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Efficiency evaluation of a membrane bioreactor to remove emerging pollutants from a hospital effluent based on the combined use of *in vitro* and *in vivo* bioassays and targeted chemicals analyses

Nicolas Creusot¹, Claire Albasi³, Nicolas Manier¹, Emmanuelle Maillot-Maréchal¹, Pascal Pandard¹, Jean-Marc Porcher¹, Caroline Martin², Isaribel Quesada³, Hélène Budzinski², Selim Aït-Aïssa¹

¹ INERIS, Institut National de l'Environnement Industriel et des Risques, Verneuil en Halatte, France;

² EPOC/LPTC, UMR 5805-CNRS, Université Bordeaux I, Talence, France ;

³ Université de Toulouse; INPT, UPS Laboratoire de Génie Chimique, Toulouse, France

E-mail contact: nicolas.creusot@ineris.fr

1. Introduction

Hospital effluents have been identified as an important source of various chemical classes including pharmaceuticals, hormones, transformation products and metabolites, diagnostic agents, detergents and disinfectants, among others. Because of potential associated risk for humans and non-targeted species (e.g. genotoxicity, endocrine disruption), elimination of such potential bioactive compounds has become an increasing issue of concern [1]. Conventional treatments designed for removing carbon, nitrogen and phosphorous compounds is often insufficient to eliminate all the active micropollutants. In particular, chemical diversity of endocrine disrupting compounds (EDCs) limits their removal. Therefore, new processes have emerged these last years, including membrane bioreactor (MBR) that seems to guarantee higher removal efficiencies for most compounds including EDCs [2].

Removal efficiency of active pollutants is mostly evaluated using targeted chemical analyses. However, given the diversity and trace level concentrations of known and unknown active compounds that are present in sewage effluent, it seems impossible to analyse all of these chemicals. To address this challenge, bio-analytical tools are increasingly used because of their capacity to detect and quantify (i.e. Toxi-Equivalent) all compounds presenting similar mechanism of action (e.g. binding to nuclear receptors). In addition, *in vivo* bioassays can be deployed for better evaluation of integrative effects on whole organisms at different trophic levels [3].

In the present study, we aimed to evaluate the efficiency of an MBR pilot for the elimination of (1) EDCs using a battery of *in vitro* bioassays (i.e. reporter cell lines) covering a panel of endocrine activities (2) various pharmaceuticals classes using LC-MS-MS analysis, in hospital effluents. In parallel, standardized ecotoxicological tests were performed to characterize ecotoxic potency of treated and non treated effluents (i.e. acute and chronic toxicity). All these tools were deployed on several sampling campaigns in order to evaluate potential seasonal fluctuation in terms of both toxicity and identity of bioactive chemicals.

2. Materials and methods

Sampling and extraction

Sampling campaigns were performed in 2013 based on 24 h average sampling. For *in vitro* bio-analytical tools and chemical analyses, non-treated and MBR-treated effluents were filtered (GF/C, 1.2 µm) and then extracted using solid phase extraction based on Oasis-HLB cartridges (200mg, 6cc) with a sequential elution (10 mL of dichloromethane, 10 mL dichloromethane:methanol (50:50) and 10 mL of methanol). Extracts were evaporated to dryness and redissolved in 1 mL of acetonitrile. SPE extracts were resuspended in DMSO for reporter gene based cell lines assays. Standardized ecotoxicological tests were performed on settled effluents.

Bioassays

Bioassays	Organism	Endpoint
MELN	human cell line (MCF-7)	Activation of estrogen receptor (ER)/Luciferase
MDA-kb2	human cell line (MDA-MB-53)	Activation of androgen/glucocorticoid receptors (AR, GR)
HG5LN-hPXR	human cell line (HeLa)	Activation of Pregnane X receptor (PXR) /Luciferase
PLHC-1	fish cell line	Activation of Aryl hydrocarbon receptor (AhR) /EROD
Bacteria luminescence inhibition test (ISO 11348-3)	<i>Vibrio fischeri</i>	bioluminescence (30 min)
Algal growth inhibition test (ISO 8692)	<i>Pseudokirchneriella subcapitata</i>	Growth (cell number after 72 h)
<i>Daphnia</i> acute immobilization test (ISO 6341)	<i>Daphnia magna</i>	Mobility (48h)
<i>Ceriodaphnia</i> chronic toxicity test (ISO 20665)	<i>Ceriodaphnia dubia</i>	Reproduction, mortality (7/8 d)
Chronic rotifer toxicity testgrowth (ISO 20666)	<i>Brachionus calyciflorus</i>	Population growth (48h)

Table 1: *In vitro* mechanism based- and standardized ecotoxicity bioassays used in this study

Chemical analyses

Targeted chemical screening, based on LC-MS/MS system, was directed towards antibiotics, anti-inflammatory drugs, anticancer drugs, anti-depressants and disinfectants.

3. Results and discussion

All the investigated endocrine activities were detected in the non-treated effluent. Estrogenic and androgenic activities (10 ng E2-eq/L and 100 ng-DHT-eq/L range) were quite similar to those previously reported in other hospital effluents [2, 4] whereas GR (>100 ng-Dex-eq/L) and PXR-like activities (10 µg-SR-eq/L range) were close to those found in industrial effluents [5]. For several activities, up to 2-fold fluctuations between sampling campaigns were observed. After MBR treatment, an overall reduction of the endocrine activities (except for PXR) was noted. In addition, significant cytotoxicity was observed in the MDA-kb2 cell line; this was suppressed after MBR. Hospital effluents are known to contain some anticancer drugs acting as cytotoxic agents. Altogether, these results illustrate specific removal of such agents by MBR treatment while EDCs were only partly eliminated.

Chemical analyses revealed the occurrence of broad range of pharmaceutical classes including cytostatic anticancer drugs and antibiotics but also metabolites (e.g. oxazepam) in the non-treated effluents. Overall, concentrations were similar to those reported in other studies (e.g. 10 µg/L range for anti-cancer drugs ifosfamide, cyclophosphamide, methotrexate) [6, 7], except for very high amount of the antibiotic ciprofloxacin (up to 1mg/L). MBR treatment allowed total, partial or no removal of targeted pharmaceuticals classes.

Ecotoxicological tests showed significant effects at several trophic levels in the non-treated effluents. The low sensitivity of tests on rotifers and bacteria should be noticed compared with tests on microcrustaceans. Contrary to endocrine activities, there is no marked inter-campaigns variation of the ecotoxic effects suggesting that others compounds than EDCs contribute to this global toxicity. Finally, in the same way than endocrine activities, MBR treatment significantly reduces the toxicity in all the organisms.

4. Conclusions

Altogether, our results confirm the relevance of the combined use of biological and chemical analyses for an holistic characterization of hospital effluent contamination by a broad range of active chemicals. We show that hospital effluent is a source of high amount of EDCs presenting various activities, i.e. ER, AR and GR, but also of antibiotics, anticancer drugs, etc. We report also that this complex mixture of bio-active compounds leads to global toxicity for exposed organisms at different trophic level confirming hazard for aquatic ecosystems.

In addition, both qualitative and quantitative time-fluctuation observed of both chemical and toxicity data may render more difficult efficient removal. Nevertheless, one major outcome is the ability of MBR treatment to reduce both endocrine disrupting activity and global toxicity although some persisted after treatment. To tackle this issue, different tertiary treatments (i.e. UV vs fenton vs ozonation) are under investigation. In parallel, effect directed analysis (EDA) combining mechanisms based *in vitro* and *in vivo* tools, fractionation and high resolution mass spectrometry will be used in order to isolate and identify the EDCs that resist to treatments. First data on tertiary treatments and EDA will be presented.

5. References

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