styrene-divinylbenzene LiChrolut EN cartridge (200 mg, 6 mL, Merck-Millipore) for nonionic polar compounds. Samples were loaded in the C18 cartridge at 5 mL/min and the residual water was acidified to pH 3 and percolated in the LiChrolut EN cartridge. Different elution solvents were used for desorption of the two cartridges. In order to obtain fractions with different polarities, C18 cartridges were eluted with 2 × 5 mL of hexane (Fraction A), 2 × 5 mL of dichloromethane/hexane (4:1, v/v) (Fraction B) and 2 × 5 mL or MeOH/dichloromethane (9:1, v/v) (Fraction C). The LiChrolut EN cartridge was eluted with 2 × 5 mL of MeOH (Fraction D). All fractions were individually evaporated with a gentle stream of N_2 and reconstituted to a final volume of 1 mL in the appropriate mobile phase prior to LC-MS analysis. Samples from Raw CBW, pretreated (C/F) CBW and CBW treated by solar photo-Fenton were extracted following this procedure (Ponce-Robles et al., 2017).