UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Departamento de Ingeniería Química

Feasibility of membrane bioreactors for the removal of Pharmaceutical and Personal Care Products present in sewage

Memoria presentada por:

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Para optar al grado de Doctor por la Universidad de Santiago de Compostela

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Departamento de Ingeniería Química

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Informan,
Que la memoria titulada "Feasibility of membrane bioreactors for the removal of Pharmaceutical and Personal Care Products present in sewage" que, para optar al grado de Doctor en Ingeniería Química y Ambiental, presenta Don Rubén Reif López, ha sido realizada bajo nuestra inmediata dirección en el Departamento de Ingeniería Química de la Universidad de Santiago de Compostela.

Y para que así conste, firman el presente informe en Santiago de Compostela, Mayo de 2011.

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Objectives and summary

The Water Framework Directive (2000/60/EC) promotes a long-term progressive reduction of contaminant discharges to the aquatic environment in sewage. It is well-known that inputs of metals and organic contaminants to the urban wastewater system occur from different sources (domestic, commercial and urban runoff) and available literature has quantified their extent and importance. During the last years significant progress has occurred in eliminating the input of pollutants from these sources as reflected in the significant reductions reported for potentially toxic elements concentrations in sewage sludge and surface waters. The list of pollutants of interest comprises substances such as metals (cadmium, chromium, copper…) and organic compounds (PAHs, PCBs…) but nowadays, a ‘new generation’ of contaminants is being detected in different water compartments at significantly lower concentration levels, for that reason considered as micropollutants.

20 years ago, the development of the low pressure/submerged filtration systems boosted the development of membrane bioreactor (MBR) technologies for treating municipal or industrial wastewater. These systems combine the unit operations of biological treatment, secondary clarification and filtration into a single process, producing a high quality effluent suitable for any discharge and reuse purposes and are being now accepted as a technology of choice, widely applied in different regions of the world. Apparently, MBR advantages might help to mitigate the continuous release of micropollutants into the aquatic environment, also considering their current market size and growth projections. MBRs are especially important in Japan, with 66% of the total of installations. 98% of these plants work with aerobic biological processes, and 55% of the systems are equipped with submerged membrane modules. Presently, more than 800 installations are in operation only in Europe, and many more are under construction. Only in Spain, the number of sewage treatment plants implementing MBR technology has been multiplied by 4 from 2002 to 2005. Despite the high quality of the effluent produced after MBR treatment, different factors have traditionally impaired the competitiveness of these systems. The most relevant was the high operational costs associated to the energy demand and membrane cleaning/replacement procedures.

Thanks to many researching efforts throughout the last years, the main drifters for the quick widespread of the MBR technology have been the reduction in modules prices and an increased energy efficiency, mainly due to improvements in the design and operation practices.

Pharmaceuticals on their own (from the Latin *pharmaceuticus* and the Greek *pharmakeutikos*), also known as pharmaceutically active compounds (PhACs), have been defined as chemical substances that are used for diagnosis, treatment (cure/mitigation), or for prevention of diseases. This definition covers both prescription and over-the-counter drugs. Personal Care Products such as soaps, perfumes, disinfectants and sunscreen agents are used to alter or improve physiological or physical status. All these substances are largely consumed in modern societies and during the last decade several studies have reported their worldwide occurrence in different environmental compartments (surface waters, groundwaters, soils, sediments, etc.). PPCPs have been detected in extremely low concentrations, ranging from the ng/L to the low µg/L level. Only thanks to the recent developments in analytical techniques, particularly the gas/mass chromatography (GC/MS) and liquid/mass chromatography (LC/MS), the presence of a wide number of these substances has been completely proven, and several routes into the aquatic environment have been identified:

- Unmetabolized fractions of pharmaceuticals consumed by humans as well as their metabolites entering raw sewage via urine and faeces and by improper disposal over the toilets
- Drugs of veterinary use and metabolites reach soils after excreta, eventually finishing in groundwaters and aquifers.
- Application of sewage sludge as a fertilizer represents and additional entry route into the environment. This route is mainly followed in the case of compounds which have tendency to be associated with the solid fraction of sewage.
- Hospital wastewater, which usually contains higher concentrations of specific pharmaceuticals such as antibiotics, anti-cancer agents or iodinated contrast media.
- Personal care products and their ingredients which are discharged after their use in wastewater.

Therefore, most of PPCPs are released as original compounds and/or metabolites, entering the wastewater treatment plants where they undergo different fate as a funcion of their physical-chemical properties and biodegradability:

1. The substance will be ultimately mineralized to carbon dioxide and water. This concerns very few compounds, e.g. aspirin

2. The lipophilic and/or not readily degradable compound will partially remain in the particulate phase following a sorption mechanism.

3. The substance will be total or partially degraded during the biological treatment step, most probably following co-metabolic pathways (due to their low concentrations).

4. Most recalcitrant PPCPs will remain unchanged after the different stages of the treatment process, passing the wastewater treatment plant and ending up in the receiving waters, eventually becoming pseudo-persistent because their elimination/transformation rates are usually countered by their constant replenishment.

5. Depending on the flow of air getting in contact with wastewater, type of aireation and Henry coefficient, a fraction of a compound might be stripped with the off-gas in the aeration tank.

At this time, little information exists regarding the human health impacts of these substances since toxicity studies for pharmaceuticals mostly come from hypersensitivity, overdose and abuse effects which require concentrations clearly higher than the typically measured in the aquatic environment. Regarding the public health, it is also important to highlight that only in the worst-case scenario PPCPs are found in drinking water, mainly due to the efficiency of the drinking water treatment plants. PPCPs are rarely found even in the low ng/L range in drinking waters and hence, harmful effects derived from its consumption are not expected. On the contrary, aquatic ecosystems are subjected to a constant input of these substances, which arises concerns due to the possibility that stationary concentrations might be achieved in particularly sensitive areas. Increasing evidence suggests that chronic exposure to biologically active substances might be hazardous, in despite of the low concentrations in which they occur. Moreover, complex mixtures of these substances are usually found (until the date, more than 150 PPCPs have been identified in different water compartments), that could give place to synergistic effects. The presence of, for example, steroids and other Endocrine Disrupting Compounds (EDCs) has been linked to reproductive malfunction or feminization of fishes. Another well-known example, consequence of the abuse in the use of antibiotics, is the development or proliferation of resistant strains of bacteria, being also of relevance in this case the contribution of antibiotics from stockbreeding activities. Moreover, cumulative effects on the metabolism of non-target organisms should also be considered.

The factors mentioned above constitute the reason why currently there are no specific regulations establishing the maximum levels of concentrations for these substances at the outlet of sewage treatment plants and therefore, PPCPs are considered as emerging contaminants:

"Pollutants not currently included in programs of routine monitoring of quality of the waters, although they can be candidates for future regulation depending of the research concerning his ecotoxicity, potential hazardous effects for the health, public perception and of the data about their presence in different environmental compartments". (6th EU Framework Programme project NORMAN).

Major therapeutic groups of PPCPs commonly detected in wastewater treatment plant effluents are antibiotics, antiepileptic, tranquilizers, anti-inflammatories, X-ray contrast media, contraceptives, musk fragrances and several cosmetic ingredients. In this work, the selection of a representative group of PPCPs was based on the following criteria: a wide range of substances found at measurable levels in STP effluents, substances commonly prescribed belonging to different therapeutic groups, substances comprising different physical-chemical properties and therefore behaviour/fate throughout sewage treatment processes, and availability of reliable analytical methods to detect them in complex matrices such as wastewater.

Conventional water treatment processes are designed for the removal of organic matter and, in some cases, nitrogenous compounds. Such treatment technologies cannot fully and systematically remove many PPCPs, mainly due to their poor biodegradability. MBRs, which are in fact a modification of the CAS process, allow a major flexibility for the operation of the biological process. There are three relevant characteristics of the MBR technology that are of particular interest for the elimination of different organic micropollutants, particularly those of moderate biodegradability:

- MBRs permit to control the Sludge Retention Time (SRT). Previous works in this line suggest that this parameter exert a significant influence in the adaptation of the microorganisms to a continuous input of PPCPs. Therefore longer SRTs will increase the capacity of the biomass to remove recalcitrant substances.

- It is possible to work with high concentrations of biomass, which allows to enhance the biological treatment within a reduced space. Additionally, developed biomass during a MBR process present some differences in terms of physical properties compared with biomass developed in conventional systems. For example, a higher surface area of MLSS, directly related to the floc-structure, which probably might increase some enzymatic activities.

- Considering the high quality of the final effluent suitable for reuse purposes in many cases, a further post-treatment (for example, nanofiltration, ozonation process or filtration through granular activated carbon columns) might be more efficient, due to the lack of substances that could interfere in such processes (organic matter, colloids, suspended solids,etc.).

Therefore, the aim of this doctoral thesis was the evaluation of the MBR technology for eliminating a specific category of organic micropollutants: Pharmaceutical and Personal Care Products (PPCPs). The selection of substances of interest comprised 11 pharmaceutically active compounds from five therapeutic classes (anti-inflammatory drugs, antibiotics, anti-depressants, tranquilizers and anti-epileptics), 3 polycyclic musk fragrances characterised for their wide usage in detergents, soaps and perfumes, 2 natural estrogens and a synthetic hormone, considered endocrine disruptors.

The first approach of this work (**Chapter 1**) consisted of a literature overview explaining the different removal mechanisms PPCPs undergo during sewage treatment and the different operational parameters and factors which influence the degree of removal achieved. Besides, a broad description of the substances studied is provided, detailing their most relevant physical-chemical properties which influence their fate and behaviour along sewage treatment. The chapter finishes with an analysis of the influence of different sewage treatment technologies available, mainly focused in the comparison of results achieved with the Conventional Activated Sludge (CAS) process and MBRs.

In **Chapter 2**, the materials and experimental methods employed to carry out the experimental work of this doctoral thesis are described. Firstly, the methods employed to analyse the properties of the liquid phase and conventional parameters (organic matter, nitrogen, temperature, solids content, pH and dissolved oxygen content) used for wastewater and sludge characterization are detailed. They are followed by the techniques for the analytical determination of PPCPs in liquid and solid samples.

Chapter 3 consisted in a preliminary study about the occurrence of PPCPs in municipal wastewater and their fate and behaviour along the different units of sewage treatment. This research was carried out in a wastewater treatment plant placed in Northwest England, where a fully instrumented pilot-plant was operated at its premises. Usually, CAS units consists of a pretreatment step for grit, fat and grease removal followed by primary treatment, where most suspended solids are eliminated as primary sludge. During both steps, a fraction of PPCPs is expected to be removed by sorption onto the particulate phase. Then, secondary treatment where the elimination of some PPCPs is achieved following two different mechanism: biological degradation and/or sorption onto secondary sludge. Finally, the final effluent is obtained after secondary settling step. In the studied system the processes of organic matter and ammonia removal take place simultaneously and it truly represents the technology more commonly used in sewage treatment plants. In order to get more insight into the mechanisms responsible for the PPCPs elimination throughout the different treatment units of the pilot-plant, mass balances for each quantified PPCP were calculated. The methodology consisted of a two-days sampling campaign where liquid samples at the inlet and outlet of each one of the considered units were immediately processed after collection. Although no solid samples were collected, the amount of PPCPs which might be present on this phase was estimated with distribution coefficients from the literature. The most frequently detected PPCPs were anti-inflammatory drugs and musk fragrances. The mass balances permitted to calculate the degree of elimination achieved in each one of the units of the plant, which was useful in order to elucidate their behaviour along sewage treatment. Additionally, the plant treated a stream of returning liquors from the sludge centrifuge unit, which was also sampled and therefore considered in the PPCPs mass balances, since substantial concentrations of them were also detected in the mentioned stream. Considering the results obtained, the daily output of PPCPs that a medium-sized STP might release into the aquatic environment was calculated. For example, in the case of diclofenac, one of the most recalcitrant substances considered in this study, a daily release of 1.5 kg/d was estimated only considering the liquid phase.

In **Chapter 4** the fate of selected PPCPs during MBR treatment was aimed to be assessed. For this purpose, a MBR was operated indoors, at the premises of the School of Engineering (University of Santiago de Compostela). Feeding consisted on a synthetic influent which reproduced the typical characteristics of a medium strenght municipal wastewater. In this chapter, the solid-phase was not considered, since solids content in the feeding was negligible and therefore, the elimination of each one of the considered PPCPs could be calculated in abcense of these data. The highest transformation (>90%) was determined for the anti-inflammatories IBP and NPX whereas CBZ, DCF and DZP were poorly removed. Surprisingly, musk fragrances elimination was only moderate (50-60%) compared with results achieved in conventional systems (>80%). Antibiotics showed a different fate. For example, SMX removal was intermediate, TMP was recalcitrant (<20%) whereas ROX and ERY were efficiently removed. These results showed slightly improved removals comparing with CAS systems with the exception of musk fragrances. However, a deeper study about the influence of the different operational parameters was not considered at this stage of the research. Another relevant aspect to improve was the composition of the feeding, which directly influenced the developed MBR biomass. Therefore, the pilot-plant was set outdoors at the premises of a full-scale sewage treatment plant, and was fed with settled sewage (**Chapter 5**) for an extended period of operation. The varying parameters that were studied at this stage of the research were the Mixed Liquor Solids Concentration (MLSS), the temperature of the biomass and the adaptation of the microorganisms to a continuous input of the selected PPCPs. Differences in the behaviour and fate were observed depending on the substance considered. For example, sulfamethoxazole removal was moderate (50-75%) and particularly influenced by the mixed liquor suspended solids (MLSS) concentration. The elimination of other antibiotics strongly increased during the operation of the MBR, probably due to biomass adaptation. Operating conditions did not influence the elimination of hormones, ibuprofen and naproxen, which were almost completely eliminated (90-99%). Similarly, the removal of carbamazepine, diazepam and diclofenac was not influenced by the operating conditions although their elimination was incomplete (20-50%). Elimination of fragrances varied significantly between operational periods: low eliminations were observed in the winter period whereas eliminations up to 70% were measured during samplings carried out in warmer periods. Sludge age, temperature and physical-chemical characteristics of the MBR sludge might exert influence on the observed eliminations.

In **Chapter 6**, a direct comparison between MBR and CAS systems was carried out by parallel operation of the MBR studied in the previous chapter and a lab-scale activated sludge unit. Different parameters and conditions such as temperature, pH, MLSS, HRT and SRT were maintained at similar values in both systems. Additionally, the influence of HRT and SRT on PPCPs removal was checked in both systems. HRT influences the contact time of the soluble components of the PPCPs in STPs, affecting the biological activity of the activated sludge. Its decrease has been shown to adversely affect the overall quality of treatment. Hence, higher HRT may be preferable for more effective elimination of micropollutants in STPs. The SRT is considered a critical operational parameter commonly used for STPs design and can be optimised during secondary wastewater treatment in order to achieve better elimination of micropollutants. Longer SRTs allow the growth of slowly growing bacteria, subsequently leading to the formation of diverse ecology of microorganisms with wider spectrum of physiological and adaptation characteristics. The main differences between the CAS and MBR systems, in terms of PPCPs removal, were found for compounds which concentrations in solid-phase were higher such as musk fragrances or antidepressants. Distribution coefficients for each one of the considered PPCPs were calculated for the two sludges. Although the differences were low, the CAS sludge had slighty higher coefficients for many substances. In general, the performance of the CAS for the elimination of compounds sorpted onto the solid phase was remarkably higher. Interestingly, the effect of the sludge purges performed to control the SRT was related to the performance of both systems since, after intensive period of purges, the overall elimination efficiencies of both bioreactors increased, and this effect was more marked in the MBR. Another influencing parameter was the SRT. After decreasing this parameter to values below 10 d, the elimination of many substances such as antibiotics was severely reduced. In this specific case, the MBR performance was superior compared to CAS. However, the main conclusion of this chapter is that the differences between both technologies eliminating recalcitrant substances are low, and the upgrade of a conventional treatment plant to MBR is not justified merely in terms of micropolutants removal.

Chapter 7 considered a different aspect of the membrane bioreactors technology: the effect of the membrane filtration step. Therefore, 3 side-stream modules coupled to a MBR and a submerged hollow fiber membrane were simultaneously tested during three sampling periods. Some of the studied PPCPs were already present in sewage from Cranfield University (UK). However, in order to work with substances representing a broader range of physical-chemical properties, 5 more PPCPs were spiked into the MBR mixed liquor. Additionally, the biological performance of the system was also tested, considering the operational parameters (pH, temperature and HRT) which varied during the different sampling campaigns. The highest transformation was achieved for ibuprofen (>98%) and naproxen (75 and 91%). On the contrary, carbamazepine elimination was poor (36 and 47%). Different fate was observed depending on the sampling period in the case of diclofenac, sulfamethoxazole and erythromycin since their elimination steadily increased. Therefore, a combination of acidic pH values (measured during the third sampling period), warm temperatures and a prolonged period of operation with a continuous input of PPCPs seemed to be the optimum conditions to maximize PPCPs removal. However, a complete depletion of the micropollutant content in sewage was never achieved. Analysis of the mixed liquor supernatant showed lower concentrations of galaxolide and diclofenac compared with the produced permeates. Therefore, additional data gathered from the operation of the MBR operated in Silvouta was used in order to confirm this behaviour. MBR performance removing PPCPs from the liquid phase was not dependant on membrane material or configuration at any extent, whereas in the case of musk fragrances and diclofenac, the filtration step seemed to contribute to increase their concentration in permeates thus reducing their overall elimination from the liquid phase.

With the different works carried out and reported along the present doctoral thesis, the knowledge about some of the key aspects of the use of MBRs for PPCPs removal has been considerably enhanced. Therefore it is considered that the obtained knowledge, summarised along the present section, will make easier the decision of implementing MBR or CAS processes in order to treat different types of wastewaters, always considering their micropollutants content. In case a MBR is the technology of choice, this research also permits to decide the optimum parameters and operational strategies in order to maximize their efficiency in terms of PPCPs removal.

Objetivos y resumen

La directiva europea del agua (2000/60/EC) tiene como uno de sus objetivos prioritarios el promover una progresiva reducción de los niveles de contaminantes presentes en las aguas residuales vertidas al medio ambiente acuático. En este sentido, durante los últimos años se han producido avances significativos en la eliminación de los aportes de contaminantes vertidos, como se refleja en las reducciones significativas de la concentración de elementos potencialmente tóxicos en lodos de depuradora y aguas superficiales. La lista de contaminantes de interés comprende sustancias como metales (cadmio, cromo, cobre…) y compuestos orgánicos (PAHs, PCBs) pero actualmente, una nueva generación de contaminantes está siendo detectada en diferentes medios acuáticos en niveles de concentración significativamente inferiores, considerados por tanto microcontaminantes.

Hace 20 años, el desarrollo de sistemas de filtración de baja presión/sumergidos potenció el desarrollo de las tecnologías basadas en biorreactores de membrana (MBRs) para el tratamiento de aguas residuales industriales. Estos sistemas, que combinan las unidades de operación de tratamiento biológico, sedimentación y filtración en un único proceso produciendo un efluente de alta calidad apto para descarga y reuso, están siendo aceptados como una tecnología viable, ampliamente utilizada en diversas partes del mundo. Aparentemente, las ventajas de los MBR podrían contribuir a mitigar la liberación continua de microcontaminantes al medio ambiente acuático, teniendo en cuenta además su cuota de mercado actual y las perspectivas de crecimiento. Los MBR son de especial importancia en Japón, con un 66% del total de instalaciones. El 98% de ellas trabajan con procesos biológicos aerobios, y el 55% están equipadas con módulos de membrana sumergida. Actualmente, más de 800 instalaciones están operativas solamente en Europa, y muchas más están en fase de construcción. En España, el número de estaciones depuradoras que han implementado la tecnología de membranas se ha multiplicado por 4 en el período 2002-2005. A pesar de la alta calidad del efluente generado en un MBR, diversos factores han dificultado la competitividad de esos sistemas. El más importante ha sido el alto coste operacional asociado al consumo energético. Gracias a los esfuerzos desarrollados en materia de investigación a lo largo de los últimos años, el uso de MBRs se ha visto impulsado gracias a la reducción de precios de los módulos de filtración y el incremento en la eficiencia energética, debidos a las significativas mejoras en el diseño y prácticas operacionales.

Los fármacos (del latin *pharmaceuticus* y el griego *pharmakeutikos*), tambien conocidos como compuestos farmacológicamente activos, han sido definidos como sustancias químicas empleadas para el diagnóstico, tratamiento (cura/alivio), y/o para prevención de enfermedades. Esta definición cubre tanto los medicamentos de prescripción cómo los que se adquieren sin receta. Los productos de cuidado personal como jabones, perfumes, desinfectantes y bloqueadores solares se utilizan para alterar o mejorar el estado fisiológico o físico. Estos productos se utilizan en grandes cantidades en sociedades modernas y durante la última década, varios estudios han constatado su presencia a nivel mundial en diferentes compartimentos medioambientales (aguas superficiales o subterráneas, suelos, sedimentos…). Las sustancias conocidas como PPCPs (Pharmaceutical and Personal Care Products) comprenden ambos tipos de compuestos y se han detectado en concentraciones extraordinariamente bajas, desde nanogramos hasta microgramos por litro. Gracias a los recientes avances en las metodologías analíticas, particularmente en la cromatografía de gases/masas (GC/MS) y de líquidos/masas (LC/MS), la presencia de un número cada vez mayor de estas sustancias se ha demostrado pudiéndose identificar las principales rutas que siguen hasta llegar al medio ambiente acuático:

- Fracciones de fármacos metabolizadas en mayor o menor medida tras su consumo humano que entran al agua residual bruta por medio de la orina y heces. Igualmente, medicamentos sobrantes o caducados que son desechados inadecuadamente por el desagüe.
- En el caso de antibióticos de uso veterinario, la fracción no metabolizada del fármaco también pueden acabar incorporándose a suelos tras ser excretada, alcanzando en algunos casos aguas superficiales o subterráneas.
- El uso de lodos de depuradora como fertilizantes representa una ruta adicional de entrada al medioambiente. Los compuestos que siguen esta ruta son aquellos que tienen una mayor tendencia a estar asociados a la fracción sólida del agua residual.
- Agua residual de hospitales que habitualmente contiene concentraciones más altas de fármacos específicos como antibióticos, medios de contraste iodados y agentes anticancerígenos.
- Productos de cuidado personal y sus ingredientes que son descargados al agua residual tras su uso.

De este modo, la mayoría de los PPCPs son liberados tanto en forma del compuesto original como en forma del metabolito, entrando estas sustancias a las plantas de tratamiento de aguas residuales, donde siguen un destino diferente en función de sus propiedades físico-químicas y su biodegradabilidad:

1. La sustancia puede ser mineralizada a dióxido de carbono y agua. Esto afecta a muy pocos compuestos, como por ejemplo el ácido acetilsalicílico.

2. Los compuestos lipofílicos y/o poco biodegradables permanecerán parcialmente asociados a la fase particulada mediante mecanismos de adsorción.

3. La sustancia será total o parcialmente degradada durante la etapa de tratamiento biológico, más probablemente siguiendo rutas de degradación co-metabólicas debido a sus bajas concentraciones.

4. Los PPCPs más recalcitrantes permanecerán inalterados después de las distintas etapas del proceso de tratamiento, acabando en las aguas superficiales que reciben el efluente tratado y pudiendo en algunos casos ser 'pseudo-persistentes', debido a que sus tasas de eliminación/transformación son habitualmente contrarrestadas por su constante reabastecimiento.

5. En función del caudal de aire en contacto con el agua residual, el tipo de aireación y el coeficiente de la Ley de Henry, una fracción de compuesto podría ser volatilizada en el tanque de aireación.

En este momento, hay poca información disponible en lo referente a posibles impactos en la salud pública debido a que los estudios de toxicidad efectuados hacen referencia a casos de hipersensibilidad, sobredosis y abuso, que requieren de concentraciones claramente más altas que las típicamente medidas en aguas. Es importante destacar que sólo en el peor de los casos se pueden encontrar trazas de PPCPs en aguas destinadas a consumo humano, debido a la eficacia de las plantas de producción de agua potable. En este tipo de aguas, los PPCPs son muy raramente cuantificables, y sólo en concentraciones en el rango de ng/L. Por tanto, no se espera ningún efecto pernicioso derivado de su consumo. Por contra, los ecosistemas acuáticos están sujetos a una entrada constante de estas sustancias, lo que está generando preocupación debido a la posibilidad de que se puedan alcanzar concentraciones estacionarias en áreas especialmente sensibles. Cada vez hay más evidencias científicas que sugieren que una exposición crónica a sustancias biológicamente activas podría ser perjudicial independientemente de los bajos niveles de concentración medidos. Es más, en las aguas se pueden encontrar mezclas complejas de estas sustancias (hasta la fecha más de 150 PPCPs han sido detectados en distintos compartimentos acuáticos), lo que podría generar la aparición de efectos sinérgicos. Por ejemplo, la presencia de disruptores endocrinos (EDCs) se ha vinculado con problemas de disfunción de la función reproductora o de feminización en algunas especies de peces. Otro caso bien conocido, consecuencia del abuso en el uso de antibióticos, es el desarrollo y proliferación de especies de bacterias resistentes, siendo en este caso de especial importancia la contribución de antibióticos procedentes de actividades de ganadería. Además, los efectos debidos a la acumulación de estas sustancias en organismos no-objetivo deberían ser igualmente considerados.

Los factores anteriormente mencionados explican que actualmente no haya una reglamentación específica que establezca niveles máximos de concentración a la salida de las plantas de tratamiento. Por esto, los PPCPs son considerados como contaminantes emergentes, tal y como recoge la siguiente definición:

"Contaminantes no actualmente incluidos en programas de monitorización rutinaria de la calidad de las aguas, aunque pueden ser candidatos para futuros reglamentos, dependiendo de las investigaciones realizadas en lo referente a su ecotoxicidad, posibles efectos adversos para la salud, opinión pública y de los datos sobre su presencia en los distintos compartimentos acuáticos". (6º programa MARCO de la UE, proyecto NORMAN).

Los grupos terapéuticos de PPCPs más comúnmente detectados en plantas de tratamiento son anti-inflamatorios, antibióticos, antiepilépticos, tranquilizantes, medios de contraste, anticonceptivos, fragancias sintéticas y varios ingredientes de cosméticos. En este trabajo se seleccionó un grupo representativo de PPCPs basándose en los siguientes criterios: trabajar con un amplio grupo de sustancias presentes en niveles detectables en efluentes de depuradoras urbanas, que abarquen los principales grupos terapéuticos así como los más comúnmente recetados, sustancias que tengan distintas propiedades físico-químicas y por tanto que presenten distinto comportamiento y grado de eliminación durante los procesos de tratamiento de aguas residuales, y la disponibilidad de métodos analíticos de confianza para medir su concentración en matrices complejas como es el caso del agua residual.

Los procesos de tratamiento convencionales están diseñados para la eliminación de materia orgánica y en algunos casos de nitrógeno. Estas tecnologías no pueden alcanzar la eliminación completa de muchos de los PPCPs, principalmente debido a su pobre biodegradabilidad. Los MBR, que de hecho son una modificación del proceso convencional, permiten una mayor flexibilidad de cara a la operación del proceso biológico. Hay tres aspectos fundamentales de la tecnología MBR de particular interés de cara a la eliminación de distintos contaminantes orgánicos, particularmente aquellos de biodegradabilidad moderada:

- Los MBRs permiten controlar perfectamente el tiempo de retención celular (TRC). Trabajos previos en esta línea sugieren que este parámetro ejerce una influencia significativa de cara a la adaptación de los microorganismos a un aporte continuo de PPCPs. De este modo, TRCs más altos incrementarán la capacidad de la biomasa para eliminar sustancias recalcitrantes.

- Es posible trabajar con altas concentraciones de biomasa, lo que permite mejorar la eficacia del tratamiento biológico en un espacio más reducido. Adicionalmente, la biomasa desarrollada durante un proceso MBR presenta propiedades físicas diferentes con respecto a la biomasa generada en sistemas convencionales, como una mayor superficie específica, propiedad directamente relacionada con la estructura de sus flóculos, lo cual podría incrementar su actividad enzimática.

- Teniendo en cuenta la alta calidad del efluente final, apto para reuso en muchos casos, su post-tratamiento (por ejemplo, nanofiltración, ozonización o filtración a través de columnas de carbón activo granular) podría ser más eficiente que el mismo proceso tratando un efluente sin filtrar, debido a la nula presencia de sustancias que puedan interferir con esos procesos (materia orgánica, sólidos suspendidos, materia coloidal, etc.).

De este modo, el objetivo de esta tesis doctoral es la evaluación de los MBR para la eliminación de una categoría específica de microcontaminantes orgánicos, los PPCPs. La selección de sustancias de interés comprende 11 compuestos farmacológicamente activos de 5 grupos terapéuticos distintos (anti-inflamatorios, antibióticos, antidepresivos, tranquilizantes y antiepilépticos), 3 fragancias sintéticas caracterizadas por su elevado uso en detergentes, jabones y perfumes, 2 estrógenos naturales y una hormona sintética, sustancias consideradas como disruptores endocrinos.

El primer objetivo de este trabajo (**Capítulo 1**) consistió en una revisión de la literatura disponible, explicando en profundidad los distintos mecanismos de eliminación que los PPCPs pueden sufrir durante los procesos de tratamiento de aguas residuales urbanas, así como los distintos parámetros operacionales y factores que influyen en el grado de eliminación alcanzado. Además, se incluye una amplia descripción de las sustancias estudiadas detallando sus propiedades físico-químicas más importantes de cara a estudiar su comportamiento. El capítulo finaliza con un análisis de las diferentes tecnologías de tratamiento disponibles, principalmente enfocado a la comparación de resultados obtenidos en sistemas convencionales y MBRs de cara a la eliminación de PPCPs.

En el **Capítulo 2** se explican los materiales y métodos experimentales utilizados para llevar a cabo el trabajo experimental de esta tesis doctoral. En primer lugar, los métodos utilizados para analizar propiedades de la fase líquida y parámetros convencionales (materia orgánica, nitrógeno, temperatura, contenido en sólidos, pH y oxígeno disuelto) utilizados para la caracterización del agua residual y los lodos. A continuación se detallan las técnicas utilizadas para la determinación de PPCPs en muestras líquidas y sólidas.

El **Capítulo 3** consistió en un estudio preliminar sobre la presencia de PPCPs en aguas residuales urbanas y su comportamiento y destino final en las distintas unidades del tratamiento de aguas residuales. Esta investigación fue llevada a cabo en una planta de tratamiento de aguas residuales situada en el Noroeste de Inglaterra, donde una planta piloto completamente instrumentada fue operada en sus instalaciones. Las unidades de tratamiento convencional suelen constar de un pretratamiento para la eliminación de grasas, aceites y arenas, seguidas a continuación de un tratamiento primario en el que la mayoría de los sólidos suspendidos son eliminados en forma de lodo primario. Durante ambos pasos, una fracción de los PPCPs puede eliminarse por adsorción o absorción en la fase particulada. Durante el tratamiento secundario o biológico, la eliminación de PPCPs se consigue fundamentalmente mediante dos mecanismos diferentes: degradación biológica y/o adsorción-absorición en el lodo secundario. Tras la sedimentación secundaria, se obtiene el efluente final del proceso. En el sistema estudiado en este capítulo se dan de forma simultánea la eliminación de materia orgánica y la nitrificación, y representa fielmente la tecnología más comunmente utilizada en estaciones depuradoras. Con la finalidad de obtener más información acerca de los mecanismos responsables de la eliminación de PPCPs en las distintas unidades de tratamiento, se calcularon balances de materia para cada PPCP detectado y cuantificado en las distintas muestras. La metodología consistió en una campaña de muestreo de dos días alternos en la que se tomaron muestras líquidas a la entrada y salida de cada una de las unidades de la planta piloto. Las muestras fueron inmediatamente procesadas tras su recolección. Aunque no se tomaron muestras de lodo primario o secundario, la cantidad de PPCPs presentes en la fase sólida se estimó mediante coeficientes de distribución obtenidos de trabajos previamente publicados. Los PPCPs más comúnmente detectados fueron anti-inflamatorios y fragancias sintéticas. Los balances de materia permitieron calcular el grado de eliminación alcanzado en cada una de las unidades de la planta, lo que fue útil para elucidar su comportamiento a lo largo de todo el proceso de tratamiento. Además, la planta trató una corriente de licores de retorno provenientes del proceso de centrifugación de lodos. Esta corriente fue también muestreada y considerada en los balances de materia, ya que contenía cantidades significativas de PPCPs. Teniendo en cuenta los resultados obtenidos, la salida diaria de PPCPs de una instalación de tratamiento de tamaño medio fue estimada. Por ejemplo, en el caso del diclofenac, una de las sustancias más recalcitrantes, se estimó una emisión de 1.5 kg/d, considerando sólamente la fase líquida.

En el **Capítulo 4**, se evaluó el rendimiento de un MBR de cara a la eliminación de los PPCPs seleccionados. El equipo utilizado fue operado en las instalaciones de la Escuela de Ingenieria (Universidad de Santiago de Compostela). La alimentación consistió en un medio sintético que simulaba la composición típica de un agua residual urbana de carga media. En este capítulo, la fase sólida no fue considerada, dado que el contenido en sólidos de la alimentación sintética es nulo y por lo tanto, la eliminación de cada uno de los PPCPs podía ser calculada sin ese dato. El porcentaje de eliminación más alto (>90%) se obtuvo en el caso de los anti-inflamatorios ibuprofeno y naproxeno mientras que la carbamazepina, el diclofenac y el diazepam no fueron eliminados significativamente. Sorprendentemente, la eliminación de fragancias sintéticas fue sólo moderada (50-60%) en comparación con los resultados obtenidos en sistemas convencionales (>80%). Los antibióticos mostraron distinto comportamiento. Por ejemplo, el sulfametoxazol fue parcialmente eliminado, el contenido en trimetoprim no se redujo en cantidades significativas (<20%), mientras que la roxitromicina y la eritromicina fueron eficazmente eliminadas. Estos resultados muestran una leve mejoría comparados con los obtenidos en sistemas convencionales, a excepción de las fragancias sintéticas. En cualquier caso, en esta etapa de la investigación no se llevó a cabo un estudio más detallado sobre la influencia de los diversos parámetros operacionales. Otro aspecto importante para mejorar los resultados y aproximarlos a la situación real en plantas de tratamiento es el reemplazo del medio sintético como alimentación por un agua residual real. El uso de medios sintéticos influencia directamente el desarrollo de la biomasa en el MBR. Por esto, el MBR a escala piloto fue puesto en funcionamiento en las instalaciones de una depuradora de aguas residuales urbanas a escala real y fue alimentado con agua residual sedimentada (**Capítulo 5**) a lo largo de un periodo de operación extendido. En esta etapa de operación se estudió la concentración de sólidos en el licor de mezcla, la temperatura de la biomasa y la adaptación de los microorganismos al aporte continuo de PPCPs. En función de la sustancia considerada, se observaron diferencias significativas en su comportamiento y en el grado de eliminación alcanzado. Por ejemplo, el sulfametoxazol fue parcialmente eliminado (50-75%), con una influencia significativa de la concentración de sólidos en el reactor. La eliminación de los otros antibióticos se vio incrementada a lo largo de la operación del MBR, probablemente debido a efectos de aclimatación de la biomasa. Por contra, ningún parámetro operacional ejerció influencia en la eliminación de hormonas, ibuprofeno y naproxeno, que fueron prácticamente eliminados en todos los muestreos (90-99%). Análogamente, las condiciones de operación no afectaron a la eliminación de sustancias recalcitrantes como la carbamazepina, el diazepam y el diclofenaco, y su eliminación fue incompleta (20-50%). La eliminación de fragancias varió sensiblemente entre los distintos periodos operacionales: durante el invierno las eficacias de eliminación fueron bajas mientras que en periodos de operación con temperaturas más elevadas las eficacias de eliminación se incrementaron hasta un 70%. Factores como la edad celular, la temperatura y las propiedades físico-químicas de la biomasa desarrollada en los MBR podrían ejercer también influencia en estos resultados.

En el **Capítulo 6**, una comparación directa entre el MBR a escala piloto y un biorreactor de lodos activos convencional a escala laboratorio fue llevada a cabo mediante operación paralela de ambos sistemas. Los diferentes parámetros operacionales como la temperatura, el pH, la concentración de biomasa, el tiempo de retención hidráulico (TRH) y la edad celular fueron mantenidos en valores similares en los dos sistemas. Además, a lo largo de la operación de ambos sistemas, se probaron 4 TRHs distintos, y dos TRCs. Por un lado, el TRH influencia el tiempo de contacto de los PPCPs solubles en la fase líquida con la biomasa y su disminución por tanto podría afectar negativamente a la calidad general del tratamiento biológico. Por tanto, valores más altos de este parámetro pueden ser deseables para una eliminación más efectiva de microcontaminantes en procesos de depuración. El TRC es considerado un parámetro de operación crucial que se tiene en cuenta de cara al diseño de las plantas de tratamiento y que resulta fundamental para el tratamiento biológico, de cara a obtener mejores eficacias de eliminación de microcontaminantes. Esto se debe a que los tratamientos biológicos operados con TRC altos permiten la proliferación de bacterias de más lento crecimiento, desarrollándose una biocenosis más diversa que puede cubrir un mayor espectro de características fisiológicas y de adaptación. De este modo, las principales diferencias encontradas en ambos sistemas se corresponden con compuestos cuyas concentraciones en la fase sólida fueron significativamente más altas, como es el caso de las fragancias sintéticas o los antidepresivos. Los coeficientes de distribución sólido-líquido para cada uno de los PPCPs estudiados fueron calculados con los dos tipos de lodo generado. Aunque las diferencias encontradas fueron pequeñas, el lodo del sistema convencional presentaba valores ligeramente superiores de estos coeficientes en la mayoría de los casos. En general, la capacidad del sistema convencional para eliminar compuestos presentes en concentraciones significativas en la fase sólida (principalmente fragancias) fue más alta. Se encontró también una relación entre el efecto de las purgas de lodo que se efectuaron para el control del TRC y la capacidad de eliminación de estas sustancias. De este modo, tras periodos de purgas intensivas, la eficacia general de ambos reactores eliminando fragancias se vio incrementada y este efecto fue más evidente en el caso del MBR. Otro factor de elevada influencia fue el TRC. Cuando se trabajó con valores de este parámetro por debajo de los 10 días, la eliminación de muchas sustancias, como es el caso de los antibióticos, se vio sevrramente afectada. Sólo en este caso concreto, la capacidad del MBR para eliminar estas sustancias fue claramente superior a la del CAS. En cualquier caso, la principal conclusión de este capítulo es que las diferencias entre ambas tecnologías de cara a la eliminación de sustancias recalcitrantes son reducidas, y por lo tanto la mejora de un sistema convencional incorporando el proceso MBR no estaría justificada desde el punto de vista de la eliminación de microcontaminantes.

El **Capítulo 7** consideró un aspecto diferente de la tecnología de biorreactores de membrana: el efecto del proceso de filtración. De este modo, 3 modulos en configuración side-stream acoplados a un MBR operado con un módulo de membrana sumergida de fibra hueca fueron evaluados simultáneamente a lo largo de tres periodos de muestreo. Algunos de los PPCPs estudiados ya estaban presentes en el agua residual generada en la Universidad de Cranfield (UK). En cualquier caso, con el objetivo de trabajar con sustancias representativas del mayor rango posible de propiedades físico-químicas distintas, 5 PPCPs fueron adicionados continuamente al licor mezcla durante la operación del biorreactor. Adicionalmente, la eficacia del proceso biológico también fue evaluada, considerando diversos parámetros operacionales (pH, temperatura y TRH) que fueron variando a lo largo de los distintos periodos de muestreo. El porcentaje de transformación más elevado se consiguió para el ibuprofeno (>98%) y el naproxeno (75 y 91%). Por el contrario, la eliminación de carbamazepina fue baja (36 y 47%). La eliminación de diclofenaco, sulfametoxazol y eritromicina aumentó paulatinamente con el tiempo de operación y por tanto dependió del periodo de muestreo considerado. Por lo tanto, la combinación de valores de pH ácidos (medidos durante el muestreo final), temperaturas cálidas y un tiempo de operación prolongado con una adición continua de PPCPs fueron las condiciones óptimas para maximizar la eliminación de PPCPs. En cualquier caso, la eliminación completa del contenido en microcontaminantes del agua residual nunca fue conseguida. Los análisis de los distintos permeados generados en el proceso mostraron concentraciones más elevadas de galaxolide y diclofenac que las medidas en el sobrenadante del licor de mezcla. Por tanto, datos adicionales provenientes de la operación del MBR en Silvouta sirvieron para confirmar este comportamiento. La capacidad del MBR para eliminar PPCPs de la fase líquida no dependió del material de las membranas o su configuración mientras que en el caso de fragancias y diclofenac, la etapa de filtración aparentemente contribuyó a incrementar su concentración en los permeados, reduciendo su eliminación global de la fase líquida.

Con los trabajos llevados a cabo y expuestos a lo largo de la presente tesis doctoral, se ha profundizado sensiblemente en el conocimiento sobre algunos aspectos clave sobre el uso de MBRs para la eliminación de PPCPs. Por lo tanto, se considera que la información generada es valiosa de cara a la toma de decisiones a la hora de implantar un proceso MBR o un proceso CAS para el tratamiento de distintos tipos de aguas residuales cuando se considera su contenido en contaminantes emergentes, y también para establecer parámetros óptimos y estrategias de operación de MBRs que permitan optimizar su funcionamiento en términos de eliminación de estas sustancias.

Obxectivos e resumo

Os fármacos (do latin *pharmaceuticus* e o grego *pharmakeutikos*), tamén coñecidos como compostos farmacolóxicamente activos, foron definidos como sustancias químicas empregadas para o diagnóstico, tratamento (cura/alivio), e ou para prevención de enfermidades. Esta definición cobre tanto os medicamentos de prescrición como os que se adquiren sen receita. Os produtos de coidado persoal como xabóns, perfumes, desinfectantes e bloqueadores solares utilízanse para alterar ou mellorar o estado fisiológico ou físico. Estes produtos utilízanse en grandes cantidades nas sociedades modernas e, durante a última década, varios estudos constataron a súa presenza a nivel mundial en diferentes compartimentos ambientais (augas superficiais ou subterráneas, chans, sedimentos, etc.) As sustancias coñecidas como PPCPs (Pharmaceutical and Persoal Care Products) comprenden ambos tipos de compostos e foron detectadas en concentracións extraordinariamente baixas, dende nanogramos até microgramos por litro. Grazas aos recentes avances nas metodoloxías analíticas, particularmente na cromatografía de gases/masas (GC/MS) e de líquidos/masas (LC/MS), determinouse a presenza dun número cada vez maior destas sustancias podéndose ademais identificar as principais rutas que seguen até chegar ao medio ambiente acuático:

- Fraccións de fármacos metabolizadas en maior ou menor medida tras o seu consumo humano, e que entran á auga residual bruta por medio dos ouriños e feces. Igualmente, medicamentos sobrantes ou caducados que son vertidos inadecuadamente polo desaugadoiro.

- No caso de antibióticos de uso veterinario, a fracción non metabolizada do fármaco tamén poden acabar incorporándose a chans tras ser excretada, alcanzando nalgúns casos augas superficiais ou subterráneas.

- Auga residual de hospitais que habitualmente conteñen concentracións máis altas de fármacos específicos coma antibioticos, medios de contraste iodados e axentes anticanceríxenos.

- Produtos de coidado persoal e os seus ingredientes, que son descargados á auga residual tralo seu uso.

Deste xeito, a maioría dos PPCPs son liberados como o composto orixinal ou en forma de metabolito, entrando ás plantas de tratamento de augas residuais, onde seguen un destino diferente en función das súas propiedades físico-químicas e a súa biodegradabilidade:

1. A sustancia pode ser mineralizada a dióxido de carbono e auga. Isto afecta a moi poucos compostos, por exemplo á aspirina.

2. Os compostos lipofílicos pouco biodegradables permaneceran parcialmente asociados á fase particulada mediante un mecanismo de adsorción ou absorción.

3. A sustancia será total ou parcialmente degradada durante a etapa de tratamento biolóxico, máis probablemente seguindo rutas de degradación cometabólicas debido ás súas baixas concentracións.

4. Os PPCPs máis recalcitrantes permanecerán inalterados despois das distintas etapas do proceso de tratamento, acabando nas augas superficiais que reciben o efluente tratado e podendo nalgúns casos ser 'pseudo-persistentes', debido a que as súas taxas de eliminación ou transformación son habitualmente contrarrestadas polo seu constante reabastecimiento.

5. En función do caudal de aire en contacto coa auga residual, o tipo de aireación e o coeficiente da Lei de Henry, unha fracción do composto podería ser volatilizada no tanque de aireación.

Neste momento, hai pouca información dispoñible no referente a posibles impactos na saúde pública debido a que os estudos de toxicidade efectuados fan referencia a casos de hipersensibilidade, sobredoses e abuso, que requiren de concentracións claramente máis altas que as tipicamente medidas en augas. É importante destacar que só no peor dos casos pódense atopar trazas de PPCPs en augas destinadas a consumo humano, debido á eficacia das plantas de produción de auga potable. Neste tipo de augas, os PPCPs son moi raramente cuantificabeis, e só en concentracións no rango de ng/L. Polo tanto, non se espera ningún efecto pernicioso derivado do seu consumo. Por contra, os ecosistemas acuáticos están suxeitos a unha entrada constante destas sustancias, o que está a xerar preocupación debido á posibilidade de que se poidan alcanzar concentracións estacionarias en áreas especialmente sensíbeis. Cada vez hai máis evidencias científicas que suxiren que unha exposición crónica a sustancias biolóxicamente activas podería ser prexudicial independentemente dos baixos niveis de concentración medidos. É máis, nas augas pódense atopar mesturas complexas destas sustancias (até a data máis de 150 PPCPs foron detectados en distintos compartimentos acuáticos), o que podería xerar a aparición de efectos sinérgicos. Por exemplo, a presenza de disruptores endocrinos (EDCs) vinculouse con problemas de disfunción da función reprodutora ou de feminización nalgunhas especies de peces. Outro caso ben coñecido, consecuencia do abuso no uso de antibióticos, é o desenvolvemento e proliferación de especies de bacterias resistentes, sendo neste caso de especial importancia a contribución de antibióticos procedentes de actividades de gandería. Ademais, os efectos debidos á acumulación destas sustancias en organismos non obxectivo deberían ser igualmente considerados.

Os factores anteriormente mencionados explican que actualmente non haxa unha regulamentación específica que estableza niveis máximos de concentración á saída das plantas de tratamento. Por isto, os PPCPs son considerados como contaminantes emerxentes, tal e como recolle a seguinte definición:

"Contaminantes non actualmente incluídos en programas de monitorización rutinaria da calidade das augas, aínda que poden ser candidatos para futuros regulamentos, dependendo das investigacións realizadas no referente á súa ecotoxicidade, posíbeis efectos adversos para a saúde, opinión pública e dos datos sobre a súa presenza nos distintos compartimentos acuáticos". (6º programa MARCO da UE, proxecto NORMAN).

Os grupos terapéuticos de PPCPs máis comunmente detectados en plantas de tratamento son anti-inflamatorios, antibióticos, antiepilépticos, tranquilizantes, medios de contraste, anticonceptivos, fragrancias sintéticas e varios ingredientes de cosméticos. Neste traballo seleccionouse un grupo representativo de PPCPs baseándose nos seguintes criterios: traballar cun amplo grupo de sustancias presentes en niveis detectables en efluentes de depuradoras urbanas, que abarquen os principais grupos terapéuticos así como os máis comunmente recetados, sustancias que teñan distintas propiedades físico-químicas e polo tanto que presenten distinto comportamento e grao de eliminación durante os procesos de tratamento de augas residuais, e a dispoñibilidade de métodos analíticos de confianza para medir a súa concentración en matrices complexas como é o caso da auga residual.

Os procesos de tratamento convencionais están deseñados para a eliminación da materia orgánica e nalgúns casos de nitróxeno. Estas tecnoloxías non poden alcanzar a eliminación completa de moitos dos PPCPs, principalmente debido á súa baixa biodegradabilidade. Os MBR, que de feito son unha modificación do proceso convencional, permiten unha maior flexibilidade de cara á operación do proceso biolóxico. Hai tres aspectos fundamentais da tecnoloxía MBR de particular interese de cara á eliminación de distintos contaminantes orgánicos, particularmente aqueles de biodegradabilidad moderada:

- Os MBRs permiten controlar perfectamente o tempo de retención celular (TRC). Traballos previos nesta liña suxiren que este parámetro exerce unha influencia significativa de cara á adaptación dos microorganismos a unha entrada continua de PPCPs. Deste xeito, TRCs máis altos poderían conducir á mellora da capacidade da biomasa para eliminar sustancias recalcitrantes.

- E posible traballar con altas concentracións de biomasa, o que permite mellorar a eficacia do tratamento biolóxico nun espazo máis reducido. Adicionalmente, a biomasa desenvolvida durante un proceso MBR presenta propiedades físicas diferentes con respecto á biomasa xerada en sistemas convencionais, como unha maior superficie específica, propiedade directamente relacionada coa estrutura dos flóculos desenvolvidos, o cal podería incrementar a súa actividade encimática.

- Tendo en conta a alta calidade do efluente final, apto para reuso en moitos casos, o seu post-tratamento (mediante por exemplo, nanofiltración, ozonización ou filtración a través de columnas de carbón activo granular) podería ser máis eficiente que o mesmo proceso tratando un efluente sen filtrar, debido á nula presenza de sustancias que poidan interferir con eses procesos (materia orgánica, sólidos suspendidos, materia coloidal, etc.).

Deste xeito, o obxectivo desta tese doutoral é a avaliación dos MBR para a eliminación dunha categoría específica de microcontaminantes orgánicos, os PPCPs. A selección de sustancias de interese comprende 11 compostos farmacolóxicamente activos de 5 grupos terapéuticos distintos (anti-inflamatorios, antibióticos, antidepresivos, tranquilizantes e antiepilépticos), 3 fragrancias sintéticas caracterizadas polo seu elevado uso en deterxentes, xabóns e perfumes, 2 estróxenos naturais e unha hormona sintética, sustancias consideradas como disruptores endocrinos.

O primeiro obxectivo deste traballo (**Capítulo 1**) consistiu nunha revisión da literatura dispoñible, explicando en profundidade os distintos mecanismos de eliminación que os PPCPs poden sufrir durante os procesos de tratamento de augas residuais urbanas, así como os distintos parámetros operacionais e factores que inflúen no grao de eliminación alcanzado. Ademais, inclúese unha ampla descrición das sustancias estudadas detallando as súas propiedades físico-químicas máis importantes de cara a estudar o seu comportamento. O capítulo finaliza cunha análise das diferentes tecnoloxías de tratamento dispoñibles, principalmente enfocado á comparación de resultados obtidos en sistemas convencionais e MBRs de cara á eliminación de PPCPs.

No **Capítulo 2** explícanse os materiais e métodos experimentais utilizados para levar a cabo o traballo experimental desta tese doutoral. En primeiro lugar, os métodos utilizados para analizar as propiedades da fase líquida e parámetros convencionais (materia orgánica, nitróxeno, temperatura, contido en sólidos, pH e osíxeno disolto) utilizados para a caracterización da auga residual e os lodos. A continuación detállanse as técnicas utilizadas para a determinación de PPCPs en mostras líquidas e sólidas.

O **Capítulo 3** consistiu nun estudo preliminar sobre a presenza de PPCPs en augas residuais urbanas e o seu comportamento e destino final nas distintas unidades do tratamento de augas residuais. Esta investigación foi levada a cabo nunha planta de tratamento de augas residuais situada no Noroeste de Inglaterra, onde unha planta piloto completamente instrumentada foi operada nas súas instalacións. As unidades de tratamento convencional adoitan constar dun pretratamento para a eliminación de graxas, aceites e areas, seguidas a continuación dun tratamento primario no que a maioría dos sólidos suspendidos son eliminados en forma de lodo primario. Durante ambos pasos, unha fracción dos PPCPs pode eliminarse por adsorción ou absorción na fase particulada. Durante o tratamento secundario ou biolóxico, a eliminación de PPCPs conséguese fundamentalmente mediante dous mecanismos diferentes: degradación biolóxica e/ou sorción no lodo secundario. Trala sedimentación secundaria, obtense o efluente final do proceso. No sistema estudado neste capítulo danse de forma simultánea a eliminación de materia orgánica e a nitrificación, e representa fielmente a tecnoloxía máis comunmente utilizada en estacións depuradoras. Coa finalidade de obter máis información acerca dos mecanismos responsábeis da eliminación de PPCPs nas distintas unidades de tratamento, calculáronse balances de materia para cada PPCP detectado e cuantificado nas distintas mostras. A metodoloxía consistiu nunha campaña de mostraxe de dous días alternos na que se tomaron mostras líquidas á entrada e saída de cada unha das unidades da planta piloto. As mostras foron inmediatamente procesadas trala súa recolección. Aínda que non se tomaron mostras de lodo primario ou secundario, a cantidade de PPCPs presentes na fase sólida estimouse mediante coeficientes de distribución obtidos de traballos previamente publicados. Os PPCPs máis comunmente detectados foron antiinflamatorios e fragrancias sintéticas. Os balances de materia permitiron calcular o grao de eliminación alcanzado en cada unha das unidades da planta, o que foi útil para elucidar o seu comportamento ao longo de todo o proceso de tratamento. Ademais, a planta tratou unha corrente de licores de retorno provintes do proceso de centrifugación de lodos. Esta corrente foi tamén mostreada e considerada nos balances de materia, xa que contiña cantidades significativas de PPCPs. A partir dos resultados obtidos estimouse a saída diaria de PPCPs dunha instalación de tratamento de tamaño medio. Por exemplo, no caso do diclofenac, unha das sustancias máis recalcitrantes, estimouse unha emisión de 1.5 kg/d, considerando somentes a fase líquida.

No **Capítulo 4**, avaliouse o rendemento dun MBR de cara á eliminación dos PPCPs seleccionados. O equipo utilizado foi operado nas instalacións da Escola de Enxeñaría da Universidade de Santiago de Compostela. A alimentación consistiu nun medio sintético que simulaba a composición típica dunha auga residual urbana de carga media. Neste capítulo, a fase sólida non foi considerada, dado que o contido en sólidos da alimentación sintética é nulo e por tanto, a eliminación de cada un dos PPCPs podía ser calculada sen ese dato. A porcentaxe de eliminación máis alta (>90%) obtívose no caso dos antiinflamatorios ibuprofeno e naproxeno mentres que a carbamazepina, o diclofenac e o diazepam non foron eliminados significativamente. Sorprendentemente, a eliminación de fragrancias sintéticas foi só moderada (50-60%) en comparación cos resultados obtidos en sistemas convencionais (>80%). Os antibióticos mostraron distinto comportamento. Por exemplo, o sulfametoxazol foi parcialmente eliminado, o contido en trimetoprim non foi reducido en cantidades significativas (<20%), mentres que a roxitromicina e a eritromicina foron eficázmente eliminadas. Estes resultados mostran unha leve melloría comparados cos obtidos en sistemas convencionais, fóra das fragrancias sintéticas. En calquera caso, nesta etapa da investigación non se levou a cabo un estudo máis detallado sobre a influencia dos diversos parámetros operacionais. Outro aspecto importante para mellorar os resultados e aproximalos á situación real en plantas de tratamento é a substitución do medio sintético como alimentación por unha auga residual real. O uso de medios sintéticos influencian directamente o desenvolvemento da biomasa no MBR. Por isto, o MBR a escala piloto foi posto en funcionamento nas instalacións dunha depuradora de augas residuais urbanas a escala real e foi alimentado con auga residual sedimentada (**Capítulo 5**) ao longo dun período de operación estendido. Nesta etapa de operación seguiuse a concentración de sólidos no licor de mestura, a temperatura da biomasa e a adaptación dos microorganismos á entrada continua de PPCPs. En función da sustancia considerada, observáronse diferenzas significativas no seu comportamento e no grao de eliminación alcanzado. Por exemplo, o sulfametoxazol foi parcialmente eliminado (50-75%), cunha influencia significativa da concentración de sólidos no reactor. A eliminación dos outros antibióticos veuse incrementada ao longo da operación do MBR probablemente debido a efectos de aclimatación da biomasa. Por contra, ningún parámetro operacional exerceu influencia na eliminación de hormonas, ibuprofeno e naproxeno, que foron practicamente eliminados en todas as mostraxes (90-99%). Analogamente, as condicións de operación non afectaron á eliminación de sustancias recalcitrantes como a carbamazepina, o diazepam e o diclofenaco, e a súa eliminación foi incompleta (20-50%). A eliminación de fragrancias variou sensiblemente entre os distintos períodos operacionais: durante o inverno as eficacias de eliminación foron baixas mentres que en períodos de operación con temperaturas máis elevadas as eficacias de eliminacion incrementáronse até un 70%. Factores como a idade celular, a temperatura e as propiedades físico-químicas da biomasa desenvolvida en MBR poderían exercer tamén influencia nestes resultados.

No **Capítulo 6**, unha comparación directa entre o MBR a escala piloto e un biorreactor de lodos activos convencional a escala laboratorio foi levada a cabo mediante operación paralela de ambos sistemas. Os diferentes parámetros operacionais como a temperatura, o pH, a concentración de biomasa, o tempo de retención hidráulico (TRH) e a idade celular foron mantidos en valores similares nos dous sistemas. Ademais, ao longo da operación de ambos sistemas, probáronse 4 TRHs distintos, e dous TRCs. Por unha banda, o TRH influencia o tempo de contacto dos PPCPs solubles na fase líquida coa biomasa e a súa diminución por tanto podería afectar negativamente á calidade xeral do tratamento biolóxico. Por tanto, valores máis altos deste parámetro poden ser desexables para unha eliminación máis efectiva de microcontaminantes en procesos de depuración. O TRC é considerado un parámetro de operación crucial que se ten en conta de cara ao deseño das plantas de tratamento e que resulta fundamental para o tratamento biolóxico, de cara a obter mellores eficacias de eliminación de microcontaminantes. Isto débese a que os tratamentos biolóxicos operados con TRC altos permiten a proliferación de bacterias de máis lento crecemento, desenvolvéndose unha biocenosis máis diversa que pode cubrir un maior espectro de características fisiológicas e de adaptación. Deste xeito, as principais diferenzas atopadas en ambos sistemas correspóndense con compostos cuxas concentracións na fase sólida foron significativamente máis altas, como é o caso das fragrancias sintéticas ou os antidepresivos. Os coeficientes de distribución sólido-líquido para cada un dos PPCPs estudados foron calculados cos dous tipos de lodo xerado. Aínda que as diferenzas atopadas foron pequenas, o lodo do sistema convencional presentaba valores lixeiramente superiores destes coeficientes na maioría dos casos. En xeral, a capacidade do sistema convencional para eliminar compostos presentes en concentracións significativas na fase sólida (principalmente fragrancias) foi máis alta. Atopouse tamén unha relación entre o efecto das purgas de lodo que se efectuaron para o control do TRC e a capacidade de eliminación destas sustancias. Deste xeito, tras períodos de purgas intensivas, a eficacia xeral de ambos reactores eliminando fragrancias veuse incrementada e este efecto foi máis evidente no caso do MBR. Outro factor de elevada influencia foi o TRC. Cando se traballou con valores deste parámetro por baixo dos 10 días, a eliminación de moitas sustancias, como é o caso dos antibióticos, viuse severamente afectada. Só neste caso concreto, a capacidade do MBR para eliminar estas sustancias foi claramente superior á do CAS. En calquera caso, a principal conclusión deste capítulo é que as diferenzas entre ambas tecnoloxías de cara á eliminación de sustancias recalcitrantes son reducidas, e polo tanto a mellora dun sistema convencional incorporando o proceso MBR non estaría xustificada dende o punto de vista da eliminación de microcontaminantes.

O **Capítulo 7** considerou un aspecto diferente da tecnoloxía de biorreactores de membrana: o efecto do proceso de filtración. Deste xeito, 3 módulos en configuracion side-stream axustados a un MBR operado cun módulo de membrana mergullada de fibra oca foron simultaneamente avaliados ao longo de tres períodos de mostraxe. Algúns dos PPCPs estudados xa estaban presentes na auga residual xerada na Universidade de Cranfield (UK). En calquera caso, co obxectivo de traballar con sustancias representativas do maior rango posible de propiedades físico-químicas distintas, 5 PPCPs foron continuamente engadidos ao licor mestura durante a operación do biorreactor. Adicionalmente, a eficacia do proceso biolóxico tamén foi avaliada, considerando diversos parámetros operacionais (pH, temperatura e TRH) que foron variando ao longo dos distintos períodos de mostraxe. A porcentaxe de transformación máis elevada conseguiuse para o ibuprofeno (>98%) e o naproxeno (75 e 91%). Pola contra, a eliminación de carbamazepina foi baixa (36 e 47%). A eliminación de diclofenaco, sulfametoxazol e eritromicina aumentou paulatinamente co tempo de operación e por tanto dependeu do período de mostraxe considerada. Por tanto, a combinación de valores de pH acedos (medidos durante a mostraxe final), temperaturas cálidas e un tempo de operación prolongado cunha adición continua de PPCPs foron as condicións óptimas para maximizar a eliminación de PPCPs. En calquera caso, a eliminación completa do contido en microcontaminantes da auga residual nunca foi conseguida. As análises dos distintos permeados xerados no proceso mostraron concentracións máis elevadas que as medidas no sobrenadante do licor de mestura. Por tanto, datos adicionais provenientes da operación do MBR en Silvouta serviron para confirmar este comportamento. A capacidade do MBR para eliminar PPCPs da fase líquida non dependeu do material das membranas ou da súa configuración mentres que no caso de galaxolide e diclofenac, a etapa de filtración aparentemente contribuíu a incrementar a súa concentración nos permeados, reducindo a súa eliminación global da fase líquida.

Cos traballos levados a cabo e expostos ao longo da presente tese doutoral, profundouse sensiblemente no coñecemento sobre algúns aspectos crave sobre o uso de MBRs para a eliminación de PPCPs. Por tanto, considérase que a información xerada é valiosa de face á toma de decisións á hora de implantar un proceso MBR ou un proceso CAS para o tratamento de distintos tipos de augas residuais cando se considera o seu contido en contaminantes emerxentes, e tamén para establecer parámetros óptimos e estratexias de operación de MBRs que permitan optimizar o seu funcionamento en termos de eliminación destas sustancias.

Chapter 1

Introduction[1]

Summary

This introduction addresses the problem of micropollutants removal in sewage treatment plants trying to identify the main factors influencing their fate and behaviour. Firstly the most significant groups of substances that are continuously emitted into the environment are presented and the physical-chemical properties and biodegradability of representative compounds are discussed. This information is crucial to understand the main removal mechanisms occurring in sewage treatment plants, such as sorption, biodegradation and chemical transformation, as well as the distribution pathways of micropollutants once released into the environment. Key operational factors which influence the removal of these compounds, including the use of additives, temperature, biomass concentration and characteristics (microbial diversity, structure, etc.), as well as hydraulic and sludge retention time, are identified. A discussion focused on comparison of data corresponding to several configurations of activated sludge systems and membrane biological reactors is presented. So far, it is not clear how the type of technology affects micropollutants removal. A number of conclusions trying to explain the influence of different factors and some guidelines useful to enhance the removal of micropollutants in sewage treatment plants are presented.

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[1] Part of this chapter has been published as:

Francisco Omil, Sonia Suárez, Marta Carballa, Rubén Reif & Juan M. Lema. Criteria for designing sewage treatment plants for enhanced removal of organic micropollutants. Xenobiotics in the Urban Water Cycle. Fatta-Kassinos, Bester, Kümmerer (eds.), Springer, pages 283-306.

Outline

1.1. Introduction

Organic micropollutants refer to a wide group of carbon containing chemical compounds, mainly of xenobiotic nature, created by industrial processes either intentionally or as by- products, such as pharmaceuticals, personal care products, hormones, pesticides, brominated flame retardants, plasticizers, perfluorinated compounds, etc. Some of these substances are being considered for inclusion in the list of Persistent Organic Pollutants (POPs), i.e. compounds that are resistant to environmental degradation through biological, chemical or photochemical processes, thus capable of long-range transport, bioaccumulation in human and animal tissue, biomagnification in food chains, and exerting potential significant impacts on human health and the environment (Katsoyiannis and Samara 2007, Clarke et al. 2008, Stockholm Convention on Persistent Organic Pollutants 2009). Moreover, a significant number of these substances, those defined as Endocrine Disrupting Compounds (EDCs), may exert estrogenic activity on various higher organisms (Kester et al. 2000). During the last decade, the focus of environmental research has been extended from the more "classic" POPs such as organochlorine pesticides or Polychlorinated Biphenyls (PCBs) to the so called "emerging contaminants" such as Pharmaceuticals and Personal Care Products (PPCPs).

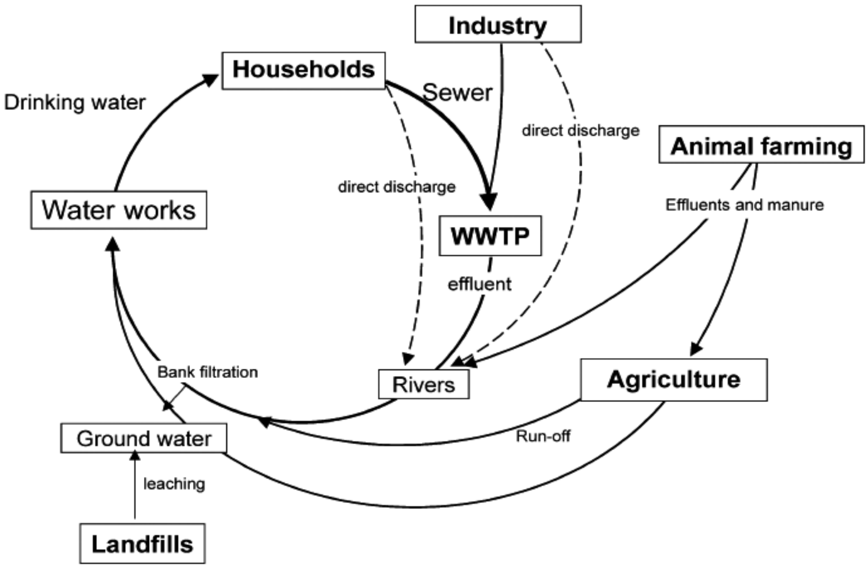


Figure 1-1. Main sources and routes PPCPs follow into the environment (Adapted from Jimenez, 2009)

Recent advances in analytical techniques, mainly related to the increasing use of Liquid Chromatography (LC) coupled with Mass Spectrometry (MS), have enabled the possibility of determining a wide variety of micropollutants which, although denoted as "emerging" because information about occurrence is fairly recent, have been discharged into the environment along decades, mainly in water bodies (Ternes 2007). Although PPCPs are present at low concentrations, many of them raise considerable toxicological concerns, either as sole compounds or also when present as components of complex mixtures.

The objective of this chapter is to present the main removal mechanisms that take place throughout Sewage Treatment Plants (STP), since, as shown on Figure 1-1, municipal wastewaters represent a significant emission source of micropollutants (Neumann et al. 2002, Joss et al. 2005). Most of the existing units operate with variations of the well known Conventional Activated Sludge (CAS) process. However, one innovative technology that is nowadays gaining popularity is the Membrane Biological Reactor (MBR). Postreatment methods, such as activated carbon or through ozone or advanced oxidation technologies, although very interesting as a polishing step leading to almost complete removal of these substances, can be considered as an "externality" of the common primary-secondary treatment, and are not discussed in this chapter.

1.2. Types of PPCPs, physico-chemical properties and biodegradability

It would be unrealistic to address the problem of PPCPs which can be found in wastewaters considering each one of the hundreds of different existing compounds. On the contrary, the scope here is to select some representative compounds belonging to the main groups of PPCPs and by considering their physico-chemical and biological properties, trying to understand the main mechanisms involved in their removal in Sewage Treatment Plants (STPs). Figure 1-2 shows the chemical structures of selected representative organic micropollutants.

1.2.1. Types of PPCPs

Among the wide number of substances that can be present in sewage, the following groups can be considered as the most important and representative according to the following criteria: i) wide occurrence in sewage reported in different areas of the world; ii) available analytical methodology; iii) high consumption; and, iv) research works already available.

Endocrine-Disrupting Compounds (EDCs)

This group comprises a diverse range of both natural and synthetic chemicals which include hormones, phthalates (used in the manufacture of plastics), alkylphenols (present in detergents and surfactants and discharged from industrial or municipal treatment systems), polychlorinated biphenyls (PCB; formerly used in electrical equipment), dioxins (released from incinerators), organochlorine pesticides and organohalogens (used as flame retardants). The hormones 17α-ethinylestradiol (EE2), ethynilestradiol (E2) and estrone (E1) can be selected as representative EDCs found in environmental water compartments. Because the types of substances that may alter endocrine function are so diverse, many substances included in the following groups below may also cause endocrine disruption at a certain extent.

Pharmaceuticals

Several pharmaceutical compounds belonging to different therapeutical classes have been detected in urban wastewaters and their fate and behaviour along STP treatment varies according to the type of compound. In this work, carbamazepine (CBZ), diazepam (DZP), ibuprofen (IBP), naproxen (NPX), diclofenac (DCF), sulfamethoxazole (SMX), erythromycin (ERY), roxithromycin (ROX), trimethoprim (TMP), fluoxetine (FLX) and citalopram (CTL) are used to illustrate the different behaviours observed in STPs.

Personal Care Products (PCPs)

The choice of personal care products (PCPs) was based on their high annual usage in a wide range of household products and concern over their possible effects on human and aquatic organisms (US National Library of Medicine, 2008). Polycyclic musk fragrances such as galaxolide (HHCB), tonalide (AHTN) and celestolide (ADBI) are selected as representative compounds.

1.2.2. Physico-chemical properties and biodegradability

Once a micropollutant is discharged into wastewater, it will be distributed between the different environmental compartments (e.g. surface water, soil, sediment) according to its physico-chemical properties, including the solubility, volatility, acidity, lipophilicity and sorption potential. Moreover, its persistence will depend on its resistance to be degraded biologically or abiotically. Table 1-1 shows the physico-chemical characteristics and biodegradability of selected PPCPs.

Volatility

The selected micropollutants (Table 1-1) posses very low Henry coefficients (H<10-5), except musk fragrances ($H= 7.3\cdot10^{-1} - 1.3\cdot10^{-4}$). Ternes and Joss (2006) observed that a significant amount of a compound will be stripped in a bioreactor with fine bubble aeration if H>0.003. Therefore, it can be concluded that stripping process is in general not relevant for the removal of micropollutants during wastewater treatment.

Acidity

Acidity (i.e. dissociation constant pKa) indicates if a specific ionic interaction is or not relevant for the sorption potential of a given compound. Moreover, for compounds containing functional groups which can be protonated and deprotonated, the pH value might play a crucial role.

For most of the compounds considered in this work, a specific ionic interaction is not relevant, since the molecule is non-ionised at the ambient neutral pH value. Only for acidic pharmaceuticals such as ibuprofen (pKa of 4.9-5.7) or diclofenac (4.1-4.2) a higher fraction might be negatively charged at neutral pH, and thus electrostatic interactions with positively charged molecules, such as coagulants, could be expected.

Lipophilicity
The octanol-water partition coefficient (Kow) is an indication of the substance hydrophobicity (lipophilicity) and thus it indicates if a specific compound tends to migrate from the aqueous phase into the liphophilic cell membrane of lipid fraction of the biomass. Jones et al. (2002) have observed that compounds exert: i) low sorption potential if log Kow <2.5, ii) medium sorption potential if log Kow between 2.5 and 4.0, and, iii) high sorption potential if log Kow > 4.0. From the compounds considered in this work, the highest Kow values were measured for the polycyclic musk fragrances.

Sorption potential
The sorption potential of a given compound is indicated by the solid-water distribution coefficient (Kd), which combines two driving forces for sorption: acidity and lipophilicity. Ternes and Joss (2006) indicated that only compounds having Kd values higher than 500 L kg-1 will be sorbed significantly onto sludge during primary and secondary treatment. In the case of sludge treatment, Carballa et al. (2007) showed that the limit of relevance below which sorption can be neglected is around Kd < l L kg^{-1}, since the sorbed amount is not only dependent on the distribution coefficient but also on the concentration of solids.

From data available in literature (Table 1-1), it can be observed that galaxolide and tonalide exert a high sorption potential followed by the hormones 17α ethinylestradiol and estradiol while the other compounds tend to remain in the water phase.

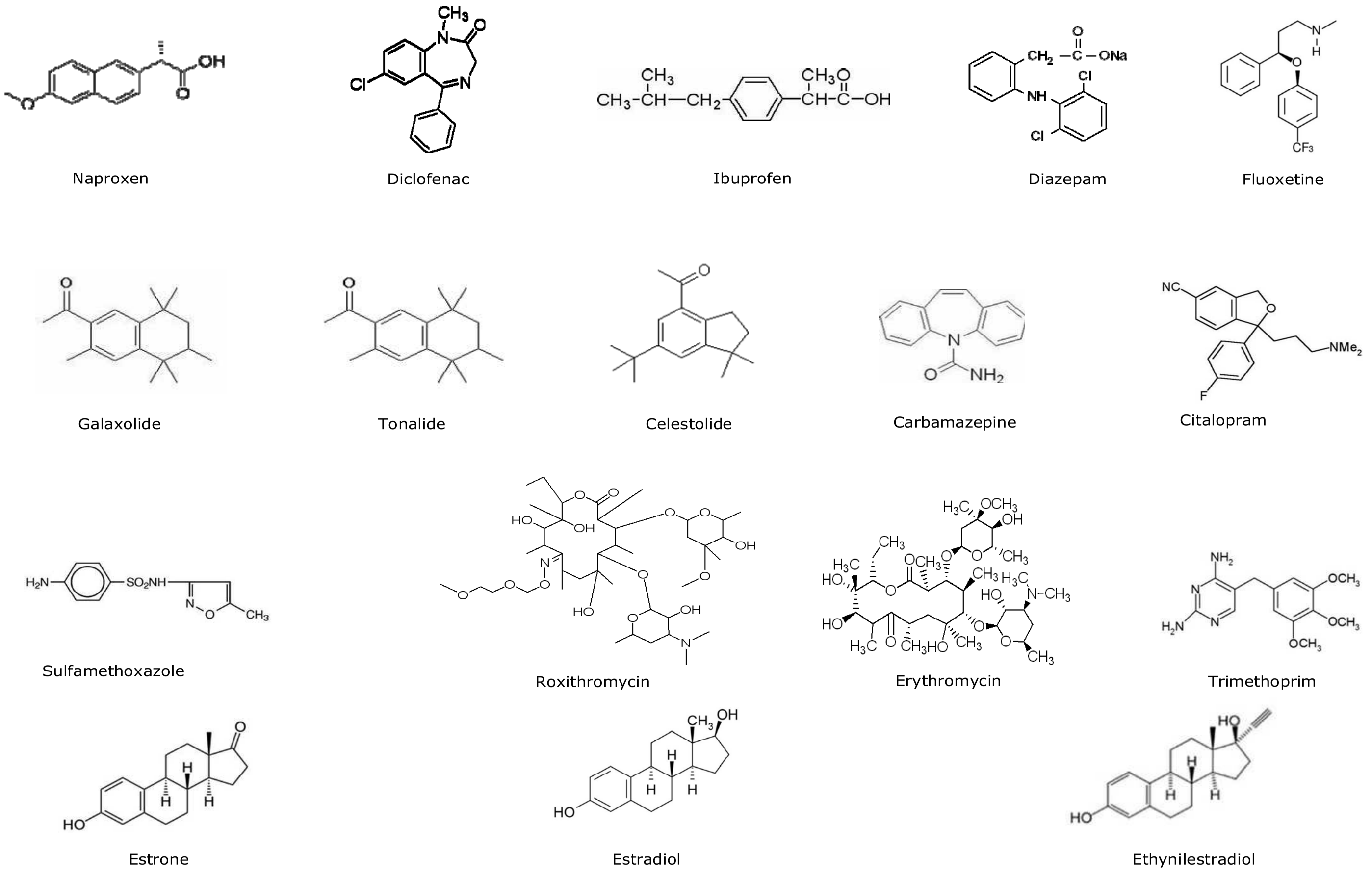
Biodegradability
Biodegradability of micropollutants will depend on their bioavailability, i.e. the potential of microorganisms to interact with them, which is related to the solubility of trace pollutants in the aqueous phase (in general very low), but also on the chemical structure of the compound. In this way, complex structures or toxic groups will make the breaking down of molecules more difficult. In order to have a measurement of the degree of biodegradability of micropollutants, kinetic constants (kbiol) can be experimentally determined. As a consequence of the very low

substrate concentrations, the approach commonly used to model the kinetic behaviour of micropollutants is pseudo first-order degradation (Joss et al. 2006) with direct proportionality of the transformation rate to the soluble substance concentration, as well as to the sludge concentration, although in a system operated under steady-state conditions the sludge concentration can be considered as constant. From Table 1-1, it can be observed that many PPCPs did not reach the minimum kbiol in the examined system required for any degree of degradation to occur (0.1 L g^{-1}SS d^{-1}). The biodegradation of the other listed compounds will strongly depend on the reactor configuration and only ibuprofen and the hormones are expected to be easily degraded in STPs (9-35 L g^{-1}SSd^{-1}).

Table 1-1. Physico-chemical characteristics and biodegradability of selected PPCPs

Category	Compound	S	H	pKa	log K_{ow}	log K_d	k_{biol}
EDCs	17α-ethinylestradiol	4.8 - 11.3	$7.9\cdot10^{-12}$	10.5 - 10.7	2.8 - 4.2	2.3 – 2.8	7 - 9
	Estradiol	3.6	$1.5\cdot10^{-9}$	10.4	3.9 - 4.0	2.3 - 2.8	300-800
	Estrone	30	$1.6\cdot10^{-8}$	10.4	3.1 - 4	2.4 - 2.6	200-300
PhACs	Carbamazepine	17.7	$1.1\cdot10^{-7}$	7.0-13.9	2.3 - 2.5	0.1 – 1.7	< 0.01
	Diazepam	50	$1.5\cdot10^{-7}$	3.3-3.4	2.8-3	1.3 - 1.6	~0.02
	Ibuprofen	21	$1.5\cdot10^{-7}$	4.9 - 5.7	3.5 - 4.5	0.9 – 1.4	9 – 35
	Naproxen	16	$1.4\cdot10^{-8}$	4.2	3.2	1.1 - 1.4	0.4 - 1.9
	Diclofenac	2.4	$1.9\cdot10^{-10}$	4.1-4.2	4.5	1.2 - 2.7	<0.1
	Fluoxetine	60	$3.6\cdot10^{-6}$	10.1	1.6	-	-
	Citalopram	31	$1.1\cdot10^{-9}$	9.6	2.9 - 3.7	2	-
	Sulfamethoxazole	610	$6.4\cdot10^{-13}$	5.6 - 6.0	0.5 - 0.9	1.2 – 2.6	< 0.1
	Erythromycin	1.4	$2.2\cdot10^{-27}$	8.9	2.5-3.0	2.2	0.5 - 1
	Roxithromycin	0.02	$2.0\cdot10^{-29}$	9.2	2.8	1.5 - 2.2	<0.3
	Trimethoprim	400	$9.8\cdot10^{-13}$	6.6-7.2	0.9-1.4	2.3	-
PCPs	Galaxolide	1.8	$1.3\cdot10^{-4}$	-	5.9 - 6.3	3.3 – 4.1	<0.03
	Tonalide	1.2	$5.1\cdot10^{-3}$	-	5.7	3.7 - 4.2	<0.02
	Celestolide	0.22	$7.3\cdot10^{-1}$	-	5.4 - 6.6	3.7 - 3.9	-

MW: molecular weight; s: solubility in water (mg·L^{-1}); H: Henry coefficient (atm·m^3·mol^{-1}); pKa: dissociation constant; K_{ow}: octanol-water partition coefficient; K_d: sludge-water distribution coefficient; k_{biol}: pseudo first-order degradation constant (L·g^{-1}SS·d^{-1}). [a]K_{oc} values in sediments. [b]Solubility of the K salt. [c]Biodegradability constant in d^{-1}. US National Library of Medicine, Toxicology Data Network 2008 (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB); US National Library of Medicine, ChemIDplus Advanced 2008 (http://chem.sis.nlm.nih.gov/chemidplus); Liu et al. 2005, Penteado et al. 2006, Gómez-Gutiérrez et al. 2007, Ben et al. 2008, EFSA 2008, Kasprzyk-Hordern et al. 2008, Suárez et al. 2008. "-": data not available.



Naproxen

Diclofenac

Ibuprofen

Diazepam

Fluoxetine

Galaxolide

Tonalide

Celestolide

Carbamazepine

Citalopram

Sulfamethoxazole

Roxithromycin

Erythromycin

Trimethoprim

Estrone

Estradiol

Ethynilestradiol

Figure 1-2. Chemical structures of selected representative organic micropollutants

1.3. Removal mechanisms in Sewage Treatment Plants

There are four possible removal mechanisms of PPCPs in STPs, which are sorption to solids, stripping (volatilisation) and biological and chemical transformation, although as stated previously, volatilisation of the micropollutants considered can be in general neglected.

1.3.1. Sorption

Sorption onto particulate matter is an important removal mechanism when the tendency of organic micropollutants to partition onto primary and secondary sludge is high. Two mechanisms, showed in Figure 1-3, are assumed to be relevant for sorption onto particulate matter (Ternes et al. 2004):

- Absorption: process in which molecules present in a given fluid enter into another bulk phase. It refers to the interactions of the aliphatic and aromatic groups of a compound with the lipophilic cell membrane of the microorganisms and the lipid fractions of the sludge. It is related to the substance's lipophilicity, characterized by the octanol-water partition coefficient (Kow)

- Adsorption: it is the physical adherence or binding of ions and molecules onto the surface of another molecule. It refers to the electrostatic interactions of positively charged groups of chemicals with the negatively charged surfaces of the microorganisms, and thus it is related to the tendency of a substance to be ionized or dissociated in aqueous phase, which is characterized by the dissociation constant (Ka). In general, negatively charged molecules, such as the anionic species of acidic compounds (e.g. naproxen), will not adsorb, whereas cationic species of other PPCPs (e.g. trimethoprim) will be more favored due to Van der Waals-type interactions.

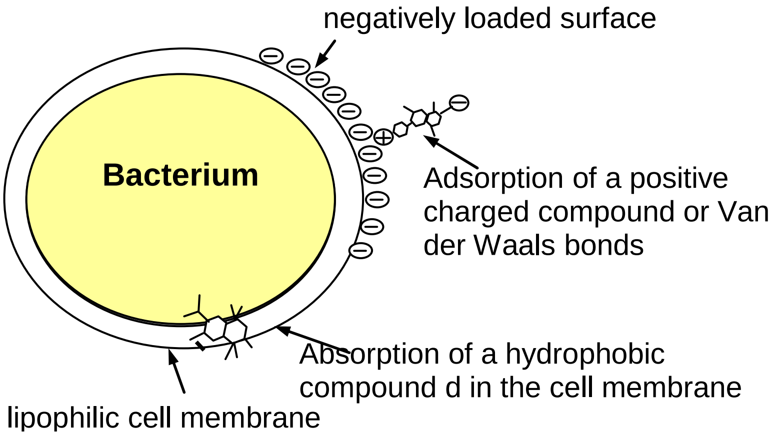


Figure 1-3. PPCPs sorption mechanism: absorption and adsorption

A common approach to determine the fraction of PPCPs sorbed onto sludge is the use of the solid–water distribution coefficient (Kd, in L kg^{-1}), defined as the ratio between the concentrations in the solid and liquid phases at equilibrium conditions.

1.3.2. Biodegradation

Biodegradation is the most important process resulting in transformation (structural changes) of organic micropollutants and it can vary from partial transformation to complete mineralisation. During biotransformation, metabolites are produced, whose character in terms of toxicity and fate in the environment may be different from the parent compounds. The same may be applied to conjugates. It is expected that deconjugation (conversion back into the original compounds) occurs in sewers, in STPs (mainly during primary treatment) or in the environment.

Biological degradation rates show big differences between compounds. There are few studies focused specifically on biological degradation of PPCPs. Joss et al. (2006) have determined pseudo first-order degradation kinetics (kbiol) for a large number of compounds under aerobic conditions with biomass from urban STPs. According to these degradation constant values, three groups of compounds can be differentiated into: (i) hardly biodegradable, with kbiol <0.1 L g^{-1}SS d^{-1}; (ii) highly biodegradable, with kbiol >10 L g^{-1}SS d^{-1}; and (iii) moderately biodegradable with 0.1<kbiol<10 L g^{-1}SS d^{-1}.

1.3.3. Chemical transformation

Apart from chemical oxidation that can be applied as post-treatment using Advanced Oxidation Processes (AOPs), the main chemical transformation that can occur along the STP is the deconjugation of certain micropollutants. Other natural processes such as photo- degradation are not relevant in STPs, although it should be considered in low turbidity waters such as rivers or lakes (Matamoros et al. 2008).

1. 4. Factors affecting removal of different types of compounds

In sewage treatment plants a number of separation processes (settling, volatilization, adsorption, etc.) and biochemical reactions take place and accordingly there is a vast number of factors that may affect the final results. Furthermore, it is important to realise that physical characteristics of sludge might influence biochemical reactions rate, considering the diffusion-reaction process, and also the development of particular biocoenosis.

1.4.1. Use of additives (e.g. coagulants, activated carbon)

Some modifications, such as the use of chemical additives, can be implemented in STPs in order to improve solid and fat separation and, consequently, to enhance the removal of those substances with high sorption properties. The basis is that natural partitioning between micropollutants and particles can be influenced by the presence of substances, such as coagulants, commonly used in some activated sludge processes for phosphorus co-precipitation.

Coagulation-flocculation has been shown to be a suitable pretreatment option for decreasing at some extent the amount of certain type of micropollutants in urban or hospital wastewaters. Carballa et al. (2005) showed that the use of ferric and aluminium salts enhances the removal of substances with high sorption properties, such as musk fragrances, up to 50-70%. Furthermore, the presence of trivalent cations could enhance the elimination of acidic compounds (such as naproxen) by ionic or quelating interactions. Similar conclusions were obtained by Suárez et al. (2009) working with hospital wastewaters, who found high removal efficiencies for fragrances such as galaxolide (>90%) which was attributed to their strong lipophilic character. This explains also the fact why better results were obtained in wastewater streams with higher fat content. For other compounds, such as the acidic and slightly lipophilic diclofenac (which is mainly deprotonated at pH-values from 6.5-7.5), the conclusion between both works points to a correlation between removal efficiencies and coagulant doses applied, probably related to the establishment of covalent interactions between the deprotonated pharmaceutical and the trivalent cations of coagulants that enhance adsorptive interactions. This fact may also explain the higher removal efficiencies reported for diclofenac in STP in which phosphorus is precipitated during secondary treatment with the addition of inorganic (ferric) salts (Suárez et al. 2008).

In a similar way, flotation systems have shown to produce similar results as coagulation-flocculation, which could be improved when treating wastewaters with high fat content (Carballa et al. 2005).

Powdered or granular activated carbon (PAC and GAC) have been shown to effectively enhance sorption of organic micropollutants like pesticides or taste and odour compounds (Newcombe et al. 1997). The most common application of these adsorbents is as a final step in drinking water plants or as postreatment in STP effluents. Although there are differences based on the type of activated carbon, all of them have been proven to significantly remove a large number of micropollutants especially in waters containing very low amounts of dissolved organic matter. In this way, Nowotny et al. (2007) obtained high eliminations of compounds such as Bisphenol A, carbamazepine, galaxolide or triclosan treating effluents from STPs, and even the highly polar compound iopromide could be highly removed when applying 10-fold higher dosages of activated carbon.

On the other hand, it is commonly considered that the adsorbability of micropollutants is significantly lower when they are in competition with background organic matter (Snyder et al. 2007), as would be the case of direct activated carbon additions into biological aeration tanks. In this case activated carbon efficiency could be greatly reduced by the presence of other organic compounds which compete for binding sites and can block pores within the activated carbon structure.

However, this configuration has been successfully used to reduce the toxicity caused by certain substances in the activated sludge process, as well as to maintain a lower concentration of potential harmful compounds that might inhibit the process. Although there is no information concerning micropollutants removal so far, the addition of powdered activated carbon enhanced removal efficiency of organic pollutants such as phenol and cyanide due to the combined action of biodegradation and adsorption processes (Papadimitriou et al. 2009). There are also interesting results in the use of hybrid systems which combine coagulation, activated carbon adsorption and MBR in order to achieve higher eliminations of certain organic pollutants such as dyes, which appear of interest for the removal of micropollutants (Lee et al. 2006).

Recent works treating the effluents containing the persistent carbamazepine in an CAS system showed that no significant removal was found until 500 mg/L of GAC was added directly in the aeration tank. Previous operation with 100 mg/L caused no effect, and the further increase of GAC concentration up to 1000 mg/L caused an increase in the removal efficiency of that compound to up to 43% (Serrano, 2008).

1.4.2. Temperature

Seasonal variations in removal rates of some micropollutants in full scale STPs were reported in several studies when comparing the total loads of compounds in the influent and effluent (Vieno et al. 2005, Castiglioni et al. 2006), in a way that higher removals were measured at higher temperatures. Considering the target substances individually, Castiglioni et al. (2006) reported that removal of some compounds was positively affected by summer temperatures (average 18.6ºC) compared to winter values (average 9.7ºC), while for other micropollutants the behaviour was similar during both seasons. However, in none of the mentioned studies, the different efficiencies in micropollutant removals could be exclusively attributed to the effect of operation temperature, since in Vieno et al. (2005) a simultaneous inhibition of nitrifying bacteria was observed, while in Castiglioni et al. (2006) the comparison was performed between data from different STPs.

It is also known that temperature can affect sorption and biodegradation rates of micropollutants during sewage treatment. For most compounds, equilibrium sorption decreases with increasing temperature (Hulscher and Cornelissen 1996), while microbial activity is enhanced at higher temperatures. However, taking into account that biological treatment in common STPs is based on activated sludge, which is typically operated at Hydraulic Retention Times (HRT) in the range of 4-24 h, sorption equilibrium can be assumed to be reached independently of operation temperature (Ternes et al. 2004).

Regarding microbial activity, the effect of temperature is dependent on the magnitude of the pseudo first-order degradation constant of the considered compound in the following way: i) highly biodegradable substances, such as natural estrogens (estrone (E1) and 17α-estradiol (E2)) and ibuprofen (FLX), are transformed to a high degree independently of operation conditions; ii) hardly biodegradable compounds such as carbamazepine (CBZ) is not removed even at higher temperatures and iii) the effect of temperature is especially noticeable for micropollutants with moderate kbiol as for example erythromycin (ERY) and sulfamethoxazole (SMX). The enzymatic mechanism responsible for degradation of certain micropollutants is often not activated as long as there is readily degradable carbon or nutrient sources available, which is the common situation in STPs. This was reported by Drillia et al. (2005) who found that the antibiotic sulfomethoxazole was used as carbon and nitrogen source by an enriched consortium. However, it was degraded whenever there was a depletion of carbon and nitrogen or both in the medium, while in the presence of acetate and ammonium nitrogen (alternative carbon and nitrogen sources, respectively), sulfomethoxazole was not degraded and remained intact.

Acclimation phenomena are also possible to occur, during which microorganisms present in a given system are able to degrade at a larger extent certain pollutants after a period of time due the establishment of a more diverse biocoenosis with broader physiological capabilities. The possibility that existing microorganisms in biological processes can acclimate to the presence of xenobiotic compounds by broadening their enzymatic spectrum was pointed out by several authors under different operational conditions (Najean et al. 1990, Layton et al. 2000, Zwiener et al. 2000, Chin et al. 2005). These effects might explain the SRT influence over the elimination of specific substances. Presently, there is little information available concerning the identification and isolation of enzymes present in the activated sludge which might be involved in biodegradation pathways of organic micropollutants. Further research must be accomplished in order to identify cometabolic routes as well as enzymatic oxidation kinetics.

1.4.3. Biomass concentration and structure

The structure of biomass is expected to have an impact on the removal of micropollutants, as it affects mass transfer between the target compound and microorganisms (i.e. the availability of the compound), the viability of bacteria and their enzymatic activity, although this aspect should be further investigated. Several operational parameters, such as SRT, composition of the treated wastewater, selection of technology, etc., may have an impact on the structure of the biomass developed in the system. This was illustrated by Cicek et al. (2001) when comparing the characteristics of biomass developed in a membrane bioreactor operated under different SRT, where the lowest SRT led to the highest biomass production rates, biomass viability and overall enzymatic activity. Similarly, in a previous study, Cicek et al. (1999) reported different biomass structure depending on the technology used for wastewater treatment, indicating that MBR sludge was composed of smaller flocs and contained many free-living bacteria, while the CAS system was composed of larger flocs and higher amounts of filamentous organisms. Additionally, biomass developed in the MBR had a higher viable fraction and a consistently higher overall activity than the CAS.

1.4.4. Hydraulic Retention Time (HRT)

Taking into account that HRT determines the mean residence time of soluble compounds inside the system, the biological degradation kinetics will be the key point in establishing if this parameter does or not affect the efficiency of the process. An example of this was illustrated by Huang et al. (2008) for the degradation of plasticizer di-(2-ethylhexyl)phthalate (DEHP) in an anaerobic-anoxic-aerobic activated sludge reactor, in which the overall removal efficiency of the compound was independent of the considered HRT, although the contribution of the individual processes to that removal was influenced by the HRT. The study revealed that anaerobic degradation of DEHP increased from 14% to 23% when the HRT of the reactor was incremented from 5 h to 14 h, indicating that the process with the slowest kinetics was positively influenced by higher HRT. Similarly, Tauxe-Wuersch et al. (2005) determine the influence of HRT on the removal of acidic drugs in full-scale STP with primary and secondary treatment showing that the behaviour depended on the physico-chemical properties of the compound: i) hardly biodegradable compounds as clofibric acid and diclofenac were not removed independently of HRT; ii) ibuprofen, which is a soluble compound with a moderate biological degradation constant (kbiol 9-35 $L.gSS^{-1}.d^{-1}$), was principally removed during biological treatment with efficiencies varying from 0% to 79%.

These differences were attributed to the fact that the three STPs did not have the same HRT and a correlation was obtained indicating that an increased residence time resulted in higher ibuprofen degradation. Additionally it was observed that absolutely no removal of this compound was observed during a heavy-raining week; iii) ketoprofen can be partially removed during primary treatment according to its moderate lipophilicity (log Kow 3.1), although the efficiency showed to depend on the HRT of the primary sedimentation tank.

1.4.5. Sludge Retention Time

As shown in Clara et al. (2005), SRT of biological reactors may influence the removal efficiency of degradable micropollutants, such as bisphenol A, ibuprofen, bezafibrate and natural estrogens, for which a positive effect on their removal was observed when working at higher SRT, although only until a critical value of 10 days. Considering that SRT defines the mean residence time of bacteria inside the system and influences consequently the microbial diversity developed in biological reactors, a minimum SRT of 10-15 days was proposed as necessary to ensure the development of a diverse biocoenosis, which comprises nitrification, denitrification, and phosphorus removal. This main conclusion in Clara et al. (2005) implies that once the growth of the whole set of microorganisms which may be involved in wastewater treatment is guaranteed, SRT does not further influence the removal of micropollutants.

Transformation of galaxolide and tonalide (musk fragrances) during biological treatment in a nitrifying/denitrifying pilot plant of around 90% was reported in Suárez (2008), although it had been previously shown that removal of these compounds was mainly driven by absorption (Bester 2004, Joss et al. 2005, Kupper et al. 2006). These differences could be attributed to the higher SRT applied to pilot plant operation, since musks are highly lipophilic compounds, implying that the retention time inside the reactor is determined by SRT, rather than by HRT. This fact explains how compounds with a very low kbiol (<0.03 L.gSS^{-1}.d^{-1}) can be biologically transformed during the secondary treatment step.

1.5. Does technology influence micropollutants removal?

Although the experience is still limited, it can be pointed out that there is an impact of the selected technology on xenobiotic substances removal. In this way, Miège et al. (2008) compared removal rates of a wide number of micropollutants in different technologies (pretreatment plus primary treatment, activated sludge with and without nitrogen treatment, membrane biological reactors, etc.). Figure 1-2, adapted from Miège et al. (2008), compares the average removal rates reported for different micropollutants in CAS systems and in other technologies.

As a main conclusion, activated sludge processes with nitrogen and phosphorus removal appears to be one of the most efficient technologies, together with MBRs combined with nitrogen elimination, in terms of micropollutants removal. However, conclusions can not be generalised because in some cases the number of data are quite limited. For example only 2 and 4 sets of data are available for fixed biomass and submerged biofilters respectively. Moreover, it is still not clear if such systems succeed in enhancing micropollutants removal when compared with conventional systems working with similar operational parameters.

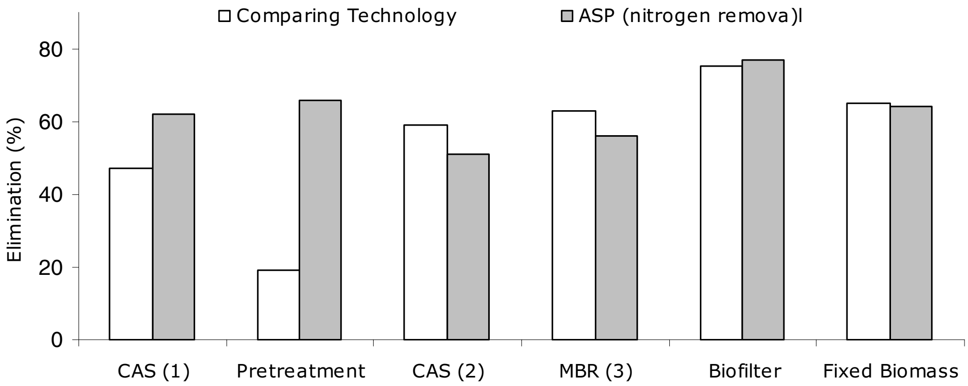


Figure 1-2. Removal efficiencies (%) corresponding to the compilation of data of more than 50 different PPCPs obtained for CAS process with nitrogen treatment compared with various treatment processes. (CAS 1): without nitrogen treatment; (CAS 2): nitrogen and phosphorus removal; (MBR 3): nitrogen removal (adapted from Miège et al., 2008).

MBR technology, which is based in the combination of a suspended biomass biological reactor and a membrane microfiltration or ultrafiltration step, has gained wider acceptance throughout the industry. One of its major advantages is the potential production of a high quality effluent which can be even appropriate for water reclamation. However, membrane filtration does not enhance the elimination of most micropollutants by means of a size-exclusion mechanism. There is quite generalised tendency that consider MBRs as a definite technology for urban wastewater treatment, including micropollutants removal. Although the know-how accumulated in the last years by these systems has been enormous and a number of well-known advantages are now clearly stated, some of the limitations of these systems (such as capital and energy costs) should be also considered. Moreover the MBR results reported so far concerning micropollutants are not significant enough to clearly show its advantage. In fact, both technologies have been shown to achieve comparable results in terms of micropollutants removal when operating at similar conditions. Table 1-2 summarizes information from different studies.

Table 1-2. Average removal efficiencies for 39 hydrophilic xenobiotics in MBR and CAS treatment (adapted from Weiss and Reemtsma 2008). "ana-anx-aer": anaerobic-anoxic-aerobic. SRT in d, HRT in h, MLSS in $g \cdot L^{-1}$. Legend for removal efficiencies: <20% (-);20-40% (+);40-90% (++); >90% (+++).

	Removal		**Operating Conditions**			
Substance	**MBR**	**CAS**		**MBR**	**CAS**	**Ref.**
BTSA	++	+	***Scale***	lab	full	[1]
Btri	++	+	***SRT***	26-102	15	
5-TTri	++	-	***HRT***	7 - 14	18	
4-Ttri	-	-	***Redox***	aerobic	ana-anx-aer	
1,6-NDSA	+	-	***MLSS***	5	5	
1-NDSA	+++	+++				
2,6-NDSA	-	+				
bayrepel-acid	+++	+	***Scale***	pilot	full	[2]
DEET	++	-	***SRT***	15 and 65	7	
MCPP	+	-	***HRT***	6.7	12	
TCEP	+	+	***Redox***	aerobic	aerobic	
carbamazepine	-	-	***MLSS***	3 and 12	2	
LAS C9-C13	+++	+++				
phenazone	++	+	***Scale***	pilot	full	[3]
acetylaminoantipyrin	++	+	***SRT***	∞	n.m.	
formylaminoantipyrin	++	-	***HRT***	10	9	
17b-estradiol	+++	+++	***Redox***	aerobic	aerobic	
estrone	+++	+++	***MLSS***	11 -20	4	
17a-ethinylestradiol	+++	++				
propyphenazone	++	-				
benzothiazole	++	+	***Scale***	lab	full	[4]
2-aminobenzothiazole	++	+	***SRT***	400	12 -14	
2-methylthiobenzothiazole	-	-	***HRT***	7 and 10	22	
BTSA	-	-	***Redox***	aerobic	ana-anx-aer	
2-mercaptobenzothiazole	++	++	***MLSS***	15	4.6	
2-hydroxybenzothiazole	+++	++				
clofibric acid	++	++	***Scale***	pilot	full	[5]
diclofenac	++	+	***SRT***	15-26	15	
ketoprofen	+++	++	***HRT***	18	18	
ibuprofen	+++	+++	***Redox***	ana-anx-aer	ana-anx-aer	
mefenamic acid	+++	++	***MLSS***	13	4	
naproxen	+++	++				
CDEA	+++	+++	***Scale***	pilot	full	[6]
NP	+++	+++	***SRT***	8 and 26	15	
NP1EO	+++	+	***HRT***	11 and 18	18	
NP2EO	+++	++	***Redox***	ana-anx-aer	ana-anx-aer	
NP3EO + NP15EO	+++	++	***MLSS***	13	13	
OP2EC	++	+				

Only one reference ([1]: Weiss and Reemtsma 2008, [2]: Zuehlke et al. 2006, [3]: Bernhard et al. 2006, [4]: Gonzalez et al. 2007, [5]: Kloepfer et al. 2006 and [6]: Kimura et al. 2007) per compound is given since no significant differences were found in the different studies reported, with the sole exception of benzothiazole-2-sulfonate (BTSA), which was partially eliminated in the work of Weiss et al. (2008) and no significant removal was reported by Kloepfer et al. (2004). Several substances were identified as recalcitrant (-) in both treatments, for example 4-Ttri (4- Tolyltriazole), 1,3-NDSA, 2-methylthiobenzothiazole and carbamazepine. When elimination is not feasible through a biodegradation pathway and sorption is not relevant due to the physico-chemical properties of the pollutant, it is not expected that any technology based on biodegradation will succeed in removing these substances. Similarly, those compounds exhibiting a rapid degradation rate (+++) such as ibuprofen, 1-NDSA and hormones, were efficiently removed in both conventional and MBR systems.

There is a number of compounds with slower degradation kinetics (+ or ++), for which removal efficiencies are affected by the type of reactor. In fact, removal efficiencies corresponding to more than 15 compounds listed in Table 1-2 increased by at least a 25% when MBR was used, although a complete depletion was hardly achieved. The explanation for the increased efficiency should be mainly attributed to the higher sludge concentration and higher sludge retention time rather than the hydraulic retention time that was not, in general, so different between the two technologies.

It is important to have in mind that, up to now, no additional works have managed to operate CAS and MBR systems with the same operational parameters. Other factors might play a crucial role, such as the different biomass structure found in MBRs (Massé et al. 2006), the higher enzymatic activity derived from a more important cell lysis, better conditions to achieve acclimation or the development of a stable population of nitrifiers, among others. However, a direct link between MBR biomass properties and micropollutants removal has not been established.

1.6. Conclusions: guidelines to enhance the removal of micropollutants in STPs

A number of conclusions can be derived from the joint analysis of all factors discussed along this paper. For this, two main characteristics of each micropollutant, biodegradability (kbiol) and partition coefficient between the solid and liquid phase (Kd) are considered. In Table 1-3 a summary of those conclusions, giving some examples, is presented.

- Compounds with high kbiol, such as 17β-estradiol, are very well transformed independently of operational conditions.

- The extension of transformation of compounds with moderate kbiol and low Kd values, such as ibuprofen, depends on the HRT.

- Compounds with low kbiol and high Kd values, such as musk fragrances, are retained in the aeration tank by sorption and significantly transformed when the SRT is high enough to permit biological degradation.

- Compounds with moderate kbiol and Kd values, such as 17α-ethinylestradiol, are moderately transformed during biological treatment, being the removal efficiency positively affected by higher SRT.

- Compounds with low kbiol and Kd values, such as carbamazepine, are not removed and not biotransformed regardless of operational conditions.

- Transformation of compounds with moderate kbiol, as for example fluoxetine, depends on factors affecting their pseudo-first order kinetics, such as temperature and sludge concentration.

- Microbial diversity influence degradation of certain micropollutants which showed to be recalcitrant (low kbiol) in CAS processes, as observed for diclofenac.

- Removal of acidic substances, as diclofenac, which are partially deprotonated at neutral pH, can be enhanced by the addition of cationic coagulants.

- Removal of ionisable substances during primary treatment can be improved by pH control. This is the case of carbamazepine, for which the protonation of its amide group improves its removal through adsorption.

- Removal of lipophilic compounds (high Kow), such as musks, can be enhanced during primary treatment in presence of fat that favours absorption.

Most of the research dealing with micropollutants removal in STP has been focussed on the determination of efficiency of different technologies or different working conditions whereas there is very scarce information about the scientific background concerning the main removal mechanisms (volatilisation, sorption, chemical and biological transformation), the influence of the characteristics of the target compound (physico-chemical and biodegradability) and the role of biomass properties (microbial diversity, structural conformation, enzymatic activity, etc.). In order to be able to develop new strategies for the successful treatment of micropollutants, more detailed knowledge about those issues is still required.

Table 1-3. Factors affecting removal in biological treatment.
Removal efficiency <20% (-); 40-70% (+); >80% (++).

	Removal	Influencing Factors	Example
$k_{biol}\downarrow$ $K_d\downarrow$	-	None, as not degraded	Carbamazepine, diazepam
$k_{biol}\downarrow\uparrow$ $K_d\downarrow$	++	HRT	Ibuprofen
$k_{biol}\uparrow$ $K_d\downarrow\uparrow$	++	None, as quickly degraded	17β-estradiol
$k_{biol}\downarrow$ $K_d\uparrow$	++	SRT	Galaxolide
$k_{biol}\downarrow\uparrow$ $K_d\downarrow\uparrow$	+	SRT	17α-ethinylestradiol
$k_{biol}\downarrow\uparrow$	+	T, VSS	Fluoxetine, citalopram, naproxen
$k_{biol}\downarrow$	+	Sludge type	Diclofenac
Ionisable	+	Coagulants, pH	Diclofenac
$K_{ow}\uparrow$	++	Fat content	Galaxolide

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Chapter 2

Materials and Methods

Summary

The analytical methods used in this work are described in this chapter, including conventional chemical parameters used for wastewater characterisation, as well as the analysis of Pharmaceutical and Personal Care Products (PPCPs) in both liquid and solid phase.

From the conventional chemical parameters included in this work, Total and Soluble Chemical Oxygen Demand (COD_T and COD_S) and Total and Volatile Suspended Solids (TSS and VSS) were determined following Standard Methods (APHA, 1999). These are therefore not further described in this chapter. Other parameters, such as nitrogen in the form of ammonia ($N\text{-}NH_4^+$) and nitrate ($N\text{-}NO_3^-$) have been measured by analytical procedures optimised in our laboratories and are thus described in detail throughout this chapter.

Descriptions of the analysis of PPCPs in both liquid and solid-phase and the solid-phase extraction (SPE) and solid-phase microextraction methodologies are also provided, including polycyclic musk fragrances (galaxolide, tonalide and celestolide), neutral pharmaceuticals (carbamazepine and diazepam), acidic pharmaceuticals (ibuprofen, naproxen and diclofenac), anti-depressants (fluoxetine and citalopram), estrogens (17β-estradiol, estrone and 17α-ethinylestradiol) and antibiotics (roxithromycin, sulfamethoxazol, trimethoprim and erythromicyn).

The biomass was characterised also by means of the technique of Scanning Electron Microscopy (SEM). The identification of the different populations present in the biomass samples was carried out by Fluorescent In Situ Hybridization (FISH) and the particle size distributions were determined with a laser particle size analyzer. However, these specific analytical methods were used in a single part of the work, and consequently are described in the corresponding chapters, as well as the experimental set-ups.

Outline

2.1. Conventional chemical analysis

2.1.1. Nitrogen

In waters and wastewaters, the forms of nitrogen of greatest interest are, in order of decreasing oxidation state, nitrate, nitrite, ammonia and organic nitrogen. All these forms, as well as nitrogen gas (N_2), are biochemically interconvertible and they are the components of the nitrogen cycle. Total oxidised nitrogen is the sum of the nitrate and nitrite forms. Nitrate generally occurs in trace quantities in surface waters, but it may attain high levels in some groundwaters or effluents of nitrifying biological treatment plants (up to 30 mg $N\text{-}NO_3^-$/L). A limit of 10 mg $N\text{-}NO_3^-$/L has been imposed on drinking water to prevent disorders. Nitrite is an intermediate oxidation state of nitrogen, either in the oxidation of ammonia or in the reduction of nitrate. Such oxidation and reduction may occur in wastewater treatment plants, water distribution systems and natural waters.

Ammonia nitrogen

Ammonia nitrogen is determined by a colorimetric method. It is based on the reaction of NH_3 with HClO and phenol, forming a strong-blue compound (indophenol) which can be colourimetrically determined using a spectrophotometer (Shimadzu UV-1603, UV-Visible) at 635 nm.

Reagents:

a. Solution 1: Phenol-nitroprusiate: 15 g of phenol and 0.05 g of sodium nitroprusiate are added to 250 mL of buffer solution (30 g $Na_3PO_4 \cdot 12H_2O$, 30 g $Na_3C_6H_5O_7 \cdot 2H_2O$ and 3 g EDTA per liter, adjusted to pH 12).

b. Solution 2: Hipochloride: 15 mL of commercial bleach are mixed with 200 mL of NaOH 1 N and filled up to 500 mL with distilled water.

To 2.5 mL of sample (diluted if necessary to get a maximum concentration of 1 mg $N\text{-}NH_4^+$/L), 1 and 1.5 mL of solution 1 and 2, respectively, are added. After waiting 45 min at room temperature, the concentration of $N\text{-}NH_4^+$ is measured in a spectrophotometer at 635 nm. The quantification is done with a 6-8 points calibration curve in the range of 0-1 mg $N\text{-}NH_4^+$/L, using NH_4Cl as standard.

Nitrite

Nitrite concentration in wastewater is determined following the method 4500-NO_2^--B described in Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WPCF, 1999).

Nitrate

Nitrate concentration in wastewater is determined following the method 4500-NO_3^--B described in Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WPCF, 1999).

2.1.2. Inorganic anions: NO_2^-, NO_3^-, Cl^-, PO_4^{3-}, Br^-, $S_2O_3^{2-}$ and SO_4^{2-}

Nitrite (NO_2^-), nitrate (NO_3^-), chloride (Cl^-), bromide (Br^-) phosphate (PO_4^{3-}), sulphate (SO_4^{2-}) and thiosulphate ($S_2O_3^{2-}$) are determined simultaneously by ionic chromatography using a Metrohm 861 Advanced Compact IC equipped with a column Metrosep A Supp5-250 and a 853 CO_2 Supressor. The liquid sample (minimum volume of 10 mL) and the mobile phase are introduced with a Metrohm 838 autosampler into a sample loop and forced to migrate through the column. The mobile phase is a buffered aqueous solution of sodium carbonate (3.2 mM) and sodium bicarbonate (1 mM). It carries the sample from the loop onto the column, which packing consists of polyvinyl alcohol with quaternary ammonium groups (particle size 5 µm). After their passage through the column, the target analytes (anions or cations) are detected by conductivity measurements.

Four to six calibration points for each ion in the range of 0.05-150 mg/L are used for the quantification of the samples. Previously to the analyses, the samples are filtrated through 0.45 mm membrane (Millipore). A typical chromatogram is shown in Figure 2-1.

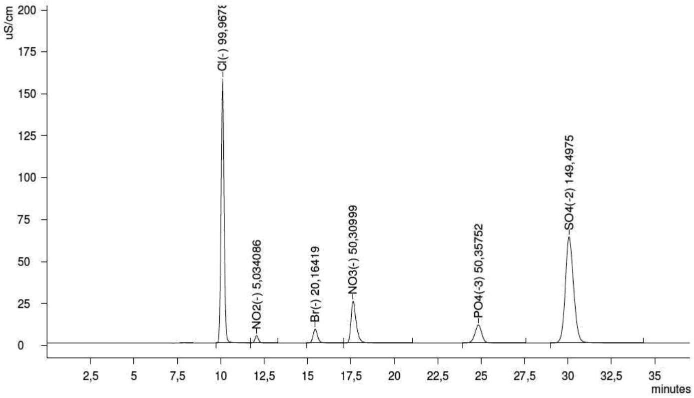

Figure 2-1. Ion chromatogram which shows the retention times of the detected anions

2.1.3. Other control parameters: pH and dissolved oxygen (DO)

pH

The pH is one of the key parameters measured in wastewater treatment systems, since its control is important to maintain the biological activity of the microorganisms involved in the treatment process. The pH measurements were performed with an electrode (Crison Instruments, S.A., 52-03) equipped with an automatic compensatory temperature device (Crison Instruments, S.A., 21-910-01) and connected to a measure instrument (pH mV-1). The sensibility of the instrument is 1 mV, corresponding to 0.01 pH units. The electrode is calibrated at room temperature with two standard buffer solutions of pH 7.02 and 4.00.

Dissolved Oxygen (DO)
A dissolved oxygen probe (AQUALITYC, model OXI-921) connected to a meter (M-Design Instruments TM-3659) was used to monitor DO concentration in the reactor.

2.2. PPCPs analysis

The analysis of PPCPs in aqueous samples comprises filtration, extraction, sample preparation, derivatisation (if needed) and detection. In order to avoid interferences caused by suspended solids, raw samples were filtered over glass fibre filters (APFC04700 or AP4004705, Millipore). In the case of MBR permeate samples, filtration step was not necessary. The sample extraction was performed with two different methodologies. The most frequently used was the Solid Phase Extraction (SPE), which is a separation process by which the analytes present in an aqueous sample are sorbed in a solid phase material and then desorbed by elution with an organic solvent. In order to increase the number of samples extracted, a SPE 24-Position vacuum manifold (Phenomenex, USA) was used (Figure 2-2).

Figure 2-2. Manifold used in the Solid Phase Extraction (SPE) step for simultaneous concentration of different samples

The Solid Phase MicroExtraction (SPME) was used with non-filtered samples and allows the quantitative determination of analytes in both liquid and solid-phase (total load), using small coated silica fibres which are dipped into the aqueous sample for a pre-determined time (Figure 2-3). 10 mL of sample were immersed in a bath at 100ºC for 5 min to equilibrate temperature. SPME methodology was only used for the analysis of musk fragrances (García-Jares et al., 2002). In the case of solid samples (primary or biological sludge), the ultrasonic solvent extraction (USE) methodology was necessary as a previous step, in order to extract PPCPs sorbed onto the sludge (Ternes et al., 2005). 0.5-1 g of sludge was sequentially extracted with 6 and 3 mL of methanol and 2 times with 3 mL of acetone. For each extraction step, the slurry was ultrasonicated for 15 min. Then, it was centrifuged at 3600 rpm for 5 min and the supernatant was collected. The solvent fractions were finally combined and the resulting volume was reduced down to 1 mL in a BUCHI device (40ºC, 150 mbar). The resulting extract was redissolved in 100 mL of Milli-Q water prior to solid-phase extraction. For some compounds, a derivatization step prior to the final quantification is also needed to assure the substance stability along the detector. Liquid or Gas Chromatography coupled to Mass Spectrometry (LC-MS or GC-MS, respectively) was used for the final quantification (Figure 2-4).

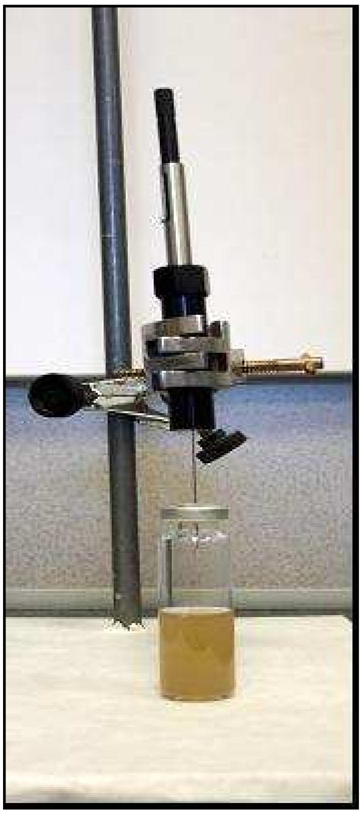

Figure 2-3. Set-up used in the Solid Phase Micro-Extraction (SPE) step for the determination of the total load of musk fragrances

Figure 2-4. Varian Saturn 2100T GC/MS/MS used for the determination of anti-inflammatories, musk fragrances, carbamazepine and diazepam.

2.2.1. Polycyclic Musk Fragrances and neutral compounds (CBZ and DZP)

Two different extraction methods have been used to determine polycyclic musk fragrances (Galaxolide: HHCB, Tonalide: AHTN and Celestolide: ADBI), depending on the objective: the SPME and the SPE.

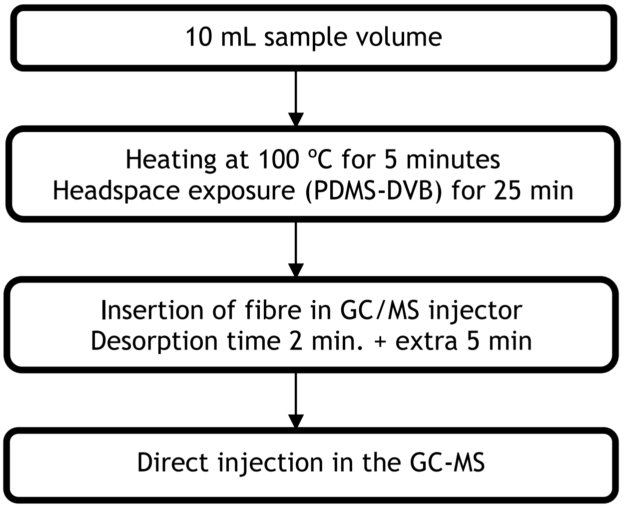

Figure 2-5. Scheme of the SPME method for polycyclic musks

10 mL of sample were immersed in a bath at 100ºC for 5 min to equilibrate temperature. Then, the PDMS-DVB (65 µm polydimethylsiloxane-diviylbenzene, Supelco, USA) was exposed to the headspace over the sample (HS-SPME) for 25 min. Once the exposition finished, the fibre was immediately inserted into the GC injector and the chromatographic analysis was carried out. Desorption time was set at 2 min, although an extra period of 5 min was considered to avoid carryover effect. The SPE method (Figure 2-6) was used for the determination of the soluble load in liquid samples. 500 mL of wastewater was filtered through glass fibre filters, adjusted to pH 2.5 with HCl 1 N and spiked with the surrogate standard (meclofenamic acid and dihydrocarbamazepine). Afterwards, 100 mL of sample were used for the enrichment in the case of sewage samples and 250 mL for effluents and permeates. SPE was performed in OASIS HLB 60 mg 3cc cartridges (preconditioned by flushing 3 mL ethyl-acetate, 3 mL methanol and 3 mL Milli-Q water adjusted to pH 2.5) with a flow rate of ~15 mL/min. Then, the cartridges were dried completely by a nitrogen stream for 45 min and the analytes eluted with 3 mL of ethyl-acetate. PCB-30 (2,4,6-trichlorobiphenyl) was added as internal standard to the final extract. Finally, the GC/MS detection was carried out in a CP 3900 chromatograph (Walnut Creek, CA, USA) equipped with a split–splitless injector and connected to an ion-trap mass spectrometer (Varian Saturn 2100 T).

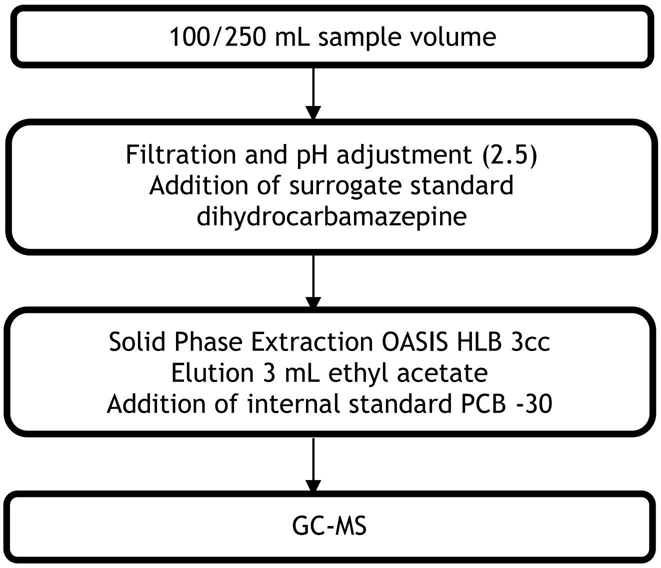

Figure 2-6. Scheme of the SPE method for musks and neutral pharmaceuticals

2.2.2. Acidic pharmaceuticals

For the acidic pharmaceuticals (Ibuprofen: IBP, Naproxen: NPX and Diclofenac: DCF), the analytical method (Figure 2-7) used is based on Rodriguez et al. (2003):

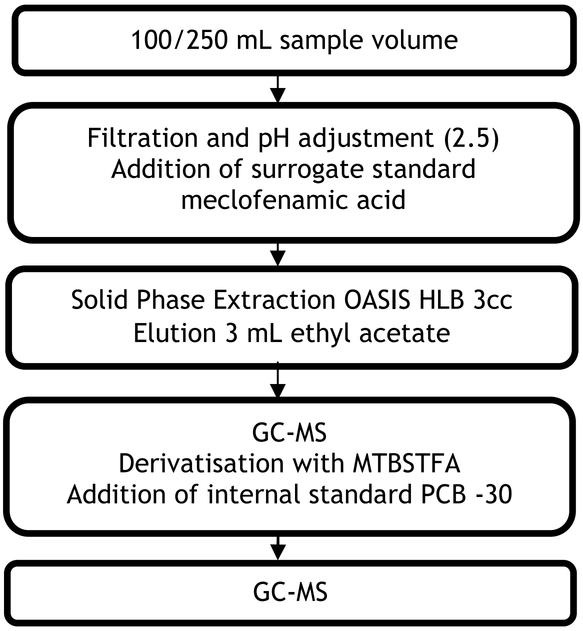

Figure 2-7. Scheme of the analytical method for acidic pharmaceuticals

Filtration, extraction and elution steps were simultaneously performed with that of fragrances and neutral pharmaceuticals (Figure 2-2). A fraction (800 µL) of the 3 mL-extract from the SPE cartridge was derivatised with 200 µL of MTBSTFA (N-Methyl-N-(*tert.*-buthyldimethylsilyl) trifluoroacetamide at 60ºC for 1 hour. Afterwards, PCB-30 was added as internal standard and detection by GC/MS/MS was carried (Varian Saturn 2100 T).

The operating conditions of the GC/MS/MS for fragrances, neutral and acidic compounds determination are summarised in Table 2-1:

Table 2-1. Operating conditions of GC and MS detection

	Fragrances and Neutral compounds		**Acidic compounds**
	Total load	*Soluble load*	*Soluble load*
		Injector split-splitless	
Splitless time	1 min	1 min	1 min
Injector temperature	260°C	250°C	280°C
Gas flow (He)	1 mL/min	1 mL/min	1 mL/min
Pressure pulse	No	30 PSI (1 min)	No
Injector time/ volume	8 min	1 µL	1 µL
Solvent	Ethylacetate	Ethylacetate	Ethylacetate
		GC temperatures	
Initial temperature	60°C	60°C	50°C
Initial time	2 min	2 min	1 min
1st ramp	10°C·min^{-1}	10°C/min	10°C/min
Final temperature	250°C	250°C	180°C
Isothermal time	0 min	0 min	7 min
2nd ramp	20°C·min^{-1}	20°C/min	10°C/min
Final temperature	280°C	280°C	230°C
Isothermal time	9.5 min	9.5 min	25 min
3rd ramp	-	-	20°C/min
Final temperature	-	-	250°C
Isothermal time	-	-	5 min
		MS parameters	
Ionization mode	EI	EI	EI
Filament current	20 µA	20 µA	10 µA
Ion trap temperature	220°C	220°C	220°C
Transfer line temperature	280°C	280°C	280°C
Multiplicador voltage	1700-1750 V	1700-1750 V	1700-1750 V
Scan velocity	0.76 s·scan^{-1}	0.76 s/scan	1 s/scan
Mass spectrum	45-400 m/z	45-400 m/z	100-330 m/z (10-25 min) 140-420 m/z (25-57 min)
m/z quantification	HHCB, AHTN (243) ADBI (229)	HHCB, AHTN (243) ADBI (229) CBZ (193+236) DZP (256+283)	IBP (263) NPX (287) DCF (352+354+356)

2.2.3. Anti-depressants and antibiotics

The chemical analysis for the determination of the concentration of fluoxetine (FLX), citalopram (CTL), sulfamethoxazole (SMX), trimethoprim (TMP), roxithromycin (ROX) and erythromycin (ERY) was performed as published by Vanderford et al. (2003). 250 ml of filtered sewage or permeate/effluent samples were passed through an Oasis HLB 60 mg cartridge (approximately at 15–20 mL/min) that had been sequentially pre-conditioned with methanol, methyl tert-butyl ether and Milli-Q water adjusted at the same pH that the sample (3 mL each). The elution step was performed with a mixture of methanol (1.5 mL) and methyl tert-butyl ether (1.5 mL) (Figure 2-8). Subsequently, all samples were analysed using a Agilent Liquid Chromatograph API 4000 G1312A equipped with a binary pump and an autosampler HTC-PAL. Separation was carried out with a column Phenomenex Sinergy 4u Max-RP 80A de 250 x 4.6 mm (particle size 4 µm) and the detection was performed with a triple quadruple Mass Spectrometer (MS) (Applied Biosystems, Foster City, CA). The mobile phase used was a mixture of two solutions: H_20 with formic acid (FA, 0.1 %) and methanol (100%). Other procedures involving optimization of the MS, selection of best ionization source and mode for each analyte, source polarity and adjustments were carried out according to the method published by Vanderford et al. (2003).

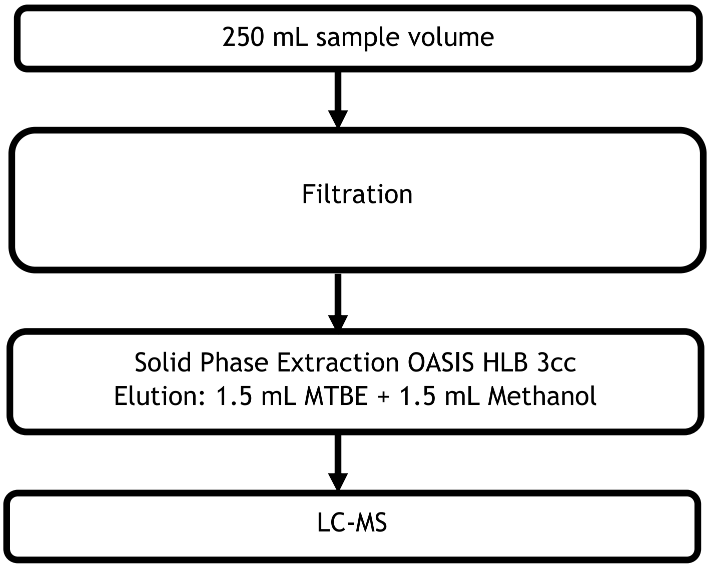

Figure 2-8. Scheme of the analytical method for anti-depressants and antibiotics

2.2.4. Estrogens

For the hormones estrone (E1), 17β-estradiol (E2) and 17α-estradiol (EE2), the filtration, extraction and elution step was simultaneously performed with that of antibiotics and antidepressants (Figure 2-8). A fraction (800 µL) of the 3 mL-extract from the SPE cartridge was analyzed by LC/MS according to the parameters described in Table 2-2:

Table 2-2. Operating conditions of LC/MS

Injection parameters

	Antibiotics and antidepressants	Hormones
Mobile Phase	A: H_20 + formic acid (0.1 %) B: methanol (100%)	A: H_20 + formic acid (0.1 %) B: methanol (100%)
Injector temperature	450 ºC	450 ºC
Pressure pulse	147 bar	156 bar
Equilibration time	17 min	7 min
Injection volume	5 µL	10 µL

m/z quantification

Antibiotics and antidepressants		Hormones	
SMX	254+156+92.2	E2	255+159.1+133.1
TMP	291.1+260.9+230	EE2	279.2+133.1+159.2
ROX	837.7+679.3+158.2	E1	271.1+133.2+159.2
ERY	734.4+158.3+576.3		
FLX	310.1+44.1+148.2		
CTL	325.05+109.2+262.2		

Gradient Elution

Antibiotics and antidepressants				Hormones			
time (min)	Flow Rate (µL/min)	A (%)	B(%)	time (min)	Flow Rate (µL/min)	A (%)	B(%)
0	700	85	15	0	700	30	70
3.5	700	85	15	2	700	12	88
10	700	20	80	15	700	6	94
13	700	20	80				
13.1	700	10	90				
21	700	10	90				

2.2.5. Limits of Detection (LOD) and Quantification (LOQ)

Table 2-3 summarises the LOD and LOQ for the analytical methods applied in the present work.

Table 2-3. Limits of Detection (LOD) and Quantification (LOQ) for the analytical methods used in the determination of PPCPs (sample volume: 250 mL)

PPCP	LOD (ng/L)	LOQ (ng/L)	PPCP	LOD (ng/L)	LOQ (ng/L)
HHCB	8	24	E1	4	12
AHTN	8	24	E2	4	12
ADBI	8	24	EE2	4	12
CBZ	400	1200	SMX	2	6
DZP	200	600	ROX	0.4	1.2
IBP	25	75	TMP	2	6
NPX	25	75	ERY	0.4	1.2
DCF	100	300			
FLX	0.4	1.2			
CTL	0.4	1.2			

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Chapter 3

Occurrence and fate of selected PPCPs in a conventional sewage treatment plant located in north west UK[1]

Summary

The occurrence and fate of Pharmaceutical and Personal Care Products during sewage treatment have been studied in a pilot-scale plant consisting of primary settler (2.85 m^3), aeration tank (1.845 m^3) and secondary settler (0.5 m^3). The plant, placed in a sewage treatment plant in NW of the UK, was fed with both raw sewage and liquors produced after sludge centrifugation. The studied unit truly reproduces the activated sludge process, with the advantage that during the sampling week it was completely devoted to this study.

Six different locations along the water line of the plant were sampled and analyzed in order to detect fragrances and pharmaceutically active compounds. The stable operation of the system and the data gathered during the sampling days enabled the possibility to estimate PPCPs mass balances which were useful in order to confirm their behaviour and final fate along the treatment process. The possible influence of treating the return liquor on conventional parameters removal is of interest since most of previous research does not consider the additional treatment of high-charged streams and consequently it was studied. Concerning PPCPs removal along the different units of the pilot plant, different behaviour was observed depending on the physical-chemical characteristics of the considered substances. Anti-inflammatories underwent a degradation process and were almost completely removed and musk fragrances were partially removed by sorption onto the suspended solids separated in the primary settler and on the biomass existing in the aeration tank, due to their strong lipophilic characteristics.

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Outline

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3.1. Introduction

Over the last decade, the occurrence of trace amounts of pharmaceutical products and other chemical ingredients from cosmetics in lakes, rivers and even tap water has become an increasing concern (Ashton et al. 2004; Heberer 2002; Tanabe 2005 and Ternes 1998). All these chemicals are often referred as Pharmaceutical and Personal Care Products (PPCPs) and they are used in large quantities throughout the world (Kummerer 2000). Only in the last 10 years have analytical methods become sufficiently sensitive to detect and quantify PPCPs in spite of their low concentrations (ppb or ppt level). Since then, a few chronic ecotoxicological effects on organisms have been reported so far (Fent et al. 2006). A well known example is the dramatic decrease of vulture species population in India, caused by traces of the anti-inflammatory drug diclofenac which is present on carrion (Oaks et al. 2004). This has led to the supposition that similar effects might be occurring in surface waters, where aquatic organisms are continuously exposed to complex mixtures of micropollutants and their metabolites. Unfortunately, there is a lack of information available regarding PPCPs potential effects at environmentally relevant concentrations that might be exerted in the aquatic environment. Some studies carried out so far have identified the development of antibiotic microbial resistance in the environment (Göbel et al. 2007) and potential endocrine disrupting effects as being those of chief concern.

As a consequence, the general knowledge about PPCPs fate has been gradually improved. Nowadays, it is well known that most of these compounds are released into the environment through many different pathways. Most relevant are excretions via urine or faeces into the sewage system of unmetabolized fractions of drugs, or into fields in the case of veterinary drugs (Ashton et al. 2004), and the rinsing off of cosmetics during shower. The flushing of unused or expired medications down the toilet or sink and the discharge of hospital wastewater may also be relevant.

Eventually, PPCPs reach Sewage Treatment Plants (STPs) where they may be removed by volatilisation, sorption to either suspended solids or biological sludge and chemical or biological transformation. The dominant mechanism will differ depending on the physical-chemical properties of the substance and its biodegradability. These properties are considered a key aspect concerning the diverse behaviour observed for micropollutants in STPs (Suarez et al. 2008). Presently, recalcitrant PPCPs are released into surface water, considered as their final fate since plants are not specifically designed to achieve their significant removal (Heberer et al. 2002). Most of the research on this field has been carried out during normal operation of full-scale treatment plants considering only the liquid phase of both raw influent and final effluent to estimate overall removal rates but missing the influence of the different removal mechanisms. A few studies considered different sampling points along the studied STP, involving intensive sampling campaigns to generate mass balances (Carballa et al. 2004), but the size of these locations and the daily variations of incoming crude sewage flow makes the generation of consistent results challenging. Pilot or lab scale studies in better controlled conditions, working with synthetic sewage where micropollutants are spiked, have also been performed (Joss et al. 2006), improving the general knowledge about biodegradability of these substances by estimating their biodegradation constants (Kbiol). Whilst providing more precise data, such lab-based studies are not necessarily representative of processes operated at larger scale. Moreover, the use of synthetic media in some cases instead of municipal sewage as feeding might not be representative of the real behaviour of these substances along sewage treatment since actual full scale works would also be expected to receive both PPCP parent compounds and their metabolites and conjugates, the fate of which may differ from the parent compounds.

Additionally, many works have been carried out using significantly different values of operational parameters such as Hydraulic Retention Time (HRT) and Sludge Retention Time (SRT) which are known to influence the removal capacity of the treatment processes, making difficult to draw general conclusions when comparing different research works. Therefore, many of the gathered results are subjected to a high uncertainty, as can be easily observed comparing removal efficiencies from different works. For example, ibuprofen (IBP) and naproxen (NPX) are considered polar substances that easily undergo biological transformation, but reported removal rates ranges between 60-90% and 40-90% respectively (Ternes, 1998; Nakada et al., 2006; Gomez et al., 2007, Stumpf et al., 1999, Zwiener et al., (2000) and Carballa et al., (2004). Diclofenac (DCF) available data is even more dispersed and contradictory since reported removal rates ranged from 0% up to 75% (Ternes, 1998; Stumpf et al., 1999; Zwiener et al., 2000; Clara et al., 2005a; Gomez et al., 2007).

In the case of musk fragrances, they are substances characterized by a high lipophillicity which enables their removal from the liquid phase following a sorption mechanism onto either suspended solids or biological sludge. However, a further degradation or volatilisation might be achieved due to their retention inside the aeration tank but there is not a general consensus about this possibility and again, reported removal rates are affected by a high variability (39-90% for galaxolide (HHCB) and 53-96% for tonalide (AHTN) according to Kanda et al., 2003; Bester, 2004 and Kupper et al., 2006.

It is thus important to confirm previous research and to extend understanding of the processes involved in PPCPs removal along the different STP unit operations performing experiments in conditions and under operational parameters similar to those of the actually set on conventional sewage works considering every stream entering or leaving the different units involved in the sewage treatment process. Simultaneous treatment of the return liquor produced after sludge centrifugation, also known as sludge reject water, is of particular interest since nowadays increasingly stricter environmental legislation is requiring that many existing STPs improve their final effluent quality incorporating technologies able to cope with the simultaneous elimination of organic matter and nutrients, mainly nitrogen and phosphorus. These streams are characterized by high ammonia content and therefore a low COD/N ratio and its treatment might mean a potential improvement for STPs since reject waters from sludge digestion might contain around 10-30% of the nitrogen load entering to the treatment plant.

Examples of typical sludge reject water composition can be found at Ghyoot et al., 1999 and Wett et al, 1998. However, the composition of such streams in terms of micropollutants and their hypothetical influence in the final effluent quality is usually missed. This work aims to provide more extensive knowledge on the occurrence and fate of PPCPs in sewage treatment processes, and in particular biological treatment, primary and secondary clarification, under strictly controlled conditions by means of a fully instrumented pilot plant operating at the premises of a full-scale STP treating raw sewage and a stream of the liquor produced after primary and excess humus sludge treatment, recycled into the aeration tanks and which was considered as a relevant sampling point due to its possibly high PPCPs content.

3.2. Materials and methods

3.2.1. Pilot-scale activated sludge plant

A diagram of the pilot plant used in this work and photographs are shown in Figure 3-1 and 3-2. The activated sludge tank received both settled sewage and the return liquor which were brought in to the plant weekly from the on-site works centrifuge. The composition of the sludge before centrifugation was 59% of primary sludge, 23.4% of secondary sludge and 17% of sludge from the intermediate settlement tanks. To assist centrifugation, liquid polymer (Allied Colloids) was added to the sludge.

In order to operate the biological unit with a HRT of 6 h, the flow of crude sewage into primary clarifier was maintained at around 300 L/h, return liquor stream was fed at 9 L/h and RAS rate was set at 1. Throughout the sampling campaign, samples for PPCPs analysis were collected twice a day on each one of the six sampling locations, during two alternate days. Sampling points were the crude sewage, settled sewage, mixed liquor suspended solids (MLSS) supernatant, return activated sludge (RAS), final effluent and return liquor. Routine physical-chemical analysis was carried out on a daily basis to assess the performance of the pilot plant with respect to aerobic carbonaceous removal and nitrification. These analyses include Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solid (TSS) and ammonia. On-site test facilities were available to carry out operational testing such as conductivity, pH and dissolved oxygen for plant monitoring purposes.

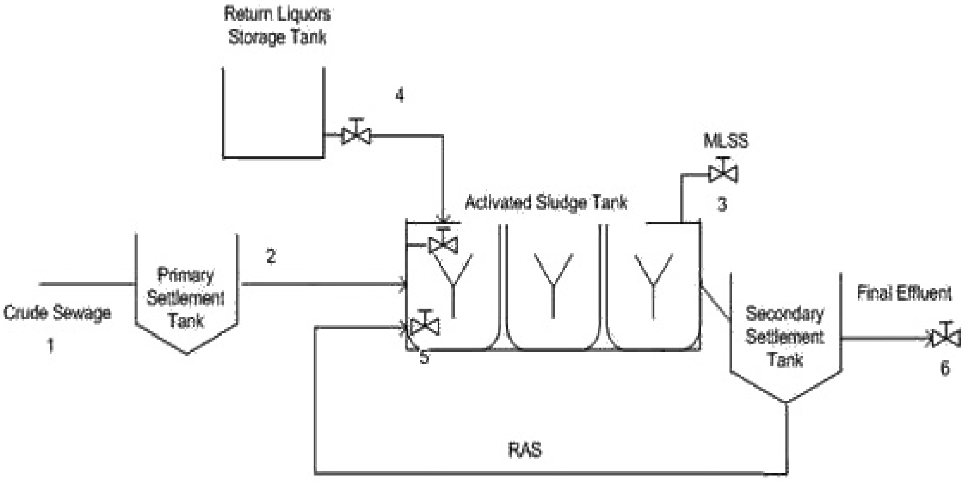

Figure 3-1. Flow sheet of the pilot plant and considered sampling points

3.2.2. Analytical methods

Total suspended solids (TSS) were determined according to standard methods (Methods for the Examination of Waters and Associated Materials, 105, HMSO, 1980). Total and soluble chemical oxygen demand (COD and CODf respectively) and ammonia (NH_4^+) were determined using a Spectroquant Cell Test and measured on a Nova 60 model spectrophotometer (Merck, West Drayton, UK). The total and soluble biochemical oxygen demand (BOD and BODf respectively) was determined according to the standard method (Methods for the Examination of Waters and Associated Materials, 130, HMSO, 1988). Conductivity and pH were measured using a Jenway 3540 pH & Conductivity Meter (Jenway, Dunmow, UK) according to the standard method (Methods for the Examination of Waters and Associated Materials, 14, HMSO, 1978).

Figure 3-2. Pilot-scale plant consisting of aeration tanks (A), primary settlers (B) and secondary settler (C)

A portable lab was used to immediately perform the pre-treatment steps and the solid-phase extraction (SPE) for PPCPs analysis (Figure 3-3). Substances considered in this work were galaxolide (HHCB), tonalide (AHTN), celestolide (ADBI), ibuprofen (IBP), naproxen (NPX), diclofenac (DCF), carbamazepine (CBZ) and diazepam (DZP).

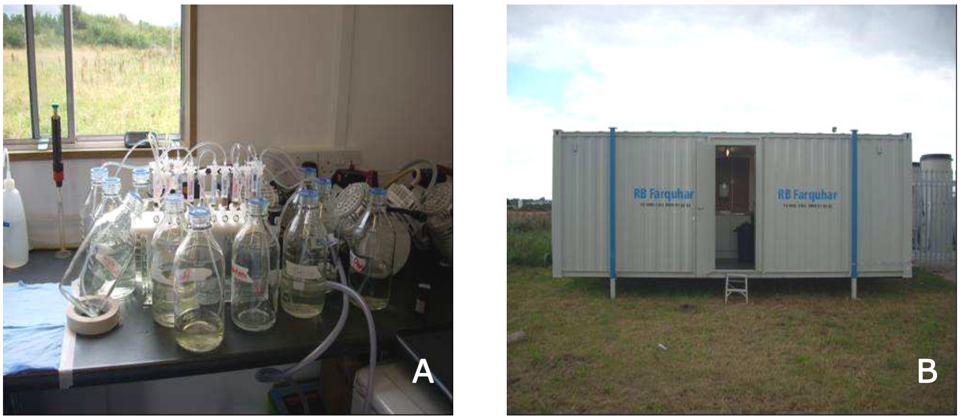

Figure 3-3. Demonstration of the SPE step for the enrichment step (A) and portable lab for on-site sampling processing (B).

Sample treatment for PPCPs analysis consisted of a pre-filtration step through glass-fibre filters (0.45 µm) immediately after sample collection (Figure 3-4), followed by filtration through nitrate cellulose membrane filters and addition of sodium diazide as biocide, in order to avoid further biological degradation.

Figure 3-4. Material for pre-filtration and filtration step.

PPCPs content was determined after solid-phase extraction (SPE) of 100 mL pre-treated samples for sewage (crude or settled) and return liquor, or 250 mL samples for MLSS or RAS supernatant and final effluent samples, using 60 mg

OASIS HLB cartridges (Waters, Milford, MA, USA). Cartridges were eluted with 3 mL of ethyl acetate. SPE extract was divided in two fractions for the direct determination of the soluble content of carbamazepine, diazepam and fragrances. The second fraction was used for the determination of anti-inflammatory drugs following silylation. GC/MS was used to determine the concentration of the investigated compounds in the SPE extract. Every sample was analyzed by duplicate in the GC/MS and the results were averaged. More detailed information about the analysis of the soluble content of anti-inflammatory compounds, CBZ, DZP and musk fragrances can be found at Rodríguez et al. (2003).

3.2.3. Mass balances calculations

Prior to determining mass balances, PPCPs concentrations sorbed onto sludge were estimated using only solid-water distribution coefficients from the literature (Kd in L·Kg^{-1}) (Table 3-1). This parameter, defined as the ratio between the concentration in the solid and liquid phases at equilibrium conditions, can reasonably predict PPCPs sorption in STP processes (Schwarzenbach, 2003). Due to the high variability of reported distribution coefficients and considering that these parameters might be matrix dependant, a selection criteria was followed: Chosen Kd values were always experimentally determined, avoiding the selection of coefficients estimated with theoretical calculations. Table 3-1 shows the parameters that were chosen, distinguishing between primary or secondary sludge and returning liquor, and other physical-chemical properties.

Table 3-1. PPCPs detected and their physical-chemical properties (Suarez et al., 2008). Kd values (L/Kg) from: Ternes et al. 2004; Urase and Kikuta 2005. Kbiol values (L·gSS^{-1}·d^{-1}) from Joss et al., 2006.

PPCP	**Therapeutic class**	**Pka**	**K_{biol}**	**Primary**	**Secondary**	**Return Liquor**
Ibuprofen	Anti-inflammatory	4.9-5.2	9 - 35	<1.3	0.9	1.1
Naproxen		4.2	0.4-1.9	1.1	1.1	1.1
Diclofenac		4.1-4.2	<0.1	2.7	1.2	1.95
Galaxolide	Fragrances	-	<0.03	3.7	3.3	3.5
Tonalide		-	<0.02	3.7	3.4	3.55

In carrying out mass balances, some assumptions had to be made:

- Since no data were found in the literature, PPCPs concentration sorbed onto return liquor was estimated using a mean Kd value calculated from the ones reported for both primary and secondary sludge.

- The primary Kd value for NPX was assumed to be the same as that for secondary.

- Since the IBP, DCF and AHTN concentrations measured in certain samples were below its detection or quantification limit, mass balances which depended on these samples were calculated using this limit values.

Total mass fluxes for PPCPs load on each stream (Figure 3-5) were calculated according to the next expression:

$$m = Qin(S + X) \qquad \text{[Eq. 1]}$$

Where m is the mass flux of PPCP (μg PPCP/d) entering or leaving a specific unit of the STP, Q is the sum of incoming flows (L/day), S is the PPCP concentration in the liquid phase (μg PPCP/L) and X (μg PPCP/L) is the amount of PPCP estimated to be sorbed onto the sludge phase.

3.3. Results and discussion

3.3.1. Conventional parameters

For the correct development of this research, it was crucial to work with the pilot-plant in similar conditions to correctly operated full-scale STPs. 8 weeks after seeding with sludge from the on-site activated sludge bioreactor, a stable MLSS concentration and acclimatisation (>96% nitrification) was achieved. Only then, the sampling campaign was carried out. Tables 3-2 and 3-3 show the values for conventional parameters measured in different sampling points and general sludge quality parameters during the sampling week, which confirms the normal operation of the system.

Dissolved oxygen content was always kept high enough (~6 mg/L) to guarantee the development of a stable population of heterothropic and nitrifying bacteria. Temperature and pH were not controlled, but their values (16 ºC and 7 respectively) are representative of the normal situation in full-scale STPs. The settleability of sludge, measured in terms of the Sludge Volumetric Index (SVI) was in the common range for a conventional activated sludge plant and therefore, after secondary settling, the level of solids in the final effluent was low (<76 mg/L), which was a necessary condition to maintain an extended retention time of over 150 days, optimum for the development of slowly growing bacteria such as nitrifiers. COD and ammonia overall removals were high, reaching efficiencies up to 80 and 98% respectively thus confirming that both biological processes (carbon removal and nitrification) occurred efficiently.

Table 3-2. Conventional parameters measured along the different locations of the pilot plant (Units: mg/L)

Stream	BOD	BOD_f	COD	COD_f	NH^{4+}	TSS
Settled Sewage	111.5	55.8	299.2	132.8	24	83.4
Final Effluent	10.5	2.7	56	25.9	0.4	32
RAS	-	-	-	-	-	9818
Aeration Tank	1922	47.4	6499	59.5	-	4870

Table 3-3. Physical-chemical parameters measured in the pilot plant

	T (ºC)	pH	DO (mg/L)	ORP (mV)	SVI
Aeration Tank	16.6	7.2	6	66.4	101.3
Secondary Settler	16.6	7.1	2.2	16.4	-

3.3.2. Occurrence of selected PPCPs in the pilot plant.

Table 3-4 displays average concentrations measured for PPCPs detected during the two sampling days in the different locations, with levels ranging from 0.1 to 7.5 μg·L^{-1}, together with the detection/quantification limits and the removal rates from the liquid phase.

The musk fragrance ADBI was not found at any sample and, on the contrary, HHCB and AHTN were found at substantial levels (2.0 and 0.9 ppb respectively). These two fragrances comprise about 95% of the EU market and 90% of the USA market for all polycyclic musks (HERA, 2004). The musk fragrance ADBI was not found at any sample and, on the contrary, HHCB and AHTN were found at substantial levels (2.0 and 0.9 ppb respectively). These two fragrances comprise about 95% of the EU market and 90% of the USA market for all polycyclic musks (HERA, 2004). The ratio between the detected levels of HHCB and AHTN (2-3) is slightly lower compared with many previous works. For example, Reiner et al., (2007) compared concentrations detected in two different STPs for both musk fragrances and found that influent concentrations of HHCB were 4.5 to 6 times higher than the AHTN concentrations. However, those results and the ones presented on this work are indicative of the greater production and use of HHCB compared with AHTN.

Similarly to AHTN, the tranquilliser DZP was not found at any sample. This substance is not normally detected in STPs, and very few authors managed to detect concentrations even in the low ppt range (Castiglioni et al., 2006). CBZ was found in some locations during the first day of sampling, but the levels detected were always below the quantification limit of the analytical method (1.4 ppb), which is particularly high for this substance. Considering that typically reported concentrations for CBZ, are similar or below this value (Clara et al., 2004), this result might be expected. However, it is interesting to mention that the detected levels were always higher in the final effluent, MLSS and RAS compared with the crude and settled sewage streams, leading to apparent negative removal rates. Other authors found a similar behaviour for CBZ and other substances such as antibiotics or β-blockers along sewage treatment. A possible explanation is the cleavage of glucuronide conjugates of those pharmaceuticals by enzymatic processes in the treatment plant (Lishman et al., 2006 and Vieno et al., 2006).

Table 3-4. Concentrations of PPCPs (μg/L) detected along the different units of the pilot plant, removal rates and standard deviations. (LOD = detection limit; LOQ = quantification limit; n = number of samples; n.a. = not available)

Sampling Point	IBP	n	NPX	n	DCF	n	HHCB	n	AHTN	n
Crude Sewage	7.5		3		<0.1		1.59		0.7	
SD	0.67	3	0.42	3	n.a	3	0.34	3	0.2	2
Primary Effluent	7.5		3		<0.1		1.54		0.7	
SD	1.4	8	0.5	8	n.a	8	0.36	8	0.2	4
Return Liquor	4.6		1.7		<0.1		0.69		<0.023	
SD	0.9	4	0.3	4	n.a	4	0.29	4	n.a	4
MLSS Supernatant	<0.08		0.2		1.2		1.06		0.37	
SD	n.a	3	n.a.	1	0.309	3	0.08	3	0.03	3
RAS	<0.08		0.2		1.2		0.96		0.36	
SD	*n.a*	3	n.a.	1	0.43	3	0.05	3	0.01	3
Final Effluent	0.2		0.2		1.1		1.07		0.37	
SD	0.08	2	0.01	3	0.33	4	0.06	4	0.01	4
Removal Rate (%)	98		93		0-45		33		48	
LOD	0.03		0.03		0.1		0.02		0.02	
LOQ	0.08		0.08		0.3		0.07		0.07	

Moreover, CBZ has been proposed as a suitable marker for anthropogenic influences on the aquatic environment, due to its high recalcitrant character (Clara et al., 2004). Therefore, in case CBZ is generated along biological treatment following a cleavage mechanism, it would not undergo a further degradation process, leading to higher outlet concentrations.

IBP was the pharmaceutical ingredient detected at the highest levels in sewage samples compared to the rest of the targeted pharmaceuticals, which is consistent with consumption rates reported for many UE countries (Carballa et al., 2008). On the other hand, DCF was not detected in the sewage (crude and settled) and return liquor samples whereas it was quantified in the MLSS, RAS and final effluent samples. Similarly to CBZ, increased effluent concentrations were also found by other authors (Lishman et al., 2006). Apart from the presence of conjugates on sewage, analytical issues based on acidic (pH=2) solid-phase extraction of matrix-prone samples such as sewage effluents have been reported for this substance. (Reddersen et al., 2003). To avoid this problem and based in previous experiences, no pH adjustment was performed prior to SPE. This may have led to underestimation of DCF concentration. As a consequence, it was not feasible to calculate accurately its removal efficiency and to estimate mass balances along the different pilot plant units for this substance, and the results presented are estimations based on its detection limit and literature data.

In this work, DCF was found to have the second highest mean concentration in the final effluent samples. Ashton et al. (2004) investigated the occurrence of several pharmaceuticals, including IBP and DCF, in several STP effluents and surface waters from the UK. The range of DCF concentrations detected in the final effluent of the pilot plant (0.8-1.4 ppb) is consistent with this previous research in STPs from the UK. On the other hand, IBP concentrations, which ranged from 0.1-0.3 ppb, were significantly lower than the ones reported by Ashton et al., (2004). However, comparing with treated wastewaters of different countries such as France, Greece, Italy, Sweden or Canada (Andreozzi et al., 2003 and Metcalfe et al., 2004), strong variations are observed between median and maximum IBP concentrations among the different countries. Whereas prescription rates and usage profiles may differ strongly from country to country, a different operation and technology of the studied STPs and factors associated to the analytical methodologies and sampling protocols used in the mentioned works are indeed decisive in the data variability, confirming the necessity of carrying out works in this field with better controlled conditions and following similar operational criteria.

Regarding the calculated removal efficiencies from the liquid phase for the different compounds, IBP and NPX were almost completely eliminated (98 and 93% respectively). In the case of DCF, it was not possible to calculate a reliable removal rate, as mentioned before. Considering that typically reported DCF concentrations in raw sewage are usually in the range of 0.5-2 ppb (Alder et al., 2006; Rosal et al., 2009) the removal rate of DCF in the studied pilot plant might be in the range of 0-45%, confirming its recalcitrant character. Musk fragrances removal from the liquid phase was from moderate to intermediate (30-50%). In this case, biodegradation mechanisms are not expected to play a major role in the removal of these substances due to their low kbiol value (Joss et al., 2006). On the contrary, musk fragrances are lipophillic substances with high solid-water distribution coefficients and consequently, they tend to be attached onto the particulate phase. Therefore, these substances represent a good example of the importance of considering both liquid and solid phase in order to determine overall removal efficiencies, as will be discussed in the following section.

3.3.3. Mass balances of PPCPs

Mass balances calculations permits to identify the removal mechanisms involved in each PPCP removal along sewage treatment and to estimate overall removal efficiencies for each compound. Main removal mechanisms are biodegradation, sorption and volatilization (Suarez et al., 2008). The latter is influenced by the Henry´s coefficient, which is only relevant for the musk fragrance ADBI. In the remaining compounds, volatilization only accounts for less than 2% of removal (Suarez et al., 2008). ADBI was not detected during the sampling campaign and therefore, the influence of the volatilization mechanism will not be considered in this work.

Figure 3-5 shows calculated mass balances considering both liquid and solid phase, which were calculated with the average values from the two sampling days, and Figure 3-6 shows the overall removal efficiencies. Crude Sewage represents the load of PPCPs in the crude sewage stream. Primary effluent load is calculated based on the settled sewage stream. RAS, settled sewage and return liquor are incorporated into the calculation of the activated sludge tank influent stream (Biological in), MLSS supernatant is the only stream considered for the activated sludge tank effluent (Biological out), which match up with the incoming load into the secondary settler (not shown). The load following secondary clarification (Settler out) is calculated from the final effluent and RAS samples and the Final Effluent stream was calculated considering only the values from the final effluent samples.

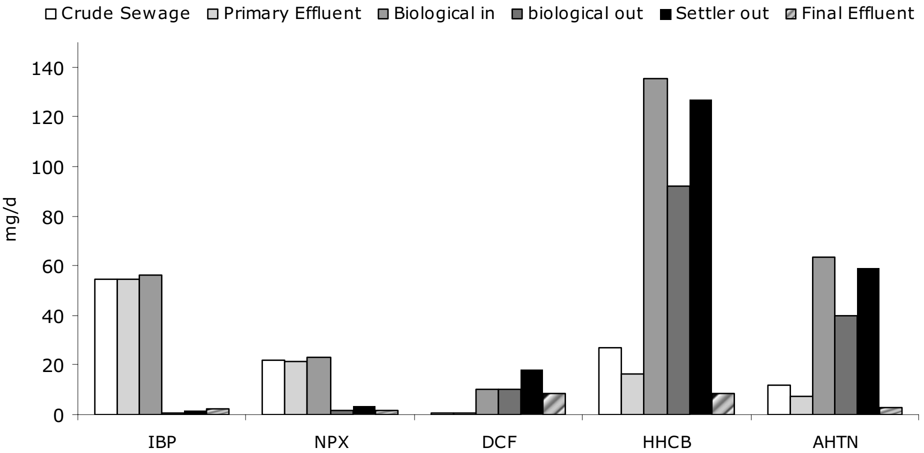

Figure 3-5. Mean mass balances of PPCPs calculated along the different units of the studied STP

For the two main groups of substances, the behaviour observed depended on their physical-chemical properties. Ibuprofen incoming load (54.6 mg/d) was the highest of all PPCPs and its elimination took place mainly along biological treatment (98%), confirming the biological degradation as its main removal mechanism. No differences were observed when comparing its removal rate from the liquid phase with the overall removal calculated after incorporating solid-phase data, which permits to confirm that IBP is a polar substance with no tendency to be sorpted onto solids.

Similar behaviour was observed for NPX in terms of overall removal and sorption behaviour. In despite of its incoming load (21.9 mg/d), which was the half of the amount of IBP at the inflow, NPX removal rate was slightly lower (93%). According to Joss et al., (2006), the biological degradation constant of this pharmaceutical is moderate (1-1.9 $L\cdot gSS^{-1}\cdot d^{-1}$), one order of magnitude below the IBP constant (21-35 $L\cdot gSS^{-1}\cdot d^{-1}$). Therefore, longer HRTs or higher MLSS concentrations are necessary for achieving significant NPX removals. In this work, the established HRT of 6 h might be low, but the MLSS concentration (~5 g/L) was high enough to remove this substance in a significant rate.

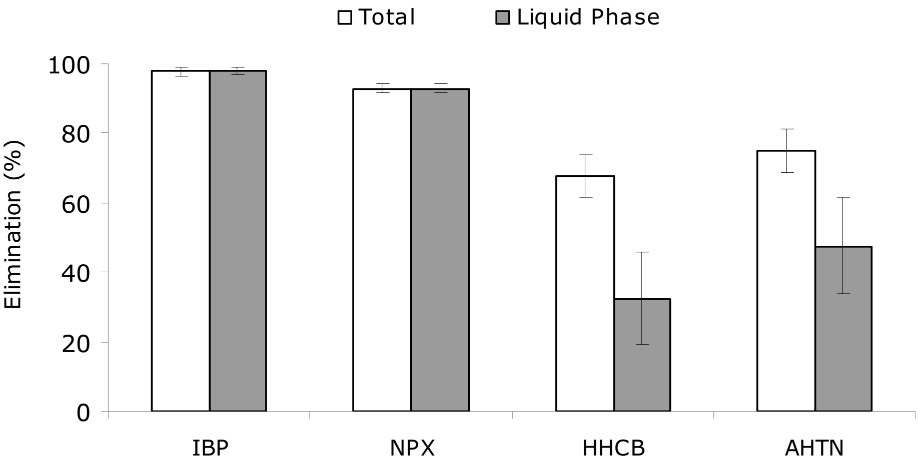

Figure 3-6. Global removal efficiencies calculated for PPCPs

Diclofenac mass flow load in the final effluent stream was considerably higher (8.4 mg/d) in comparison with IBP and NPX. Considering the reported consumption rates and occurrence data for this compound, it can be assumed that its incoming load was lower compared to those of IBP and NPX and therefore its removal might be estimated as low or negligible along the complete treatment process. This finding is also confirmed by its low degradation constant and distribution coefficient (Table 3-1). Considering that MLSS and SRT were high enough, the only possibility to enhance the DCF removal along biological treatment might be to establish considerably longer HRTs, which indeed might affect the overall output of the STP, mainly in economic terms. For this recalcitrant substance, the implementation of a further post-treatment step might help to attenuate its release into the aquatic environment.

For both polycyclic musk fragrances, a certain degree of removal was achieved after primary settling. This behaviour might be due to their strong lipophillic character and indicates that removal following a sorption mechanism occurs along every stream which contains suspended solids (Carballa et al., 2005). Interestingly, a marked increase in HHCB and AHTN incoming loads into the aeration tank was observed because of the influence of the return liquor and particularly the RAS stream. As a difference with the pharmaceuticals, a significant reduction of the fragrances load was observed comparing the biological out and final effluent streams, due to the solids separation after secondary settling. After this final step, the total removal rates achieved in the pilot plant were 68% for HHCB and 75% for AHTN (Figure 3-6), which are significantly higher when compared with the liquid phase data. A certain degree of biodegradation might also be achieved in despite of the low Kbiol values reported for musk fragrances (Table 3-1), as a consequence of longer retention times inside the reactor due to their association with solids. This supposition is based on works which detected HHCB-lactone, which is product of HHCB oxidation, in treated effluents (Reiner et al., 2007). Nevertheless, the calculated mass balances of this work indicate that the key removal mechanism for fragrances is the sorption onto the particulate phase, primarily during the biological treatment step.

Despite the degree of removal achieved, the loads in the final effluent for HHCB and AHTN were 8.3 and 2.9 mg/d respectively, which are considerably higher than the measured for IBP and NPX (1.2 and 1.6 mg/d respectively) and similar to DCF load, in the case of HHCB. Considering that the average flow rate of the full-scale STP where this research was carried out is 54,000 m^3/d, the estimated release of HHCB or DCF, would be of 63 g per day only in the discharged liquid stream from this site. In order to draw additional conclusions about the strategies that should be followed in order to attenuate the release of PPCPs into the aquatic environment, a direct comparison among removal rates reported in this work and previous research carried out by Carballa et al., 2004 in a STP located in north west Spain can be done, since most of the studied substances and analytical methodologies were similar. Interestingly, there are significant differences: IBP and NPX eliminations from the liquid phase were somewhat lower and fragrances removal rates were particularly high compared with our work. In this latter case, the elimination of HHCB and AHTN along pre-treatment and primary treatment steps accounted for half of their overall removal rates along the STP whereas in our work most of the removal took place mainly along secondary treatment. As a feasible explanation, the full-scale STP incorporated an additional pre-treatment step (based on screenings and grit and fat removal) and more efficient primary settlers which enabled to work with longer HRTs along primary treatment. On the other hand, the removal rates of IBP and NPX were considerably higher in our work (65 and 50% versus 93 and 99% respectively). In this case, the main difference between both biological treatments is based on the SRT, which is considered an influencing parameter in terms of PPCPs removal (Clara et al., 2005b). In the full-scale STP, no ammonia removal was achieved since a low SRTs of 1-3 days was established, whereas the correct design and operation of our pilot plant enabled to work with extended SRTs of 150 days. This operational strategy permitted to enhance the overall treatment quality, improving significantly the removal rates of IBP and NPX.

The results clearly shows that is necessary to enhance the sewage treatment quality, optimising the operation of existing plants and upgrading them with nutrients removal or recently developed treatment technologies such as membrane bioreactors, able to work with extended SRT at low HRTs, or with ozone post-treatment, which is known to degrade significantly recalcitrant substances such as diclofenac.

3.3.4. Return liquor treatment

Table 3-5 shows conventional parameters analysis performed during the sampling week on the return liquor which were directly fed into the aeration tank. Comparing with data reported for reject water from sludge digestion (Ghyoot et al., 1999; Wett et al., 1998), nitrogen concentrations were lower but, on the contrary, COD and TSS values were significantly higher. With respect to the treated settled sewage stream, considerably higher values were detected for the measured parameters in the return liquor.

In despite of this, the overall treatment capacity of the pilot plant was always excellent in terms of COD and ammonia removal with no apparent impact on its normal operation, which confirms the benefits of treating this kind of streams in the conventional biological treatment. Regarding the studied PPCPs, their concentrations in the return liquor were roughly the half of the measured in the crude sewage stream (Table 3-4) with the exception of the musk fragrance AHTN, which concentration was below detection limit. Therefore, the detected levels confirm that a biological treatment of the return liquor is beneficial also in terms of PPCPs removal and its influence on the overall treatment can be considered negligible, considering that the flow rate of this stream was significantly lower compared with the main stream of settled sewage coming into the aeration tank.

Table 3-5. Conventional analysis of the return liquor in mg/L

Day	TSS	BODt	BODf	CODt	CODf	NH4+
Monday	1110	1540	777	2940	1680	89.8
Tuesday	840	1880	789	3000	1560	88.2
Wednesday	780	1520	458	2950	1540	84.8
Thursday	790	1250	582	2810	1800	92
Friday	1060	1020	581	3010	1820	86.8
Mean	916	1442	637	2942	1680	88
SD	157	325	142	80	130	3

3.4. Conclusions

The pilot plant described in this work, based on the activated sludge system, has proven to be very effective for a combined treatment of both urban sewage and return liquor from sludge centrifugation. Moreover, COD removal was always high, a nitrification rate up to 96% was easily achieved and no adverse effects were observed after treating the return liquor stream.

Eight different PPCPs were analyzed in sewage samples and along the inflow/outflow of the different units of the pilot plant. Only AHTN and DZP remained below the detection limit, and CBZ was found in a few discrete samples. After estimating amounts of PPCPs sorpted onto solids, complete mass balances were calculated. The analysis of PPCPs behaviour along the different units helped to ascertain the two main removal mechanisms involved. Anti-inflammatory drugs were mainly removed inside the activated sludge tank, most probably by biological degradation, whereas musk fragrances removal occurred by sorption onto solids and arose in the primary or secondary sludge.

PPCPs levels in the return liquor from sludge centrifugation were approximately the half of the crude sewage, which confirms the benefits of treating such streams since no apparent influence on the overall removal of conventional parameters or PPCPs was observed.

In general, results observed in this work corroborate some of the previously reported after intensive sampling in full-scale treatment plants. Almost complete removal rates were observed for IBP, NPX, intermediate in the case of HHCB and AHTN, and low or negligible removal was estimated for the acidic drug DCF. The calculated mass balances for the outflow load of the full-scale STP permitted to estimate a release of PPCPs in the range of 9-63 g per day, depending on the substance considered. This estimation confirms that the development of enhancement strategies in existing plants should be a priority since it might help to attenuate the release of micropollutants in the water cycle, though new treatment and post-treatment technologies such as membrane bioreactors or ozonation are likely to continue to be explored.

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Chapter 4

Fate of Pharmaceutical and Cosmetic Ingredients during the Operation of a MBR Treating Sewage[1]

Summary

Municipal wastewaters contain many organic compounds, among them active ingredients as pharmaceuticals and personal care products (PPCPs), which are used in large quantities throughout the world. Most of these compounds come either from domestic sewage or from hospital or industrial discharges and enter municipal Sewage Treatment Plants (STPs). However, these plants have not been specifically designed to remove these trace polluting compounds.

The dynamics of twelve PPCPs in a membrane bioreactor (MBR) have been studied when treating synthetic sewage. These micropollutants are spiked into the synthetic feeding at environmentally relevant concentrations ranging from 10 to 20 μg/L.

Taking into account previous researches, the MBR is operated at an extended Solid Retention Time (SRT), since a high value of this parameter is considered as crucial for the removal of these micropollutants. Under these conditions, different fates are observed depending on PPCPs characteristics. Hydrophobic organic substances, like musk fragrances, are partially sorbed onto the sludge. This explains the partial removal observed in the reactor, with an overall efficiency around 50%. Other substances, like the anti-inflamatories ibuprofen and naproxen, are not sorbed but they are eliminated almost completely (98 and 84% of removal, respectively). On the other hand, substances like carbamazepine or diclofenac show a recalcitrant character and their elimination from the effluent is very limited, below 9%.

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[1] Part of this chapter has been published as:

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Outline

4.1. Introduction

There is a rising concern about the occurrence and persistence of active substances such as the ingredients of Pharmaceutical and Personal Care Products (PPCPs) in the aquatic environment, due to their specific characteristics. High worldwide consumption of pharmaceuticals provides a continuous release of the present substances or their metabolites into the environment, mainly via human or animal excretions (Ashton et al. 2004) Most of them show a recalcitrant behaviour and are not easily removed from wastewaters in STPs (Ternes 1998; Heberer 2002). Pharmaceutical compounds are designed to produce a biological activity on human beings or animals. As a result, there are possible side-effects on aquatic ecosystems still not cleared.

Although some studies have shown the toxic potential of several pharmaceutical ingredients, the knowledge in this field is still limited. For example, some synthetic polycyclic musks are considered as potential endocrine disruptors. Estrogenic effects and antiestrogenic activities have been observed even at very low concentrations. Besides, they are lipophilic and consequently may bioaccumulate in biota (Tanabe 2005). In this sense, recent research showed high concentrations of the fragrance galaxolide (up to 4100 ng/L) and other polycyclic musks in blood plasma of healthy adults (Hutter et al. 2005). In the case of antibiotics, its widespread use is closely linked with the rise of antibiotic resistance, and diseases that were considered eradicated, such as tuberculosis, are now making a comeback (Göbel et al. 2007).

Recent improvements on chemical analytical methodologies have enabled to detect extremely low concentrations of xenobiotics. As a consequence, worldwide studies have been carried out over the last decade reporting concentrations of PPCPs up to several micrograms per litre in surface or groundwater, rivers, streams and sewage. STPs and hospital effluents were identified as important emission sources of PPCPs into the aquatic environment (Ternes 1998; Heberer 2002; Metcalfe el al. 2003; Ashton et al. 2004; Carballa et al. 2005; Gómez et al. 2007). Micropollutants pass along the units of STPs with different fate, according to their structure and physico-chemical properties, thus several works have been carried out in order to characterise their overall removal efficiency. Regarding the biological degradation of pharmaceutical compounds, musk fragrances and estrogens, a classification scheme based on batch experiments with sewage sludge taken from a STP has been proposed recently. Among 35 different organic micropollutants, only 4 of them were removed in a significant extension (Joss et al. 2006). Besides, experiments in lab-scale activated sludge plants were performed in order to find the main operation parameters which could affect PPCPs removal. No complete elimination rates were achieved in any case. Sludge retention time (SRT) and acclimation of the biomass were pointed as key issues to improve removal efficiencies when biological mechanisms are involved (Suárez et al. 2005). In conclusion, traditional and modern STPs equipped with activated sludge process have not been designed to remove micropollutants efficiently (Carballa et al. 2005). This is a matter of high concern, as PPCPs can reach water sources used for drinking water production (Heberer 2002). Therefore, it is important to test new technologies for urban wastewater treatment.

Presently, membrane bioreactors constitute a promising technology in industrial and urban wastewater treatment. Membrane filtration retains suspended solids that are usually washed out in biological reactors coupled with secondary settlers. As a result, it is feasible to work with high biomass concentrations and to control SRT accurately, obtaining a high quality permeate-effluent (Suárez et al. 2005). High solids concentration inside the reactor might improve removal rates of lipophilic substances following a mechanism of sorption onto the sludge, and a longer SRT could favour the slowly growing bacteria, improving this way the biodiversity of microorganisms inside the MBR (Göbel et al. 2007) and achieving a complete adaptation to the presence of PPCPs (Clara et al. 2005).

This work is focussed on the fate of twelve micropollutants as well as the assessment of their potential toxic effects exerted on activated sludge in a submerged membrane bioreactor (MBR). This system was operated with synthetic sewage at long SRT and high biomass concentrations. MBR efficiency in terms of ammonia and COD removal were also tested, as well as membrane performance by measuring Transmembrane Pressure (TMP).

4.2. Materials and methods

4.2.1. MBR pilot-scale plant

The MBR used in this work (Figures 4-1 and 4-2) is a pilot scale unit with a liquid capacity of 220 litres and equipped with a Zenon ZeeWeed-10 submerged hollow fibre membrane module. Its main characteristics are an average pore size of 0.04 µm and a nominal surface area of 0.9 m^2. This unit comprises an extended aeration device with air diffusers located on the bottom header, where air is supplied by a blower (MEDO LA-120) capable of provide air flow rates up to 120 $L \cdot min^{-1}$ in order to ensure the required level of oxygen for biological oxidation and to facilitate membrane scouring. Besides, a 22 L tank is available to receive permeate, which is finally discharged by gravity. The membrane module is connected to a micro gear pump (micropump magnetic drive, 83047, series 120, Idex corporation) capable of reversing speed, serving both as permeate and backwash pump.

Figure 4-1. Membrane Module ZeeWeed-10 and Bioreactor

The filtration cycle, i.e. permeate production time and backwash duration is controlled with a timing device (Allen-Bradley Multifunctional Digital Timer, 700-HX). The chosen cycle was adjusted to 15 minutes of permeate production followed by 45 seconds of backwashing.

The MBR was inoculated with 2.5 g VSS/l of biomass obtained from a full scale Conventional Activated Sludge (CAS) which treats industrial wastewater from fish-meal and fish-oil production. During the whole operational period, Temperature and pH were monitored but not adjusted to selected values in order to maintain the same operating conditions as in full scale plants, varying in the range of 18–24 ºC and 7.5–8.5, respectively. Devices for the measurement of dissolved oxygen, established at 2–3 ppm, and a Bourdon-Type manometer to monitor the transmembrane pressure are available.

Feeding System

The MBR was fed with a synthetic medium which simulates domestic sewage. It consisted of a mixture of two streams: tap water and a concentrate. The mixing of both streams takes place at the inlet of the MBR. The concentrate was stored in a stainless steel tank with a capacity of 165 L, and was fed into the reactor with a separate peristaltic pump (Masterflex® L/S Economy Drive, 2-200 rpm) at a flow around 3 $mL{\cdot}min^{-1}$.

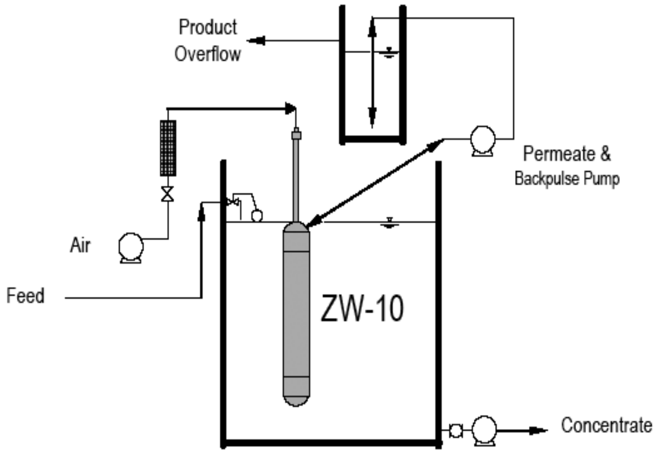

Figure 4-2. Flow sheet of the MBR pilot-scale plant

The average composition after the mixing is shown on Table 4-1 and tries to reproduce the chemical characteristics of a medium charged urban wastewater. The pH of the feed was adjusted to circumneutral values with the help of concentrated sulphuric acid. A solution of several trace metals which serve as nutrients for biomass was also added. PPCPs were spiked into the concentrate in order to ensure environmentally relevant concentrations inside the MBR.

Table 4-1. Average composition of the synthetic feed

Compounds	**Concentration (mg·L^{-1})**	**Trace solution**	**Concentration (g·L^{-1})**
CH_3COONa	490	$FeCl_3 \cdot 6H_2O$	1.5
NH_4Cl	150	H_3BO_3	0.15
Na_2HPO_4	25	$CuSO_4 \cdot 5H_2O$	0.03
KH_2PO_4	12	KI	0.03
$NaHCO_3$	200	$ZnSO_4 \cdot 7H_2O$	0.12
Trace solution	0.1 mL/L	$CoCl_2 \cdot 6H_2O$	0.15
		$MnCl_2 \cdot 4H_2O$	0.12

4.2.2. Analytical methods

Feeding and permeate samples, collected regularly, were analysed for conventional parameters (soluble COD, solids, nitrogen) following Standard

Methods. PPCPs were determined following the methods described in Chapter 2. Samples were collected along different days during a week in order to get an integrated sample. For this purpose, glassware was always used for sampling in order to avoid sorption of lipophillic pollutants. Samples were mixed, stored in aluminium containers and kept at 4ºC prior to the solid-phase extraction and analysis. Substances considered in this work and their calculated concentrations in the liquid phase (ppb) at the inlet are shown in Table 4-2.

Table 4-2. Concentrations of PPCPs in the feed.

Compound	**Concentration (μg/L)**	**Compound**	**Concentration (μg/L)**
Anti-inflammatories		*Antibiotics*	
Ibuprofen (IBP)	10	Trimethoprim (TMP)	10
Naproxen (NPX)		Roxithromycin (ROX)	
Diclofenac (DCF)		Sulfamethoxazole (SMX)	
Anti-epileptic		Erythromycin (ERY)	
Carbamazepine (CBZ)	20	*Musks*	
Tranquilliser		Galaxolide (HHCB)	20
Diazepam (DZP)	20	Tonalide (AHTN)	
		Celestolide (ADBI)	

4.3. Results and discussion

4.3.1. Conventional parameters and membrane performance

The MBR was started with the inoculation of nitrifying sludge and the feeding of synthetic wastewater. During a start up period of 2 months, the hydraulic retention time (HRT) was maintained at 1 day, and stable conditions were obtained. The reactor showed excellent performance in terms of soluble COD and ammonia removal (mainly due to nitrification), with elimination rates up to 95 and 99% respectively.

In a second step, the HRT was lowered to 12 hours maintaining the same efficiencies (Figure 4-3). After the start-up period, PPCPs were spiked into the synthetic feeding. No inhibitory effects were observed in biomass growth, COD degradation and nitrification, as can also be deducted from Figure 4-3.

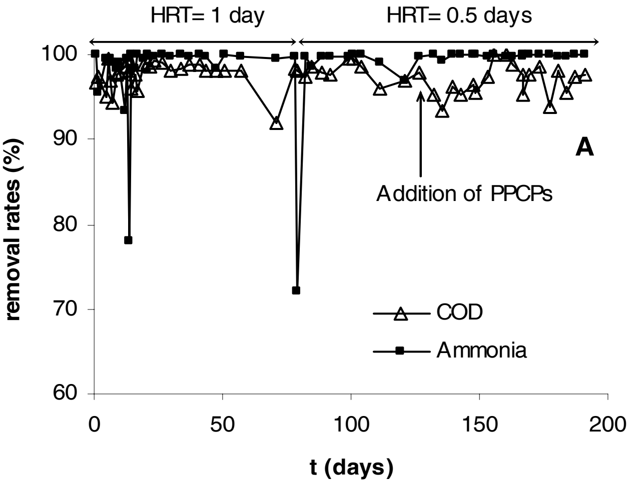

Figure 4-3. Influence of HRT and PPCPs addition on ammonia and COD removal.

Biomass content grew from 2.5 to 12 g VSS/l in only four months (Figure 4-4). Then, limitations in the oxygen transfer efficiency were observed and the system was purged regularly in order to keep a biomass concentration of 10 g SSV/L, which is compatible with the maintenance of 2 mg/L of dissolved oxygen. The SRT under these conditions amounted to 72 days, high enough to ensure the nitrification process and the acclimation of the microorganisms to the PPCPs presence, probably enhancing their biological removal.

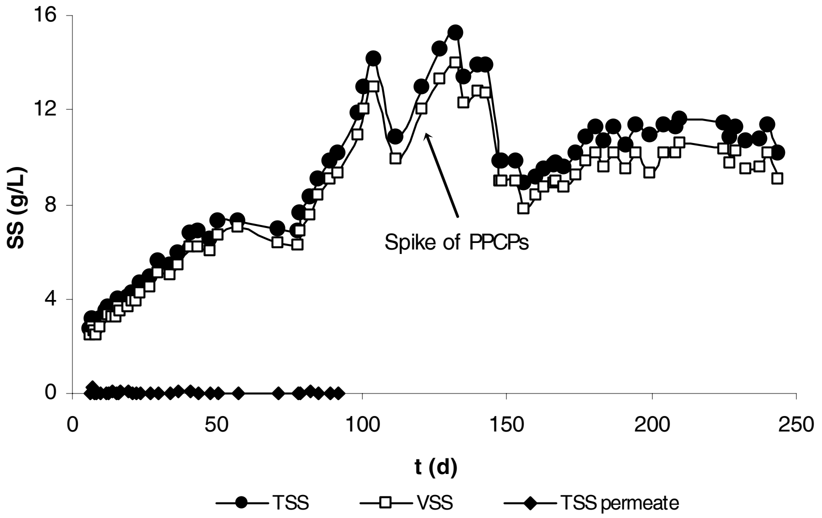

Figure 4-4. Biomass growth along the MBR operation

Transmembrane pressure was also monitored (Figure 4-5) during the whole operation of the MBR. A significant increase of this paramater was observed when the applied flux was increased up to 20.8 L/m^2·h, beyond typical MBR operating fluxes. However, no chemical or mechanical cleaning was required during the initial operational period until day 225, when a simple chemical backwashing was performed by adding sodium hypochloride into the permeate tank. Afterwards, transmembrane pressure gradually decreased for a few days. The furhter increase of this parameter, from day 250, clearly indicated that the system was operating above critical flux. Therefore, the liquid capacity of the MBR was modified in the following chapters in order to operate with a wider variety of HRTs without compromising membrane integrity.

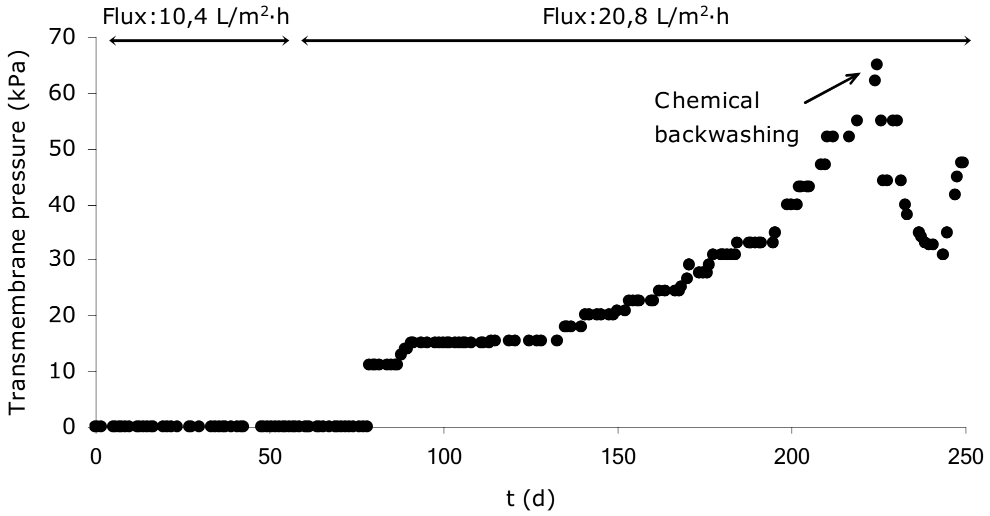

Figure 4-5. Evolution of transmembrane pressure during the operation period

4.3.2. PPCPs elimination

After the start-up period, PPCPs were spiked into the synthetic feeding. No inhibitory effects were observed in biomass growth, COD degradation and nitrification, as can be deducted from figure 4-3. The reactor was maintained under these conditions for two additional months, in order to allow the biomass to acclimate to the presence of PPCPs. When a SRT of 72 days was achieved, samples of feeding and permeate were collected and analyzed.

Figure 4-6 shows the measured concentration of PPCPs in the feeding and permeate. Strong variations among the different compounds and therapeutic groups are detected, ranging from diclofenac, whose concentration was maintained in the same level in the permeate and feeding, up to ibuprofen, that was almost undetected in the outflow. These eliminations observed are expected to be mainly due to mechanisms of sorption and biological degradation on the sludge. Besides, lipophilic compounds might also be sorbed on the surface of the membrane. Size-exclusion can not be considered since the molecular weight cut-off of the considered substances is too low for an ultrafiltration membrane.

The acidic pharmaceuticals naproxen and especially ibuprofen were almost completely removed, with elimination rates up to 84 and 98%, respectively. These results are similar or slightly higher than other previously reported in STPs equipped with conventional systems (Metcalfe et al. 2003; Gómez et al. 2007). For example, Suarez et al. (2005) reported elimination rates of 68 and 82% respectively, working with a lab-scale activated sludge plant. Kimura et al. (2005) found better results for naproxen when working with MBR technology and no clear differences in the case of ibuprofen.

In the case of the tranquilliser diazepam and the antiepileptic carbamazepine, elimination data are also in good agreement with results found in previous studies. For both substances, poor removal rates were measured (9% for carbamazepine and 26% for diazepam). Joss et al. (2006) studied PPCPs biodegradation in batch experiments with sludge taken from MBR and CAS systems. Results obtained for these substances were less than 20% of biological removal for both compounds. Again, removal rates measured in this MBR system were slightly higher. Thus, it can be concluded that they are not easily degraded by biological treatment in a MBR, even working at a high SRT. Similarly, diclofenac was not removed at any significant extent. Kimura et al. (2005) also reported low elimination rates for diclofenac comparing MBR with other systems, suggesting that the presence of chlorine on its structure might enhance its persistence.

The results obtained for the removal of fragrances were around 46-56%. These values are not very high taking into account the outstanding lipophilic character of these substances and thus their affinity to be sorbed onto the sludge. Carballa et al. (2005) found removal rates around 40% for musk fragrances during the primary treatment of an urban STP, where no biodegradation mechanisms are expected. Taking into account the removal achieved in the biological reactor, the average removal efficiencies obtained in the whole treatment were in the range of 80-85%. On the other hand, the works carried out by Clara et al. (2005) with a MBR pilot plant and several STPs treating real sewage indicate surprisingly a high percentage of biodegradation operating with 27 days of SRT. The difference between these results and those presented in this work might be attributed to a better acclimation achieved under these conditions, since our MBR has been operated with PPCPs only during two months. Moreover, the sludges used as inoculum were not previously acclimated. Further analysis will be carried out in order to check this possibility.

In the case of antibiotics, macrolides roxithromycin and erythromycin were removed at a high extent (77 and 91% respectively), and sulfamethoxazole was half-removed (52%). On the other hand, trimethoprim showed low elimination rates (36%). Göbel et al. (2007) compared the performance of different wastewater treatment technologies, including a membrane bioreactor operated at different SRT. Results from both works are similar for erythromycin and sulfamethoxazole when working with sludge age ranging from 60 to 80 days, but substantial differences were found in the case of trimethoprim, where removal rates up to 85% were measured. Again, more acclimation time might be needed for this substance.

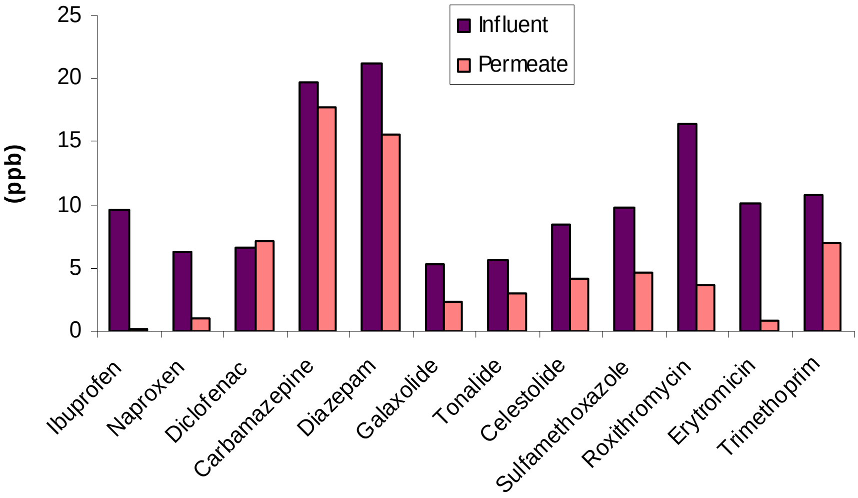

Figure 4.5. Concentrations of selected PPCPs in the MBR influent and permeate

4.4. Conclusions

In this work, a MBR was operated in order to evaluate its performance in terms of PPCPs removal treating synthetic sewage. Removal rates observed for COD and ammonia were always above 95%. Changes in operational parameters such as HRT did not affect the quality of the permeate, and the spike of PPCPs into the feeding had no effect on the performance of this system. SRTs in the range of 44-72 days were maintained, with VSS of 10 g/L. The ultrafiltration membrane showed excellent performance, although TMP was increased progressively, due to membrane fouling.

Ibuprofen, naproxen and erythromycin were almost completely removed from the influent, with elimination rates slightly better than other works previously reported, possibly due to the high SRT achieved. A partial removal was observed with sulfamethoxazole and musk fragrances. On the other hand, carbamazepine, diazepam, diclofenac and trimethoprim remained at high concentrations in the permeate.

According to the short period that this system has been treating PPCPs in the influent (2 months) and the source of the inoculum used (without any previous contact with these micropollutants), it is expected that acclimation phenomena might occur and lead to better results in the coming months. However, it is also recommended to carry out further experiments using spiked municipal sewage as feeding, and testing different operational parameters and conditions in order to accurately simulate conditions similar to those usually set in full-scale sewage treatment plants.

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Chapter 5

Influence of temperature, biomass concentration and adaptation on PPCPs removal with MBRs[1]

Summary

The aim of this work was to evaluate the performance of a MBR treating sewage after primary treatment spiked with micropollutants (hormones, cosmetic ingredients and pharmaceuticals) with different physical-chemical properties. The MBR was monitored during a prolonged period of operation in order to investigate the influence of operational parameters such as temperature and biomass concentration. Acclimation phenomena were considered, since the long period of operation might allow the establishment of a more diverse biocoenosis adapted to the presence of these substances. Differences in the behaviour and fate were observed depending on the substance considered. For example, sulfamethoxazole removal was moderate (50-75%) and particularly influenced by the mixed liquor suspended solids (MLSS) concentration. The elimination of other antibiotics strongly increased during the operation of the MBR, probably due to biomass adaptation. Operating conditions did not influence the elimination of hormones, ibuprofen and naproxen, which were almost completely eliminated (90-99%). Similarly, the removal of carbamazepine, diazepam and diclofenac was not influenced by the operating conditions although their elimination was incomplete (20-50%). Elimination of fragrances varied significantly between operational periods: low eliminations were observed in the winter period whereas eliminations up to 70% were measured during summer samplings. Sludge age, temperature and physical-chemical characteristics of the MBR sludge might exert influence on the observed eliminations.

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[1] This chapter has been submitted as:

Reif, R., Omil, F., Lema, J.M. Influence of temperature, biomass concentration and adaptation on PPCPs removal with membrane bioreactors.

Outline

5.1. Introduction

Micropollutants such as Pharmaceutical and Personal Care Products (PPCPs) and Endocrine Disrupting Compounds (EDCs) are being detected ubiquitously in the aquatic environment. Although denoted as "emerging" because information about occurrence is fairly recent, PPCPs have been discharged into the environment for decades, mainly in water bodies (Ternes, 1998). Ever since, many studies have been conducted in order to identify the main sources and pathways these substances follow before reaching aquatic environments (Reif et al., 2010). For example, many prescription and over-the-counter drugs are flushed down the toilet. Other compounds cannot be fully metabolized by the body and are excreted soon after administration (Stackelberg el al., 2004; Weigel et al., 2004). An important source that should not be underestimated is the emissions from livestock activities. For example, Sedlak et al. (2007) identified cattle as the main source of 17α-estradiol, estrone and other steroids to surface waters and therefore in agricultural watersheds. Their maximum concentrations measured were comparable or even higher than concentrations detected in STPs effluents. In spite of the different pathways these substances follow, the final result is the same: biologically resistant contaminants or their metabolites end up in surface waters. Considering that the removal of these substances is not enforced by local governments and no regulations currently require monitoring or public reporting of their presence in the water supply, most of the existing STPs do not presently pursue their elimination and more than 80 types of PPCPs have been found in sewage effluents, surface water, ground water or even drinking water. Consequently, PPCPs occurrence is raising concerns regarding their unique characteristics and the scarcity of information available relating to their environmental risk. Some adverse ecological responses have been described to date, such as the development of pathogenic microorganisms resistant to antibiotics (Dantas et al., 2006) or the toxicity of the parasiticide ivermectin to crustaceans exposed at ppt levels (Garric et al., 2007). Moreover, it should not be ignored that organisms living in effluent-dominated systems are subjected to chronic exposure to complex mixtures of PPCPs which may interact with unknown effects (Brooks el al., 2006).

The use of membranes for wastewater treatment is gaining acceptance throughout the industry and nowadays a number of indicators suggest that membrane bioreactors (MBRs) are being accepted increasingly as the technological choice (Judd, 2006). MBRs main advantage is the potential production of a high quality effluent, due to the combination of an intensive biological treatment followed by an ultrafiltration (UF) step, overcoming clarification. Presently, MBRs are also being tested in terms of micropollutants removal due to their ability to achieve high sludge retention times (SRTs) and biomass concentration. These parameters are known to exert influence on the overall quality of treatment, including the removal of nutrients and persistent substances. In fact, some studies dealing with pilot-scale MBRs have found improved removal rates for some substances. For example, Miège et al., (2008) compiled micropollutant removal efficiencies from 113 research papers finding that MBR processes usually provide 15% higher removal rates when compared with conventional nitrogen removal processes. Even so, the information available is scarce and contradictory since it has been also reported that CAS systems operated with long SRTs might achieve similar performances (Clara et al., 2005).

The effect of the filtration step is usually discarded because typical membrane pore sizes are at least a factor of 100 larger than the molecular sizes of most of the micropollutants studied (Radjenovic el al., 2009). Nevertheless, literature data assessing the performance of different UF membranes in terms of PPCPs removal is again very limited and some differences may be expected in the elimination of hydrophobic compounds following sorption mechanisms in the cake layer deposited onto the membrane surface, which is known to depend on the membrane material, the components in the activated sludge bulk and the cleaning procedures.

The particular structure of the biomass developed in the MBR process has also been deeply studied. Some enzymatic activity might be increased due to smaller activated sludge flocs and higher specific surface area of the biomass, which are typical characteristics of MBR biomass. These specific properties might improve the mass transfer conditions enhancing the availability of micropollutants susceptible to microbial biodegradation (Fatone, 2009). Nonetheless, substances that are recalcitrant to the biological process will remain unaltered after MBR or CAS treatment.

This work aims to provide more extensive knowledge on the behaviour and fate of 17 PPCPs with different physical-chemical properties during the operation of a submerged hollow-fibre MBR, which was operated for 10 months under strictly controlled conditions at the premises of a full-scale STP. The influence of the temperature, mixed liquor solids concentration (MLSS) and microorganisms adaptation on the observed removal rates was studied during the different sampling campaigns.

5.2. Materials and methods

5.2.1. Pilot-scale MBR: feeding and spiking system

The MBR was equipped with a Zenon ZW-10 hollow fibre membrane module (average pore size: 0.04 µm) submerged in a 180 L aeration tank (additional information can be found at Chapter 2). It was placed upon the premises of the municipal STP of Silvouta (Santiago de Compostela, Figure 5-1), which treats low-charged waters of 100,000 population equivalents, mainly household discharges but also important discharges from three hospitals and a university campus.

Figure 5-1. Picture of the Silvouta STP

The set-up of this chapter (Figure 5-2) consists of a 1 m^3 tank which served as a primary settler, a mixing tank and the pilot-scale MBR. The primary sedimentation tank received its sewage from the primary sewage reservoir of the STP. Sodium bicarbonate solution was constantly added at the upper part of the tank in order to inoculate the primary sewage with the aim to maintain an alkalinity level of 200mg/L of $NaHCO3$. The settling tank provided the supernatant sewage that was pumped to the mixing tank, which consisted of a stainless steel drum of capacity 165 L. It was equipped with a automatically operated liquid level regulator consisting of two platinum electrodes separated at a distance of 100 cm apart, corresponding to the upper and lower permissible levels of liquid in the mixing tank.

Figure 5-2. Picture showing the primary settler (left) and the mixing tank (right) with the spiking pump and PPCPs solution

PPCPs solution was placed in a 120 mL amber coloured glass bottle in order to prevent photodegradation and sorption. This bottle was filled twice a week. During operation, a spiking pump maintained continuous inoculation of the PPCPs into the mixing tank while the influent pump simultaneously pumped supernatant sewage from the primary settling tank. As soon as the pumped sewage gets to the upper liquid level of this platinum bar, it automatically triggers off all pumps. Pumping automatically resumes when the level of the sewage in the tank decreases to the lower limit of the bar. A polyethylene pipe connected to the mixing tank served as route through which the sewage from the tank was being served to the MBR.

The MBR was continuously fed with settled sewage spiked with PPCPs at concentrations ranging from 1.0 up to 40 μg/L. The pilot-plant was operated at extended SRT conditions in order to favour sludge adaptation and biodegradation kinetics, and with a HRT of 24 hours which ensured sustainable operation of the membrane module, below critical flux. Therefore, throughout the period of operation of this pilot MBR, no excess sludge withdrawal was performed except the small samples that was being collected for analysis, and that resulted to gradual increase in the biomass concentration in the reactor until a nearly constant value was attained. The filtration cycle consisting of 15 min of permeate production followed by 45 s of backwashing, and the chemical and physical cleanings that were performed in a regular basis contributed to avoid significant flux drops associated with membrane fouling during the complete operational period.

The treated sewage was continuosly spiked with the following PPCPs at environmentally relevant concentrations: tranquilliser: diazepam (DZP, 20 ppb); antiepileptic: carbamazepine (CBZ, 20 ppb); antibiotics: sulfamethoxazole, erythromycin, trimethoprim and roxithromycin (SMX, ERY, TMP and ROX, 10 ppb); antidepressants: fluoxetine and citalopram (FLX and CTL, 10 ppb); musk fragrances: galaxolide, tonalide and celestolide (HHCB, HHTN and ADBI, 40 ppb), anti-inflammatory drugs: ibuprofen, naproxen and diclofenac (IBP, NPX and DCF, 10 ppb) and the hormones estradiol, ethynilestradiol and estrone (E2, EE2 and E1, 1 ppb). Selected pharmaceuticals and hormones were purchased from Sigma-Aldrich. Musk fragrances were generously supplied by Ventós (Spain). Influent, permeate and sludge samples for PPCPs analysis were collected as time-proportional according to the HRT of 24 hours set in the MBR. Glassware and aluminium bottles were used for sampling and storage.

5.2.2. Analytical methods

PPCPs and conventional analysis in the liquid phase were carried out as described in Chapter 2. During each sampling campaign, samples of biological sludge were also collected in order to determine the amount of PPCPs sorbed onto the solid fraction using the methodologies of ultrasonic solvent extraction (USE) for antibiotics, antidepressants and hormones (Ternes et al., 2005) and the solid-phase microextraction (SPME) for musk fragrances (García-Jares et al., 2002).

FISH was performed according to the procedure described by Amann et al. (1995) with 4% paraformaldehyde solution. The specific oligonucleotide probes used were: (1) EUB338I, specific for Bacteria domain, (2) Alf1b specific for Alphaproteobacteria, (3) Bet42a specific for Betaproteobacteria, (4) Neu653 specific for Nitrosomonas spp. Probes Bet42a, NEU653 and NIT3 were used in a 1:1 ratio together with their specific probe competitors. After in situ hybridization cells were stained with 4,6-diamidino-2-phenylindole (DAPI) (0.5 mgmL21) for 10 min. Fluorescence signals were recorded with an acquisition system coupled to an Axioskop 2 epifluorescence microscope (Zeiss, Germany).

5.3. Results and discussion

5.3.1. Conventional parameters

The STP of Silvouta treats low-charged waters of 100,000 population equivalents, mainly household discharges but also important discharges from three hospitals and a university campus. Different physical-chemical parameters were monitored during the operation of the MBR (Table 5-1) in order to ensure that the conditions were optimum for sewage treatment. Dissolved oxygen levels were always maintained above 2 mg/L, minimum value considered to ensure the necessary amount of oxygen for the aerobic processes and for the correct development of nitrifying bacteria. Temperature was monitored but not controlled and was influenced by the ambient temperature variations since the MBR was placed outdoors. MLSS content inside the bioreactor gradually increased along the whole operational period and, before the last sampling campaign, a considerable amount of biomass (~60%) was purged from the MBR in order to study the influence of this parameter on PPCPs removal under similar temperature conditions. With the exception of the low value measured during the April sampling campaign, the pH was relatively constant, in the range of 7.2-9, optimal for the development of nitrifying bacteria (Metcalf & Eddy, 2003).

Table 5-1. Summary of physical-chemical parameters monitored in the MBR

Period	T (ºC)	MLVSS (mg/L)	DO (mg/L)	pH
October	18	1.4	-	7.2
December	12	3.8	-	7.2
February	9	5.5	12.3	7.7
April	15	5.2	6.5	6
June	19	8.8	5	7.4
July	19	3.1	6	7.6

The elimination of organic matter (expressed as COD), ammonia and solids parameters were continuously monitored during the whole operational period of the MBRs (Table 5-2 and Figure 5-2), focusing on the solids content, organic matter and nutrients. MBR performance in terms of total and soluble COD removal was excellent, always above 90% removal. Due to the efficiency of the ultrafiltration process, the amount of suspended solids in the final permeate was always negligible (< 0.7 mg·L-1). The concentration of nitrogen was followed in the form of ammonia, nitrite and nitrate (nitrite and nitrate data not shown)

Table 5-2. Summary of MBR performance in terms COD and ammonia concentrations (mg·L^{-1})

Period	Total COD$_{in}$	Soluble COD$_{in}$	Soluble COD$_{out}$	N-NH$_4^+$ $_{in}$	N-NH$_4^+$ $_{out}$	TSS$_{in}$
October	220	112	27	19	1	101
December	526	314	25	20	3	154
February	587	158	17	15	0	374
April	636	188	25	18	0	707
June	447	266	25	30	1	208
July	344	202	34	20	0	115

Ammonia content in the sewage was from moderate to low, and it was successfully removed following two mechanisms: Transformation into nitrate by nitrifying bacteria or assimilation for biomass growth, as can be confirmed by the levels of nitrate normally detected in permeate. Moreover, nitrite content was negligible, confirming that the two stages of the nitrification process were occurring during the biological treatment process.

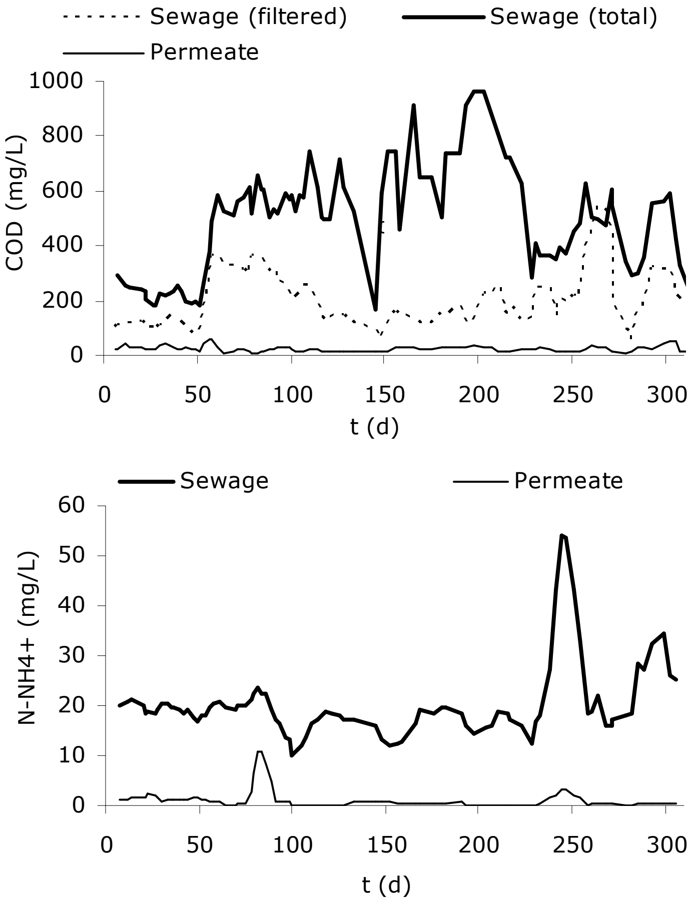


Figure 5-2. COD and ammonia elimination during the MBR operation

5.3.2. Fate of PPCPs in the pilot scale MBR

The biomass developed in the MBR was grown from inoculated sludge from a STP implementing a Biodenipho® process, in order to ensure the presence of nitrifying bacteria. Two preliminary sampling campaigns (October and December) for PPCPs analysis in the liquid phase were carried out during the first months of operation. When stable operation was achieved for the MBR, samplings were performed in order to calculate removal efficiencies throughout the different periods, considering both liquid and solid phase data.

Previous studies have shown that during sewage treatment, PPCPs undergo processes like biodegradation, during which they are transformed by microorganisms, or sorption onto sludge (primary or secondary). The extent of these processes depends on the physical-chemical characteristics of the compound

(Suarez el al., 2008; Omil et al., 2010). More particularly, the efficiency of PPCPs biodegradation mainly depends on their inherent biodegradability properties. Joss et al., 2006 estimated the biodegradation constants (kbiol) for many substances, including most of those considered in this research. The value of those constants should be considered as estimative for this study since differences in the behaviour of continuous and batch bioreactors are expected, although their relative kbiol values allow establishing a comparison between the observed biodegradation rates for the different PPCPs. According to the different patterns observed and the influence exerted by the operational parameters, results were categorized into 3 different groups of PPCPs: easily removed, influenced by operation parameters and PPCPs which did not follow a specific trend.

PPCPs easily removed

Figure 5-3 shows average removal rates for the three hormones and the anti-inflammatories IBP and NPX together with their reported kbiol values (Joss et al., 2006).

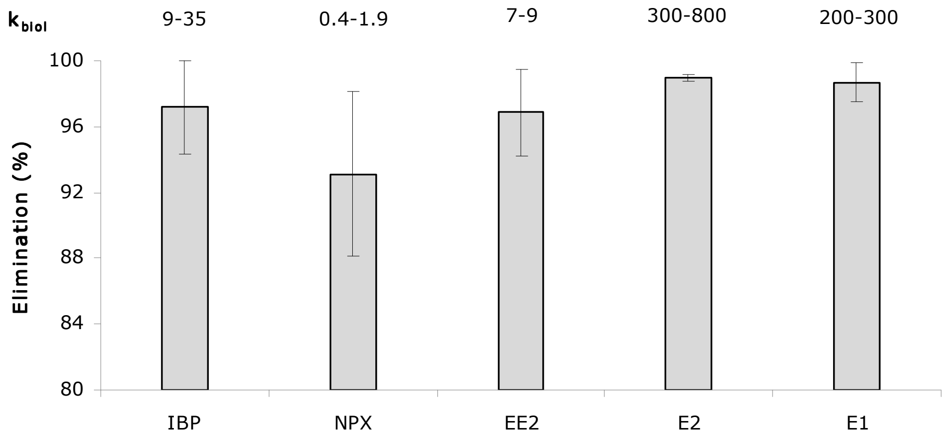


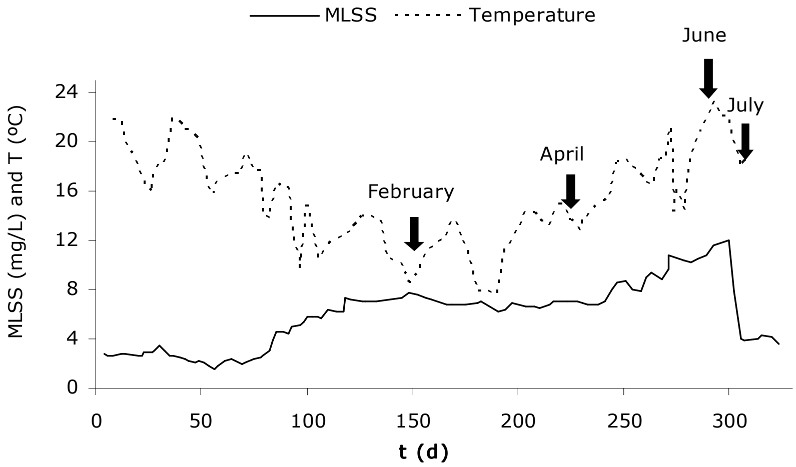
Figure 5-3. PPCPs easily removed during MBR treatment and Kbiol (L·gss-1·d-^{1}) values.

These substances were almost completely removed during the MBR treatment process, with no apparent influence of the operational parameters. In general, calculated removal rates, considering both liquid and solid phase, were above 90% for each compound. A strong relationship between the removal rate and the kbiol value can be observed. The highest removal rate was achieved for the hormone E2, which is also the compound with the highest kbiol value whereas the lowest rate was observed for the anti-inflammatory NPX, which also has the lowest kbiol value. According to Joss et al., (2006), pharmaceuticals follow pseudo-first order kinetics.

Therefore, the sludge concentration should be directly correlated to the transformation rate. This effect, which should be obvious for substances with moderate kbiol such as NPX, is only perceived slighty in this study, probably due to the HRT of 24 h set in the MBR and the extended SRT, which were long enough to achieve significant removals independently of the MLSS levels. Therefore, the effect of MLSS and temperature was not particularly relevant in the case of these substances. In general, observed results are in accordance with the findings described in the literature for conventional systems and MBRs (Kimura et al., 2005; Kreuzinger et al., 2004; Radgenovic et al., 2007; Reif et al., 2008).

Influence of the operational parameters

Figure 5-4 shows the evolution of the MLSS concentration and seasonal variations of the temperature during the complete operational period of the MBR.



MLSS	5.5	5.2	8.8	3.1
T	9	15	19	19

Figure 5-4. Evolution of MLSS and temperature in the MBR; (↓= PPCPs analysis)

Additionally, Figure 5-5 shows the removal achieved for the substances which showed different behaviour depending on the sampling period considered and, consequently, may be influenced by the acclimation phenomena, MLSS and/or temperature variations: antibiotics (SMX, ERY, ROX and TMP) and the antidepressant FLX. For these substances we will focus on sampling periods after December, when the solid phase was also considered and stable operation of the MBR was achieved.

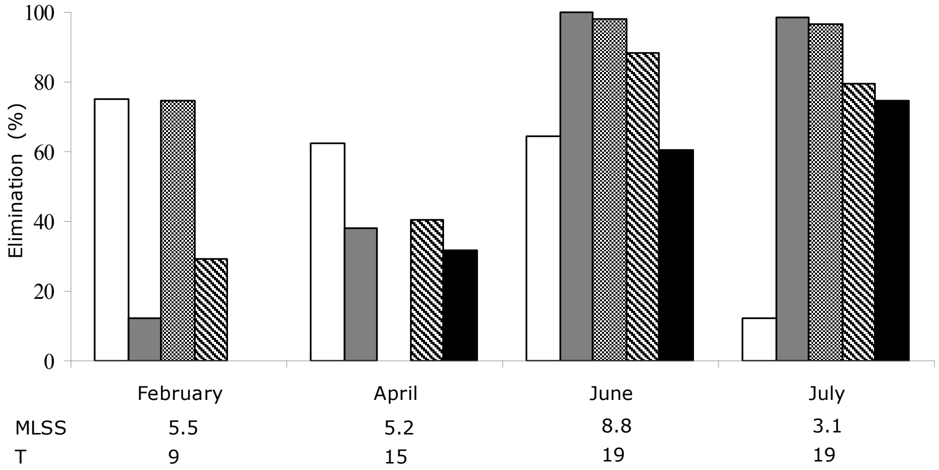


Figure 5-5. Influence of MLSS levels, temperature and adaptation on PPCPs removal from sewage. SMX (☐), ERY (▪), ROX (▩), TMP (▧), FLX (■)

The calculated removal rates depended on the mechanism which is expected to play a major role in the elimination of each substance: biodegradation or sorption. As it is well-known, the sorpted fraction of a substance is directly related to its solid-water distribution coefficient (Ternes et al., 2004), which depends on its physical-chemical properties but might also depend to a certain extent on sludge physical properties such as hydrophobicity or particle size. Normally, low kd values have been reported for the antibiotics and, to our knowledge, kd values for FLX have not been reported. Table 5-3 compares the coefficients calculated in this research with reported data. In the case of FLX, Johnson et al., (2005) predicted a very strong chemical organic carbon adsorption coefficient (koc) (Table 5-3) which could support the kd data from our study, since considerable amounts of this compound were detected in primary and secondary sludge. It is also important to mention that kd values from literature were obtained from conventional activated sludge units (with the exception of ROX) whereas in our research, data corresponds to MBR sludge.

Table 5-3. Comparison of calculated kd values (L/Kg) with literature data ([a]Göbel et al., 2005; [b]Johnson et al., 2005; [c]Jones et al., 2002; [d]Joss et al., 2005)

	SMX	ERY	ROX	TMP	FXL
Calculated Kd	4 - 39	11 - 66	66 - 102	27 - 108	1173 - 1419
Literature Kd	[a]87 - 425	[a]0, [c]165	[d]470	[a]159 - 257	[b]$\log K_{oc} = 5.3$

Therefore, some differences might be expected, considering that the biomass developed in MBRs usually present some unique characteristics, such as smaller mean particle size or poor settling properties. The coefficient values clearly show that the studied antibiotics have low or negligible sorption to sludge biomass, as also demonstrated by Batt et al., (2007) with abiotic experiments.

In general, the elimination rate of the considered compounds increased with time. This tendency was expected considering the evolution of the three parameters studied: MLSS content was constantly increasing due to cellular growth and the contribution of primary sludge; temperature increased gradually after the winter period; and, the adaptation effect improved as a consequence of a more diverse biocoenosis developed during the operation of the system. Most of the studied substances are not readily biodegradable, with kbiol values rangin from 0.1 to 1 (L/gss/d) such as SMX, ERY and ROX (Joss et al., 2006). No kbiol is available for TMP and the reported data regarding its removal potential is scarce and contradictory, with removal rates ranging from negligible values up to 70%. For example, Göbel et al., 2007 found elimination values ranging from -13 to 31% in full-scale activated sludge plants and Junker et al. (2006) with experiments using 14C-Labeled TMP in a lab-scale STP confirmed its low biodegradability during biological treatment. On the contrary, Batt et al., (2006) estimated removal rates that ranged from 25 to 75% depending on the type of sludge that was used as inoculum. This information might be indicative of the influence of operational conditions in the removal of TMP. As shown in Figure 5-5, elimination rates for SMX and TMP increased following the same trend as MLSS concentration inside the reactor. Only during the February and April sampling periods, MLSS content did not change significantly, but TMP and ERY removal increased again whereas SMX experienced a slight decrease. In the case of ROX, its removal efficiency for the April sampling period could not be calculated due to analytical issues. Nevertheless, this substance follows the same trend, with increasing removal efficiency throughout the operation of the reactor.

After the intensive purge of sludge (day 302), SMX removal was particularly affected, decreasing to even lower efficiency levels than those calculated during the initial sampling period. This finding confirms that SMX removal rate may be influenced by the MLSS concentration. TMP, ROX and ERY removal rates, however, did not change significantly after the purge. Consequently, the influence of the MLSS concentration can be considered low or negligible for these compounds. Regarding the temperature and comparing results from the February and July sampling campaigns, it is obvious that TMP, ERY and ROX elimination rates steadily increased during the operation of the reactor. Theoretically, the increase in temperature may explain this finding but elimination rates estimated with data from the preliminary sampling of October were poor (5, 9 and 35 % respectively) and during this period the temperature of the mixed liquor was high (18ºC). Therefore, biomass acclimation effects might be more relevant than the increase in temperature. In the case of FLX, there is an evident lack of information regarding its behaviour and fate during sewage treatment. As mentioned above, this substance has strong affinity for the organic carbon portion of soils and sediments. Similarly, Kwon et al., 2006 corroborated this sorption tendency and performed biodegradation tests, concluding that FLX is resistant to microbial degradation. Therefore, the sorption removal mechanism onto sludge may play a significant role on its elimination during sewage treatment, and acclimation effects might not be relevant in this case. Apparently, FLX removal is not influenced by MLSS since similar removal rates were achieved with varying MLSS concentrations (June vs July). On the contrary, temperature might exert influence since no removal was observed during preliminary and February samplings.

The elimination of musk fragrances (HHCB, AHTN and ADBI) also varied substantially and might also be affected by the seasonal variations of temperature, but considering their particular physical-chemical properties, their behavior and fate will be discussed separately in the following section.

The case of musk fragrances: HHCB, AHTN and ADBI

The behaviour of these substances has been particularly studied through the sewage treatment. The partition to sludge has been identified as their main removal mechanism (Suarez et al., 2008) and in this research, This behaviour was confirmed by measuring the concentration of musk fragrances in the sludge phase (Table 5-4).

Calculated log kd values were similar or higher to previously reported results (Ternes et al., 2004; Kupper et al., 2006; Reiner et al., 2007) showing the high affinity of these substances to partitioning onto solids. Nevertheless, the calculated ranges present a great variability between different sampling campaigns. Distribution coefficients might vary depending on the type of sludge or physical-chemical conditions such as temperature, which might affect the dynamics of the sludge, the solubility in water of the target compounds and therefore, the sorption equilibrium. Moreover, some studies reported significant changes on the sludge developed on MBRs with respect to the sludge typically present in CAS systems (Massé et al., 2006) which might affect its sorption potential. For example, McArdell et al., (2005) compared kd values from conventional activated sludge plants, a fixed bed biofilm reactor and a MBR, finding that the sludge from the latter tended to sorb more strongly than conventional systems, possibly due to higher hydrophobicity and/or the smaller floc size.

Table 5-4. Liquid (µg/L) and solid-phase concentrations (µg PPCP/g TSS) of musk fragrances and calculated log Kd ranges for the biological sludge.

	HHCB			AHTN			ADBI		
	In	Out	Biol.	In	Out	Biol.	In	Out	Biol.
October	31	4	33	14	1	19	20	2	20
December	14	14	85	5	5	45	9	8	36
February	17	23	65	8	7	44	12	17	36
April	32	14	124	20	9	127	27	14	83
June	9	4	25	7	5	21	9	5	17
July	11	4	21	8	3	22	11	3	18
log kd	3.4 - 3.9			3.6 - 4.3			3.3 - 3.8		

In: liquid-phase sewage; Out: permeate; Biol.: biological sludge

In this study, the MBR was seed with CAS sludge and therefore some of its main characteristics might have experienced changes along its operation. In order to corroborate this trend, the particle size distribution of the biomass was followed during the operation of the MBR. However, these aspects will be discussed in the following section.

In Figure 5-6, removal efficiency for musk fragrances increased during the operation of the MBR with the exception of AHTN in the June sampling. In order to explain this behaviour, adaptation effects can be ruled out since biodegradation is not the dominant removal mechanism. An apparent correlation with the temperature measured in the mixed liquor can be observed, particularly comparing samplings from February and April (Figure 5-6). This effect is also detected when comparing data from the preliminary sampling of October, when the temperature of the mixed liquor was 18 ºC and the maximum degree of musk elimination of this work was achieved (above 85% for the three compounds). However, this sampling was carried out a few days after the starting-up of the MBR, when the biomass was still the typically present in CAS systems. During the winter period (February sampling), negligible removal was observed in some cases while in the summer period, with warmer temperatures and biomass completely adapted to the MBR conditions (after more than 300 days of operation), significant removals were achieved.

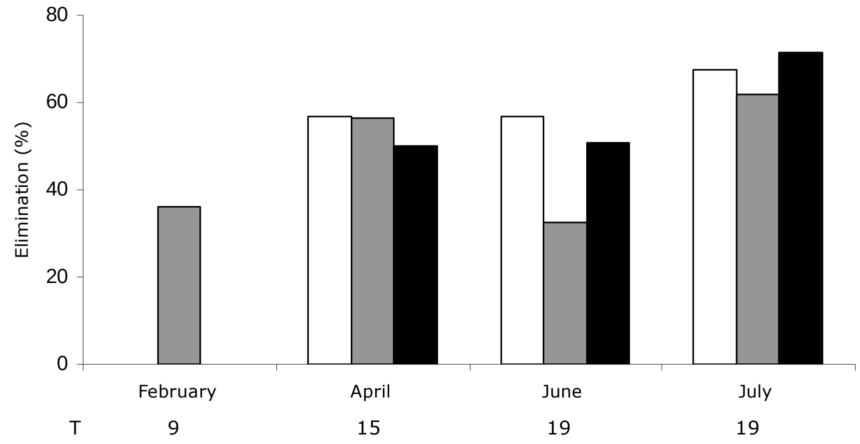


Figure 5-6. Musk Fragrances removal efficiencies. HHCB (□), AHTN (▨), ADBI (■)

In samplings of June and July the elimination of fragrances increased in spite of the similar value of temperatures. Different effects could be considered in order to explain this behaviour. In the first place, the mentioned changes of the physical properties of the MBR biomass might negatively influence the removal of lipophilic substances, which is in contradiction with the findings of McArdell et al., (2005). Secondly, it should be considered that distribution coefficients involve two different sorption mechanisms: adsorption and absorption. In general, adsorption is favoured at low temperatures while the solubility of lipophilic compounds in the cell wall (absorption) increases with the temperature. Therefore, it can be inferred that the dominant mechanism might be absorption. Finally, regarding the differences observed in June/July sampling periods, it is important to observe the dramatic purge of biomass (Figure 5-4) between the two sampling campaigns as the sorption potential of the biomass might be considered as exhausted and the newly generated sludge after the purge could enhance the overall sorption capacity of the system. Results from the preliminary sampling of October and previous research seems to confirm this trend, since reported removals of musk fragrances are significantly higher in conventional systems (Suarez et al., 2010). Further research is necessary in order to confirm this trend, for example by parallel operation of MBR and CAS systems under strictly similar conditions.

Recalcitrant PPCPs

Table 5-5 shows the removal efficiency for the remaining substances studied in this research and their calculated distribution coefficients. The antiepileptic CBZ and the tranquiliser DZP were only spiked during the last months of operation of the MBR. These substances have been considered as recalcitrant along sewage treatment (Clara et al., 2004; Reif et al., 2008; Serrano et al., 2010) and do not present tendency to be associated with the particulate phase (Suarez et al., 2008). The concentration of CBZ extracted from the sludge was below the detection limit of the methodology. In the case of DZP, its concentration present on sludge was low although measurable, in good agreement with previous research (Clara et al., 2004; Castiglioni et al., 2006; Ternes et al., 2004). Considering these two factors, T and MLSS concentration do not influence their removal significantly. However, intermediate removal rates were achieved during the last sampling campaign for both substances, which are unusually high compared with previously reported data.

Table 5-5. Overall removal efficiency (%) for DCF, CBZ, DZP and CTL

	DCF	CBZ	DZP	CTL
February	38	-	-	57
April	18	-	-	-
June	41	22	34	56
July	20	53	43	51
Average	29	38	39	55
SD	12	-	-	3
kd sludge (L/Kg)	nd	nd	131	410

nd: not detected

The antidepressant CTL also presented intermediate removal efficiencies, similar in the 3 sampling periods. In this case, neither MLSS concentrations nor temperature influenced its elimination. As mentioned before, this substance presents intermediate sorption behaviour which may be responsible of the observed elimination. The anti-inflammatory DCF is a substance widely studied due to its recalcitrant behaviour and high consumption rates (Alder et al., 2006). However, contradictory data are usually reported with overall removals ranging from null values up to 70% (Ashton et al., 2004; Rosal et al., 2010). Its mean removal efficiency (24%) seems to confirm its recalcitrant characteristics with no significant influence of the operational parameters and its levels on sludge were below the detection limit.

5.3.3 Biomass characteristics

Sludge characteristics might experience changes along MBR treatment due to factors such as the complete retention of solids inside the bioreactor or the operation at extended SRT. Some works dealt with these specific properties (Wang et al., 2007; Pollice et al., 2007, Chang and Kim, 2005; Chu and Li, 2005) with the aim to extend the understanding of the membrane fouling mechanisms, which have usually been a significant drawback for the growth of the MBR market. Considering that a particularly relevant removal mechanism for micropollutants removal is the sorption onto sludge, a study of the differences among MBR and CAS biomass might help to elucidate if the specific physical-chemical characteristics developed in MBR biomass might explain the differences among MBR and CAS technologies in terms of micropollutants removal. In this work, some biomass physical characteristics were measured during the operation of the MBR: the specific filtration resistance (α) and particle size distributions (Table 5-6). It is difficult to establish a comparison among different works, since contradictory trends are usually reported. For example, Cicek et al., 1999 found high α values around 10^{15} m/kg in MBRs.

Table 5-6. Physical characteristics of the MBR biomass

	Particle Size Distribution (µm)			α	MLSS
	d(0.1)	d(0.5)	d(0.9)	$(m\cdot kg^{-1})$	$(g\cdot L^{-1})$
October	12.9	51.2	250.4	$2.23\cdot10^{9}$	2.6
December	19.8	67	168.1	$2.01\cdot10^{10}$	3.84
February	13.3	56.9	165.2	$3.68\cdot10^{9}$	7.02
May	9.24	34.76	108.9	-	9.2

On the other hand, Pollice et al., 2007 reported similar values to the ones reported on this work, within the range of 10^{10}-10^{12} m/kg, also in good agreement with data reported by Wang et al., 2007. The first measure was performed soon after the starting-up of the MBR, when the biomass properties were similar to the ones measured in a typical CAS sludge. α increased during the initial period of operation, from October to December. During that period the solids content within the MBR slightly increased. Then, when the solids experienced a substantial increase, α decreased again, showing a weak correlation with the MLSS content. Wang et al., 2007 suggested that clusterings of Extracellular Polimeric Substances (EPS) and Soluble Microbial Products (SMP), named Biopolymer Clusters (BPC), might be responsible of the increase of α, since significantly different amounts of BPCs were found in the sludge cake deposited on the membrane surface and the bulk sludge. Previous works found that many operating parameters affect the EPS characteristics in the sludge. More concretely, the operation at extended SRT has been pointed out as the most significant (Hernandez Rojas et al., 2005; Massè et al., 2006), since decreased levels of EPS were found at this conditions. In the case of the SMP, Cabassud et al., (2004) compared CAS and MBR sludge in terms of EPS and SMP content, and found higher levels of SMP in the MBR sludge whereas EPS levels were similar. Kimura et al., (2009) also found a relationship between the SRT and the characteristics of SMP, but using non-conventional analytical methods such as excitation-emission matrix (EEM) fluorescence spectroscopy.

Regarding the particle size distribution, MBR mean floc size decreased along the operation of the MBR, particularly for the d(0.9) value, as can be observed in Table 5-6 and Figure 5-7.

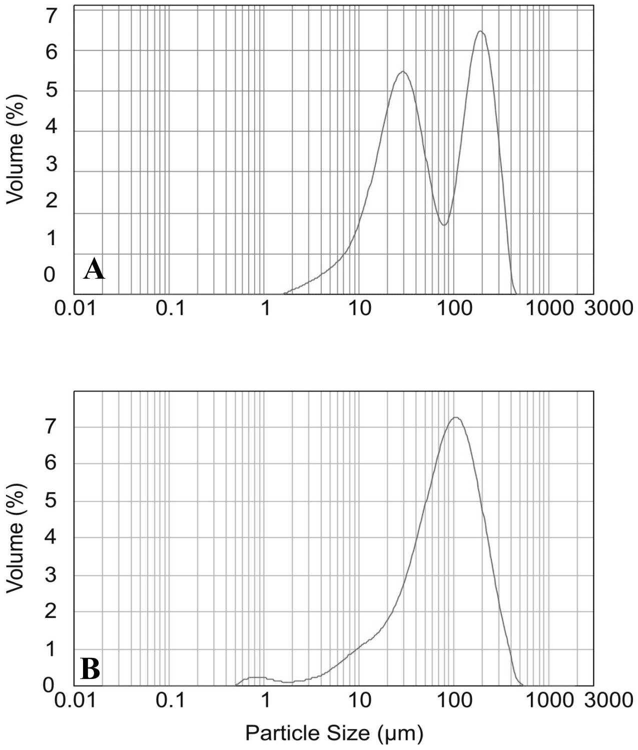


Figure 5-7. MBR biomass particle size distribution during the starting-up (A) and after 10 months of operation (B)

Similarly, Durante et al., (2006) found low particle sizes as a consequence of the hydraulic stress and the progressive decrease of the F/M applied, typical of MBRs with increasing MLSS levels as in the case of our work. Wisniewski et al., (1998) also mentioned the tangential flow along the membrane as a relevant factor which contributes to increase the shear stress, inducing changes in the settleability of the sludge. According to Massè et al., (2006), the decrease in floc size may be associated to a more compact floc structure due to fact that the small particles (dispersed bacteria and small colonies) have a higher density than the large flocs with more bridging between biopolymers. Based in previous research and considering the measures performed in this work, it can be concluded that MBR biomass indeed present some specific characteristics, as a result of the combination of different factors such as the operation at extended SRT, the F/M ratio and the sheer stress induced by the membrane filtration process.

Understanding the influence of these differences might be relevant in order to explain the potential of the MBR technologies for PPCPs removal when compared with conventional technologies.

Finally, the FISH methodology was also successfully applied in order to confirm the presence of *Alphaproteobacteria* and *Betaproteobacteria* in the sludge during the starting up of the MBR and before ending its operation (Figures 5-8 and 5-9).

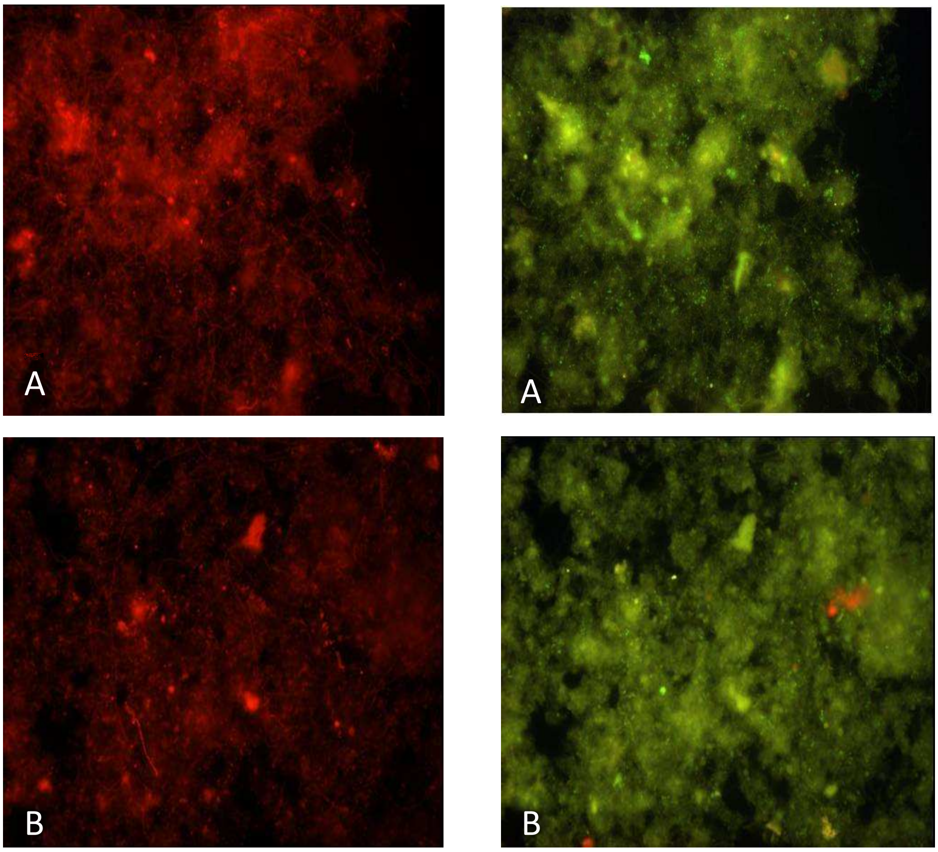


Figure 5-8. Presence of *Alphaproteobacteria* (in green) in the activated sludge used as inoculum (A) and after the operation of the MBR (B). Scans in red shows microorganisms corresponding with the Eubacteria domain (1000x)

Alphaproteobacteria presence along the operation of the MBR was demonstrated by comparing the FISH probes ALF1 (in green) with the non-specific EUB338 corresponding with the bacterial domain (in red). Most relevant bacteria from activated sludge cultures belonging to this domain are the nitrite-oxidizers *Nitrobacter ssp.* and the heterotrophs *Paracoccus*. Considering that the conditions established in the MBR were appropriate for the removal of organic matter and nitrogen (as demonstrated by the COD and ammonia removal rates measured along the MBR operation), the detected bacteria might belong to both genus.

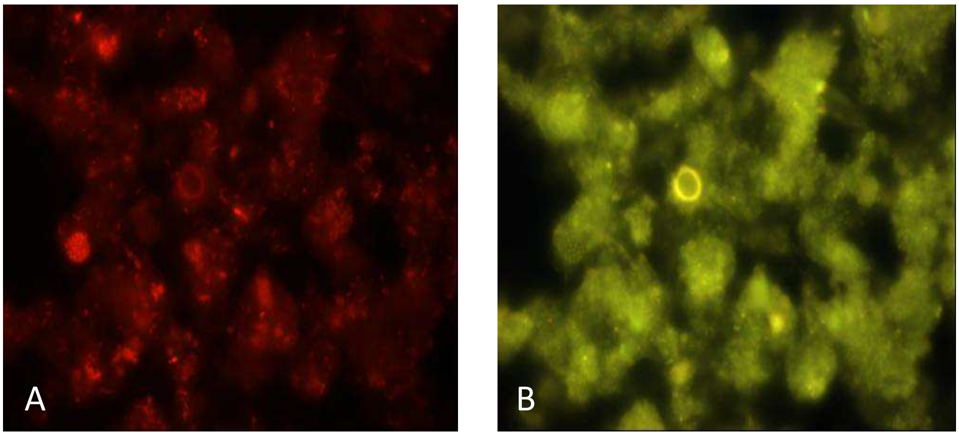


Figure 5-9. Presence of *Betaproteobacteria* in the activated sludge after the operation of the MBR (A) and photo of a negative FISH analysis performed with the NEU653 probe (B). (1000x)

Interestingly, the presence of *Betaproteobacteria* was only confirmed in sludge samples from the final period of operation of the MBR. Additionaly, the presence of three different species of ammonia oxidizing bacteria (AOB), *Nitrosomonas Europaea*, *Nitrosomonas Eutropha* and *Nitrosococus Mobilis*, could not be confirmed, since FISH analysis with their specific probe (NEU653) did not show fluorescence. Therefore, the detected *Betaproteobacteria* might belong to different AOBs genus or more probably to the heterotrophs *Comamonas*, which presence is usual in activated sludge cultures.

5.4. Conclusions

The physical-chemical properties and biodegradability of the compounds considered in this study and the different parameters considered allow gathering the following conclusions, summarized in Table 5-7, regarding the potential of the MBR technology for the removal of PPCPs:

IBP, NPX and the three hormones (E2, EE2 and E1) were almost completely removed during the MBR treatment independently of the operational conditions. According to the low concentration of substance present on sludge, these PPCPs were removed following biodegradation mechanisms.

Musk fragrances and FLX elimination varied significantly depending on the operational period considered, ranging from poor to moderate-high. Absorption onto sludge was identified as their main removal mechanism. A combination of different effects might explain the differences observed among the different sampling periods: Temperature of the mixed liquor, sludge age (modified by the sludge purges) and the physical properties of the MBR biomass.

The lowest elimination was observed for CBZ, DZP, CTL and DCF. Comparing with reported data for conventional systems, particularly for CBZ, results were surprisingly higher than expected. With the exception of CTL, sorption was not relevant for removing these substances, and the temperature and MLSS did not influence their removal at any extent.

The elimination of the antibiotics SMX and TMP was from moderate to high and increased along the operation of the MBR. In both cases and considering their low biodegradability constants, MLSS might slightly influence their elimination. However, in the case of TMP, a combination of this factor with acclimation effects might also explain the degree of removal achieved.

In the case of ERY and ROX, high removal of the parent compound was observed, but only after several months of operation. MLSS and temperature did not influence their removal and consequently, adaptation effects might explain the observed behaviour.

Table 5-7. Classification scheme of the different compounds according to their behaviour, fate and to the influence exerted by MLSS, Tª and acclimation on their removal.

PPCP	Sorption	Removal	Tª	MLSS	Acclimation
IBP	-	++	no	no	no
NPX	-	++	no	no	no
DCF	-	-	no	no	no
CBZ	-	-+	no	no	no
DZP	-	-	no	no	no
HHCB	++	+	yes	no	no
AHTN	++	++	yes	no	no
ADBI	++	++	yes	no	no
SMX	-	+	no	yes	no
ERY	-	++	no	no	yes
ROX	-	++	no	no	yes
TMP	-	+	no	yes	yes
EE2	-	++	no	no	no
E2	-	++	no	no	no
E1	-	++	no	no	no
FLX	+	+	yes	no	no
CTL	+	-+	no	no	no

Removal: (-) < 40%; (-+) 40-65%; (+) 65-90%; (++) 90-100%

Sorption: (-) Poor; (+) Moderate; (++) High

If an influence of temperature, MLSS concentration or the acclimation effect on the transformation degree is observed it is indicated as (yes) or (no)

5.5. References

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Chapter 6

Are Membrane Bioreactors more efficient than Conventional Activated Sludge Technologies for removing PPCPs from Sewage?[1]

Summary

The performance of two parallely-operated systems, a conventional activated sludge unit and a membrane bioreactor, was compared in terms of 17 pharmaceutical and personal care products removal. Their performance was tested with different HRTs (8-24 h) and SRTs (>20 d and <10 d) maintaining their working conditions (MLSS, pH, temperature, DO) at similar values. The elimination of polar compounds such as diazepam, carbamazepine or diclofenac was rarely above 30% in both systems. On the other hand, ibuprofen or naproxen exhibited higher removals (> 85%) with no significant amount retained in solids, thus indicating that biodegradation was the key mechanism. Comparing both technologies, no significant differences were observed for many of the PPCPs with the exception of fragrances and anti-depressants, which were removed at a higher extension in the conventional system. The most significant impact was observed during the operation at low SRT, when the MBR achieved improved results.

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Reif, R., Barcón, T., Omil, F. and Lema, J.M. Are Membrane Bioreactors more efficient than Conventional Activated Sludge Technologies for removing PPCPs from Sewage?. Bioresource Technology.

Outline

6.1. Introduction

Previous research has shown that more than 80 classes of Pharmaceutical and Personal Care Products (PPCPs) and their metabolites are being detected in sewage effluents, surface water, ground water and even drinking waters (Ternes 1998; Suarez et al., 2008; Reif et al., 2010). In fact, in the last decade many studies have been carried out dealing with the occurrence, fate and elimination of these micropollutants throughout sewage treatment processes but also focusing on the potential adverse ecological responses which might arise due to the discharge of these substances into the environment (Nunes et al., 2005).

Nowadays, it is well-known that the main input of PPCPs into the urban wastewater system occurs mainly from domestic sources, particularly by involuntary means such as excretion and bathing. Subsequently, from the discharged effluents, PPCPs gain entrance into the hydraulic cycle and the aquatic environment, eventually becoming pseudo-persistent because their elimination or transformation rates are countered by their constant replenishment (Heberer, 2002; Barceló and Petrovic, 2008), since the design criteria of current sewage treatment plants (STPs) does not incorporate treatment steps specifically devoted to the elimination of these substances. Additionally, hydrophobic organic contaminants such as musk fragrances are largely transferred to the sewage sludge during sewage treatment with potential implications for the use of sludge or its correct disposal.

The major therapeutic groups of PPCPs commonly detected in wastewater treatment plants are antibiotics, antiepileptics, anti-inflammatories, tranquilizers, X-rays contrast media, contraceptives, musk fragrances and cosmetics (Ternes, 1998; Heberer, 2002). Since the European Union Water Framework Directive (2000/60/EC) aims to promote sustainable water use and the long-term progressive reduction of contaminant discharges into the aquatic environment in urban wastewater, it is crucial to develop strategies for improving the overall quality of sewage treatment.

On the one hand, previous research has demonstrated that the operational parameters of the STPs might influence the elimination of persistent substances at a certain extension. These parameters include the hydraulic residence time (HRT), solid retention time (SRT), mixed liquor suspended solids concentration (MLSS), pH, redox conditions and temperature (Barceló and Petrovic, 2008; Suarez et al., 2008). Optimizing these parameters together with a good knowledge of the physical and chemical properties of each compound might help to determine the best strategy to follow in order to achieve better eliminations. On the other hand, the testing of new technologies for wastewater treatment might also help to minimize the release of micropollutants into the aquatic environment.

Currently, a leading-edge technology, the membrane bioreactor (MBR), is also being tested in terms of micropollutants removal due to its ability to produce high quality effluents. Such systems combine the biological process with a membrane filtration step within one process unit, overcoming clarification and producing a high quality effluent. Regarding their efficiency in terms of PPCPs removal, the results reported so far are not significant enough to clearly show its advantage with respect to CAS systems and it might be crucial to understand if they can help to mitigate the presence of micropollutants in effluents.

Some studies have compared the performance of CAS and MBR systems. For some specific substances, the reported eliminations were higher in the MBRs (+25%), but this increase might be attributed mainly to the optimum conditions that were set in these systems, particularly the biomass concentration and SRT (Omil et al., 2010). Additionally, many of these studies were carried out comparing pilot or lab-scale MBRs with full-scale sewage treatment works, which are not easily controllable for developing accurate sampling strategies and long-term experiments. Another substantial difference between both technologies is the different physical properties of the biomass developed in CAS and MBR systems.

Nowadays, it is known that sludge characteristics experience changes during MBR treatment due to factors such as the complete retention of solids inside the bioreactor, the operation at extended SRT or the effect of the membrane filtration process (Judd, 2006). As an example, Massé et al., (2006) found different structural conformation of biomass in MBRs which have an effect, for example, on its settling properties. Nowadays, research is focussed on specific biomass properties such as the specific cake resistance, floc characteristics, viscosity, hydrophobicity and surface charge (Chang and Kim, 2005; Chu and Li, 2005; Pollice et al., 2007) with the aim to extend the understanding of membrane fouling mechanisms, considered a significant drawback for the growth of the MBR market. However, a deeper understanding of the influence of these aspects might indeed be relevant in order to explain the potential of the MBR technologies for PPCPs removal when compared with conventional technologies, considering that such systems are notably more expensive than traditional wastewater facilities.

The purpose of this work is to compare the efficiency of MBR and CAS technologies operated with similar conditions treating sewage. Different values of HRT and SRT were simultaneously tested in both systems, and their overall performance was assessed in terms of PPCPs removal efficiencies.

6.2. Materials and methods

6.2.1. Experimental set-up and operation methodology

Part of the set-up of this study was located at the premises of the Silvouta STP located in Santiago de Compostela (NW Spain). It consisted of a primary settling step, a mixing tank where PPCPs were continuously spiked and the MBR pilot-scale plant. Feeding system was already explained in the previous chapter and the main novelty consisted in the lab-scale CAS (Figure 6-1) placed at the premises of the School of Engineering (University of Santiago de Compostela) which was fed with spiked sewage from the Silvouta mixing tank.

Figure 6-1. Lab-scale CAS after 3 months of operation.

The CAS is composed of an aerated tank of 2.5 L capacity and a clarifier unit of 1 L capacity. Figure 6-2 consists of a diagram detailing the overall set-up used for the purposes of the CAS-MBR comparison described in this chapter.

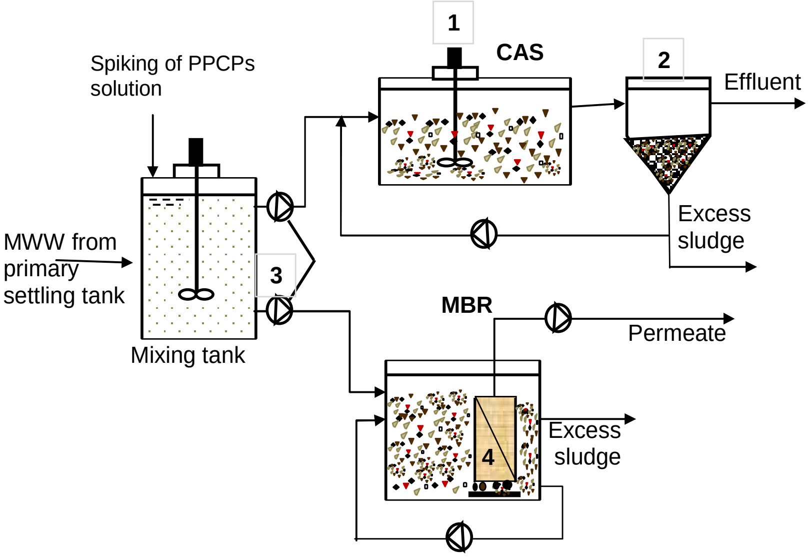

Figure 6-2. Experimental set-up showing the schematic diagram of the mixing tank, the MBR and the CAS systems

The operation of the CAS involved the continuously mixing of the biomass with the influent wastewater and the returned activated sludge in the aeration tank. Aeration was continuous in the tank to maintain uniform oxygen supply and loading. The recirculation of the biomass from the sedimentation tank to the reactor was achieved by means of a prismatic pump (P-2: Maxima, ® L/S 1-100 rpm, China). The rate at which this recirculation occurs was determined through manual adjustment to suit the performance of the plant. The aeration of the reactor was supplied by means of an aeration pump (Elite802, Rolf C. Hagen Inc. China). The CAS plant was allowed to stand with constant biomass recirculation and aeration for 5 days to enhance acclamation of the biomass to the new environment. The flow of the influent into the reactor was achieved by means of an influent pump (Model: 7554-85, Barrington, USA) supplied by Cole-Parmer Instrument Company, UK.

The biomass was completely adapted to the presence of micropollutants since the MBR was previously operated and fed with spiked sewage during an extended period (Chapter 5). The biomass developed during the MBR process was also used as an inoculating source to start-up the CAS system. The methodology to carry out this research consisted of studying the influence of different conditions on PPCPs elimination in both bioreactors during an extended period of operation. The MBR and CAS systems were parallel-operated at varying SRT (high and low) and HRT

(24, 20, 12 and 8 hours) conditions and 6 sampling campaigns were carried out. Biological sludge was purged from both systems on a regular basis in order to operate with similar biomass concentration, maintaining at all times a SRT above 20 days. After 5 months of operation, sludge purges were performed more intensively in order to study the impact of decreasing the SRT below 10 days. Subsequently, no purges were carried out during 2 months in order to carry out a final sampling campaign for PPCPs analysis which enabled to test the capacity of the CAS and the MBR to remove PPCPs operated in optimum conditions (extended SRT and 12 hours of HRT) and low temperatures.

The treated sewage was continuosly spiked with the following PPCPs at environmentally relevant concentrations: tranquilliser: diazepam (DZP, 20 ppb); antiepileptic: carbamazepine (CBZ, 20 ppb); antibiotics: sulfamethoxazole, erythromycin, trimethoprim and roxithromycin (SMX, ERY, TMP and ROX, 10 ppb); antidepressants: fluoxetine and citalopram (FLX and CTL, 10 ppb); musk fragrances: galaxolide, tonalide and celestolide (HHCB, HHTN and ADBI, 40 ppb), anti-inflammatory drugs: ibuprofen, naproxen and diclofenac (IBP, NPX and DCF, 10 ppb) and the hormones estradiol, ethynilestradiol and estrone (E2, EE2 and E1, 1 ppb). Selected pharmaceuticals and hormones were purchased from Sigma-Aldrich. Musk fragrances were generously supplied by Ventós (Spain). Influent, permeate and sludge samples for PPCPs analysis were regularly collected as time-proportional according to the HRT set in both systems. Glassware and aluminium bottles were used for sampling and storage.

6.2.2. Analytical methods

PPCPs, conventional analysis and particle size measures were carried out as described in Chapters 2 and 5. During each campaign, samples of biological sludge were also collected in order to determine the amount of PPCPs sorbed onto the solid fraction using the methodologies of ultrasonic solvent extraction for antibiotics, antidepressants and hormones (Ternes et al., 2005) and the solid-phase microextraction for musk fragrances (García-Jares et al., 2002).

Morphological studies of the biomass were performed with a scan electron microscope (Digital SEM Leica 440 at 20 kV) controlled with a computer system and with a magnification capacity ranging from 15 to 290000 folds. The sludge sample was washed three times for 10 minutes with phosphate buffer 0.05 N at pH 7.4 and subsequently fixed with a solution of glutaraldehyde 3% in phosphate buffer overnight. After fixation the sample was dehydrated using ethanol solutions with increasing ethanol concentrations (30, 50, 70 and 100%). Later the sample was shaded with gold and observed under the scan electron microscope.

Mass balances of the amount of each substance entering and leaving the MBR and CAS were determined in order to calculate total removal efficiencies considering both liquid and solid phase data. The calculation methodology can be found at Reif et al., 2010. Likewise, the amount of PPCP sorbed onto the biological sludge was used for calculating the solid-water distribution coefficient for each substance, according to the expression described by Ternes et al., 2005.

6.3. Results and discussion

6.3.1. Conventional parameters

A summary of the main operational parameters set during the different sampling campaigns can be found in Table 6-1.

Table 6-1. Operational conditions set on the CAS and MBR systems

Days of Operation		Tª (ºC)	MLVSS (g/L)	SRT	HRT (h)	pH	DO (mg/L)
(I): 23	CAS	21	2.2	high	24.3	7.9	8.2
	MBR	18.1	2.7		23.6	7.6	5.4
(II): 49	CAS	24.3	3.7	high	19.2	7.8	7.2
	MBR	21.8	4.3		18.7	7.2	6.2
(III): 20	CAS	23.6	3.8	high	12	7.8	7.3
	MBR	20	6.4		12.4	7	3
(IV): 19	CAS	20.5	4.5	high	8.4	6.07	6.9
	MBR	15	4.5		8.5	8.2	4.5
(V): 52	CAS	13.3	2.1	low	8.1	7.6	9.4
	MBR	14	2.5		8.1	7.3	7.5
(VI): 62	CAS	10.5	4.7	high	12.2	7.3	7.1
	MBR	13.3	7.8		11.9	7.4	6.5

Conventional parameters, mainly COD (total and soluble), nitrogen (ammonia and nitrate) and solids concentration were regularly followed at the inlet and outlet of both reactors. CAS and MBR performance in terms of COD removal was excellent (90-95%). Slightly higher efficiencies were always measured in the MBR permeate compared with CAS effluent, most probably due to the effect of membrane rejection. Ammonia removal was also high, above 95%, with no significant differences among the six operational periods. Only one exception was observed during the low SRT period, when the sludge purges become more intensive. Under those conditions, CAS performance removing ammonia slightly decreased. Regarding the solids concentrations, its content in MBR permeate was negligible and the clarifier from the CAS separated them efficiently.

6.3.2. Fate of PPCPs in the MBR and CAS pilot-scale plants

Table 6-2 shows a classification of PPCPs according to the degree of removal achieved and globally summarizes the main results of this research.

Table 6-2. Classification scheme of the results gathered in both bioreactors regarding PPCPs elimination

	MBR						**CAS**					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI
Tª (ºC)	18	22	20	15	14	13	21	24	24	21	13	14
HRT (h)	24	20	12	8	8	12	24	20	12	8	8	12
SRT	high	high	high	high	low	high	high	high	high	high	low	high
IBP	+	++	++	++	++	++	+	++	++	++	++	++
NPX	++	++	++	++	+	++	++	++	++	++	-	++
DCF	-	-	-	-	-	-	-	-+	-	-+	-	-
CBZ	-+	-	-	-	-	-	-+	-+	-	-	-	-
DZP	-+	-	-	-	-	-	-+	-+	-	-	-	-
HHCB	-+	-	-	-	-	+	++	+	+	+	+	++
AHTN	-+	-	-	-	-	+	++	+	-+	+	+	++
ADBI	-+	-	-	-	-+	+	++	+	+	+	+	++
SMX	-	-+	-+	-+	-	-+	-	-	-+	+	-	+
ERY	++	++	++	++	+	++	++	++	++	++	-	-+
ROX	++	++	++	+	-+	++	+	+	++	++	-	+
TMP	+	-+	+	+	+	++	-	-	+	+	-	+
FLX	+	-	-+	+	-	+	++	+	+	-+	+	-+
CTL	-	-	-	-+	-	-	+	-	-+	-+	-	-+
EE2	++	++	++	+	-+	+	++	++	++	++	+	++
E2	++	++	++	++	++	++	++	++	++	++	+	++
E1	++	++	++	++	+	++	++	++	++	++	+	++

Influence of HRT

The influence of HRT was not relevant for most of the PPCPs studied (Table 6-3). Only substances that are usually considered recalcitrant such as carbamazepine or diazepam showed a slight dependency on this parameter since their eliminations decreased simultaneously with the HRT, despite the increase of biomass concentration in both systems. Considering their slow degradation kinetics (Joss et al., 2006) and low tendency to be associated with the solid fraction (Ternes et al., 2005), this finding appears to be reasonable. In fact, the lowest outlet concentrations were achieved during the first operational period with the longest HRT tested (24 h). Nevertheless, the degree of removal achieved during the sampling period corresponding with the lowest HRT set in both systems (8 hours) increased, which is contradictory since the lowest HRT should correspond with the lowest elimination.

Table 6-3. Influence of HRT for the elimination of PPCPs

		Elimination (%)				
HRT (h)	System	CBZ	DZP	ROX	EE2	FLX
24	MBR	55	56	96	92	67
20		23	36	97	91	24
12		14	9	97	94	41
8		30	29	88	79	66
24	CAS	58	56	88	95	95
20		40	52	84	91	83
12		4	28	94	96	82
8		31	39	95	95	65

Elimination rates measured for these two substances were unusually high during the first sampling period, up to 56% in the case of CBZ. With the exception of the MLSS levels which were moderate during this initial period, the other parameters were optimum for the removal of hardly biodegradable substances: long HRT, warm temperatures (factors which are directly linked with biodegradation kinetics) and extended SRT operation. Moreover, the biomass was completely adapted to the presence of both substances, since the MBR was started several months earlier to the first sampling period, and the biomass developed was used as inoculum for the CAS system. The combination of these factors might explain the high degree of elimination achieved. This explanation is also supported considering that this trend was observed in both bioreactors and no relevant differences in the eliminations were observed between them. However, as HRT decreased, results were in better agreement with previous researching works which observed their persistence throughout the biological treatment (Clara et al., 2004; Joss et al., 2006, Reif et al., 2008)

Other PPCPs such as ROX, EE2 and FLX also showed a weak dependence on the HRT, but this behaviour was different depending on the technology considered and therefore the influence of this parameter cannot be confirmed in this case.

Influence of SRT

In this part of the research, SRT influence was assessed comparing the results achieved during the 4th and 5th operational periods due to the intensive sludge purges performed in both systems during the 5th period. This permitted to work at significantly low values for this parameter (below 10 days). Figure 6-3 shows the comparison between both systems under these conditions.

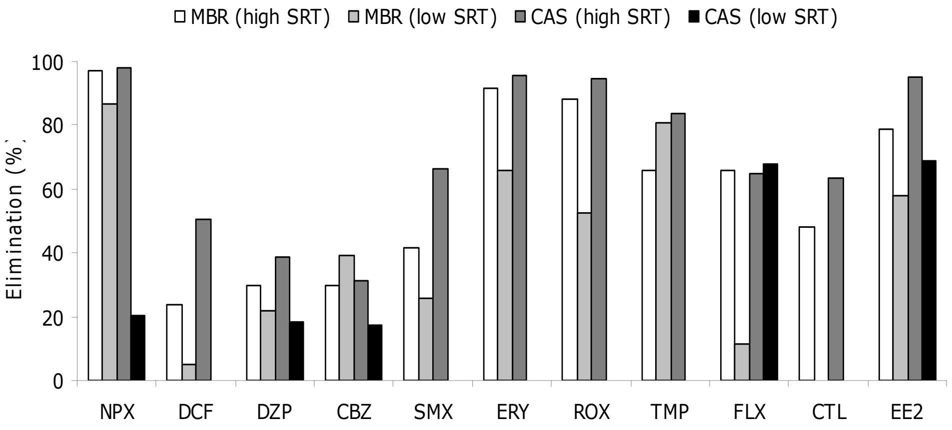

Figure 6-2. Influence of the SRT for the elimination of selected PPCPs

The removal efficiency of many substances was severely reduced, down to negative values in some cases, after reducing the SRT in the CAS system. In the case of the MBR, the antidepressant CTL was the only substance whose elimination completely ceased under these conditions. The elimination of the remaining substances was strongly affected, but negative removals were never achieved. Additionally, different behaviours were observed depending on the substance considered and its physical-chemical properties. The effect of the temperature of the mixed liquor should also be considered in order to compare CAS and MBR data, since the CAS location was indoors until period IV (Tª: 20.5 ºC) and then was placed outdoors for the remaining periods (Tª period V: 13.3 ºC) in order to eliminate any difference with the MBR operation.This might influence results observed in period IV, when difference of 5.5 Cº was observed between both bioreactors, but this difference is low and it might only slightly favour reactive processes in the CAS system.

Anti-inflammatories: Ibuprofen, naproxen and diclofenac

NPX removal slightly decreased in the MBR after changing the SRT whereas in the CAS it was reduced from 98 down to 20%. The biodegradability of this pharmaceutical is moderate and consequently, particularly affected by operating conditions and factors such as microorganisms adaptation. The elimination of ibuprofen was always >85% in both systems (data not shown), and the variations of SRT or HRT did not affect its removal from sewage to any extent. In the case of DCF, its recalcitrant characteristics have been well-documented in literature (Clara et al., 2005; Bernhard et al., 2006; Yu et al., 2006), although some works also reported high removals during conventional treatment (Stumpf et al., 1999; Radgenovic et al., 2007). In this case, the performance of the CAS system was better than the MBR, particularly when it was operated at high SRT. At low SRT, the performance of both systems decreased and again, the influence of this parameter was more pronounced in the CAS.

Tranquillizer and antiepileptic: Diazepam and carbamazepine

The behaviour and fate of these two PPCPs was fairly similar in both systems. In general terms, their elimination during the different sampling periods was low-moderate. After decreasing SRT, the elimination of DZP dropped significantly in the CAS (-22%) and slightly in the MBR (-7%). Interestingly, CBZ removal in the MBR was not particularly affected after SRT reduction, since its elimination even increased (+9%), whereas the opposite trend was observed in the CAS (-14%).

Similarly to the effect of HRT variations, it can be stated that the elimination of substances with slow biodegradation kinetics is easily influenced by the variation of relevant operating conditions such as SRT. As a consequence, it might be possible to improve their elimination during a biological treatment process, but most probably it would not be feasible from an economical perspective, since HRT, temperature and MLSS levels should be substantially higher than those typically set in conventional systems and even in MBRs. Even given these conditions, complete elimination of CBZ and DZP would never be achieved.

Antibiotics: sulfamethoxazole, erythromycin, roxithromycin and trimethoprim

The elimination of the antibiotics SMX, ERY and TMP was again severely reduced in the CAS and only moderately in the case of the MBR with the exception of TMP, whose removal slightly increased in the MBR after decreasing the SRT, but not in a significant way. Considering that the SRT influenced the removal of these substances, it might be pointed out that, again, the development of a broader biocoenosis, mainly nitrifying bacteria, might be beneficial for the removal of some recalcitrant substances. In the case of TMP, this was also demonstrated by previous research (Batt et al., 2006; Göbel at al., 2007). Morever, the results achieved in this research highlight a relevant advantage of the MBR systems. Due to the complete retention of biomass, nitrifying conditions are easily achievable and sustainable even during an intensive period of biomass purges.

Antidepressants: fluoxetine and citalopram

Interestingly, the removal of antidepressants followed a different trend. After decreasing the SRT, the elimination of FLX in the MBR dropped substantially whereas in the CAS no significant impact was noticed. In the case of CTL, both systems were completely ineffective in removing this pharmaceutical from sewage when the SRT was decreased. However, its elimination was also slightly higher in the CAS system. To our knowledge, very few authors have studied the behavior of both substances during sewage treatment. However, there is increasing evidence regarding their tendency to partition onto sludge (Johnson et al., 2005). Therefore, their elimination might be feasible mainly following a sorption process and biomass properties might play a significant role. However, considering the significant impact of the SRT, the biodegradation might also be relevant. Therefore, a certain parallelism between CTL and EE2 can be observed, since both substances have intermediate sorption potential (data shown in next section) and their elimination might be carried out as a combination of biodegradation and sorption processes. SRT influence was more evident in CTL removal, and consequently, the biodegradation process might be more relevant. In contrast, sorption might be the dominant mechanism in the case of EE2.

Hormones: estradiol, ethynilestradiol and estrone

The elimination of estradiol and estrone was almost complete in the MBR and the CAS. Previous works also confirmed their high biodegradability potential during sewage treatment (Bernhard et al., 2006; Yu et al., 2006; Radjenovic et al., 2007). However, SRT reduction has a small effect on the elimination of estrone in the MBR and of estrone and estradiol in the CAS (data not shown).

A slightly different behaviour was observed for the hormone EE2 since its elimination was equally high but the impact after reducing the SRT was more evident (-21% and -26% for the MBR and CAS respectively) and fairly similar in both systems. Previous research dealing with EE2 biodegradation suggests that the two main removal mechanisms play a significant role in its removal and confirms the results achieved. On the one hand, the sorption onto solids is moderate-high. This aspect has also been demonstrated in this work and will be discussed in the following section. On the other hand, its biodegradation is feasible and almost complete under nitrifying conditions (De Gusseme et al., 2009; Clouzot et al., 2010), an aspect that is also corroborated in this work considering that the removal of EE2 was negatively affected when the SRT was reduced down to values which were not suitable for the development of nitrifying bacteria.

6.3.3. Influence of biomass properties on elimination of highly sorbed compounds

Previous studies have identified differences in the conformation and structure of the biomass developed during a MBR process compared with CAS (Massè at al., 2006), although it is not clear if these differences affect characteristics such as the biomass sorption potential or the biodegradation kinetics. In this research, the most significant differences between CAS and MBR results were found for biodegradation of polar substances at low SRT, and for the elimination of compounds which tend to sorb onto sludge, independently of the operational conditions. To gain some knowledge about that issue, we have determined the Kd values of the sludges obtained from the parallely-operated CAS and MBR systems. To our knowledge, no previous results comparable to that have been reported. In Table 6-4 we present the results of the two reactors in six different operating periods. The main objective of performing this wide number of solid phase analysis was to gather information regarding the potential of each technology for the elimination of lipophilic substances which undergo a sorption mechanism by calculating their solid-water distribution coefficients. As it can be observed, most of the values are consistent in different sampling campaigns for each of the considered substances. The concentrations of the three anti-inflammatories, the antiepileptic CBZ and the hormone E2 extracted from the sludge samples were below the detection limit of the analytical methodology and therefore they are not listed in the table.

Given the complexity and uncertainty of the analytical methodology for PPCPs extraction from biological sludge, in some cases the measured concentrations might be affected by different analytical and operational factors. Therefore, statistical analysis software (SPSS) was applied in order to reject unreliable data (95 % confidence). These data, showed in bold, were not considered for log Kd calculations. CAS sludge shows a slightly higher tendency to sorb lipophilic substances since the calculated coefficients are moderately higher when compared with the Kd values measured for the MBR sludge in many cases. Despite this trend, the observed differences in terms of Kd values between both technologies were low. Therefore, 3 additional results (shown in italics) were identified as being considerably higher in the CAS system. Therefore, these values were not considered for log Kd calculation. The substances with higher Kd are the musk fragrances and the antidepressant FLX, followed by the hormones E1 and EE2, and CTL, which present intermediate Kd values. Sorption was not particularly relevant for antibiotics and DZP, in good agreement with previously reported data for conventional systems (Ternes et al., 2005; Göbel et al., 2005; Joss et al., 2005). Apparently, no specific trend or influence of the operational conditions can be deducted when comparing the results achieved between different operational periods. Figure 6-3 shows the comparison of the removals achievd in each technology for the most lipophilic substances during the different sampling campaigns of this research. The overall elimination degree achieved for musk fragrances, FLX, CTL and EE2, was usually higher in the CAS, which is in agreement with their Kd values. It is worth to observe in Figure 6-3 that FLX, CTL and in a less extent EE2 are better removed in CAS than MBR when operating at low SRT (period V).

Table 6-4. Kd values (L/kg) calculated for PPCPs detected on MBR and CAS sludge samples.

	Period	SMX	ERY	ROX	TMP	FLX	CTL	EE2	E1	DZP	HHCB	AHTN	ADBI
MBR	I	40	nd	56	64	4250	860	1111	1302	160	**9852**	**10245**	**6316**
	II	52	nd	0	53	1872	606	740	nd	105	3270	2246	1492
	III	30	125	**248**	86	1129	410	1820	**5707**	86	2501	2315	2082
	IV	49	13	17	44	3147	744	823	1125	161	3404	3446	2372
	V	23	20	46	64	3143	596	339	406	71	3302	2944	2084
	VI	13	72	70	96	1676	391	602	1170	46	1678	2530	1201
log Kd		1.1-1.7	1.1-2.1	1.2-1.8	1.7-2.0	3.0-3.6	2.6-2.9	2.5-3.3	2.6-3.1	1.7-2.2	3.2-3.5	3.3-3.5	3.1-3.4
CAS	I	76	nd	13	85	**18652**	**3851**	**3794**	nd	nd	na	na	na
	II	57	nd	32	135	6882	1306	nd	nd	312	2636	2745	1818
	III	**3444**	nd	30	51	*17449*	1846	1407	nd	**3725**	4860	3237	2150
	IV	*1002*	9	136	106	6597	1398	1693	nd	312	3614	2883	2249
	V	1	22	13	100	*20490*	1291	373	426	98	3919	4036	2381
	VI	14	47	123	152	2328	493	917	2711	145	6346	**6565**	**7443**
log Kd		0.0-1.9	0.9-1.7	1.1-2.1	1.7-2.1	3.4-3.8	2.7-3.3	2.6-3.2	2.6-3.4	2.0-2.5	3.4-3.8	3.4-3.6	3.3-3.4

nd: not detected on the sludge sample; na: not analyzed due to sample inavailability

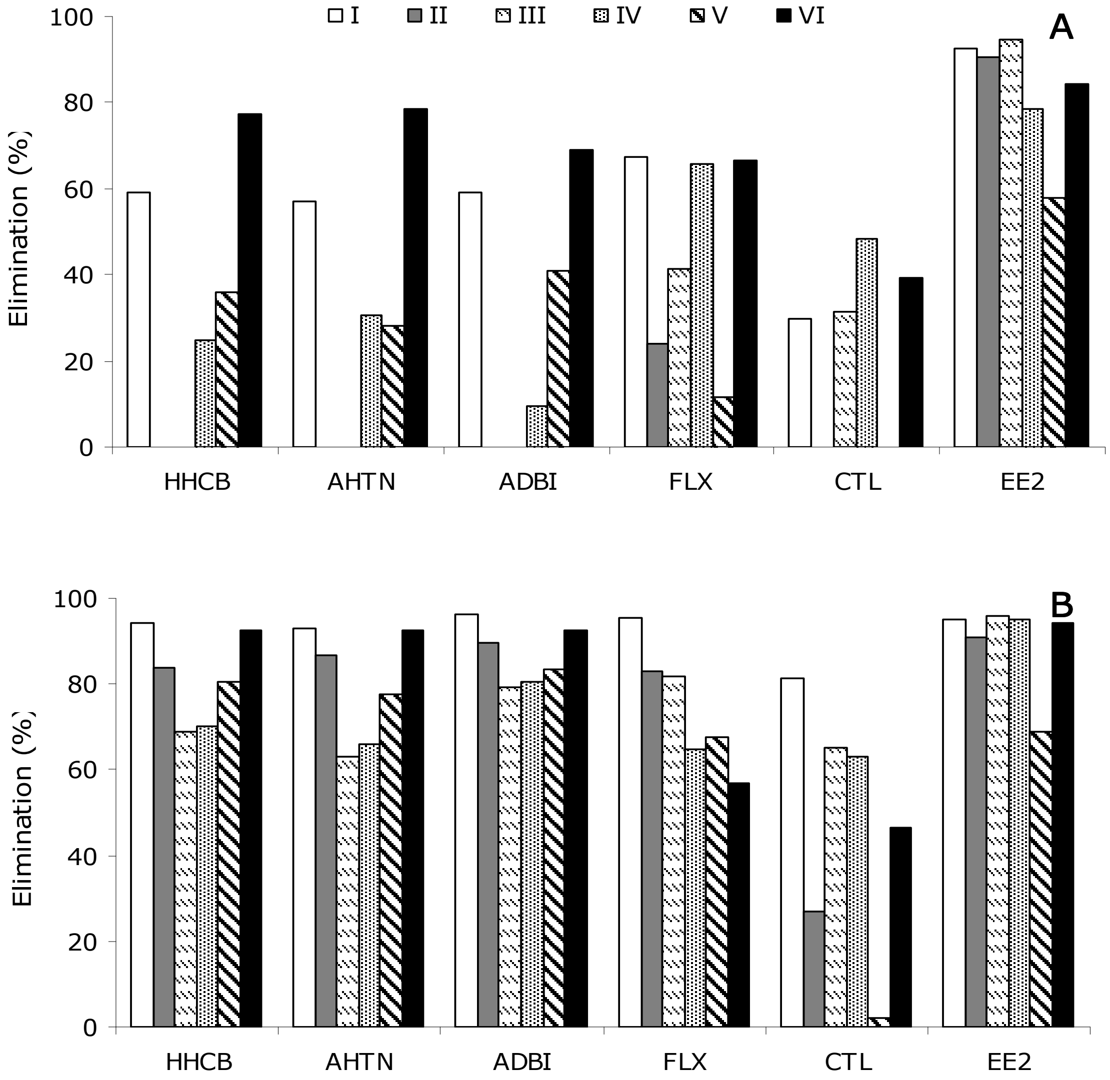

Figure 6-3. Elimination of PPCPs with high affinity to solids (A): MBR; (B): CAS

This effect could be explained in terms of the slightly increased distribution coefficients measured in the CAS sludge. In this case, PPCPs sorption to biomass might compensate the negative influence of the low SRT operation. The differences are less important in the case of fragrances due to the higher Kd values of both sludges. Regarding the influence of the changes in the operational parameters, it was clearly less relevant for the elimination of musks in the CAS.

Comparing both graphs, it is clear that the CAS system always achieved higher eliminations of the considered PPCPs, being this tendency maintained throughout the different operational periods. This conclusion would be only valid for a CAS with an efficient solid-liquid separation unit. This observed behaviour is hardly explained by variations of Kd values (not particularly different between them) or the HRT, since after its decrease (period III-period IV) the removal efficiencies even increased. Regarding the effect of the mixed liquor temperature, the variations of this parameter were not so different between periods I-IV. Therefore, our explanation is mostly based on the sludge purge strategy. In Figure 6-4, the suspended solids lost in the final effluent, as well as the sludge purged in both reactors are shown.

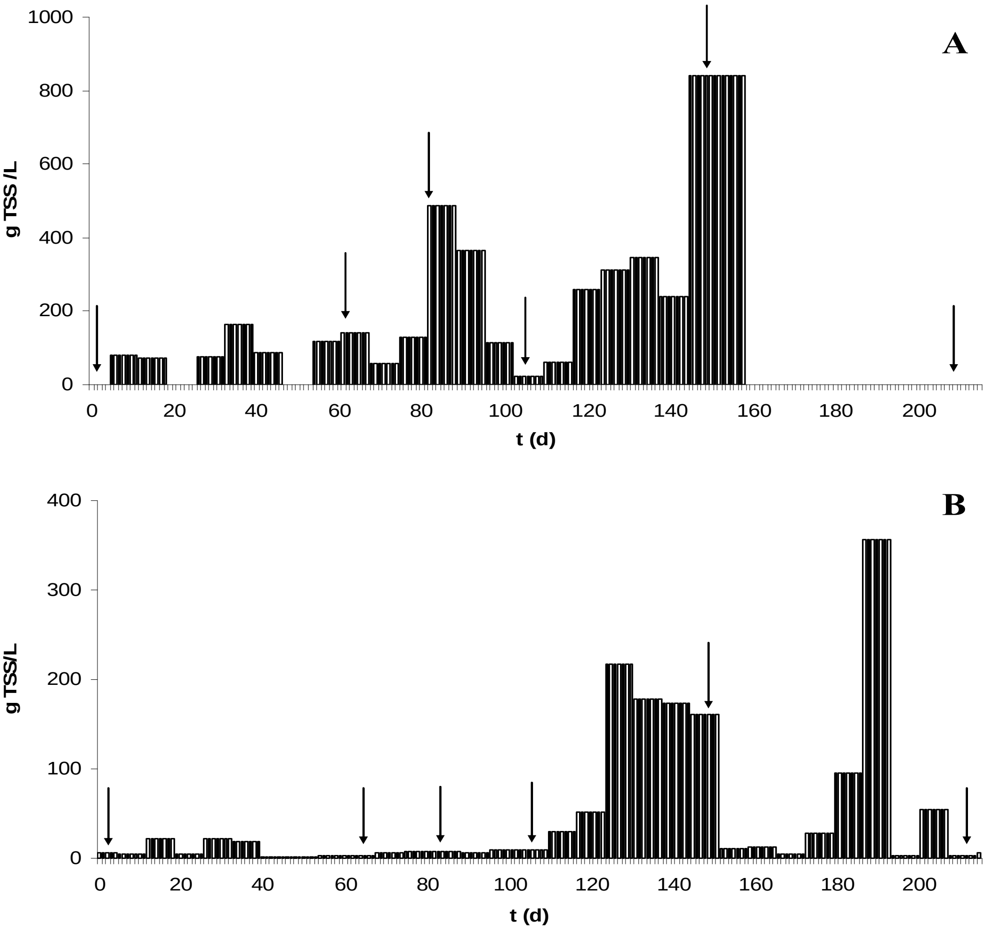

Figure 6-4. Amount of biological sludge purged during the operation of the (A) MBR and the (B) CAS.

It can be observed that the best results were always obtained after high intensity purge periods. For example, the highest elimination for the 3 fragrances was obtained during the first sampling period in which the CAS had been inoculated with biomass from the MBR, which had an intensive purge during the previous weeks (not shown in Figure 6-4). The same pattern is also observed by comparing the eliminations achieved during periods IV (high SRT), V (low SRT) and VI (high SRT), since the removal efficiencies of musk fragrances during these periods were constantly increasing. This effect was evident in both technologies, but in the case of the MBR these variations were more abrupt. After the purge, a fresher sludge with potentially higher sorption properties is developed, which can definitely contribute to an enhanced removal. Moreover, the release rate of compounds following a desorption mechanism might be lower due to the sludge purges. This effect is also evident comparing data from Table 6-4 (period I), where the highest values of Kd were obtained.

Nevertheless, the differences observed between both technologies might be better understood studying the unique physical-chemical characteristics of the sludge developed in each bioreactor. In this work, the particle size distributions were determined and compared during the operation of the CAS and MBR systems (Table 6-5 and Figure 6-5).

Table 6-5. Particle size distribution median values for MBR and CAS biomass

	Particle Size Distribution (µm)					
	d(0.1)		**d(0.5)**		**d(0.9)**	
	MBR	CAS	MBR	CAS	MBR	CAS
July	9.24	10.75	34.76	47.08	108.94	139.02
September	9.06	17.73	28.44	53.62	82.75	248.89
December	12.41	37.9	65.31	122.03	187.36	305.17
February	13.63	55.38	74.15	134.15	225.09	286.31

The first measure of the particle size was performed soon after the starting-up of the CAS, when the biomass properties were similar to the ones measured in a typical MBR sludge. Interestingly, the CAS mean floc size increased in the course of its operation, particularly for the d(0.9) value, as can be observed in Table 6-5 and Figure 6-5, achieving typical values for conventional systems (Massè at al., 2006). Regarding the MBR biomass, its mean floc size was always small, although it slightly increased with time. A feasible explanation of the differences observed between MBR and CAS biomass in terms of particle sizes was proposed by Durante et al., (2006), who found that MBR low particle sizes were a consequence of the hydraulic stress and the progressive decrease of the food to microorganism ratio applied, typical of MBRs with increasing MLSS levels as in the case of our work. Wisniewski et al., (1998) also mentioned the tangential flow along the membrane as a relevant factor which contributes to increasing the shear stress, inducing changes in the settleability of the sludge. According to Massè et al., (2006), the decrease in floc size may be associated with a more compact floc structure, due to fact that the small particles (dispersed bacteria and small colonies) have a higher density than the large flocs, with more bridging between biopolymers.

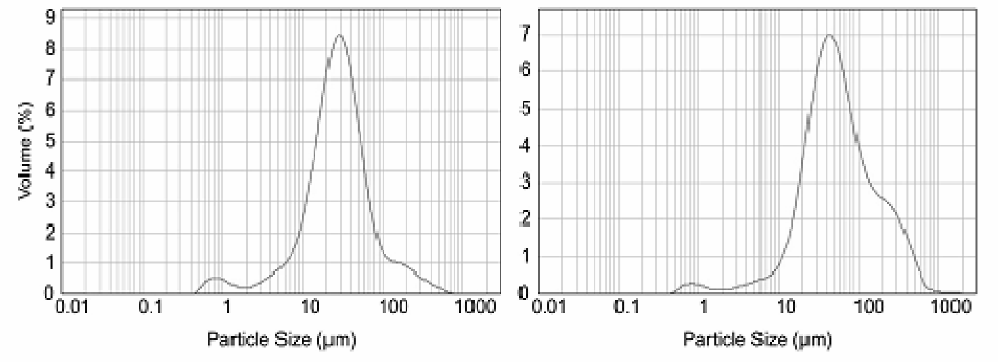

Figure 6-5. Particle Size distributions measured for MBR (left) and CAS (right) on period IV.

Figure 6-6 shows the morphology of MBR and CAS sludge thanks to SEM methodology, with the aim of supporting the previous findings. The structure observed consists of compact and well defined macro-flocs. Dispersed bacteria might also be present, as indicated by the particle size measures, but they remain hidden within the complex structure. However, these pictures clearly illustrate that the morphology of the CAS and MBR biomass is considerably different. Based on previous research and considering the measures performed in this work, it can be concluded that MBR biomass indeed present some specific characteristics, which might be a result of the combination of different factors such as the operation at extended SRT, the F/M ratio. Considering that operational parameters were similar in CAS and MBR, these characteristics might explain the different performance observed for the elimination of highly sorbed compounds.

However, the study of other factors should also be undertaken. For example, the different mechanisms involved during a membrane filtration process might influence the membrane performance in terms of rejection of hydrophobic compounds. According to Ozaki et al., (2008), PPCPs with high hydrophobicity, which is estimated with their log Kow values, might interact with membrane surface following an adsorption process. Then, these compounds might be transported through the membrane, contributing to increase their concentration in the final permeate. However, it remains unclear if PPCPs already sorpted to sludge might follow this mechanism.

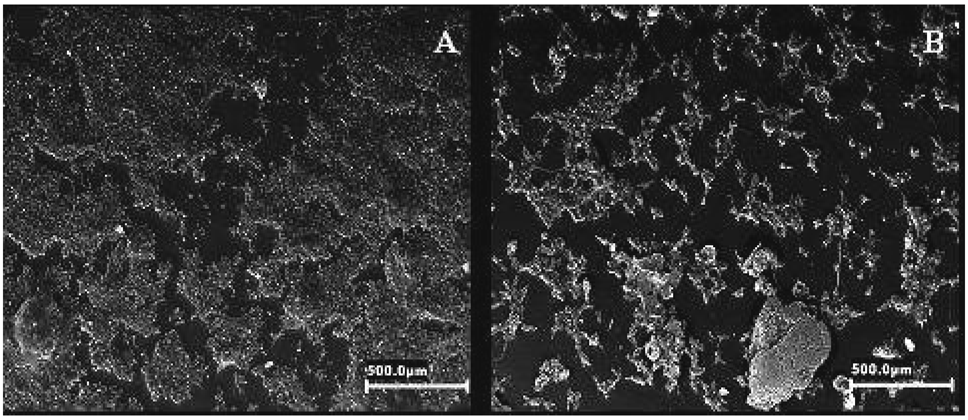

Figure 6-6. SEM scans obtained with sludge from (A) MBR and B) CAS on period VI

Additionally, other parameters such as membrane composition, pore size or the dipole moment of the considered PPCP might influence the overall performance of the MBR. Therefore, a deeper understanding of the influence of all these aspects might indeed be relevant in order to explain the potential of MBR technology for enhancing the elimination of micropollutants. Further research should include batch sorption tests with different types of sludges and trials with membrane modules comprising a variety of configurations, materials and pore size ranges, in order to elucidate the role of these factors on the elimination of PPCPs from sewage.

6.4. Conclusions

PPCPs elimination was tested in CAS/MBR systems. Differences were not significant for most of them with the exception of lipophillic substances. 7 PPCPs (IBP, NPX, ERY, ROX, E2, EE2 and E1) were almost completely removed in both bioreactors. HRT influence was negligible whereas the most significant impact on PPCPs removal was observed in both plants at low SRT. Only under these conditions, the performance of the MBR was clearly better. However, the main outcome of the present work is to show that the upgrading of STPs with MBR technology would not be justified only in terms of PPCPs removal efficiencies.

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Chapter 7

Towards a better understanding of PPCPs removal mechanisms on Membrane Bioreactors[1]

Summary

The performance of a pilot-scale MBR equipped with 4 different membranes (multi-tubular, flat sheet and hollow fibre modules) treating sewage from Cranfield University was studied in terms of PPCPs removal. This system truly represents two representative MBR configurations: side-stream and submerged modules. The used membranes and materials are also similar to the ones typically used in MBR applications. Their simultaneous long-term testing and comparison for assessing PPCPs elimination is completely novel and useful for a deeper understanding of the mechanisms involved in PPCPs elimination during MBR treatment.

The performance of the modules was not particularly distinct between them and no specific pattern was observed. The highest transformation was achieved by ibuprofen (>98%) and naproxen (75 and 91%). On the contrary, carbamazepine elimination was poor (36 and 47%). Different fate was observed depending on the sampling period in the case of diclofenac, sulfamethoxazole and erythromycin since their elimination steadily increased. Acclimation effects and different operational parameters such as temperature or pH might explain these results. Analysis of the mixed liquor supernatant showed higher concentrations of sulfametoxazole, diclofenac and galaxolide when compared to the final permeates. Therefore, additional data gathered from the operation of the MBR in Santiago de Compostela was used in order to confirm this behaviour. MBR performance removing PPCPs from the liquid phase does not seem to be dependent on membrane material or configuration at any extent, whereas in the case of fragrances and diclofenac, the filtration step seemed to increase their concentration in permeates thus reducing their overall elimination from the liquid phase.

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[1] This chapter has been submitted as:

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Towards a better understanding of PPCPs removal mechanisms on Membrane Bioreactors. Journal of Hazardous Materials.

Outline

7.1. Introduction

Micropollutants such as Pharmaceutical and Personal Care Products (PPCPs) and Endocrine Disrupting Compounds (EDCs) are being detected ubiquitously in the aquatic environment. Nowadays, no regulations require monitoring or public reporting of the presence of PPCPs in Sewage Treatment Plants (STPs) effluents, their main emission pathway. Therefore, their elimination is not considered a prioritary objective. As a consequence, more than 150 types of PPCPs are found in different water compartments.

Membrane Bioreactors (MBRs) have improved the overall quality of sewage treatment due to the combination of an intensive biological step followed by micro or ultrafiltration (UF), overcoming clarification. Presently, MBRs are also being tested in terms of micropollutants removal due to their ability to operate with long sludge retention times (SRTs) and high biomass concentrations, parameters which are known to exert influence on the removal of nutrients and hardly biodegradable substances. For example, Miège et al. (2008) compiled data from 113 research papers finding that MBR processes usually provide 15% higher removal rates when compared with conventional processes. Even so, the information available is scarce and contradictory since it has been also reported that Conventional Activated Sludge (CAS) systems operated with long SRTs might achieve similar performances (Clara et al., 2005; Lesjean et al., 2010).

Usually, two removal mechanisms are considered as the most influential: biological degradation and sorption onto solids, whereas rejection has not been studied since typical UF pore sizes are a factor of 100 larger than the molecular sizes of most of the micropollutants (Radjenovic el al., 2009). Research dealing with nanofiltration (NF) or reverse osmosis (RO) membranes treating different types of water matrices has provided a deeper insight regarding the different rejection mechanisms which a solute can undergo during the filtration step (Yoon et al., 2006; Alturki et al., 2010).

Besides size-exclusion, two mechanisms, which might also influence PPCPs separation from the liquid phase in MBRs, have been identified: electrostatic repulsion, mainly influenced by the pH of the mixed liquor and the pKa of the considered PPCP (Urase et al., 2005), and hydrophobic interactions (Yoon et al., 2006), relevant for non-ionisable PPCPs with lipophillic characteristics (high Kow values).

This research aims to provide more extensive knowledge on the feasibility of the MBR technology for PPCPs removal. Therefore, the behaviour and fate of PPCPs with different physico-chemical properties was assessed in a pilot-scale MBR equipped with 4 different membrane modules operated simultaneously under strictly controlled conditions at the premises of Cranfield University pilot plant hall. The influence of operational parameters, biomass adaptation and the performance of the different modules was studied during the different sampling campaigns. In order to gather additional information regarding the effect of the membrane filtration step on the removal of diclofenac and highly sorbed compounds such as musk fragrances, data from another pilot-scale MBR operated in Santiago de Compostela (Spain) obtained for an extended period was also considered.

7.2. Materials and methods

7.2.1. Membrane Bioreactors description

The MBR operated in Cranfield University consisted of different full-scale sized membranes (multi-tubular (MT), flat sheet (FS) and two hollow fibre (HF1 and HF2) modules) operated in parallel as air-lift side-stream mode from a 2.2 m^3 aeration tank. Due to permanent failure of module HF1 after the first sampling campaign, it was replaced by module HF2, with different characteristics (Table 7-1). Additionally, an internal submerged hollow fiber module (sHF) enabled the operation of the side-stream modules decoupled from the hydraulic overall performance of the MBR. More detailed information can be found at Moreau et al. (2009). Figure 7-1 shows a picture and the scheme of the pilot plant.

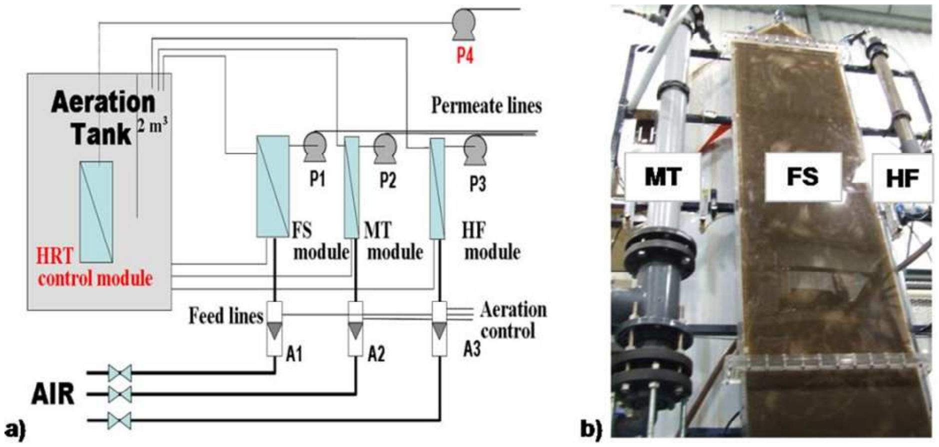

Figure 7-1. Diagram of the MBR operated in Cranfield University (a) and photography of the 3 side-stream modules (b)

The MBR operated in Santiago de Compostela was equipped with a Zenon ZW-10 hollow fibre membrane module submerged in an aeration tank of 180 L (additional information available at Chapters 3 and 4). It was placed in the premises of a municipal STP and was continuously fed with settled sewage spiked with PPCPs.

Table 7-1. Main characteristics of the membrane modules used in both MBRs

				Flow regime (s)		
Module	**Type**	**Material**	**Pore Size (µm)**	**On**	**Off**	**Bw**
Cranfield						
sHF	submerged HF	Polyvinylidene fluoride	0.03		continous	
MT	airlift-sidestream	Polyvinylidene fluoride	0.03	540	50	10
FS	airlift-sidestream	Polyvinylidene fluoride	0.08	540	60	0
HF1	airlift-sidestream	Polysulfone	0.05	540	50	10
HF2	airlift-sidestream	Polyethersulfone	0.04	540	50	10
Santiago						
HF	submerged HF	Polyvinylidene fluoride	0.04	900	0	45

7.2.2. Analytical methods and sampling estrategy

Chemical Oxygen Demand (COD) was determined using a Spectroquant Cell Test and measured on a Nova 60 model spectrophotometer. Mixed Liquor Suspended Solids (MLSS), dissolved oxygen (DO) content and pH were determined according to standard methods.

Selected PPCPs and properties are detailed in Table 7-2. Some of them were continuously spiked into the mixed liquor with the aim of studying a wider range of compounds. pKa values were taken from Suarez et al. (2008) and Tadkaew et al. (2010). Kd values were calculated with sludge at circumneutral pH from the MBR operated in Santiago de Compostela (Chapters 4 and 5), with the exception of values in brackets, which were obtained from Suarez et al. (2008).

Three sampling campaigns (two days each) were carried out. Five discrete samples were taken in aluminium bottles between 8:00 and 20:00 on each day. Sampling points were: sewage, permeates and mixed liquor supernatant from the MBR tank obtained after centrifugation at room temperature in PTFE containers. After collection, samples were immediately filtered through glass-fibre and nitrate cellulose membrane filters (0.45 µm). PPCPs content in the liquid phase was determined following the methodologies explained in Chapter 2.

Table 7-2. Studied PPCPs present in sewage and/or spiked in the mixed liquor (HHCB and CBZ were spiked after the first sampling period)

Substance	**Classification**	**Acronym**	**Spike**	**pKa**	**Kd (L/Kg)**
Ibuprofen	Anti-inflammatories	IBP	no	4.41	(8)
Naproxen		NPX	no	4.2	-
Diclofenac		DCF	yes	4.18	(16)
Galaxolide	Cosmetic ingredients	HHCB	yes	-	2831
Tonalide		AHTN	no	-	2696
Carbamazepine	Tranquilizer	CBZ	yes	13.94	(1)
Sulfametoxazole	Antibiotics	SMX	yes	5.81	34
Erythromycin		ERY	yes	8.9	57
Roxythromycin		ROX	no	9.2	73
Trimethoprim		TMP	no	6.6-7.2	68

7.3. Results and discussion

7.3.1. Removal of organic matter and nitrogen

Table 7-3 shows data regarding the operation of the MBR. The HRT was gradually reduced during the operation of the MBR and SRT was not controlled (average value: 10 d). Temperature increased in accordance with its seasonal variations. DO levels were particularly low during II and III periods, probably due to high aeration demand for biological activity of heterotrophic bacteria. Although such levels were not appropriate for the correct development of nitrifiers, low pH values measured in III shows an acidification process as a result of nitrifying activity. In fact, a decrease in the performance of the system removing organic matter in form of COD and N-NH4+ was observed during this period whereas their elimination was higher during previous samplings.

Table 7-3. MBR operational parameters, performance and mean particle size

		pH	DO (mg/L)	Tª (ºC)	MLSS (g/L)	HRT (h)	COD (%)	N-NH4+ (%)
December	I	7.1	3.1	12	3.4	24	97.2	98.6
February	II	7.8	0.2	15	6.1	12	95.9	97.9
June	III	4.4	0.9	21	4	8	84.6	92.8

7.3.2. Comparison of membrane modules performance

PPCPs levels measured in permeates are shown in Figure 7-2. The only difference between each sampling day was the membrane flux of the side-stream modules (9 LMH first day and 18 LMH on second day). However, no effect of this parameter was observed and consequently, data obtained during the two days were averaged.

Different degree of rejection might be expected depending on the physical-chemical characteristics of the PPCPs. According to their pKa values (Table 7-2), two groups are distinguished: ionisable (IBP, NPX, DCF and SMX) and non-ionisable (HHCB, AHTN, CBZ, ERY, TMP and ROX). The rejection of dissociated substances might be influenced by pH through an electrostatic repusion mechanism. During periods I and II, pH of the mixed liquor was circumneutral and then acidic during III (Table 7-3). At neutral pH, ionisable compounds exist predominantly in their hydrophillic form.

According to Yoon et al. (2006) hydrophobic interactions with membranes might also influence PPCPs rejection and consequently, non-ionisable PPCPs with high Kow values such as musk fragrances might absorb onto membrane surface. This phenomenon is interesting since solutes might eventually pass through the membrane, resulting in higher permeate concentrations (Ozaki et al., 2008). Comparing concentration levels measured during the different periods, it is obvious that the performance of the modules was not particularly distinct (Figure 7-2) and no trend can be observed since differences between compounds were in most cases lower than the analytical uncertainty associated with organics trace analysis.

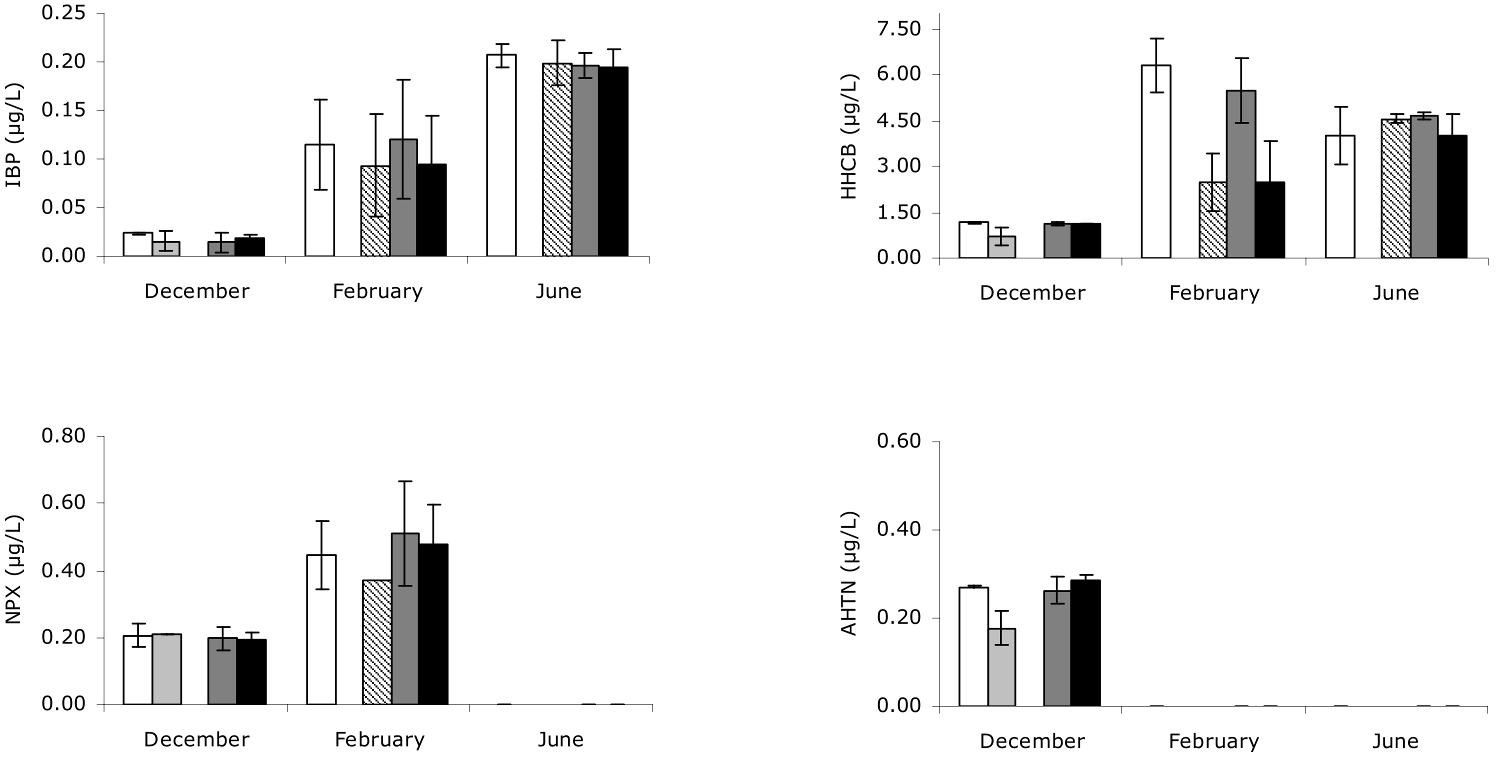

Figure 7-2. Comparison of PPCPs concentrations measured in the different permeates (1)

□ iHF ▨ HF1 ▧ HF2 ■ MT ■ FS

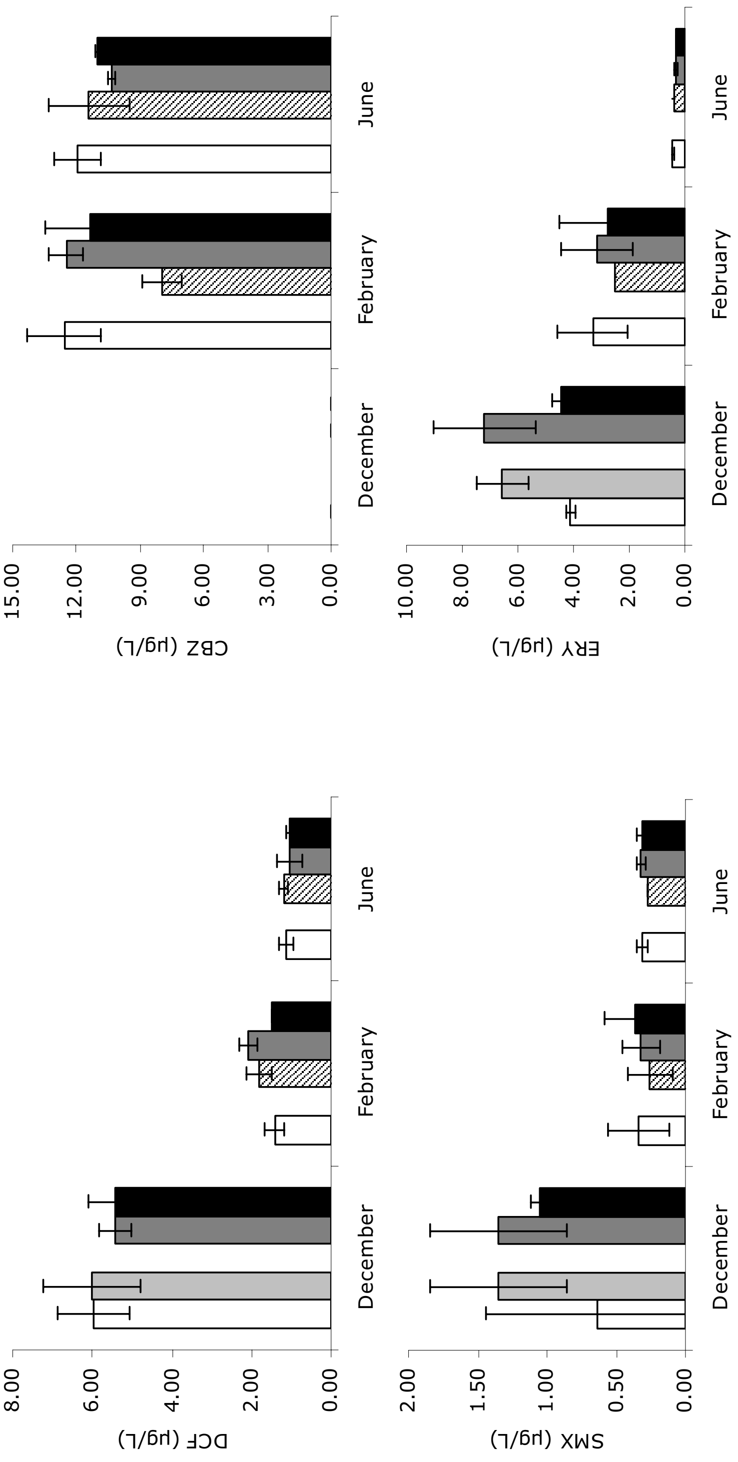

Figure 7-2. Comparison of PPCPs concentrations measured in the different permeates (2)

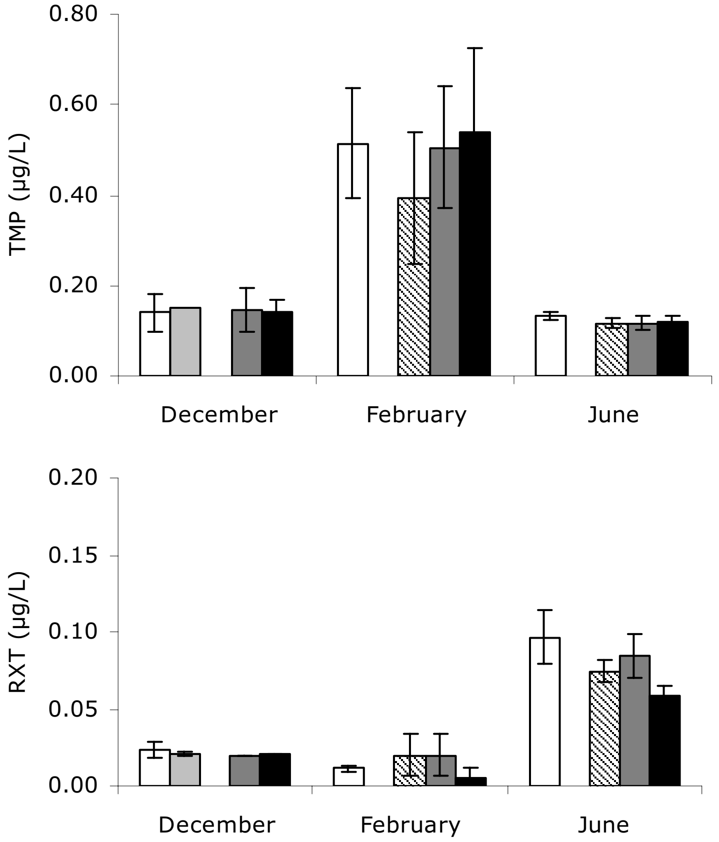

Figure 7-2. Comparison of PPCPs concentrations measured in the different permeates (3)

7.3.3. Influence of operational parameters and filtration step

IBP was highly degraded (<98%) in good accordance with reported values (Tadkaew et al., 2011). In the case of NPX, slightly higher concentrations were measured in both permeate and supernatant (Figure 7-3) since its biodegradability is moderate (Joss et al., 2006) thus depending more on operational conditions (75 and 91% of elimination). On the contrary, DCF behaviour was rather different: During period I, its removal was negligible. During II and III periods, its elimination increased up to 80% in spite of decreasing HRT values. According to Urase et al. (2005) low pH (period III) might enhance the elimination of ionisable compounds such as DCF or SMX. In our work, the behaviour of the latter was similar to DCF, although its elimination was from intermediate (44%) to high (90-95%).

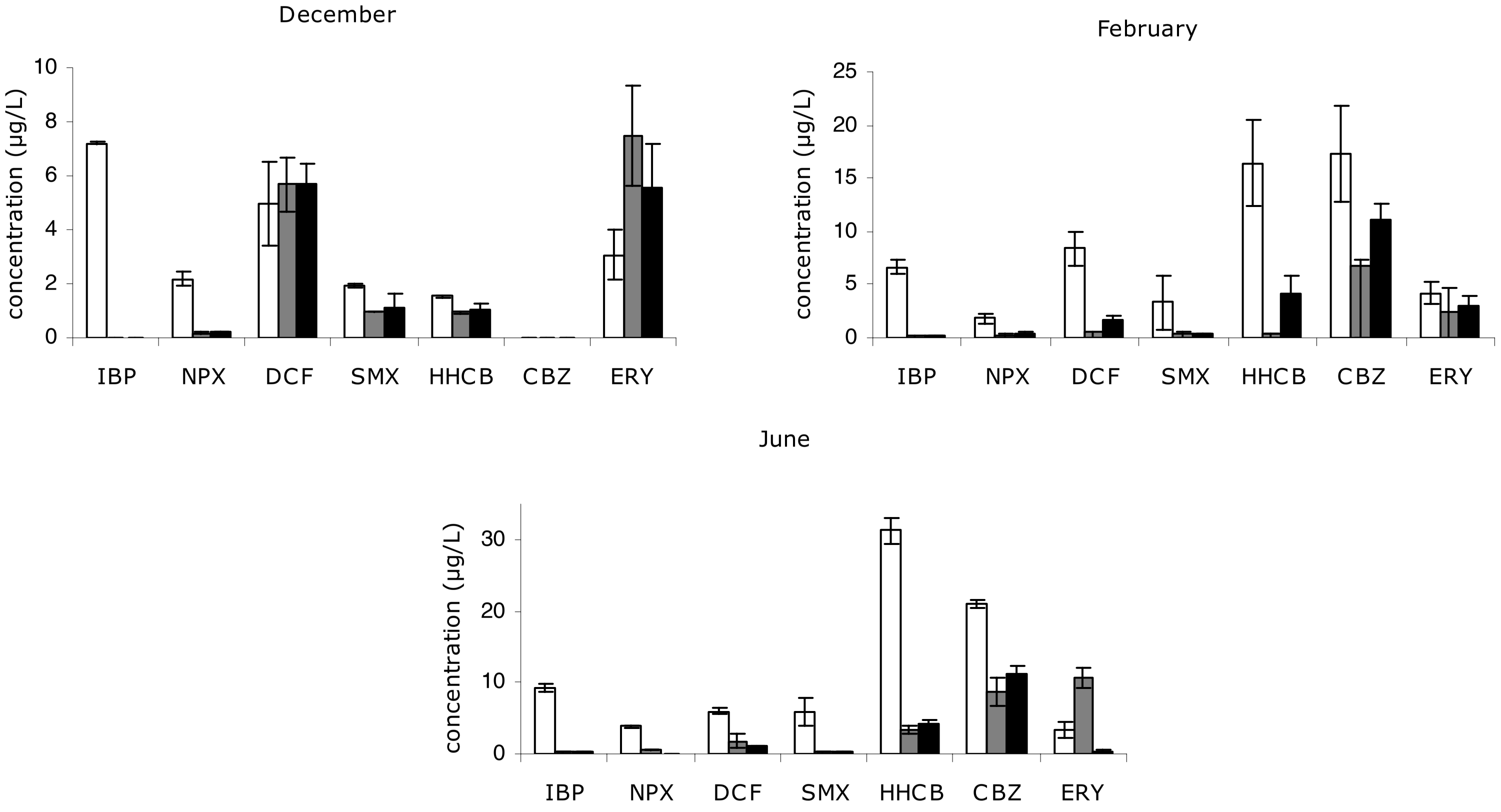

Figure 7-3: PPCPs concentrations measured in the different sampling points

□ Feed ▨ MLSS supernatant ■ Permeate

No apparent explanation can be found for the high elimination achieved during period II for for DCF, since pH was 7.8. However, elimination data for this compound tends to be contradictory (Clara et al., 2005). CBZ elimination was poor, though higher than previously reported values. Its pKa is high enough to dismiss the influence of pH on its removal whereas its Kd value (Table 7-2) is low enough to consider its sorption onto solids as negligible. As a consequence, operational conditions did not exert influence on its removal (36 and 47%). HHCB elimination is expected to occur by sorption onto solids (Suarez et al., 2008). Its elimination was incomplete, although it steadily increased (33, 75 and 86%). Temperature values might explain this behaviour since absorption equilibrium and reactive processes might be favoured with warmer temperatures. During period I, ERY concentration in MLSS and permeates was even higher compared with input data. However, MLSS data during period III was contradictory compared with permeate levels and should not be considered. Analytical issues during the determination of this compound or the presence of conjugates in sewage which might be converted back into their original unconjugated forms during treatment (Lishman et al., 2006) might explain these results. During period II ERY elimination was poor whereas in June it increased up to 89%. Considering its high pKa value (8.9), acidic conditions during this period should not exert influence. Therefore, warmer temperatures and adaptation of the biomass might again explain the degree of elimination achieved.

Studying the filtration step, DCF was found at higher concentrations in permeates during periods I and II. This behaviour was also observed in the case of SMX and HHCB. According to Yoon et al., (2006) hydrophobic compounds might be retained at a significant extent in UF membranes. However, it might not be feasible to compare both works since those experiments were not carried out with sludge. In order to confirm the observed trend, data from a larger number of analyses in MLSS and permeate obtained during the operation of another MBR are shown in Figure 7-4. Again, DCF and SMX concentrations were higher in permeates, but not at every sampling point. CBZ differences between supernatant and permeate were low. However, in 5 out of 8 analysis, permeate concentrations were lower compared with supernatant levels, showing the opposite tendency. On the contrary, HHCB was detected in higher concentrations in almost every permeate sample. Previous research with NF/RO membranes mention that diffusion of hydrophobic substances through the bulk membrane material might lead to the permeation of the solute (Ozaki et al., 2008). If the solute is accumulated in solids due to a continuous input, such mechanism might lead to increased permeate concentrations, affecting the overall performance of the MBR eliminating hydrophobic compounds thus counteracting the positive effect of membrane retention. However, biomass yield and the disposal of sludge might also influence the levels detected in permeates.

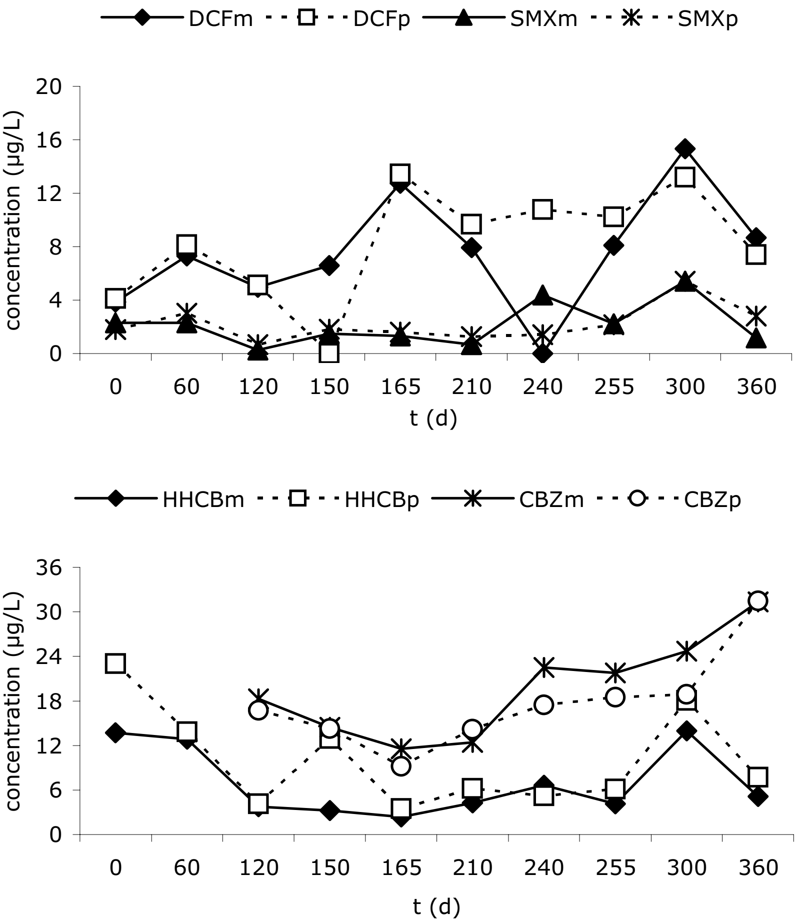

Figure 7-4. Comparison of PPCPs concentrations measured in MLSS supernatant (m) and permeate (p) during the operation of the MBR (Spain)

7.4. Conclusions

The simultaneous testing of 4 different UF modules and the performance of a MBR for studying the elimination of PPCPs from sewage resulted in the following conclusions:

- None of the types of modules considered (tubular, flat-sheet and hollow fiber), the MBR configuration (side-stream or submerged) or permeate fluxes showed different performance eliminating PPCPs from the liquid phase.

- MBR treatment did not achieve a complete elimination of many PPCPs. Their removal varied depending on the considered period thus confirming the influence of operational parameters such as pH, temperature or biomass adaptation.

- SMX, DCF or HHCB were found at higher concentrations in permeate compared with levels measured in MLSS supernatant. Therefore, the UF step might decrease PPCPs overall removal efficiency from the liquid phase, but only in some cases.

- Further research is necessary in order to link PPCPs physico-chemical properties with the rejection efficiency of UF modules during the filtration step.

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General conclusions

This research aims at contributing to the enhancement of the knowledge concerning the presence of Pharmaceutical and Personal Care Products (PPCPs) in wastewater treatment plants effluents and the use of an emerging technology, membrane bioreactors (MBRs), for their elimination during sewage secondary treatment. The specific conclusions of the present doctoral thesis are listed below.

1. Occurrence and fate of PPCPs in municipal wastewater treatment plants

Samples from the inflow and outflow of the different units of a CAS pilot plant placed in a Sewage Treatment Plant in NW UK were analysed in order to detect the presence of eight different PPCPs. Only the fragrance celestolide (ADBI) and the tranquilizer diazepam (DZP) remained below the detection limit of the analytical methodology used, and the antiepileptic (CBZ) was only found in a few discrete samples below quantification limit. In general, PPCPs were found in levels ranging from 0.1 to 7.5 μg/L. Anti-inflammatory drugs ibuprofen (IBP) and naproxen (NPX) were mainly removed inside the activated sludge tank (98 and 93% respectively), most probably by biological degradation, whereas galaxolide (HHCB) and tonalide (AHTN) elimination, which was moderate (68% for HHCB and 75% for AHTN) occurred by sorption onto solids (primary and secondary sludge). Low or negligible removal was estimated for the acidic drug DCF, which was released in the final effluent of the pilot plant with a mass flow load of 8.4 mg/d. The calculated mass balances for the outflow load permitted to estimate a release of PPCPs in the range of 9-63 g per day in the discharge stream from the full-scale STP, depending on the substance considered. Therefore, the development of enhancement strategies in existing plants should be a priority in order to help to attenuate the release of micropollutants in the water cycle. In this sense, new treatment technologies such as membrane bioreactors constitute a powerful alternative which is worth to be explored.

2. Elimination of PPCPs with MBRs

Preliminary studies were carried out in a MBR which used a synthetic influent, simulating the composition of a medium-charged urban wastewater. The list of PPCPs was enlarged with four antibiotics: sulfamethoxazole (SMX), erythromycin (ERY), roxithromycin (ROX) and thrimetoprim (TMP). The acidic pharmaceuticals naproxen and especially ibuprofen were almost completely removed, with elimination rates up to 84 and 98%. In the case of CBZ and DZP, their removal accounted for 9% and 26% respectively whereas results obtained for the removal of fragrances were around 46-56%. Elimination of fragrances was not very high taking into account the outstanding lipophilic character of these substances and thus their affinity to be sorbed onto the sludge. In the case of antibiotics, macrolides ROX and ERY were removed at a high extent (77 and 91% respectively), and SMX was half-removed (52%). On the other hand, TMP showed low elimination rates (36%). According to the short period that this system had been treating a feeding spiked with PPCPs and the source of the inoculum used (without any previous contact with these micropollutants), it is expected that acclimation phenomena might occur with more extended periods of operation, which could lead to the improvement of these results.

3. Influence of operational parameters

In this part of the research, the MBR was operated at the premises of a municipal STP for an extended period. 5 more PPCPs were considered. On the one hand, the hormones estradiol (E2), ethynilestradiol (EE2) and estrone (E1) considered as Endocrine Disrupting Compounds (EDCs). On the other hand, two antidepressants, fluoxetine (FXL) and citalopram (CTL). Solid phase (primary and secondary sludge) was also considered for PPCPs analysis.

IBP, NPX and the three hormones (E2, EE2 and E1) were almost completely removed during the MBR treatment (>90%) independently of the operational conditions. Musk fragrances and FLX elimination varied significantly depending on the operational period considered, ranging from poor to moderate-high and significant amounts were measured on the solid-phase, confirming sorption onto sludge as their main removal mechanism. A combination of effects might explain the differences observed among the different sampling periods: Temperature of the mixed liquor, sludge age (modified by the sludge purges) and the physical properties of the MBR biomass.

The elimination of the antibiotics SMX and TMP was from moderate to high and increased along the operation of the MBR. Considering their low biodegradability constants, MLSS concentration might slightly influence their elimination. After several months of operation, ERY and ROX degree of elimination increased considerably. No specific influence of the MLSS concentration and temperature was observed. Therefore, adaptation effects might explain the observed behaviour. The lowest elimination was observed for CBZ, DZP, CTL and DCF. However, CBZ elimination was above 50% at certain periods of operation, being this data surprisingly higher than expected. With the exception of CTL, sorption was not relevant for removing these substances, and the temperature and MLSS did not influence their removal at any extent.

HRT influence was negligible for most of the PPCPs whereas low SRT operation (below 10 d) negatively affected the removal of DCF, SMX, ERY, ROX, TMP, EE2 and CTL. The most relevant impact on the removal of lipophilic compounds was caused by the amount of sludge purged previously to each sampling period, which turned out to be beneficial. Therefore, the depletion of sorption sites in the MBR biomass might explain the low elimination degree achieved in the case of highly sorbed compounds such as musks and antidepressants.

4. Comparison between conventional activated sludge (CAS) bioreactors and MBRs

The removal of most of the studied substances was not significantly different between both technologies. However, some relevant differences were found. On the one hand, the elimination of PPCPs in conventional systems was found to be particularly sensitive to adverse conditions such as low values of SRT and temperature. On the other hand the elimination of lipophilic substances, more specifically the musk fragrances HHCB, AHTN and ADBI was always significantly higher in the conventional system, independently of the sampling period considered.

5. Effect of the membrane filtration step

Different membrane modules, configurations and fluxes were tested and the effect of the filtration step was studied by comparing PPCPs concentrations measured in the mixed liquor supernatant with those detected in permeates. None of the types of modules considered (tubular, flat-sheet and hollow fiber), configurations (side-stream or submerged) or permeate fluxes showed different performance eliminating PPCPs from the liquid phase. SMX, DCF or HHCB were found at higher concentrations in permeate compared with levels measured in MLSS supernatant. Therefore, the UF step might decrease PPCPs overall removal efficiency from the liquid phase, but only in some cases.

6. Recomendations

The knowledge about MBR technology gathered from this research confirms that nowadays, upgrading existing STPs based in the CAS system with MBR technology would not be justified only in terms of micropollutants removal, since CAS systems correctly operated for nitrogen removal might be able to remove PPCPs at a similar degree. However, parameters that have shown to exert a strong influence on the elimination of such compounds are definitely easier to control in

MBRs, which also have many other advantages, mainly based on the quality of the final effluent generated, suitable for reuse purposes in many cases. Considering these aspects, the conclusions of this work should not constitute an obstacle for the widespread of the MBR technology for sewage treatment. On the contrary, this work can be considered as a starting point which sets the future trends in MBR research that should be followed in order to optimize their ability to remove organic micropollutants. For example, further research might be focussed on the enhancement of biomass sorption properties. In this line, promising results are currently being obtained with hybrid processes which combine sorption onto activated carbon within a single MBR unit. Moreover, the combination of current research with the field of microbiology might lead to significant improvements, since the complete retention of microorganisms inside the process tank might allow to develop sustainable populations of microorganisms specialized in PPCPs degradation.

Conclusiones generales

El objetivo fundamental de este trabajo de investigación consiste en mejorar el conocimiento sobre la presencia de productos farmacéuticos y de cuidado personal (PPCPs) en efluentes de estaciones depuradoras de aguas residuales urbanas (STPs), y fundamentalmente sobre el uso de una tecnología emergente, los biorreactores de membrana (MBRs) para mejorar su eliminación durante el proceso de tratamiento biológico. A continuación se especifican las conclusiones principales de esta tesis doctoral.

1. Presencia y destino final de PPCPs en estaciones depuradoras de aguas residuales urbanas

Se analizaron muestras de la entrada y salida de las distintas unidades de una planta piloto convencional de lodos activos ubicada en las dependencias de una depuradora en el Reino Unido con el fin de detectar la presencia de ocho PPCPs diferentes. Los resultados indicaron que únicamente los niveles de la fragancia celestolide (ADBI) y el tranquilizante diazepam (DZP) permanecieron por debajo del límite de detección de la metodología analítica utilizada, mientras que el antiepiléptico CBZ únicamente estuvo por debajo del límite de cuantificación en muestras puntuales. En general se encontraron concentraciones de PPCPs desde 0.1 hasta 7.5 µg/L. Los antiinflamatorios ibuprofeno (IBP) y naproxeno (NPX) fueron eliminados prioritariamente en el biorreactor de lodos activos (98 y 93% respectivamente), probablemente mediante un proceso de degradación biológica mientras que el galaxolide (HHCB) y el tonalide (AHTN), cuyas eficacias de eliminación fueron más moderadas (68% para HHCB y 75% para AHTN) se eliminaron siguiendo un proceso de sorción en lodos primarios y secundarios. En el caso del diclofenaco (DCF) se encontró poca o nula eliminación cifrándose su caudal másico en el efluente final en 8.4 mg/d. Los balances de materia que fueron calculados para las diferentes sustancias estudiadas permitieron estimar una liberación en el efluente final de la depuradora a escala real de entre 9-63 g por día, dependiendo de la sustancia considerada. Por lo tanto, el desarrollo de estrategias de mejora en plantas de tratamiento ya existentes debería ser considerado como una prioridad, dado que podría contribuir a atenuar la continua emisión de microcontaminantes al medio ambiente acuático. Por otra parte, es necesario considerar el estudio del potencial de nuevas tecnologías de tratamiento como los MBR.

2. Eliminación de PPCPs mediante biorreactores de membrana

Se llevaron a cabo una serie de estudios preliminares en un MBR que utilizó como alimentación un medio sintético que simulaba las propiedades de un agua residual urbana de carga media. La lista de PPCPs fue incrementada con cuatro antibióticos: sulfametoxazol (SMX), eritromicina (ERY), roxitromicina (ROX) y trimetoprim (TMP). Los fármacos de propiedades ácidas naproxeno y especialmente ibuprofeno fueron prácticamente eliminados (84 y 98% respectivamente). En el caso de la CBZ y el DZP, su eliminación fue de 9% y 26% respectivamente, mientras que los resultados obtenidos para la eliminación de fragancias estuvieron en torno a un 46-56% en todos los casos. Estos porcentajes de eliminación no fueron muy elevados, considerando su elevado carácter lipofílico y por lo tanto su afinidad para la sorción en sólidos. En el caso de los antibióticos, los macrólidos ROX y ERY fueron eficazmente eliminados (77 y 91% respectivamente), y el SMX fue parcialmente eliminado (52%). Por otra parte, el TMP mostró una baja eficacia de eliminación. De acuerdo al corto periodo durante el cual este sistema estuvo tratando la alimentación con adición de PPCPs y la fuente del inóculo (sin contacto previo con estos microcontaminantes), se espera que pueda darse el fenómeno de adaptación de la biomasa tras un periodo de operación más extenso, mejorándose así los resultados obtenidos.

3. Influencia de los parámetros operacionales.

En esta parte de la investigación, el MBR fue trasladado a las dependencias de una depuradora de aguas urbanas para ser operado durante un periodo de tiempo extendido. Cino PPCPs adicionales fueron considerados. Por una parte, las hormonas estradiol (E2), etinilestradiol (EE2) y estrona (E1) consideradas como compuestos disruptores endocrinos (EDCs). Por otra parte, dos antidepresivos: fluoxetina (FXL) y citalopram (CTL). La fase sólida (lodo primario y secundario) fue también muestreada y analizada para determinar su contenido en PPCPs.

IBP, NPX y las tres hormonas fueron casi completamente eliminados (>90%) independientemente de los parámetros operacionales. Los porcentajes de eliminación de fragancias sintéticas y FLX variaron significativamente, de bajo-moderado hasta alto. En este caso, se encontraron en la fase sólida cantidades muy elevadas, confirmando que la sorción en el lodo es su principal mecanismo de eliminación. Una combinación de distintos efectos podría explicar las diferencias observadas entre las distintas campañas de muestreo: La temperatura del licor de mezcla, la edad celular (modificada mediante purgas de lodo) y las propiedades físico-químicas de la biomasa MBR. La transformación de los antibióticos SMX y TMP fue de moderada a alta a lo largo de la operación del MBR. Considerando sus bajas constantes de biodegradación, la concentración de sólidos suspendidos del licor de mezcla podría ejercer cierta influencia en su eliminación. Tras varios meses de operación, el grado de eliminación de ERY y ROX se vio incrementado de forma considerable, aunque no se observó una influencia específica de la concentración de lodos y temperatura. Por tanto, los fenómenos de aclimatación de la biomasa podrían explicar este comportamiento. Los porcentajes de transformación más bajos fueron medidos para CBZ, DZP, CTL y DCF. En cualquier caso, la eliminación de CBZ estuvo por encima del 50% en determinados momentos, siendo éste valor por encima de lo esperable, teniendo en cuenta el carácter recalcitrante de esta sustancia. A excepción del CTL, la sorción no fue un mecanismo importante para la eliminación de estas sustancias. Además, ni la temperatura ni la concentración de sólidos influenciaron su eliminación.

La influencia del HRT fue baja o nula para la mayoría de PPCPs, mientras que la operación a bajo SRT (por debajo de 10 días) afectó negativamente a la eliminación de DCF, SMX, ERY, ROX, TMP, EE2 y CTL. El efecto más acusado en la eliminación de sustancias lipofílicas fue causado por la cantidad de loso purgado antes de cada campaña de muestreo, lo cual resultó ser beneficioso. De este modo, el agotamiento de centros de sorción en la biomasa MBR podría explicar el bajo porcentaje de eliminación conseguido en el caso de compuestos fácilmente eliminables por sorción, como son las fragancias y los antidepresivos.

4. Comparación entre sistemas convencionales de lodos activos y biorreactores de membrana

El porcentaje de eliminación de la mayoría de compuestos estudiados no fue significativamente distinto entre ambas tecnologías. En cualquier caso, se pudieron encontrar algunas salvedades. Por una parte, la eliminación de PPCPs en sistemas convencionales fue particularmente sensible a la operación en condiciones adversas, como son los valores bajos de temperatura ambiental o SRT. Por otra parte, la eliminación de sustancias lipofílicas, más específicamente las fragancias policíclicas HHCB, AHTN y ADBI, fue siempre más elevada en el sistema convencional con independencia del periodo de muestreo considerado.

5. Efecto del proceso de filtración por membranas

Se evaluaron distintos módulos de membrana, configuraciones y flujos de permeado mediante comparación de los niveles de concentración de PPCPs medidos en el sobrenadante del licor de mezcla con los encontrados en permeados. Ninguno de los módulos considerados (tubular, lamina plana y fibra hueca), configuraciones (side-stream o sumergida) o flujos de permeado (alto/bajo) mostraron distinto rendimiento en la eliminación de PPCPs de la fase líquida. SMX, DCF or HHCB fueron encontrados en concentraciones más altas en algunos permeados con respecto a los niveles medidos en el sobrenadante. Por lo tanto, el proceso de ultrafiltración podría contribuir a la disminución de la eficacia de eliminación de PPCPs de la fase líquida, aunque solamente en algunos casos concretos.

6. Recomendaciones

El grado de conocimiento sobre la tecnología MBR que se ha alcanzado durante esta investigación confirma que, hoy por hoy, la mejora de plantas de tratamiento convencional mediante tecnología de membranas no estaría justificada desde el punto de vista de la eliminación de contaminantes emergentes, dado que los sistemas convencionales correctamente operados para la eliminación de nitrógeno podrían ser capaces de eliminar PPCPs con similar eficiencia que los MBRs. En cualquier caso, algunos parámetros que han demostrado ejercer una fuerte influencia en la eliminación de estas sustancias son definitivamente más sencillos de controlar en MBRs. Estos sistemas además presentan otras ventajas adicionales, principalmente basadas en la calidad del efluente final generado, apto para reutilizar. Considerando de forma global todos estos aspectos, las conclusiones de esta investigación no deberían constituir ningún obstáculo de cara a la aplicación de la tecnología de membranas para el tratamiento de aguas residuales. En todo caso, este trabajo puede ser considerado como un punto de partida que marca las líneas de investigación futuras que deberían ser seguidas de cara a la optimización de la capacidad de los MBR para eliminar microcontaminantes orgánicos. Por ejemplo, las nuevas líneas de trabajo podrían enfocarse a la mejora de las propiedades de sorción de la biomasa. En esta línea, los procesos híbridos que combinan la sorción en carbón activado con los tratamientos MBR están obteniendo resultados prometedores. Igualmente, la combinación de la información generada con MBRs con el campo de la microbiología podría llevar a mejoras significativas, ya que la posibilidad de retener completamente la biomasa generada permitiría el desarrollo y proliferación de poblaciones microbianas más especializadas en la degradación de PPCPs y otros compuestos recalcitrantes, optimizando así la etapa de tratamiento biológico.

Conclusións xerais

O obxectivo fundamental deste traballo de investigación consiste en mellorar o coñecemento sobre a presenza de produtos farmacéuticos e de coidado persoal (PPCPs) en efluentes de estacións depuradoras de augas residuais urbanas (STPs), e fundamentalmente sobre o uso dunha tecnoloxía emerxente, os biorreactores de membrana (MBRs), para mellorar a súa eliminación durante o proceso de tratamento biolóxico. A continuación especifícanse as conclusións principais desta tese doutoral.

1. Presenza e destino final de PPCPs en estacións depuradoras de augas residuais urbanas

Analizáronse mostras da entrada e saída das distintas unidades dunha planta piloto convencional de lodos activos situada nas dependencias dunha depuradora no Reino Unido, co fin de detectar a presenza de oito PPCPs diferentes. Os resultados indicaron que únicamente os niveis da fragrancia celestolide (ADBI) e o tranquilizante diazepam (DZP) permaneceron por baixo do límite de detección da metodoloxía analítica empregada, namentras que o antiepiléptico CBZ únicamente estivo or debaixo do límite de cuantificación en mostras puntuais. En xeral, encontráronse concentracións de PPCPs desde 0.1 até 7.5 µg/L. Os antiinflamatorios ibuprofeno (IBP) e naproxeno (NPX) foron eliminados prioritariamente no biorreactor de lodos activos (98 e 93% respectivamente), probablemente mediante un proceso de degradación biolóxica mentres que o galaxolide (HHCB) e o tonalide (AHTN), cuxas eficacias de eliminación foron máis moderadas (68% para HHCB e 75% para AHTN), elimináronse seguindo un proceso de sorción en lodos primarios e secundarios. Pouca ou nula eliminación foi calculada para o composto de propiedades acedas diclofenaco (DCF), que foi liberado no efluente final da planta piloto cun caudal másico de 8.4 mg/d. Os balances de materia que foron calculados para as diferentes sustancias estudadas permitiron estimar unha liberación no efluente final da depuradora de entre 9-63 g por día, dependendo da sustancia considerada. Polo tanto, o desenvolvemento de estratexias de mellora en plantas de tratamento xa existentes debería ser considerado como unha prioridade, dado que podería contribuír a atenuar a continua emisión de microcontaminantes ao medio ambiente acuático. Por outra banda, é necesario considerar o estudo do potencial de novas tecnoloxías de tratamento coma os MBR.

2. Eliminación de PPCPs mediante biorreactores de membrana

Leváronse a cabo unha serie de estudos preliminares nun MBR que utilizou como alimentación un medio sintético que simulaba as propiedades dunha auga residual urbana de carga media. A lista de PPCPs foi incrementada con catro antibióticos: sulfametoxazol (SMX), eritromicina (ERY), roxitromicina (ROX) e trimetoprim (TMP). Os fármacos de propedades acedas naproxeno e especialmente ibuprofeno foron practicamente eliminados (84 e 98% respectivamente). No caso da CBZ e o DZP, a súa eliminación foi de 9% e 26% respectivamente, mentres que os resultados obtidos para a eliminación de fragrancias estiveron ao redor dun 46-56% en todos os casos. Estas porcentaxes de eliminación non foron moi elevadas, considerando o seu elevado carácter lipofílico e polo tanto a súa afinidade para a sorción en sólidos. No caso dos antibióticos, os macrolidos ROX and ERY foron eficazmente eliminados (77 e 91% respectivamente), e o SMX foi parcialmente eliminado (52%). Por outra banda, o TMP mostrou unha baixa eficacia de eliminación. De acordo ao curto período durante o cal este sistema estivo a tratar a alimentación con adición de PPCPs e a fonte do inóculo (sen contacto previo con estes microcontaminantes), espérase que poida darse o fenómeno de adaptación da biomasa tras un período de operación máis extenso, mellorándose así os resultados obtidos.

3. Influencia dos parámetros operacionais

Nesta parte da investigación, o MBR foi trasladado ás dependencias dunha depuradora de augas urbanas para ser operado durante un período de tempo estendido. Cinco PPCPs adicionais foron considerados. Por unha banda, as hormonas estradiol (E2), etinilestradiol (EE2) e estrona (E1) consideradas como compostos disruptores endocrinos (EDCs). Por outra banda, dous antidepresivos: fluoxetina (FXL) e citalopram (CTL). A fase sólida (lodo primario e secundario) foi tamén muestreada e analizada para determinar o seu contido en PPCPs.

IBP, NPX e as tres hormonas foron case completamente eliminados (>90%) independentemente dos parámetros operacionais. As porcentaxes de eliminación de fragrancias sintéticas e FLX variaron significativamente, de baixo-moderado até alto. Neste caso, atopáronse na fase sólida cantidades moi elevadas, confirmando que a sorción no lodo é o seu principal mecanismo de eliminación. Unha combinación de distintos efectos podería explicar as diferenzas observadas entre as distintas campañas de mostraxe: A temperatura do licor de mestura, a idade celular (modificada mediante purgas de lodo) e as propiedades físico-químicas da biomasa

MBR. A transformación dos antibióticos SMX e TMP foi de moderada a alta ao longo da operación do MBR. Considerando as súas baixas constantes de biodegradación, a concentración de sólidos suspendidos do licor de mestura podería exercer certa influencia na súa eliminación. Tras varios meses de operación, o grao de eliminación de ERY e ROX veuse incrementada de forma considerable, aínda que non se observou unha influencia específica da concentración de lodos e temperatura. Polo tanto, os fenómenos de aclimatación da biomasa poderían explicar este comportamento. As porcentaxes de transformación máis baixos foron medidos para CBZ, DZP, CTL e DCF. En calquera caso, a eliminación de CBZ estivo por encima do 50% en determinados momentos, sendo este valor por encima do esperable, tendo en conta o carácter recalcitrante desta sustancia. A excepción do CTL, a sorción non foi un mecanismo importante para a eliminación destas sustancias. Ademais, nin a temperatura nin a concentración de sólidos influenciaron a súa eliminación.

A influencia do HRT foi baixa ou nula para a maioría de PPCPs, mentres que a operación a baixo SRT (por baixo de 10 días) afectou negativamente á eliminación de DCF, SMX, ERY, ROX, TMP, EE2 e CTL. O efecto máis acusado na eliminación de sustancias lipofílicas foi causado pola cantidade de lodo purgado antes de cada campaña de mostraxe, o cal resultou ser beneficioso. Deste xeito, o esgotamento de centros de sorción na biomasa MBR podería explicar a baixa porcentaxe de eliminación conseguido no caso de compostos facilmente eliminables por sorción, como son as fragrancias e os antidepresivos.

4. Comparación entre sistemas convencionais de lodos activos e biorreactores de membrana

A porcentaxe de eliminación da maioría de compostos estudados non foi significativamente distinto entre ambas tecnoloxías. En calquera caso, puidéronse atopar algunhas excepcións. Por unha banda, a eliminación de PPCPs en sistemas convencionais foi particularmente sensible á operación en condicións adversas, como son os valores baixos de temperatura ambiental ou SRT. Por outra banda, a eliminación de sustancias lipofílicas, máis especificamente as fragrancias policíclicas HHCB, AHTN e ADBI, foi sempre máis elevada no sistema convencional con independencia do período de mostraxe considerada.

5. Efecto do proceso de filtración por membranas

Avaliáronse distintos módulos de membrana, configuracións e fluxos de permeado mediante comparación dos niveis de concentración de PPCPs medidos no sobrenadante do licor de mestura cos atopados en permeados. Ningún dos módulos considerados (tubular, lamina plana e fibra oca), configuracións (side-stream or mergullada) ou fluxos de permeado (alto/baixo) mostraron distinto rendemento na eliminación de PPCPs da fase líquida. SMX, DCF or HHCB foron atopados en concentracións máis altas nalgúns permeados con respecto aos niveis medidos no sobrenadante. Polo tanto, o proceso de ultrafiltración podería contribuír á diminución da eficacia de eliminación de PPCPs da fase líquida, aínda que sómente nalgúns casos concretos.

6. Recomendacións

O grao de coñecemento sobre a tecnoloxía MBR que se alcanzou durante esta investigación confirma que, neste momento, a mellora de plantas de tratamento convencional mediante tecnoloxía de membranas non estaría xustificada desde o punto de vista da eliminación de contaminantes emerxentes, dado que os sistemas convencionais correctamente operados para a eliminación de nitróxeno poderían ser capaces de eliminar PPCPs con similar eficiencia que os MBRs. En calquera caso, algúns parámetros que demostraron exercer unha forte influencia na eliminación destas sustancias son definitivamente máis sinxelos de controlar en MBRs. Estes sistemas ademais presentan outras vantaxes adicionais, principalmente baseadas na calidade do efluente final xerado, apto para a súa reutilización. Considerando de forma global todos estes aspectos, as conclusións desta investigación non deberían constituír ningún obstaculo de cara á aplicación da tecnoloxía de membranas para o tratamento de augas residuais. En todo caso, este traballo pode ser considerado como un punto de partida que marca as liñas de investigación futuras que deberían ser seguidas de cara á optimización da capacidade dos MBR para eliminar microcontaminantes orgánicos. Por exemplo, as novas liñas de traballo poderían enfocarse á mellora das propiedades de sorción da biomasa. Nesta liña, os procesos híbridos que combinan a sorción en carbón activado cos tratamentos MBR están a obter resultados prometedores. Igualmente, a combinación da información xerada con MBRs co campo da microbioloxía podería levar a melloras significativas, xa que a posibilidade de reter completamente a biomasa xerada permitiría o desenvolvemento e a proliferación de poboacións microbianas máis especializadas na degradación de PPCPs e outros compostos recalcitrantes, optimizando así a etapa de tratamento biolóxico.

List of Publications

1. Journal publications

- R. Reif, S.Suarez, F. Omil, and J. M. Lema (2008). Fate of pharmaceuticals and cosmetic ingredients during the operation of a MBR treating urban wastewaters. *Desalination* (221) 511-517.

- R. Reif, A. Santos, S.J. Judd, J.M. Lema and F. Omil (2011). Occurrence and fate of Pharmaceutical and Personal Care Products in a sewage treatment works. *Journal of Environmental Monitoring,* 13(1) 137-144.

- R. Reif, A. Besancon, K. Le Corre, B. Jefferson, J.M. Lema, F. Omil (2011). Comparison of PPCPs removal on a paralell-operated MBR and AS system and evaluation of effluent post-treatment on vertical flow reed beds. *Water Science and Technology* (Accepted).

- Santos, R. Reif, P. Barton, J. N. Lester, F. Coulon, P. Hillis and S. J. Judd (2011). Fate and removal of permethrin during conventional activated sludge treatment. *Water Science and Technology* (Accepted).

2. Articles in preparation

- R. Reif, F. Omil F. and J.M. Lema (2011). Influence of temperature, biomass concentration and adaptation on PPCPs removal with membrane bioreactors. (In preparation).

- R. Reif, T. Barcón, F. Omil and J.M. Lema. (2011) Are membrane bioreactors more efficient than conventional activated sludge technologies for removing PPCPs from sewage? Submitted to *Bioreource Technology.*

- S. Suarez, R. Reif, Omil, F. and J.M. Lema. (2011). Fate and removal of Pharmaceuticals and Personal Care Products (PPCPs) in a conventional activated sludge treatment process. Submitted to *Water Research.*

3. Book chapters

- F. Omil, S. Suárez, M. Carballa, R. Reif, J.M. Lema (2010). Criteria for designing sewage treatment plants for enhanced removal of organic micropollutants. Xenobiotics in the Urban Water Cycle: Mass Flows, Environmental Processes and Mitigation Strategies. 283-306. SpringerLink.

- S. Suárez, M. Carballa, R. Reif, D. Serrano, J.M. Lema and F.Omil (2010). Mass Balances of Organic Micropollutants in Sewage Treatment Plants. Innovative Technologies for Urban Wastewater Treatment Plants. 191-216. ISBN: 978-84-693-3992-3.

4. Contribution to congress

- R. Reif, S. Suarez, F. Omil, J.M. Lema. Fate of pharmaceutical and cosmetic ingredients during the operation of a MBR treating sewage. Oral presentation. Desalination and the environment. Halkidikki (Greece) on 22-25 April 2007.

- R. Reif, S. Suarez, F. Omil and J.M. Lema. Efficiency of a pilot-scale MBR for the removal of organic micropollutants from sewage. Oral presentation. E2M-3+3 Méditerrannée.Tlemcen (Algeria) on 22-24 May 2008.

- R. Reif, S. Suarez, F. Omil and J.M. Lema. Performance of a pilot scale MBR for the removal of PPCPs from sewage when using different inoculums sources. Oral presentation. 4th International meeting on Biotechnology (BIOTEC). Granada (Spain) on 17-19 September 2008.

- R. Reif, A. Santos, S.J. Judd, J.M. Lema and F. Omil F. Occurrence and fate of selected PPCPs in a conventional sewage treatment plant located in North West UK. Oral presentation. Third International Meeting on Environmental Biotechnology and Engineering (3IMEBE). Palma de Mallorca (Spain) 21-25 September 2008.

- J.M. Lema, S. Suárez, R. Reif, M. Carballa and F. Omil. Factors affecting the biodegradation of micropollutants in sewage treatment plants. Oral presentation. 13th International Biotechnology Symposium & Exhibition (IBS-2008). Dalian (China) on 12-17 October 2008.

- R. Reif, A. Besancon, K. Le Corre, B. Jefferson, J.M. Lema, F. Omil. Comparison of PPCPs removal on a paralell-operated MBR and AS system and evaluation of effluent post-treatment on vertical flow reed beds. Oral presentation. International Conference on Xenobiotics in the Urban Water Cycle - XENOWAC 2009. Paphos (Chipre) on 11-13 March 2009.

- R. Reif, S. Esplugas, J.M. Lema and F. Omil. Elimination of PPCPs from municipal sewage, MBR permeate and secondary effluent using a post-ozonation system. Oral presentation. VII ANQUE International Congress. Integral Water Cycle: Present and Future. "A Shared Commitment". Oviedo (Spain) on 13-15 June 2010.

- R. Reif, F. Omil and J.M. Lema. Are Membrane Bioreactors more efficient than Conventional Activated Sludge Technologies for removing PPCPs from Sewage?. Poster presentation. Conferencia Nacional de Jóvenes Profesionales del Agua de España (Spain National Young Water Professionals Conference, Spain NYWPC). Barcelona (Spain) on 16-18 June 2010.

- R. Reif, F. Omil and J.M. Lema. Comparison of conventional and innovative sewage treatment technologies simultaneously operated with varying operating conditions. Oral presentation. Biotec 2010. Pamplona (Spain) on 29th September - 1 October 2010.

- R. Reif, F. Omil and J.M. Lema. Application of MBR and Ozonation Technologies for Enhancing the Removal of Micropollutants from Municipal Sewage. Oral presentation. IWA Regional Conference and Exhibition on Membrane Technology and Water Reuse. Istambul (Turkey) on 18-22 October 2011.

- G. Rodriguez-García, R. Reif, A. Hospido, M.T.Moreira and G. Feijoo. A comparison between conventional and novel wastewater treatment technologies: Activated Sludge and Submerged Membrane Biorreactor. Poster presentation. Water & Industry 2011 International Conference. Valladolid (Spain) on 1 - 4 May 2011.

- M. Garrido-Baserba, R. Reif, M. Poch. A knowledge management methodology for the integrated assessment of WWTP during conceptual design. Oral presentation. Watermatex. San Sebastian (Spain) on 20-22 June 2011.

- R. Reif, F. Omil and J.M. Lema. Comparison of the efficiency of MBR and CAS technologies for the removal of PPCPs from sewage. Platform presentation. Micropol & Ecohazard 2011. Sidney (Australia) on 11-13 de July 2011.

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