“WASTEWATER TREATMENT BY SOLAR IRRADIATION AND OZONE BASED PROCESSES. EVALUATION BY ADVANCED ANALYTICAL AND MICROBIOLOGICAL TECHNIQUES”

SUMMARY

Usually, industrial effluents pose a serious risk to the environment and human health and their remediation is impeded by the presenceof toxic and bio-recalcitrant organic compounds. Selection of the best treatment option needs a detailed study based on different analytical techniques and bioassays, that permits also a consistent comparison and/or combination of different treatment processes. In this PhD work, the problem of water resources contamination by toxic and biorecalcitrant compounds has been studied, based on a case study of a complex industrial wastewater coming from boiling natural cork prior to its commercialization.

Conventional analyses (DOC, COD, turbidity, total nitrogen, dissolved iron, total polyphenols content, TSS, VSS), advanced analytical tools (ionic chromatography, high resolution liquid chromatography with UV-Diode array detection or coupled to mass spectrometry, etc.) and microbiological techniques (plate count, opticalmicroscopy, DNA extraction, qPCR or metagenomic techniques) as well as biodegradability and toxicity (acute and chronic) tests (Respirometry, BioFix®Lumi, Artoxkit, Protoxkit or Daphtoxkit) have been applied in order to get an exhaustive characterization of the selected wastewater, as well as the evaluation of the proposed treatments. The development of specific protocols and techniques has been faced with the aim of being the basis for future advanced studies of the treatment, evaluation and analysis of complex wastewater.

Advanced analytical methodologies (high sensitivity and selectivity) have been developed, enabling qualitative and quantitative analysis for a detailed industrial wastewater characterization. The studies have been focused on target and non-target contaminants screening, using chromatographic techniques coupled to mass spectrometry. With this objective, an analytical method using liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed for the extraction and quantification of target compounds (pesticides) in sewage sludge from industrial activities. Pesticides have been recognized as relevant environmental contaminants. Although agricultural practices have been traditionally considered the main source of water contamination by pesticides. In recent years the attention has been paid in other nonagricultural sources, such as urban wastewater treatment plants. In addition to their reported presence in treated effluents, which contribute to their spread in the aquatic environment, some pesticides tend to accumulate in sewage sludge during wastewater treatment.

The developed method was based on the application of QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) protocol for the solid phase of the sludge and a solid phase extraction (SPE) for the supernatant, both followed by an LC-MS/MS

analysis. QuEChERS method emerged in 2003 in the area of multiresidue pesticide analysis in food and agricultural products becoming nowadays the most recognized and extensive methodology used in this field. Because of its proven ability to efficiently extract organic compounds of a wide range of polarities, the QuEChERS method has recently expanded to environmental applications by analyzing not only pesticides but also other compounds of environmental concern in soil, sediments and water. These applications confirm it as a powerful multi-residue/multi-matrix technique. Both methods (SPE and QuEChERS) were satisfactorily validated for most of the compounds investigated, showing very often recoveries higher than 80%. Limits of quantification were lower than 40 ng/L in the aqueous phase and below 40 ng/g in the solid phase for the majority of the analytes. The method was successfully applied for the quantification of a group of ten selected compounds in sewage sludge coming from an agro-food industry and an urban WWTP. Results demonstrated that high concentration of pesticides can accumulate in the industrial sludge.

Advanced analytical methodologies have also been applied to high complex and unknown nature cork boilingwastewater (CBW). Treatment of CBW and other industrial wastewaters need the identification of organic constituents that can influence the applied treatment line, task very often not possible using traditional searching MS databases. As in other fields of knowledge, the application of advanced searching techniques such as metabolomics can be an alternative. In this work, an advanced analytical method has been developed through the use of an HRMS (High Resolution Mass Spectrometry) system for the detection of compounds showing significant changes during the treatment line (appear, disappear, etc.). The methodology consisted in a sequential solid phase extraction (SSPE) step followed by an analysis in a Q-TOF system. SSPE consisted of using an octadecylsilica (C18) cartridge for nonionic organic compounds in series with a styrene-divinylbenzene cartridge for nonionic polar compounds in order to fractionate the analytes into different polarity groups and eliminate possible matrix effects. The results were analyzed using multivariate analysis software, finding a number of significant masses that presented a well-defined behavior in the process. So, the use of this non-target approach showed an effective strategy to explore, classify and detect unknown compounds in different types of complex wastewater.

As previously indicated, a strategy that allows the effective treatment of complex wastewater generated in the cork processing industry has been developed. Cork industry makes an important contribution to the economy of the countries surrounding the Mediterranean Sea. Cork stoppers are the main product of cork industry and one of the key processing steps in cork industry is the boiling of cork in water. This operation deals with cleaning/disinfection of the material and leads to an increase of volume and improves its workability. Boiling step is usually carried out for at least 1 hour at temperature near 100ºC. A second boiling operation is sometimes required after stabilization of the boiled cork. This cork-processing step gives rise to high volumes (140-1200 L/ton of cork) of so named cork boiling wastewater (CBW), with a high organic load of 1000-4000 mg/L. The composition of CBW depends on the type of cork and the number of boiling cycles, which also differs from company to company. CBW is characterized by an acidic pH and substantial content of phenolic compounds and tannins. This residue has a polluting potential and it is a serious environmental hazard due to the toxicity and biorecalcitrant nature of its organic content. Despite it, these effluents are usually discharged into the environment without any treatment or into municipal wastewater treatment plants (MWTPs), lowering the efficiency of activated sludge, and thereby causing a real environmental impact on surface and underground aquatic/soil systems. Therefore it is necessary to search for efficient treatment solutions. In this work, combination of physical-chemical treatments (coagulation/flocculation), advanced oxidation processes (ozonation and solar photo-Fenton) and biological treatments (activated sludge) have been studied.

First of all, the adaptation of an aerobic biological system for the treatment of raw CBW was studied. The main objective was to evaluate the effectiveness of an adapted biological system as well as clearly identify its limitations to face CBW decontamination. Biological assays simulating a Batch aerobic (Bio)Reactor were performed in a cylindrical reactor tank provided with a porous air diffuser placed at the bottom of the reactor. Air was directly injected in order to keep dissolved oxygen concentration close to saturation values, providing also a correct agitation of the whole system. With the aim to determine changes in microbiological population present in conventional activated sludge once fed with CBW, microbial community variation was evaluated using optical microscopy, plate count, DNA extraction, qPCR and metagenomics. Other macroscopic parameters also determined were DOC, COD, TSS, VSS, ionic content or chronic toxicity (using respirometric assays). The variation of microorganisms species throughout the biological adaptation showed a diminution of the total DNA concentration, general bacteria (16S) and amino oxidant bacteria (AOB) (jointly with a reduction of TSS and VSS), being worthy to mention the appearance of filamentous bacteria after 31 hours of contact with CBW. Other microbiological species died while a few others arose, indicating a possible adaptation of the biological system, accompanied of a DOC reduction. Nevertheless, the important increase of total nitrogen and other ions (phosphate and potassium) concentration coming from the intracellular fluid suggested cell membranes breakage and therefore a malfunction of the adapted biological system. This effect, accompanied of chronic toxicity measured by respirometric assays (increasing from 23% to 73%), confirmed the non-viability of the adaptation of activated sludge to raw CBW. These results demonstrated the added value of complementing classical microbiological analysis (plate counting and optical microscopy) with new advanced techniques such as qPCR and metagenomics. In addition, it has been concluded that a wide microbial analysis approach is crucial to predict the behavior of new biological systems, specifically created or adapted from conventional activate sludge, in order to face the remediation of complex/industrial wastewaters such as CBW.

After demonstrating the impossibility of adapting a biological system to this type of industrial wastewater (mainly due to the development of chronic toxicity), it was proposed to partially treat the wastewater by applying different AOPs (solar photo-Fenton and ozone) to increase their biocompatibility for subsequently complete the treatment in an also adapted post-aerobic biological system. Prior to the application of these AOPs, a physic-chemical pre-treatment using coagulation-flocculation (C/F) was optimized. This pre-treatment aimed to stabilize industrial wastewater, to eliminate suspended solids (accompanied to a small elimination of dissolved organic carbon), as well as to decrease turbidity. The C/F treatment would increase the efficiency of the subsequent photochemical oxidation treatment, mainly due to the decrease of suspended solids and turbidity. C/F study included different commercial coagulants and flocculants as well as two coagulants commonly used for industrial wastewater pre-treatment, $FeCl_3$ anhydrous and Ca $(OH)_2$. The results indicated that the most effective treatment was $FeCl_3$ at 0.5g/L, achieving a compromise between high efficiency in turbidity and DOC removal and reagent consumption. After pre-treatment with $FeCl_3$, a residual concentration of Fe (III) remained in solution which would be beneficial for a subsequent photo-Fenton process. However, for the application of an ozonation treatment, lower efficiency could be observed due to the formation of very stable iron-organic complexes hardly to oxidize by ozone. It was therefore decided to select $FeCl_3$ at 0.5 g/L for photo-Fenton processes and the commercial coagulant ECOTAN BIO at 1g/L for ozonation purposes.

Solar photo-Fenton experiments were performed in a compound parabolic collector (CPC) solar pilot plant with a temperature control system specially designed for solar photocatalytic applications installed at Plataforma Solar de Almería (Spain). The total irradiated area is 4.16 m^2, total illuminated volume inside the absorber tubes of 44.6 L (V_i) and a total volume of 75 L (V_T). Ozonation system was an Anseros PAP-pilot (Anseros Klaus Nonnenmacher GmbH, Germany) that provide up to 3.6 g ozone/h. The ozonation contact column has a total volume of 20 L. The system operates in batch mode and it is equipped with two non-dispersive UV analysers to measure inlet and outlet ozone concentration in gas phase, a flow meter for inlet air regulation and two oxygen generators from air. Global results showed less efficiency in the ozonation processes compared to solar photo-Fenton processes even when H_2O_2 was added to ozone test to improve hydroxyl radicals' generation. High acute toxicity values persisted at the end of ozonation in comparison with photo-Fenton experiments. According with this, solar photo-Fenton treatment was selected as the best option for the treatment of CBW after a proper C/F pre-treatment.

The final step in the proposed treatment line consisted in the adaptation of an aerobicbiological treatment for CBW partially treated by C/F and solar photo-Fenton process. Assays were performed simulating a Batch aerobic (Bio)Reactor in a cylindrical reactor tank as previously described. The effectiveness of the biological treatment was also evaluated by microbiological techniques (optical microscopy, plate count, DNA extraction and qPCR) and toxicological techniques. In this case, the decrease of TSS and VSS jointly with a significant release of ions evidenced the bacterial flocs broke-up and the destabilization of the biological system. DNA concentration was always in concordance with the decrease of TSS and VSS. Optical microscopy showed complete deflocculation, with damaged flocs and died microbial species. So, global results showed again the impossibility of adapting a biological system to this specific partially oxidized industrial wastewater, and therefore, the need to apply AOPs till the complete mineralization of CBW organic content.

In general, the different methodologies described in this work are promising for their application in the study of complex and biorecalcitrant industrial wastewaters, giving a broad perspective on how to approach these problems making aholistic and multidisciplinary use of knowledge based on advanced analytical chemistry, microbiology and chemical engineering.