UNIVERSIDADE DE SANTIAGO DE COMPOSTELA
Departamento de Ingeniería Química

Strategies for the treatment of municipal and hospital wastewaters containing Pharmaceutical and Personal Care Products

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

Santiago de Compostela, Diciembre de 2007

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Informan:
Que la memoria titulada "Strategies for the treatment of municipal and hospital wastewaters containing Pharmaceutical and Personal Care Products" que, para optar al grado de Doctor en Ingeniería Química, Programa de Doctorado en Ingeniería Química y Ambiental, presenta Doña Sonia Suárez Martínez, 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, diciembre de 2007.

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Objetivos y Resumen

Los compuestos farmacéuticos y de cuidado personal (PPCPs) incluyen un amplio número de sustancias que se consumen en grandes cantidades en las sociedades modernas. Suelen abarcar compuestos activos presentes en la formulación de productos farmacéuticos, ingredientes de productos de cuidado personal (champús, lociones corporales, protectores solares, etc.) y hormonas naturales (ej. estrona, estradiol, estriol) y sintéticas (ej. etinilestradiol). En general, se trata de un grupo de compuestos muy amplio en lo que respecta a su estructura molecular, propiedades físico-químicas y persistencia. En la actualidad, se están utilizando miles de principios activos para sintetizar un número mucho mayor de medicamentos. Tomando como ejemplo el ámbito de la UE, se están consumiendo aproximadamente 3000 sustancias diferentes sólo en medicina humana, a las que hay que sumar un número muy importante de fármacos de uso veterinario. En cuanto a las perspectivas para los próximos años, se prevé un incremento en el consumo de medicamentos, principalmente debido al envejecimiento paulatino de la población, aunque también a la mejora en la calidad de vida de la población en general.

Estos compuestos se han detectado en diferentes compartimentos medioambientales, tales como ríos, lagos, aguas subterráneas, sedimentos, etc. La falta de información concluyente sobre el potencial impacto ecotoxicológico derivado de la presencia de este tipo de compuestos en el medioambiente ha convertido este tema en un asunto puntero de la investigación relacionada con las ciencias e ingeniería del medioambiente. La creciente preocupación se basa fundamentalmente en el hecho de que este tipo de sustancias se han diseñado específicamente para producir un efecto biológico en los pacientes a los que se les administran, incluso en concentraciones muy bajas.

Los PPCPs pueden llegar a las aguas subterráneas y/o superficiales por medio de un complejo entramado de vías, aunque la principal corriente de transporte la constituyen las Aguas Residuales Urbanas (ARU), a las que llegan los medicamentos después de ser metabolizados en mayor o menor medida y finalmente excretados por la orina y las heces. La utilización de productos de cuidado personal (champús, perfumes, cremas etc.), así como el vertido directo de los medicamentos sobrantes o caducados a los desagües, es otra vía de entrada importante a tener en cuenta. Los fármacos de uso veterinario, junto con los aditivos presentes en los piensos (antibióticos, hormonas, etc.), también pueden acabar contaminando las aguas, a través de la utilización de los estiércoles como fertilizante. Las industrias productoras de compuestos farmacéuticos pueden ser otro origen de vertido de estos compuestos a los medios acuáticos próximos. Sin embargo, con las normativas cada vez más estrictas y la implementación de tecnologías limpias en los

procesos de fabricación, cabe esperar una contribución mínima de esta vía al vertido de PPCPs en los próximos años. Por último, los hospitales, a parte de tener un consumo de agua muy elevado, vierten en general aguas muy complejas debido a la multitud de compuestos utilizados (agentes de diagnóstico como los medios de contraste, anestésicos como los alquil-fenoles, desinfectantes como alcoholes, formaldehído y clorofenoles) y a la gran cantidad de fármacos que se consumen en dichas instalaciones.

Debido a las bajas concentraciones en las que se encuentran estos compuestos en las aguas residuales (µg/L o ng/L) y a la complejidad de la estructura molecular de algunos PPCPs, las tecnologías convencionales implementadas en las Estaciones de Tratamiento de Aguas Residuales y Potables (EDAR y ETAP, respectivamente) no son lo suficientemente eficaces para completar su eliminación, lo que conlleva una descarga no controlada de PPCPs a los medios acuáticos superficiales y subterráneos. Por lo tanto, los productos farmacéuticos y sus metabolitos podrían alcanzar medios de abastecimiento de agua e incluso la cadena alimenticia. Esto justifica el creciente número de trabajos de investigación que se están centrando en la evaluación del riesgo para el ecosistema e incluso para la salud humana, derivado de la exposición a este tipo de compuestos. Sin embargo, esto último resulta muy improbable en base a dos aspectos: i) La mayoría de los compuestos investigados no se detectaron en aguas potables y, en los casos en los que su presencia se pudo medir, los niveles de concentración detectados están en el rango de los ng/L; ii) Para una ingestión de agua potable media de 2 L/d y una concentración de compuesto farmacéutico de 1 µg/L (rango normal en ARU y mil veces superior al rango detectado en aguas potables), harían falta unos 550 años para consumir la dosis media de un fármaco adquirido en farmacia (400 mg). Con respecto a la toxicidad en organismos acuáticos que habitan en aguas superficiales contaminadas con PPCPs, los trabajos publicados sobre la toxicidad aguda de algunos PPCPs indican que el riesgo en este sentido es improbable. Sin embargo, existe una falta de información sustancial en lo que respecta a los efectos crónicos que la exposición continuada a muy bajas concentraciones de PPCP puede suponer para dichos organismos. Sería conveniente que los estudios de toxicidad abarcasen el ciclo de vida completo de los organismos acuáticos, ya que ésta es la duración real de la exposición; pues este contacto continuado podría producir cambios importantes, pero imperceptibles en períodos cortos, y por lo tanto difíciles de distinguir de la evolución natural. Merecen una atención especial, por su relevancia, aquellos compuestos que pueden alterar el sistema nervioso y endocrino de los organismos expuestos, tales como los estrógenos y los antidepresivos, además de aquellos que tengan tendencia a bioacumularse en los organismos acuáticos, como por ejemplo las fragancias, para las cuales se midieron concentraciones 600 veces mayores en los tejidos de peces expuestos a ellas que las que se encontraron en las propias aguas.

Con la tecnología implementada en las EDAR más modernas, se asegura una eliminación eficaz del carbono y del nitrógeno presente en las ARU, además de un estricto control microbiano. Sin embargo, estas instalaciones están recibiendo un gran número de contaminantes traza, incluyendo los PPCPs, para los que las tecnologías de tratamiento convencionales no han sido diseñadas específicamente. Los datos disponibles en bibliografía relativos a las eficacias de eliminación de PPCPs alcanzadas en EDAR varían enormemente de un trabajo a otro, aunque demuestran que su eliminación es a menudo incompleta. La eliminación de PPCPs durante el tratamiento de ARU tiene lugar por medio de un conjunto de mecanismos, tales como la volatilización, la adsorción en el fango y la transformación biológica y/o química. Para determinar cuál es la contribución relativa de cada uno de estos mecanismos, es esencial recopilar la máxima información posible sobre las características físico-químicas de las sustancias consideradas, incluyendo su acidez, carácter lipofílico, volatilidad y potencial de adsorción. Conseguir incrementar la eliminación global de PPCPs en las EDAR pasa por buscar nuevas estrategias de tratamiento, que incluyan modificaciones en las condiciones de operación, la implementación de nuevas tecnologías o la incorporación de un proceso avanzado de post-tratamiento.

El objetivo de esta Tesis consistió en investigar el comportamiento de los PPCPs y su eficacia de eliminación en distintos procesos involucrados en la depuración de ARU, incluyendo tratamientos primarios, secundarios y post-tratamientos. La selección de compuestos se ha hecho en base a criterios de consumo, riesgos potenciales asociados al compuesto, sus propiedades físico-químicas y a la existencia de métodos analíticos fiables. La lista de PPCPs finalmente seleccionada incluye compuestos farmacéuticos de cinco grupos terapéuticos diferentes (antibióticos, antidepresivos, anti-inflamatorios, tranquilizantes y antiepilépticos), hormonas, incluyendo los dos estrógenos naturales estrona (E1) y 17β-estradiol (E2) y la hormona sintética 17α-etinilestradiol (EE2), utilizada como ingrediente activo en los anticonceptivos, el medio de contraste de rayos X iopromide (IPM), y, como productos de cuidado personal, tres fragancias policíclicas.

El primer objetivo de este trabajo consistió en analizar la situación en España en lo que respecta a la presencia de PPCPs en las aguas residuales. Con este fin se han llevado a cabo unas exhaustivas campañas de muestreo en una ciudad del noroeste de España, de aproximadamente 125.000 habitantes, durante los años 2004, 2005 y 2006, tal y cómo se detalla en el Capítulo 3 de este trabajo. El agua residual recogida en estas campañas de muestreo representa cuatro orígenes diferentes: i) agua residual municipal; ii) efluentes procedentes de tres hospitales diferentes; iii) la corriente de entrada y iv) la corriente de salida de la estación depuradora de la ciudad. Estas muestras se caracterizaron incluyendo parámetros físico-químicos convencionales y la concentración de los PPCPs considerados en este trabajo. Los resultados de estos análisis indicaron que las aguas residuales

municipales de la ciudad pueden clasificarse como moderadamente contaminadas. Por el contrario, los efluentes hospitalarios presentaron en general una contaminación significativamente más elevada, con concentraciones máximas medidas para los parámetros convencionales que triplicaban los estándares fijados para aguas residuales municipales concentradas. En lo que respecta a los PPCPs, se han medido concentraciones de naproxeno (NPX) hasta 40 veces más elevadas en los efluentes de hospital en una de las campañas de muestreo realizadas. Del conjunto de PPCPs considerados, las concentraciones más altas se midieron para el ibuprofeno (IBP) y NPX, mientras que los compuestos EE2, fluoxetina (FLX) y citalopram (CTL) se encontraron en general en niveles inferiores al límite de detección de los métodos analíticos utilizados. Sin embargo, cabe destacar, que las pocas detecciones positivas para estas sustancias, se dieron en corrientes de origen hospitalario.

El comportamiento de PPCPs durante un proceso biológico de tratamiento de aguas residuales convencional ha sido objeto de estudio en el Capítulo 4. Con este propósito, se ha puesto en marcha una planta piloto de lodos activos formada por un tanque anóxico acoplado a otro aerobio, para la eliminación conjunta de materia orgánica y nitrógeno. En esta planta se ha realizado de forma periódica un seguimiento detallado de los PPCPs seleccionados para esta Tesis, con el fin de analizar en primer lugar la evolución en la concentración de PPCPs en la fase líquida y, posteriormente, para aplicar un balance de materia más detallado. En esta segunda etapa se han considerado los diferentes mecanismos de eliminación involucrados en el tratamiento biológico de aguas (la volatilización, la adsorción y la degradación). Los compuestos carbamazepina (CBZ), diazepam (DZP) y diclofenac (DCF) han demostrado una gran resistencia al tratamiento biológico, permaneciendo prácticamente inalterados durante su paso por la planta piloto. Por el contrario, se midieron unos porcentajes de eliminación muy elevados (> 80%), para galaxolide (HHCB), tonalide (AHTN), FLX, IBP, NPX y los estrógenos naturales E1 y E2. La adsorción ha demostrado jugar un papel importante en la biotransformación de las fragancias galaxolide y tonalide, que habían mostrado previamente cierta resistencia a la biodegradación. Esto se debe, muy probablemente, a que el compuesto adsorbido sobre el lodo permanece retenido durante un tiempo mayor en la planta, favoreciendo de este modo su transformación. La eliminación de la tercera fragancia considerada en el estudio, celestolide (ADBI), fue en gran parte consecuencia del carácter altamente volátil de este compuesto. De hecho, la volatilización de ADBI en el tanque aeróbico supuso hasta un 45% de su eliminación global. Otro aspecto incluido en este capítulo fue el análisis de la influencia de algunas condiciones de operación, tales como la temperatura, el Tiempo de Retención Celular (TRC) y la relación de recirculación interna, sobre el proceso. El TRC mostró ejercer un efecto sobre el grado de transformación de aquellos compuestos con potencial de adsorción, probablemente al realzar la retención del

compuesto dentro de la planta y consecuentemente su disponibilidad para la degradación biológica. Este efecto fue especialmente importante para sustancias que presentaban cinéticas de transformación biológica lentas, como por ejemplo el sulfametoxazol (SMX), para el que el grado de eliminación aumentó hasta un 25% al sobrepasar los 40 d de TRC. Para algunos compuestos se ha observado un efecto positivo al incrementar la temperatura de operación (de aproximadamente 16ºC a 21ºC), llegando a mejoras en la eficacia de eliminación de hasta un 32% para el caso del SMX. Durante los primeros meses la planta piloto ha estado operando con una relación de recirculación interna de 3, que se ha subido posteriormente hasta 4 para realzar la eliminación de nitrógeno. Este incremento tuvo una influencia positiva en la eliminación de IBP, NPX, FLX y CTL, aunque no afectó a la transformación de estrógenos, CBZ, DZP y DCF.

Mientras que en el Capítulo 4 la contribución de las condiciones de oxidación-reducción (redox) anóxicas y aerobias en la eliminación de PPCPs se determinó por aplicación de balances de materia, en el Capítulo 5 se analizó este efecto de forma experimental. Para ello se han puesto en marcha dos reactores a escala de laboratorio, operando uno de ellos en condiciones típicas de nitrificación aerobia y el otro en un ambiente anóxico desnitrificante. Se ha hecho un seguimiento de las concentraciones de los compuestos seleccionados en la fase líquida y se han aplicado balances de materia al proceso considerando la contribución de la volatilización, la adsorción y la transformación a la eliminación de PPCPs. Los compuestos FLX, estrógenos naturales y fragancias se han transformado eficazmente tanto en condiciones aerobias (> 76%) como anóxicas (> 65%), mientras que el NPX, el EE2 y los antibióticos roxitromicina (ROX) y eritromicina (ERY) sólo se han eliminado significativamente en el reactor aerobio (> 82%). El antidepresivo CTL se ha degradado con un eficacia intermedia tanto en condiciones aerobias como anóxicas (> 62% y > 41%, respectivamente). Algunos compuestos se mostraron altamente resistentes a la transformación biológica, incluyendo la CBZ, el DZP y los antibióticos SMX y trimetoprim (TMP). En este capítulo también se ha analizado la influencia de algunas condiciones de operación, tales como la temperatura, el TRC y la adaptación y concentración de biomasa. La eliminación de DCF en el reactor aerobio se vio influenciada positivamente por el desarrollo de una biomasa nitrificante, llegando a eliminaciones de hasta un 74 %. De manera similar, en el reactor anóxico se ha logrado transformar de manera eficiente el IBP (75%) transcurrido un período de adaptación de 340 días.

En el Capítulo 6, se han evaluado los procesos de coagulación-floculación y de flotación para el pretratamiento de efluentes hospitalarios. En este capítulo se ha incorporado un nuevo compuesto a la selección inicial de PPCPs. Se trata del medio de contraste iopromide (IPM), cuyo consumo en hospitales es muy relevante. El trabajo realizado incluyó ensayos discontinuos de coagulación-floculación llevados a cabo en un dispositivo experimental conocido como "Jar-Test", complementados

posteriormente con la puesta en marcha de una planta piloto de coagulación-floculación en continuo. Además, tanto el efluente hospitalario como la corriente de salida de esta planta de coagulación continua, han sido tratados en una celda de flotación. En general, la flotación de los efluentes hospitalarios condujo a resultados ligeramente peores comparados con la coagulación en lo que respecta a la eliminación de Sólidos en Suspensión Totales (SST) y PPCPs. Sin embargo, cuando la flotación se aplicó como post-tratamiento del efluente de la planta de coagulación continua, se logró mejorar la eficacia global del proceso. La eliminación de SST en el pretratamiento fue muy eficaz, alcanzando eficiencias máximas de 88%, 72% y 97% para la coagulación discontinua, la flotación de los efluentes de hospital y la combinación de la coagulación y la flotación, respectivamente. Para el caso de la Demanda Química de Oxígeno total (DQO) la eficiencia de los procesos estaba condicionada por la fracción de materia orgánica particulada en el agua residual, que era la fracción que se eliminaba principalmente en el proceso, mientras que la materia orgánica en suspensión permanecía en el efluente. De los PPCPs seleccionados, IPM, CBZ y DZP fueron los compuestos más persistentes, mientras que para las fragancias y el DCF se alcanzaron eliminaciones muy elevadas. Para los antibióticos se han medido eficacias de eliminación negativas. Este hecho podría deberse a que la concentración de antibiótico determinada analíticamente en el agua residual hospitalaria antes de su tratamiento estaba por debajo de la concentración real, lo que podría ser una consecuencia de que una fracción del antibiótico estuviera confinada dentro de partículas de heces (por ejemplo para los macrólidos) o parcialmente metabolizada (por ejemplo el SMX). Para el resto de compuestos, NPX e IBP, la reducción en la concentración alcanzada en el proceso fue moderada.

En el Capítulo 7, se ha investigado la eficiencia del ozono para el tratamiento del agente antimicrobiano triclosan (TRI) y del fármaco antidepresivo FLX. Para ello se han determinado las constantes cinéticas de segundo orden, k_{O3}, para la reacción del O_3 con las especies básicas y ácidas del TRI y de la FLX. Aunque se midieron valores muy elevados de k_{O3} para las especies desprotonadas de cada uno de los compuestos (k_{O3} = 5.1 (± 0.1) $\times$ 10^8 $M^{-1}s^{-1}$ para TRI aniónico y k_{O3} = 1.1 (± 0.1) $\times$ 10^6 $M^{-1}s^{-1}$ para FLX neutra), sólo el TRI reacciona rápidamente a pHs cercanos al neutro (las constantes cinéticas aparentes de segundo orden, $k_{app,O3}$, a pH 7, fueron 3.8 $\times$ 10^7 $M^{-1}s^{-1}$ para TRI y 9.6 $\times$ 10^2 $M^{-1}s^{-1}$ para FLX). El modelado cinético del proceso indica que el O_3 ha reaccionado con TRI y FLX por ataque electrofílico a su grupo fenol y amina, respectivamente. En la segunda parte de este trabajo se ha estudiado la oxidación por ozono de TRI y FLX en un efluente secundario procedente de dos plantas convencionales de lodos activos. El TRI se ha oxidado con una eficiencia relativamente alta, tal y como se esperaba dada su alta reactividad con O_3. Para este compuesto se ha logrado una reducción en su concentración de prácticamente un 100% aplicando una dosis de ozono de 4 mg/L ($8.3 \cdot 10^{-5}$ mol/L) a

un agua residual que contenía materia orgánica en concentraciones de 7.5 mg/L de COD y de aproximadamente un 58 % para una dosis de ozono de 6 mg/L ($1.3 \cdot 10^{-4}$ mol/L) cuando el agua residual tratada presentaba un contenido en materia orgánica algo superior (12.4 mg/L de COD). La transformación de FLX fue menos eficaz, lo cual se justifica en base a la baja reactividad con O_3 que presenta cuando se trabaja a pHs cercanos a la neutralidad. Esta cinética más lenta permitió que la caída en la concentración de FLX fuera evaluada en función del tiempo de reacción para su modelado. En esta parte del trabajo se confirmó que los valores de la k_{O3} determinados en agua destilada pueden aplicarse en el modelado de la oxidación de FLX en agua residual.

Obxectivos e Resumo

Os compostos farmacéuticos e de coidado persoal (PPCPs) inclúen un amplo número de compostos que se consumen en grandes cantidades nas sociedades modernas. Estes compostos detectáronse en diferentes compartimentos ambientais, tales como ríos, lagos, augas subterráneas, sedimentos, etc. A falta de información concluinte sobre o potencial impacto ecotoxicolóxico derivado da presencia deste tipo de compostos no medioambiente converteu este tema nun asunto punteiro da investigación relacionada coas ciencias e enxeñería do medioambiente. A crecente preocupación baséase fundamentalmente no feito de que este tipo de sustancias foron deseñadas especificamente para producir un efecto biolóxico nos pacientes aos que se lles administran, ata en concentracións moi baixas. Ademais, debido ás baixas concentracións nas que se atopan estes compostos nas augas residuais (µg/L ou ng/L) e á complexidade da estructura molecular dalgúns PPCPs, as tecnoloxías convencionais instaladas nas Estacións de Tratamento de Augas Residuais e Potables (EDAR e ETAP, respectivamente) non son o suficientemente eficaces para completar a súa eliminación, o que leva a descargas non controladas de PPCPs aos medios acuáticos superficiais e subterráneos. Polo tanto, os productos farmacéuticos e os seus metabolitos poderían alcanzar medios de abastecemento de auga e ata a cadea alimenticia. Isto xustifica o crecente número de traballos de investigación que se están centrando na avaliación do risco para o ecosistema e ata para a saúde humana, derivado da exposición a este tipo de compostos.

Coa tecnoloxía implementada nas EDAR máis modernas, asegúrase unha eliminación eficaz do carbono e do nitróxeno presente nas Augas Residuais Urbanas (ARU), ademais dun estricto control microbiano. Con todo, estas instalacións están recibindo un gran número de contaminantes traza, incluíndo os PPCPs, para os que as tecnoloxías de tratamento convencionais non foron deseñadas especificamente. Os datos dispoñibles en bibliografía relativos ás eficacias de eliminación de PPCPs alcanzadas na EDAR varían enormemente dun traballo a outro, aínda que demostran que a súa eliminación é a miúdo incompleta. A eliminación de PPCPs durante o tratamento de ARU ten lugar por medio dun conxunto de mecanismos, tales como a volatilización, a adsorción no lodo e a transformación biolóxica e/ou química. Para determinar cal é a contribución relativa de cada un destes mecanismos, é esencial recompilar a máxima información posible sobre as características físico-químicas das sustancias consideradas, incluíndo a súa acidez, carácter lipofílico, volatilidade e potencial de adsorción. Conseguir incrementar a eliminación global de PPCPs nas EDAR pasa por buscar novas estratexias de tratamento, que inclúen modificacións nas condicións de operación, a implementación de novas tecnoloxías ou a incorporación dun proceso avanzado de post-tratamento.

O obxectivo desta Tese consistiu en investigar o comportamento dos PPCPs e a súa eficacia de eliminación en distintos procesos involucrados na depuración de ARU, incluíndo tratamentos

primarios, secundarios e post-tratamentos. A selección de compostos fíxose en base a criterios de consumo, riscos potenciais asociados ao composto, as súas propiedades físico-químicas e á existencia de métodos analíticos fiables. A lista de PPCPs finalmente seleccionada inclúe compostos farmacéuticos de cinco grupos terapéuticos diferentes (antibióticos, antidepresivos, anti-inflamatorios ,tranquilizantes e antiepilépticos), hormonas, incluíndo os dous estróxenos naturais estrona (E1) e 17β-estradiol (E2) e a hormona sintética 17α-etinilestradiol (EE2), utilizada como ingrediente activo nos anticonceptivos, o medio de contraste de raios X iopromide (IPM), e, como productos de coidado persoal, tres fragrancias policíclicas.

O primeiro obxectivo deste traballo consistiu en analizar a situación en España no que respecta a presencia de PPCPs nas augas residuais. Con este fin leváronse a cabo unhas exhaustivas campañas de mostraxe nunha cidade do noroeste de España, de aproximadamente 125.000 habitantes, durante os anos 2004, 2005 e 2006, tal e como se detalla no Capítulo 3 deste traballo. A auga residual recollida nestas campañas de mostraxe representa catro orixes diferentes: i) auga residual municipal; ii) efluentes procedentes de tres hospitais diferentes; iii) a corrente de entrada e iv) a corrente de saída da estación depuradora da cidade. Estas mostras caracterizáronse incluíndo parámetros físico-químicos convencionais e a concentración dos PPCPs considerados neste traballo. Os resultados destas análises indicaron que as augas residuais municipais da cidade poden clasificarse como moderadamente contaminadas. Pola contra, os efluentes hospitalarios presentaron en xeral unha contaminación significativamente máis elevada, con concentracións máximas medidas para os parámetros convencionais que triplicaban os estándares fixados para augas residuais municipais concentradas. No que respecta aos PPCPs, medíronse concentracións de naproxeno (NPX) ata 40 veces máis elevadas nos efluentes de hospital nunha das campañas de mostraxe realizadas. Do conxunto de PPCPs considerados, as concentracións máis altas medíronse para o ibuprofeno (IBP) e NPX, mentres que os compostos EE2, fluoxetina (FLX) e citalopram (CTL) atopáronse en xeral en niveis inferiores ao límite de detección dos métodos analíticos utilizados. Con todo, cabe destacar, que as poucas detección positivas destas sustancias, déronse en correntes de orixe hospitalario.

O comportamento de PPCPs durante un proceso biolóxico de tratamento de augas residuais convencional foi obxecto de estudio no Capítulo 4. Con este propósito, púxose en marcha unha planta piloto de lodos activos formada por un tanque anóxico conectado a outro aerobio, para a eliminación conxunta de materia orgánica e nitróxeno. Nesta planta realizouse de forma periódica un seguimento detallado do conxunto dos PPCPs seleccionados para esta Tese, para analizar en primeiro lugar a evolución na concentración de PPCPs na fase líquida e, posteriormente, para aplicar un balance de materia máis detallado. Nesta segunda etapa consideráronse os diferentes mecanismos de eliminación involucrados no tratamento biolóxico de augas (a volatilización, a adsorción e a degradación). Os compostos carbamazepina (CBZ), diazepam (DZP) e diclofenac (DCF) mostraron unha grande resistencia ao tratamento biolóxico, permanecendo practicamente inalterados durante o seu paso pola planta piloto. Pola contra, medíronse unhas porcentaxes de eliminación moi elevadas (> 80%), para galaxolide (HHCB), tonalide (AHTN), FLX, IBP, NPX e os estróxenos naturais E1 e E2. A adsorción demostrou xogar un papel importante na biotransformación das fragrancias galaxolide e tonalide, que mostraran

previamente certa resistencia á biodegradación. Isto débese, moi probablemente, a que o composto adsorbido sobre o lodo permanece retido durante un tempo maior na planta, favorecendo deste xeito a súa transformación. A eliminación da terceira fragrancia considerada no estudo, celestolide (ADBI), foi en gran parte consecuencia do carácter altamente volátil deste composto. De feito, a volatilización de ADBI no tanque aerobio supuxo ata un 45 % da súa eliminación global. Outro aspecto incluído neste capítulo foi a análise da influencia dalgunhas condicións de operación, tales como a temperatura, o Tempo de Retención Celular (TRC) e a relación de recirculación interna, sobre o proceso. O TRC mostrou exercer un efecto sobre o grao de transformación daqueles compostos con potencial de adsorción, probablemente ao realzar a retención do composto dentro da planta e consecuentemente a súa dispoñibilidade para a degradación biolóxica. Este efecto foi especialmente importante para sustancias que presentaban cinéticas de transformación biolóxica lentas, por exemplo o sulfametoxazol (SMX), para o que o grao de eliminación aumentou ata un 25% ao exceder os 40 d de TRC. Para algúns compostos observouse un efecto positivo ao incrementar a temperatura de operación (de aproximadamente 16ºC a 21ºC), chegando a melloras na eficacia de eliminación de ata un 32% para o caso do SMX. Durante os primeiros meses a planta piloto estivo operando cunha relación de recirculación interna de 3, que se subiu posteriormente ata 4 para realzar a eliminación de nitróxeno. Este incremento tivo unha influencia positiva na eliminación de IBP, NPX, FLX e CTL, aínda que non afectou á transformación de estróxenos, CBZ, DZP e DCF.

Mentres no Capítulo 4 a contribución das condicións de oxidación-reducción (redox) anóxicas e aerobias na eliminación de PPCPs determinouse por aplicación de balances de materia, no Capítulo 5 analizouse este efecto de forma experimental. Para iso puxéronse en marcha dous reactores a escala de laboratorio, operando un deles en condicións típicas de nitrificación aerobia e o outro nun ambiente anóxico desnitrificante. Fíxose un seguimento das concentracións dos compostos seleccionados na fase líquida e aplicáronse balances de materia ao proceso considerando a contribución da volatilización, a adsorción e a transformación na eliminación de PPCPs. Os compostos FLX, estróxenos naturais e fragrancias transformáronse eficazmente tanto en condicións aerobias (> 76%) como anóxicas (> 65%), mentres que o NPX, o EE2 e os antibióticos roxitromicina (ROX) e eritromicina (ERY) só se eliminaron significativamente no reactor aerobio (> 82%). O antidepresivo CTL degradouse cunha eficacia intermedia tanto en condicións aerobias como anóxicas (> 62% e > 41%, respectivamente). Algúns compostos mostráronse altamente resistentes á transformación biolóxica, incluíndo a CBZ, o DZP e os antibióticos SMX e trimetoprim (TMP). Neste capítulo tamén se analizou a influencia dalgunhas condicións de operación, tales como a temperatura, o TRC e a adaptación e concentración de biomasa. A eliminación de DCF no reactor aerobio viuse influenciada positivamente polo desenvolvemento dunha biomasa nitrificante, chegando a eliminacións de ata un 74 %. De xeito similar, no reactor anóxico logrouse transformar de xeito eficiente o IBP (75%) transcorrido un período de adaptación de 340 días.

No Capítulo 6, avaliáronse os procesos de coagulación-floculación e de flotación para o pretratamento de efluentes hospitalarios. Neste capítulo incorporouse un novo composto á selección inicial de PPCPs. Trátase do medio de contraste iopromide (IPM), cuxo consumo en

hospitais é moi relevante. O traballo realizado incluíu ensaios descontinuos de coagulación-floculación levados a cabo nun dispositivo experimental coñecido como "Jar-Test", complementados posteriormente coa posta en marcha dunha planta piloto de coagulación-floculación en continuo. Ademais, tanto o efluente hospitalario como a corrente de saída desta planta de coagulación continua, tratáronse nunha unidade de flotación. En xeral, a flotación dos efluentes hospitalarios conduciu a resultados lixeiramente peores comparados coa coagulación no que respecta á eliminación de Sólidos en Suspensión Totais (SST) e PPCPs. Con todo, cando a flotación se aplicou como post-tratamento do efluente da planta de coagulación continua, lograrouse mellorar a eficacia global do proceso. A eliminación de SST no pretratamento foi moi eficaz, alcanzando eficiencias máximas de 88%, 72% e 97% para a coagulación descontinua, a flotación dos efluentes de hospital e a combinación da coagulación e da flotación, respectivamente. Para o caso da Demanda Química de Osíxeno total (DQO) a eficiencia dos procesos estaba condicionada pola fracción de materia orgánica particulada na auga residual, que era a fracción que se eliminaba principalmente no proceso, mentres que a materia orgánica en suspensión permanecía no efluente. Dos PPCPs seleccionados, IPM, CBZ e DZP foron os compostos máis persistentes, mentres que para as fragrancias e o DCF alcanzáronse eliminacións moi elevadas. Para os antibióticos medíronse eficacias de eliminación negativas. Este feito podería deberse a que a concentración de antibiótico determinada analiticamente na auga residual hospitalaria antes do seu tratamento estaba por debaixo da concentración real, o que podería ser unha consecuencia de que unha fracción do antibiótico estivese confinada dentro de partículas de feces (por exemplo para os macrólidos) ou parcialmente metabolizada (por exemplo o SMX). Para o resto de compostos, NPX e IBP, a reducción na concentración alcanzada no proceso foi moderada.

No Capítulo 7, investigouse a eficiencia do ozono para o tratamento do axente antimicrobiano triclosan (TRI) e do fármaco antidepresivo FLX. Para iso determináronse as constantes cinéticas de segunda orde, k_{O3}, para a reacción do O_3 coas especies básicas e acedas do TRI e da FLX. Aínda que se mediron valores moi elevados de k_{O3} para as especies desprotonadas de cada un dos compostos (k_{O3} = 5.1 (± 0.1) $\cdot 10^8$ $M^{-1}s^{-1}$ para TRI aniónico e k_{O3} = 1.1 (± 0.1) $\cdot 10^6$ $M^{-1}s^{-1}$ para FLX neutra), só o TRI reacciona rapidamente a pH próximo ao neutro (as constantes cinéticas aparentes de segundo orde, $k_{app,O3}$, a pH 7, foron 3.8 $\cdot 10^7$ $M^{-1}s^{-1}$ para TRI e 9.6 $\cdot 10^2$ $M^{-1}s^{-1}$ para FLX). O modelado cinético do proceso indica que o O_3 reaccionou con TRI e FLX por ataque electrofílico aos seus grupos fenol e amina, respectivamente. Na segunda parte deste traballo estudouse a oxidación por ozono de TRI e FLX nun efluente secundario procedente de dúas plantas convencionais de lodos activos. O TRI oxidouse cunha eficiencia relativamente alta, tal e como se esperaba dada a súa alta reactividade con O_3. Para este composto lograrouse unha reducción na súa concentración de practicamente un 100% aplicando unha dose de ozono de 4 mg/L ($8.3 \cdot 10^{-5}$ mol/L) a un auga residual que contiña materia orgánica en concentracións de 7.5 mg/L de COD e de aproximadamente un 58% para unha dose de ozono de 6 mg/L ($1.3 \cdot 10^{-4}$ mol/L) cando a auga residual tratada presentaba un contido en materia orgánica algo superior (12.4 mg/L de COD) . A transformación de FLX foi menos eficaz, o cal se xustifica en base á baixa reactividade con O_3 que presenta cando se

traballa a pHs próximos á neutralidade. Esta cinética máis lenta permitiu que a caída na concentración de FLX fose avaliada en función do tempo de reacción para o seu modelado. Nesta parte do traballo confirmouse que os valores da k_{O3} determinados en auga destilada poden aplicarse no modelado da oxidación de FLX en auga residual.

Objectives and Summary

Pharmaceuticals and Personal Care Products (PPCPs) comprise a wide number of compounds largely consumed in modern societies that have been detected in different environmental compartments (rivers, lakes, groundwaters, sediments, etc.). The lack of conclusive information about the potential impact derived from their occurrence, fate and ecotoxicological effects has converted this topic into an emerging issue. Special concern arises from the fact that these substances have been specifically designed to produce a biological effect upon intake by the patients, even at very low concentrations. Additionally, due to their low concentrations in wastewaters (ppb or ppt) and the complex structure of some of them, common technologies used in Sewage and Drinking Water Treatment Plants (STPs and DWTPs, respectively) may not be efficient enough to accomplish their removal, leading to an uncontrolled discharge to the aquatic environment. Consequently, drugs and their metabolites can enter water supplies and even the food chain, which gives rise to concern and leads to an increasing amount of research focusing on risk assessments in order to evaluate their possible impact on the ecosystem and even on human health.

Modern STPs can effectively accomplish carbon and nitrogen removal, as well as microbial pollution control. However, these installations are receiving a large number of different trace polluting compounds, such as PPCPs, for which conventional treatment technologies have not been specifically designed. The reported overall removal rates of PPCPs in full-scale STPs vary strongly and they clearly show that their elimination is often incomplete. Different removal mechanisms are responsible for PPCPs depletion in STPs, including volatilisation, sorption to sludge and biological and/or chemical transformation. Information about physico-chemical characteristics of these substances, such as acidity, lipophilicity, volatility and sorption potential is a useful tool to establish the relative contribution of each. In order to enhance the overall removal of PPCPs in STPs it is necessary to look for new strategies, including modification of operating conditions, implementation of new technologies or incorporation of advanced post-treatment steps.

The aim of this work was to study the fate and behaviour of a representative group of PPCPs during different treatment technologies commonly applied in STPs, including primary, secondary and post-treatments. The selection of compounds has been based on criteria of prescription amounts, associated risks, physico-chemical properties and the existence of reliable analytical methods. The list of PPCPs

included in this research consisted of pharmaceuticals from five different therapeutic classes (antibiotics, anti-depressants, anti-inflammatory drugs, tranquilizers and anti-epileptics), hormones, including the two natural estrogens estrone (E1) and 17β-estradiol (E2) and the synthetic hormone used in contraceptive drugs 17α-ethinylestradiol (EE2), the X-ray contrast media iopromide (IPM), and as cosmetic ingredients three polycyclic musk fragrances.

The first approach of this work was to analyse the situation in Spain concerning the occurrence of PPCPs in wastewater. For this purpose in Chapter 3, an exhaustive sampling campaign was carried out during the years 2004, 2005 and 2006 in a city of NW Spain of approximately 125,000 inhabitants, including wastewater samples from four different origins: i) municipal wastewater; ii) effluents from three different hospitals; and iii) the influent and iv) the effluent from the STP of the city. Conventional physico-chemical parameters as well as the concentration of PPCPs were determined in the different samples. The characterisation of the wastewaters showed that, while municipal sewage could be classified as moderately polluted, hospital effluents were in general stronger polluted and maximum concentrations for conventional wastewater parameters were at least 3-fold higher than standard values for concentrated municipal sewage. In terms of PPCPs, hospital effluents showed up to 40-fold higher concentrations of naproxen (NPX) during one sampling campaign. From the whole set of PPCPs monitored, highest concentrations were measured for ibuprofen (IBP) and NPX, whereas EE2, fluoxetine (FLX) and citalopram (CTL) were generally not detected in the wastewaters sampled, although the few positive detection of anti-depressants were found for hospital streams.

The fate and behaviour of PPCPs during a conventional biological wastewater treatment process was aimed to be assessed in Chapter 4. For this purpose, an extensive monitoring of selected PPCPs was carried out in a denitrifying/nitrifying pilot plant. The occurrence of PPCPs in the liquid phase was determined in a first step, which was further complemented with a detailed mass balance, where the most relevant removal mechanisms during biological treatment have been considered (volatilisation, sorption and degradation). Carbamazepine (CBZ), diazepam (DZP) and diclofenac (DCF), remained unaltered during their passage through the pilot plant, whereas the highest transformation (>80%) has been determined for galaxolide (HHCB), tonalide (AHTN), FLX, IBP, NPX and natural estrogens (E1 and E2). Sorption has shown to play an important role in the biotransformation of the musks galaxolide and tonalide, which had previously shown not to be easily biodegraded, probably by enhancing their retention inside the pilot plant. The removal of the third fragrance considered, celestolide (ADBI), was highly due to volatilisation in the aerobic tank, which supposed up to 45% of its overall elimination. The influence of some operational conditions, such as temperature, Sludge Retention Time (SRT) and the internal recirculation flow on the process has been evaluated. The SRT had only an effect on the transformation degree of

compounds with a significant sorption potential, presumably because it enhances the retention of the compound inside the plant and consequently its availability for biological degradation. This effect was especially important for substances, such as sulfamethoxazole (SMX), with slow biological transformation kinetics, where the removal efficiency increased up to a 25% when working at SRT above 40 d. The positive effect of warm temperatures (~21ºC) comparing to moderate ones (~16ºC), was observed in some cases, with increases in the removal of up to 32% in the case of SMX. During the first months the pilot plant has been working at an internal recirculation rate of 3 that was afterwards increased to 4, in order to enhance nitrogen removal. This increase had a positive influence on the removal of IBP, NPX, FLX and CTL, whereas it did not affect the transformation of estrogens, CBZ, DZP and DCF.

While in Chapter 4 the contribution of anoxic and aerobic redox conditions to the removal of PPCPs was determined by means of mass balances, this effect was experimentally analysed in Chapter 5. For that purpose, two lab-scale reactors have been set-up, one working at typical nitrifying aerobic conditions and the other in a denitrifying anoxic environment. Depletion of selected compounds on the basis of the concentrations in the liquid phase was followed and mass balances considering the contribution of volatilisation, sorption and transformation were applied. The compounds FLX, natural estrogens and musk fragrances were transformed to a large extent under aerobic (>76%) and anoxic (>65%) conditions, whereas NPX, EE2 and the antibiotics roxithromycin (ROX) and erythromycin (ERY) were only significantly transformed in the aerobic reactor (>82%). The anti-depressant CTL was moderately biotransformed under both, aerobic and anoxic conditions (>62% and >41%, respectively). Some compounds showed high resistance to biological transformation, as CBZ, DZP, and the antibiotics SMX and trimethoprim (TMP). Additionally, the influence of some operational conditions, such as temperature, Sludge Retention Time (SRT) and biomass adaptation and concentration, was analysed. Removal of DCF in the aerobic reactor was positively affected by the development of nitrifying biomass and increased up to 74%. Similarly, efficient anoxic transformation of IBP (75%) was determined after an adaptation period of 340 days.

In Chapter 6, coagulation-flocculation and flotation processes have been considered for the pre-treatment of hospital wastewater. The iodinated contrast media iopromide (IPM) was a compound included in this part of the work, according to its relevant consumption in hospitals. Batch coagulation-flocculation assays have been performed in a Jar-Test device, which where afterwards complemented with the set-up of a continuous coagulation-flocculation pilot-scale plant. Additionally raw hospital wastewater as well as the effluent from this continuous coagulation plant has been treated in a flotation cell. In general, flotation of raw wastewater led to slightly worse results compared to batch coagulation regarding both, Total

Suspended Solids (TSS) and PPCPs removal, although, when applied to the effluent obtained from the coagulation pilot plant, the overall efficiency of the process was positively affected. Removal of TSS during pre-treatment was very effective reaching maximum efficiencies of 88%, 72% and 97% for batch coagulation, raw wastewater flotation and combined coagulation-flotation, respectively. In the case of total Chemical Oxygen Demand (COD) the efficiency of the processes was dependant on the fraction of particulate organic matter, which was the fraction that was considerably removed, whereas soluble organic matter was normally not eliminated. From the selected PPCPs, IPM, CBZ and DZP were the most persistent compounds, whereas fragrances and DCF were eliminated to a high degree. For NPX and IBP the decrease in concentration was in between the previous substances. In the case of antibiotics negative removals have been generally measured. This could be partially attributed to the fact that the concentration of antibiotic measured in the hospital wastewater before the treatment was below its real concentration, because a fraction of the antibiotic was enclosed in faeces particles (e.g. macrolides) or partly metabolised (e.g. SMX).

In Chapter 7, the efficiency of ozone for the treatment of the antimicrobial agent triclosan (TRI) and the antidepressant drug FLX has been investigated. For that purpose, second-order rate constants, k_{O3}, were determined for reaction of O_3 with each of TRI's and FLX's acid-base species. Although very high values of k_{O3} were measured for the deprotonated species of each target compound (k_{O3} = 5.1 (± 0.1) × 10^8 $M^{-1}s^{-1}$ for anionic TRI and k_{O3} = 1.1 (± 0.1) × 10^6 $M^{-1}s^{-1}$ for neutral FLX), only TRI was fast reacting at circumneutral pH (the pH-dependent, apparent second-order rate constants, $k_{app,O3}$, were 3.8 × 10^7 $M^{-1}s^{-1}$ for TRI and 9.6 × 10^2 $M^{-1}s^{-1}$ for FLX at pH 7). Kinetic modelling indicated that O_3 reacted with TRI and FLX via electrophilic attack at their phenol and neutral amine moieties, respectively. Afterwards, TRI and FLX oxidation during ozonation of secondary effluent samples from two conventional activated sludge treatment plants was also investigated. TRI was oxidized with relatively high efficiency during wastewater ozonation, due to its high reactivity toward O_3. Nearly 100% TRI depletion was achieved for a 4 mg/L ($8.3 \cdot 10^{-5}$ mol/L) O_3 dose applied to a wastewater containing 7.5 mg/L of DOC, and ~58% TRI depletion for dosage of 6 mg/L ($1.3 \cdot 10^{-4}$ mol/L) O_3 to a wastewater containing 12.4 mg/L of DOC. Fluoxetine transformation was less efficient, due to its low reactivity toward O_3 at the circumneutral pH. Consequently, FLX loss could be followed as a function of time, which confirmed that k_{O3} values determined in pure waters could be used to model FLX oxidation in wastewater.

Chapter 1

Introduction[1]

Summary

The presence of bioactive micropollutants such as Pharmaceuticals and Personal Care Products (PPCPs) in different environmental compartments (rivers, lakes, groundwaters, sediments, etc.) is an emerging issue due to the lack of existing information about the potential impact derived from their occurrence, fate and ecotoxicological effects. Due to their low concentrations reported in wastewaters (ppb or ppt) and the complex structure of some of them, common technologies used in Sewage and Drinking Water Treatment Plants (STPs and DWTPs, respectively) may not be efficient enough to accomplish their removal. Information about physico-chemical characteristics such as acidity, lipophilicity, volatility and sorption potential is a useful tool to understand the different removal patterns observed. In order to perform an accurate overall mass balance along the different units of a given STP, it is necessary to gather information not only about the presence of micropollutants in the aqueous phase, but also on the fraction sorbed onto solids. Since only some PPCPs are very efficiently eliminated in common STP configurations, it is necessary to look for new strategies in order to enhance their removal, including modification of operating conditions (e.g. solids retention time), implementation of new technologies (e.g. membrane bioreactors) or incorporation advanced post-treatment steps (e.g. oxidation, adsorption, membrane filtration).

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

Suarez, S., Carballa, M., Omil, F., Lema, J.M. (2008) How are pharmaceutical and personal care products (PPCPs) removed from urban wastewaters. Reviews in Environmental Science and Bio/Technology. Published on-line http://www.springerlink.com/content/x2p2g6j025733352/?p=fcfafe6831c94fddae6f124ee2815a9e&pi=3

Outline

1.1. The concern about PPCPs in the environment

Pharmaceuticals and Personal Care Products (PPCPs) constitute a group of a wide number of compounds largely consumed in modern societies which, until recently, have not been of major concern with regard to their environmental effects. When these substances are freely discharged into the environment, they could cause some impact on aquatic and terrestrial organisms, since they have been specifically designed to produce biological effects even at very low concentrations. In addition, some of them are bioaccumulative. After intake, drugs are generally absorbed by the organism and further subjected to metabolic reactions, where the chemical structure of the active molecule is modified. Two main pathways can be distinguished during metabolism: i) phase I, where hydrolysis, oxidation, reduction, alkylation and dealkylation reactions occur; and ii) phase II, where conjugates, mainly glucuronides and sulfonates, are formed in order to enhance excretion. However, a significant fraction of the parent compound leaves human or animal organisms unmetabolised via urine or faeces.

Because of the large variety of chemical structures of these micropollutants and the very low concentrations at which they are present in the environment (µg/L or ng/L), a considerable effort is being made in order to develop methodologies to quantify and assess their occurrence, chemical properties and degradability potential. The analytical methods are based on advanced chromatography (GC or LC) coupled to mass spectrometry (MS/MS), thus being very costly, time consuming and requiring a high expertise. These methods have been principally applied to wastewater (Ternes, 1998; Kanda et al., 2003; Fahlenkamp et al., 2004; Johnson et al., 2005), but also to surface or groundwater (Boyd et al., 2003; Drewes et al., 2003).

PPCPs, coming either from domestic sewage, hospital wastewaters or industrial discharges, reach STP influents. During the treatment in STPs, a distribution between the dissolved and the solid fraction, including primary and secondary sludge, will take place. This partition is especially relevant for the most lipophilic compounds. Therefore, the release of non-degraded PPCPs into the environment will occur with the final effluent of the plant, as well as with the excess sludge, which has been reported to contain pharmaceutical substances (Khan and Ongerth, 2002; Kupper et al., 2004; Kinney et al., 2006). If sewage sludge is disposed of on agricultural lands, PPCP pollution will not only reach surface water, but also groundwater (Figure 1-1).

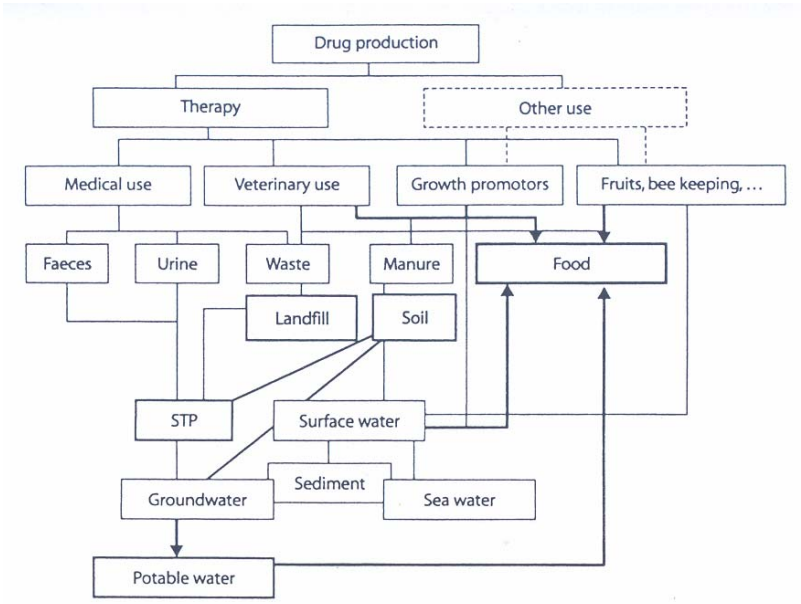


Figure 1-1. Sources and distribution of PPCPs in the environment (Kümmerer, 2004).

Drugs and their metabolites can enter water supplies and even the food chain, which gives rise to concern and leads to an increasing amount of research focusing on risk assessment in order to evaluate their possible impact both on the ecosystem and even on human health (Flippin et al., 2007; Balk and Ford, 1999; Stuer-Lauridsen et al., 2000; Pomati et al., 2006; Liebig et al., 2006; Jones et al., 2002; Lindberg et al., 2007). In order to perform accurate risk assessments, environmental concentrations in the different compartments, as well as the effect that PPCPs could exert onto exposed organisms, have to be determined. From the data available concerning toxicity of PPCPs (Henry et al., 2004; Schreurs et al., 2004; Flippin et al., 2007; Hong et al., 2007) acute effects on aquatic organisms are not expected, except for spills, while very little information is available about chronic exposures, in particular with respect to biological targets (Fent et al., 2006). This lack of knowledge should be overcome in the coming years by assessing long-term effects in order to determine the possible damages on aquatic organisms exposed to PPCPs via wastewater discharges during their whole life.

In general, chronic toxicity studies found in the current literature indicate that the Lowest Observed Effect concentrations (LOEC) in standard laboratory organisms are around two orders of magnitude higher than concentrations found in STP effluents (Fent et al., 2006), thus indicating little risk for aquatic organisms in surface water. For example, ibuprofen has shown to alter the pattern of Japanese

medaka reproduction when exposed to levels in the range of 1-100 µg/L (Flippin et al., 2007), chronic toxicity of Selective Serotonin Reuptake Inhibitors (SSRIs) has been characterised by a Non Observable Effect Concentration (NOEC) for daphnids of 9 µg/L (Henry et al., 2004), and for diclofenac adverse effects (cellular toxicity and estrogenic effects) on Japanese medaka were detected at 1 µg/L (Hong et al., 2007). In the case of antibiotics, the main concern about their release into natural waters is related to the potential for development of microbial resistance to these antibiotics, which means that an increasing number of infections could no longer be treated with the current medicines. In this sense sulfonamide- and trimethoprim-resistant bacteria have been identified in rivers in the U.S. (Lindberg et al., 2005), and in the case of STP effluents more than 70% of the bacteria have manifested an insensitivity against at least one antibiotic, including penicillin, bacitracin, tetracycline or erythromycin (Hirsch et al., 1999).

Regarding risk assessment, a particular emphasis should be paid to those PPCPs that affect the nervous or endocrine system, as well as to compounds that can bio-accumulate in the aquatic biota. This is the case of the fragrances galaxolide and tonalide, whose concentration measured in fish was 600 times higher than the nominal test doses applied (Schreurs et al., 2004). Moreover, the complex mixtures that exist in the environment, which may produce synergistic effects, should also be considered.

There was no regulation concerning the risk associated to pharmaceuticals in the environment until recently. The European Commission released a draft guideline (Directive 2001/83/EC) specifying that an authorization for a medicinal product for human use must be accompanied by an environmental risk assessment (EMEA, 2005). On the other hand, in the U.S.A. an environmental assessment report has to be provided in those cases where the pharmaceutical concentration in the aquatic environment is ≥1µg/L (FDA-CDER, 1998).

1.2. Selection of compounds

The complete list of PPCPs selected for the present work included pharmaceuticals from five different therapeutic classes (antibiotics, anti-depressants, anti-inflammatory drugs, tranquilizers and anti-epileptics), hormones including the two natural estrogens E1 and E2 and the synthetic hormone used in contraceptive drugs (EE2), one X-ray contrast media (IPM), and as cosmetic ingredients three polycyclic musk fragrances have been selected (Table 1-1).

Table 1-1. Therapeutic class, CAS, chemical structure and consumption rates for selected PPCPs.

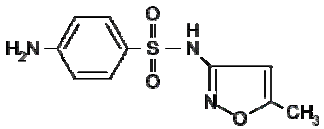
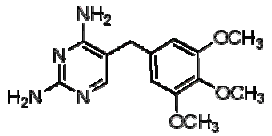
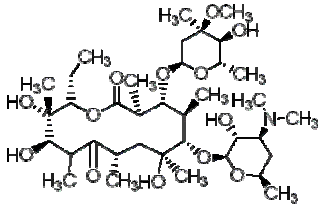
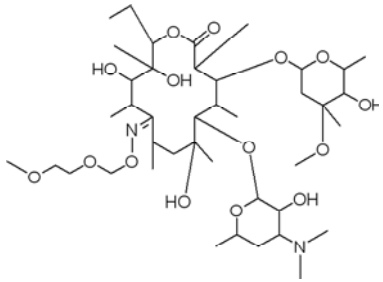
Therapeutic class	Compound	CAS	Formula	Structure	Consumption (g/capita.year)
Antibiotics	Sulfamethoxazole (SMX)	723-46-6	$C_{10}H_{11}N_3O_3S$		0.12 Austria (1999) 0.93 Germany (1995) 0.07 Spain (2006)
	Trimethoprim (TMP)	738-70-5	$C_{14}H_{18}N_4O_3$		0.07 Switzerland (1999) 0.18 Germany (1995) 0.03 Spain (2006)
	Erythromycin (ERY)	114-07-8	$C_{37}H_{67}NO_{13}$		0.03 Switzerland (1999) 1.15 UK (2000) 0.06 Spain (2006)
	Roxithromycin (ROX)	80214-83-1	$C_{41}H_{76}N_2O_{15}$		0.02 Switzerland (1999) 0.07 Germany (1995) 0.002 Spain (2006)

Table 1-1. continues

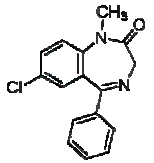
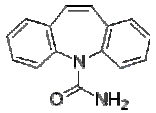
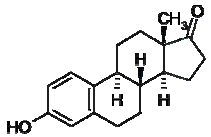
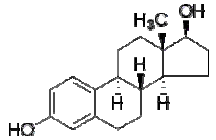
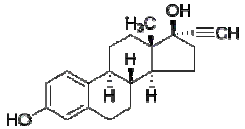
Therapeutic class	Compound	CAS	Formula	Structure	Consumption (t/year)
Tranquilizer	Diazepam (DZP)	439-14-5	$C_{16}H_{13}ClN_2O$		0.015 Austria (1999) 0.016 UK (2000) 0.018 Spain (2006)
Anti-epileptic	Carbamazepine (CBZ)	298-46-4	$C_{15}H_{12}N_2O$		0.07 Switzerland (2004) 1.07 Germany (2000) 0.34 Spain (2006)
Hormones	Estrone (E1)	53-16-7	$C_{18}H_{22}O_2$		--
	17β-Estradiol (E2)	50-28-2	$C_{18}H_{24}O_2$		--
	17α-Ethinylestradiol (EE2)	57-63-6	$C_{20}H_{24}O_2$		5.10^{-4} UK (2000) $1.7.10^{-5}$ Spain (2006)

Table 1-1. continues

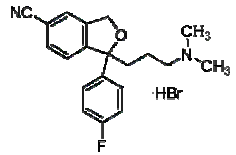
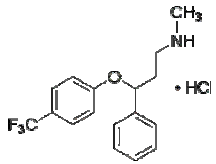
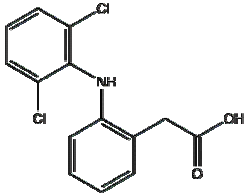
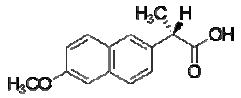
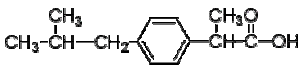
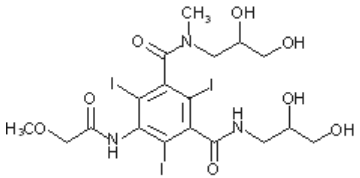
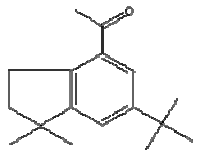
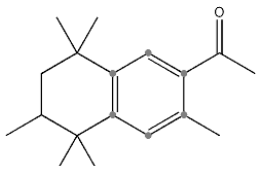
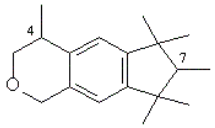
Therapeutic class	Compound	CAS	Formula	Structure	Consumption (t/year)
Anti-depressants	Citalopram (CTL)	059729-33-8	$C_{20}H_{21}FN_2O$		0.07 Denmark (1997) 0.03 Spain (2006)
	Fluoxetine (FLX)	054910-89-3	$C_{17}H_{18}F_3NO$		0.03 UK (2000) 0.08 Spain (2006)
Anti-inflammatory	Diclofenac (DCF)	15307-86-5	$C_{14}H_{11}Cl_2NO_2$		0.23 Switzerland (2004) 1.05 Germany (2001) 0.53 Spain (2006)
	Naproxen (NPX)	22204-53-1	$C_{14}H_{14}O_3$		0.23 Switzerland (2004) 1.03 UK (2000) 0.54 Spain (2006)
	Ibuprofen (IBP)	15687-21-1	$C_{13}H_{18}O_2$		0.03 Italy (2001) 4.21 Germany (2001) 4.57 Spain (2006)

Table 1-1. continues

Therapeutic class	Compound	CAS	Formula	Structure	Consumption (t/year)
Contrast media	Iopromide (IPM)	73334-07-3	$C_{18}H_{24}I_3N_3O_8$		0.67 Austria (2003) 0.79 Germany (1999) 0.11 Spain (2006)
Fragrances	Celestolide (ADBI)	13171-00-1	$C_{17}H_{24}O$		<0.03 Europe (2000)
	Tonalide (AHTN)	1506-02-1	$C_{18}H_{26}O$		0.49 Europe (2000)
	Galaxolide (HHCB)	1222-05-5	$C_{18}H_{26}O$		1.95 Europe (2000)

Hirsch et al. (1999); Stuer-Lauridsen et al. (2000) ; Beausse (2004); Webb (2004); Clara et al. (2005b); Gobel et al. (2005); Fent et al. (2006); Kupper et al. (2006); Suarez (2007)

Table 1-2. Physico-chemical properties (ordered by acidity) and biodegradability kinetics of selected PPCPs.

PPCP	s	H	pKa	log K_{ow}	log K_d			k_{biol}
					Primary	*Biological*	*Digested*	
DZP	50	$1.5 \cdot 10^{-7}$	3.3-3.4	2.8-3	1.6	1.3	-	~0.02
DCF	2.4	$1.9 \cdot 10^{-10}$	4.1 - 4.2	4.5	2.7	1.2	1.7	< 0.1
NPX	16	$1.4 \cdot 10^{-8}$	4.2	3.2	-	1.1	1.3 - 1.4	0.4 – 1.9
IBP	21	$6.1 \cdot 10^{-6}$	4.9 - 5.2	3.1 - 4.0	< 1.3	0.9	1.4	9 – 35
SMX	610	$2.6 \cdot 10^{-11}$	1.8+5.2	0.9	-	2.4	1.2 - 1.4	< 0.1
TMP	400	$9.8 \cdot 10^{-13}$	6.6 - 7.2	0.9-1.4	-	2.3	-	-
CBZ	17.7	$4.4 \cdot 10^{-9}$	7	2.4 - 2.9	< 1.3	0.1	1.5 - 1.7	< 0.01
ERY	1.4	$2.2 \cdot 10^{-27}$	8.9	2.5 – 3.0	-	2.2	-	0.5 - 1
ROX	0.02	$2.0 \cdot 10^{-29}$	9.2	2.8	-	2.2	1.5 - 1.9	< 0.3
CTL	31	$1.1 \cdot 10^{-9}$	9.6	2.9 - 3.7	-	2.0	-	-
FLX	60	$3.6 \cdot 10^{-6}$	10.1	1.6	-	0.7	-	-
E1	30	$1.6 \cdot 10^{-8}$	10.4	3.1 -4	-	2.4	2.4 - 2.6	200 - 300
E2	3.6	$1.5 \cdot 10^{-9}$	10.4	3.9 - 4.0	-	2.8	2.3 - 2.5	300 - 800
EE2	11.3	$3.3 \cdot 10^{-10}$	10.5 - 10.7	3.7 - 4.0	2.4	2.5	2.3 - 2.6	7-9
IPM	23.8	$4.1 \cdot 10^{-27}$	-	- 2	< 0.7	1.0	1.0 - 1.2	1 – 2.5
ADBI	0.22	$7.3 \cdot 10^{-1}$	-	5.4 - 6.6	3.7	3.9	-	-
AHTN	1.2	$5.1 \cdot 10^{-3}$	-	5.7	3.7	3.4	3.9 - 4.2	<0.02
HHCB	1.8	$5.4 \cdot 10^{-3}$	-	5.9	3.7	3.3	3.9 - 4.1	<0.03

s: solubility in water ($mg \cdot L^{-1}$); H: Henry coefficient ($\mu g \cdot m^{-3}$ air/$\mu g \cdot m^{-3}$ wastewater); pKa: dissociation constant; K_{ow}: octanol-water partition coefficient; K_d: solid-water distribution coefficient; k_{biol}: pseudo first-order degradation constant ($L \cdot gSS^{-1} \cdot d^{-1}$). Syracuse Research Corporation (SRC); Stuer-Lauridsen et al., 2000; Jones et al., 2002; Brooks et al., 2003; Ricking et al., 2003; Kummerer, 2004; Ternes et al., 2004; Theiss, 2004; Jjemba, 2006; Kupper et al.,2006; Ternes and Joss, 2006; Vasskog et al., 2006; Carballa et al., 2007d.

The selection of the compounds considered in this Thesis has been based on several criteria: i) A wide range of commonly prescribed therapeutics classes should be represented; ii) For each therapeutic class the most used pharmaceuticals have been selected. In fact, most of the selected pharmaceuticals are included in the top 200 RxList of the FDA, while ibuprofen (IBP), diazepam (DZP) and citalopram (CTL) were in between the 25 most used pharmaceuticals in Denmark (Stuer-Lauridsen et al., 2000), and erythromycin (ERY), trimethoprim (TMP), diclofenac (DCF), IBP, fluoxetine (FLX), CTL and DZP were among the top prescribed antibacterial, analgesic and mental health drugs for the UK (NHS, 2005); iii) Drugs that arise special concern about their effects on aquatic organisms, such as anti-depressants, hormones and antibiotics have been considered; iv) Compounds with different physico-chemical properties have been selected (Table 1-2) in order to analyse their influence during wastewater treatment: v) The existence of analytical methods that ensured a sensitive and reliable detection of the compounds during the different experiments.

Knowledge of the physico-chemical properties of PPCPs is crucial for a clearer analysis of the complex processes that can occur during their passage through STPs. Table 1-2 shows the properties for the selected substances, which will be used to elucidate the main removal mechanisms involved in the different treatment techniques considered in chapters 4 to 6 of the present work.

1.3. Removal mechanisms

The possible removal mechanisms of PPCPs in STPs include volatilisation, sorption to solids and biological and chemical transformation.

1.3.1. Sorption

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

$$K_d = \frac{C_{sorbed}}{SS \cdot C_{dissolved}} \qquad \text{[Eq. 1-1]}$$

where C_{sorbed} is the sorbed PPCP concentration onto sludge (µg/L), $C_{dissolved}$ the dissolved concentration of the compound (µg/L) and SS the suspended solids concentration (kg/L).

This coefficient takes into account the two main sorption mechanisms (Ternes et al., 2004):

- *Absorption*, which refers to the interactions of the aliphatic and aromatic groups of a compound with the lipophilic cell membrane of the

microorganisms and with the lipid fractions of the sludge. It is related to the substance's lipophilicity, characterized by the octanol-water partition coefficient (K_{ow}).

- *Adsorption*, which refers to the physical adherence or binding of ions and molecules onto the surface of another molecule. It is related 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 acidic compounds dissociated at circumneutral pH (e.g. naproxen), will not adsorb, whereas positively charged substances (e.g. cationic carbamazepine) will be more favoured due to Van der Waals-type interactions.

Therefore, the sorption potential of PPCPS is a function of both, their lipophilic character (K_{ow}) and acid dissociation constant (pKa). The following cases studies illustrate the effect of these phenomena:

- Fragrances belong to the substances listed in Table 1-2 with the lowest solubilities in water (< 2 mg/L), being this characteristic reinforced by their strong lipophilic character as indicated by the high log K_{ow} values (4.6 – 6.6). Both issues explain their high log K_d values (3.3–4.2) based on absorption.
- The case of the hormones included in Table 1-2 is similar to that of fragrances, although the interaction with sludge will be weaker, since they are less hydrophobic (log K_{ow} of 3.1–4.0) and have consequently lower sorption coefficients (log K_d of 2.3-2.8).
- The sorption capacity of the antibiotic trimethoprim (TMP) is similar to that of the previously cited hormones, although in this case the interaction with sludge is mainly driven by adsorption, since this compound is not lipophilic, but at circumneutral pH the dicationic species of TMP supposes about 50% of the total TMP concentration.

Experimental data on PPCPs concentrations in sludge are very scarce, probably due to the difficulties of solid samples analysis. To overcome this problem, the use of K_d values appears to be a useful tool to predict the distribution between both phases. However, an accurate determination of this coefficient is required, because relative small deviations in its value can lead to quite different conclusions concerning the removal mechanism responsible for the elimination of a particular compound (Carballa et al., 2007a).

1.3.2. Volatilisation

The fraction of compound volatilized in the aeration tank (ϕ) depends on the flow of air getting in contact with wastewater (q_{air}, in m^3 air/m^3 wastewater), type of aeration and Henry coefficient (H, in $\mu g \cdot m^{-3}$air/$\mu g \cdot m^{-3}$wastewater), as shown in Equation 1-2.

$$\phi = \frac{C_{dissolved} \cdot H \cdot q_{air}}{C_{dissolved} + C_{dissolved} \cdot H \cdot q_{air} \cdot C_{dissolved} + K_d \cdot SS} = \frac{H \cdot q_{air}}{1 + H \cdot q_{air} + K_d \cdot SS} \qquad \text{[Eq. 1-2]}$$

Taking into account the typical air flow rates used in a Conventional Activated Sludge (CAS) systems (5-15 m^3 air/m^3 wastewater), as well as the Henry coefficient for the different PPCPs (Table 1-2), losses due to stripping are completely negligible for all selected pharmaceuticals and estrogens, almost negligible for AHTN and HHCB and only significant for ADBI.

1.3.3. Biological transformation

Although the microbiota developed in STPs may have been exposed to many micropollutants for a long time, the effective biological removal of these substances is conditioned by singular factors. The concentration of micropollutants in municipal wastewater is around 5 orders of magnitude below its Chemical Oxygen Demand (COD), thus biological degradation most probably occurs by co-metabolism. Moreover, the chemical structure of some PPCPs is very complex and strong, as for example in the case of the X-ray contrast media iopromide, which has been designed for remaining unaltered during its application and is thus mainly excreted unchanged (Bourin et al., 1997).

There are few studies focused specifically on biological degradation of PPCPs. Because of the low concentrations of these trace pollutants, the depletion can be described as first-order reaction. In fact, Joss et al. (2006) have determined pseudo first-order degradation kinetics (k_{biol}) for a large number of compounds. They performed batch experiments for 48 h with fixed biomass concentrations (0.5 g VSS/L), where the selected PPCPs were spiked at a concentration of 3 µg/L. According to these degradation constant values, three groups of compounds can be differentiated: i) hardly biodegradable, with k_{biol} < 0.1 L/g SS·d; ii) highly biodegradable, with k_{biol} > 10 L/g SS·d; and, iii) moderate biodegradable with 0.1 < k_{biol} < 10 L L/g SS·d.

In any case, these degradation constants should not be taken as fixed values, since the biodegradability of PPCPs can be influenced by different factors, such as the type or adaptation of the sludge involved in the treatment (chapter 4 and 5).

1.4. Fate of PPCPs in Sewage Treatment Plants

1.4.1. Overall removal

Modern STPs can effectively accomplish carbon and nitrogen removal, as well as microbial pollution control. However, these installations receive also a large number of different trace polluting compounds, such as PPCPs, for which conventional treatment technologies have not been specifically designed.

The reported overall removal rates of PPCPs in full-scale STPs vary strongly and they clearly show that their elimination is often incomplete (Table 1-3). As a consequence, a significant fraction is discharged with the final effluent into the aquatic environment or sorbed onto the primary and secondary sludge, whose deposition on land can be another significant pathway of releasing these substances in the environment.

Table 1-3. Overall removal efficiencies for PPCPs in STPs.
(++) High (+) Medium (-) None

PPCP	Removal efficiency (%)	Sorption	Biodegradation
HHCB	64 – 85	++	+
AHTN	63 – 90	++	+
ADBI	~80	++	+
DCF	59 – 75	+	-/+
E1	(-80) – 99	+	++
E2	30 – 100	+	++
EE2	(-18) – 98	+	+
IBP	60 – 95	-	++
CBZ	0 – 45	-	-

Ternes, 1998; Stumpf et al.,1999; Ternes et al., 1999b; Baronti el al., 2000; Bester, 2004; Carballa et al., 2004; de Mes et al., 2005; Kupper et al., 2006; Nakada et al., 2006; Gómez et al., 2007.

Most of these studies report removal of the parent compounds from the aqueous phase by comparing influent and effluent concentrations, without distinguishing between the three major fates of a substance in STPs: a) degradation to lower molecular weight compounds, b) physical sequestration by solids (and subsequent removal as sludge), and c) hydrolysis of conjugates yielding the parent compound. Therefore, by simply following disappearance of a substance from the liquid phase it is not possible to conclude if it was structurally altered.

From Table 1-3, it can be seen that CBZ is a persistent substance since it is neither subject to degradation nor to sorption. Reported overall removal efficiencies

of DCF, IBP and fragrances are quite similar for several STPs all over the world (Germany, Brazil, Spain, Switzerland, Japan, etc.), thus indicating that the specific configuration of each plant seems not to be a relevant parameter affecting the elimination of these compounds. Concerning hormones, the reported removal efficiencies of E1, E2 and EE2 vary strongly between studies. Different behaviours have been observed: i) an increase along the passage through the STP (Baronti et al., 2000; Carballa et al., 2004); ii) no significant removal (Ternes et al., 1999a); and, iii) efficiencies higher than 80% (Ternes et al., 1999b; de Mes et al., 2005; Nakada et al., 2006). Although it is not fully elucidated which factors could explain these deviations, since in many cases there are not enough operational data reported, some observations can be underlined: i) the temperature of the process can influence the removal efficiency achieved, according to enhanced microbial activities at higher temperatures. This could be the factor that explained the huge differences (of up to 80% in the case of E1 and EE2) between the absolute removal of estrogens measured in a German and a Brazilian STP in Ternes et al. (1999b), where the average temperature during the sampling was -2ºC and above 20ºC, respectively; ii) different kinetic behaviours (k_{biol} in Table 1-2), since E2 is almost completely oxidized to E1 in less than 3 hours, the further oxidation of E1 is slower (50% after 24 hours) and EE2 is not appreciable removed even after 48 hours (Ternes et al., 1999a). Therefore, a minimum Hydraulic Retention Time (HRT) is needed to accomplish the complete removal of hormones; and, iii) discrepancies related to the conjugated fractions present in the raw influent of STPs, since it is not clear where deconjugation occurs. The general pattern assumed is that glucuronides are mainly cleaved in the sewer system, while sulphonates remain unaltered until primary treatment.

1.4.2. Primary treatment

Primary treatment comprises the removal of suspended solids and fat using sedimentation and flotation units. Therefore, although some degradation can also occur, sorption is the main mechanism involved in the removal of chemicals during primary treatment, and consequently, only those substances with sorption potential (Table 1-2) are prone to be eliminated.

Accordingly, highly lipophilic musk fragrances (log K_d ~ 3.7) were in general removed to a significant extent, namely 30-50% (Carballa et al., 2004), 15-51% (Simonich et al., 2002) and 0-40% (Artola-Garicano et al., 2003), whereas hydrophilic pharmaceuticals (carbamazepine and ibuprofen) remain unaltered (log K_d < 1.3). Moreover, an increase in the concentrations at the inlet of the primary clarifier compared to the raw influent of the STP is sometimes observed, indicating a potential contribution of the supernatants from the sludge treatment processes (Carballa et al., 2004; Khan and Ongerth, 2004) to the concentration of fragrances.

Concerning estrogens, little removal (7% for E1 and 0% for E2) was observed at an enhanced (with $FeCl_3$ addition) primary STP in Australia (Braga et al., 2005). In contrast, Matsui et al. (2000) stated that estrogenic activity decreased along the treatment, with a small reduction of 10-15% during primary treatment. In a Spanish STP (Carballa et al., 2004), 17β-estradiol was partially eliminated (around 20%), whereas in a German STP, EE2 and E2 were eliminated during primary treatment, up to 35 and 29%, respectively (Andersen et al., 2003).

Two processes are expected to be responsible for the fate of estrogens during primary treatment: cleavage of conjugates together with the contribution of supernatants from sludge processes will increase their concentrations after primary treatment (Andersen et al., 2003; Carballa et al., 2004), whereas their quite high log K_d values (~2.5) suggest that sorption could take place in appreciable extent. Therefore, the overall fate of estrogens during primary treatment will depend on several factors, such as sewer configuration (affecting the fraction of deconjugated compounds arriving at STPs), wastewater characteristics (mainly solids content and hydrolytic enzymatic activity) and environmental parameters (pH, temperature).

Some modifications can be implemented in STPs in order to improve solids and fat separation, such as the use of chemical additives, and, consequently, enhance removal of substances with high sorption properties. The basis is that natural PPCPs-particles partitioning can be influenced by the presence of other substances in the medium or modified by the addition of some chemicals (coagulants, flocculants, tensoactives, etc.).

Carballa et al. (2005) showed that the use of coagulants (ferric and aluminium salts) improves the removal of substances with high sorption properties, such as musk fragrances and diclofenac up to 50-70%, which is related to the increased solids separation. Besides, the presence of trivalent cations could enhance the elimination of acidic compounds (e.g. naproxen) by ionic or quelating interactions. In laboratory-scale flotation units, musks have shown to be removed to a greater degree (35–60%), followed by diclofenac (20–45%) and, to a lesser extent, carbamazepine (20–35%) and ibuprofen (10–25%). Wastewaters with high fat content proved to have a positive effect on musks and neutral compounds removal (Carballa et al., 2005).

1.4.3. Biological treatment

Parameters influencing the removal of PPCPs

The widest used biological treatment technology in large urban areas is CAS, operating at HRT of 4-24 h. Many CAS plants operate exclusively under aerobic conditions, although a number of installations were upgraded in order to include also anoxic zones for nutrient removal. More recently, membrane technology has been incorporated to biological treatment in order to substitute secondary settlers. The biomass developed in such systems is characterized by higher sludge retention

times (SRT) and smaller floc size, which might influence the removal efficiency of micropollutants. Up to the present, research regarding the removal of PPCPs in Membrane Bioreactors (MBR) is limited to lab- and pilot-scale plants and, if operated at similar SRT, generally no difference regarding the removal of PPCPs has been observed with respect to CAS plants (Clara et al., 2005a; Joss et al., 2005).

The vast majority of data published in the field of PPCPs removal from wastewater refer to full-scale STPs, where only the raw influent and final effluent is sampled in order to measure the soluble concentration of the considered PPCP. Therefore, only the overall removal efficiency including primary and secondary treatment can be determined. Some authors considered different sampling points in full-scale STPs allowing to distinguish the removal efficiency of the primary and secondary treatment step (Carballa et al., 2004; Kupper et al., 2006), while others performed the sampling in the influent and the effluent of the biological reactor (Joss et al., 2004; 2005; Jones et al., 2007). Additional information about the behaviour of PPCPs in biological reactors can be obtained from experiments carried out in lab- and pilot-scale plants (Zwiener et al., 2000; Clara et al., 2004; Joss et al., 2004; Clara et al., 2005a; Joss et al., 2005; Suarez et al., 2005). More detailed studies considering the different removal mechanisms for PPCPs are less frequent, although there are some works dealing with the importance of sorption and volatilisation (Bester, 2004; Joss et al., 2004; Clara et al., 2005a; Joss et al., 2005; Kupper et al., 2006).

There are evidences that some operating parameters such as HRT, SRT, redox conditions and temperature may affect PPCPs removal. HRT was shown to affect elimination of ibuprofen and ketoprofen (Tauxe-Wuersch et al., 2005), in a way that lower removal was observed for shorter HRT. This effect was also observed during heavy rain periods, when rainwater caused the decrease of HRT (Ternes, 1998). Concerning SRT, increased values have shown to improve removal for most PPCPs (Clara et al., 2005a), although beyond 25-30 d this parameter is not significant anymore. Regarding redox conditions, different removal efficiencies have been observed for anaerobic, anoxic and aerobic conditions (Joss et al., 2004). Finally, operating at higher temperatures may also influence the removal of PPCPs in a positive way, as shown for example in Ternes et al. (1999b) when comparing the efficiencies obtained for the removal of estrogens in a German and a Brazilian STP. Nitrification in the aerobic tank appears to be positive for EE2 removal (Vader et al., 2000), as well as operating at higher temperatures (Ternes et al., 1999b).

Case studies

Fragrances illustrate the coexistence of the three mechanisms involved in their removal: volatilisation, sorption and biodegradation. Volatilisation in aeration tanks represents a minor removal pathway in the case of HHCB and AHTN (< 5%), whereas the fraction of ADBI lost by volatilisation could account for a 25 % when an

aeration flow of 10 m^3 air/m^3 wastewater and a biomass concentration of 2 g SS/L is applied (Equation 1-2). Due to their strong lipophilic character, sorption onto sludge will be very significant. In fact, it has been shown that for AHTN sorption was the only mechanism responsible for its removal (Bester, 2004; Joss et al., 2005), although Kupper et al. (2006) associated 43% of the depletion observed to degradation. In the case of HHCB, a certain biological degradation was observed (16-50%) and partially confirmed by the detection of one metabolite, HHCB-lactone (Bester, 2004; Joss et al., 2005; Kupper et al., 2006). The third musk considered, ADBI, showed a similar behaviour as the other two in Kupper et al. (2006), although these data could not be confirmed by other works due to lack of available literature. Taking into account all these mechanisms, musk removals from the liquid phase in the range of 50-75% were reported (Carballa et al., 2004; Joss et al., 2005; Kupper et al., 2006).

The acidic compounds ibuprofen and diclofenac show a different behaviour. Although both have a low affinity for solids, they differ in their biodegradation kinetic coefficients (Table 1-2). Ibuprofen exhibits high values, in the range of 9-35 L/g SS·d (Joss et al., 2006), whereas the biodegradability of diclofenac is very low (< 0.1 L/g SS·d). High removal of ibuprofen (>90%) has been confirmed by Suarez et al. (2005), Joss et al. (2005) and Jones et al. (2007), although there are also some lower eliminations reported (50-70%, according to Carballa et al., 2004; Zwiener et al., 2000). On the other hand, Suarez et al. (2005), Joss et al. (2005) and Clara et al. (2005a) confirmed the low biodegradation of diclofenac. The higher overall removal efficiencies reported in Table 1-3 might be partially attributed to the elimination of sludge during the primary treatment (log K_d 2.7), but also to an enhanced sorption to sludge during secondary treatment upon the addition of inorganic salts for phosphorus precipitation (Ternes, 1998; Clara et al., 2005a). Removal of DCF could be significantly improved by favouring the development of nitrifying biomass, as indicated in chapter 5 of this Thesis.

Removal efficiencies reported for E1, E2 and EE2 in CAS plants are in the range of 49-99%, 88-98% and 71-94%, respectively (Andersen et al., 2003; Joss et al., 2004). Redox conditions seem to influence their removal, since most of the elimination of E1 and E2 was reported to already occur in the denitrifying step of a STP, whereas EE2 depletion was only observed during the aerobic process (Andersen et al., 2003). These observations were confirmed by batch experiments, showing that: i) degradation of E1 and E2 takes place in anaerobic, anoxic and aerobic environments, but at significant different rates (Joss et al., 2004); ii) oxidation of E2 is faster than of E1 (Table 1-2); and, iii) EE2 was only significantly removed under aerobic conditions and at slower rates than natural estrogens (Table 1-2). The sorption potential of estrogens, according to their K_d (Table 1-2) is in between that of fragrances, which are the most lipophilic compounds from the selected list, and that of the most acidic compounds (DZP, DCF, NPX and IBP). A

rough estimation indicates that for a CAS system operating with 2 g SS/L, 83% of these compounds are present onto sludge. This enhanced retention of estrogens in the aeration tank facilitates their degradation and, consequently, the fraction of estrogens present in the purged sludge is almost negligible (<10% according to Andersen et al., 2003 and Joss et al., 2004).

Summarizing, in the biological treatment, the following case studies can be distinguished according to the k_{biol} and K_d values (Table 1-2) of a particular compound:

- Compounds with high k_{biol} and low K_d values, such as ibuprofen, are very well transformed independently of SRT and HRT.
- Compounds with low k_{biol} and high K_d values, such as musk fragrances, are retained in the aeration tank by sorption and significantly transformed when the SRT is long enough to accomplish biological degradation.
- Compounds with high k_{biol} and medium K_d values, such as natural estrogens, are moderately transformed independently of HRT and slightly dependant on SRT.
- Compounds with low k_{biol} and K_d values, such as carbamazepine, are not removed nor biotransformed regardless HRT and SRT.

Table 1-4. Factors affecting removal in biological treatment.

	HRT	SRT	Example
$k_{biol}\downarrow$ $K_d\downarrow$	-	-	CBZ, DZP
$k_{biol}\downarrow$ $K_d\uparrow$	-	+	HHCB
$K_{biol}\uparrow$ $K_d\downarrow$	-	-	IBU
$K_{biol}\downarrow\uparrow$ $K_d\downarrow\uparrow$	+/-	+/-	EE2
$k_{biol}\uparrow$ $K_d\downarrow\uparrow$	-	+/-	E1, E2

1.4.4. Sludge treatment

As stated previously, some micropollutants are sorbed onto sludge during wastewater treatment. The behaviour of PPCPs during sludge anaerobic digestion is not clear and even contradictory according to literature. Some authors indicate that PPCPs exhibit some resistance to anaerobic biodegradation. For example, Khan and Ongerth (2002) stated that most PPCPs persist in the aqueous fraction of digested sludge. Andersen et al. (2003) detected similar inlet and outlet loads of estrogens in an anaerobic digester, concluding that estrogens were not degraded appreciably under methanogenic conditions. Matsui et al. (2000) observed that 17β-estradiol concentrations and estrogen activity of the dewatering liquid from the sludge

treatment were higher than those of the influent to the plant. Johnson and Williams (2004) reported that strictly anaerobic desulphating strains are capable of cleaving estrone-3-sulphate and 17β-estradiol-3-sulphate, thus increasing their concentrations during this step. In contrast, other authors reported the opposite. For example, Holbrook et al. (2002) found that between 51% and 67% of the estrogenic activity contained in the influent wastewater was either eliminated during the wastewater or biosolids treatment processes. Kreuzinger et al. (2004) indicated that anaerobic digestion accelerates the breakdown of natural estrogens. There are also some indications of AHTN and HHCB degradation (around 40%) during sludge digestion (Van de Plassche and Balk, 1997). Carballa et al. (2007b) studied the behaviour of several PPCPs during anaerobic digestion of sewage sludge under mesophilic and thermophilic conditions at different SRT. The highest removal efficiencies were achieved for musks and the natural estrogens (50-95%), while for other compounds (eg. Ibuprofen), the values ranged between 20 and 60%, except for carbamazepine, which showed no elimination.

The main factors which could affect anaerobic biotransformation are biomass adaptation, SRT, temperature and pretreatment. For example, in Carballa et al. (2007c), DCF was removed by 80% after an initial period of sludge adaptation, whereas no influence of SRT and temperature on PPCPs removal was in general observed. The use of pre-treatments (alkaline, thermal and ozonation) was considered in Carballa et al. (2006 and 2007b), where only a minor impact on the removal was observed, leading only the ozonation process to some removal of carbamazepine (up to 60% in thermophilic range) in comparison with the absence of elimination in the conventional process.

1.4.5. Post-treatment

Post-treatment techniques, such as ozonation, membrane filtration and sorption on activated carbon may be effective for completing the removal of the most recalcitrant PPCPs, although it would be also costly to implement, with estimated costs in the range of 0.01-0.04 €/m^3 for ozonation and one order of magnitude higher for the other two techniques (Ternes and Joss, 2006).

Ozonation and Advanced Oxidation Processes (AOP)

Only a limited number of STPs apply ozonation for post-treatment (Paraskeva and Graham, 2002) to their secondary effluents, although it has proven to be a very effective tool for PPCPs removal (Huber et al., 2003; 2005; Suárez et al., 2007). Second-order rate constants reported for the reaction with ozone (O_3) are very high, with values for EE2 and E2 around 7×10^9 $M^{-1}\cdot s^{-1}$ and in the order of 10^5-10^6 $M^{1}\cdot s^{-1}$ for DCF and CBZ (Huber et al., 2003), therefore these compounds are expected to be completely transformed during ozonation. On the other hand, the same study indicates a low second-order rate constant for IBP, between 1 and 10 $M^{-1}\cdot s^{-1}$. The explanation for this behaviour is the absence of reactive groups towards

ozone in its chemical structure, since ozone is a very selective oxidant that typically exhibits rapid reaction kinetics with a relatively small number of functional moieties (activated aromatic rings, neutral alkylamines, double bonds, and thiols).

During wastewater ozonation, micropollutants can be directly oxidized by O_3 or by hydroxyl radicals (HO•) which are formed during ozone decay. Most PPCPs considered by Huber et al. (2003) have shown very high second-order rate constants for the reaction with HO•, around 10^9 $M^{-1}\cdot s^{-1}$. Therefore, those compounds that react rapidly with O_3 will be predominantly oxidized by direct reactions, whereas the rest will be oxidized by the HO• formed, although this latter oxidation mechanism is expected to be fairly effective, since most of the HO• is scavenged by the wastewater matrix. The suitability of ozonation for the post-treatment of STP effluents has been shown in Ternes et al. (2003), where the most abundant compounds still present in the effluent after biological treatment (CBZ, DCF and HHCB) were eliminated at very high efficiencies (>93%).

If the objective is to oxidize ozone-resistant compounds, ozone has to be transformed into HO• radicals (one of the most powerful oxidants), thus transforming the process into an Advanced Oxidation Process (AOPs). The goal of any AOP design is to generate and use HO• as a strong but non-selective oxidant. The easiest way to transform a conventional ozonation process into an AOP is to add hydrogen peroxide or by using UV irradiation. However, Ternes et al. (2003) observed only a slight increase in the oxidation efficiency when comparing AOPs to conventional ozonation. The reason is that the combination of O_3 with H_2O_2 or UV radiation during wastewater treatment leads to a limited enhancement of HO• formation, since the organic matter present already catalyses their formation.

An additional advantage of applying an ozonation based post-treatment step is that the final effluent is disinfected previous to its discharge into receiving waters. In most STPs where disinfection of its final effluent is mandatory, usually chlorination or UV irradiation is applied, although these techniques exhibit lower oxidation capacities.

Membrane filtration

Microfiltration (MF) or Ultrafiltration (UF) membranes are used for wastewater tertiary treatment in order to obtain a high-quality final effluent that can be employed for groundwater recharge or reused for agricultural applications (Pollice et al., 2004). Passing the wastewater through this type of membranes ensures an efficient elimination of suspended matter and disinfection, but it is generally not able to retain PPCPs by size exclusion (lower limit pore size around 500 Da), which explains the similar efficiencies of MBR and CAS regarding PPCPs removal (Clara et al., 2005b). Snyder et al. (2007) confirmed that the vast majority of PPCPs spiked to a secondary effluent were not rejected when passing through an UF system, although estrogens (E2, E1 and EE2) were well removed (91-99%) which was

attributed to their relatively high sorption properties, even though other compounds as for example HHCB did not follow this pattern. Although the technical feasibility of membranes has been demonstrated, their implementation is still limited because of the high investment and operational costs involved (Alonso et al., 2001). If membrane filtration is required as a post-treatment technique for an efficient removal of micropollutants, Nanofiltration (NF) and Reverse Osmosis (RO) constitute an interesting alternative (Snyder et al., 2007). Currently these processes are almost exclusively applied in drinking water treatment facilities, whereas their application during wastewater treatment is scarce. Reported data illustrate that DCF can be efficiently removed from municipal sewage effluents using membrane filtration (Heberer, 2002). In addition, the combination of MF or UF with RO as secondary effluent post-treatment seems to be very efficient for the removal of PPCPs (Drewes et al., 2002; Snyder et al., 2007). An interesting strategy for combining MF or UF with RO is to perform the biological treatment in a MBR followed by a RO system, which has been operated at pilot scale by Snyder et al. (2007). This concept was successful to eliminate recalcitrant compounds, such as DCF and CBZ, which were mainly removed in the RO unit, while very biodegradable compounds, as IBP, have been already eliminated in the MBR.

Activated carbon

Powdered and Granular Activated Carbon (PAC and GAC) has been commonly used for sorption of organic micropollutants like pesticides or taste and odour compounds (Ternes and Joss, 2006). The main removal mechanism is based on hydrophobic interactions, wherefore the treatment is specially suited to non-polar organic compounds. Data available in the literature for the sorption potential of PAC and GAC for PPCPs were obtained from batch experiments of individual compounds in MilliQ water for the determination of the corresponding sorption isotherms or kinetic parameters, as well as from the performance of the sorption processes during drinking water treatment.

The main advantage of activated carbon processes is that no by-products are generated. In addition, the regeneration and final disposal of the adsorbent leads to a complete oxidation of sorbed PPCPs, since it is performed at temperatures higher than 650ºC (Ternes and Joss, 2006). Ternes et al. (2002) studied the removal efficiency of a GAC pilot plant treating groundwater spiked with selected PPCPs and found CBZ as the compound with the highest sorption capacity, but also DCF was efficiently removed. The sampling campaigns carried out in real drinking water treatment works confirmed that GAC processes are very effective in the removal of these compounds. Also Snyder et al. (2007) reported that both, PAC and GAC, are capable of removing endocrine disruptors, pharmaceuticals and personal care products by more than 90% in drinking water facilities. In the same study, experiments following the jar-test methodology with 5 mg/L of PAC for the

treatment of surface water spiked with more than 60 PPCPs, reported the following sorption capacity for the selected compounds: IBP (15%) < DCF (37%) < HHCB (55%) < CBZ, E1, EE2 (75-78%) < E2 (85%).

1.5. Conclusions

Although there are still a lot of uncertainties about the fate of PPCPs along the different units of STPs, a number of conclusions can be drawn:

- √ PPCPs have been reported to be present in different environmental water compartments all over the world, including rivers, lakes, groundwaters and wastewaters.
- √ Common STP technologies are only able to achieve high removal efficiencies for a limited number of compounds, either due to sorption (AHTN, HHCB, ADBI) or transformation (ibuprofen). Other compounds show a remarkable persistent behaviour, as carbamazepine, which is neither sorbed nor biotransformed. Therefore, these substances are being continuously discharged into the environment through STP effluents.
- √ Information about physico-chemical properties (volatilisation, sorption, dissociation, etc.) for each PPCP should be considered, since it is a valuable tool to understand, and even predict, the removal mechanisms involved.
- √ It is not enough to determine these substances only in the liquid phase, since a significant fraction can be sorbed onto solids (sludge), which is especially important in the case of lipophilic substances such as fragrances, EE2, etc.
- √ Overall mass balances should also consider the conjugated fractions of PPCPs arriving at STPs, which could also be depending on sewer configurations, wastewater characteristics and environmental factors, such as pH or temperature.
- √ Biological transformation of PPCPs is not only a function of their biodegradation rate constants (k_{biol}), but also on their solid-water distribution coefficient (K_d), since compounds with significant K_d values will be removed when the SRT in the aeration tank is enough to accomplish their biological degradation, as occurs with musks. However, once a certain limit value of HRT and SRT is exceeded, the removal efficiencies are not enhanced anymore.
- √ The presence of inorganic salts (coagulation-flocculation in primary treatment or phosphate precipitation during biological treatment) could improve the elimination of acidic compounds due to precipitation of trivalent salts.
- √ Advanced post-treatment units (ozone, AOPs, activated carbon, membranes) may constitute an interesting option to further remove these micropollutants in STPs.

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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).

From the conventional chemical parameters included in this work, Total and Soluble Chemical Oxygen Demand (COD_T and COD_S), Total and Volatile Solids (TS and VS), Total and Volatile Suspended Solids (TSS and VSS), nitrite and nitrate concentrations were determined following Standard Methods (APHA, 1999) and are therefore not further described in this chapter. Other parameters, such as Total (TN), Inorganic (IN) and Total Kjeldhal Nitrogen (TKN), nitrogen in the form of ammonia ($N\text{-}NH_4^+$), Total Organic and Inorganic Carbon (TC, TOC, TIC) and several inorganic anions (NO_2^-, NO_3^-, Cl^-; PO_4^{3-} and SO_4^{2-}) have been measured by analytical procedures optimised in our laboratories and are thus described in detail throughout this chapter.

A description of the analysis of PPCPs is 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, estriol and 17α-ethinylestradiol), antibiotics (roxithromycin, sulfamethoxazol, trimethoprim and erythromicyn) and the X-ray contrast media (iopromide).

The specific analytical methods used in a single part of the work are described in the corresponding chapter, 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.

Organic nitrogen is defined functionally as the organically bound nitrogen in the tri-negative oxidation state, but it does not include all organic nitrogen compounds. Analytically, organic nitrogen and ammonia can be determined together and have been referred to as "Total Kjeldahl Nitrogen" (TKN), a term that reflects the technique used in their determination.

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.

Total (TN), Inorganic (IN) and Total Kjeldhal Nitrogen (TKN)

TKN was determined in a total organic nitrogen analyzer (Rosemount-Dohrmann DN-1900) equipped with a quimioluminiscence detector with two channels. One channel determines the Total Nitrogen (TN) by oxidation at high temperature and the other determines the Inorganic Nitrogen (IN) by a chemical reduction. TKN is determined as the difference between TN and IN.

All the nitrogen present in the water can be catalytically oxidised to nitrous oxide (NO). The process for TN determination goes by in two steps. The first step is a catalytic oxidation (Cu as catalyst) in the combustion tube at 850°C and with pure oxygen (1 atm) as carrier gas. The second one is the chemical reduction of residual NO_2 with H_2SO_4 at 80ºC and catalyzed by $VaCl_3$. For the IN determination, only the second step (chemical reduction) is used. The NO obtained in the two steps is dried and forced to react with O_3 producing an unstable excited state NO_2^*. The change back of this oxide to its fundamental state emits a proton, from which the determination of TN and IN is carried out by quimioluminiscence using a multiplicator tube. The instrument is calibrated with a certified standard solution (KNO_3, 20 mg N/L) using a response factor method.

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. Total Organic and Inorganic Carbon (TC, TOC, TIC)

Organic carbon in water and wastewater may include a variety of organic compounds in different oxidation states. Total Organic Carbon (TOC) is a more convenient and direct expression of total organic content than either Biological (BOD) or Chemical Oxigen Demand (COD), but does not provide the same kind of information. Unlike BOD or COD, TOC is independent of the oxidation state of the organic matter and does not measure other organically bound elements, such as nitrogen and hydrogen, and inorganics that can contribute to the oxygen demand measured by BOD and COD (APHA-AWWA-WPCF, 1999).

To determine the quantity of organically bound carbon, the organic molecules must be broken down and converted to a single carbon molecular form that can be measured quantitatively.

TOC was determined by a Shimadzu analyzer (TOC-5000) as the difference between the Total Carbon (TC) and the Inorganic Carbon (IC). The instrument is connected to an automated sampler (Shimadzu, ASI-5000-S). TC is determined from the amount of CO_2 produced during the combustion of the sample at 680°C, using platinum immobilised over alumina spheres as catalyst. The IC is obtained

from the CO_2 produced in the chemical decomposition of the sample with H_3PO_4 (25%) at room temperature. The CO_2 produced is optically measured with a nondispersive infrared analyzer (NDIR) after being cooled and dried. High purity air is used as carrier gas with a flow of 150 mL/min. Four-point calibration curve in the range of 0-1 g C/L, using potassium phthalate as standard for TC and a mixture of sodium carbonate and bicarbonate (Na_2CO_3/$NaHCO_3$, 3:4 w/w) for IC, is used for the quantification.

2.1.3. Inorganic anions: NO_2^-, NO_3^-, Cl^-, PO_4^{3-} and SO_4^{2-}

Nitrite (NO_2^-), nitrate (NO_3^-), chloride (Cl^-), phosphate (PO_4^{3-}) and sulphate (SO_4^{2-}) are determined simultaneously by capillary electrophoresis using a Waters Capillary Ion Analyzer (CIA). Sodium sulphate (0.01 M) is used as electrolyte (Vilas-Cruz et al., 1994). Besides, an electro-osmotic modifier (50 mL/L) CIA-Pak™ OFM Anion BT Waters (Ewing et al., 1989; Heiger, 1992) is also added. The sample is forced to migrate through a capillary (melting silica covered with poliimida, 60 cm long and 45 μm of internal diameter) kept at 25°C by the application of an electric current. Depending on the ratio charge/mass of the ion, the migrating time is different. A hydrostatic injection (10 cm height for 30 seconds) and an indirect detection (UV, 254 nm, 240 kV, 16-22 μA) are used.

Four to six calibration points for each ion in the range of 3-100 mg/L are daily used for the quantification of the samples. Previously to the analyses, the samples are filtrated through 0.45 μm membrane (Millipore).

2.2. PPCP analysis

The analysis of PPCPs comprises filtration (if only the liquid phase is considered), extraction, sample preparation, derivatisation (if needed) and detection. In order to avoid interferences caused by suspended solids, between 0.6 and 1 L of the raw sample was filtered over glass fibre filters (APFC04700 or AP4004705, Millitpore). Sample extraction consisted of Solid Phase Extraction (SPE) or Solid Phase MicroExtraction (SPME) and was principally used as pre-concentration technique of PPCPs prior to their quantitative determination. For some compounds, a derivatization step prior to the final quantification is 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.

2.2.1. Polycyclic Musk Fragrances (PMF)

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.

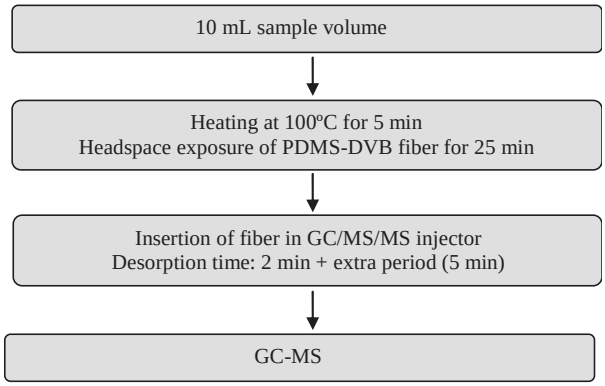

Figure 2-1. Scheme of the SPME method for polycyclic musks.

The SPME method (Figure 2-1) allows the determination of the total load of PMF in the sample and it was only performed for musks (García-Jares et al., 2002). 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.

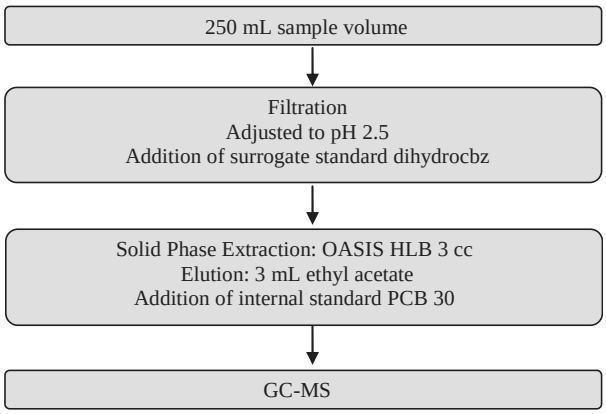

Figure 2-2. Scheme of the SPE method for musks and neutral pharmaceuticals.

The SPE method (Figure 2-2) was used for the determination of the soluble load of PMF in liquid samples. 300 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, 250 mL of sample were used for the enrichment, which 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).

2.2.2. Neutral pharmaceuticals

Neutral pharmaceuticals (Carbamazepine: CBZ and Diazepam: DZP) were simultaneously determined with PMF by means of the SPE method (Figure 2-2).

2.2.3. Acidic pharmaceuticals

For the acidic pharmaceuticals (Ibuprofen: IBP, Naproxen: NPX and Diclofenac: DCF), the analytical method (Figure 2-3) used is based on Rodriguez et al. (2003). The filtration, extraction and elution step was simultaneously performed with that of PMF 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 was carried (Varian Saturn 2100 T).

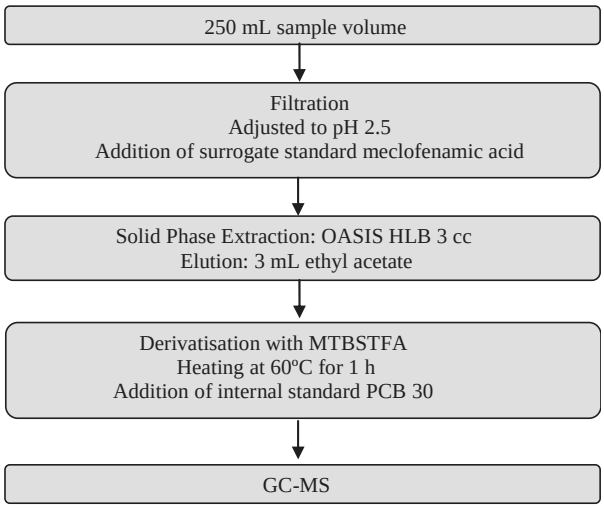

Figure 2-3. Scheme of the analytical method for acidic pharmaceuticals.

The operating conditions of the GC-MS for PCM, 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	260ºC	250ºC	280ºC
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	60ºC	60ºC	50ºC
Initial time	2 min	2 min	1 min
1st ramp	10ºC·min^{-1}	10ºC/min	10ºC/min
Final temperature	250ºC	250ºC	180ºC
Isothermal time	0 min	0 min	7 min
2nd ramp	20ºC·min^{-1}	20ºC/min	10ºC/min
Final temperature	280ºC	280ºC	230ºC
Isothermal time	9.5 min	9.5 min	25 min
3rd ramp	-	-	20ºC/min
Final temperature	-	-	250ºC
Isothermal time	-	-	5 min
		MS parameters	
Ionization mode	EI	EI	EI
Filament current	20 µA	20 µA	10 µA
Ion trap temperature	220ºC	220ºC	220ºC
Transfer line temperature	280ºC	280ºC	280ºC
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)

2.2.4. Anti-depressants

Fluoxetine's (FLX) and citalopram's (CTL) analytical determination has been carried out according to Lamas et al. (2004). Analyses were carried out on a Varian 3400 GC, equipped with a split/splitless injector, coupled to a Varian Saturn 3 ion trap mass spectrometer (Varian Chromatography Systems, Walnut Creek, CA, USA). Experimental parameters were: column, CP-SIL 8 CB 30 m, 0.25 mm i.d., 0.25 µm film; temperature program, 60ºC for 2 min, heated to 250ºC at 25ºC/min, heated

to 280ºC at 10ºC/min, and finally heated to 292ºC at 1.5ºC/min (total analysis time, 25.6 min). Helium was employed as carrier gas at an initial head column pressure of 8 psi. Injector was programmed to return to the split mode after 2 min from the beginning of a run. Injector temperature was held constant at 270ºC. Trap and transfer line temperatures were 220 and 292ºC, respectively. The mass spectrometer was used in the positive electron impact mode at 70 eV with automatic gain control. A mass range of m/z 43–420 was scanned, and the detector was turned off for the first 11 min of the run. The quantifications ions (m/z) were 44 and 58 for fluoxetine and citalopram, respectively.

Water samples were filtered through glass fibre filters and placed in 22-mL headspace vials. To improve the extraction a derivatisation process was carried out with potassium hydrogen carbonate and acetic anhydride (acetylation). Afterwards the vial was sealed with an aluminium cap and a Teflon-faced septum, immersed in a water bath at 100ºC and let to reach an equilibrium state for 5 min before SPME. The fiber (PDMS-DVB) was than exposed to the sample under magnetically stirring during 30 min and afterwards immediately inserted into the GC injection port. Desorption time was set at 3 min (Figure 2-4).

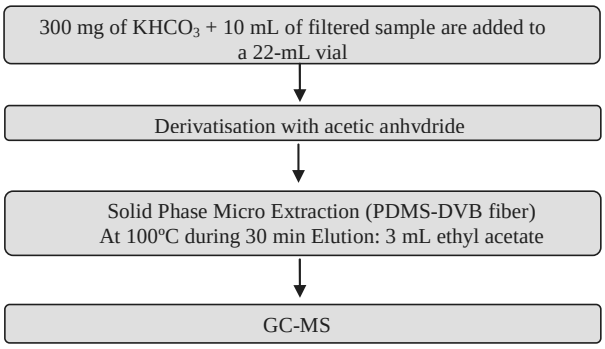

Figure 2-4. Scheme of the analytical method for anti-depressants.

2.2.5. Estrogens

Estrone (E1), 17β-estradiol (E2), estriol (E3) and 17α-ethinylestradiol (EE2) have been analysed according to Quintana et al. (2003). Samples were filtered and the pH was adjusted to 6 using 0.1 or 1 M HCl solutions. Then methanol (1%) and the internal standard, 17β-estradiol-d4 (75 ng/l), were added to the samples. The samples were subsequently passed through an Oasis HLB 60 mg cartridge (approximately at 15–20 mL/min) that had been sequentially pre-conditioned with ethyl acetate, methanol and Milli-Q water adjusted at the same pH that the sample (3 ml each). Cartridges were then dried with a nitrogen stream for 30 min and eluted with 3 mL of ethyl acetate. At this step, a dark extract was obtained; therefore, the final volume was reduced to approximately 0.3 mL and further cleaned-up by passing it through a 500 mg Sep-Pak silica cartridge (previously

conditioned with 5 mL of ethyl acetate). Analytes were then eluted with 10 mL of ethyl acetate, and the extract reduced to 0.1 mL and derivatised with MSTFA at 85ºC for 100 min. After that, they were cooled to room temperature and injected in the chromatographic system (Figure 2-5).

GC–MS–MS analysis was carried out using a Varian CP 3800 gas chromatograph equipped with a BP-1 type capillary column (30 m×0.32 mm i.d., df: 0.17 μm) connected to ion-trap mass spectrometer (Varian Saturn 2000) with capacity to perform MS–MS analysis. Injections (1–2 μL) were performed in the splitless mode with a purge time of 1 min. In both columns the silylated compounds were separated using the following oven program: 1 min at 50ºC, first ramp at 20ºC/min to 220ºC (held 17 min), second ramp at 20ºC/min to 250ºC (held for 20 min). The GC–MS interface and the ion trap temperature were set at 250 and 200ºC, respectively. Mass spectra were obtained in the m/z interval 100-550, using electron impact ionization (70 eV). The quantifications ions (m/z) were: 257, 326 + 285, 324 and 193 for E1, E2, E3 and EE2, respectively.

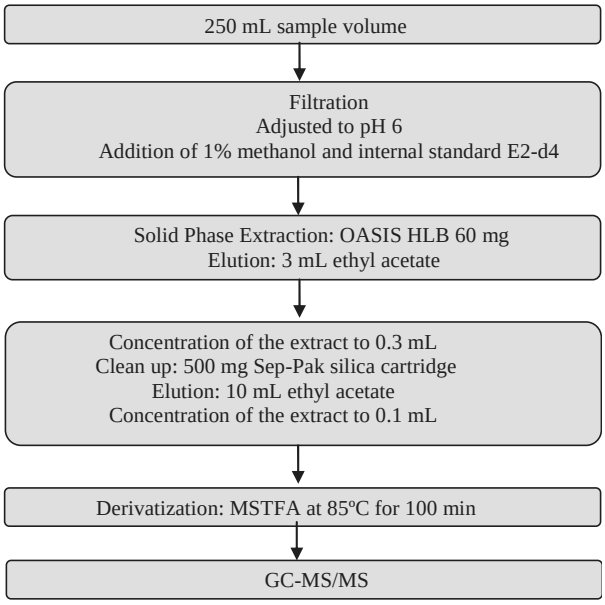

Figure 2-5. Scheme of the analytical method for estrogens.

2.2.6. Antibiotics and Iopromide

These two groups of compounds were analysed by the Austrian Federal Environment Agency (Figure 2-6) and comprised four antibiotics (roxithromycin: ROX, sulfamethoxazol: SMX, trimethoprim: TMP and erythromicyn: ERY) and the X-ray contrast media (iopromide: IPM).

In our group the samples were collected in glass or aluminium bottles and immediately prefiltered (glass fibre prefiltres, AP4004705 Millipore), supplied with a

pinch of sodium azide and stored in the freezer. The samples were sent frozen to Austria via urgent mail, in order to ensure their conservation.

The analytical procedure followed for the detection of antibiotics is summarised in Figure 2-6, while for iopromide direct injection into LC-MS/MS was performed, since concentrations in the mg/L range were found in the wastewaters analysed.

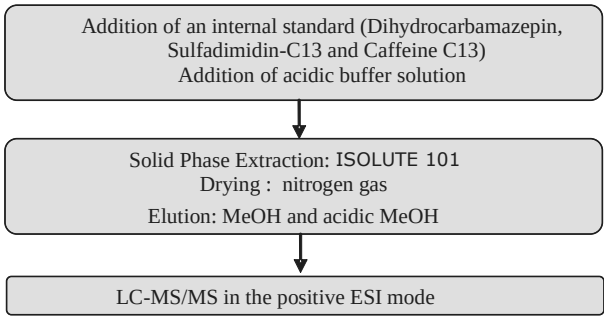

Figure 2-6. Scheme of the analytical method for antibiotics.

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

Table 2-1 summarises the LOD and LOQ for the analytical methods applied in the present work.

Table 2-1. Limits of Detection (LOD) and Quantification (LOQ) for the analytical methods used in the determination of PPCPs.

PPCP	LOD (ng/L)	LOQ (ng/L)	PPCP	LOD (ng/L)	LOQ (ng/L)
HHCB	23	70	E1	0.7	2
AHTN	23	70	E2	0.7	2
ADBI	23	70	E3	0.7	2
CBZ	470	1400	EE2	1.7	5
DZP	230	700	SMX	2.6	10
IBP	27	80	ROX	1.2	4.1
NPX	27	80	TMP	2.9	10
DCF	100	300	ERY	1.2	4.1
FLX	17	50	IPM	2,600	10,000
CTL	15	45			

2.3. References

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Chapter 3

Occurrence of Pharmaceuticals and Personal Care Products (PPCPs) in hospital and municipal wastewaters

Summary

An intensive sampling campaign has been carried out during the years 2004, 2005 and 2006 in Santiago de Compostela, NW Spain, including municipal wastewater, effluents from three different hospitals and the influent and effluent from the STP of the city. Conventional physico-chemical parameters as well as the concentration of 19 Pharmaceutical and Personal Care Products (PPCPs) were analysed in the different samples. Among PPCPs that were analysed were: three musk compounds, four hormones, one X-ray contrast media and pharmaceuticals from 5 different therapeutic classes (anti-epileptics, anti-depressants, anti-inflammatories and antibiotics).

The characterisation of wastewaters showed that, while municipal sewage could be classified as moderately contaminated, hospital effluents were in general stronger polluted and maximum concentrations for conventional wastewater parameters were at least 3-fold higher than standard values for concentrated municipal sewage. In terms of PPCP, the highest concentration of anti-inflammatory drugs, CBZ, DZP, ADBI and natural estrogens has always been detected in hospital effluents. In fact, maximum concentrations in hospital wastewater for IBP, NPX and CBZ of 74.7, 192 and 41.8 ppb, respectively have been measured, whereas the maximum level for these compounds in urban wastewater was below 9 ppb. In the case of NPX, up to 40-fold higher concentrations were measured in hospital effluents compared to the municipal wastewater collected in the same sampling campaign. A second characteristic of hospital streams was its higher variability concentrations of PPCPs compared to municipal wastewaters.

From the whole set of PPCPs monitored, highest concentrations were measured for IBP and NPX, whereas EE2, FLX and CTL were generally not detected in the wastewaters sampled, although the few positive detections of anti-depressants were found for hospital streams.

Outline

3.1. Introduction

First studies concerning the occurrence of pharmacologically active compounds in the environment have been already published in the seventies, focussing on clofibric acid, the active metabolite of blood lipid regulating drugs (Garrison et al., 1976; Hignite and Azarnoff, 1977). Nevertheless, it was not until ten years ago when pollution of aquatic systems with Pharmaceutical and Personal Care Products (PPCPs) became one of the emerging issues in environmental chemistry and as a matter of public concern. An illustration of the advances made about this topic is the present knowledge of more than 80 identified compounds detected in sewage effluents, surface water and even ground and drinking water (Heberer, 2002a). In any case, this is only a small proportion of the overall amount of PPCPs consumed, since in the EU around 3000 different substances are being used in medicines at present, and thousands of different chemicals are incorporated in personal care products, such as skin and dental care products, soaps, sunscreen agents etc. (Ternes et al., 2004).

The predominant therapeutic classes of pharmaceuticals include: analgesic/anti-inflammatory drugs; lipid-regulators; antibiotics; beta-blockers; anti-epileptics and hormones. The concentrations for these compounds in municipal wastewater are in the µg/L or ng/L range. For example, the compounds ibuprofen (IBP), naproxen (NPX) and diclofenac (DCF) are frequently reported anti-inflammatory drugs with maximum concentrations measured in municipal sewage of 170, 5 and 3.6 µg/L, respectively (Lindqvist et al., 2005; Bendz et al., 2005; Gomez et al., 2007). Among antibiotics, the most prevalent in the environment are macrolides, fluoroquinolones and sulfonamides, whereas tetracyclines or penicillins have been found only in some cases and generally at low concentrations (Beausse, 2004). Concentrations of antibiotics in municipal wastewater are commonly at least one order of magnitude below that of anti-inflammatory drugs (Gobel et al., 2005; Bendz et al., 2005), with maximum concentrations reported for the macrolides roxithromycin (ROX) and erythromycin (ERY) around 40 and 190 ng/L, respectively, and 0.6 µg/L for the sulfonamide sulfamethoxazole (SMX). Carbamazepine (CBZ) is an anti-epileptic pharmaceutical frequently detected in sewage, ground and even in drinking water (Heberer, 2002a). Concentrations of CBZ in the influents and effluents of Sewage Treatment Plants (STPs) can reach the µg/L level (Castiglioni et al., 2005; Bendz et al., 2005), and it has even been detected in ground water at concentrations of 1.1 µg/L and in drinking water at 30 ng/L (Heberer, 2002a). The widespread detection of CBZ is presumably related to its high persistence during conventional sewage treatment, since it is neither subject to degradation nor to adsorption, and only ozonation seems to be an adequate tool for its elimination (Clara et al., 2004). This qualifies it as a suitable marker for anthropogenic influences on the aquatic environment.

Research concerning the occurrence of estrogenic compounds is mainly derived from the concern about endocrine disrupting effects exerted by STP discharges into surface waters. In fact, the natural hormones 17β-estradiol (E2) and estrone (E1) and the synthetic hormone 17α-ethynylestradiol contained in contraceptive agents, have been identified as prime contributors to the estrogenic character of STP effluents and, particularly E2 exerted at least two orders of magnitude higher estrogenic activity than other potential endocrine disrupters like nonylphenols or PCBs (Ternes et al., 1999b; de Mes et al., 2005). When these compounds are classified according to their estrogenic potencies, EE2 would be the most important one, followed by E2 and E1, while by far the less potent estrogen is the natural hormone estriol (E3). If typical effluent concentrations are included in the assessment of endocrine disrupting potencies, EE2 would still be the most important endocrine disrupter, although the overall impact of E2 would appear less significant than that of E1 (Johnson and Sumpter, 2001). Natural estrogens are excreted at 106, 14 and 32 mg/day of conjugated (glucuronides and sulphonides) E3, E2 and E1 in female urine (D'Ascenzo et al., 2003) leading to concentrations of 80-380, 10-150 and 10-130 ng/L, respectively, of free estrogens at the inlet of STP, after deconjugation in the sewer systems.

Among personal care products, musk compounds such as galaxolide (HHCB) tonalide (ADBI) or celestolide (ADBI), have been included in several STP surveys (Bester, 2004; Peck and Hornbuckle, 2004; Joss et al., 2005; Clara et al., 2005b; Kupper et al., 2006). These compounds have been detected in freshwater fish and even in human tissues, presumably as a consequence of their high bio-accumulation potencies, and exhibit weak estrogenic effects (Bester, 2004; Schreurs et al., 2004), which are the two main causes for concern. The highest concentrations in STP influents have been reported for HHCB, followed by AHTN and ADBI with maximum reported levels of 13, 2.6 and around 0.2 µg/L (Kupper et al., 2006; Reiner et al., 2007).

It is of high importance to identify and characterise the different sources of PPCPs into municipal wastewater. At present time, the two most important points of human pharmaceutical consumption are households and hospitals, although amounts of substances emitted by hospitals are often neglected when Predicted Environmental Concentrations (PEC) are calculated (Kummerer, 2001), probably as a consequence of scarce information available about consumption and emission patterns in hospitals.

Hospitals are in general intensive consumers of water, thus generating significantly higher wastewater flows than conventional households (400-1200 L/bed·d versus 100 L/capita·d), loaded with microorganisms, heavy metals, pharmaceuticals, toxic chemicals and radioactive elements. The direct discharge of these effluents into urban sewerage systems, without preliminary treatment, constitutes a potential risk to the environment, since conventional STPs have not

been designed for this specific purpose.

Among pharmaceuticals specifically consumed in hospitals are cytostatic agents (ifosfamide and cyclophosphamide), although a fraction of the administered dose could be excreted at home by out-patients. Expected concentration in hospital effluents are in the range of 5-50 µg/L, although a high variability of emissions has been observed (Kummerer, 2001). Consumption of antibiotics in hospitals can also be very significant compared to their overall use. For example in Germany, in 1999, the contribution was of 26%, which explains the high concentrations (up to 100 µg/L) reported for several antibiotics, such as β-lactams, fluoroquinolones, sulfonamides and trimethoprim in hospital effluents (Kummerer, 2001; Lindberg et al., 2004; Brown et al., 2006). Hospital can neither be neglected as contributors of Adsorbable Organic Halogen Compounds (AOX) in urban wastewaters, contained in X-ray contrast media, solvents, disinfectants, cleaners and drugs containing chlorine (Kummerer, 2001).

The aim of the present work was to determine the occurrence of PPCPs in urban and hospital wastewaters in Santiago de Compostela, NW Spain, with three large hospitals, the effluents of which join the sewer system of the city. Conventional physico-chemical wastewater parameters, as well as the concentration of selected PPCPs, have been monitored in hospital effluents, in sewage from domestic origin and at the inlet and outlet of the STP that treats the wastewater of the city.

3.2. Materials and methods

3.2.1. Wastewater

A sampling campaign has been carried out during the years 2004, 2005 and 2006 in Santiago de Compostela, a city of approximately 125,000 inhabitants, which includes a major University campus and three important hospitals (Figure 3-1). Wastewater samples from four different origins have been considered, including municipal wastewater, hospital effluents and the influent (SP5) and the effluent (SP6) from the STP of the city. The sampling point representative of wastewater from domestic origin (SP1) is located before any discharge from hospitals and collects sewage from a residential area comprising also part of the University campus. Hospital effluents have been collected at a hospital with a capacity of around 750 beds and outpatient consultation for all medical specialities (SP2), whereas the other two hospitals considered are mainly dedicated to dermatologic (SP3) and psychiatric and orthopaedic (SP4) consultations, with approximately 90 and 290 beds, respectively.

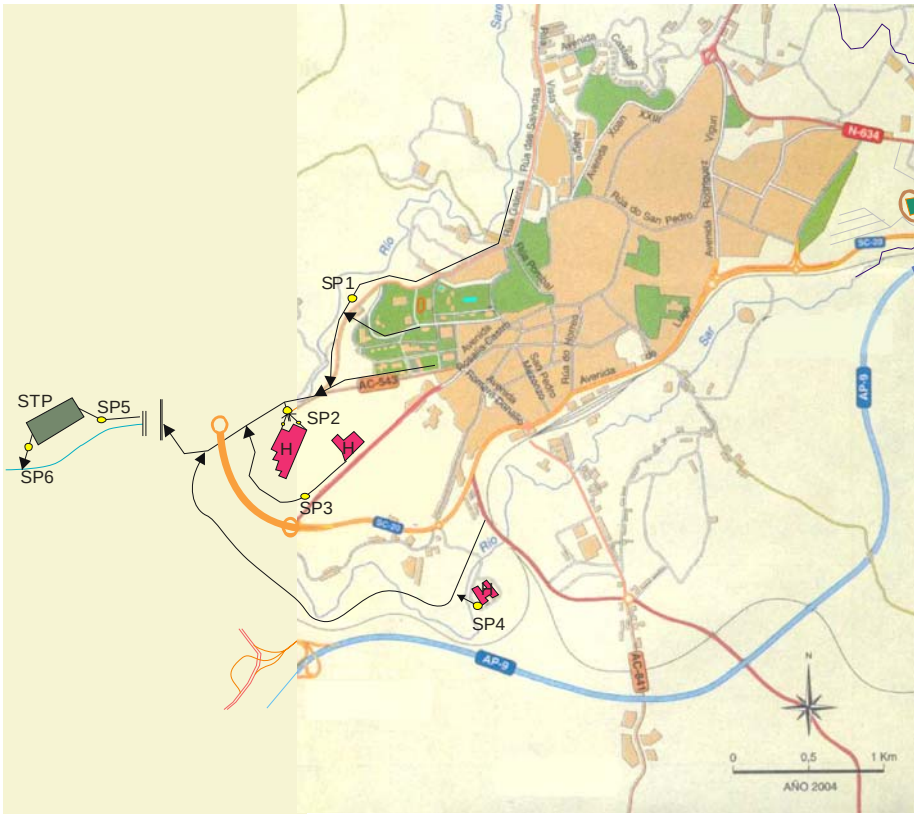

Figure 3-1. Location of the considered sampling points.

3.2.2. Sampling

Eight sampling campaigns have been carried out during the 13th and 21st of April 2004, the 15th and 22nd of September 2004, the 2nd and 9th of February 2005 and the 16th and 23rd of June 2005, representing duplicates of the four different seasons of the year. Integrated water samples were obtained by mixing 24 liquid samples collected each hour by an automatic device, with the exception of the first sampling were the integration period comprised only 12 hours (from 5 a.m. to 5 p.m.). From September onwards the Hydraulic Retention Time (HRT) of the STP (16 h) was taken into account in the sampling of the influent and effluent of the plant.

In a second part of this work, a more detailed sampling of the main hospital (SP2) has been performed, considering the two points of discharges from the building separately: S1 which comprises wastewater from hospitalised patients, surgery, laboratories, radiology and general services and S2 which consists of wastewater from radiotherapy and outpatient consultation. In this case sampling had to be performed manually, although still an integration over 24 h has been considered.

Figure 3-2. Individual sampling of S1 and S2 in SP2.

3.2.3. Analytical methods

Total and Soluble Chemical Oxygen Demand (COD_T and COD_S), Total and Volatile Solids (TS and VS), Total and Volatile Suspended Solids (TSS and VSS), nitrite and nitrate concentrations were determined following Standard Methods (APHA, 1999). The concentrations of amoniacal nitrogen, chlorine, sulphate, phosphate, Total Inorganic and Organic Carbon (TIC and TOC) and Total Kjeldahl Nitrogen (TKN) was determined according to chapter 2.

The concentration of PPCPs was determined following the methods described in chapter 2. The integrated samples were prefiltered (glass fibre prefiltres, AP4004705 Millipore). For the analysis of antibiotics, a pinch of sodium azide was added to the filtered sample before its storage in the freezer, where it was kept until analysed by the Austrian Federal Environment Agency. For the rest of compounds, samples were analysed within one week, thus storage in the fridge was sufficient.

3.3. Results and discussion

3.3.1. Conventional parameters

Wastewater collected during the sampling campaigns was characterised including conventional parameters such as the content of solids, organic matter, nutrients and different salts, as shown in the annex of this chapter (Table I). This information has been summarised in Table 3-1, providing global ranges for each parameter in the different sampling points, as well as for the group of municipal and hospital wastewater samples. Additionally, a statistical analysis based on the building of histograms was used to identify the Most Frequent Range (MFR) for the different parameters in the considered wastewater types.

Table 3-1. Characterisation of the wastewaters regarding conventional parameters in the different sampling points, outlining the sub-categories of Municipal (MWW) and Hospital Wastewater (HWW), as well as the whole range of data (overall).

Sampling point		TS	VS	TSS	VSS	COD_T	COD_S	Cl^-	SO_4^{-2}	NO_2^-	$N\text{-}NO_3^-$	$N\text{-}NH_4^+$	$P\text{-}PO_4^{-3}$	TIC	TOC	NTK
SP1	MIN	298	148	62	35	75	21	17	10	<LOD	<LOD	5.2	<LOD	12.3	8.8	9.2
	MAX	1010	420	466	282	501	195	126	48	<LOD	1.8	37.8	4	45.2	65.7	34.1
SP2	MIN	210	65	20	18	67	11	50	<LOD	<LOD	<LOD	17.6	<LOD	13	16	6
	MAX	2909	677	339	331	2464	2277	300	57	<LOD	<LOD	80.1	4.1	154	697	74.1
SP3	MIN	447	197	77	65	327	192	35	10	<LOD	<LOD	48.6	<LOD	34.9	58.7	46.3
	MAX	845	460	292	270	765	428	98	54	<LOD	0.2	99.4	6	87.9	110	90.3
SP4	MIN	350	205	78	68	291	104	27	6	<LOD	<LOD	12.2	<LOD	14.2	26	12.9
	MAX	2955	2679	1464	1406	3585	433	77	43	<LOD	2.7	37.5	3.9	44.6	147	61.6
SP5	MIN	298	105	82	50	35	21	14	12	<LOD	<LOD	8.7	<LOD	12	6.1	8
	MAX	1255	436	350	255	575	112	96	57	<LOD	2.3	37.3	2.4	46.5	28.9	46
SP6	MIN	440	98	30	27	57	22	179	75	<LOD	3.4	0.3	<LOD	10.1	6.3	4.3
	MAX	470	231	17	17	17	14	86	33	<LOD	10.8	12.0	1	33.6	20.4	22.9
MWW (SP1, SP5)	MIN	298	105	62	35	35	21	14	10	<LOD	<LOD	5.2	<LOD	12	6.1	8
	MAX	1255	436	466	282	575	195	126	57	<LOD	2.3	37.8	4	46.5	65.7	46
	MFR	300-500	100-300	100-200	50-200	100-400	20-100	30-70	30-50	<LOD	<2.3	15-30	<LOD	25-46	10-30	20-35
HWW (SP2, SP3, SP4)	MIN	210	65	20	18	67	11	27	<LOD	<LOD	<LOD	12.2	<LOD	13	16	6
	MAX	2955	2679	1464	1406	3585	2277	300	57.3	0	2.7	99.4	6	154	697	90.3
	MFR	400-1000	200-600	100-300	100-300	300-600	100-600	50-100	<30			15-50	<LOD	<90	<200	30-70
Overall	MIN	210	60	7	6.5	17	11	14	0	<LOD	<LOD	0.3	<LOD	10.1	6.1	4.3
	MAX	2955	2679	1464	1406	3585	2277	300	75	<LOD	10.8	99.4	6	154	697	90.3
	MFR	400-800	100-400	100-300	100-300	200-600	<600	30-100	<50	<LOD	<2	<40	<LOD	<50	<100	<70

*Concentrations in mg/L. MFR: Most Frequent Range, where at least 50% of data were located. LOD: Limit of Detection (Chapter 2).

Considering SP1 and SP5 as representative samples of municipal wastewater and comparing their characteristics with standard values (Henze, 1995; Sincero, 2003), urban wastewaters could be classified as moderately polluted (MFR in Table 3-1), although the maximum values measured for the content of solids was within the range of strongly polluted sewage. These peaks in total and suspended solids were measured for sample SP1 collected during February 2005, which was a very dry period, and for sample SP5 from June 2005, where the TS and VS load of hospital stream SP2 could have contributed to the composition of SP5 (Annex, Table I).

Hospital effluents were, in general, stronger polluted than municipal sewage (Table 3-1) and maximum concentrations of TS, TSS and COD were at least 3-fold higher than standard values for concentrated municipal sewage (Henze, 1995; Sincero, 2003). Apart from that, the variability in the composition of hospital effluents was significantly larger than for municipal sewage. The majority of data regarding TSS concentration in hospital wastewaters were in the range of 100-300 mg/L, similar to what had been reported by Kajitvichyanukul and Suntronvipart (2006), although higher than the concentrations reported by Chiang et al. (2003) which are closer to the minimum concentrations measured in the present work for hospitals. On the other hand, in Gautam et al. (2007) the content of suspended solids reached up to 531 mg/L, which is still below the maximum value determined in this study. Focusing on the input of organic matter from hospitals effluents, the bulk of data were in the range 300-600 mg/L, although up to 2500-3500 mg/L of total COD have been detected in those streams, which was considerably higher than the concentrations reported in the literature, which did not exceed 1350 mg/L (Chiang et al. 2003; Kajitvichyanukul and Suntronvipart, 2006). From the three hospitals considered in this work, the one that discharges at SP3 could be discarded as relevant source for conventional pollution, since this effluent could be assimilated as urban wastewater.

3.3.2. PPCPs

From the selected PPCPs, IBP, NPX, DCF, CBZ, DZP, HHCB, AHTN and ADBI have been analysed during all sampling campaigns, whereas estrogens (E1, E2, EE2 and E3) and anti-depressants (FLX and CTL) have been excluded from the sampling campaigns of SP2 performed between November 2005 and June 2006 due to their lower detection level or frequency in the previous samplings, although they were substituted by new substances, namely four antibiotics (ROX, ERY, SMX and TMP) and the contrast media IPM. As for conventional parameters, the whole set of data obtained during the present work has been included in the annex of this chapter (Table II), while Table 3-2 provides a summary, including the MFR for each PPCP.

Table 3-2. Concentrations of PPCPs for the different sampling points, outlining the sub-categories of Municipal (MWW) and Hospital Wastewater (HWW), as well as the whole range of data (overall).

Sampling point		IBP	NPX	DCF	CBZ	DZP	HHCB	AHTN	ADBI	E1	E2	E3	ROX	ERY	SMX	TMP	IPM
SP1	MAX	8.60	6.30	<LOQ	<LOQ	<LOD	2.87	4.49	2.01	97	25	182	n.a.	n.a.	n.a.	n.a.	n.a.
	MIN	1.51	1.33	<LOD	<LOD	<LOD	0.15	0.05	<LOD	27	<LOD	40					
SP2	MAX	74.7	25.8	4.0	41.8	1.10	2.57	1.96	3.38	168	56	1552	0.25	2.10	12.0	1.70	1600
	MIN	0.76	1.13	<LOD	<LOD	<LOD	0.13	<LOD	<LOD	5	7	104	<LOD	<LOD	0.08	0.16	<LOD
SP3	MAX	34.5	192	4.04	18.6	<LOD	1.07	0.86	0.49	98	28	353	n.a.	n.a.	n.a.	n.a.	n.a.
	MIN	1.10	4.17	<LOD	<LOD	<LOD	0.22	<LOD	<LOD	40	11	29					
SP4	MAX	16.6	24.1	2.09	27.4	<LOD	0.78	0.26	0.98	43	10	77	n.a.	n.a.	n.a.	n.a.	n.a.
	MIN	2.74	1.54	<LOD	<LOD	<LOD	0.15	<LOD	<LOD	<LOD	<LOD	<LOD					
SP5	MAX	7.85	6.53	<LOQ	<LOQ	<LOD	1.09	0.61	<LOQ	44	11	194	n.a.	n.a.	n.a.	n.a.	n.a.
	MIN	2.19	2.02	<LOD	<LOD	<LOD	0.35	<LOD	<LOD	6	<LOD	38					
SP6	MAX	2.50	4.06	<LOQ	<LOQ	<LOD	0.76	0.34	<LOQ	32	<LOD	<LOD	n.a.	n.a.	n.a.	n.a.	n.a.
	MIN	0.21	0.62	<LOD	<LOD	<LOD	0.27	0.20	<LOD	2	<LOD	<LOD					
MWW (SP1, SP5)	MAX	8.60	6.53	<LOQ	<LOQ	<LOD	2.87	4.49	2.01	97	25	194	n.a.	n.a.	n.a.	n.a.	n.a.
	MIN	1.51	1.33	<LOD	<LOD	<LOD	0.15	<LOD	<LOD	6	<LOD	38					
	MFR	5-8	1.3-6.5	<LOQ	<LOD	<LOD	0.6-1.2	0.2-0.6	<LOD	20-50	<11	38-194					
HWW (SP2, SP3, SP4)	MAX	74.7	192	4.04	41.8	1.10	2.57	1.96	3.4	168	56	1552	0.25	2.10	12.0	1.70	1600
	MIN	0.76	1.13	<LOD	<LOD	<LOD	0.13	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.08	0.16	<LOD
	MFR	3-25	3-15	<LOD	<LOQ	<LOD	0.2-0.5	0.1-0.4	<LOD	<110	4-30	<300	<LOD	<0.7	<0.7	0.2-0.9	700-1100
Overall	MAX	74.7	192	4.04	41.8	1.10	2.87	4.49	3.38	168	56	1552	0.25	2.10	12.0	1.70	1600
	MIN	0.21	0.62	<LOD	0.03	<LOD	0.13	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.08	0.16	<LOD
	MFR	1-10	2-7	<LOD	<LOD	<LOD	0.2-0.9	0.1-0,5	<LOD	5-50	<15	<130	<LOD	<0.7	<0.7	0.2-1.7	700-1100

*Concentrations in μg/L, except for estrogens (E1, E2 and E3) in ng/L. Most frequent range (MFR) is defined as those, where at least 50% of data were located. n.a. Not analysed. LOD Limit of Detection (Chapter 2)

Apart from the compounds included in Table 3-2, the synthetic hormone EE2 and the anti-depressants FLX and CTL have also been monitored, although they were generally not detected in the wastewaters sampled. More concretely, EE2 was not detected in any of the samples considered, CTL gave 4 positive results, 3 of which in SP 4 (0.22-0.50 µg/L) and the other in SP 3 (0.40 µg/L), and FLX was only detected in 2 samples, both collected in SP 3 (0.15-0.47 µg/L).

3.3.2.1. Occurrence of PPCPs in municipal wastewater

The highest concentrations of the selected PPCPs have been measured for the anti-inflammatory drugs IBP and NPX, for which several µg/L of compound have been detected in all municipal wastewater samples (SP1 and SP5) collected, in agreement with STP influent concentrations provided by other authors (Lindqvist et al., 2005; Bendz et al., 2005).

From the considered fragrances, HHCB was detected in all samples, AHTN in 13 out of 14 samples, whereas ADBI was in general not detected. The concentrations of HHCB and AHTN were around 1 µg/L, being the ratio of HHCB:AHTN between 2 and 3, similar to what had been reported by Bester (2004), where a shift in the application pattern of these musks towards increasing HHCB:AHTN ratios comparing to the 1:1 value of earlier years had been already indicated. Somewhat higher levels of HHCB have been reported in STP influents in recent studies performed by Kupper et al. (2006) and Reiner et al. (2007), while concentrations of AHTN were similar to those measured in the present work.

Natural estrogens, E1, E2 and E3, as well as the contraceptive agent, EE2, have been followed along the different sampling points. The two hormones E1 and E3 were detected in all samples analysed, at concentrations between 6-97 and 38-194 ng/L, respectively, therefore at least one order of magnitude below fragrances. On the other hand, EE2 was below the LOD during the whole sampling campaign. The third natural estrogen, E2, was found in almost all considered water samples, although at lower concentrations (MFR<11 ng/L). Similar tendencies for free natural estrogen concentrations have been reported for STP influents (Baronti et al., 2000; Onda et al., 2003; D'Ascenzo et al., 2003; Nakada et al., 2006), which is furthermore directly related to their excretion pattern in female urine, with E1 being the most abundant estrogen excreted by cycling women, and, in the case of pregnant women, being the levels of E3 and of E1 almost 2 and 1 order of magnitudes higher, respectively, than of E2 (Baronti et al., 2000; D'Ascenzo et al., 2003). The absence of EE2 in the considered municipal wastewater samples had been already reported by Carballa et al. (2004) for SP5. In general, concentrations reported for STP influents were in the low ng/L range (Baronti et al., 2000; de Mes et al., 2005; Clara et al., 2005a), according to the significant lower consumption of this drug (kg/year) compared to other pharmaceuticals such as antibiotics, anti-

inflammatories or anti-epileptics (t/year, Hirsch et al., 1999; Ternes et al., 1999a; Clara et al., 2005b).

The rest of PPCPs included in the monitoring of municipal wastewater (DCF, CBZ, DZP, FLX and CTL) were not detected or could not be quantified in any of the samples. In a previous sampling of SP5 performed by Carballa et al. (2004), the concentrations of DZP, CBZ, DCF were as well below the LOD. Results obtained for DZP were not surprising, taking into account the low consumptions reported for this compound (Clara et al., 2005b; Fent et al., 2006) and that only negligible amounts of a dose are excreted unchanged, since it is almost completely transformed into its main metabolite desmethyldiazepam, and to a minor extent to temazepam and oxazepam, which are excreted primarily in the urine conjugated as glucuronides (Klotz, 1977). Nevertheless, detections of DZP in STP influents of up to 1.2 µg/L and in the effluents of 0.7 µg/L have been reported by Fent et al. (2006). In the case of CBZ and DCF, low concentrations in municipal sewage, similar to the detection limits of the analytical methods employed in this work (chapter 2), have been measured (Lindqvist et al., 2005; Bendz et al., 2005; Nakada et al., 2006; Gomez et al., 2007), although one order of magnitude higher levels of DCF have also been reported (Gomez et al., 2007). Regarding the two anti-depressants (FLX and CTL) only one reference about their concentrations in urban wastewater has been found (Vasskog et al., 2006), where the low detection level of FLX has been confirmed, although somewhat higher concentrations of CTL (maximum of 612 ng/L) were measured.

Theoretical concentrations of the considered PPCPs could be estimated (Table 3-3) from national consumption rates provided by the Spanish Ministry of Health for pharmaceuticals and EE2, whereas in the case of natural hormones excretion rates and population distribution in the considered city according to sex and age, following data of the Spanish National Institute of Statistics, have been considered. Due to lack of Spanish consumption figures for fragrances, data for Europe have been extrapolated to Spanish population. For the calculations, Equation 3-1 was applied to pharmaceuticals, EE2 and musk compounds:

$$C_{calc} = \frac{A \cdot P \cdot E \cdot 10}{Q} \qquad \text{[Eq. 3-1]}$$

where, C_{calc} is the theoretically calculated concentration of the pharmaceutical compound in municipal wastewater (µg/L), A is the pharmaceutical consumption rate per inhabitant and year (g/capita.y), P is the number of inhabitants of the city, E is the amount of pharmaceutical excreted unmetabolised by humans (%) and Q is the flow rate of municipal wastewater (m^3/y).

In the case of estrogens, the methodology described in Johnson et al. (2000) was followed. Excretion rates of natural hormones was dependent on gender, and in the case of females additionally divided into menstruating females (15-49 years

old), post-menopause (above 49 years) and pregnant women (8.12/1000 inhabitants). The calculation was performed according to Equation 3-2:

$$C_{calc} = \frac{\sum P_i \cdot E_i}{Q} \qquad \text{[Eq. 3-2]}$$

where, C_{calc} is the theoretically calculated concentration of natural estrogens in municipal wastewater (ng/L), E_i is the excretion rate of naturals estrogen for one specific group (µg/capita.d), P_i is the number of inhabitants in the city pertaining to that specific group, and Q is the flow rate of municipal wastewater (m^3/d).

Table 3-3. Estimated concentrations of PPCPs in municipal wastewater, according to their consumption and excretion rates.

PPCP	Consumption in Spain (g/capita.y)	Excretion rates[(1)]	Calculated concentration[(2)]
IBP	4.57	15	1.9
DCF	0.53	15	0.22
NPX	0.54	10	0.15
CBZ	0.34	3	0.03
DZP	0.02	1	0.001
ROX	$1.9 \cdot 10^{-3}$	63	0.003
ERY	0.06	44-70	0.07-0.12
SMX	0.07	10-15	0.02-0.03
TMP	0.03	50-60	0.04-0.05
FLX	0.08	<10	0.02
CTL	0.03	10	0.01
IPM	0.11	100	0.31
HHCB	1.92	100[(3)]	5.3
AHTN	0.48	100[(3)]	1.3
ADBI	0.03	100[(3)]	0.1
EE2	$1.7 \cdot 10^{-5}$	26	0. 012
E1	-	3.9-600	9.4
E2	-	1.6-259	4.1
E3	-	1-6000	51

[(1)] In % for pharmaceuticals, EE2 and musk compounds and in µg/capita.d for natural estrogens

[(2)] Concentrations in µg/L, except for estrogens (E1, E2, E3 and EE2) in ng/L.

[(3)] Fragrances are not ingested, thus the value considered for E is 100%.

Comparing the calculated concentrations (Table 3-3) with the measured ranges for MWW (Table 3-2) a good concordance can be observed for all compounds, except for NPX, for which the minimum measured concentration is almost one order of magnitude higher than the predicted one. However, taking into account that only

the fraction of unmetabolised parent compound has been considered in the prediction, and that around 60% of NPX can be excreted as glucuronide, this higher levels of NPX may be associated to the cleavage of these conjugates in the sewer system, as β-glucoronidase enzymes are reported to be commonly present in sewers (Johnson and Sumpter, 2001).

Antibiotics and IPM have not been analysed in municipal wastewater. Comparing the predicted concentrations with previously reported data for STP influents, similar ranges for TMP and SMX have been found in Bendz et al. (2005), although almost one order of magnitude higher concentrations have been detected in sewage from Switzerland (Gobel et al., 2005), although this could be attributed to the higher consumption of these antibiotics in Switzerland compared to Spain. Additionally, it is known that 50% of the administered dose of SMX is excreted as its metabolite, N^4-acetylsulfamethoxazole, which could be hydrolysed back in the sewer system leading to an increased level of SMX at the inlet of the STP (Gobel et al., 2005). The consumption per capita of ROX in Switzerland was around 10 fold higher than in Spain, which was in agreement with the one order of magnitude higher concentration reported for this compound in Gobel et al. (2005) compared to the predicted concentration in Table 3-3. Concentrations of IPM in municipal wastewater in the range of 6-9 μg/L have been reported (Ternes and Hirsch, 2000; Carballa et al., 2004), although this concentration is expected to vary in a wide range taking into account that this compound is generally not removed in STPs (Ternes and Hirsch, 2000; Carballa et al., 2004) and that high variability of concentrations reported for STP effluents, with maximum levels of 11 μg/L, but median concentrations 0.75 μg/L (Ternes and Hirsch, 2000).

3.3.2.2. Occurrence of PPCPs in hospital wastewater

Wastewater consumption in the three hospitals included in the sampling campaigns was 429±63, 50±26 and 236±27 m^3/d for the hospitals discharging at SP2, SP3 and SP4, respectively. This means that water consumption per bed in hospitals was in the range of 580-820 L/bed·d, which is consistent with previously reported data for France (750 L/bed·d, CLIN Paris-Nord, 1999), and even somewhat lower than the specific consumption determined in an Indian hospital (1200 L/bed·d, Gautam et al., 2007). In any case, the average water consumption of hospitals was significantly higher when compared with that of common households (~100 L/capita·d).

Concerning PPCP concentrations measured within the samplings (Table 3-2) it is worth to note that the overall maximum levels for the three anti-inflammatory drugs (IBP, NPX and DCF), CBZ, DZP, ADBI and the three natural estrogens (E1, E2 and E3) have always be detected in hospital effluents. A second characteristic of hospital effluents was related to the wide range of concentrations measured during the different samplings, indicating that these types of streams are significantly less homogeneous than municipal wastewater. For example, for IBP concentrations in

the range of 0.8-75 µg/L have been measured in the present work (Table 3-2), which was very similar to the trend reported in Gomez et al. (2006), where between 1.5 and 151 µg/L of IBP were found in the hospital effluent sampled. In this same survey of Gomez et al. (2006), DCF and FLX have also been monitored and once more the results were consistent with those obtained in the present work, but this was not the case for CBZ concentrations, since in Gomez et al. (2006) only 0.03-0.07 µg/L have been found, whereas up to 42 µg/L were measured in the present research.

The comparison of municipal and hospital wastewater in terms of PPCP concentrations has been graphically represented in Figure 3-3 for two sampling campaigns where the differences were pronounced, although the complete set of figures has been included in the annex of the chapter.

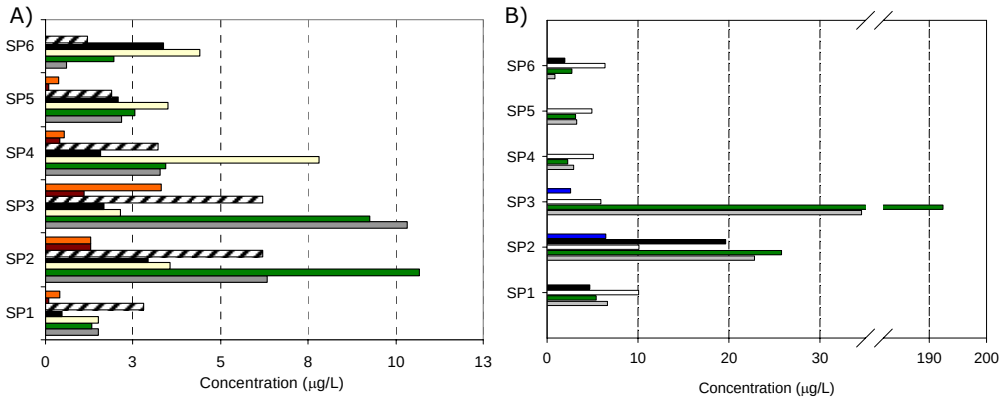

Figure 3-3. Concentration profile of PPCPs in the different SP from A) April 21st and B) June 23st. IBP (■), NPX (■), HHCB·10 (□), AHTN·10 (■), ADBI·10 (■), E1·100 (◪), E2·100 (■) and E3·10 (■).

In Figure 3-3 A (data from April 21st), higher PPCP concentrations have been detected for all compounds considered, especially in SP2 and SP3, being the differences in concentrations from 2 fold (for E1) up to 13 fold (for E2) higher in these streams when compared to the municipal wastewater upstream (SP1). In some occasions, as that illustrated in Figure 3-3 B for NPX (data from June 23st), the differences could be even higher, in this specific case, almost 40 fold higher concentrations of this pharmaceutical have been detected in SP3 than in SP1.

These influences could also be analysed in terms of mass flows, according to Equation 3-3:

$$HC_i = \frac{Q_H \cdot C_{i,H}}{Q_{SP5} \cdot C_{i,SP5}} \cdot 100 \qquad \text{[Eq. 3-3]}$$

where HC is the contribution of hospital effluents to the concentration of the PPCP (i) at the inlet of the STP (%), Q_H and Q_{SP5} are the flow rates of the wastewater discharged at the hospital considered and the total flow reaching the municipal STP, respectively (m^3/d), whereas $C_{i,H}$ and $C_{i,SP5}$ are the concentration of the considered PPCPs (i) at those locations (µg/L).

Table 3-4. Contribution of hospital effluents to the concentrations of PPCP in the influent of the STP (HC_i according to Equation 3-3).

Sampling Campaign	Sampling Point	IBP	NPX	HHCB	AHTN	E1	E2	E3
April 21st	SP2	2	3	1	1	2	9	2
	SP3	0	0	0	0	0	1	0
	SP4	0	0	1	0	0	1	0
June 23rd	SP2	4	5	1	55	n.a.	n.a.	n.a.
	SP3	1	3	0	0			
	SP4	0	0	0	0			
Sept. 15th	SP2	1	2	0	0	0	2	2
	SP4	0	0	0	0	2	0	0
Sept. 22nd	SP2	0	1	0	1	3	4	15
	SP4	0	1	0	0	0	0	0

For the two samplings represented in Figure 3-3, the contribution of hospitals was in general negligible (<10%) with the exception of AHTN discharge at SP2 which was one order of magnitude higher than its concentration at the STP inflow. As already observed in the concentration profiles, the hospital discharging at SP4 was the one with the lowest influence on STP influent concentrations (Table 3-4). From the data of Figure 3-3 B, the concentration of NPX in SP3 was outlined, although in terms of mass flows, it was the hospital responsible for the concentration of NPX in SP2 that was responsible to a higher degree for the overall discharge of this compound (the calculated HC_{NPX} was 3% for SP3 and 5% for SP2). The results for IBP were similar to those for NPX, being the highest concentrations contained in SP3, although the highest contribution was identified for SP2 (HC_{IBP} 1% and 4% for SP3 and SP2, respectively). By far, the highest influence of natural estrogens on municipal wastewater was related to hospital discharge SP2.

In other sampling campaigns, as those represented in Figure 3-4 (data from September 15th and 22nd in A and B, respectively), the concentration profiles among the different SP were more homogeneous, being the discharges of hospitals, in particular SP2, only more concentrated regarding natural estrogens (E2 and to a bigger extent E3), which is also reflected in the calculated HC_i of Table 3-4. The higher influence observed for estrogens could be related to the fact that the hospital responsible for the effluent from SP2 is where pregnant women (who present 2 and 3 orders of magnitude higher excretion rates for E2 and E3, respectively) make their routine check-ups and give birth.

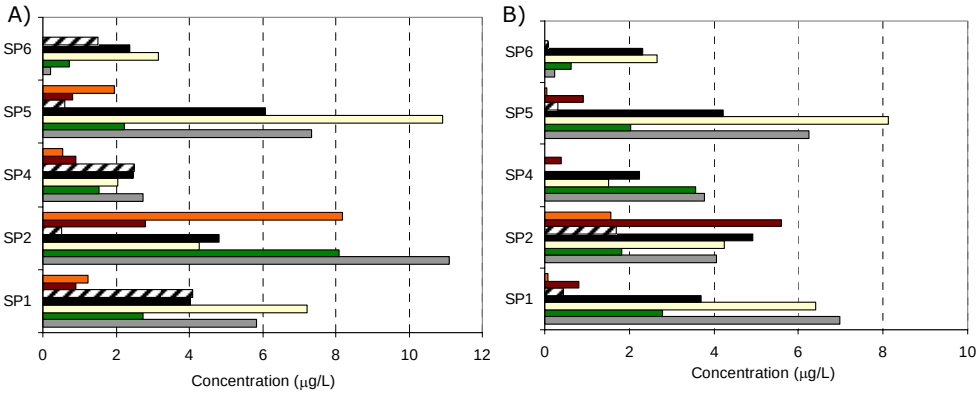

Figure 3-4. Concentration profile of PPCPs in the different SP from A) September 15th and B) September 22nd. IBP (■), NPX (■), HHCB·10 (□), AHTN·10 (■), E1·100 in A and E1·10 (◪), E2·100 (■) and E3·10 in A and E3·1 in B(■).

For antibiotics and IPM, concentrations have only been followed in SP2, although if these data were compared with the calculated concentrations in municipal wastewater according to PPCP consumptions (Table 3-3), at least one order of magnitude higher concentrations have been detected in SP2 for ERY, SMX and TMP. Hospital effluents surveyed in previous works contained antibiotics in the range of 0.01-13, 0.01-7.6 and 0.01-0.03 μg/L for SMX, TMP and ERY, respectively (Lindberg et al., 2004; Brown et al., 2006; Gomez et al., 2006). Except for ERY, for which concentrations of up to 2 μg/L have been measured in the current work, the results obtained were in agreement with these previously reported data (Table 3-2).

In the case of IPM maximum concentrations above 1 mg/L have been measured in several occasions, which, taking into account the dilution of the hospital effluent upon discharge into municipal sewage, would led to a maximum expected concentration in municipal wastewater of 5.3 μg/L, thus still one order of

magnitude higher than the concentration of 0.3 µg/L calculated according to IPM consumption rates (Table 3-3). A possible explanation for this discrepancy could be the fact that for the calculations of municipal wastewater concentrations in Table 3-3, homogeneous consumption of PPCPs has been assumed, which is not the pattern for IPM intake, since it is exclusively administered in hospitals and excreted almost unchanged after a short retention time (~2 h) at or close to the hospital itself. In fact, the concentration of 5.3 µg/L of IPM estimated in this work is consistent with data reported by Ternes and Hirsch (2000), where several µg/L of this compound were measured in STP effluents, which, taking into account that this compound was generally not transformed during wastewater treatment (Ternes and Hirsch, 2000; Carballa et al., 2004), would lead to similar concentrations in STP influents. Apart from that, concentrations of IPM in hospital effluents in the ppm range were not surprising, considering that for European hospitals concentrations of Adsorbable Organic Halogen Compounds (AOX) of up to 8 mg/L have been reported, which were mainly associated to chlorinated and iodinated compounds (AOCl and AOI, respectively), and, furthermore, being AOI mainly caused by X-ray contrast media (Kümmerer, 2004).

3.3.2.3. Removal of PPCPs in STP

A rough estimation of removal efficiencies achieved for the selected PPCPs in the STP of the city was performed applying Equation 3-4:

$$\text{Removal}\,(\%) = \frac{C_{i,SP5} - C_{i,SP6}}{C_{i,SP5}} \cdot 100 \qquad \text{[Eq. 3-4]}$$

where $C_{i,SP6}$ is the concentration of the considered PPCPs (i) at SP6 (µg/L).

Removal efficiencies for the compounds commonly detected during the sampling campaigns have been represented in Figure 3-5. It has to be noted that only elimination from the liquid phase was contemplated, without distinguishing between sorption, volatilisation or transformation.

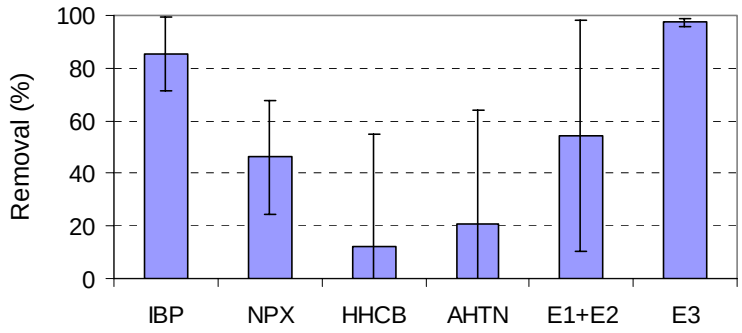

Figure 3-5. Removal of PPCPs in the STP.

The most efficiently removed compounds from Figure 3-5 were IBP and E3 (>85%), consistent with previously reported data (D'Ascenzo et al., 2003; Johnson et al., 2005; Clara et al., 2005b; Nakada et al., 2006; Gomez et al., 2007). The two natural estrogens E1 and E2 have been analysed in combination, taking into account that E2 is very quickly transformed into E1 in aerobic processes (Johnson and Sumpter, 2001), leading to an average removal of 54%, which was in between the removal reported by Carballa et al. (2004) and D'Ascenzo et al. (2003). Similar removal has been measured for NPX, in agreement with the results obtained in a previous sampling of the same STP (Carballa et al., 2004), although in the lower part of the ranges for NPX removal reported in the literature (Joss et al., 2005; Lindqvist et al., 2005). The results observed for the two fragrances were the most surprising ones, taking into account the low average removal determined when compared to other results (Carballa et al., 2004; Bester, 2004; Kupper et al., 2006) and the high variations between the different sampling campaigns. The factors that are thought to affect removal of PPCPs and could partially explain the discrepancies between results of different authors will be discussed in detail in chapters 4 and 5 of the present work.

3.3.2.4. Occurrence of PPCPs in STP effluents

The two anti-inflammatory drugs IBP and NPX were those detected at the highest concentration in the effluent from the STP included in the sampling (Table 3-2). For IBP and DCF levels in the range of 0.1-28 µg/L and 0.1-2.2 µg/L, respectively, have been reported in the literature for STP effluents (Lindqvist et al., 2005; Bendz et al., 2005; Gomez et al., 2007), thus in line with the present results, although at the lower part of the wide range in the case of IBP.

From the musk compounds, ADBI was less frequently detected in SP6 than HHCB and AHTN, being the concentrations of the latter between 0.2-0.8 µg/L. In the monitoring of fragrances performed by Ricking et al. (2003) and by Kupper et al. (2006) similar trends have been observed, although higher concentrations of HHCB, up to 3.7 µg/L, have also been detected in STP effluents (Reiner et al., 2007).

From the natural estrogens included in this study, only E1 has been found after the passage of the wastewater through the STP at concentrations of 2-32 ng/L, in agreement with results obtained elsewhere (Castiglioni et al., 2005; de Mes et al., 2005; Young, 2004). Estradiol and EE2 have been detected in STP effluents in other researches, although at low concentrations (<9 ng/L according to de Mes et al., 2005).

The antiepileptic CBZ has only been detected once, which implies that its concentration was at least 0.5 µg/L (LOD). The presence of this compound in STP effluents was not surprising according to its high resistance to conventional wastewater treatment processes. In fact it has been detected in the µg/L range in several STP discharges (Heberer, 2002a; Castiglioni et al., 2005; Bendz et al., 2005) and even in drinking water traces of CBZ were identified (Heberer, 2002a).

Diazepam is less frequently detected in effluents from STP and, in any case, maximum concentrations were clearly below 100 ng/l (Castiglioni et al., 2005; Heberer, 2002a).

Monitoring of STP effluents is essential in order to evaluate the potential impact of their discharge into surface waters, especially in those places with low surface water flows. In several works a direct correlation between the discharges from municipal STPs and the concentrations of PPCPs in surface waters was determined (Hirsch et al., 1999; Heberer, 2002b; Lindqvist et al., 2005).

For this particular situation, the risk derived from the discharge of STP effluents containing PPCPs to aquatic organisms could be roughly evaluated following a procedure based on the basic concept of environmental risk assessment (EC, 2003), that consists of comparing a predicted or measured environmental concentration (PEC or MEC) with a Predicted No Effect Concentration (PNEC). A risk characterisation ratio (PEC or MEC/PNEC) higher or equal to 1 means that the risk for the environment is unacceptable, thus risk management has to be contemplated. The PECs have been estimated from the concentrations of PPCPs in SP6, starting with the worst-case assumption of no surface water dilution (PEC = $C_{i,SP6}$) and taking the maximum concentration measured during the samplings. The PNECs have been taken from the literature (Balk and Ford, 1999; Webb, 2004; Young et al., 2004; de Mes et al., 2005; Lindqvist et al., 2005) and once again, the worst case has been always considered.

Table 3-5. Calculation of the risk characterisation ratio for those PPCPs detected in the effluent of the STP.

PPCP	PEC	PNEC	Risk ratio
IBP	2.5	5	0.5
DCF[(1)]	0.3	116	0.003
NPX	4.1	128	0.03
CBZ[(1)]	1.4	0.42	3.3
DZP[(2)]	0.2	4.3	0.05
FLX[(2)]	20	26	0.8
CTL[(2)]	0.020	3.9	0.005
HHCB	0.8	6.8	0.1
AHTN	0.3	3.5	0.09
EE2[(2)]	5	0.1	50
E1	32	3-5	11
E2[(2)]	2	1	2
E3[(2)]	2	>5	<0.4

Concentrations in µg/L, except for E1, E2, E3, EE2 and FLX in ng/L.
[(1)] LOQ has been considered; [(2)] LOD has been considered

Risk characterisation ratios from Table 3-5 indicated that under worst-case assumptions potential risk to the aquatic organisms would be exerted by CBZ, EE2, E1 and E2 discharges. For these compounds the risk evaluation should be further refined concerning the PEC or the PNEC. If the default surface water dilution factor from the EU (EC, 2003) was considered in the PECs, STP effluent concentrations were reduced one order of magnitude when discharged into surface water, which would reduce the PEC/PNEC ratio below 1 for CBZ and E2. In the case of CBZ, estimated concentration in surface water after dilution was 0.14 µg/L which would be consistent with the maximum level of this compound reported for different rivers (60-90 ng/L according to Vieno et al., 2006; Gros et al., 2007; Kim et al., 2007), although maximum concentrations up to the µg/L range have also been reported in the literature, not only for surface water, but also for groundwater (Heberer, 2002a). The PEC for E2 would be reduced to 0.2 ng/L after incorporating the dilution factor, which is in the range of surface water concentrations found in Baronti et al. (2000), although concentration in the higher ng/L level have also frequently been reported (de Mes et al., 2005), thus no definite conclusion about the risk associated to E2 exposure could be made.

In the case of estrone the PNEC used was based on a limited dataset and therefore considered as a provisional value (Young, 2004). The surface water concentration estimation of 3.2 ng/L seems coherent with measured levels in river water (Baronti et al., 2000; de Mes et al., 2005; Kim et al., 2007), leading to a risk

ratio close to 1 (0.6-1.0), thus indicating a potential risk for the aquatic environment.

For EE2, the refined PEC was 0.5 ng/L, which should be reconfirmed by measurements in river water, since previously reported data vary within a wide range of concentrations (0.04-4.3 ng/L according to Baronti et al., 2000; Heberer, 2002; de Mes et al., 2005), which would still lead to a PEC/PNEC of 5 indicating potential risk. In any case, it is worth to note that this PNEC was derived from the most sensitive aquatic species that was fish, to protect them from vitellogenin induction (Young, 2004).

3.4. Conclusions

Municipal wastewaters collected during the sampling campaigns could be classified as moderately polluted, whereas hospital effluents were in general stronger contaminated and maximum concentrations of TS, TSS and COD were at least 3-fold higher than standard values for concentrated municipal sewage.

From the 19 PPCPs included in the survey, the synthetic hormone EE2 and the anti-depressants FLX and CTL were generally not detected, and in the few cases were the anti-depressants could be identified it was in the effluents from hospital origin.

Municipal wastewater contained highest concentrations of the anti-inflammatory drugs IBP and NPX, for which several µg/L of compound have been detected in all samples collected. From the considered fragrances, HHCB and AHTN were detected in almost all samples at concentrations around 1 µg/L, whereas ADBI was in general not detected. The natural estrogens E1 and E3 were detected in all samples analysed, at concentrations between 6-97 and 38-194 ng/L, respectively, therefore almost one order of magnitude below fragrances, although the third natural estrogen considered, E2, was found at lower concentrations (in general <11 ng/L). The rest of PPCPs included in the monitoring of municipal wastewater (DCF, CBZ, DZP, EE2, FLX and CTL) were not detected or could not be quantified in any of the samples considered.

The water consumption per bed in hospitals was in the range of 580-820 L/bed·d, thus significantly higher than that of common households. It is worth to note that the overall maximum levels for IBP, NPX, DCF, CBZ, DZP, ADBI and the three natural estrogens (E1, E2 and E3) have always been measured in hospital effluents. In fact, maximum concentrations in hospital wastewater for IBP, NPX and CBZ of 74.7, 192 and 41.8 ppb, respectively have been measured, whereas the maximum level for these compounds in urban wastewater was below 9 ppb. In the case of IPM concentrations in the mg/L range have been detected in several samplings. The most pronounced difference between municipal and hospital wastewater within one sampling campaign has been measured for NPX in June 2005, with concentrations of 3-6 µg/L and 160-190 µg/L, respectively. Apart from

that hospital effluents were significantly less homogeneous than municipal wastewaters regarding the content of PPCPs.

From the three hospitals considered in this work, the one that discharged at SP3 was the less polluted concerning conventional contaminants, and could be perfectly assimilated as urban wastewater, but for the high concentrations of some PPCPs detected in that stream.

Removal of PPCPs from the liquid phase during their passage trough the STP has been calculated. The most efficiently removed compounds were IBP and E3 (>85%), followed by E1+E2 and NPX (~50%) and, finally by the two fragrances HHCB and AHTN for which high variations between results from different sampling campaigns have been observed, as well as a quite low average removal (<20%).

In agreement with the analysis of municipal wastewater, the two anti-inflammatory drugs, IBP and NPX, were those detected at the highest concentration in the effluent from the STP. From the musk compounds, ADBI was less frequently detected than HHCB and AHTN, being the concentrations of the latter between 0.2-0.8 µg/L. From the natural estrogens included in this study, only E1 has been found after the passage of the wastewater through the STP at concentrations of 2-32 ng/L. These concentrations have been used to evaluate the potential risk derived from the discharge of the STP effluent into the receiving river, concluding, under worst-case assumptions, that CBZ, EE2, E1 and E2 could exert a potential adverse effect on aquatic organisms.

3.5. References

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3.6. Annex

Table I. Characterisation of the wastewaters regarding conventional parameters in the different sampling points.

Table II. Concentrations of PPCPs among sampling points during the different sampling campaigns.

Table III. Contribution of hospital effluents to the concentrations of PPCP in the influent of the STP (HC_i according to Equation 3-3).

Figure I. Concentration profile of PPCPs in the different SP from sampling campaigns of February 2nd (A) and 9th (B) and of June 16th (C).

Table I. Characterisation of the wastewaters regarding conventional parameters in the different sampling points.

Sampling campaing	Sampling point	TS	VS	TSS	VSS	COD_T	COD_S	Cl^-	SO_4^{-2}	NO_2^-	$N\text{-}NO_3^-$	$N\text{-}NH_4^+$	$P\text{-}PO_4^{-3}$	TIC	TOC	NTK
April 2004	SP1	475	195	107	100	163	96	126	48	<LOD	0.3	37.2	4.0	45.2	19.9	29.5
		298	170	62	35	75	21	17	37	<LOD	1.4	5.2	0.7	12.3	8.8	9.2
	SP2	580	343	162	157	479	257	50	25	<LOD	0.0	32.4	2.0	33.1	46.7	27.6
		533	332	112	110	392	248	88	19	<LOD	0.0	17.6	4.1	88.1	26.2	33.6
	SP3	845	460	292	270	765	393	66	49	<LOD	0.0	86.7	6.0	78.3	90.6	67.6
		537	277	157	65	467	344	50	54	<LOD	0.2	61.7	2.0	34.9	99.5	46.3
	SP4	808	375	360	272	463	182	52	43	<LOD	0.0	30.1	3.4	39.4	31.4	25.5
		440	311	200	140	291	189	27	31	<LOD	0.0	13.6	1.1	17.5	26.0	12.9
	SP5	298	105	82	50	35	26	32	27	<LOD	2.3	8.7	0.7	18.2	6.1	8.0
		300	165	147	100	140	21	15	17	<LOD	1.0	9.7	0.8	12.8	9.5	10.5
	SP6	440	98	30	27	57	22	179	75	<LOD	3.4	11.3	1.0	33.6	6.3	10.3
		470	231	17	17	17	14	86	33	<LOD	10.8	0.3	0.0	11.7	7.3	4.3
September 2004	SP1	480	196	192	163	315	26	74	35	<LOD	<LOD	24.9	<LOD	31.8	15.5	21.5
		318	250	146	134	195	34	69	48	<LOD	1.8	27.9	2.1	26.6	14.8	25.4
	SP2	792	474	270	178	843	198	139	57	<LOD	<LOD	49.0	<LOD	43.5	70.8	45.1
		646	464	236	222	575	148	161	50	<LOD	<LOD	33.9	3.9	33.8	45.2	34.2
	SP3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SP4	484	224	196	179	550	118	73	22	<LOD	<LOD	17.8	<LOD	24.0	42.2	18.4
		2955	2679	1464	1406	3585	433	77	21	<LOD	2.7	37.5	3.9	14.2	147	41.8
	SP5	612	270	267	233	525	73	49	22	<LOD	<LOD	24.8	<LOD	30.9	27.6	22.5
		724	436	350	255	575	80	96	57	<LOD	<LOD	25.6	2.4	38.6	28.9	24.5
	SP6	368	92	20	20	47	20	104	49	<LOD	2.6	9.6	<LOD	15.3	7.9	6.5
		274	130	22	20	32	20	112	68	<LOD	<LOD	7.7	<LOD	19.8	8.9	8.5

Table I. continues

Sampling campaing	Sampling point	TS	VS	TSS	VSS	COD_T	COD_S	Cl^-	SO_4^{-2}	NO_2^-	$N\text{-}NO_3^-$	$N\text{-}NH_4^+$	$P\text{-}PO_4^{-3}$	TIC	TOC	NTK
February 2005	SP1	1010	420	466	282	501	195	74	42	<LOD	<LOD	24.2	<LOD	41.1	53.0	29.2
		414	228	172	145	223	63	31	36	<LOD	<LOD	28.6	<LOD	38.1	65.7	33.2
	SP2	997	420	271	251	545	254	159	<LOD	<LOD	<LOD	51.1	<LOD	46.1	66.5	44.1
		1213	543	275	249	744	537	227	18	<LOD	<LOD	25.7	<LOD	83.6	187	74.1
	SP3	570	280	160	145	436	428	60	16	<LOD	<LOD	66.5	<LOD	54.9	108	53.3
		447	273	154	140	327	192	35	27	<LOD	<LOD	48.6	<LOD	52.8	110	55.1
	SP4	497	220	78	68	382	285	58	19	<LOD	<LOD	34.5	<LOD	39.9	50.8	43.9
		490	293	114	104	400	301	44	21	<LOD	<LOD	23.1	<LOD	44.6	140	61.6
	SP5	427	170	191	134	187	49	40	44	<LOD	2.0	16.3	<LOD	12.0	16.9	22.8
		463	270	196	166	305	90	32	36	<LOD	1.5	27.4	<LOD	46.5	22.8	46.0
	SP6	303	60	8	8	25	12	60	40	<LOD	1.8	9.3	<LOD	10.1	9.4	9.0
		267	83	7	6	25	22	37	45	<LOD	<LOD	12.0	<LOD	17.7	20.4	22.9
June 2005	SP1	612	265	225	145	240	163	56	11	<LOD	<LOD	37.8	<LOD	41.8	22.8	34.1
		340	148	111	85	255	59	50	10	<LOD	<LOD	18.4	<LOD	27.1	16.9	19.3
	SP2	780	290	175	153	370	293	188	11	<LOD	<LOD	48.8	<LOD	57.9	30.8	51.6
		1105	528	285	265	901	101	300	8	<LOD	<LOD	80.1	<LOD	83.9	23.1	66.9
	SP3	623	373	183	165	604	283	98	10	<LOD	<LOD	99.4	<LOD	87.9	58.7	90.3
		495	197	77	77	439	338	78	30	<LOD	<LOD	91.4	<LOD	65.3	83.3	71.0
	SP4	673	414	218	209	802	281	66	6	<LOD	<LOD	12.2	<LOD	32.9	31.4	30.2
		350	205	114	112	471	104	54	6	<LOD	<LOD	28.5	<LOD	31.0	33.4	33.2
	SP5	608	274	254	195	398	112	62	12	<LOD	<LOD	37.3	<LOD	42.3	19.3	38.8
		1255	400	129	113	267	72	14	33	<LOD	<LOD	16.4	<LOD	<LOD	25.8	21.0
	SP6	240	90	13	13	19	12	28	5	<LOD	0.7	9.7	<LOD	17.2	7.9	11.6
		311	67	36	27	32	33	47	6	<LOD	<LOD	9.7	<LOD	20.7	14.6	12.7

Table I. continues

Sampling campaing	Sampling point	TS	VS	TSS	VSS	COD_T	COD_S	Cl^-	SO_4^{-2}	NO_2^-	$N\text{-}NO_3^-$	$N\text{-}NH_4^+$	$P\text{-}PO_4^{-3}$	TIC	TOC	NTK
November 2005	SP2-S1	735	510	339	331	2464	2277	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	78	697	58
		210	105	20	18	67	11	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	28	16	20
	SP2-S2	2157	350	225	205	504	164	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	154	42	67
		363	65	126	28	67	67	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	13	29	6
March 2006	SP2-S1	558	415	131	117	700	459	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	24	163	48
		518	305	151	143	224	129	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	28
	SP2-S2	2351	476	130	107	392	182	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	73
		1208	390	63	52	336	267	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	22
June 2006	SP2-S1	838	546	265	244	1012	588	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	25	183	54
		901	677	302	290	1084	487	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	46	162	66
	SP2-S2	2909	611	218	209	1571	1255	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	110	410	65
		2632	656	224	210	805	554	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	90	195	54

* All concentrations expressed in mg/L. n.a. Not analysed. LOD Limit of detection (Chapter 2)

Table II. Concentrations of PPCPs among sampling points during the different sampling campaigns.

Sampling campaing	Sampling point	IBP	NPX	DCF	CBZ	DZP	HHCB	AHTN	ADBI	ROX	ERY	SMX	TMP	IPM
November 2005	SP2-S1	21.7	12.6	4.00	0.54	1.1	2.57	1.61	3.38	0.25	0.066	0.510	0.87	1400
		5.60	5.34	<LOD	<LOD	<LOD	0.72	0.13	0.067	n.a.	n.a.	n.a.	n.a.	790
	SP2-S2	74.7	18.1	<LOQ	0.047	<LOD	0.79	0.38	<LOD	<LOD	<LOD	0.260	0.16	260
		0.76	1.13	<LOD	<LOD	<LOD	0.13	0.050	<LOQ	n.a.	n.a.	n.a.	n.a.	76
March 2006	SP2-S1	23.2	6.69	<LOD	0.13	<LOD	0.40	0.39	<LOD	<LOD	2.1	0.46	0.71	1600
		16.6	6.10	<LOD	0.14	<LOD	0.39	0.36	<LOD	<LOD	0.21	0.75	0.16	1100
	SP2-S2	22.3	3.54	0.50	0.026	<LOD	0.43	0.40	<LOD	<LOD	0.710	12	1.70	<2.6
		7.98	4.74	<LOD	0.62	<LOD	0.26	0.33	<LOD	<LOD	<LOD	9.8	0.70	110
June 2006	SP2-S1	19.9	20.6	0.95	$0.25^{(1)}$	$0.035^{(1)}$	0.38	0.15	<LOD	$<LOD^{(1)}$	$0.64^{(1)}$	$0.08^{(1)}$	$0.26^{(1)}$	$780^{(1)}$
		19.4	10.5	<LOD			0.30	0.14	<LOD					
	SP2-S2	13.8	10.8	<LOD	$0.18^{(1)}$	$0.027^{(1)}$	0.27	0.15	<LOD	$<LOD^{(1)}$	$0.20^{(1)}$	$0.10^{(1)}$	$0.22^{(1)}$	$1100^{(1)}$
		10.0	24.2	<LOD			0.20	0.10	<LOD					

Table II. continues

Sampling campaing	Sampling point	IBP	NPX	DCL	CBZ	DZP	HHCB	AHTN	ADBI	E1	E2	E3
April 2004	SP1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	97	15	182
		1.51	1.33	<LOQ	<LOD	<LOD	0.15	0.048	n.a.	28	1	40
	SP2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	72	21	573
		6.32	10.7	<LOQ	<LOD	<LOD	0.36	0.29	n.a.	62	13	129
	SP3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	95	28	353
		10.3	9.26	<LOD	<LOD	<LOD	0.21	0.17	n.a.	62	11	330
	SP4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	43	9	77
		3.26	3.42	<LOD	<LOD	<LOD	0.78	0.16	n.a.	32	4	55
	SP5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	31	2	74
		2.19	2.56	<LOQ	<LOD	<LOD	0.35	0.21	n.a.	19	1	38
	SP6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	32	<LOD	<LOD
		0.60	1.96	<LOQ	<LOD	<LOD	0.44	0.34	n.a.	12	<LOD	<LOD
September 2004	SP1	5.84	2.74	<LOQ	<LOQ	<LOD	0.72	0.40	<LOQ	41	9	122
		6.97	2.78	<LOQ	<LOQ	<LOD	0.64	0.37	<LOQ	43	8	73
	SP2	11.1	8.10	<LOQ	<LOQ	<LOD	0.43	0.48	<LOQ	5	28	818
		4.05	1.83	<LOQ	2.33	<LOD	0.42	0.49	<LOD	168	56	1552
	SP3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SP4	2.74	1.54	<LOQ	<LOQ	<LOD	0.20	0.25	<LOQ	25	9	53
		3.78	3.56	<LOQ	<LOD	<LOD	0.15	0.22	<LOD	<LOD	4	<LOD
	SP5	7.33	2.22	<LOQ	<LOQ	<LOD	1.09	0.61	<LOQ	6	8	194
		6.24	2.02	<LOQ	<LOQ	<LOD	0.81	0.42	<LOD	31	9	59
	SP6	0.21	0.74	<LOQ	<LOD	<LOD	0.31	0.24	<LOQ	15	<LOD	<LOD
		0.23	0.62	<LOQ	<LOQ	<LOD	0.27	0.23	<LOD	9	<LOD	<LOD
February 2005	SP1	7.61	6.30	<LOD	<LOD	<LOD	0.52	0.29	<LOD	27	<LOD	42
		5.74	4.06	<LOQ	<LOD	<LOD	0.60	0.30	<LOD	93	25	155
	SP2	5.37	7.86	<LOD	7.21	<LOD	0.48	0.31	<LOD	93	19	195
		3.70	14.8	<LOD	41.8	<LOD	0.37	0.37	<LOD	26	7	104
	SP3	7.46	4.17	4.04	18.6	<LOD	1.07	0.86	<LOD	98	24	187
		1.10	28.7	1.92	<LOD	<LOD	0.67	0.75	<LOD	40	11	29
	SP4	5.38	12.6	<LOD	8.03	<LOD	0.23	0.23	<LOD	<LOD	10	11
		7.44	24.1	2.09	27.4	<LOD	0.39	0.26	0.983	<LOD	<LOD	<LOD
	SP5	5.90	6.53	<LOQ	<LOD	<LOD	0.59	0.33	<LOD	30	<LOD	93
		7.28	5.35	<LOQ	<LOD	<LOD	0.94	0.41	<LOD	44	11	108
	SP6	0.21	4.06	<LOQ	<LOD	<LOD	0.72	0.28	<LOD	2	<LOD	<LOD
		2.50	2.20	<LOQ	<LOD	<LOD	0.76	0.27	<LOD	7	<LOD	<LOD

Table II. continues

Sampling campaing	Sampling point	IBP	NPX	DCF	CBZ	DZP	HHCB	AHTN	ADBI	E1	E2	E3
June 2005	SP1	8.60	6.29	<LOD	<LOD	<LOD	2.87	4.49	2.01	n.a.	n.a.	n.a.
		6.65	5.41	<LOD	<LOD	<LOD	1.01	0.47	<LOQ	n.a.	n.a.	n.a.
	SP2	4.88	10.0	<LOD	<LOD	<LOD	0.70	<LOD	<LOQ	n.a.	n.a.	n.a.
		22.8	25.8	<LOD	<LOD	<LOD	1.01	1.96	0.65	n.a.	n.a.	n.a.
	SP3	17.8	156	<LOQ	<LOQ	<LOD	0.89	<LOD	0.49	n.a.	n.a.	n.a.
		34.6	192	<LOD	<LOQ	<LOD	0.59	<LOD	0.26	n.a.	n.a.	n.a.
	SP4	16.6	3.18	<LOD	<LOD	<LOD	0.47	<LOD	0.16	n.a.	n.a.	n.a.
		2.94	2.28	<LOD	<LOD	<LOD	0.51	<LOD	<LOQ	n.a.	n.a.	n.a.
	SP5	7.85	5.07	<LOD	<LOD	<LOD	0.79	0.357	<LOQ	n.a.	n.a.	n.a.
		3.27	3.14	<LOD	<LOD	<LOD	0.49	<LOD	<LOD	n.a.	n.a.	n.a.
	SP6	0.40	2.39	<LOD	<LOD	<LOD	0.73	0.25	<LOQ	n.a.	n.a.	n.a.
		0.87	2.73	<LOD	<LOD	<LOD	0.64	0.20	<LOD	n.a.	n.a.	n.a.

(1) In the two sampling campaigns of June CBZ, DZP, antibiotics and IPM were analysed in a mixture of SP2-S1 and SP2-S2, at a ratio equivalent to their relative discharge (SP2-S1/SP2-S2 ~ 3.2)

* All concentrations expressed in µg/L, except for estrogens (E1, E2 and E3) in ng/L.

Table III. Contribution of hospital effluents to the concentrations of PPCP in the influent of the STP (HC_i according to Equation 3-3).

Sampling Campaign	Sampling Point	IBP	NPX	HHCB	AHTN	E1	E2	E3
February 2nd	SP2	1	1	0	1	2	6	1
	SP3	0	0	0	0	0	1	0
	SP4	0	1	0	0	0	1	0
February 9th	SP2	0	2	0	1	0	0	1
	SP3	0	0	0	0	0	0	0
	SP4	0	1	0	0	0	0	0
June 16th	SP2	0	1	1	0	n.a.	n.a.	n.a.
	SP3	0	2	0	0			
	SP4	1	0	0	0			

n.a. Not analysed

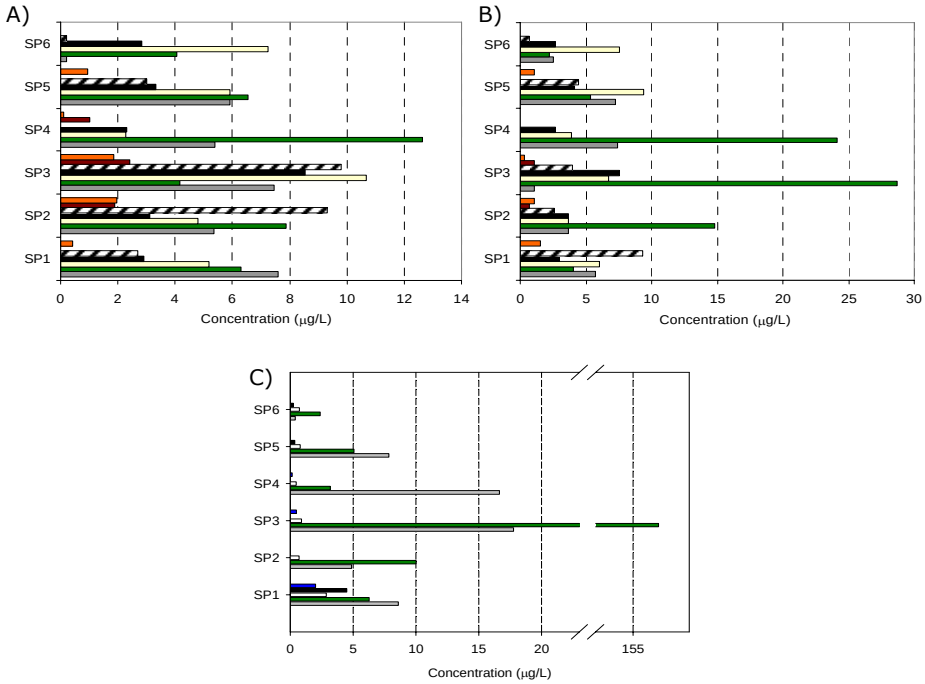

Figure I. Concentration profile of PPCPs in the different SP from sampling campaigns of February 2nd (A) and 9th (B) and of June 16th (C). IBP (■), NPX (■), HHCB·10 in A and B, HHCB in C (□), AHTN·10 in A and B, AHTN in C (■), E1·100 (◪), E2·100 (■) and E3·10 (■).

Chapter 4

Fate and removal of Pharmaceuticals and Personal Care Products (PPCPs) in a conventional activated sludge treatment process[1]

Summary

The fate and behaviour of 16 Pharmaceutical and Personal Care Products (PPCPs) during a conventional biological wastewater treatment process was assessed in a denitrifying/nitrifying pilot plant. Three musk compounds (galaxolide (HHCB), tonalide (AHTN) and celestolide (ADBI)), two hormones (the natural 17β-estradiol (E2) and the synthetic 17α-ethinylestradiol (EE2)) and pharmaceuticals of 5 different therapeutic classes (anti-epileptic: carbamazepine (CBZ), tranquiliser: diazepam (DZP), anti-depressants: fluoxetine (FLX) and citalopram (CTL), anti-inflammatories: ibuprofen (IBP), naproxen(NPX) and diclofenac (DCF) and antibiotics: sulfamethoxazole (SMX), roxithromycin (ROX), trimethoprim (TMP) and erythromicyn (ERY), have been considered, so as to represent case studies of compounds with substantially different physico-chemical properties.

The occurrence of the selected compounds on the basis of the concentrations in the liquid phase was determined in a first step, which was further complemented with a detailed mass balance, where the most relevant removal mechanisms during biological treatment have been considered (volatilisation, sorption and degradation).

The worst case was represented by CBZ, DZP and DCF, which remained unaltered during their passage through the pilot plant, whereas the highest transformation (>80%) has been determined for HHCB, AHTN, FLX, IBP, NPX and natural estrogens. Sorption has shown to play an important role in the biotransformation of the two musk compounds, which had previously shown not to be easily biodegraded, probably by enhancing their retention inside the pilot plant. The removal of the third fragrance considered (ADBI) was highly influenced by volatilisation in the aerobic tank, which supposed up to 45% of its overall elimination.

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

S. Suárez, M. Ramil, F. Omil and J.M. Lema (2005). Removal of pharmaceutically active compounds in nitrifying–denitrifying plants. Water Science and Technology **52**, 9-14.

Outline

4.1. Introduction

The introduction of the activated sludge process as wastewater treatment technology dates from 1913 (Johnson and Sumpter, 2001). Nowadays, it can be said that Sewage Treatment Plants (STPs) are designed for an efficient removal of organic matter. In fact, a large STP is able to treat up to 30,000 t/h of wastewater containing 300 mg/L BOD in a few hours with an efficiency higher than 97%, thus releasing a final effluent with BOD concentrations below 10 mg/L. The most widely used systems are Conventional Activated Sludge (CAS) units, operated at a Hydraulic Retention Time (HRT) of 4-14 hours, and biological filters, mostly used in small villages and operated at HRT of 0.5 hours (Johnson and Sumpter, 2001). More recently, in the last two decades, important progresses regarding the simultaneous elimination of organic matter and nutrients have been achieved, in some cases driven by stricter legal requirements. For example, in 1996 the Spanish Government introduced discharge limits for nitrogen and phosphorus (R.D. 509/1996), although only affecting sensitive areas, and four years later the "DIRECTIVE 2000/60/EC establishing a framework for Community action in the field of water policy" specified as ultimate aim to achieve the elimination of priority hazardous substances. It states that, when identifying priority hazardous substances, account should be taken of the precautionary principle, relying in particular on the determination of any potentially adverse effects of the product and on a scientific assessment of the risk.

Definitely, what can be seen is that in the last decades, when trying to improve the quality of water, the main focus shifted from conventional pollutants (organic matter, solids and nutrients) to more specific xenobiotic compounds, some of which detected at the low µg/L level and therefore described as micropollutants. These include between others aromatic hydrocarbons (Long et al., 1998), sulphonated compounds (Di Corcia et al., 1999) and, more recently, Pharmaceuticals and Personal Care Products (PPCPs).

Nowadays, the occurrence of PPCPs in urban wastewaters from all over the world is demonstrated (Ternes, 1998; Stumpf et al., 1999; Carballa et al., 2004; de Mes et al., 2005; Hua et al., 2006; Nakada et al., 2006). The resulting contamination of the aquatic media, including ground and surface water, depends mainly on the removal efficiency of STPs regarding these compounds. In fact, the direct relation that exists between the presence of PPCPs in surface water and the discharge of STP effluents has been evidenced in several works (Heberer et al., 2002; Stumpf et al., 1999), which is of special concern when the proportion of the discharge is significant with respect to the natural water flow. Some PPCPs can indeed be used as markers for municipal sewage in surface water, as for example caffeine, coprostanol or carbamazepine (Heberer et al., 2002; Clara et al., 2004b). There are numerous works that evidence that the present STPs are not designed for

the complete elimination of this type of substances (Ternes et al., 1999b; Baronti el al., 2000; Bester, 2004; Kupper et al., 2006; Gómez et al., 2007), with variable removal efficiencies depending on the compound, but also on the treatment plant considered.

Parameters such as HRT, SRT, redox conditions and temperature are thought to affect the removal of PPCPs. The HRT represents the mean time that the liquid phase remains within the treatment process. It was shown to affect elimination of ibuprofen and ketoprofen (Tauxe-Wuersch et al., 2005), in a way that lower removal was observed for shorter HRTs. Similarly, Drewes et al. (2002) concluded that facilities employing longer HRTs during treatment showed significant lower effluent concentration for analgesic drugs and gemfibrozil. On the other hand, the SRT determines the mean residence time of microorganisms in the reactor, consequently only organisms which are able to reproduce themselves during this time can be retained and enriched in the system. According to this definition, high SRTs allow the enrichment of slowly growing bacteria and consequently, the establishment of a more diverse biocoenosis with broader physiological capabilities (Clara et al., 2005a). Generally speaking, activated sludge systems without nitrification work at SRTs between 4 and 5 days, for nitrification and nitrogen removal between 8 and 20 days, depending on the aerobic/anoxic-volume ratio, and for nitrogen removal and simultaneous sludge stabilization around 25 days are installed in the plant (Clara et al., 2004b). For several PPCPs a positive effect on their removal has been observed when working at higher SRT and a critical value for this parameter of 10 days was identified (Clara et al., 2005a). Regarding redox conditions and temperature, differences in the removal efficiencies for some PPCPs have been reported (Ternes et al., 1999b; Joss et al., 2004).

The vast majority of data published in the field of removal of PPCPs from wastewater refer to full-scale STPs, where only the raw influent and final effluent is sampled, in order to measure soluble concentrations of the considered PPCPs. Therefore, only the overall removal efficiency including primary and secondary treatment can be determined, without distinguishing between sorption, volatilization or transformation. There are some exceptions of works dealing with the importance of sorption and volatilization (Bester, 2004; Joss et al., 2004; Clara et al., 2005a; Joss et al., 2005; Kupper et al., 2006), authors that considered different sampling points in full-scale STPs, therefore allowing to distinguish the removal efficiency of the primary and secondary treatment step (Carballa et al., 2004; Kupper et al., 2006), and research where sampling was limited to the influent and the effluent of the biological reactor (Joss et al., 2004; Joss et al., 2005; Jones et al., 2007). Additional information about the behavior of PPCPs in biological lab- and pilot-scale plants is also available, although much less frequent (Zwiener et al., 2000; Clara et al., 2004; Clara et al., 2005a; Suarez et al., 2005) and with samples taken exclusively from the influent and effluent.

The aim of the present work was to perform a detailed study of the fate and behavior of 16 PPCPs in a pilot plant that represents the most common technology used in full-scale STPs. The reactor was fed with a synthetic medium in order to maintain a complete control of the system and to avoid the complexity of real wastewater, such as the presence of conjugates, metabolites or colloidal solids that could interfere with the reliable quantification of the considered substances in the influent. An extensive sampling including the different streams of the system was carried out so as to evaluate the influence of the different redox conditions (anoxic and aerobic) on the transformation of selected micropollutants. Additionally, the effect of temperature and installed SRT on the performance of the system was analyzed.

4.2. Materials and methods

4.2.1. Activated sludge treatment plant

The experimental equipment used is an activated sludge system divided into a first anoxic and a second aerobic zone, supplied with a secondary sedimentation tank (Figure 4-1). The total useful volume of the reactor is 30 L, of which 40% correspond to the anoxic fraction and the rest to the aerobic compartment.

Figure 4-1. Activated sludge pilot plant.

Feeding system

The reactor was fed with a synthetic medium that consisted of an on-line mixture of tap water and a concentrate at a ratio 9:1. Tap water was stored in a stainless steel tank with a capacity of 160 L and impelled to the reactor with and average flow rate of 27 L/d by means of a peristaltic pump (P-1: Masterflex® Console Drive, 1-100 rpm). Once this pump was calibrated, the flow rate was additionally checked following the decrease in the water level inside the storing tank (by means of an external calibrated glass tube). The concentrate was held in an aluminium tank of 30 L useful volume and fed into the reactor with a separate peristaltic pump (P-2: Masterflex® L/S Economy Drive, 2-200 rpm) at a flow around 3 L/d (Figure 4-2). This flow was maintained with a regular calibration of the pump and checked following the decrease in the weight of the storing tank. The resulting HRT was 1 day.

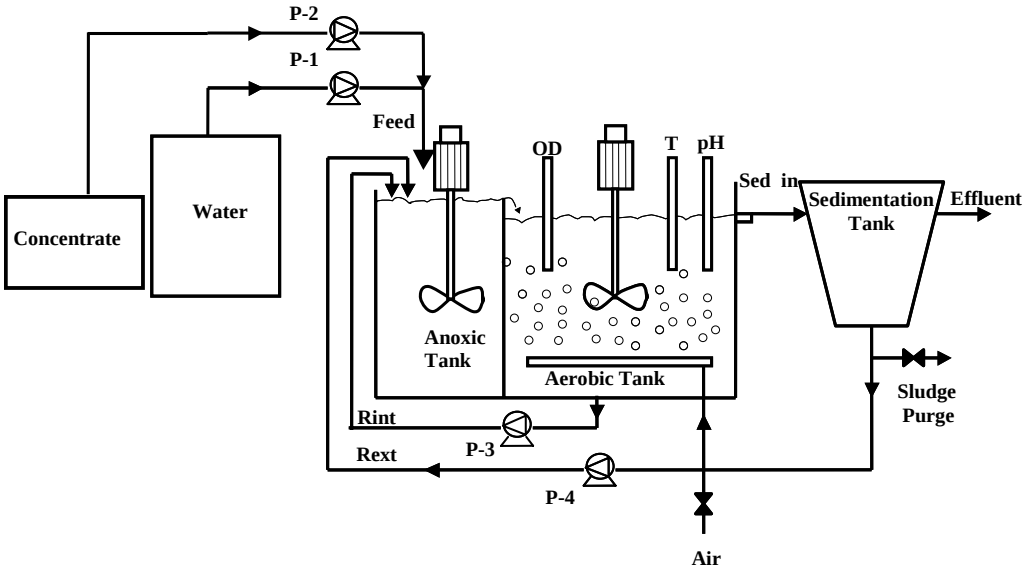

Figure 4-2. Schematic diagram of the activated sludge pilot plant.

The composition of the resulting mixture from these two streams tried to reproduce the chemical characteristics of a medium charged urban wastewater with an average composition of 500 mg/L of COD, 40 mg/L of N-NH_4 and 8 mg/L of P-PO_4 (Table 4-1).The pH of the feed was adjusted to 7 with the help of concentrated sulphuric acid.

Table 4-1. Composition of the synthetic feed and of the trace solution.

Compounds in the fed	Concentration (mg/L)	Compounds in the trace solution	Concentration (g/L)
CH_3COONa	619	$FeCl_3 \cdot 6H_2O$	1.5
NH_4Cl	153	H_3BO_3	0.15
Na_2HPO_4	24.3	$CuSO_4 \cdot 5H_2O$	0.03
KH_2PO_4	11.8	KI	0.03
$NaHCO_3$	200	$ZnSO_4 \cdot 7H_2O$	0.12
Trace solution(1)	0.1	$CoCl_2 \cdot 6H_2O$	0.15
		$MnCl_2 \cdot 4H_2O$	0.12

(1) Concentration in mL/L

The wastewater was introduced into the anoxic tank where the denitrifying process takes place. Heterotrophic bacteria are responsible for the removal of nitrogen, since they utilize nitrate for the oxidation of organic matter in the absence of oxygen (Equation 4-1).

$$8\ NO_3^- + 5\ NaCH_3CO_2 \rightarrow 10\ CO_2 + 4\ N_2 + H_2O + 5\ NaOH + 8\ OH^- \qquad [Eq.\ 4\text{-}1]$$

After passing the anoxic compartment the wastewater flowed into the aerobic tank where the nitrification process and the oxidation of organic matter occurred. Nitrification consists in the conversion of ammonium into nitrates (Equation 4-2) by means of autotrophic microorganisms (principally *Nitrosomonas*, *Nitrospira* and *Nitrobacter*).

$$NH_4^+ + 2\ O_2 \rightarrow NO_3^- + 2\ H^+ + H_2O \qquad [Eq.\ 4\text{-}2]$$

The residual organic matter is degraded by heterotrophic microorganisms (Equation 4-3).

$$CH_3COONa + 2\ O_2 \rightarrow 2\ CO_2 + H_2O + NaOH \qquad [Eq.\ 4\text{-}3]$$

Internal and external recirculation

In the selected pre-denitrifying system there is a need of internal nitrate recirculation from the aerobic to the anoxic tank. This internal recirculation ratio was initially set at 3, but increased to 4 after ten months of operation in order to enhancing the removal efficiency of nitrogen. A peristaltic pump (P-3: RS 255-9598, 135 rpm) controlled by a timer (LOGO, SIEMENS) was used for the recirculation.

The final effluent from the plant consisted of the outlet stream of the aerobic compartment from which suspended solids have been separated using a sedimentation tank. An external recirculation stream returns the biomass retained in this tank to the inlet of the plant. This external recirculation ratio was fixed at 0.5. A peristaltic pump (P-4: RS 255-9627, 20 rpm) connected to the same controller as the internal recirculation pump was used (LOGO, SIEMENS). The external recirculation stream was also used for the purging of sludge when a specific SRT was sought. In that case, the purging was carried out manually once per day.

According to the previous description, the reactor can be classified as a single-sludge system, where the biomass is formed by an association of autotrophic and heterotrophic microorganisms that are activated alternatively according to the conditions of the two different compartments.

Aeration and homogenization systems

Both, the aerobic and anoxic tank are supplied with a mechanical paddle type stirrer with a fixed speed motor (RS 255-9611, 40 rpm). In the aerobic tank air is supplied by a series of diffusers connected to a compressed-air line. A pressure reduction valve is used to maintain the dissolved oxygen level at the desired level (>3 mg/L).

Figure 4-3. Details of the activated sludge pilot plant.

Instrumentation

The physical parameters that are measured on-line are temperature, pH and dissolved oxygen level. The electrodes used are specially designed for continuous operations, gaining robustness, but loosing precision with respect to those used for punctual measurements. The selected instruments were acquired from Hanna Instruments (HI 146-00) for temperature and Desin Instruments for pH (EPHM10) and dissolved oxygen.

Inoculation and start-up

The pilot plant was inoculated with 2 g VSS /L taken from the Biodenipho® biological reactor of the STP of Milladoiro where COD, nitrogen and phosphorus removal was achieved.

During the start-up period the reactor was fed with the synthetic wastewater described in Table 4-1. The SRT was maintained at 30 d and controlled by daily sludge purges. This stage lasted 3.5 months, which was more than 3 times the implemented SRT. In that way the development of a diversified biota, including slowly- growing bacteria, was ensured and stable operational condition for the plant were achieved.

After this initial stage, the first group of PPCPs was incorporated to the reactor at a level close to the environmental one (Table 4-2).

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

Compound	Concentration (ppb or $\mu g \cdot L^{-1}$)	Compound	Concentration (ppb or $\mu g \cdot L^{-1}$)
Anti-depressants:		*Antibiotics:*	
Fluoxetine (FLX)	20	Trimethoprim (TMP)	
Citalopram (CTL)		Roxithromycin (ROX)	10
Estrogens:		Sulfamethoxazole (SMX)	
β-Estradiol (E2)	10	Erythromycin (ERY)	
α-Ethynylestradiol (EE2)			
Anti-inflammatories:		*Musks:*	
Ibuprofen (IBP)	10	Galaxolide (HHCB)	40
Naproxen (NPX)		Tonalide (AHTN)	
Diclofenac (DCF)		Celestolide (ADBI)	
Anti-epileptic:	20	*Tranquilliser:*	20
Carbamazepine (CBZ)		Diazepam (DZP)	

Operation strategy

The pilot plant has been operating continuously during 3 years. The main operational parameters, namely HRT, composition of the synthetic feed (excluding PPCPs) and dissolved oxygen level, have been maintained constant during the whole process.

Temperature was not controlled and varied therefore according to the ambient temperature. Consequently two operation periods could be differentiated, corresponding to moderate/low (winter) temperatures (14-18ºC) and warmer (summer) values (18-23ºC).

As indicated previously, the internal recirculation ratio was set at 3 during the first 10 months and increased to 4 afterwards.

The SRT varied between <20 d, 20-40 d and >40 d. In some cases this variation was due to natural fluctuations of VSS concentrations inside the pilot plant and in the final effluent, whereas in other cases it was manipulated through purges. In the latter case, the sludge was purged from the external recirculation at a flow (Q_{Purge}, L/d) determined according to Equation 4-4:

$$SRT = \frac{X_{Reactor} \cdot V_{Reactor}}{Q_{Purge} \cdot X_{Purge} + Q_{Effluent} \cdot X_{Effluent}} \qquad \text{[Eq. 4-4]}$$

where $X_{Reactor}$, X_{Purge} and $X_{Effluent}$ are the biomass concentrations (g VSS/L) inside the reactor, in the purge and in the final effluent, respectively, $Q_{Effluent}$ is the effluent flow (L/d) and $V_{Reactor}$ is the useful volume of the plant (L).

After the start-up period, a first group of PPCPs, including anti-depressants, estrogens, anti-inflammatories, the anti-epileptic drug and the tranquilliser, were added to the feed. Seven months later, the mixture was completed with three fragrances and the following month with the four antibiotics.

Sampling

Samples were collected from five different points of the pilot plant (Figure 4-2), in order to fully characterize its performance. These points correspond to the feed, medium of the anoxic and the aerobic compartment, external recirculation and final effluent. Internal recirculation was not sampled since it comes directly from the aerobic compartment. Samples were analysed weekly for conventional operation parameters (COD, SS, nitrogen). Regarding the concentration of PPCPs, two samples per month were taken during the first four months and afterwards the sampling frequency was reduced to one campaign every 1-2 months.

4.2.2. Analytical methods

Soluble Chemical Oxygen Demand (COD), Total and Volatile Suspended Solids (TSS and VSS), nitrite and nitrate concentrations were determined following Standard Methods (APHA, 1999). Amoniacal nitrogen was determined according to section 2.

The concentration of PPCPs was determined following the methods described in chapter 2. The samples were collected in glass or aluminium bottles and immediately prefiltered (glass fibre prefiltres, AP4004705 Millipore). For the analysis of antibiotics, a pinch of sodium azide was added to the filtered sample before its storage in the freezer, where it was kept until analysed by the Austrian Federal Environment Agency. For the rest of compounds, samples were analysed within one week, thus storage in the fridge was sufficient.

4.2.3. Mass balances

The main removal processes to be considered for PPCPs during their passage through the pilot plant are biological stripping, sorption and degradation.

Stripping

Due to the intensive aeration in the aerobic compartment, stripping could be a removal pathway for PPCPs. However, it also depends on Henry's coefficient (H) of the given compound, which is below 10^{-6} for all the selected substances except for fragrances, and on its sorption potential onto solids. According to Rogers (1996),

the compounds with $H<10^{-4}$ and ratio $H/K_{OW}<10^{-9}$ exhibit negligible volatilisation. Otherwise, following this same empirical approach, the three fragrances considered, with $H > 0.005$ and $H/K_{OW}>7\cdot10^{-9}$, could be significantly removed by volatilisation.

Therefore, the influence of volatilisation has been evaluated for fragrances following the approach described below. Inside a fully mixed reactor, the soluble air concentration can be assumed constant and in equilibrium with the gas phase. This equilibrium is described by Henry's law as follows:

$$K_H = \frac{p_j}{Sj} \qquad \text{[Eq. 4-5]}$$

where, K_H is the Henry's law constant ($atm\cdot m^3/mol$), p_j is the partial pressure of compound j in the gas phase (atm) and S_j its soluble concentration in the water phase (mol/m^3).

The Henry's law constant can be converted into its dimensionless version (H) applying the ideal gas law:

$$H = \frac{K_H}{R \cdot T} = \frac{C_{j,air}}{C_{j,dissolved}} \qquad \text{[Eq. 4-6]}$$

where, R is the universal gas constant (0.082 $atm\cdot L/mol\cdot K$), T is the temperature (K), $C_{j,air}$ is the concentration of compound j in the gas phase ($\mu g/L_{air}$) and $C_{j,dissolved}$ its concentration in the water phase ($\mu g/L_{water}$).

Assuming no degradation, the total concentration of compound j ($C_{j,total}$) that gets into the reactor is distributed as follows:

$$C_{j,total} = C_{j,dissolved} + C_{j,sorbed} + C^*_{j,air} \qquad \text{[Eq. 4-7]}$$

where, $C_{j,sorbed}$ is the sorbed concentration of compound j onto the sludge (described in Equation 4-11), and $C^*_{j,air}$ is the concentration that leaves the reactor during aeration, based on the volume of wastewater treated, all expressed in µg/L. $C^*_{j,air}$ is obtained by multiplying $C_{j,air}$ by the aeration applied per unit of wastewater treated (q_{air}, L_{air}/L_{ww}):

$$C^*_{j,air} = C_{j,air}\cdot q_{air} = H\cdot C_{j,dissolved}\cdot q_{air} \qquad \text{[Eq. 4-8]}$$

thereby, the relative fraction stripped to the gas phase can be calculated as follows:

$$\frac{C^*_{j,air}}{C_{j,total}} = \frac{H \cdot q_{air}}{1 + K_{d,j}\cdot SST_i + H \cdot q_{air}} \qquad \text{[Eq. 4-9]}$$

Applying equation 4-9 for the three fragrances, in order to calculate the influence of volatilisation on their removal in the worst case, assuming that the applied air flow (q_{air}) is 15, which is the upper limit for conventional sludge systems (5-15 L_{air}/L_{ww}, Joss et al., 2006) and a sludge concentration inside the reactor (SST_i) of 1.5 g SST/L, which is the lowest value observed during the operation of

the reactor, the influence of stripping was not significant for Galaxolide and Tonalide (<2%), but it was for Celestolide (~50%). Therefore, for this latter compound it will be taken into account in the mass balances as follows:

$$F_{j,Stripped} = C_{j,air} \cdot q_{air} \cdot Q_{Feed} \qquad \text{[Eq. 4-10]}$$

Where $F_{j,Stripped}$ is the mass flow (µg/d) of compound j removed by volatilisation and Q_{Feed} the flow rate treated in the pilot plant (L/d).

Sorption

The fraction of compound sorbed to the sludge will be estimated assuming sorption equilibrium (Ternes et al., 2004a) according to equation 4-11:

$$K_{d,j} = \frac{C_{j,sorbed}}{C_{j,dissolved} \cdot SST_i} \qquad \text{[Eq. 4-11]}$$

where, $C_{j,sorbed}$ is the sorbed concentration of compound j onto the sludge (µg/L), $K_{d,j}$ the solid–water distribution coefficient of compound j (L/kg), SST_i the suspended solids concentration in stream i (kg/L) and $C_{j,dissolved}$ the dissolved concentration of compound j (µg/L).

The different $K_{d,j}$ values considered in the mass balances are taken from bibliography (Table 1-2). Priority was always given to experimentally determined values, although in the case of the two anti-depressants (FLX and CTL) this parameter had to be estimated from their K_{OW}, following the procedure described in Jones et al. (2002). Finally, in the case of fragrances (HHCB, AHTN and ADBI) the $K_{d,j}$ was calculated for the pilot plant considered, since both, the total and the soluble concentration of musk compounds, has been determined.

Mass balance

The total mass flow of compound j in stream i, is the sum of the amount present in the liquid phase and the fraction sorbed on the sludge particles (Equation 4-12):

$$F_{j,i} = F_{j,iLiq} + F_{j,iSol} = C_{j,i}Q_i + Q_i K_{d,j} SST_i C_{j,i} = C_{j,i}Q_i\left(1 + K_{d,j}SST_i\right) \qquad \text{[Eq. 4-12]}$$

where, $F_{j,iLiq}$, $F_{j,iSol}$ and $F_{j,i}$ are the mass flow rates of compound j in stream i in the liquid phase, sorbed to the sludge and the total flow, respectively (all in µg/d) and Q_i is the flow rate of stream i (L/d).

Total mass flow for each trace pollutant considered (Table 4-2) was determined for the different external flows of the pilot plant (Figure 4-2), including feed, internal recirculation (Rint), external recirculation (Rext), sludge purge, inflow of the settler (Sed in) and effluent.

Some flow rates were measured experimentally, namely for the concentrate, tap water used for dilution, internal recirculation, external recirculation and sludge purge. The other were calculated as follows.

The flow rate of the feed (Q_{Feed}) was calculated as the sum of the flow of the concentrate and the tap water used for dilution. This stream, diluted with the external and internal recirculation constitutes the inflow of the anoxic tank (Equation 4-13):

$$Q_{Anox} = Q_{Feed} + Q_{Rext} + Q_{Rint} \qquad \text{[Eq. 4-13]}$$

where, $Q_{Anox,}$ Q_{Rext} and Q_{Rint} are the flow rates of the inlet to the anoxic compartment and the external and internal recirculation, respectively (L/d). The wastewater passes at the same flow rate, Q_{Anox}, from the anoxic to the aerobic tank.

From the aerobic compartment, part of the wastewater is returned to the head of the plant as internal recirculation and the rest goes to the sedimentation tank (Equation 4-14):

$$Q_{Sed\ in} = Q_{Anox} - Q_{Rint} \qquad \text{[Eq. 4-14]}$$

where, $Q_{Sed\ in}$ is the flow at which the wastewater goes from the aerobic tank to the settler (L/d).

Finally, the flow rate of the effluent is approximately equal to the one of the feed, since the effect of purging can be considered as negligible.

The difference between the total flow of compound j that enters one compartment of the pilot plant, including the dissolved and sorbed fraction ($F_{j,i}$), and the flow that leaves this compartment, including volatilisation, can be attributed to biological transformation, assuming steady state conditions for the pilot plant (Equation 4-15):

$$E_{j,Anox} = \frac{\left(F_{j,Feed} + F_{j,Rint} + F_{j,Rext}\right) - F_{j,Anox\ out}}{F_{j,Feed}} \cdot 100$$

$$E_{j,Aer} = \frac{F_{j,Anox\ out} - \left(F_{j,Sed\ in} + F_{j,Rint}\right) - F_{j,stripped}}{F_{j,Feed}} \cdot 100$$

$$E_{j,Sed} = \frac{F_{j,Sed\ in} - \left(F_{j,Effluent} + F_{j,Rext} + F_{j,Purge}\right)}{F_{j,Feed}} \cdot 100 \qquad \text{[Eq. 4-15]}$$

$$\Rightarrow\ E_{j,Plant} = \frac{F_{j,Feed} - \left(F_{j,Effluent} + F_{j,Purge}\right) - F_{j,Stripped}}{F_{j,Feed}} \cdot 100$$

where, $E_{j,Anox}$, $E_{j,Aer}$ and $E_{j,Sed}$ are the removal efficiencies for compound j, calculated for the anoxic, aerobic and sedimentation tank, respectively and $E_{j,Plant}$ the global efficiency of the plant (%). The term ($F_{j,Feed}$ + $F_{j,Rint}$ + $F_{j,Rext}$) constituts the influent to the anoxic tank of the pilot plant ($F_{j,Anox\ in}$), whereas $F_{j,Anox\ out}$ represents the

outflow from this tank. Similarly, ($F_{j,Sed\ in}$ + $F_{j,Rint}$) is the outlet of the aerobic tank ($F_{j,Aer\ out}$) and ($F_{j,Effluent}$ + $F_{j,Rext}$ + $F_{j,Purge}$) the total discharge of the sedimentation tank ($F_{j,Sed\ out}$).

4.3. Results and discussion

4.3.1. Conventional operation parameters

Dissolved oxygen concentration and temperature were measured periodically in the aerobic compartment, whereas pH was analysed in both, the anoxic and aerobic tank. The evolution of these parameters during the whole operation period is shown in Figure 4-4.

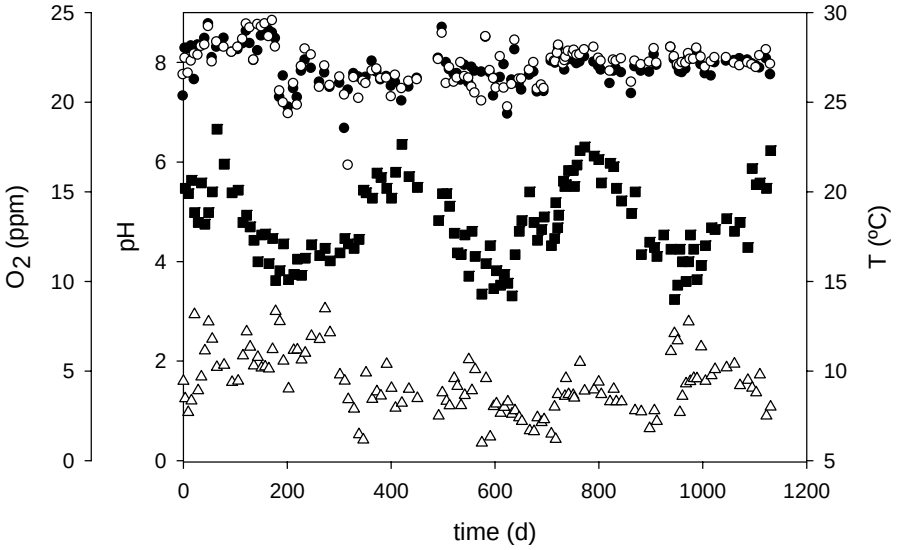

Figure 4-4. Temperature (■), pH in the anoxic (●) and aerobic compartment (○) and dissolved oxygen (△) measured in the pilot plant.

As shown in Figure 4-4, the pH inside the pilot plant was relatively constant during the complete operational period and very similar in both compartments. No adjustment of pH was performed, since the natural value observed (~8) is in the range of optimal pH for nitrifying bacteria (7.2-9, Metcalf & Eddy, 2003). Denitrifying bacteria have an optimal pH around 6.5-7.5 (Metcalf & Eddy, 2003), which is a little lower than the operational value. However, complete denitrification was observed in the pilot plant.

Dissolved oxygen concentration in the aerobic compartment was maintained as high as possible during the first 10 months, to ensure maximum growth of nitrifying

bacteria. After this initial period the performance of the pilot plant was readjusted by lowering the aeration in order to maintain dissolved oxygen concentrations in the range of 2.5-4.5 ppm. In addition, the internal recirculation rate was increased from 3 to 4. Therewith the operation of the plant was optimised regarding energetic efficiency and nitrogen removal.

Temperature was not controlled, since this is the normal situation in full-scale plants. Therefore the fluctuations observed in Figure 4-4 are due to ambient temperature variations. However, this fluctuation is softened since the plant was situated indoors (14-18ºC in winter and 18-23ºC in summer).

Biomass concentration was followed along the pilot plant, including anoxic and aerobic tank, internal recirculation and final effluent (Figure 4-5).

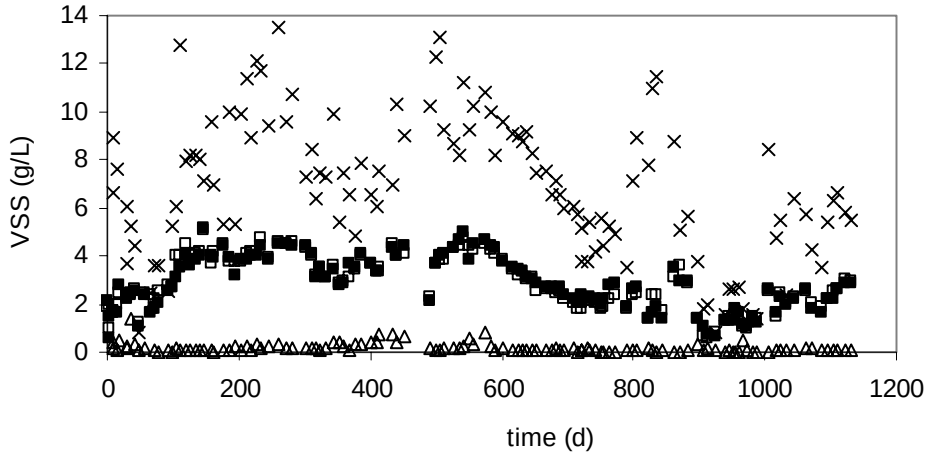

Figure 4-5. Biomass concentration, measured as VSS, in the anoxic (□) and aerobic (■) compartment of the pilot plant, as well as in the external recirculation (×) and the final effluent (△).

The reactor was inoculated with ~2 g VSS/L of sludge, which was duplicated by growth after approximately 140 d. Afterwards, sludge concentration was quite constant at 4 g VSS/L until day 590. After a period of around 130 d of decrease in the sludge concentration inside the reactor, it stabilised again at around 2 g VSS/L. This decrease in the sludge concentration could be attributed to a negative mass balance, namely the growth rate was lower than the sludge purge. This aspect is directly related to the high SRT maintained in the pilot plant that is known to lead to a lower biomass growth rate (Metcalf & Eddy, 2003; Cicek et al., 2001). At the same time, the settleability of sludge deteriorated due to growth of filamentous bacteria, thus a considerable loss of sludge within the effluent couldn't be avoided, resulting in a global loss of biomass in the pilot plant. In fact, the initial Volumetric

Sludge Index (VSI) of 74 mL/g increased up to 866 mL/g during this destabilisation and recovered afterwards to 165 mL/g, which was still a little high but couldn't be improved until the end of the plant operation. In any case, the concentration of VSS was always in the range of typical values measured in full-scale CAS treatment plants (Andersen et al., 2003; Carballa et al., 2004; Metcal and Eddy, 2003) and the performance of the settler was quite efficient, since during normal operation conditions less than 4% of the inflowing VSS left the plant with the effluent.

The concentration of nitrogen in the form of ammonia, nitrite and nitrate was also followed along the reactor. In the feed, nitrogen was added as ammonium chloride at a concentration of ~ 40 mg N/L. Dilution of the feed with the internal a external recycling leads to an approximate concentration of $N\text{-}NH_4^+$ inside the anoxic tank given by Equation 4-16:

$$C_{N-NH4_{Anox}} = \frac{Q_{Feed} \cdot C_{N-NH4_{Feed}} + Q_{R\,int} \cdot C_{N-NH4_{Aer}} + Q_{Re\,xt} \cdot C_{N-NH4_{Re\,xt}}}{Q_{Feed} + Q_{R\,int} + Q_{Re\,xt}} \qquad \text{[Eq. 4-16]}$$

where, $C_{N\text{-}NH4Feed}$, $C_{N\text{-}NH4Anox}$, $C_{N\text{-}NH4Aer}$ and $C_{N\text{-}NH4Rext}$ are the nitrogen concentrations in the feed, anoxic and aerobic tank and external recirculation (mg/L).

As shown Figure 4-6, there is a good correlation between the measured and calculated concentrations, being the former ~ 20% lower than the latter. This fraction can be assumed to be assimilated by the bacteria for growth, which is not taken into account in Equation 4-16.

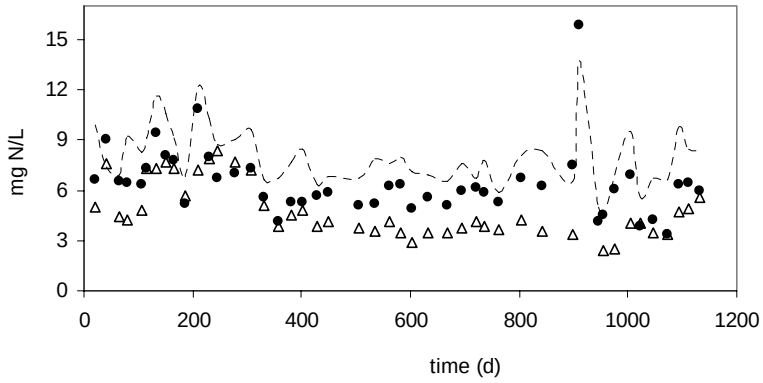

Figure 4-6. Concentration of nitrogen as ammonium in the anoxic tank experimentally measured (•) and calculated according to equation 4-16 (---). Additionally the concentration of nitrogen as nitrate in the aerobic compartment is represented (△).

Theoretically, in order to fulfil nitrogen balance in the aerobic tank of the plant, the total mass of nitrogen has to be the same in the aerobic and anoxic tank. This is due to the fact that in this compartment a transformation and not an elimination of

nitrogen occurs (Equation 4-2), with the exception of the amount of $N\text{-}NH_4^+$ used by the bacteria for growth. This means that the ratio of $C_{N\text{-}NO3Aer}$/ $C_{N\text{-}NH4Anox}$ has to be constant and equal to the ratio of the anoxic/aerobic volume (2/3). The measured concentrations of nitrate in the aerobic tank represented in Figure 4-6 are in general in good agreement with the expected values.

Overall removal efficiencies stabilised 10 days after the start-up of the plant at ~82% for nitrogen and ~95% for COD. Once the internal recirculation ratio was raised from 3 to 4 (at day 323), nitrogen removal efficiency increased up to >90%. The punctual drop observed in Figure 4-7 was derived from an accidental overload of the plant. However, the efficiency of the plant was recovered in a few days.

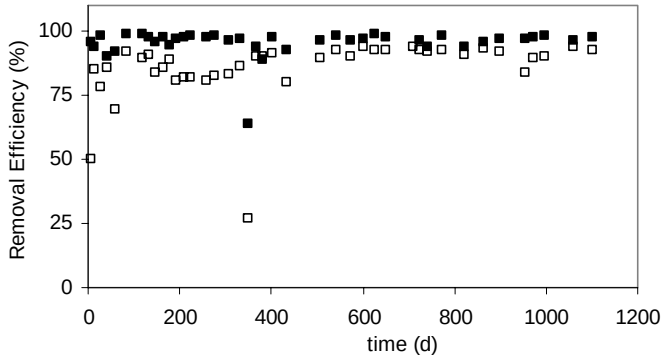

Figure 4-7. Nitrogen (□) and COD (■) removal efficiencies.

4.3.2. Fate of PPCPs in the pilot plant

The selected PPCPs have been regularly monitored along the pilot plant. The first group of pharmaceuticals added to the reactor in November 2003, comprised a) Anti-inflammatory drugs (IBP, NPX and DCF), b) neutral compounds (CBZ and DZP), c) anti-depressants (FLX and CTL) and d) hormones (EE2 and E2). Monitoring of those compounds started in January 2004 and lasted up to June 2005 for FLX, CTL, EE2 and E2 and to July 2006 for the rest of compounds. The corresponding concentration profiles are summarized in Figure 4-7, where the whole set of data has been considered in order to calculate the mean concentrations in the different sampling point, distinguishing two operation periods, the first at Rint of 3 and the second at Rint of 4.

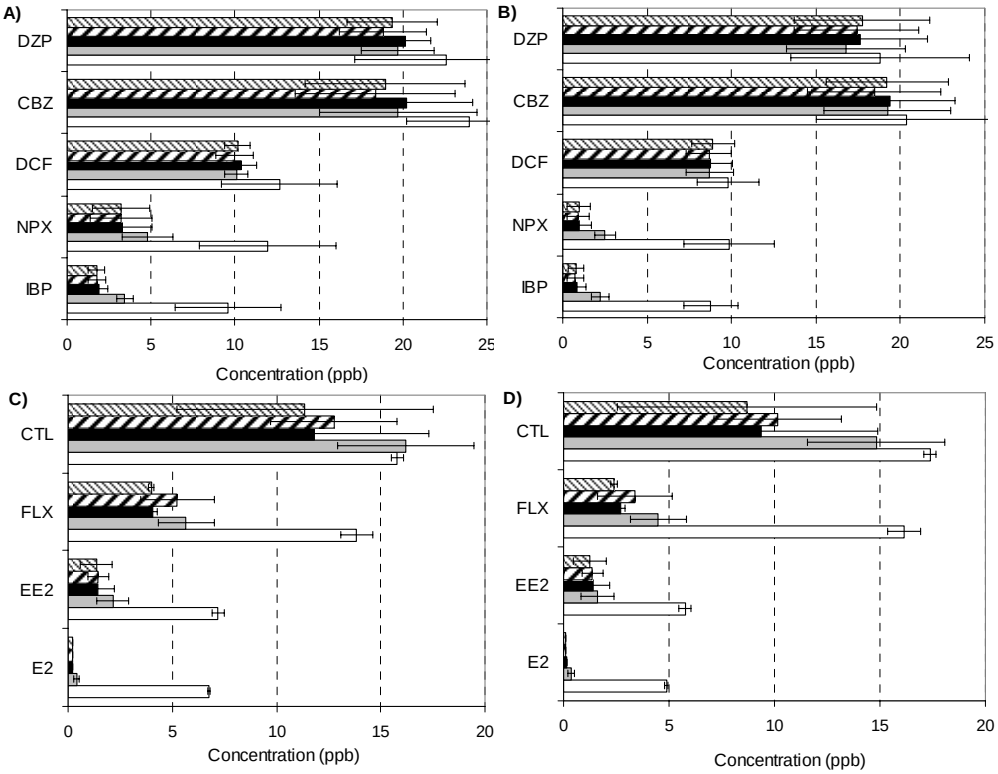

Figure 4-7. Concentration of PPCPs in the pilot plant when operating at an internal recirculation rate of 3 (A and C) and 4 (B and D), including the feed (□), anoxic (■) and aerobic (■) compartments, external recirculation stream (◪) and final effluent (▧).

These concentrations in the liquid phase (Figure 4-7) can be used in order to make a rough estimation of the overall removal efficiency achieved in the pilot plant for the different compounds, analysing the contribution of both, the anoxic and aerobic compartment, applying Equation 4-17. The results are depicted in Figure 4-8.

$$\text{Removal}(\%) = \begin{bmatrix} \text{Anoxic Tank}: & \dfrac{C_{Feed} - C_{Anox}}{C_{Feed}} \cdot 100 \\ \text{Aerobic Tank}: & \dfrac{C_{Anox} - C_{Effluent}}{C_{Feed}} \cdot 100 \\ \text{Overall}: & \dfrac{C_{Feed} - C_{Effluent}}{C_{Feed}} \cdot 100 \end{bmatrix} \qquad \text{[Eq. 4-17]}$$

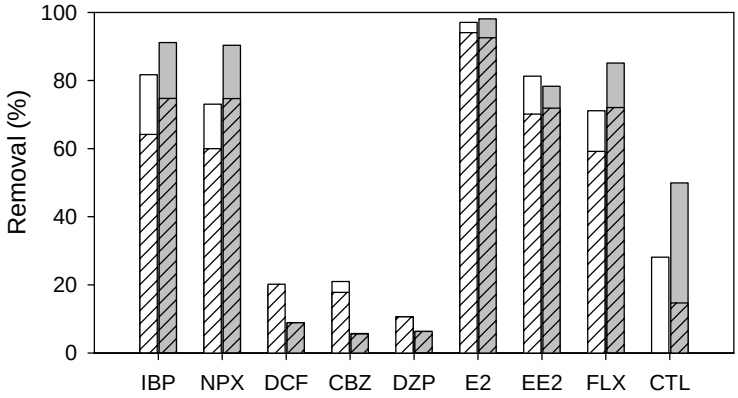

Figure 4-8. Removal of PPCPs from the liquid phase when operating at an internal recirculation rate of 3 (□) and 4 (■), including the contribution of the anoxic compartment mainly due to dilution (▨).

According to Figure 4-8, a significant decrease in PPCPs concentration occurs in the first anoxic compartment of the pilot plant, representing the dilution of the feed after its mixing with the internal and external recirculation streams an important contribution to this decrease, especially for compounds that are efficiently removed.

The increase in the internal recirculation rate led to a slight improvement of the removal efficiency of IBP, NPX, FLX and CTL, in correlation with what happened with nitrogen removal. More effective mixing in the reactor could partially be responsible for that, although it could also be due to the higher oxygen transport from the aerobic to the anoxic compartment, where the compounds would be to some extent aerobically transformed. This effect will be especially relevant for substances with moderate biological degradation constants, as has been observed in the present work.

Overall removal of IBP and NPX ranged between 82-91% and 73-90%, respectively. Very high disappearances (>80%) of IBP from the liquid phase have been previously observed in activated sludge full-scale STP of Germany, Spain, England or Japan (Ternes, 1998; Nakada et al., 2006; Gomez et al., 2007; Jones et al., 2007). Somewhat lower removal efficiencies (50-75%) have been reported by Stumpf et al. (1999), Zwiener et al. (2000) and Carballa et al. (2004). The performance of the pilot plant regarding NPX removal was slightly better than formerly reported in literature (Ternes et al., 1999; Carballa et al., 2004; Nakada et al., 2006). In some cases high variability in the fate of NPX in full scale STP have been observed (Nakada et al., 2006; Lindqvist et al., 2005), even though the applied treatment processes and operational conditions were quite similar one to another. The removal of NPX in the studied pilot plant has been increasing from 27% up to 99% during the first 300 days (Figure 4-9), indicating a possible acclimation of the bacteria to this pharmaceutical. Acclimation of biomass is known to be beneficial for anaerobic degradation of different xenobiotics (Najean et al., 1990; Thouand and Block, 1993; Chin et al., 2005), although its influence on the removal of PPCPs has only been reported in few cases. For example, Zwiener et al. (2000) observed an increase in the removal of IBP in an oxic biofilm reactor which was attributed to an adaptation of the biomass. Similarly, Ternes et al. (2004) pointed out the possibility that existing microorganisms could acclimate to the presence of PPCPs by broadening their enzymatic spectrum, in response to the lower sludge loading with bulk organics when working at higher SRT (Ternes et al., 2004b). Finally, Layton et al. (2000) observed a positive effect of using adapted microbial populations for the removal of estrogens, when comparing the performance of biosolids from a municipal plant with those from an industrial plant. On the other hand, after day 600 a clear correlation between sludge concentration in the pilot plant and the efficiency in the elimination of NPX can be observed (Figure 4-9). This confirms the hypothesis that biological transformation of pharmaceuticals follows a pseudo-first order kinetic (Joss et al., 2006), with direct proportionality of the transformation rate to the soluble substance concentration S, as well as to the sludge concentration (Equation 4-18), although the effect of the latter will only be significant for compounds with moderate biological degradation constants (kbiol in L/g of SS.d).

$$\frac{dC_{j,total}}{dt} = -k_{biol} \cdot SST \cdot C_{j,dissolved} \qquad \text{[Eq. 4-18]}$$

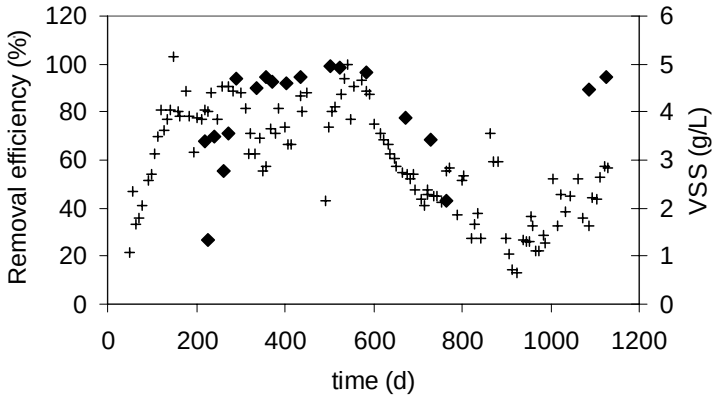

Figure 4-9. Removal of NPX in the pilot plant along the complete operation period (⬥) compared to the concentration of VSS inside the reactor (+).

Diclofenac, carbamazepine and diazepam were not significantly removed during the treatment as shown by the flat concentration profiles in Figure 4-7 A and B. Contradictory results about removal of DCF can be found in the literature, since removals of 0% to 75% are reported (Ternes, 1998; Stumpf et al., 1999; Zwiener et al., 2000; Clara et al., 2004a; Clara et al., 2005b; Lindqvist et al., 2005; Gomez et al., 2007). The reason for these discrepancies is not always clear, although some parameters such as HRT, SRT or mode of operation seem to influence the fate of DCF during biological wastewater treatment. The biological degradation constant of this pharmaceutical is below 0.1 L/g SS·d (Joss et al., 2006), therefore only those plants operating at high HRT are expected to be able to degrade it, since DCF is not retained in the reactor by adsorption (K_d 1.2 L/kg). More concretely, considering that biological degradation is governed by pseudo-first order kinetics (Joss et al., 2006), the half life of this pharmaceutical for plants working with a biomass concentration in the range of 2-4 g/L can be estimated as 2-3.5 days. This could explain the low biodegradation achieved in the investigated pilot plant where the HRT was ~1 day, as well as some discrepancies detected in the literature. For example, in Clara et al. (2005b) reported 70% removal of DCF for a STP working at a HRT of 13 d, whereas this efficiency was reduced to negligible removals for the other two STP considered which had an HRT below 1.2 days. The SRT might be a second parameter that could influence the elimination of DCF, although its influence is not completely elucidated (Clara et al., 2005a). It is worth to outline that most of the published data about DCF removals were obtained from full scale STP where the raw influent and the final effluent constituted the only sampling points, therefore including both, primary and secondary treatment. In fact, it has been shown that primary treatment processes can partially contribute to the removal of DCF during

sewage treatment (Carballa et al., 2005). The addition of inorganic salts to the biological reactor in order to achieve the removal of phosphorus by precipitation may also enhance the elimination of this pharmaceutical by sorption (Suárez et al., 2007), which could be one explanation for the 69% depletion of this compounds observed by Ternes et al. (1998).

In the case of CBZ the low removal percentage achieved upon the treatment in the pilot plant was consistent with previously reported data (Ternes, 1998; Clara et al., 2004b; Joss et al., 2005), which could make it suitable for using it as marker for anthropogenic influences on the aquatic environment according to Clara et al. (2004b).

Removal of DZP during biological treatment has not been reported previously, probably because this compound is normally not detected in raw wastewaters during the samplings investigated STPs (Carballa et al., 2004; Clara et al., 2005b). However, analysing the K_d and k_{biol} (Table 1-2) of this compound, neither sorption nor degradation is expected to be significant, which was actually confirmed in studied pilot plant.

The elimination percentages reached in the pilot plant for both estrogens considered range between 97-98% and 78-81% for E2 and EE2, respectively. Similar data have been reported previously, with removals between 87-100% for E2 (Ternes et al., 1999b; Baronti et al., 2000; Onda et al., 2003; Joss et al., 2004; Nakada et al., 2006) and in the range of 71-94% for EE2 (Ternes et al., 1999; Joss et al., 2004; Baronti et al., 2000). Nevertheless, contradictory results for EE2 removal have been reported in Clara et al. (2005a), where in some treatment facilities high values were observed, whereas in others working at comparable SRT no or only slight removal was determined. Probably the discrepancy in the results is more related to the different HRT of the plants considered, since estrogens show distinct kinetic behaviour (k_{biol} in Table 1-2), with E2 being almost completely oxidized to estrone (E1) in less than 3 hours, the further oxidation of E1 being slower (50% after 24 hours) and EE2 not being appreciably removed even after 48 hours (Ternes et al., 1999a). Therefore, a minimum HRT is needed to accomplish the complete removal of hormones. Half-lives for EE2 under aerobic conditions between 6h up to 5 days have been reported by de Mes et al. (2005) and a possible inhibition of sludge by the presence of EE2 is outlined. The previous discussion indicates the complexity of the assessment of EE2 behaviour during wastewater treatment and partially explains the high variations in removal efficiency reported (de Mes et al., 2005).

From the two anti-depressants considered, fluoxetine was found to be better removed than citalopram, with efficiencies of 71-85% and 28-50%, respectively. Information about the fate of these two pharmaceuticals during wastewater treatment has scarcely been reported, although the serotonin re-uptake inhibitor fluoxetine is apparently the most acute toxic human pharmaceutical reported so far

(Fent et al., 2006). For fluoxetine the biodegradation profile has been estimated to be >91% by Webb (2004), whereas Johnson et al. (2005) estimated removals for both anti-depressants of ~20%, thus indicating that one has to be careful when using estimations. Fluoxetine and Citalopram concentrations have been measured in the influent and effluent of three STPs in Norway (Vasskog et al., 2006) and the corresponding removal percentages have been assessed in the range of 8-70% for FLX and 29-57% for CTL. Even these data can only be considered as rough estimates, since they were obtained in one single sampling campaign. The results of the present research indicate that our pilot plant showed higher removal for FLX then the reported by Vasskog et al. (2006), whereas for CTL comparable removals have been obtained.

In January 2005 the number of PPCPs considered in this research has been broadened by incorporating a) Antibiotics (ERY, ROX, SMX and TMP) and b) Fragrances (HHCB, AHTN and ADBI). The monitoring of those compounds lasted until July 2006. The corresponding concentration profiles taking the mean value of the whole set of data for the two operation periods with a Rint of 3 and of 4, are summarized in Figure 4-10.

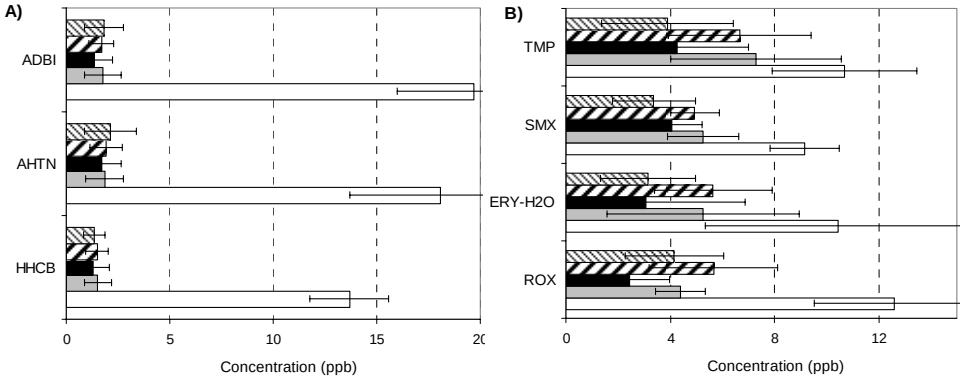

Figure 4-10. Concentration of fragrances (A) and antibiotics (B) in the pilot plant, including the feed (□), anoxic (■) and aerobic (■) compartments, external recirculation (▨) and final effluent (▧).

Erythromycin has been monitored as a degradation product where it has lost one molecule of water (ERY-H2O), since this loss is expected to occur in the samples during their analytical determination where an acidic pH was used (Hirsch et al., 1999). The complete transformation of ERY into ERY-H2O is demonstrated as the measured ERY-H2O concentration in the feed is in the order of the spiked level (10 ppb). The concentrations in the liquid phase (Figure 4-10) have been used to make an estimation of the overall removal efficiency achieved in the pilot plant for

the different compounds, indicating the contribution of both, the anoxic and aerobic compartment, according to equation 4-17. The results are depicted in Figure 4-11.

Figure 4-11 shows that while fragrances (HHCB, AHTN and ADBI) are almost completely eliminated from the liquid phase after passing the anoxic compartment, antibiotics (ROX, ERY, SMX and TMP) are gradually transformed as they pass through the plant. As will be discussed in the following paragraph dealing with mass balances of PPCPs, the high elimination of musks in the anoxic compartment is assumed to be related to sorption onto sludge, according to their high K_d values.

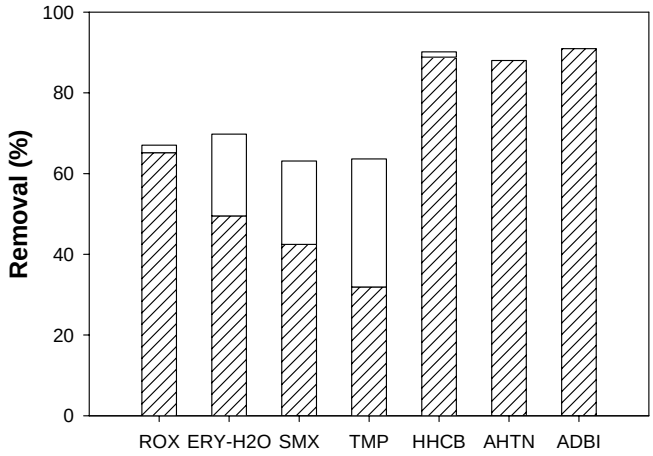

Figure 4-11. Removal of PPCPs from the liquid phase including the contribution of the anoxic compartment mainly due to dilution (▨).

Removal percentages of antibiotics achieved in the pilot plant were quite similar for the four compounds considered, reaching values of 64-70%, which are considerably higher than the data reported for the same antibiotics by Gobel et al. (2007) during full-scale wastewater treatment in activated sludge systems. For ROX maximum removal percentages of 40% have been reported by Gobel et al. (2007) and Joss et al. (2005). Both antibiotics, ERY and TMP, have shown high persistence during wastewater treatment in several full-scale monitoring campaigns (Lindberg et al., 2005; Castiglioni et al., 2006; Gobel et al., 2007). In the case of SMX, eliminations in the range of -138 up to 71% have been found in previous works (Carballa et al., 2004; Joss et al., 2005; Lindberg et al., 2005; Castiglioni et al., 2006; Gobel et al., 2007). One possible reason for these discrepancies between our results and the cited data could be related to the fact that all those reported removals refer to full-scale STPs that have been monitored during a specific time and the complexity of sewage compared to the synthetic feed used in the present research. For example, SMX is partially (~50% of the administered dose)

metabolised to *N*^{4}-acetylsulfamethoxazole, which can subsequently be transformed back to SMX during the biological treatment (Gobel et al., 2005). In fact, if this metabolite is taken into account the results for the removal increase significantly in Joss et al. (2005) and Gobel et al. (2007). On the other hand, macrolides could be partly enclosed in faeces particles, since they are mainly excreted with the bile and faeces, and released during secondary treatment (Gobel et al., 2007).

The fragrances considered in the present work (HHCB, AHTN and ADBI) were very effectively removed from the liquid phase (~90%), in concordance with the performance observed in different STP across Europe and the USA (Simonich et al., 2002; Kupper et al., 2006), although somewhat higher than the removal observed in Bester (2004) and Carballa et al. (2004), the latter if only the biological reactor is considered, since if the primary treatment is also considered the overall removal increases up to the levels measured for the pilot plant. According to the high lipophilicity of musk compounds (K_{ow}> 4.6), sorption onto sludge could play an important role in their elimination from the liquid phase. Details about the relative contribution of sorption and biodegradation will be discussed in the following paragraph.

4.3.3. Mass balances of PPCPs

The removal processes considered in the mass balances of the selected PPCPs include biological degradation, sorption and volatilisation. The relative contribution of each mechanism to the overall removal depends on the specific properties of each compound, mainly the strength of its chemical structure (related to k_{biol}), the sorption coefficient (K_d) and the Henry's coefficient (H), respectively. In the present work, volatilisation will only be considered for the mass balance of ADBI, since for the rest of compounds it supposes less than 2% and it can consequently be neglected.

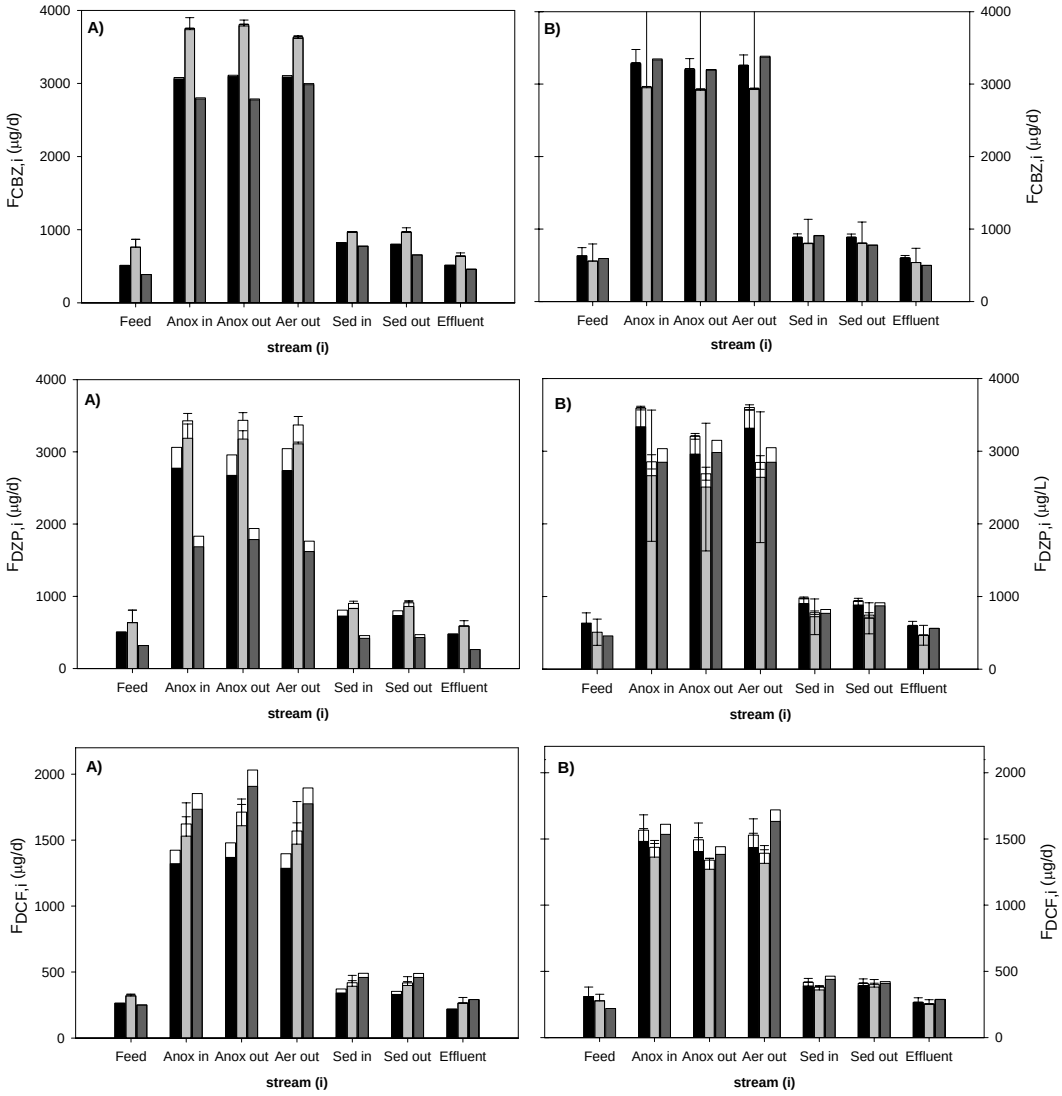

Figure 4-12. Total mass flow of CBZ, DZP and DCF in the different streams of the pilot plant working at low (A) and high (B) temperatures and at SRT of <20 d (■), 20-40 d (■) and >40 d (■). The contribution of sorption is also indicated (□).

In order to apply mass balances to the pharmaceuticals considered, total flows have been calculated according to Equation 4-12 for the different streams in the experimental set-up and the evolution in each compartment determined according to Equation 4-15. The effect of temperature (low: 14-18ºC and high: 18-23ºC) and SRT (<20 d, 20-40 d and >40 d) have been considered for all compounds, whereas the effect of the internal recirculation ratio (3 during the first 10 months and 4 afterwards) has only been analysed for those compounds whose removal seemed to be affected according to the data of their fate (IBP, NPX, FLX and CTL).

The three most recalcitrant compounds out of the selected PPCPs were CBZ, DZP and DCF, which was expected beforehand according to their low k_{biol} and K_d (Table 1-2). This is illustrated in Figure 4-12 by flat mass flow profiles along the reactor in the different compartments of the pilot plant (anoxic, aerobic and settling tank) independently of the temperature or SRT considered.

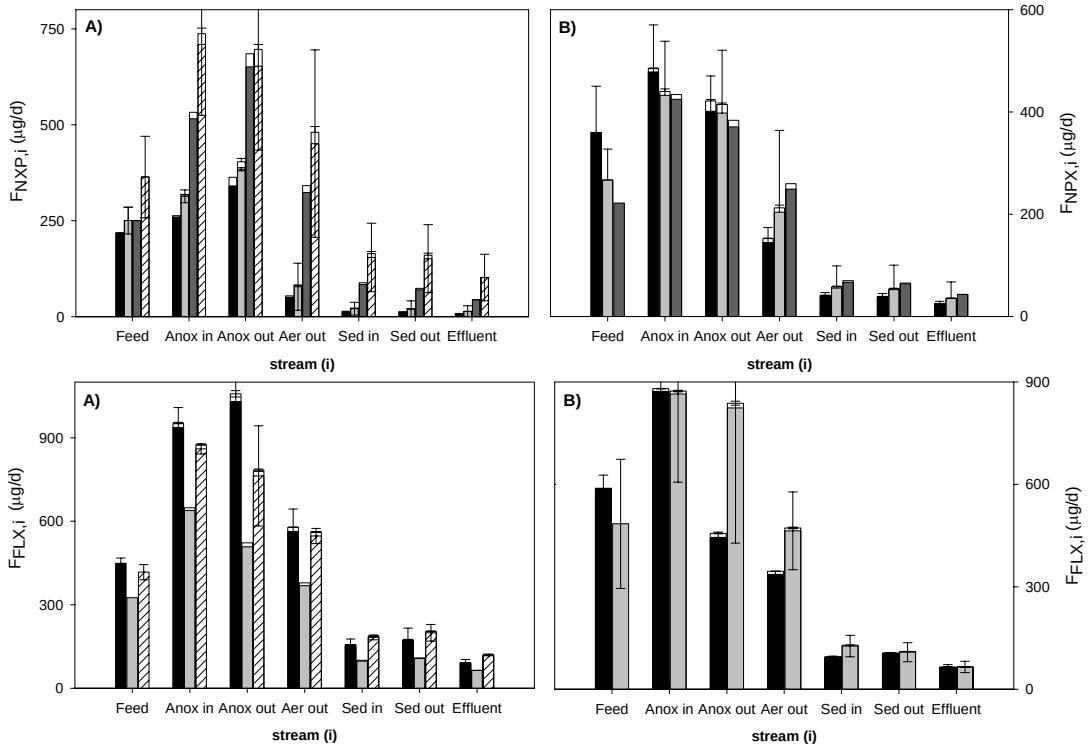

Figure 4-13. Total mass flow of NPX and FLX in the different streams of the pilot plant working at low (A) and high (B) temperatures and at SRT of <20 d (■), 20-40 d (■) and >40 d (■). The contribution of sorption (□) and Rint 3 (▨) is also indicated.

The compounds FLX and NPX are not significantly sorbed to the sludge due to their low sorption coefficient (log K_d < 1.1), but both compounds exhibited an important biological degradation in the pilot plant (79-89% for FLX and 81-96% for NPX), although when the plant was operated with an internal recirculation ratio of 3, these removals were somewhat lower, 69% for NPX and 71% for FLX. In the case of FLX, part of the transformation was already observed in the anoxic compartment of the plant, although not always (Figure 4-13). Regarding operational conditions, SRT did not exert any effect on its removal, whereas a slight increase in the efficiency of the plant (6-10%) was observed at warmer temperatures. Biological degradation observed for NPX corresponds very well with the predicted value according to the kinetic constants determined by Joss et al. (2006), since, when working with a biomass concentration in the range of 2-4 g/L and a HRT of 1 day, the resulting removal according to pseudo-first order kinetics is in the range of 86-100% (when sorption is neglected). With increasing SRT and temperature, a negative effect on the removal has been detected, which has to be attributed to inaccuracies of the experimental procedure.

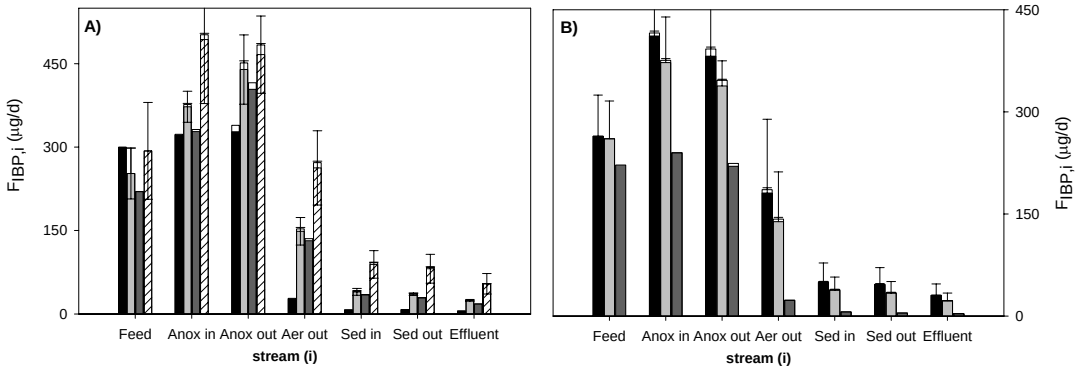

Figure 4-14. Total mass flow of IBP in the different streams of the pilot plant working at low (A) and high (B) temperatures and at SRT of <20 d (■), 20-40 d (■) and >40 d (■). The contribution of sorption (□) and Rint 3 (▨) is also indicated.

The physico-chemical characteristics of Ibuprofen are similar to those of NPX, although its biological degradation constant is more than one order of magnitude higher (k_{biol}: 9-35 L/g SS·d). Therefore, the even better transformation percentages measured for this compound (80-99%) are not surprising (Figure 4-14). Operational conditions of SRT and temperature did not affect the transformation, as had been reported beforehand by Joss et al. (2005). Therefore, the seasonal variation in IPB removal reported by Castiglioni et al. (2006), of 38% in winter (~10ºC) and 93% in summer (~19ºC), could no be confirmed in the present work, although temperature

in the pilot plant did never reach such low values. Nevertheless, not the same can be said for the redox conditions, since transformation only occurred in the aerobic tank of the plant. This dependency of IBP degradation on redox conditions has previously been reported by Zwiener et al. (2000). The behaviour of IBP plotted in Figure 4-14, corresponds very well with that reported by Clara et al. (2005b) for the full scale STP, which worked at similar conditions as the studied pilot plant. The lowest transformation degree has been measured for the first operation period, that is at Rint 3, low temperature and SRT < 20 d, which is attributed exclusively to the Rint itself, since when the same conditions were implemented at the higher recirculation rate, the resulting transformation was of 99%.

Both, the natural (E1 and E2) and synthetic (EE2) estrogens should be bio-transformed to a significant extent according to their k_{biol} (Table 1-2). In addition, their sorption coefficient is around 2.5, that is, they are slightly lipophilic and will therefore be partially sorbed onto sludge. In the present research, only the natural hormone E2 has been added to the synthetic influent, which was already partially converted into E1 in the feed storing tank of the pilot plant. This was not surprising, since E1 is reported to be completely oxidized to E2 in a few hours (Joss et al., 2004). Accordingly, the mass balances have always been applied to the sum of both, E1 and E2, instead of considering each compound individually (Figure 4-15). Natural estrogens have been almost completely transformed (>93%) in the aerobic tank of the pilot plant, whereas they passed the anoxic compartment unaltered. The worst case (93%) was observed at the lower SRT and temperature, which improved to >98% when one of this two parameter was raised. The plant was a little less efficient regarding EE2 elimination, with efficiencies between 74 and 85%, which was also achieved within its aerobic compartment. Again, the worst case corresponded to the lower SRT and temperature and could be improved in a 11% by increasing the SRT above 20 d. Some previous authors reported the effect of redox conditions on the behaviour of estrogens. For example, in Andersen et al. (2003) most of the elimination of E1 and E2 during full-scale wastewater treatment was reported to already occur in the denitrifying step, whereas EE2 depletion was only observed during the aerobic process. A similar performance was observed in batch experiments (Joss et al., 2004), showing that degradation of E1 and E2 took place in all, anaerobic, anoxic and aerobic environments, but at significant different rates, whereas EE2 was only significantly removed under aerobic conditions and at slower rates than natural estrogens. On the other hand in the same work, it was observed that the match between model calculations and measured values in STPs improved if no degradation of natural estrogens was assumed in the first anoxic reactor despite their degradation potential under those conditions, presumably due to competitive inhibition of their degradation by the influent substrate. Therefore, the results obtained in the present work are not surprising and could serve as a confirmation of this postulation. The enrichment of activated sludge in nitrifying

bacteria seems to positively influence the removal of EE2, probably through a hydroxylation of the compounds (Vader et al., 2000), which could be responsible for its important transformation in the considered pilot plant. Inside the pilot plant, around 70% of estrogens are present sorbed onto sludge which facilitates their degradation. The fraction of estrogens present in the purged sludge is between 4-17% (Figure 4-15), similar to previously reported data (<10% according to Andersen et al., 2003; Onda et al., 2003 and Joss et al., 2004).

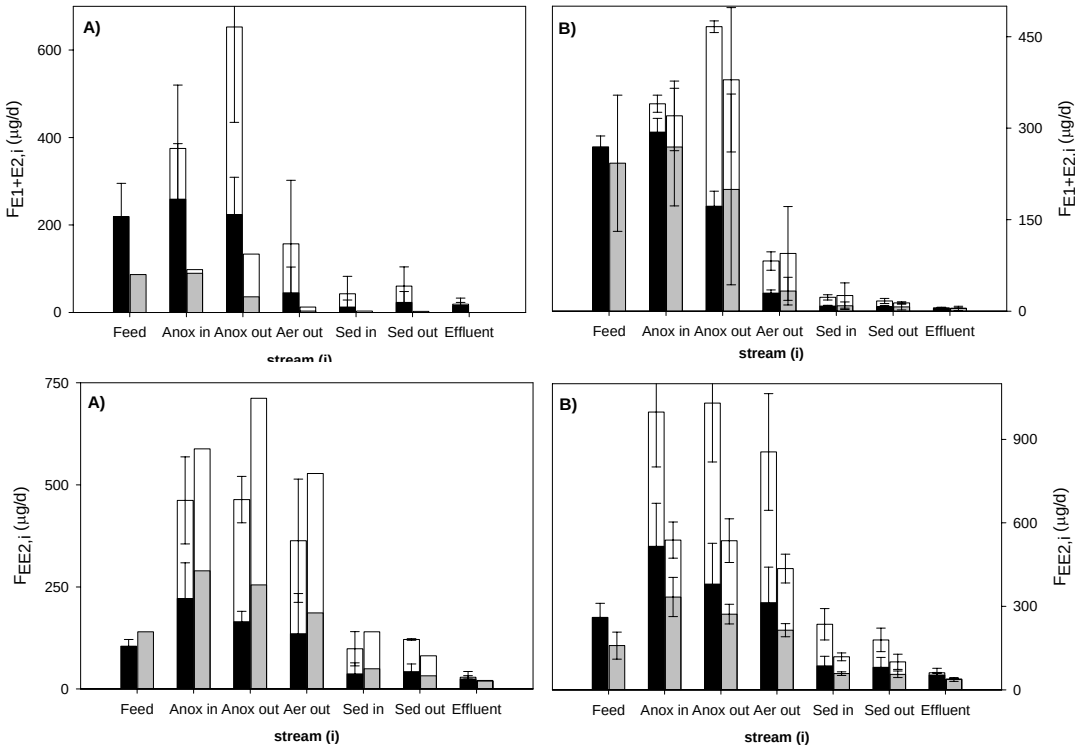

Figure 4-15. Total mass flow of E1+E2 and EE2 in the different streams of the pilot plant working at low (A) and high (B) temperatures and at SRT of <20 d (■) and 20-40 d (■). The contribution of sorption is also indicated (□).

Antibiotics (SMX, TMP, ROX and ERY) and CTL have similar sorption behaviour as estrogens, but significantly lower biodegradation constants (Table 1-2), with the exception of ERY whose sorption coefficient is negligible (Gobel et al., 2005). Even so, appreciable transformations have been observed for the four antibiotics (33-86%). The lowest efficiency of the plant corresponded to the removal of TRM and SMX, which notably increased in the case of SMX when either the SRT or the

temperature was higher (from 38% up to 63-70%). A similar positive effect of the operational temperature has been reported by Castiglioni et al. (2006) for SMX with an increase in the removal percentage from 17% in winter (9.7ºC) to 71% during summer (18.6ºC), being the removal of 38% measured in the present work at 14-18ºC in between those values. In the case of ROX and ERY the residual flow of pharmaceutical in the effluent reached 14-34% and 25-49%, respectively, with an improved performance in the case of ERY at higher operational temperatures. Partial transformation under anoxic redox conditions have only been observed in the case of ERY and TMP, although not in all experiments (Figure 4-16). The antibiotics ERY, SMX and TMP have been classified as not readily biodegradable according to a Close Bottle Test (Alexy et al., 2004), although the concentration of pharmaceuticals used in this test was one order of magnitude higher than in the environment. In fact, when the biodegradability of SMX and TMP was determined at environmental levels (Perez et al., 2005), these previous results were not confirmed, since 74% of elimination was observed for SMX within 3 days, whereas TMP was found to be easily biodegraded by nitrifying sludge, that could be also responsible for its transformation in the studied pilot plant. Transformation of ROX and SMX in full-scale CAS plants seems to be somewhat lower than the achieved in this pilot plant (Joss et al., 2005; Gobel et al., 2007), which could be a consequence of the lower SRT installed in those plants. In fact, the positive effect of SRT on the removal efficiency of the considered antibiotics seems to be noticeable above 20 d, according to Gobel et al. (2007), since in the two CAS considered in that work, with SRT of 10-12d and 21-25d, no differences between their performance has been observed, although in the MBR 2-3 times higher removals of TMP and ERY at SRT of 60-80d has been reported when comparing to SRT<33 d, and in the case of ROX these increase already occurs at the SRT of 33d. In that work it was postulated that the combination of high SRT and reduced sludge loading (F/M) may cause an increase in the biodiversity of the active biomass, which seem to have an influence on the elimination of compounds undergoing co-metabolism.

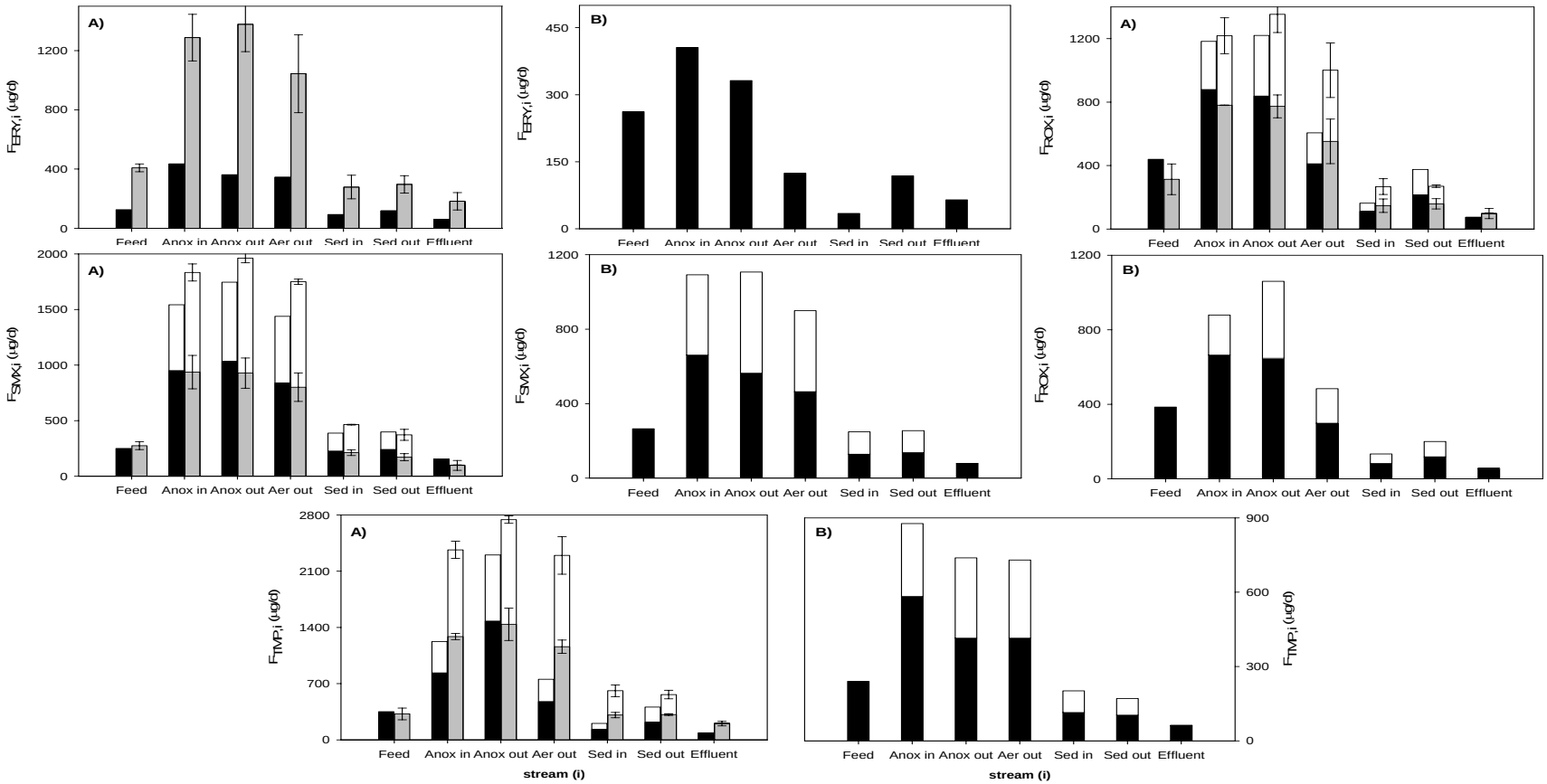

Figure 4-16. Total mass flow of ROX, ERY, SMX and TMP in the different streams of the pilot plant working at low (A) and high (B) temperatures and at SRT of 20-40 d (■),and >40 d (■). The contribution of sorption (□) is also indicated.

Transformation of CTL inside the pilot plant was 25% when operated at Rint of 3, and increased up to 47-60% when this parameter was incremented to 4. The amount of substance that leaves the plant with the effluent sorbed onto the biomass was below 6%, therefore negligible (Figure 4-17). The highest transformation degree has been observed at the higher temperature and SRT, and only under aerobic conditions. As indicated in the previous paragraph, the data about the occurrence and fate of CTL during biological wastewater treatment obtained in this work are very innovative, since, as far as the author knows, there is only one publication about the presence of this compound in the influent and effluent of three STPs in Norway (Vasskog et al., 2006), obtained during one single sampling campaign.

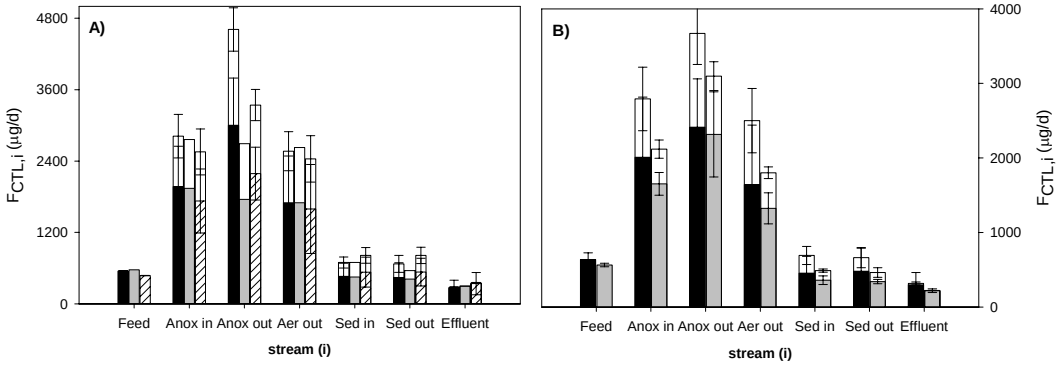

Figure 4-17. Total mass flow of CTL in the different streams of the pilot plant working at low (A) and high (B) temperatures and at SRT of <20 d (■), and 20-40 d (■). The contribution of sorption (□)and Rint 3 (▨) is also indicated.

Fragrances illustrate the coexistence of the three removal mechanisms previously mentioned: volatilisation, sorption and biodegradation. Volatilisation in aeration tanks represents a minor removal pathway in the case of HHCB and AHTN and has consequently not been taken into account, whereas this same assumption can not be made in the case ADBI, where volatilisation has been considered in the mass balances (Figure 4-18). The air flow considered for the mass balance was 5 L_{air}/L_{WW}, therefore the lower limit for CAS systems, since otherwise, negative mass balances in the aerobic tank were obtained. Fragrances were the only compounds for which sorption coefficients could be calculated from the experimental values, with the following result: $2.5 \pm 2 \cdot 10^3$ L/kg for HHCB, $3.2 \pm 7.5 \cdot 10^3$ L/kg for AHTN and $2.6 \pm 6.4 \cdot 10^3$ L/kg for ADBI. These values are in the same order as those obtained by Ternes et al. (2004a) for HHCB and AHTN, although almost one order of magnitude lower than the reported by Kupper et al. (2006) for the three compounds. The high contribution of sorption onto sludge on

the total mass flow of musk compounds in the different streams of the pilot plant is clearly shown in Figure 4-18, which is related to their strong lipophilic character. Transformation of HHCB and AHNT during biological treatment reached 81-90% and 84-94%, respectively. Despite the relative low contribution of volatilisation on the total mass flow of ADBI that left the aerobic tank (Figure 4-18), it highly influenced the degree of transformation reached in the pilot plant. In fact, the overall removal of ADBI attained in the pilot plant was in the same range as for the other two fragrances considered (85-94%), although transformation did only account for a 44-77%, the rest was lost by stripping. It has been previously shown that for AHTN sorption was the only mechanism responsible for its removal (Bester, 2004; Joss et al., 2005), although Kupper et al. (2006) associated 43% of the depletion observed to degradation. In the case of HHCB, a certain biological degradation was observed (16-50%) and partially confirmed by the detection of one metabolite, HHCB-lactone (Bester, 2004; Joss et al., 2005; Kupper et al., 2006). The third musk considered, ADBI, showed a similar behaviour as the other two in Kupper et al. (2006), although this data couldn't be confirmed by other works due to lack of available literature. In any case, transformations observed in the pilot plant were considerably higher than these reported values, which could be attributed to the higher SRT installed in the pilot plant, compared to the cited data (SRT<25 d). This assumption is based on the consideration that for lipophilic compounds, such as fragrances, the retention time inside the reactor is determined by the SRT, rather than by the HRT of the plant. This fact explains how compounds with a very low k_{biol} (Table 1-2) can be biologically transformed during the secondary treatment step. Similar biotransformation percentages to the measured in this study have been reported by Clara et al. (2005) for AHTN and HHCB in a STP working at SRT > 52 days, what supports the previous discussion. It is shown in Figure 4-18 that at least partial transformation of fragrances already occurs in the anoxic compartment of the pilot plant, indicating that redox conditions did not exert an important influence of this process.

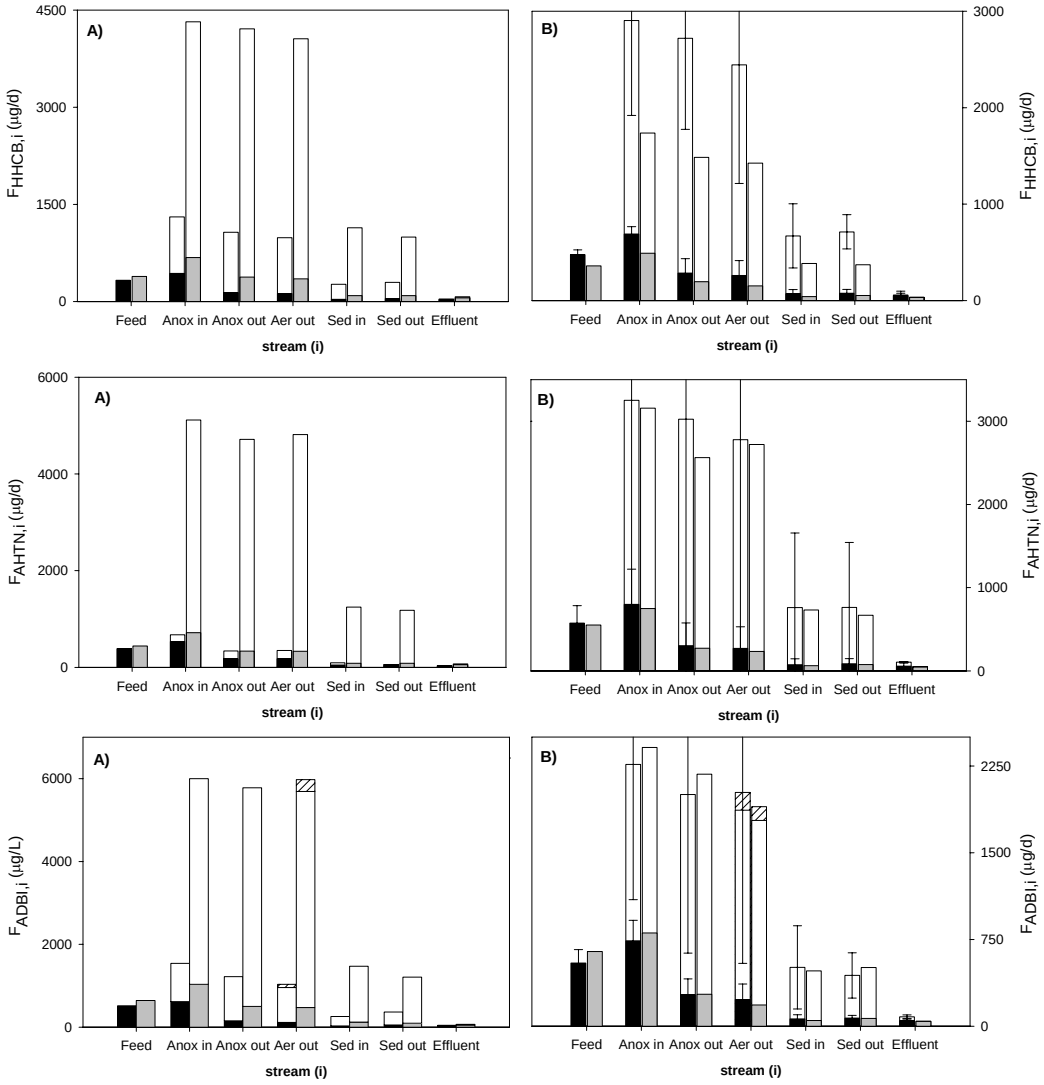

Figure 4-18. Total mass flow of HHCB, AHTN and ADBI in the different streams of the pilot plant working at low (A) and high (B) temperatures and at SRT of 20-40 d (■),and >40 d (■). The contribution of sorption (□) and volatilisation (▨) is also indicated.

4.4. Conclusions

The pilot plant considered in this research has been fed continuously with a set of 16 PPCPs, three of which are musk compounds, two hormones (the natural E2 and the synthetic EE2) and the rest pharmaceuticals of different therapeutic classes (anti-epileptics, tranquilisers, anti-depressants, anti-inflammatories and antibiotics).

The fate of PPCPs on the basis of the concentrations in the liquid phase was determined in a first step, which was further complemented with a detailed mass balance, considering the most relevant removal mechanisms during biological treatment. While the conclusions about the overall elimination efficiency of the considered compounds was similar in both types of analysis, the contribution of the anoxic compartment to the overall process is overestimated when only concentrations in the liquid phase are considered, principally due to the influence of the two types of recirculation on the inflow to this tank. In addition, when the fate of PPCPs is determined, only removal, but not transformation can be established. A clear example of the significant difference between these two concepts has been shown for ADBI, where the removal attained in the pilot plant was 85-94%, although transformation did only account for a 44-77%, the rest was lost by stripping.

In order to analyse the influence of physico-chemical properties on the fate of PPCPs during biological wastewater treatment (Table 4-3), compounds were classified according to them, as follows:

- √ The three compounds with low K_d and low k_{biol}, CBZ, DZP and DCF, were not significantly removed during the treatment.
- √ In the case of FLX, NPX and IBP high removal of the parent compound has been observed, which was associated to the good biodegradability of the compounds, since sorption of this compounds is negligible.
- √ Natural estrogens (E1+E2) have also been very efficiently removed, confirming their fast biodegradation, additionally favoured by a slight sorption capacity.
- √ On the contrary, musk fragrances (HHCB, AHTN and ADBI) are efficiently transformed inside the pilot plant, presumably due to their enhanced retention according to their lipophilicity, since the biological degradation constant of fragrances is very low.
- √ In the case of EE2 and ROX, less than 40% of the influent concentration remains in the final effluent of the pilot plant, which can be again partially attributed to their enhanced retention inside the pilot plant due to their medium sorption capacity. This could also explain the increased removal of EE2 detected at higher SRT.

√ For the rest of PPCPs, CTL, SMX, TMP and ERY, the transformation degree in the pilot plant was at least 40%, according to their moderate sorption and biodegradation potential.

Table 4-3. Summary of the transformation achieved for the considered PPCPs when working at Rint 4, according to their physico-chemical properties.

Compound	K_d	k_{biol}	Transformation			Influence	
			Anoxic	Aerobic	Overall	SRT	T
CBZ	–	–	––	––	––	no	no
DZP	–	–	––	––	––	no	no
DCF	–	–	––	––	––	no	no
FLX	–	n.a.	––/+	–/++	++	no	yes
NPX	–	–+	––	++	++	no	no
IBP	–	+	––	++	++	no	no
E1+E2	–+	+	––	++	++	yes	yes
EE2	–+	–+	––	+/++	+/++	yes	no
CTL	–+	n.a.	––	–+	–+	no	no
SMX	–+	–	––	–+/+	–+/+	yes	yes
ROX	–+	–	––	+/++	+/++	no	no
TMP	–+	n.a.	––/–+	––/+	–+/+	no	no
ERY	–+	–	–/–+	–/+	–+/+	no	yes
HHCB	+	–	–/+	–/–+	++	no	no
AHTN	+	–	–+/++	––/–+	++	no	no
ADBI	+	n.a.	–/–+	––/–	–+/+	no	no

(––) Removal <20%; (–) Removal 20-40%; log K_d < 1.4; k_{biol} < 0.1 (L/gss.d); (–+) Removal 40-60%; 1.4<log K_d < 3.3; 0.1<k_{biol} < 10 (L/g_{ss}·d); (+) Removal 60-80%;log K_d > 3.3; k_{biol} > 10 (L/g_{ss}·d); (++) Removal >80%; n.a. not available. If an influence of SRT or temperature (T) on the transformation degree was observed it is indicated as (yes) or (no).

Some operational parameters of the pilot plant, such as HRT, composition of the synthetic feed and dissolved oxygen level, have been maintained constant during the whole process, whereas temperature, SRT and the internal recirculation flow has varied, accordingly their influence on the process could be evaluated.

√ The SRT of the plant had only an effect on the transformation degree of compounds with a significant sorption potential, presumably because it enhances the retention of the compound inside the plant and consequently its availability for biological degradation. This effect is especially important for substances, such as SMX, with low k_{biol}.

√ The positive effect of warm temperature comparing to moderate ones, was only observed for FLX, E1+E2, SMX and ERY, although its influence was just significant for the two antibiotics, with an increase in their transformation of 24-32%.

√ During the first months the pilot plant has been working at an internal recirculation rate of 3, instead of 4, which had a negative influence on the

removal of IBP, NPX, FLX and CTL, whereas it didn't affect estrogens, nor the recalcitrant CBZ, DZP and DCF removals.

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Chapter 5
Continuous biodegradation of Pharmaceutical and Personal Care Products (PPCPs) under denitrifying and nitrifying conditions[1]

Summary

The fate and behaviour of 16 Pharmaceutical and Personal Care Products (PPCPs) during a conventional biological wastewater treatment process were assessed in the previous chapter. The contribution of anoxic and aerobic redox conditions, sequentially applied to remove organic matter and nitrogen from the wastewater, was determined by means of mass balances. The aim of this part of the work was to experimentally analyse these differences.

Two lab-scale reactors have been set-up, one working at pure nitrifying aerobic conditions and the other in a denitrifying anoxic environment. Depletion of selected compounds on the basis of the concentrations in the liquid phase was followed and mass balances considering the contribution of volatilisation, sorption and transformation were applied.

The compounds fluoxetine (FLX), natural estrogens (E1+E2) and musk fragrances (HHCB, AHTN and ADBI) were transformed to a large extent under aerobic (>76%) and anoxic (>65%) conditions, whereas naproxen (NPX), ethinylestradiol (EE2), roxithromycin (ROX) and erythromycin (ERY) were only significantly transformed in the aerobic reactor (>82%). The anti-depressant citalopram (CTL) was moderately biotransformed under both, aerobic and anoxic conditions (>62% and >41%, respectively). Some compounds manifested high resistance to biological transformation, as carbamazepine (CBZ), diazepam (DZP), sulfamethoxazole (SMX) and trimethoprim (TMP).

Additionally, the influence of some operational conditions, such as temperature, Sludge Retention Time (SRT) and biomass adaptation and concentration, was analysed. Removal of diclofenac (DCF) in the aerobic reactor was positively affected by the development of nitrifying biomass and increased up to 74%. Similarly, efficient anoxic transformation of IBP (75%) was determined after an adaptation period of 340 days.

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

S. Suárez, F. Omil and J.M. Lema (*submitted*) Removal of Pharmaceutical and Personal Care Products (PPCPs) under different redox conditions. Environ. Sci. Technol.

Outline

5.1. Introduction

Sewage Treatment Plants (STPs) that are designed in order to simultaneously eliminate organic matter and nitrogen from urban wastewater need to perform treatment under anoxic and aerobic conditions, which can be installed in different compartments of the plant (e.g. activated sludge plants), or be sequentially applied in one single reactor (e.g. sequential batch reactors). Heterotrophic conversion of organic matter is the main process in aerobic systems, where it is assimilated for growth (anabolism) and oxidized or mineralised (catabolism) with the consequent release of energy, at an approximate yield of ΔG^o -110 kJ/e-eqv, where O_2 acts as electron acceptor. Important genera of heterotrophic bacteria include *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Citromonas*, *Flavobacterium*, *Pseudomonas*, and *Zoogloea* (Jenkins et al., 1993). Autotrophic bacteria represent a small fraction of the biomass developed in aerobic reactors. This bacteria use an inorganic carbon source for growth, which normally consists of carbon dioxide that is being reduced through the oxidation of ammonia before its assimilation. This process occurs in two main steps, starting with ammonia oxidation to nitrite by bacteria *Nitrosomonas*, *Nitrosospira* and *Nitrosococcus*, followed by the subsequent oxidation of nitrite to nitrate by *Nitrobacter*, *Nitrococcus*, *Nitrospina* or *Nitrospira*. The energy yield of this oxidation is much lower (ΔG^o -270 kJ/mol $N\text{-}NH_4^+$ and ΔG^o -80 kJ/mol $N\text{-}NO_2^-$), which leads to low growth rates. For that reason, only plants that work at high Sludge Retention Times (SRTs between 10-15 days) promote the development of nitrifying bacteria. In addition, nitrifying bacteria are more sensitive to low oxygen concentrations than heterotrophic microorganisms, therefore dissolved oxygen concentrations should be maintained above 2 mg O_2/L to promote complete nitrification (Tillman, 1996). In anoxic ambient, facultative heterotrophic bacteria can use nitrate instead of oxygen as electron acceptor, which is known as denitrification, where $N\text{-}NO_3^-$ is removed from wastewater as nitrogen gas (N_2). It has to be outlined that bacteria preferentially utilize electron acceptors that provide the highest energy yield, which means that denitrification will only occur if no dissolved oxygen is available for bacteria.

Nowadays, removal mechanisms for conventional contaminants, fundamentally organic matter and nutrients, under different redox conditions are understood in detail and have been efficiently applied in most full-scale STPs, although not the same can be said for micropollutants. For the latter, normally only influent and effluent concentrations have been considered in the monitoring of STP (Clara et al., 2005; Joss et al., 2005; Gobel et al., 2007; Jones et al., 2007), without analysing the contribution of anoxic and aerobic conditions to the overall removal. There are some exceptions, as for example in Andersen et al. (2003) where sampling in the STP included both, effluents from denitrification and nitrification tanks, which led to the conclusion that while natural estrogens (E1 and E2) were already significantly

eliminated under denitrifying conditions, the synthetic hormone (EE2) was mainly removed in the following aerobic process. This behaviour was furthermore confirmed by kinetic experiments under aerobic, anoxic and anaerobic redox conditions (Joss et al., 2004), revealing that degradation of natural estrogens was possible under all those redox potentials, whereas EE2 could only be removed at a significant rate under aerobic conditions. For this latter compound, the enrichment of activated sludge in nitrifying bacteria could enhance its transformation into metabolites devoid of estrogenic activity (Vader et al., 2000). Additionally, biodegradation of some other pharmaceuticals (ibuprofen, diclofenac and clofibric acid) in oxic and anoxic biofilm reactors was investigated by Zwiener et al. (2000), where aerobic conditions have shown to be especially favourable for the transformation of IBP.

It is of great importance to extend the research about the behaviour of PPCPs in different redox conditions, not only to advance in the knowledge of the whole wastewater treatment process, but also in order to understand further pathways of those contaminants once released into the environment (e.g. groundwater recharge, degradation in surface water etc.). Different metabolites could be formed under aerobic and anoxic conditions, as has been reported for other pollutants, such as nonylphenol ethoxylate surfactants (Goel et al., 2003) or the pharmaceutical residue phenazone (Greskowiak et al., 2006), indicating that the pathways for anoxic and aerobic biodegradation processes are not always coincident. This could explain that for some pollutants higher removals have been observed under anoxic than under aerobic conditions, despite the lower oxidation potential of the first. This was reported by Drewes et al. (2001) in laboratory biodegradation experiments with triiodinated benzene derivatives used as X-ray contrast media, where negligible removal was observed under aerobic redox conditions, but partial removal in anoxic environments.

The objective of the present work was to evaluate the potential of aerobic and anoxic redox conditions for eliminating the 16 Pharmaceutical and Personal Care Products (PPCPs) considered in chapter 4, in order to better understand the overall removal process for such compounds in full-scale STPs.

5.2. Materials and methods

5.2.1. Denitrifying and nitrifying reactors

Two completely mixed reactors with a useful volume of 2 L, connected to a sedimentation tank of 1 L have been set-up for this part of the work (Figure 5-1). They were inoculated with biomass taken from the pilot plant described in chapter 4. One reactor has been working under anoxic conditions, whereas the other has been operating under a nitrifying aerobic ambient.

Figure 5-1. Completely mixed nitrifying (N) and denitrifying (DN) reactors.

Since these two reactors have been set-up with the aim of improving knowledge about the behaviour of PPCPs at the different redox conditions of the pilot plant (chapter 4), similar operation conditions have been considered. Thus, the reactors have been running at the same HRT of 1 d and with analogous compositions of the synthetic feed, although adapted to the requirements of the specific bacterial population that was intended to be developed. The parameters temperature, pH and SRT have not been manipulated during the whole operation and varied therefore freely, as occurred in the pilot plant of chapter 4 and also commonly in full-scale STPs.

Denitrifying reactor

The anoxic reactor was mechanically stirred (IKA® RW 20 DZM coupled to a three-bladed propeller) and capped in order to restrict the transfer of oxygen from air to the liquid phase (Figure 5-2), although not completely sealed since the nitrogen gas produced had to be evacuated. Recirculation of biomass from the settler to the reactor was carried out by means of a peristaltic pump (P-2: Masterflex ® L/S 1-100 rpm) at a flow rate of 60 L/d. The synthetic feed was stored in the fridge in a

10 L closed collapsible LDPE container and was continuously pumped into the reactor by a second peristaltic pump (P-1: Masterflex ® L/S 1-100 rpm) at a flow rate of 2 L/d. Once this pump was calibrated, the flow rate was additionally checked by following the decrease in the container's weight with time. The resulting operation parameters were a HRT of 1 d and an external recirculation ratio (Rext) of 30.

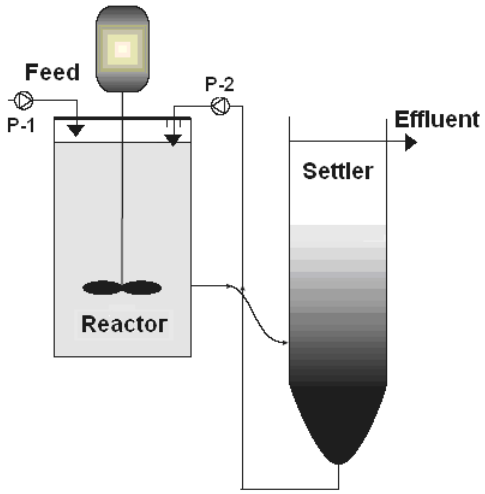

Figure 5-2. Schematic diagram of the denitrifying reactor.

The reactor was designed in order to promote a denitrification process, that is the elimination of nitrate as gaseous nitrogen (Equation 5-1), by heterotrophic bacteria. Therefore the synthetic feed incorporated an organic carbon source and nitrate with the following composition: 500 mg/L of COD, 40 mg/L of $N\text{-}NO_3$ and 8 mg/L of $P\text{-}PO_4$ (Table 5-1). The pH of the feed was initially adjusted to 7, although afterwards decreased to ~4, in order to solve two problems that were observed at the initial pH: The first was an alkaline reactor pH (>8) resulting from the denitrification process itself (Equation 5-1) and the second nitrate decomposition in the feed storing tank.

$$12NO_3^- + 19NaCH_3CO_2 + 12H^+ \rightarrow 18CO_2 + 4N_2 + 11H_2O + 19NaOH + 4C_5H_7O_2N \quad \text{[Eq. 5-1]}$$

Table 5-1. Composition of the feed for the denitrifying reactor and of the trace solution.

Compounds in the fed	Concentration (mg/L)	Compounds in the trace solution	Concentration (g/L)
$NaCH_3CO_2$	619	$FeCl_3 \cdot 6H_2O$	1.5
$NaNO_3$	240	H_3BO_3	0.15
Na_2HPO_4	24	$CuSO_4 \cdot 5H_2O$	0.03
KH_2PO_4	12	KI	0.03
Trace solution[(1)]	0.1	$ZnSO_4 \cdot 7H_2O$	0.12
		$CoCl_2 \cdot 6H_2O$	0.15
		$MnCl_2 \cdot 4H_2O$	0.12

[(1)] Concentration in mL/L.

Nitrifying reactor

In the aerobic plant oxygen was supplied at the bottom of the reactor by an air pump (Million Air MA-200) and distributed in the form of small bubbles by means of a ceramic diffuser (Figure 5-3).

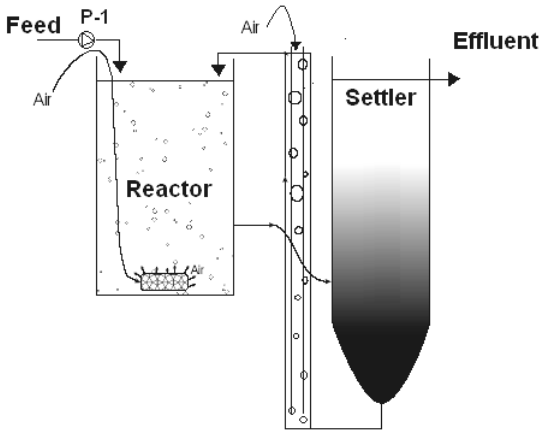

Figure 5-3. Schematic diagram of the nitrifying reactor.

Recirculation of biomass from the settler to the reactor was carried out by means of a mammut pump that was connected to an analogous air pump as those used for aeration. The synthetic feed was stored in the fridge in a 10 L closed collapsible LDPE container and was continuously pumped into the reactor by the same peristaltic pump as that of the anoxic one (P-1: Masterflex ® L/S 1-100 rpm) at a flow rate of 2 L/d and the flow rate was checked following the decrease in time of the container's weight. The operation parameters for this reactor were a HRT of 1 d and an external recirculation ratio (Rext) of 40, since no lower values could be obtained with the mammut pump used.

Table 5-2. Composition of the feed for the nitrifying reactor.

Compounds	Concentration
$NaHCO_3$	200 → 1000
NH_4Cl	153
Na_2HPO_4	24
KH_2PO_4	12
Trace solution	0.1

* All concentrations in ppm (mg/L), except trace solution (Table 5-1) in mL/L.

The process that wanted to be developed in the aerobic reactor was a pure nitrifying system where ammonia is oxidized to nitrate by autotrophic bacteria (Equation 5-2). Therefore the synthetic feed consisted of an inorganic carbon source and ammonia at analogous concentrations as the considered in the anoxic reactor and the pilot plant (chapter 4), that is 200 mg/L $NaHCO_3$, 40 mg/L of $N\text{-}NH_4$ and 8 mg/L of $P\text{-}PO_4$ (Table 5-2), although the concentration of $NaHCO_3$ had to be afterwards increased in order to compensate the generation of acidity during the nitrifying process. The pH of the feed was maintained at 7.

$$NH_4^+ + 1.86O_2 + 1.98HCO_3^- \rightarrow 0.02C_5H_7O_2N + 0.98NO_3^- + 1.88H_2CO_3 + 1.04\ H_2O \quad \text{[Eq. 5-2]}$$

Operation strategy

Both reactors have been fed without PPCPs during the first 50 days, when the aim was to achieve stabile and steady operation conditions rather than to analyse the fate and behaviour of micropollutants. This start-up period was maintained for at least one SRT, since bacterial population had to get adapted to the new redox and feeding conditions. After this initial stage, pharmaceuticals were incorporated to the feed at the concentrations indicated in Table 5-3.

Table 5-3. Concentration of PPCPs in the feeding tank.

Compound	Concentration (ppb or $\mu g \cdot L^{-1}$)	Compound	Concentration (ppb or $\mu g \cdot L^{-1}$)
Anti-depressants:		*Antibiotics:*	
Fluoxetine (FLX)	20	Trimethoprim (TMP)	
Citalopram (CTL)		Roxithromycin (ROX)	20
Estrogens:		Sulfamethoxazole (SMX)	
β-Estradiol (E2)	10	Erythromycin (ERY)	
α-Ethynylestradiol (EE2)			
Anti-inflammatories:		*Musks:*	
Ibuprofen (IBP)	10	Galaxolide (HHCB)	40
Naproxen (NPX)		Tonalide (AHTN)	
Diclofenac (DCF)		Celestolide (ADBI)	
Anti-epileptic:	20	*Tranquilliser:*	20
Carbamazepine (CBZ)		Diazepam (DZP)	

Both reactors have been continuously working during 440 d at constant operation parameters, although temperature fluctuated according to ambient values and SRT changed due to variable losses of biomass within the effluent.

The two plants were weekly sampled, including feed, reaction medium and final effluent, in order to analyse conventional parameters. Additionally, temperature (Hanna Instruments), pH (Crison) and dissolved oxygen (WTW® Oxi 340i) concentration have been determined in both reactors. After the addition of PPCPs, concentration of those compounds was monitored in the influent and effluent once per month, although the sampling frequency was reduced to one campaign every 2-3 months after the first seven months. In the case of antibiotics only two sampling campaigns have been carried out.

5.2.2. Analytical methods

Total and Volatile Suspended Solids (TSS and VSS), nitrite and nitrate concentrations were determined following Standard Methods (APHA, 1999). Amoniacal nitrogen, Total, Inorganic and Organic Carbon (TC, IC and TOC) were determined according to section 2.

The concentration of PPCPs was determined following the methods described in chapter 2. The samples were collected in glass or aluminium bottles and immediately prefiltered (glass fibre prefiltres, AP4004705 Millipore). For the analysis of antibiotics, a pinch of sodium azide was added to the filtered sample before its storage in the freezer, until analysed by the Austrian Federal Environment Agency. For the rest of compounds, samples were analysed within one week, consequently storage in the fridge was sufficient.

5.2.3. Mass balances

Mass balances were applied following the same procedure as in chapter 4 (for details see 4.2.3).

Stripping

Briefly, the influence of volatilisation has been only evaluated for the aerobic reactor, since the anoxic plant was not aerated. The relative fraction stripped to the gas phase was calculated according to Equation 5-3:

$$\frac{C^{*}_{j,air}}{C_{j,total}} = \frac{H \cdot q_{air}}{1 + K_{d,j} \cdot SST + H \cdot q_{air}} \qquad \text{[Eq. 5-3]}$$

where, $C^{*}_{j,air}$ is the concentration of compound j that leaves the reactor during aeration and $C_{j,total}$ the total concentration of compound j, both in µg/L, H the dimensionless Henry's law constant, q_{air} the aeration applied per unit of wastewater treated ($L_{air}/L_{wastewater}$), $K_{d,j}$ the solid–water distribution coefficient of compound j (L/kg) and SST the suspended solids concentration inside the reactor (kg/L).

According to Equation 5-3 in the worst case, assuming a q_{air} of 15 and a SST inside the aerobic plant of 0.8 g/L, the influence of volatilisation was not significant

for any compound (<4%, even for the relatively volatile compounds Galaxolide and Tonalide), except for Celestolide (58%). Therefore, the mass flow of compound j that leaves the aerobic reactor due to volatilisation ($F_{j,Stripped}$ in µg/d) has been calculated according to Equation 5-4 and included in the mass balances.

$$F_{j,Stripped} = C^{*}_{j,air} \cdot Q \qquad \text{[Eq. 5-4]}$$

Q in Equation 5-4 represents the flow treated in the aerobic reactor (L/d).

Sorption

Mass flow of compound j that leaves the reactors sorbed onto the solids of the final effluent ($F_{j,Sol}$ in µg/d) has been estimated applying Equation 5-5 where sorption equilibrium is assumed:

$$F_{j,Sol} = Q \cdot K_{d,j} \cdot SST_{Eff} \cdot C_{j,Eff} \qquad \text{[Eq. 5-5]}$$

where, $C_{j,Eff}$ is the dissolved concentration of compound j (µg/L) and SST_{Eff} the suspended solids concentration (kg/L), both measured in the effluent.

Sorption coefficients ($K_{d,j}$) considered in the mass balances were the same as those of chapter 4, with the exception of fragrances (HHCB, AHTN and ADBI) where this parameter has been calculated from experimental total and soluble concentrations measured in the effluent.

Total mass flow of compound j in the effluent ($F_{j,Eff}$ in µg/L) has been calculated as the sum of the flow in the liquid and the solid phase (Equation 5-6).

$$F_{j,Eff} = C_{j,Eff} \cdot Q \cdot \left(1 + K_{d,j} SST_{Eff}\right) \qquad \text{[Eq. 5-6]}$$

In the case of influents, the mass flow ($F_{j,Feed}$ in µg/L) has been calculated assuming that sorption is negligible, since the synthetic feed didn't contain solid particles (Equation 5-7):

$$F_{j,Feed} = C_{j,Feed} \cdot Q \qquad \text{[Eq. 5-7]}$$

where, $C_{j,Feed}$ is the concentration of compound j in the feed (µg/L).

Assuming steady state conditions for the reactors, biological transformation can be calculated according to Equation 5-8:

$$E_{j,Anox} = \frac{F_{j,Feed} - F_{j,Eff}}{F_{j,Feed}} \cdot 100$$
$$E_{j,Aer} = \frac{F_{j,Feed} - \left(F_{j,Eff} + F_{j,Stripped}\right)}{F_{j,Feed}} \cdot 100 \qquad \text{[Eq. 5-8]}$$

where, $E_{j,Anox}$ and $E_{j,Aer}$ are the transformation efficiencies (%) for compound j in the anoxic and aerobic reactor, respectively.

5.3. Results and discussion

5.3.1. Conventional operation parameters

The physical parameters dissolved oxygen concentration, temperature and pH were followed inside both reactors and are depicted in Figure 5-4.

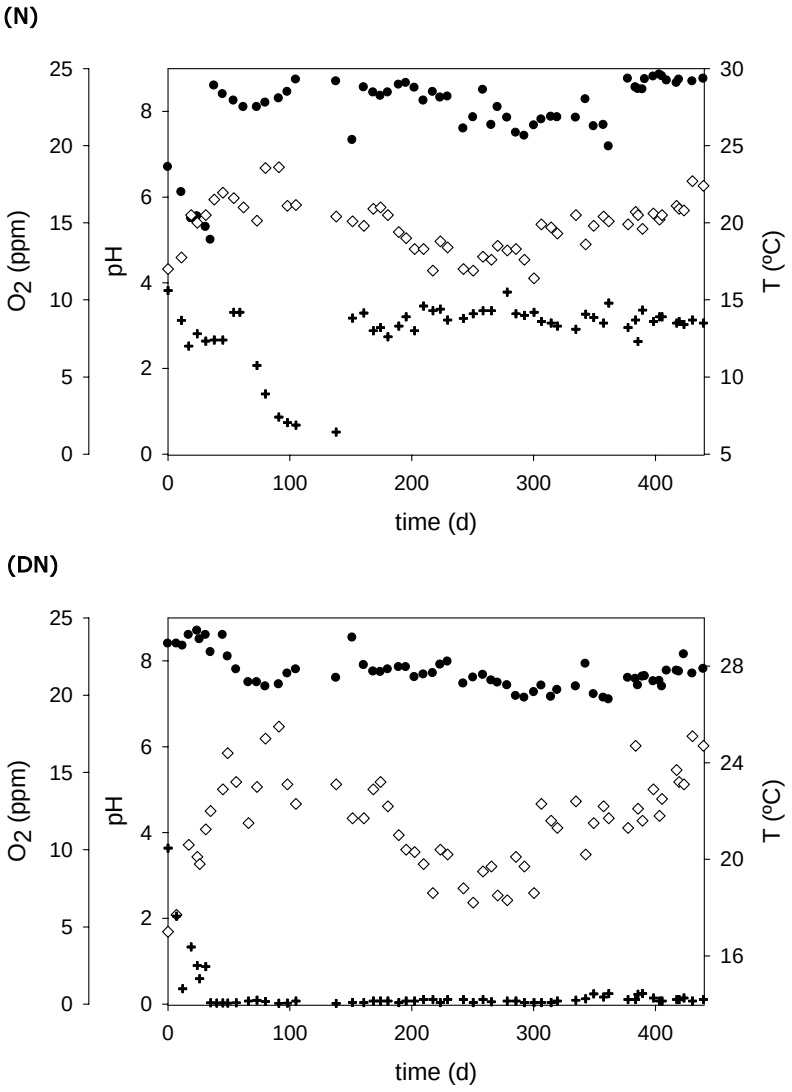

Figure 5-4. Temperature (◊), pH (●) and dissolved oxygen (+) in the nitrifying (N) and denitrifying (DN) reactor.

In Figure 5-4 (A) it can be observed how the initial decrease in pH in the aerobic reactor has been successfully compensated by increasing the concentration of $NaHCO_3$ after day 35. No further adjustment of pH was carried out, since it was naturally maintained within the optimal pH range for nitrifying bacteria (7.2-9, Metcalf & Eddy, 2003). Similarly, after decreasing the pH of the anoxic feed from 7 to ~4 at day 32, the pH inside the reactor decreased from ~8.5 to ~7.5, which is closer to the range of the optimal value for denitrifying bacteria (6.5-7.5, Metcalf & Eddy, 2003).

During the start-up period of the anoxic reactor dissolved oxygen concentration was above 1.5 mg O_2/L, which was attributed to an excessive stirring of the reactor that was open to the air at this initial stage. This problem was solved by capping the plant and lowering the stirring speed so as to assure complete homogenization. From that moment onwards, oxygen concentration was kept below 0.5 mg O_2/L, which is the usual reference point used to avoid troubleshooting in such processes (Tillman, 1996). In the case of the aerobic reactor, oxygen level was maintained at saturation (~9 mg O_2/L), since it was used for recirculation and for mixing, with the exception of the drop observed at day 70 due to a failure in the aeration system. In any case the oxygen level was still maintained above 2 mg O_2/L, which avoided problems regarding failure of the nitrification process.

Fluctuations in temperature were in the range of 16-21ºC and 21-26ºC, during winter and summer, respectively.

Both, the anoxic and aerobic reactor, were inoculated with sludge from the pilot plant of chapter 4, at an initial concentration of 1.4 and 1.1 g VSS/L, respectively. Biomass concentration has been regularly followed inside the reactors, as well as in the effluents (Figure 5-5). While in the anoxic reactor the concentration of sludge remained stable around 1.5 g VSS/L during the whole operation period, with the exception of some punctual situations (day 175 and 270) where some biomass was washed out and its concentration inside the reactor slightly decreased, this was not the case of the aerobic plant. In this latter case, biomass concentration decreased down to 0.5 g VSS/L until day 160, most probably due to the promotion of endogenous respiration of heterotrophic bacteria, that had been inoculated together with the autotrophic nitrifying bacteria from the pilot plant, in the absence of an organic matter source. After this initial stage, bacterial population stabilised and initiated a growth period between day 161 and 314, at a rate of $7.8 \cdot 10^{-3}$ g VSS/d, achieving a steady sludge concentration of 1.6 g VSS/L. The yield constant of produced biomass per amount of ammonia oxidized was 0.1 g VSS/g N-NH_4, which is in the same order as the stoichiometric parameter (Equation 5-2).

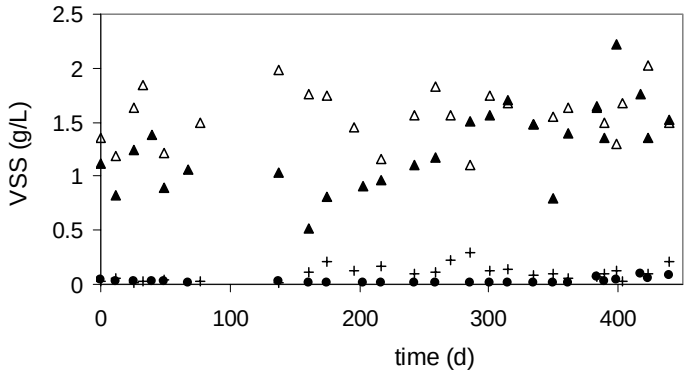

Figure 5-5. Biomass concentration, measured as VSS, inside the anoxic (△) and aerobic reactor (▲), as well as in the effluents (anoxic: + and aerobic: •).

The efficiency of the aerobic reactor regarding nitrification, calculated as $\frac{(N-NO_{3\,Effluent})-(N-NO_{3\,Feed})}{N-NH_{4\,Feed}}\cdot 100$, increased from ~40% up to ~90% during the first 30 days of operation (Figure 5-6). After this start-up period, nitrification efficiency remained stable and very close to the maximum value of 98% (Equation 5-2). The fulfilment of nitrogen balance in the reactor is shown in Figure 5-6 by the coincidence of the concentrations of N-NH_4 consumed and N-NO_3 produced.

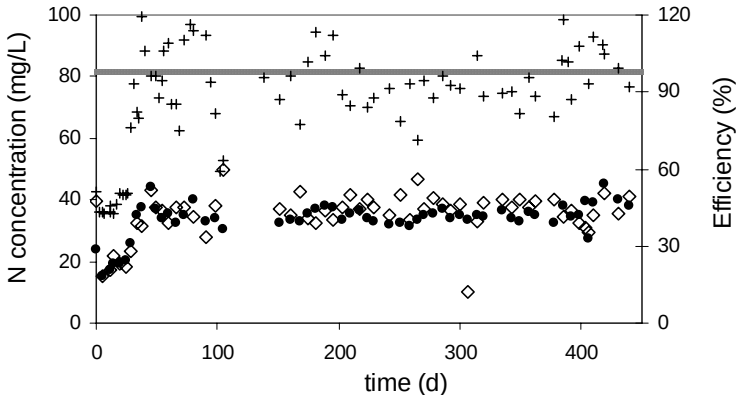

Figure 5-6. Efficiency of nitrification in the aerobic reactor (+) compared to the calculated maximum value (××××××), N-NH_4 consumed (◊) and N-NO_3 produced (•).

Similarly, the anoxic reactor showed to be very efficient in the removal of nitrate in the form of nitrogen gas, since after 50 days of start-up period, the efficiency, calculated as $\frac{(N-NO_{3Feed})-(N-NO_{3Effluent})}{N-NO_{3Feed}}\cdot 100$, stabilised around 100%. The main difficulty in the operation of this reactor was to maintain a stable concentration of nitrate in the synthetic feed, which even after the acidification and refrigeration of the feed, was not completely attained (Figure 5-7).

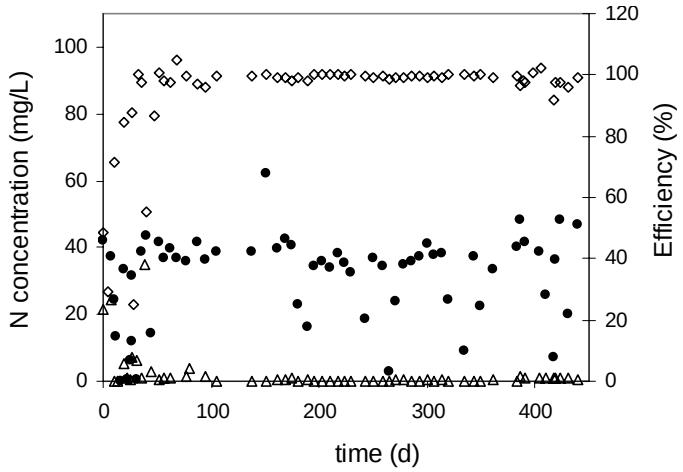

Figure 5-7. Efficiency of denitrification in the anoxic reactor (◊) and N-NO_3 concentration in the influent (•) and effluent (△) of the plant.

5.3.2. Fate of PPCPs in the anoxic and aerobic reactors. Application of mass balances

Spikes of the 16 PPCPs considered have been added to the feed of both reactors after the start-up period of 50 days. Both, influent and effluent samples, have been regularly taken in order to determine the concentration of pharmaceuticals in the liquid phase. Fragrances (HHCB, AHTN and ADBI), have been regularly analysed in the liquid phase, whereas total concentration including sorbed and dissolved fraction has been measured on only one occasion.

Fate of PPCPs in the reactors

Table 5-4 summarises mean concentrations of PPCPs measured in the influent and effluent from both reactors, as well as the removal achieved for each compound according to Equation 5-9:

$$\text{Re moval}\,(\%) = \begin{bmatrix} \text{Anoxic Reactor}: & \dfrac{C_{\text{Anox Feed}} - C_{\text{Anox Eff}}}{C_{\text{Anox Feed}}} \cdot 100 \\ \text{Aerobic Reactor}: & \dfrac{C_{\text{Aer Feed}} - C_{\text{Aer Eff}}}{C_{\text{Aer Feed}}} \cdot 100 \end{bmatrix} \qquad \text{[Eq. 5-9]}$$

where $C_{Anox\ Feed}$, $C_{Anox\ Eff}$, $C_{Aer\ Feed}$, $C_{Aer\ Eff}$ are the concentrations of PPCPs in the feed and effluent from the anoxic and aerobic reactors, respectively (ppb or µg/L). In all cases, removal was calculated from the measured influent concentration, rather than from that spiked to the feeding tank (Table 5-3). The differences between both could be attributed to losses of the spiked compounds due to degradation or sorption within the feeding system.

Table 5-4. Mean concentrations of PPCPs in the feed (C_{Feed}) and effluent (C_{Eff}) of the aerobic and anoxic reactors (ppb or µg/L). Removal from the liquid phase (%).

PPCP	Aerobic reactor			Anoxic reactor		
	C_{Feed}	*C_{Eff}*	*Removal*	*C_{Feed}*	*C_{Eff}*	*Removal*
E1+E2	6.6±1.4	0.07±0.04	99±0	6.4±0.8	1.8±0.3	72±2
IBP	8.1±1.5	0.4±0.3	95±4	8.0±0.7	5.1±2.0	37±26
HHCB	13.4±8.6	0.4±0.3	92±12	10.2±8.2	0.5±0.2	86±15
AHTN	15.5±11.0	0.7±0.3	90±13	11.7±9.8	0.9±0.1	82±16
ADBI	15.8±7.0	0.4±0.3	97±2	10.9±7.7	0.6±0.2	88±15
FLX	13.4±3.7	1.0±0.2	92±3	14.6±6.5	2.2±0.8	84±6
ROX	17.4±5.9	1.6±0.6	91±0	18.8±1.2	15.4±2.5	15±7
ERY	17.7±2.2	2.0±0.7	89±2	23.9±0.1	19.1±2.3	20±10
EE2	5.5±2.6	0.8±0.9	87±11	5.8±1.9	4.6±1.4	20±13
NPX	9.5±0.9	1.3±0.5	86±5	9.0±1.1	8.1±0.4	9±13
CTL	13.0±7.4	4.5±2.3	60±17	16.0±4.3	9.0±3.0	44±9
DCF	8.2±1.9	6.2±2.7	22±28	6.4±0.9	6.2±0.6	2±5
SMX	21.1±1.6	16.4±0.1	22±5	n.a.	n.a.	-
DZP	16.1±4.1	13.3±3.5	17±11	15.3±5.8	12.2±3.4	16±17
TMP	19.3±1.0	16.4±.1.0	14±10	n.a.	n.a.	-
CBZ	19.0±4.9	18.1±5.9	6±12	17.9±4.8	17.9±5.6	1±10

n.a. not analysed

Ten of the considered PPCPs were removed to a high degree (>86%) in the aerobic reactor, comprising hormones (E1+E2 and EE2), the anti-inflammatory drugs IBP and NPX, the three musks (HHCB, AHTN, and ADBI), the anti-depressant FLX and two antibiotics (ROX and ERY). This high efficiency of aerobic treatment plants regarding the removal of hormones, anti-inflammatory drugs and fragrances have been already reported by several authors (Baronti et al., 2000; Simonich et

al., 2002; Joss et al., 2004; Kupper et al., 2006; Nakada et al., 2006; Gomez et al., 2007), whereas eliminations previously determined for FLX, ROX and ERY (Joss et al., 2005; Castiglioni et al., 2006; Vasskog et al., 2006; Gobel et al., 2007) were significantly lower than those measured in the present work. The anoxic reactor has shown to be able to remove fragrances, FLX and natural estrogens (E1+E2) in an effective way, although in a slightly lower degree (>72%) compared to the aerobic reactor, whereas for the rest of these ten compounds removal achieved was much less effective (<37%).

The other anti-depressant, CTL, has partially been removed in both reactors (60% and 44% in the aerobic and anoxic reactor, respectively), similar to what has been observed by Vasskog et al. (2006).

The rest of pharmaceuticals (DCF, SMX, DZP, TMP and CBZ) have not been significantly transformed (<22%) by the biological treatment with neither nitrifying nor denitrifying bacteria, with the exception of the two antibiotics (SMX and TMP) whose behaviour in the anoxic reactor could not be established, since none of these compounds has been detected in the feed or effluent of the reactor, although both had been spiked to the synthetic feed. The reason for that is not completely clear, although interferences with some components of the feed could be the reason. The high persistence of these compounds has also been observed in different full-scale STP (Clara et al., 2004; Lindberg et al., 2005; Gobel et al., 2007; Lindqvist et al., 2005), with the exception of SMX where removal efficiencies reported varied in a wide range. For example, eliminations of 0-84% and (-138)-60% can be found in Castiglioni et al. (2006) and Gobel et al. (2007), respectively, which can be partially due to the complexity of real wastewaters (chapter 4).

Application of mass balances

Mass balances have been applied to each compound, according to Equations [5-4] – [5-8] and results have been graphically represented, including the contribution of biological transformation ($E_{j,Anox}$ and $E_{j,Aer}$), sorption ($F_{j,sol}/F_{j,Feed}\cdot 100$) and volatilisation in the case of ADBI in the aerobic reactor ($F_{j,Stripped}/F_{j,Feed}\cdot 100$) to the overall removal of PPCPs. Residual fraction of each compound that leaves the reactors with the effluent has been also included in the plots ($C_{j,Eff}\cdot Q/F_{j,Feed}\cdot 100$).

The operation period of both reactors has been divided according to two levels of temperature (low: 16-20ºC or 16-21ºC and high: 20-24ºC or 21-26ºC for the aerobic or anoxic reactor, respectively), as well as two levels of SRT, whose limit was determined by the fluctuations in the biomass concentration inside the reactors and in the effluents (50 and 20 days in the aerobic and anoxic reactor, respectively).

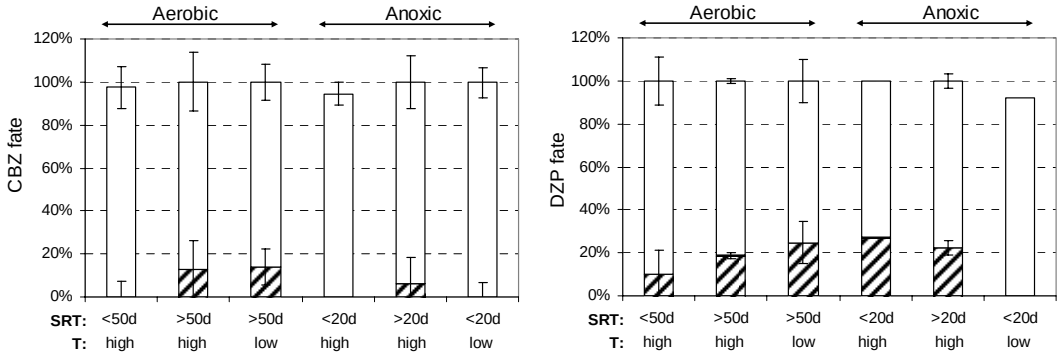

Figure 5-8. Fate of CBZ and DZP in the aerobic and anoxic reactors, indicating the contribution of biological transformation (▨), sorption (■) and release within the effluent (□).

The two most recalcitrant compounds out of the selected PPCPs were CBZ and DZP (Figure 5-8), according to what had been already observed in the pilot plant experiments (chapter 4). The third compound that showed to be very persistent during biological treatment in chapter 4 was DCF, which was confirmed by the anoxic reactor results, but not by the aerobic ones (Figure 5-9). For the latter high deviation in the aerobic data (Figure 5-9 A) led to represent the removals as a function of biomass concentration inside the plant for the different sampling dates (Figure 5-9 B). The data seem to indicate that there has been an initial adaptation period that coincides with the death and wash out of heterotrophic bacteria (~170d) during which removal of DCF increased from 0% to 25%. After that day, a correlation between sludge concentration in the reactor and elimination of DCF was observed, similarly to the behaviour of NPX in the pilot plant (chapter 4), reaching maximum removals of around 74%. The fate of DCF under anoxic and oxic conditions has been investigated by Zwiener et al. (2000) in biofilm reactors, where achieved efficiencies were below 20% under both conditions. Taking into account that those biofilm reactors had been inoculated with municipal sewage sludge and that operation stopped after only 120 days, these results are comparable to the ones obtained in the present research during the first months.

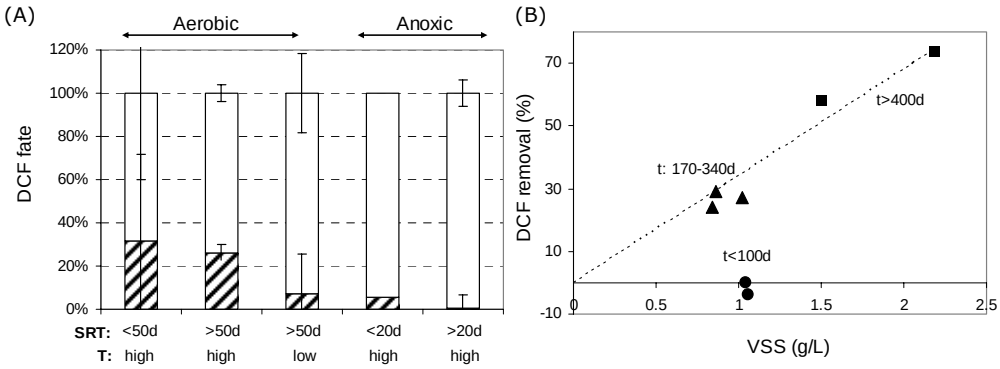

Figure 5-9. A) Fate of DCF in the aerobic and anoxic reactors, indicating the contribution of biological transformation (◪), sorption (■) and release within the effluent (□). B) Correlation between removal of DCF and biomass concentration in the aerobic reactor for the different sampling dates (t).

The compounds FLX and NPX were both transformed to a high degree in the aerobic reactor (Figure 5-10) with less than 9% and 16% of residual mass flow in the effluent, respectively. While FLX exhibited significant transformation in the anoxic treatment (79-89%), that was not the case of NPX. The observed results correspond very well with the fate of those compounds in the denitrifying-nitrifying pilot plant (chapter 4). A positive effect of increasing the SRT of the reactor has been observed for FLX in the anoxic reactor and NPX in the aerobic one, although for the latter the increase in removal was very slight (3%).

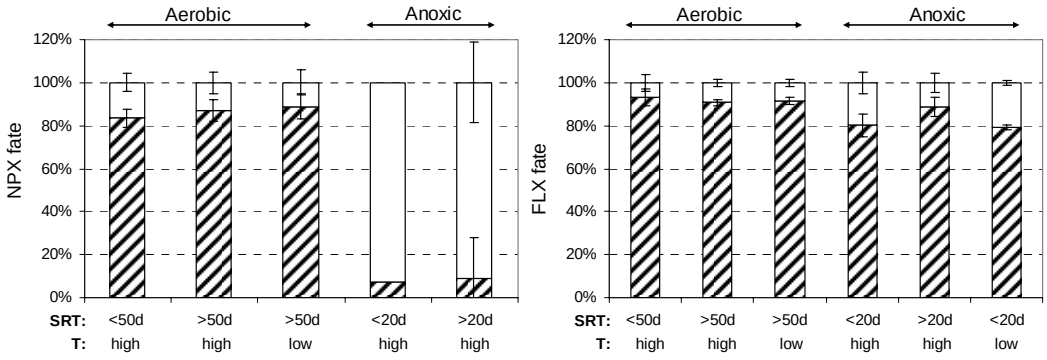

Figure 5-10. Fate of NPX and FLX in the aerobic and anoxic reactors, indicating the contribution of biological transformation (◪), sorption (■) and release within the effluent (□).

Ibuprofen and NPX exhibit both low sorption potential, although their aerobic biological degradation constant is high and moderate, respectively ($k_{biol\ IBP}$: 9-35 L/g SS·d; $k_{biol\ NPX}$: 0.4-1.9 L/g SS·d). As expected, aerobic transformation of IBP was slightly better than for NPX, between 93-96% (Figure 5-11). When data measured in the anoxic process were classified according to operational conditions of temperature and SRT, high deviation were observed (Figure 5-11 A), thus removal was plotted as a function of time (Figure 5-11 B). In this case adaptation of bacteria seem to be responsible for the wide range of transformation efficiencies measured, since it increased gradually with time from below 16% (day 0-200) up to ~45% (day 250) and finally ~75% (day 340). In any case, adaptation of bacteria seems not to be related to the pharmaceutical itself, since biomass was taken from a reactor that had already been fed with IBP for six months, but to the development of a specific denitrifying biomass with different enzymatic spectrum. In fact, in the combined anoxic/aerobic pilot plant (chapter 4), no removal of IBP in the anoxic compartment has been observed, whereas the transformation in the aerobic part reached similar levels as in the present aerobic reactor. Zwiener et al. (2000) measured removals for IBP of more than 90% under oxic and of 15% under anoxic conditions, although, as stated previously, they could have missed adaptation due to a too early stop of the reactors (120 days).

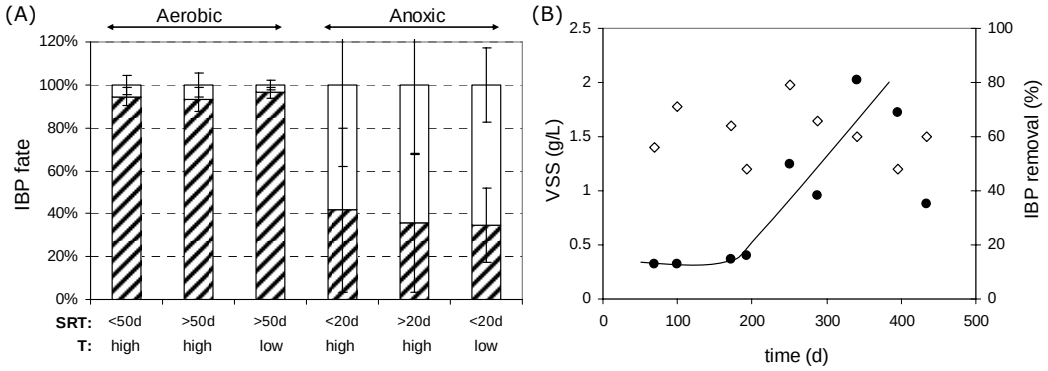

Figure 5-11. A) Fate of IBP in the aerobic and anoxic reactors, indicating the contribution of biological transformation (▨), sorption (■) and release within the effluent (□). B) Correlation between removal of IBP in the anoxic reactor (•) and its biomass concentration (◊).

Natural estrogens (E1 and E2) were highly transformed (99%) under aerobic conditions and even in the anoxic reactor transformation was significant (69-73%, Figure 5-12), according to their high biological degradation constants ($k_{biol\ aerobic}$ > 160 L/g_{SS}·d and $k_{biol\ anoxic}$ > 30 L/g_{SS}·d) determined for both compounds under both redox conditions (Joss et al., 2004). In the anoxic reactor, a slight increase in the

transformation degree was observed when increasing the SRT of the plant. In full-scale STPs transformation of natural estrogens in the anoxic compartment has been reported by Andersen et al. (2003), although in the pilot plant of chapter 4 removal only occurred in the aerobic tank of the plant. According to Joss et al. (2004), this could be attributed to a competitive inhibition of their degradation by the influent substrate, inhibition that was not observed in the current anoxic reactor. Consequently, inhibition in the pilot plant of chapter 4 could be rather attributed to degradation products that are formed upon aerobic E1 and E2 biotransformation, which are conducted back to the inlet of the pilot plant through both, the internal and external recirculation stream.

Ethinylestradiol was only transformed appreciably in the aerobic reactor (82-90%), whereas under anoxic conditions less than 26% of the parent compound was degraded (Figure 5-12). This corresponds very well with the kinetic behaviour of EE2, according to Joss et al. (2004), for different redox conditions, where it was shown that EE2 was removed at a significant rate only under aerobic conditions. This was additionally observed in combined anoxic/aerobic treatment plants (Andersen et al., 2003, pilot plant of chapter 4). Nitrifying sludge was reported to enhance transformation of EE2, via hydroxylation that converts EE2 into hydrophilic products devoid of estrogenic activity (Vader et al., 2000), although in this case the improvement of the process when enriching the sludge from the pilot plant (chapter 4) in nitrifying bacteria was very slight (~6%). The transformation efficiency for EE2 in the aerobic reactor increased an 8% when the plant was operated at the higher SRT (Figure 5-12).

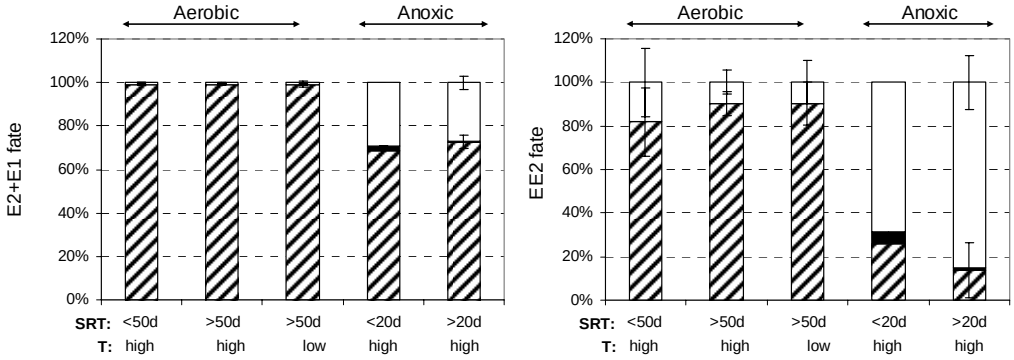

Figure 5-12. Fate of natural hormones (E1+E2) and EE2 in the aerobic and anoxic reactors, indicating the contribution of biological transformation (▨), sorption (■) and release within the effluent (□).

In the case of antibiotics only two sampling campaigns have been carried out, both at the same conditions of low temperature and SRT below 20 d (Figure 5-13). Roxithromycin and erythromycin were transformed very efficiently (~90%) in the aerobic reactor and to a larger extent than at similar conditions (lower T and SRT) in the pilot plant (chapter 4). This could be an indication of a higher affinity of nitrifying bacteria towards these compounds. On the other hand, only slight transformations of these two antibiotics have been observed in the anoxic reactor (<27%), which confirms the results for ROX and clarifies those of ERY obtained in the pilot plant experiments (chapter 4). The other two antibiotics considered (SMX and TMP) have shown a higher persistence towards aerobic biological treatment, since the maximum transformation observed was 26% and 21% for SMX and TMP, respectively. If these results are compared to those previously obtained in the pilot plant when operating at the lower temperature and SRT (chapter 4), similar results were found for SMX (38%), but significantly worse efficiencies for TMP, whose removal in the pilot plant was around 76%, although part of this transformation could have occurred in the anoxic compartment of the pilot plant. Perez et al. (2005) reported that nitrifying microorganisms were more efficient in degrading trimethoprim than sludge from a conventional aerobic process which showed a great resistance to this biodegradation, although this could not be confirmed in the present work where the opposite was observed.

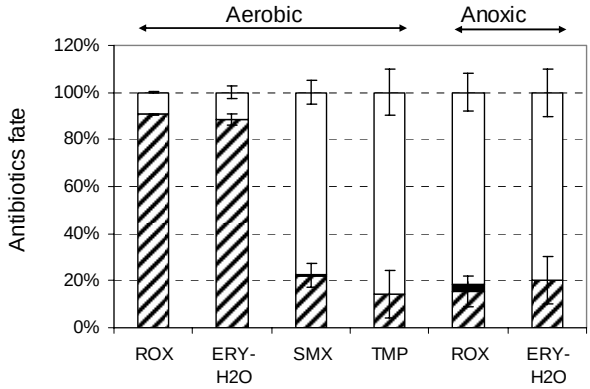

Figure 5-13. Fate of antibiotics (ROX, ERY, SMX and TMP) in the aerobic and anoxic reactors, indicating the contribution of biological transformation (▨), sorption (■) and release within the effluent (□)

Transformation of CTL in the aerobic reactor increased from 62% at the lower SRT up to 70% when this parameter was increased (Figure 5-14). Similarly, the increase in temperature led to an improvement of 4% in the performance of this reactor. The efficiency of the anoxic plant was somewhat lower, although still quite

significant (41-46%), which was surprising on the basis of the results observed in the pilot plant (chapter 4), where similar overall removals had been measured (50-60%) but no contribution of the anoxic compartment had been detected.

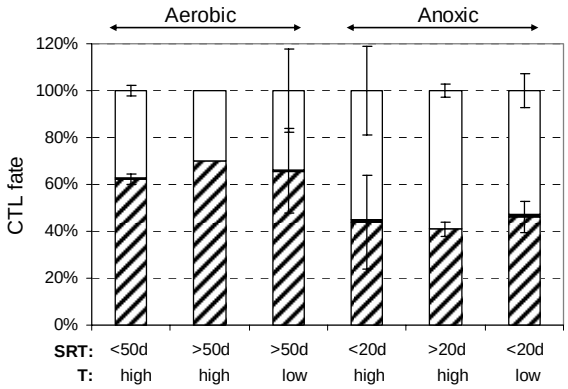

Figure 5-14. Fate of CTL in the aerobic and anoxic reactors, indicating the contribution of biological transformation (▨), sorption (■) and release within the effluent (□).

For musk compound ADBI the three removal mechanisms, volatilisation, sorption and biodegradation, have been considered in the mass balance of the aerobic reactor (Figure 5-13), whereas for the other two fragrances (HHCB and AHTN) volatilisation has been neglected. The air flow considered in Equations 5-3 and 5-4 was 5 L_{air}/L_{WW}, in order to maintain the same assumption as in chapter 4. Moreover, the sorption coefficients (K_d) applied in Equation 5-5 have been calculated from experimental values of total and soluble concentrations of fragrances, with the following result: $1.5 \cdot 10^3$ and $4.9 \cdot 10^3$ L/kg for HHCB, $2.0 \cdot 10^3$ and $3.4 \cdot 10^3$ L/kg for AHTN and $5.2 \cdot 10^2$ and $3.9 \cdot 10^3$ L/kg for ADBI, in the aerobic and anoxic reactor, respectively. These values are in the same order as those determined for the pilot plant (chapter 4), except for ADBI in the aerobic plant where the sorption coefficient was almost one order of magnitude lower. For the three compounds, determined sorption coefficients in the anoxic reactor were somewhat higher than aerobic ones, which is normally not considered when applying mass balances in full-scale STP. Therefore, it is strongly recommended to determine those coefficients for each particular situation, especially in the case of highly lipophilic compounds, at least until the different factors that exert an influence on K_d and the large range of published K_d (Ternes et al., 2004; Joss et al., 2005; Kupper et al., 2006) are completely clarified.

Transformation of HHCB and AHTN during aerobic biological treatment reached 79-99% and 76-98%, respectively, whereas in the anoxic plant the efficiency was slightly lower, in the range of 67-84% and 65-76%, respectively. Therefore, removal of these fragrances was not exclusively due to sorption, in spite of what had been reported by Bester (2004) and Joss et al. (2005) for AHTN. In fact, in the present study, the fraction of these substances that left the reactors sorbed onto the solids contained in the effluent was negligible in the aerobic reactor and below 18% in the anoxic one (Figure 5-15). Results regarding the fate of HHCB and AHTN are similar to those attained in the pilot plant (chapter 4) and confirm that these compounds can be transformed under both, anoxic and aerobic redox conditions, at very high efficiencies.

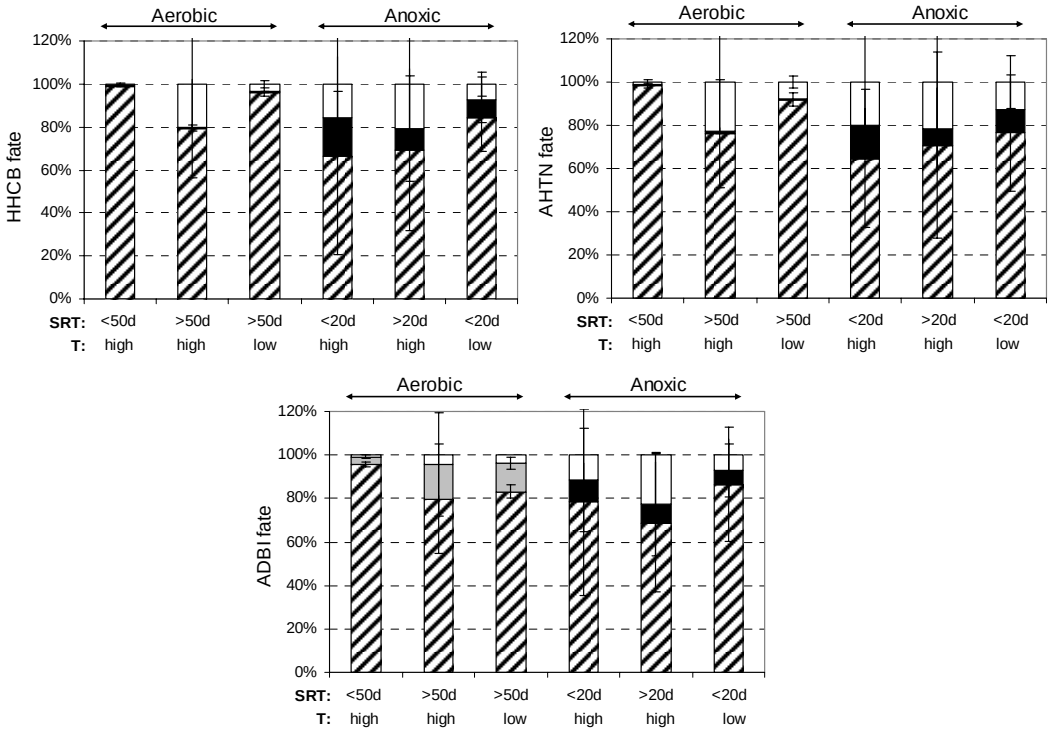

Figure 5-15. Fate of HHCB, AHTN and ADBI in the aerobic and anoxic reactors, indicating the contribution of biological transformation (◪), sorption (■), release within the effluent (□) and for ADBI volatilisation in the aerobic reactor (■).

Residual concentration of ADBI in the aerobic effluent was below 4%, being the two most significant removal pathways biological transformation (80-96%) and volatilisation (3-16%), since contribution of sorption was negligible (Figure 5-15). The anoxic reactor also demonstrated high efficiency in the overall removal of ADBI

(78-93%), although in this case sorption (7-10%) and biodegradation (69-89%) were the responsible removal processes (Figure 5-15). For this substance, the results obtained in this biodegradation experiments were slightly better than those obtained in the pilot plant (transformation of 44-77% in chapter 4).

5.4. Conclusions

Two reactors, one working at nitrifying aerobic conditions and the other in an anoxic denitrifying environment, have been fed continuously with the same set of 16 PPCPs as the pilot plant of the previous chapter, comprising musk compounds, hormones, anti-epileptics, tranquilisers, anti-depressants, anti-inflammatories and antibiotics.

Concentration of PPCPs in the liquid phase was followed in the feed and effluent from both reactors, in order to characterise their fate, as well as to analyse the contribution of sorption, transformation and volatilisation on their removal, by the application of mass balances. The major removal pathway for the selected compounds, except for fragrances, was (bio)transformation. Sorption accounted for 7-18% of total mass flow for musk compounds in the effluent from the anoxic reactor, but was negligible in the case of the aerobic plant, due to the better settling characteristics of nitrifying biomass. Volatilisation was only significant for ADBI and contributed between 3-16% to the removal of this substance.

The selected compounds could be classified according to their aerobic and anoxic biodegradability as follows (Table 5-5):

- √ Highly biodegradable under aerobic and anoxic conditions: FLX, natural estrogens (E1+E2) and musk fragrances (HHCB, AHTN and ADBI).
- √ Highly biodegradable under aerobic conditions but persistent in the anoxic reactor: NPX, EE2, ROX and ERY.
- √ Moderately biodegradable under aerobic and anoxic conditions: CTL
- √ Resistant to biological transformation: CBZ and DZP
- √ In the case of SMX and TMP only the behaviour in the aerobic reactor could be determined. Both compounds manifested low biotransformation potential.
- √ Diclofenac passed the anoxic reactor without undergoing any transformation, whereas in the aerobic reactor, after stabilisation and further development of a nitrifying biomass, removal of DCF increased up to 74%.
- √ Efficient aerobic transformation of IBP was confirmed, and even in the anoxic reactor removals of 75% could be achieved after an adaptation period of 340 days.

Moreover, the influence on the removal of PPCPs of two operation parameters, temperature and SRT, was analysed (Table 5-5). The positive effect of increasing SRT has been demonstrated in five occasions, with maximum improvement of ~10%

in the removal of FLX, CTL and EE2, whereas temperature only affected very slightly (4%) the removal of CTL.

Table 5-5. Summary of the transformations achieved for the considered PPCPs in the anoxic and aerobic reactors in comparison with those previously determined in the pilot plant experiments (chapter 4).

Compound	Transformation		Influence		Transformation in PP		
	Aerobic	Anoxic	SRT	T	Anoxic	Aerobic	Overall
CBZ	––	––	no	no	––	––	––
DZP	––	––	no	no	––	––	––
DCF	––/+	––	no	no	––	––	––
FLX	++	++	yes	no	––/+	–/++	++
NPX	++	––	yes	no	––	++	++
IBP	++	––/+	no	no	––	++	++
E1+E2	++	+	yes	no	––	++	++
EE2	++	––/–	yes	no	––	+/++	+/++
CTL	+	–+	yes	yes	––	–+	–+
SMX	–	n.a.	n.a.	n.a.	––	–+/+	–+/+
ROX	++	––	n.a.	n.a.	––	+/++	+/++
TMP	–	n.a.	n.a.	n.a.	––/–+	––/+	–+/+
ERY	++	––/–	n.a.	n.a.	–/–+	–/+	–+/+
HHCB	++	+	no	no	–/+	–/–+	++
AHTN	++	+	no	no	–+/++	––/–+	++
ADBI	++	+/++	no	no	–/–+	––/–	–+/+

(––) <20%; (–) 20-40%; (–+) 40-60%; (+) 60-80%; (++) >80%; n.a. not analysed. The influence of SRT or temperature (T) on the transformation degree is indicated as (yes) or (no).

One of the objectives of the present work was to compare the obtained results with those determined for the pilot plant where aerobic and anoxic processes were sequentially applied (chapter 4). The following conclusions can be drawn (Table 5-5):

- √ Comparable results have been obtained for CBZ, DZP, FLX, NPX, EE2, SMX, HHCB and AHTN.
- √ The considerable removal of TMP measured in the pilot plant has not been observed in the aerobic biodegradation experiment, although since part of the transformation measured in the pilot plant could already have occurred in its anoxic compartment (the results were not conclusive), anoxic biodegradability should be determined in order to make a definite conclusion.
- √ Some PPCPs were transformed to a higher degree in the present biodegradation reactors compared to the pilot plant. This was the case of DCF and ERY where the development of nitrifying bacteria in the aerobic reactor improved its efficiency. Similarly, the operation under strict anoxic conditions

highly favoured the removal of IBP, which passed the anoxic compartment of the pilot plant unaltered, but reached transformations of more than 70% in this case.

√ The results from the anoxic reactor helped to clarify which was the contribution of the anoxic compartment of the pilot plant to the overall removal of FLX and ERY that could not be established in chapter 4.

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Chapter 6
Pre-treatment of hospital wastewater by coagulation-flocculation and flotation[1]

Summary

Coagulation-flocculation and flotation processes have been considered for the pre-treatment of hospital wastewater. Twelve of the Pharmaceutical and Personal Care Products (PPCPs) considered during biological treatment (fragrances: galaxolide (HHCB), tonalide (AHTN) and celestolide (ADBI), anti-epileptics: carbamazepine (CBZ), tranquilisers: diazepam (DZP), anti-inflammatory drugs: ibuprofen (IBP), naproxen(NPX) and diclofenac (DCF), antibiotics: sulfamethoxazole (SMX), roxithromycin (ROX), trimethoprim (TMP) and erythromicyn (ERY)) have been included in this research. Additionally, the iodinated contrast media iopromide (IPM) has been incorporated in the study, since this compound is specifically used in hospitals.

In the first part of the work batch coagulation-flocculation assays have been performed in a Jar-Test device, which where afterwards complemented with the set-up of a continuous coagulation-flocculation pilot-scale plant. Additionally raw hospital wastewater as well as the effluent from this continuous coagulation plant has been treated in a flotation cell.

In general, flotation of raw wastewater led to slightly worse results compared to batch coagulation regarding both, Total Suspended Solids (TSS) and PPCPs removal, although when applied to the effluent obtained from the coagulation pilot plant the overall efficiency of the process was positively affected.

Removal of TSS during pre-treatment was very effective reaching maximum efficiencies of 88%, 72% and 97% for batch coagulation, raw wastewater flotation and combined coagulation-flotation, respectively. In the case of total Chemical Oxygen Demand (COD) the efficiency of the processes was dependant on the fraction of particulate organic matter, which was the fraction that was considerably removed, whereas soluble organic matter was normally not eliminated.

From the selected PPCPs, IPM, CBZ and DZP were the most persistent compounds, whereas fragrances and DCF were eliminated to a high degree. For NPX and IBP the decrease in concentration was in between the previous substances. Finally, for antibiotics negative removals have been generally measured.

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

S. Suárez, F. Omil and J.M. Lema (*submitted*) Pre-treatment strategies of hospital wastewater by coagulation-flocculation and flotation. Water Research

Outline

6.1. Introduction

Primary treatment in urban Sewage Treatment Plants (STPs) usually consists of primary settling where suspended solids and organic matter are partially removed from the wastewater in order to optimize its subsequent secondary biological treatment. This process can be enhanced by chemical coagulation before settling, whose main aim is to promote flocculation of fine particles into more readily settleable flocs. Coagulation may increase removal of Total Suspended Solids (TSS) up to a 20%, of Biological Oxygen Demand (BOD) and pathogens up to a 30% and in the case of phosphorus from 5-10% removal during primary settling up to 70-90% efficiencies can be attained by chemical coagulation (Vesilind, 2003). The suitability of chemical coagulation has to be analysed for each situation, since it also implies negative aspects such as an increase in primary sludge production and operational costs. Iron and aluminium salts, lime and organic polyelectrolytes are commonly used for wastewater coagulation-flocculation, acting the inorganic salts as coagulants (neutralising particle charge) and the polymers as flocculants (enhancing floc building), although formation of oxides or hydroxides from inorganic salts can also help in the building of flocs in the absence of organic polymers.

Flotation is an alternative physical treatment process aimed at separating suspended or colloidal particles from wastewater. In this case floating instead of settling of solid particles is promoted by means of introducing fine gas bubbles (normally air) into the wastewater, which after getting attached to suspended particles induce their rise to the water surface due to their lower combined specific density, where they can be removed by a skimming device. Air/particle interactions may occur by different mechanisms: i) electrical attraction; ii) air bubbles are physically trapped in the solids structure and iii) chemical interactions. There are two basic methods for dispersing air bubbles through waste streams, namely Induced Air Flotation (IAF) and Dissolved Air Flotation (DAF). In the IAF, air is drawn down the shaft of a rotor in the flotation chamber where it is dispersed into the effluent through a diffuser pipe or an aspirator at atmospheric pressure. Consequently air bubbles of around 1000 μm are formed and kept in contact with the wastewater for a residence time between 4-6 min. In DAF, air is dissolved in water under pressure, which upon release at the entrance of the flotation unit promotes the formation of microscopic air bubbles (10-120 μm) due to a decrease in the air solubility. These bubbles are effective at removing even smaller oil droplets, but require higher residence times (20-30 min) for efficient separation (Hanafy and Nabih, 2007). Air can be dissolved under pressure in the whole influent stream, although it is also frequent to pressurise only a fraction (30-50%) and feed the rest by gravity or low pressure pumps to the system, mixing both streams at the inlet of the flotation unit. A third design option is to recycle, pressurise and saturate part of the effluent (15–30%) and mix it with the influent at the inlet of the

flotation tank (Hanafy and Nabih, 2007). The main application of dissolved air flotation is the treatment of wastewater polluted with oil or fat (Vaughan et al., 2000; Hanafy and Nabih, 2007), although very recently several other application such as the treatment of effluents from the mining and mineral processing industry (Rodrigues and Rubio, 2007) or the electroplating industry (Kurniawan et al., 2006) have been reported.

Chemical addition-DAF is a combination of coagulation-flocculation and flotation, where inorganic salts and/or organic polymers are mixed with the wastewater before flotation (Vaughan et al., 2000; Mels et al., 2001).

These two processes can be applied at different stages of water treatment: i) Pre-treatment of industrial effluents before entering the municipal sewer system, as for example bakery wastewater (Liu and Lien , 2001), hospital wastewater (Gautam et al., 2007) and herbal pharmaceuticals manufacturing effluents (Jain et al., 2001); ii) Primary treatment of urban wastewater (Mels et al., 2001); iii) Tertiary treatment of urban wastewater (Chuang et al., 2006) and iv) Drinking water treatment plants, which typically combine coagulation with sand filtration, sorption with activated carbon and disinfection by ozone or chlorine.

The aim of this research was to determine the efficiency of coagulation-flocculation and flotation processes for the pre-treatment of hospital wastewater, especially focussing on the removal of 13 Pharmaceutical and Personal Care Products (PPCPs), including three musk compounds (galaxolide (HHCB), tonalide (AHTN) and celestolide (ADBI)), the anti-epileptic carbamazepine (CBZ), the tranquiliser diazepam (DZP), three anti-inflammatory drugs (ibuprofen (IBP), naproxen(NPX) and diclofenac (DCF)), four antibiotics (sulfamethoxazole (SMX), roxithromycin (ROX), trimethoprim (TMP) and erythromicyn (ERY)) and the iodinated contrast media iopromide (IPM).

Very little information is available concerning the fate and behaviour of these micro-pollutants during coagulation or flotation processes, although in the last years several researches dealing with the occurrence of PPCPs during coagulation-flocculation of drinking water have been published (Adams et al., 2002; Westerhoff et al., 2005; Seitz et al., 2006; Vieno et al., 2006; Stackelberg et al., 2007). Removal of PPCPs during primary treatment of municipal wastewater was studied by Carballa et al. (2005), where it was concluded that compounds with high sorption potentials, such as the musk compounds HHCB and AHTN and the anti-inflammatory drug DCF, can be significantly removed during both, coagulation-flocculation and flotation processes. Regarding pre-treatment of industrial effluents that may represent potential sources of pharmaceuticals in wastewaters, as pharmaceutical manufacturing companies and hospitals, information is also scarce and if is merely focussed on conventional parameters, such as COD, TSS and pathogens (Torres et al., 1997; Chiang et al., 2003; Kajitvichyanukul and Suntronvipart, 2006; Gautam et al., 2007). The purpose of this work was to

overcome this lack of information by first extensively characterise a hospital effluent (chapter 3) and afterwards analyse the suitability of standard coagulation and flotation processes for the pre-treatment of such streams.

6.2. Materials and methods

6.2.1. Wastewater

Batch coagulation-flocculation and flotation experiments were carried out with samples of hospital wastewater collected during two of the last sampling campaigns considered in chapter 3 (November 2005 and March 2006). For the assays, two types of hospital streams were considered: S1 which comprises wastewater from hospitalised patients, surgery, laboratories, radiology and general services and S2 which consists of wastewater from radiotherapy and outpatient consultation.

For the continuous pilot-scale coagulation-flocculation plant, 600 L of hospital wastewater were collected in the same sewer the day before its operation, although in this case as a mixture of stream S1 and S2.

6.2.2. Batch coagulation-flocculation experiments

Batch coagulation-flocculation experiments have been carried out in a Jar-Test device (Figure 6-1), in four 1 L glass beakers. Two types of coagulants have been considered, ferric chloride ($FeCl_3$) and aluminium sulphate ($Al_2(SO_4)_3$) and the necessity of alkalinity addition in the form of $CaCO_3$ has been evaluated.

Experimental procedure started with the filling of beakers with 850 mL of hospital wastewater, which were spiked with those PPCPs that were below the analytical detection limit during the sampling campaigns (chapter 3), at concentrations shown in Table 6-1. The corresponding dose of coagulant and alkalinity was added to each vessel, with the exception of the blank where the process was run in the absence of external reagents.

Figure 6-1. Jar-test device.

The experiment consisted of the following sequential steps: i) Coagulation: Fast stirring at 150 rpm during 3 minutes; ii) Flocculation: Gentle stirring at 50 rpm during 5 minutes; iii) Settling: Stirrers where switched off in order to allow settling of flocs during 1 hour; iv) Sampling: Supernatant was taken in order to analyse TSS, total COD and PPCP concentration.

Table 6-1. Concentration (µg/L) of PPCPs spiked to hospital wastewater.

Compound	Concentration	Compound	Concentration
IPM, IBP, NPX	0	CBZ and DZP	20
DCF and Antibiotics (SMX, TMP, ERY, ROX)	10	Fragrances (HHCB, AHTN, ADBI)	40

6.2.3. Batch flotation experiments

Dissolved air flotation assays were performed in a device composed of a 2 L pressurisation cell, where tap water was saturated with air at high pressure (5-6 bar), connected to a 1 L flotation cell that contained the wastewater sample to be treated (Figure 6-2). Same conditions with respect to the types and doses of coagulants and alkalinity as in the previous experiments have been considered.

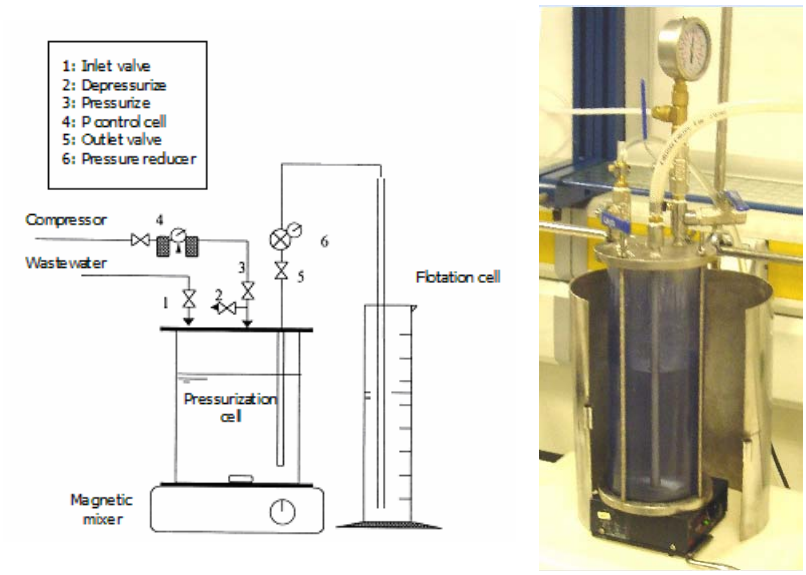

Figure 6-2. Flotation cell.

The experiment comprised: i) Sample preparation: Hospital effluents were spiked with those PPCPs that were not commonly detected in these wastewaters (Table 6-1). A volume of 700 mL was transferred to the flotation cell and supplied with the corresponding doses of coagulants and alkalinity, with the exception of the blank; ii) Saturation: Pressurisation cell was filled with water (valve 1) that was afterwards saturated with air (valve 3); iii) Flotation: Saturated water was introduced at the bottom of the flotation cell (valve 5) until a volume of 900 mL was reached. Flotation of suspended solids and fat was allowed for 1 hour; iv) Sampling: Sample was taken with a syringe from below the water surface, in order to avoid the floating layer, to analyse TSS, total COD and PPCP concentration.

6.2.4. Coagulation-flocculation pilot plant

The coagulation-flocculation pilot plant has been continuously fed with hospital wastewater that was collected the day before the experiment at the hospital sewer (Figure 6-3) and transported in a 1 m^3 storage tank to the municipal STP of Santiago de Compostela where the pilot plant experiments were carried out.

Figure 6-3. Collection and transport of hospital wastewater to feed the pilot plant.

At the STP the wastewater was spiked with PPCPs (Table 6-1) and left under continuous stirring during the whole night in order to ensure a complete homogenisation.

The pilot plant used consisted of three main sections (Figure 6-4): i) Coagulation tank of around 4.4 L equipped with a fixed-speed stirrer (200 rpm) and a pH-meter and controller, although this application was not used in order to follow the same procedure as in the batch experiments; ii) Flocculation tank with a volume of 15 L provided with a stirrer whose speed could be regulated to a maximum of 25 rpm; iii) Lamellar settler composed of 10 stainless steel (AISI-304) plates in a 35 L tank.

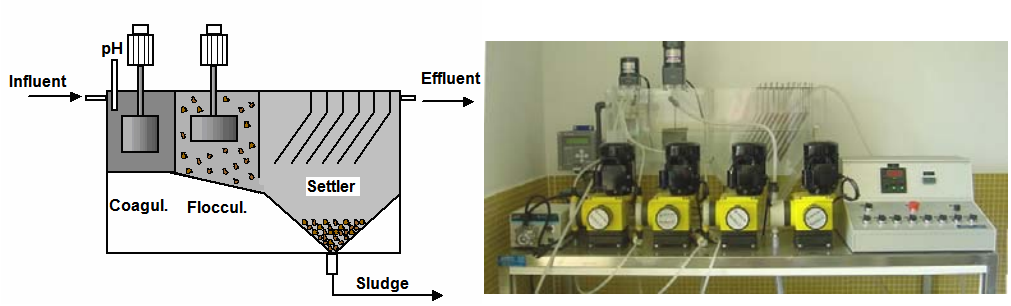

Figure 6-4. Coagulation-flocculation pilot plant.

The pilot plant was operated from a control panel composed of:

- Switch on/off of the plant.
- Emergency stop switch.
- Switch on/off of pumps and stirrers.
- Stirring speed regulator of the flocculation tank.
- Feed flow rate and pH montoring.
- Set-up of pH controller in coagulation tank.

The system was operated at a HRT of 32 min (12 min of coagulation-flocculation and 20 min of settling), with continuous addition of hospital wastewater by means of a peristaltic pump (Cole-Parmer) at a flow of 100 L/h and of coagulant ($FeCl_3$ or $Al_2(SO_4)_3$) with a dosing pump (Dosapro Milton Roy) at 3 L/h. The applied coagulant doses were of 0 and 25 mg/L for each coagulant. After 90 min of steady operation of the pilot plant (3×HRT), effluent sample was taken in order to analyse standard wastewater parameters as well as PPCPs concentration. Operation was carried out twice during two consecutive weeks of July 2006.

Figure 6-5. In-situ installation of coagulation-flocculation pilot plant.

6.2.5. Operation strategy

Optimum doses for coagulants in preliminary Jar-Test experiments were selected where only the removal of TSS at $FeCl_3$ and $Al_2(SO_4)_3$ additions in the range 0-200 ppm was analysed. Additionally, the necessity of incorporating alkalinity in the form of $CaCO_3$ in order to avoid a possible decrease in pH, as illustrated in Equation 6-1 for $FeCl_3$ (Gautam et al., 2007), was evaluated.

$$FeCl_3 + 3HCO_3^- \rightarrow Fe(OH)_{3(S)}\downarrow + CO_2 + 3Cl^-$$
$$FeCl_3 + 3H_2O \rightarrow Fe(OH)_{3(S)}\downarrow + 3HCl \qquad \text{[Equation 6-1]}$$

It was observed that only coagulant additions above 25 mg/L required a supplement of $CaCO_3$, at the same dose as the coagulant. Furthermore, coagulant doses above 50 mg/L did not lead to an additional improvement in the separation process, thus this concentration was selected as the maximum addition to be considered in further assays.

Batch coagulation-flocculation and flotation assays were performed with four different hospital wastewaters (S1 and S2 from one sampling in November 2005; samples of S1 and S2 were collected on 15th and 22nd of March 2006 and afterwards both S1 samples, as well as both S2 samples were mixed in order to obtain one representative sample of S1 and S2 corresponding to spring). The following five operation conditions regarding coagulant additions have been considered for these experiments: i) absence of reagents; ii) 25 mg/L of $FeCl_3$; iii) 50 mg/L of $FeCl_3$ and of $CaCO_3$; iv) 25 mg/L of $Al_2(SO_4)_3$ and v) 50 mg/L of $Al_2(SO_4)_3$ and of $CaCO_3$. In some cases, due to lack of wastewater, the number of experiments had to be reduced.

Continuous pilot plant experiments have been only conducted in the absence of any reagent and at the lower coagulant doses, since the improvement in the performance at the higher dose was not compensated by the increase in the consumption of additives, both coagulants and alkalinity. The effluent of this pilot plant was afterwards treated in the flotation cell in order to compare two possible pre-treatment strategies for hospital effluents: i) single coagulation-flocculation unit and ii) two step treatment by coagulation-flocculation followed by flotation.

6.2.6. Analytical methods

Total Suspended Solids (TSS) and Chemical Oxygen Demand (COD) of the unfiltered samples were determined following Standard Methods (APHA, 1999).

Concentration of PPCPs was determined following the methods described in chapter 2. Samples from the influents and effluents were collected in glass or aluminium bottles and immediately prefiltered (glass fibre prefiltres, AP4004705 Millipore). For the analysis of antibiotics and Iopromide (IPM), a pinch of sodium azide was added to the filtered sample before its storage in the freezer, until

analysed by the Austrian Federal Environment Agency. For the rest of compounds, samples were analysed within one week, thus storage in the fridge was sufficient.

6.2.7. Calculations

Removal efficiencies (E_j) for TSS, COD and PPCPs were determined according to Equation 6-2:

$$E_j = \frac{C_{j,Influem} - C_{j,Effluent}}{C_{j,Influent}} \cdot 100 \qquad \text{[Eq. 6-2]}$$

where, $C_{j,Influent}$ and $C_{j,Effluent}$ are the concentrations of compound j (mg/L or µg/L) in the influent and effluent, respectively.

Calculations were based on soluble concentrations of PPCPs, except for fragrances and DCF where total concentrations have been considered in the analysis, according to their higher K_d values (Table 1-2). For the latter, total concentrations ($C_{j,total}$) were determined applying Equation 6-3:

$$C_{j,total} = C_{j,dissolved} \cdot (1 + K_d \cdot SS) \qquad \text{[Eq. 6-3]}$$

where, $C_{j,dissolved}$ is the soluble concentration of compound j (µg/L) and SS the suspended solids content (kg/L) of the considered stream (influent or effluent). Sorption coeffients (K_d) for fragrances have been determined from experimentally measured total and soluble concentrations, whereas for DCF the value of 459 L/kg reported by Ternes et al. (2004) for primary sludge was considered.

6.3. Results and discussion

6.3.1. Batch coagulation-flocculation experiments

Removal of TSS and COD

Coagulation-flocculation processes have been designed for promoting removal of suspended solids and colloids from wastewater, which do not settle spontaneously. Typically, removal of TSS could be increased from 40-70% without coagulation up to 60-90% if a coagulant is used (Vesilind, 2003). In the case of hospital wastewaters considered in this work, suspended particles already manifested good settling properties without external addition of coagulants (69-84%), which was somewhat enhanced (4-13%) when the wastewater was coagulated with $FeCl_3$ (Figure 6-6). The second coagulant considered ($Al_2(SO_4)_3$) led to an increase in TSS in the effluent when compared to the blank, therefore concerning conventional wastewater pollutants the use of aluminium salts was not favourable.

Removal of COD was highly influenced by the fraction of total COD associated to particulate and soluble organic matter. While between 11-18% of COD was removed in stream S1 sampled in November 2005 (Figure 6-6a) where only an 8% of total COD corresponded to solid particles, removal reached up to 72% for the second S1 collected (Figure 6-6c), although for the latter solid organic matter

represented a 38%. If optimal operation conditions had to be selected on the basis of conventional wastewater parameters, it would correspond to the use of 50 mg/L of $FeCl_3$ as coagulation agent.

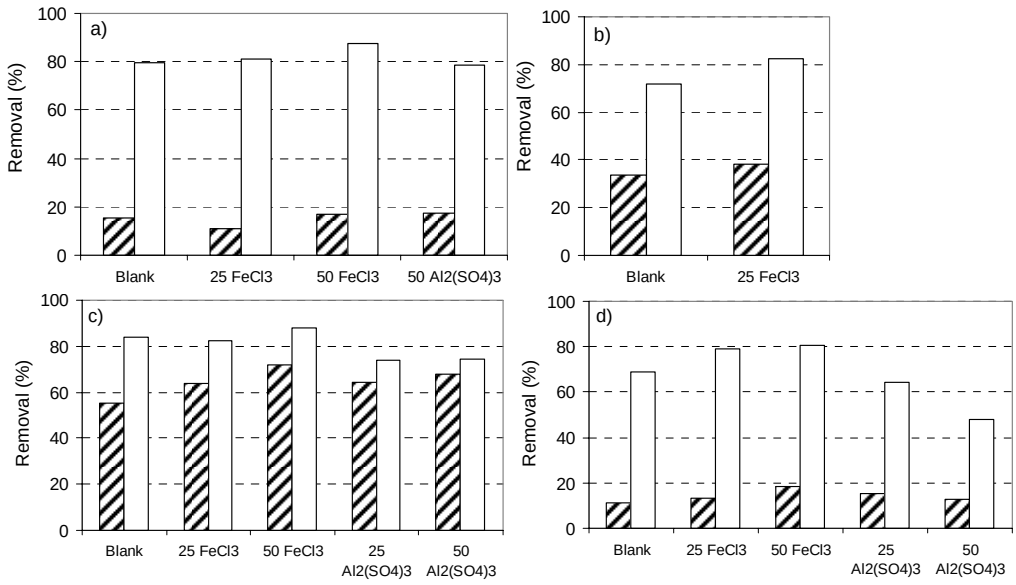

Figure 6-6. Removal of total COD (▨) and TSS (□) in hospital wastewater from a) S1 November 2005; b) S2 November 2005; c) S1 March 2006 and d) S2 March 2006.

Removal of PPCPs

Removals of PPCPs from the liquid phase achieved in the Jar-Test assays were depicted in Figure 6-7.

The compounds IPM, CBZ, DZP and IBP could generally not be eliminated from the liquid phase during the process, with the exception of the 40-45% decrease in the concentration of CBZ and DZP determined in one assay (Figure 6-7c). This behaviour is in concordance with the very low sorption tendency of these compounds, neither by adsorption nor absorption, according to their very low sorption coefficients on primary sludge (K_d < 44 L/kg, Ternes et al., 2004). The ineffectiveness of coagulation processes for the removal of CBZ and IBP in drinking water treatment plants as well as during primary treatment has been reported by several authors (Ternes et al., 2002; Carballa et al., 2005; Vieno et al., 2006). Similarly, IPM showed to be very resistant to coagulation-flocculation during drinking water treatment (Westerhoff et al., 2005; Seitz et al., 2006). Maximum removal of DZP during primary treatment did not exceed 25% even at an applied coagulant dose of one order of magnitude higher than the considered in the present work (Carballa et al., 2005).

Removal of NPX was in the range of 20-40%, which was somewhat higher than some previously reported data for primary treatment (Carballa et al., 2005) and for drinking water treatment (Boyd et al., 2003; Westerhoff et al., 2005). This anti-inflammatory drug is negatively charged at the circum-neutral pH of the wastewater (pK_a 4.2), therefore electrostatic interactions with the negatively charged surface of suspended solids (adsorption) are discarded, unless this negative charge is neutralised. When a coagulant was used, covalent interactions with the trivalent cations could be responsible for this neutralisation, although this can not explain the behaviour of blanks. For the latter, heavy metals (Pt^{+4}, Gd^{+3}) that were reported to be frequent pollutants of hospital effluents (Kummerer, 2004) could exert a similar effect as trivalent cations.

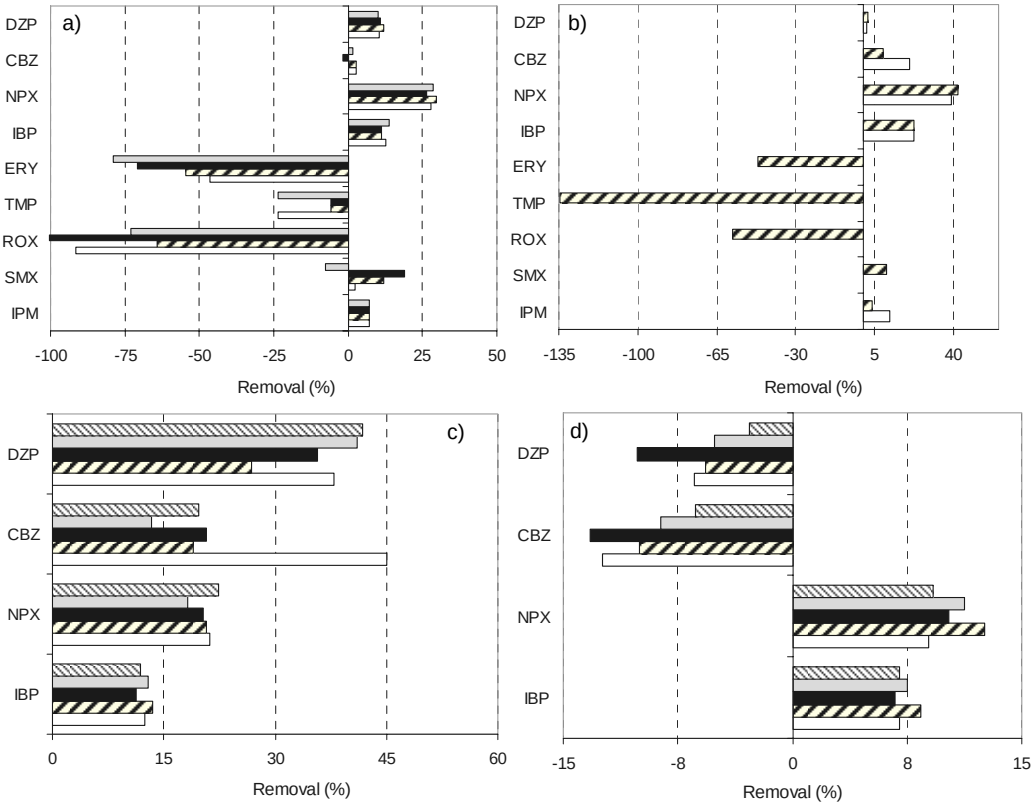

Figure 6-7. Removal of PPCPs in blank (□), at 25 ppm (◪) and 50 ppm (■) of $FeCl_3$ and at 25 ppm (▧) and 50 ppm (▩) of $Al_2(SO_4)_3$ in hospital wastewater from a) S1 November 2005; b) S2 November 2005; c) S1 March 2006 and d) S2 March 2006.

Macrolides (ROX and ERY) and trimethoprim showed negative removals during coagulation, whereas SMX concentrations were not significantly altered. For the sulphonamide, the ineffectiveness of coagulation processes has already been reported for drinking water treatment (Adams et al., 2002; Vieno et al., 2006). Taking into account that this part of the work has been carried out with wastewater and that macrolides could be partly enclosed in faeces particles, since they are mainly excreted with the bile and faeces (Gobel et al., 2007), their release during the coagulation experiment could justify their behaviour.

Musk compounds and DCF were expected to be partially sorbed onto suspended solids, according to their K_d values (Equation 6-4):

$$\frac{C_{j,sorbed}}{C_{j,total}} = \frac{K_d \cdot SS}{1 + K_d \cdot SS} \qquad \text{[Eq. 6-4]}$$

where $C_{j,sorbed}$ is the concentration of compound j sorbed onto solids (µg/L).

Sorption coefficients determined for fragrances from total and soluble concentrations in streams S1 and S2 were: 6970±3350 L/kg, 7270±2050 L/kg and 4800 L/kg for HHCB, AHTN and ADBI, respectively, which were in the range of those reported by Ternes et al. (2004) for primary sludge and Kupper et al. (2006) for raw sludge.

The minimum removal efficiency expected for these compounds could be determined with the following equation:

$$\text{Removal}(\%) = \frac{K_d \cdot SS}{1 + K_d \cdot SS} \cdot E_{TSS} \qquad \text{[Eq. 6-5]}$$

where E_{TSS} is the efficiency of the coagulation-flocculation process regarding TSS removal (%).

Both experimentally determined and calculated minimum removal efficiencies for DCF, HHCB, AHTN and ADBI during Jar-Test assays were plotted in Figure 6-8. From the data it can be observed that in general the efficiency of coagulation-flocculation, even without any coagulant addition, was twice the minimum removal efficiency expected from the settling of suspended particles, indicating an enhanced sorption of fragrances and DCF during the process.

Fragrances were removed between 60-91%, 60-97% and 50-92% for HHCB, AHTN and ADBI, respectively. The lower removal of the third compound with respect to the other two is concordant with its lower sorption coefficient. The lower limit corresponded generally with the result obtained with stream S2 from March 2006, while the upper limit with S1 from November 2005 (Figure 6-8d and a, respectively). A comparison of the physico-chemical characteristics of these streams showed that the first had the lowest (9 mg/L) whereas the second the highest (43 mg/L) fat content among the four streams. Taking into account that fragrances

have a strong lipophilic character (log K_{ow} ~6) and that sorption was mainly driven by hydrophobic interactions (absorption), enhanced removal was actually expected in streams with higher fat content. Although only slight differences have been observed regarding type of coagulant and doses applied, the use of 25 ppm of $FeCl_3$ led to optimum conditions in most cases. The results determined in the present work at very low and even without any coagulant addition, were even somewhat higher than those previously determined by Carballa et al. (2005) during primary treatment. During drinking water treatment removal of HHCB has shown to be negligible (Westerhoff et al., 2005; Stackelberg et al., 2007), although the lower fat content of this water source could explain these differences.

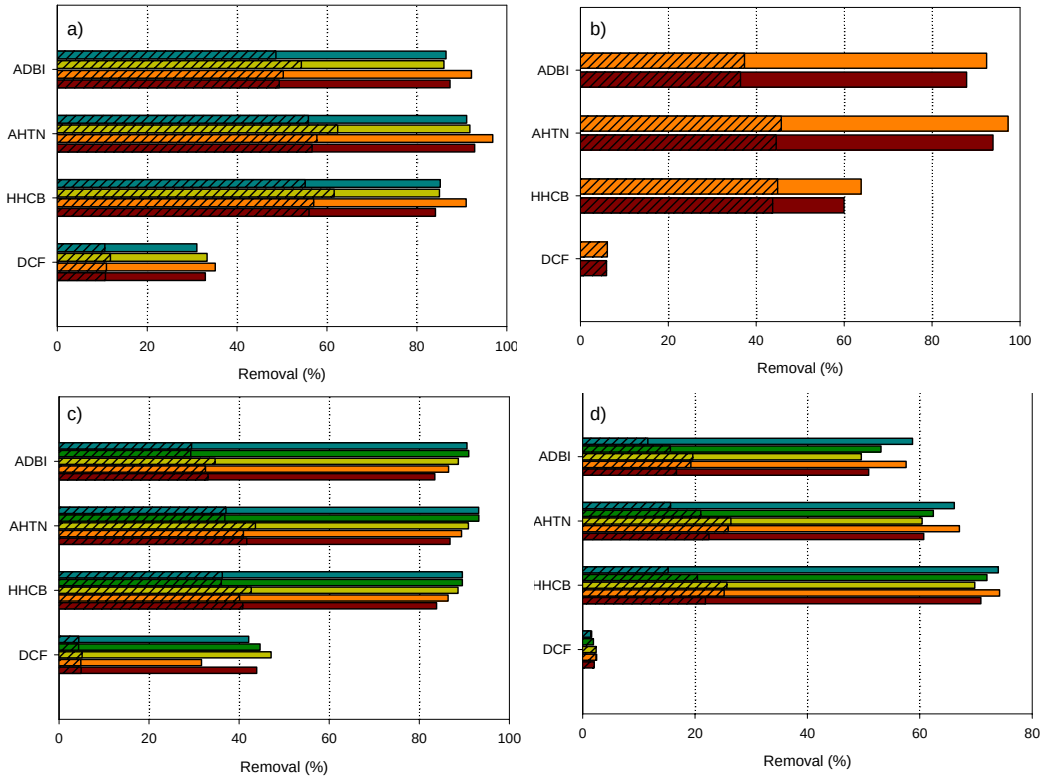

Figure 6-8. Removal of fragrances and diclofenac in blank (■), at 25 ppm (■) and 50 ppm (■) of $FeCl_3$ and at 25 ppm (■) and 50 ppm (■) of $Al_2(SO_4)_3$ in hospital wastewater from a) S1 November 2005; b) S2 November 2005; c) S1 March 2006 and d) S2 March 2006. Minimum removal efficiencies according to Equation 6-5 are indicated (▨).

Significant removal of diclofenac was only observed for S1, where the initial concentration was reduced by 31-47%. This pharmaceutical is of acidic nature (pK_a ~4) and therefore mainly deprotonated at circum-neutral pH, thereby adsorption will not occur unless this charge is neutralised. On the other hand, the compound is slightly lipophilic (log K_{ow} 4.5), consequently it could be absorbed in the lipid fraction of solids. This second characteristic could explain that the removal exclusively occurred in streams S1 whose fat content was higher than in streams S2 (25-43 mg/L vs. 9-13 mg/L, respectively). The suitability of coagulation-flocculation processes for removal of DCF was reported by Carballa et al. (2005) for primary treatment, as well as by Vieno et al. (2006) for drinking water plants, in both cases with higher efficiencies than those measured in the present work (~70%), but also working at higher coagulant doses. On the other hand, Ternes et al. (2002) reported negligible removal of DCF by flocculation using $FeCl_3$ in lab and full-scale investigations at similar doses as those applied in the present work. This seems to indicate a correlation between the removal efficiency achieved for DCF and the coagulant dose applied in the process, probably related to the establishment of covalent interactions between the deprotonated pharmaceutical and the trivalent cations of the coagulants that enhances adsorptive interactions (Carballa et al., 2005).

6.3.2. Batch flotation experiments

Removal of TSS and COD

Flotation experiments were conducted with the same wastewater and applying equal conditions as in coagulation-flocculation experiments. Data regarding removal of TSS and COD have been summarised in Figure 6-9, where a high variability when comparing efficiencies for a specific coagulant type and dose was clearly stated.

Maximum eliminations of TSS were in the range of 60-72%, whereas these upper limits were somewhat lower when focussing on COD, 16-58%, depending on the ratio of solid and soluble organic matter (Mels et al., 2001). In general, flotation led to worse separation of TSS than the previously considered coagulation-flocculation process. Results obtained in the present research were comparable to those obtained during pre-treatment of bakery wastewater by Liu and Lien (2001).

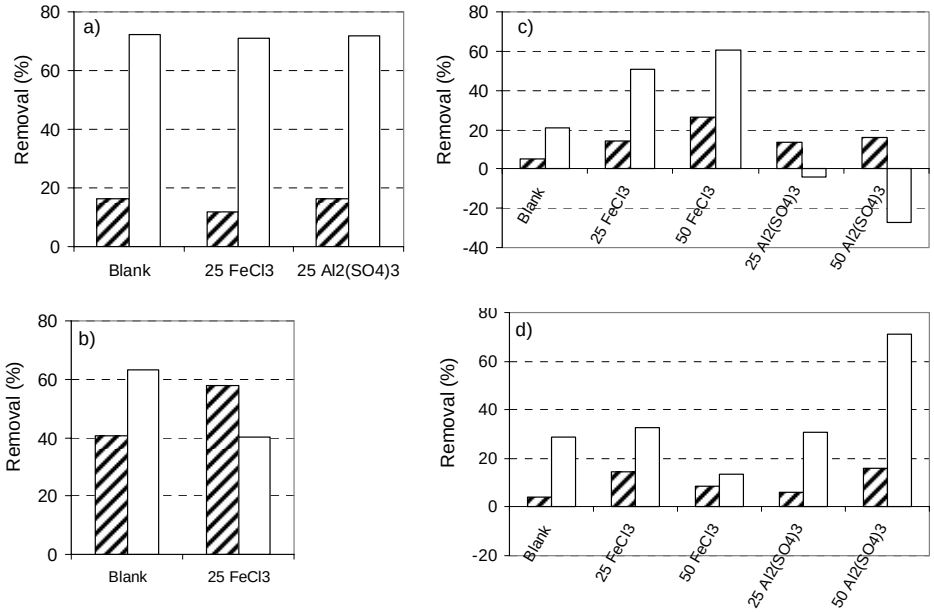

Figure 6-9. Removal of total COD (▨) and TSS (□) from hospital wastewater from a) S1 November 2005; b) S2 November 2005; c) S1 March 2006 and d) S2 March 2006.

Removal of PPCPs

Elimination of the considered micropollutants was analysed following an analogous procedure as for coagulation experiments. In a first step removal of those PPCPs with low sorption potential onto primary sludge from the liquid phase, was determined (Figure 6-10).

The behaviour of antibiotics was similar to what had been observed during coagulation that is, for macrolides (ROX and ERY) and trimethoprim negative removals have been obtained, while SMX concentrations remained almost constant.

Removal of NPX was dependant on the treated stream, since no significant decrease in its initial concentration was detected for S2 from March 2006 (Figure 6-10c), whereas up to 45% was eliminated during flotation of S1 and S2 from November 2005 (Figure 6-10a and b). These differences could partially be due to the slightly lower pH of the samples from November than those from March (7.4-7.9 and 8.5-8.7, respectively), which would led to a higher fraction of protonated NPX (pK_a 4.2) in the first that could enhance its interaction with solids, which is hindered when the compound is deprotonated. Similar results have been measured for IBP, although the maximum removal observed was somewhat lower than for NPX (<30%). These results agree very well with those reported by Carballa et al. (2005).

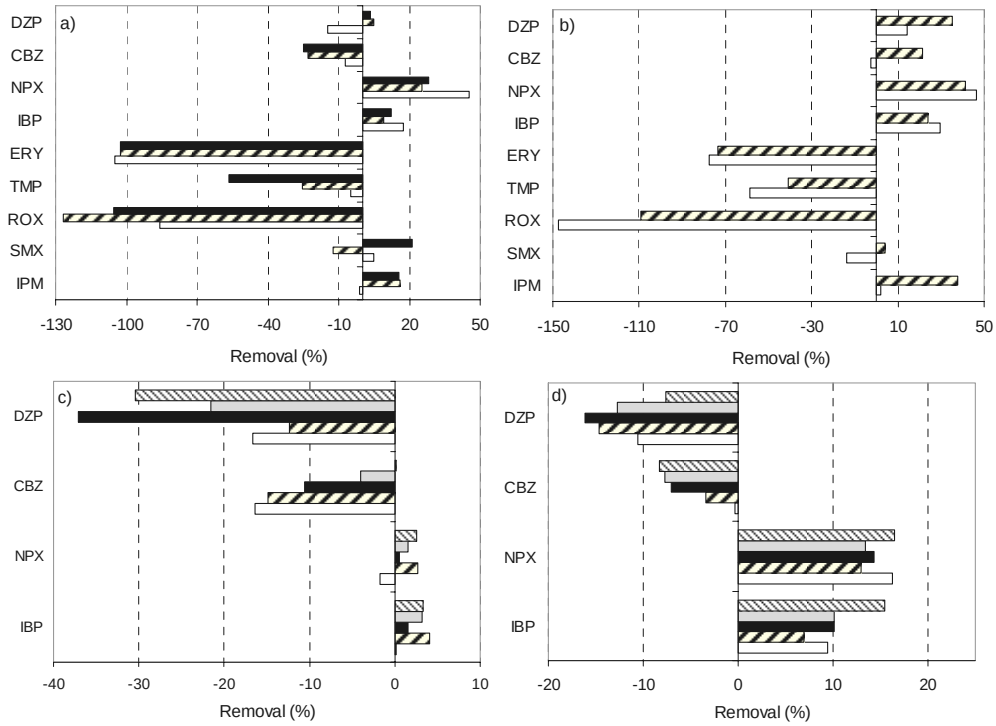

Figure 6-10. Removal of PPCPs in blank (□), at 25 ppm (◪) and 50 ppm (■) of FeCl3 and at 25 ppm (▧) and 50 ppm (▣) of Al2(SO4)3 in hospital wastewater from a) S1 November 2005; b) S2 November 2005; c) S2 March 2006 and d) S1 March 2006.

The anti-epileptic drug CBZ and the tranquiliser DZP were generally not eliminated from the liquid phase, with the exception of S2 from November (Figure 6-10b) were a depletion of up to 21 and 35%, respectively, were measured, which were somewhat lower than those reported by Carballa et al. (2005). In the case of CBZ, whose pK_a is 7, removal could depend on pH which determines the protonation degree of its amide group. In fact, removal was only observed in the sample with the lowest pH, which contains the highest portion of protanted specie which can establish covalent interaction with the negatively charged solid's surface (adsorption).

The fate of fragrances and DCF was analysed on the basis of total concentrations of the compounds (Equation 6-3) and compared with the minimum removal efficiency expected according to separation of TSS and sorption coefficients of these compounds (Equation 6-5). The corresponding results are shown in Figure 6-11. As occurred in the coagulation assays, removal of fragrances and DCF was significantly higher than expected on the basis of TSS separation, even in the

absence of external flotation additives. Removal of DCF was only observed when wastewater from November was subject to flotation, in the range 13-51% that is very close to the removal of 20-45% that had been reported by Carballa et al. (2005) for this type of treatment. Surprisingly, the highest efficiency of flotation occurred with S2 from November (Figure 6-11b) which does not correspond to the fattiest sample as occurred during coagulation, but with the most acidic one. Removal efficiency seemed to be dependant on the state of the acid-base equilibrium of this acidic compound.

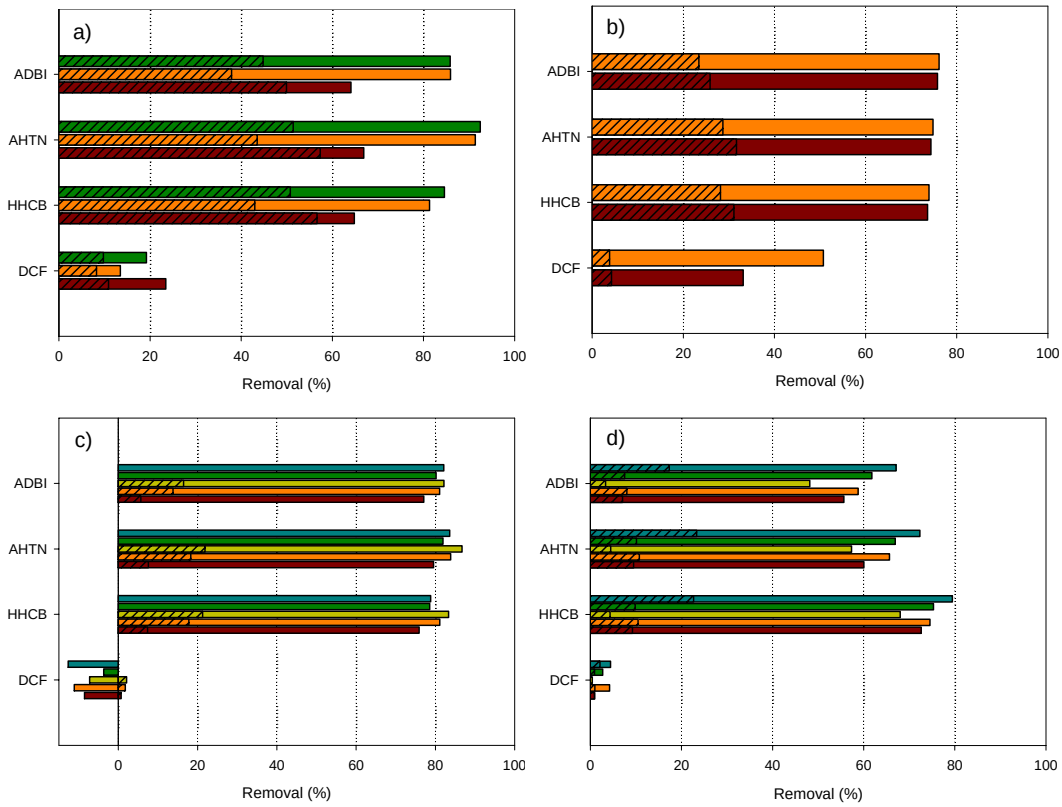

Figure 6-11. Removal of fragrances and diclofenac in blank (■), at 25 ppm (■) and 50 ppm (■) of $FeCl_3$ and at 25 ppm (■) and 50 ppm (■) of $Al_2(SO_4)_3$ in hospital wastewater from a) S1 November 2005; b) S2 November 2005; c) S1 March 2006 and d) S2 March 2006. Minimum removal efficiencies according to Equation 6-5 are indicated (▨).

As expected beforehand, highest efficiencies of flotation were measured for the most lipophilic compounds, fragrances. Removals of 65-85%, 60-93% and 56-86% were obtained for HHCB, AHTN and ADBI, respectively, being this upper limit slightly lower than those achieved by coagulation. Generally, the use of coagulants

improved the process, offering the aluminium based reagent better results than the ferric one. As occurred in coagulation experiments, the degree of musk separation correlated with the fat content of the wastewater used, which confirms that the process is driven by absorption, as had been already postulated in Carballa et al. (2005).

6.3.3. Continuous experiments

The hospital wastewater that was used to feed the coagulation-flocculation pilot plant was characterised, including TSS, COD and concentration of PPCPs (Table 6-2) after the spike.

Table 6-2. Characteristics of hospital wastewater treated in the coagulation plant.

Compound	C_I	C_{II}	Compound	C_I	C_{II}
COD	3485	1723	AHTN	8.9	8.9
TSS	1562	531	ADBI	9.2	11.7
IBP	2.8	16.1	ERY	n.a.	11
NPX	9.8	1.5	SMX	n.a.	6.6
DCF	3.2	7.1	ROX	n.a.	9
CBZ	20.2	21.3	TMP	n.a.	10
DZP	11.9	19	IPM	n.a.	6000
HHCB	10.2	14.1			

Concentrations for experiment 1 (C_I) and 2 (C_{II}) in mg/L for TSS and COD and µg/L for PPCPs. (n.a.) not analysed.

Removal of TSS and COD

The hospital effluent was first continuously treated in the coagulation-flocculation pilot plant at three different conditions: i) without external additions (blank); ii) using 25 mg/L of $Al_2(SO_4)_3$ as coagulant and iii) in the presence of 25 mg/L of $FeCl_3$. At this lower coagulant dose alkalinity addition was not necessary, which was one of the main reasons for selecting these conditions, apart from the insignificant process improvement obtained in batch experiments when working at the higher doses. The effluents of the pilot plant were afterwards treated in the batch flotation cell in order to evaluate the resulting enhancement of the pre-treatment efficiency. Results regarding removal of conventional wastewater parameters during coagulation-flocculation followed by flotation have been summarised in Figure 6-12.

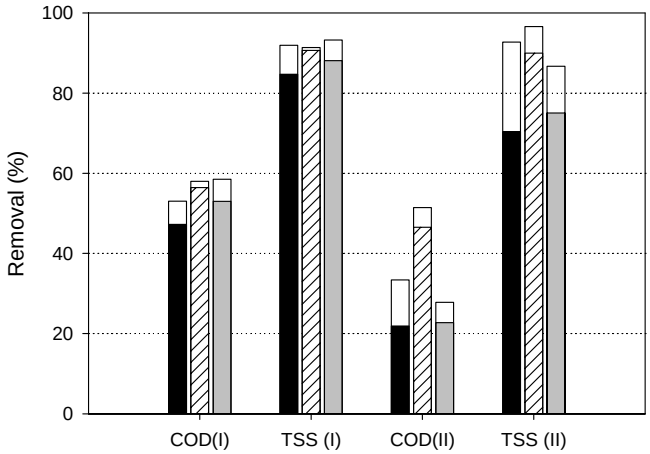

Figure 6-12. Removal of COD and TSS in the coagulation plant, during experiment I and II, in the absence of coagulants (■), at 25 ppm of $Al_2(SO_4)_3$ (▨) and at 25 ppm of $FeCl_3$ (▩). Increase in the overall removal when this process is followed by flotation (□).

Considering the coagulation and flotation processes individually, removals of TSS between 70-91% and in the range of 22-56% for COD were measured for the first, while for the second this values were significantly lower, namely 8-67% and 1-13% for TSS and COD, respectively. As observed in the batch experiments, removal of COD was dependant on the fraction of total COD attributable to solid particles (62% and 39% in wastewaters I and II, respectively). It is worth to point out that despite the high TSS concentration of the wastewater collected for the first experiment (Table 6-2), removal of TSS was still very high (85-91%) at the relatively low coagulant doses applied, compared to other works (Jain et al., 2001). The overall efficiency of the combined coagulation-flotation process was similar for both experiments (87-97%), although in the first the contribution of flotation was almost negligible (<10%), whereas in the second the slightly lower performance of the coagulation-flocculation step was compensated by better results during flotation. Although the process was very efficient without any coagulant addition, somewhat better results were achieved when the aluminium salt was incorporated. In general, these results are in good agreement with those obtained during batch treatments.

Removal of PPCPs

Occurrence of the considered PPCPs during the combined coagulation-flotation process has been depicted in Figure 6-13. In the case of antibiotics and iopromide only data about the performance of coagulation during experiment II were

available, whereas for the rest of compounds a complete analysis was performed. Results obtained during experiments I were generally very well reproduced during assay II and in concordance with the main conclusions drawn from the previous batch analyses.

The compounds which were not affected by the treatment were IPM, NPX, CBZ and DZP, which was already observed in batch experiments for all substances except for NPX were up to 42-46% depletion had been measured during both, coagulation and flotation processes. The shorter settling time installed in the continuous plant compared to batch systems (20 vs. 60 min) could be responsible for the worse efficiencies obtained in the first.

On the other hand, when $Al_2(SO_4)_3$ was added as coagulant, slight removal of IBP was observed during both experiments (21-39%) in the coagulation-flocculation pilot plant, while flotation was not effective in increasing this removal, which were somewhat better results that those obtained in Jar-Test experiments (8-22%).

As had been concluded from the batch assays, fragrances and to a lesser extent DCF were the most efficiently removed compounds from the considered PPCPS. Maximum elimination of DCF was 52 and 60% for experiment I and II, respectively, which was achieved when working at 25 mg/L of $Al_2(SO_4)_3$. The difference between both experiments was due to the performance of flotation, rather than coagulation (Figure 6-13). In the case of fragrances, while the overall maximum removal attained was very similar in both assays (86-96%), it was only achieved when using the aluminium coagulant in the second experiment, while in first one this high removal was independent of operation conditions. This was a result of the compensation of coagulation and flotation, that is, when coagulation was less efficient, it was compensated by higher efficiencies during flotation (Figure 6-13 II). The suitability of the considered pre-treatment processes for the removal of fragrances was already confirmed in batch experiments, but the continuous mode of operation additionally identified aluminium salts as better coagulants than ferric ones.

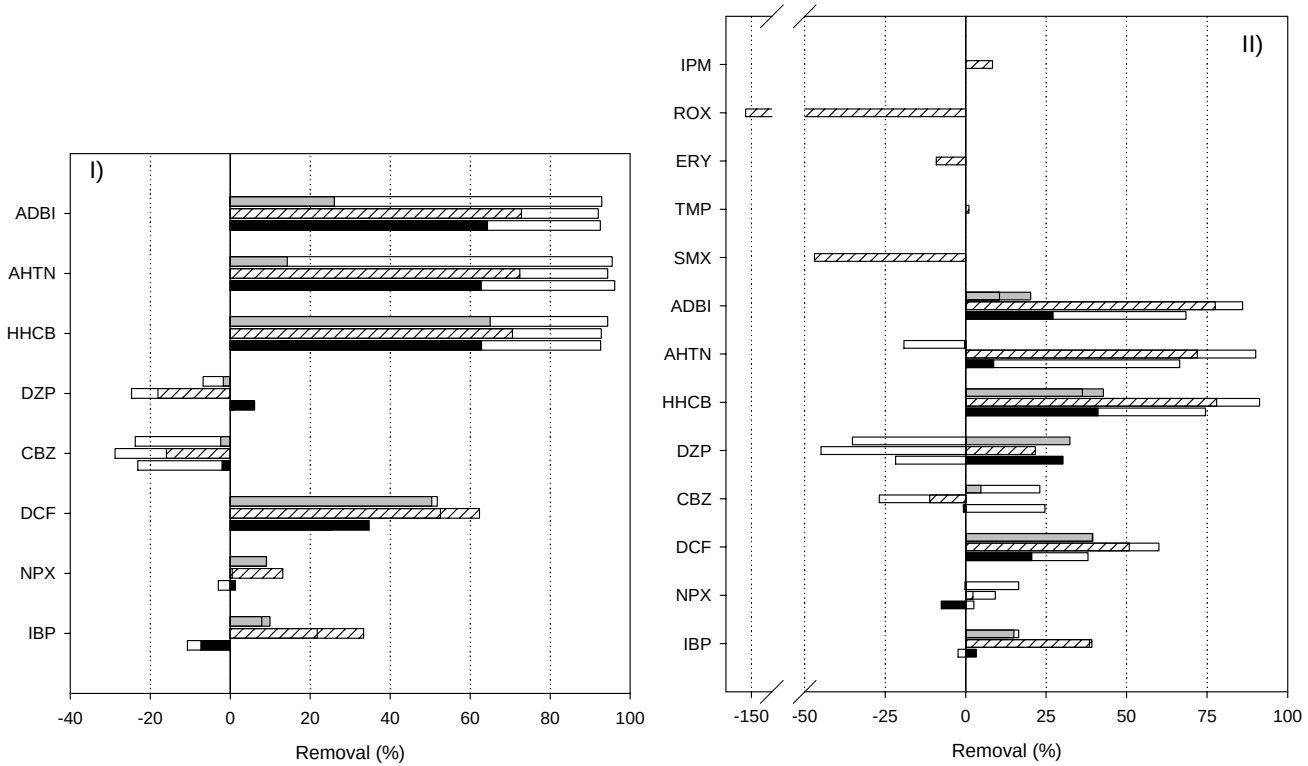

Figure 6-13. Removal of PPCPs in the coagulation plant, during experiment I and II, in the absence of coagulants (■), at 25 ppm of $Al_2(SO_4)_3$ (▨) and at 25 ppm of $FeCl_3$ (▩). Increase in the overall removal when this process is followed by flotation (□).

As in batch assays, concentrations of antibiotics increased during coagulation-flocculation, even for SMX. For the latter, a similar situation during biological treatment has been justified by the presence of N^4-acetylsulfamethoxazole, which is the main metabolite of SMX, that could have been transformed back to its parent compound (Gobel et al., 2005), although in the present research a problem with the analysis of the wastewater seems more plausible, taking into account that after a spike of 10 ppb of SMX, only 6.6 ppb have been detected in the inlet of the pilot plant, while 9.7 ppb were measured in its effluent.

6.4. Conclusions

Two pre-treatment technologies, coagulation-flocculation and flotation, have been applied to hospital wastewater in order to asses the removal of 13 PPCPs, comprising musk compounds, anti-epileptics, tranquilisers, anti-inflammatories antibiotics and contrast media. In the first part of the work batch assays have been performed, which where afterwards complemented with the set-up of a continuous coagulation-flocculation pilot plant.

During batch coagulation experiments the compounds IPM, CBZ, DZP and IBP could generally not be eliminated from the liquid phase, whereas very high removal efficiencies (>90%) have been measured for the three fragrances (HHCB, AHTN and ADBI). For NPX and DCF (<50%) the decrease in concentration was in between the previous substances. In the case of antibiotics, negative removals have been measured for macrolides (ROX and ERY) and TMP, whereas SMX concentrations were not significantly altered during the process. Enhanced sorption of fragrances and DCF during coagulation was observed when measured removals were compared with the efficiency determined from then removal of sorbed compounds through the settling of suspended particles, according to their sorption coefficients.

In general, flotation led to slightly worse results compared to coagulation regarding both, TSS and PPCPs removal, although the general tendencies observed were similar in both cases. Accordingly, highest efficiencies were measured for fragrances where maximum removals reached ~90%, followed by DCF and NPX where the upper limits attained were ~50%.

Similar conclusions have been drawn from the continuous operation with the coagulation-flocculation pilot plant, followed by treatment in the flotation cell. The overall efficiency regarding removal of TSS was in the range of 87-97%, with a contribution of flotation between 1-22%. The compounds which were not affected by the treatment were IPM, NPX, CBZ and DZP, similar to what had been observed in batch experiments except for NPX were removals higher than 40% had been measured for the optimum batch coagulation and flotation processes. In the case of IBP the opposite was observed, that is higher removals in the continuous mode of operation (21-39%). Fragrances and DCF were the most efficiently removed compounds from the considered PPCPS with maximum eliminations of ~95% and

60%, respectively achieved when the aluminium salt (25 ppm of $Al_2(SO_4)_3$) was used as coagulant.

The two main mechanisms known to be responsible for sorption of PPCPs onto suspended solids were absorption and adsorption. The first, based on lipophilic interactions was mainly responsible for the removal of fragrances, where better results were obtained in streams with higher fat content, whereas the second, based on electrostatic interactions was the driving force for the removal of ionic compounds. This was illustrated by the behaviour of CBZ during flotation, where removal was only observed in the sample with the lowest pH. A second example would be DCF, for which the removal efficiency achieved during coagulation seemed to depend on the coagulant dose applied in the process.

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Chapter 7
Fluoxetine and Triclosan oxidation during municipal wastewater ozonation[1]

Summary

Reaction kinetics have been investigated for oxidation of the antimicrobial agent triclosan (TRI) and the antidepressant drug fluoxetine (FLX) by aqueous ozone (O_3). Second-order rate constants, k_{O3}, were determined for reaction of O_3 with each of TRI's and FLX's acid-base species. Although very high values of k_{O3} were measured for the deprotonated species of each target compound (k_{O3} = 5.1 (± 0.1) × 10^8 $M^{-1}s^{-1}$ for anionic TRI and k_{O3} = 1.1 (± 0.1) × 10^6 $M^{-1}s^{-1}$ for neutral FLX), only TRI was fast reacting at circumneutral pH (the pH-dependent, apparent second-order rate constants, $k_{app,O3}$, were 3.8 × 10^7 $M^{-1}s^{-1}$ for TRI and 9.6 × 10^2 $M^{-1}s^{-1}$ for FLX at pH 7). Kinetic modelling indicated that O_3 reacted with TRI and FLX via electrophilic attack at their phenol and neutral amine moieties, respectively.

TRI and FLX oxidation during ozonation of secondary effluent samples from two conventional activated sludge treatment plants was also investigated. TRI was oxidized with relatively high efficiency during wastewater ozonation, due to its high reactivity toward O_3. Nearly 100% TRI depletion was achieved for a 4 mg/L ($8.3 \cdot 10^{-5}$ mol/L) O_3 dose applied to a wastewater containing 7.5 mg/L of DOC, and ~58% TRI depletion for dosage of 6 mg/L ($1.3 \cdot 10^{-4}$ mol/L) O_3 to a wastewater containing 12.4 mg/L of DOC. Fluoxetine transformation was less efficient, due to its low reactivity toward O_3 at the circumneutral pH. Consequently, FLX loss could be followed as a function of time, which permitted modelling of FLX oxidation with k_{O3} values determined in pure waters.

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

Suarez, S., Dodd, M.C., Omil, F., von Gunten, U. (2007) Kinetics of triclosan oxidation by aqueous ozone and consequent loss of antibacterial activity: Relevance to municipal wastewater ozonation. Water Research **41** (12), 2481-2490.

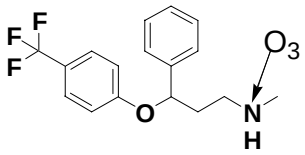
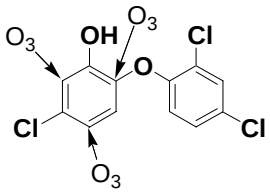
Outline

7.1. Introduction

Triclosan, 5-chloro-2-(2,4-dichlorophenoxy)phenol (Table 7-1), is used as an antimicrobial agent in a large number of skin and oral care medical and household products (soaps, creams, toothpaste, mouthwash). To a lesser extent, triclosan (TRI) is used in textiles and plastics (sportswear, bed clothes, shoes, carpets) to control the growth of disease or odour-causing bacteria. In Europe, around 350 tons of TRI are sold per year as the active ingredient of Irgasan DP 300 or Irgacare MP (Singer et al., 2002). After application, residues of TRI are expected to reach municipal wastewaters. In fact, Lindstrom et al. (2002) detected this compound in all wastewater samples analysed, at concentrations in the range of 0.6-1.3 µg/L. TRI is quite hydrophobic (log K_{OW} 4.2-5.4, Lindstrom et al., 2002; Singer et al., 2002), suggesting that it should be removed with relatively high efficiency during wastewater treatment as a consequence of partitioning onto biomass. Accordingly, removal rates achieved for TRI in Sewage Treatment Plants (STPs) can be quite high for modern, well-designed plants. For example, Singer et al. (2002) quantified a total removal (biodegradation plus sorption onto sludge) of 94%. This efficiency could be even higher if free-chlorine is used to disinfect the final effluent of the plant, since free chlorine-TRI reactions are quite fast (Rule et al., 2005). However, despite the generally high performance of modern STPs, residual TRI concentrations are common in secondary wastewater effluents, leading to TRI discharge into many receiving surface waters (Kolpin et al., 2002; Singer et al. 2002).

Fluoxetine, N-methyl-8-14-(trifluoromethyl)phenoxylbenzenepropanamine, is an antidepressant drug, commercially sold as Prozac®, that acts as a selective serotonin reuptake inhibitor in presynaptic neurons. In comparison to traditional tri-cyclic antidepressants, fluoxetine (FLX) can be prescribed in lower doses with minimal side-effects (Raggi et al., 1998), contributing in large part to its widespread use. Predicted FLX concentrations in wastewaters, taking into account human consumption and assuming no human metabolism, are 0.37 and 0.43 µg/L for the UK and USA, respectively (Webb, 2004; Brooks et al., 2003). Very little information is available regarding the fate of FLX in STPs and reported removal efficiencies vary within a wide range (8-90%, Webb, 2004; Johnson et al., 2005; Vasskog et al., 2006). In any case, the incomplete removal of this pharmaceutical is stated by its presence in STP effluents as well as in different surface waters (Kolpin et al., 2002; Metcalfe et al., 2003; Himmelsbach et al., 2006).

Table 7-1. Selected compounds and expected sites of O_3 attack

Compound	Fluoxetine (FLX)	Triclosan (TRI)
Use	Antidepressant drug	Antimicrobial agent
Structure with sites of O_3 attack		

Although TRI and FLX concentrations appear to be significantly reduced during biological wastewater treatment, their residual concentrations may still be a matter of concern. Residual concentrations of TRI in surface water warrant attention in part due to the low predicted no-effect concentration determined for the algae species *Scenedesmuss subspicatus*, which was estimated as 50 ng/L when a safety factor of 10 was considered. It is also known that TRI can induce antibacterial resistance (McMurry et al., 1998), presumably as a consequence of its broad-spectrum antibacterial activity, exerted via enzyme-specific disruption of lipid biosynthesis (Levy et al., 1999). However, the relevance of environmental TRI concentrations to development of antibacterial resistance remains unclear. Another point of concern is the reported formation of 2,8-dichlorodibenzo-p-dioxin during TRI photolysis (Latch et al., 2005). This could be of particular importance, as photolysis has shown to be the primary process by which TRI is depleted from surface waters (Tixier et al., 2002; Singer et al., 2002). At present, the potential risks for aquatic biota exposure to low concentrations of FLX are uncertain, since standard aquatic toxicity test suggest that little risk should be expected, whereas, adverse effects within female Japanese medaka have been reported at typical municipal effluent concentrations (Brooks et al., 2003; Webb, 2004).

Additional transformation of TRI via photochemical pathways (Latch et al., 2005; Tixier et al., 2002) and/or metal-oxide-mediated oxidation (Zhang and Huang, 2003), as well as of FLX via photochemical pathways (Lam et al., 2005) are expected to occur in natural environments. However, in light of possible negative interactions with aquatic biota, it may be more prudent to achieve higher removal during wastewater treatment, thus avoiding the discharge of these compounds into surface waters. Ozonation, which has proven to be an effective post-treatment technique for other pharmaceutical and personal care products (Huber et al., 2003; Huber et al., 2005), presents one possible option for wastewater post-treatment. Ozone (O_3) typically exhibits rapid reaction kinetics with a relatively small number of functional moieties, including activated aromatic rings, neutral alkylamines, double bonds, and thiols (Hoigne and Bader, 1983). TRI and FLX, which contain a

phenol and a secondary amine moiety, respectively (Table 7-1), are therefore expected to react rapidly with O_3. The present investigation was conducted to determine rate constants for the reactions of O_3 with TRI and FLX, and to apply these measurements to modelling TRI and FLX oxidation during ozonation of typical municipal wastewater effluents.

7.2. Materials and methods

7.2.1. Stock solutions

Stock solutions of TRI and FLX were prepared in Milli-Q water (Millipore), at a concentration of 100 µM for TRI and 1.16 mM for FLX. Stock solutions of O_3 (~1.5 mM) were prepared by sparging an O_3-containing gas stream through Milli-Q water that was cooled in an ice bath. The O_3-containing gas stream was produced by passing pure oxygen through an Innovatec CMG 3-4 pulsed corona-discharge O_3 generator. Working O_3 stock solutions (~0.1-0.5 mM) were prepared by diluting the saturated O_3 solution in Milli-Q water, acidified at pH ~ 4 with H_2SO_4.

7.2.2. Determination of the rate constants for reactions with ozone

Triclosan

Experiments for the determination of O_3 rate constants were performed at 23±2°C in a continuous-flow, quenched-reaction monitoring system. A multi-position syringe pump (Harvard Apparatus - Holliston, MA 22) was used to simultaneously inject the TRI and the O_3 solution at equal flow rates (ranging from 2.5 to 14 mL/min), from separate, 25 mL Hamilton gas-tight syringes, into a 60° mixing tee, coupled to a seven-point switching valve (Kintek Corporation – Austin, TX). The switching valve directed the mixed reaction solution through one of seven PTFE loops with volumes of 16.1, 35.2, 50.9, 85.2, 133.6, 169.6, and 199.3 µL. The effluent of each reaction loop was directed through a second mixing tee receiving a continuous stream of quenching reagent from a third channel of the multi-position syringe pump, in order to stop the reaction. Samples were collected from the effluent of the second mixing tee for measurement of residual TRI concentrations. Reaction times were varied (35 ms-2.4 s) by switching the reaction loop or by adjusting system flow-rate, to obtain measurements of reactant depletion with time.

Experiments were conducted under pseudo-first-order conditions of excess O_3. TRI was dissolved at 0.5-1 µM concentrations in a 10 mM phosphate buffer for the experiments conducted at pH 2 to 4, and in a 20 mM acetate buffer for those carried out at pH 4.5 to 5.5. A 10 mM solution of tert-butyl alcohol (*t*-BuOH) was added to the medium as a hydroxyl radical (•OH) scavenger. Working O_3 solutions were prepared in acidified Milli-Q water (pH ~ 4) at $[O_3] \geq 20 \times [TRI]$. Cinnamic acid (1 mM), which yields benzaldehyde in 1:1 stoichiometry upon reaction with a mole of O_3 (Leitzke et al., 2001), was used as a quenching agent. Benzaldehyde

formation was used to quantify residual O_3 concentrations. Experiments were performed at least in duplicate.

Fluoxetine

Kinetic experiments were carried out in 100 mL amber borosilicate glass bottles with a piston dispenser system screwed onto the bottle tops. The reaction solution consisted of 0.5-2 µM FLX and 10 mM *t*-BuOH dissolved in 10 mM phosphate buffer (pH 2-4 and 6.5-7) or 20 mM acetate buffer (pH 4.5-6). The reaction started with the injection of O_3 under vigorous magnetic stirring, at a concentration of at least 20-fold molar excess. Samples of the reaction solutions (3 mL) were then dispensed at regular time intervals into tubes containing a quenching agent (cinnamic acid, at 500 µM), over reaction monitoring periods ranging from 20 s for pH 7 to 1 hour for pH 2. Quenched samples were then analysed by HPLC for residual FLX concentrations. Duplicate experiments were performed at 20 °C by thermostating the reaction vessels in a constant-temperature water bath, placed on top of a magnetic stirring plate.

7.2.3. Municipal wastewater ozonation

Additional experiments were conducted with samples of secondary municipal wastewater effluent obtained from two conventional activated sludge treatment plants (one at pilot-scale, PS, and the other at full-scale, FS). Characteristic water quality parameters are shown in Table 7-2.

Table 7-2. Water quality parameters of pilot-scale (PS) and full-scale (FS) effluents.

Effluent	pH	DOC (mg/L)	Alkalinity (mM as HCO_3^-)	•OH scavenging rate (s^{-1})[a]
PS	7.9	7.5	8.1	2.5×10^5
FS	7.5	12.4	0.9	3.2×10^5

[a] Calculated at pH 8 and ambient temperature (Elovitz and von Gunten, 1999)

Triclosan

Experiments were conducted in 30 mL amber, borosilicate glass vials containing the respective wastewater spiked with TRI (0.5 µM) and *para*-chlorobenzoic acid (*p*CBA, 0.5 µM), which was used as a probe to quantify •OH exposures (Elovitz and von Gunten, 1999). Reactions were started by injecting a defined volume of O_3 stock solution covering an O_3 dose range of 0.1 to 6 mg/L ($2.1 \cdot 10^{-6}$-$1.2 \cdot 10^{-4}$ M). After 60 s of reaction time, each solution was dosed with 200 µM of cinnamic acid to quench any residual O_3 and samples were transferred to HPLC for analysis of residual TRI and pCBA concentrations. Reactions were conducted at 20ºC by thermostating the reactors in a water bath.

Fluoxetine

A procedure similar to that used for measurement of O_3-TRI reaction kinetics was used for wastewater experiments with FLX. Samples of each wastewater were spiked with FLX (0.5-1 µM) and pCBA (0.5-1 µM) and transferred into one syringe. Ozone solutions were transferred into a second syringe. Two initial O_3 concentrations were used for each of the wastewaters (2.5 and 5 mg/L ($5.2 \cdot 10^{-5}$ - $1.0 \cdot 10^{-4}$ M) for PS water; 5 and 10 mg/L ($1.0 \cdot 10^{-4}$-$2.1 \cdot 10^{-4}$ M) for FS water). After passage of the reaction solutions through the appropriate reaction loop, O_3 residuals were quenched with cinnamic acid (250 µM) contained in a third syringe. Quenched samples were collected from the system effluent and transferred to HPLC for analysis of FLX, *p*CBA and benzaldehyde.

7.2.4. Analytical Methods

Dissolved Organic Carbon (DOC) and alkalinity were determined following Standard Methods (APHA, 1999). The rest of compounds were measured by HPLC-UV, using isocratic methods with a 150×4.6 mm (5µm) Nucleosil-100 C18 column (Machery-Nagel). Mobile phases used were acetonitrile (ACN), 2 mM acetate buffer at pH 5 (Ac-Buffer) and 50 mM phosphate buffer at pH 2.2 (Ph-Buffer) depending on the compound (Table 7-3).

Table 7-3. HPLC methods description.

Compound	Flow rate (ml/min)	Mobile phase	Detection (nm)	Retention time (min)
Triclosan	0.7	80% ACN 20% Ac-Buffer	270/205	7.5
Fluoxetine	0.7	40% ACN 60% Ph-Buffer	226/205	10
Cinnamic acid, *p*CBA, and benzaldehyde	0.7	30% ACN 70% Ph-Buffer	250	12.5

7.2.5. Calculations

Kinetics of the reaction of a target compound A with ozone can be described as:

$$\frac{d[A]_{tot}}{dt} = -k_{O3} \cdot [A]_{tot} \cdot [O_3] \qquad \text{[Eq. 7-1]}$$

where k_{O3} is the second-order rate constant for the reaction ($M^{-1} \cdot s^{-1}$), [A] the concentration of the target compound (M) and [$\dot{O}_3$] the ozone concentration (M). If A is an acid or base with one pK_a, Equation 7-1 can be modified to include the reactions of each of its two acid-base species with O_3 (neutral and anionic TRI and cationic and neutral FLX):

$$\frac{d[A]_{tot}}{dt} = -\left(\alpha \cdot k_{O3,1} + (1-\alpha)\cdot k_{O3,2}\right)\cdot [A]_{tot} \cdot [O_3] \qquad \text{[Eq. 7-2]}$$

where α is the dissociation coefficient (Equation 7-3) that can be calculated from the pK_a,

$$\alpha = \frac{1}{1+\frac{10^{-pKa}}{10^{-pH}}} \qquad \text{[Eq. 7-3]}$$

and $k_{O3,1}$ and $k_{O3,2}$ represent the species-specific rate constants for reaction of O_3 with the undissociated (AH) and dissociated (A^{-1}) forms of the target compound, respectively ($M^{-1}\cdot s^{-1}$). The observed reactivity of A can be characterized at a certain pH with the apparent second-order rate constant, $k_{app,O3}$, according to Equation 7-4:

$$k_{app,O3} = \alpha \cdot k_{O3,1} + (1-\alpha)\cdot k_{O3,2} \qquad \text{[Eq. 7-4]}$$

Thus, Equation 7-2 can be rewritten as:

$$\frac{d[A]_{tot}}{dt} = -k_{app,O3} \cdot [A]_{tot} \cdot [O_3] \qquad \text{[Eq. 7-5]}$$

Under pseudo-first order conditions with an excess of O_3, $k_{app,O3}$ can be calculated from the slope of Equation 7-6:

$$\ln\left(\frac{[A]_{tot}}{[A]_{tot,0}}\right) = -k_{obs} \cdot t \qquad \text{[Eq. 7-6]}$$

where the pseudo-first-order rate constant k_{obs} (s^{-1}) is equal to $k_{app,O3}\cdot[O_3]$. With $k_{app,O3}$ determined experimentally at different pH values, and applying Equation 7-4, one can calculate the pH-independent, specific second-order rate constants $k_{O3,1}$ and $k_{O3,2}$.

Equations 7-1 to 7-6 can be used to characterize an ozonation process in which only O_3 is reacting with the target compound. That is the case of the experiments performed for the determination of TRI's and FLX's rate constants, where •OH radicals were scavenged with *t*-BuOH and O_3 remained the only oxidant. However, •OH radicals play an important role during ozonation of wastewater, which can be expressed by Equation 7-7:

$$\ln\left(\frac{[A]_t}{[A]_0}\right) = -k_{app,O_3,A}\int_0^t [O_3]\cdot dt - k_{app,\cdot OH,A}\int_0^t [\bullet OH]\cdot dt \qquad \text{[Eq. 7-7]}$$

where $k_{app,\bullet OH}$ is the apparent second-order rate constant for the reaction of •OH with A ($M^{-1}s^{-1}$).

As shown by Equation 7-7, O_3 and •OH exposures must be known to assess pollutant oxidation during wastewater ozonation processes. O_3 concentrations can

easily be measured, whereas for •OH radicals indirect methods have to be used. For the latter, •OH exposure was estimated by monitoring the depletion of an O_3-resistant compound, *p*CBA, during ozonation of each wastewater sample. $\int_0^t [\bullet OH] \cdot dt$ was then calculated according to Equation 7-8 (von Gunten, 2003).

$$\ln\left(\frac{[pCBA]_t}{[pCBA]_0}\right) = -k_{app,\cdot OH,pCBA} \int_0^t [\bullet OH] \cdot dt \qquad \text{[Eq. 7-8]}$$

Equation 7-8 was in turn used to estimate the contribution of •OH radicals to the observed oxidation of compound A during ozonation of the wastewater samples, according to Equation 7-9.

$$\ln\left(\frac{[A]_t}{[A]_0}\right) = -k_{app,O_3,A} \int_0^t [O_3] \cdot dt + \frac{k_{app,\cdot OH,A}}{k_{app,\cdot OH,pCBA}} \cdot \ln\left(\frac{[pCBA]_t}{[pCBA]_0}\right) \qquad \text{[Eq. 7-9]}$$

The fraction of total A oxidation attributable to •OH ($f_{\bullet OH}$) was calculated according to Equation 7-10. In cases for which $\int_0^t [O_3] \cdot dt$ could not be directly determined (e.g., when losses of A or consumption of O_3 by reactive matrix constituents were too fast to permit direct monitoring), $f_{\bullet OH}$ was estimated from initial and final compound concentrations.

$$f_{\bullet OH} = \frac{k_{app,\cdot OH,A} \int_0^t [\bullet OH] dt}{k_{app,O_3,A} \int_0^t [O_3] dt + k_{app,\cdot OH,A} \int_0^t [\bullet OH] dt} = \frac{\frac{k_{app,\bullet OH,A}}{k_{app,\bullet OH,pCBA}} \ln\left(\frac{[pCBA]}{[pCBA]_0}\right)}{\ln\left(\frac{[A]}{[A]_0}\right)}$$

[Eq. 7-10]

7.3. Results and discussion

7.3.1. Rate constants for reactions of TRI and FLX with O_3

Apparent second-order rate constants, $k_{app,O3}$, were determined by linear regressions of TRI and FLX depletion upon reaction with O_3 at various pH values (Equation 7-6). The averages of the corresponding measurements are presented in Figure 7-1. These data show that the $k_{app,O3}$ values for each compound increase in parallel with the degree of deprotonation of the substrate. This observation

indicates that the most reactive form of each compound is its deprotonated conjugate, that is anionic triclosan or neutral fluoxetine (Table 7-1).

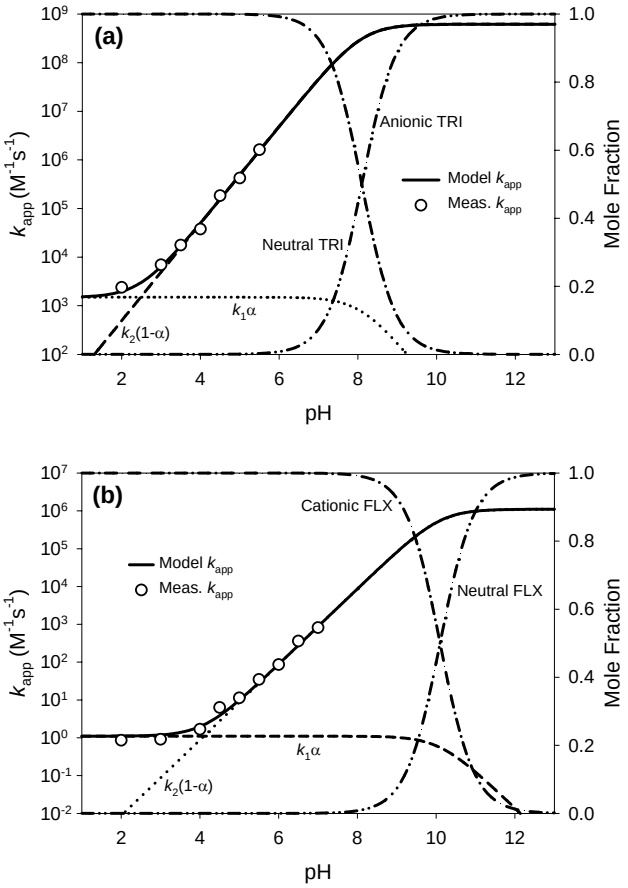


Figure 7-1. Apparent second-order rate constants for (a) TRI and (b) FLX as a function of pH.

Specific second-order rate constants for each compounds' acid-base species were determined by non-linear regression of data shown in Figure 7-1, according to Equation 7-4, and are shown in Table 7-4. These constants were used to model $k_{app,O3}$ for TRI and FLX in a larger pH range, by substitution into Equation 7-4. Model results are presented as solid lines in Figure 7-1, for comparison with measured data.

Table 7-4. Second-order rate constants for reactions of O_3 with TRI and FLX

Compound	pK_a	$k_{O3,protonated}$ ($M^{-1}s^{-1}$)	$k_{O3,deprotonated}$ ($M^{-1}s^{-1}$)	$k_{app,\ pH\ 7}$ ($M^{-1}s^{-1}$)	$t_{1/2,\ pH\ 7}$ [c] (s)
TRI	8.1[a]	1.3 (± 0.1) × 10^3	5.1 (± 0.1) × 10^8	3.8 × 10^7	4 × 10^{-4}
FLX	10.1[b]	1.1 (± 0.6)	1.1 (± 0.1) × 10^6	8.7 × 10^2	19

[a]Singer et al., 2002, [b]Brooks et al. 2003, [c]Half lives calculated at pH 7 and $[O_3]$ = 2 mg/L.

The enhancement of TRI's reactivity toward O_3 upon deprototonation is presumably a consequence of an activation of its phenol ring by the electron-donating substituent O^-. Neutral phenols generally react with O_3 at baseline rate constants between 10^2 and 10^3 $M^{-1}s^{-1}$, whereas their conjugate phenolate forms exhibit rate constants between 10^7 and 10^9 $M^{-1}s^{-1}$ (Hoigné and Bader, 1983). The k_{O3} for anionic TRI is between the rate constants measured for phenolate (1.4 × 10^9 $M^{-1}s^{-1}$) and for the anionic forms of the mono-substituted 2-chlorophenol (2 × 10^8 $M^{-1}s^{-1}$).

This can most likely be explained by a combination of electronic and steric interactions within the TRI molecule. Deactivation of the TRI phenol ring by its electron-withdrawing *m*-Cl substituent, is likely offset in part by the electron-donating effect of the *o*-phenyl ether moiety, making anionic TRI more reactive than 2-chlorophenol. However, the electron-donating effect of the *o*-phenylether moiety is most probably attenuated by steric effects derived from its relatively large molar volume, resulting in the lower reactivity of anionic TRI compared to phenolate. The hypothesis that O_3 reacts initially with TRI by electrophilic attack at the latter's phenol moiety was strongly supported (Suarez et al., 2007).

FLX appears to react with O_3 with a baseline rate constant of 1.1 $M^{-1}s^{-1}$ below pH 3, at which the cationic FLX species predominates. These data suggest that O_3 reacts slowly with cationic FLX's aromatic tolyl moiety, since the trifluoromethyl-substituted aromatic moiety is likely strongly deactivated toward electrophilic attack by O_3, and protonated amines are generally reported to be unreactive toward O_3 (Hoigne and Bader, 1983; Pryor et al., 1984; Munoz and von Sonntag, 2000). The increase in $k_{app,O3,FLX}$ to an apparent maximum of 1.1 × 10^6 $M^{-1}s^{-1}$ appears to correspond directly to an increase in the proportion of FLX present in its neutral form. Prior researchers have reported rate constants of around 10^6 $M^{-1}s^{-1}$ for various neutral secondary and tertiary amines, supporting the expectation that FLX's apparent reactivity is governed by O_3 attack at its neutral secondary amine moiety at pH > 3.

7.3.2. Wastewater ozonation

Triclosan transformation occurred too rapidly ($t_{1/2}$ < 35 ms) during ozonation of wastewater to permit direct, time-resolved reaction monitoring, therefore only overall removal at different applied ozone doses could be determined (Figure 7-2).

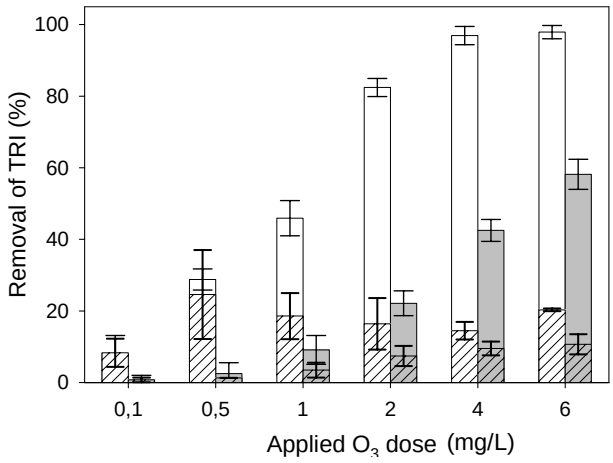


Figure 7-2. Transformation of TRI during ozonation of PS (□) and FS (■) effluents. Contribution of •OH to overall oxidation (▨).

Transformation achieved for a defined O_3 dose was higher in the pilot scale (PS) effluent than in the full scale (FS) effluent, presumably due to the higher DOC content of the latter, which competes with the target compound for O_3 and •OH radicals (Elovitz et al., 2000). For the PS effluent, application of O_3 doses higher than 4 mg/L ensures complete depletion of TRI. However, for the FS effluent, there is still considerable residual TRI (42%) even after dosing 6 mg/L of O_3, presumably due to rapid O3 and •OH scavenging by dissolved organic matter contained in this water. Apparent contributions of •OH to the overall oxidation of triclosan in these wastewaters, calculated according to Equation 7-10, decreased as the O_3 dose applied to each water was increased (Figure 7-2). Presumably, higher O_3 doses led to higher cumulative O_3 exposures, and as a consequence of the temporally decreasing yields of •OH during wastewater ozonation (Buffle et al., 2006) to lower apparent, cumulative ratios of $\int_0^t [\bullet OH]dt$ to $\int_0^t [O_3]dt$. Consequently, direct oxidation of triclosan by O_3 appeared to be enhanced at higher O_3 doses. According to prior investigations, biocidal activity of the TRI molecule is derived primarily from its phenol ring, via van der Waals and hydrogen-bonding interactions with the bacterial enoyl-acyl carrier protein reductase enzyme (Levy et al., 1999). Thus, direct oxidation of the TRI molecule by O_3 yielding oxygen addition to the phenol ring or

phenol ring opening (Mvula and von Sonntag, 2003) should reduce or eliminate this target-specific biochemical activity, what has been indeed confirmed by measuring triclosan's antibacterial activity after treatment with ozone (Suarez et al., 2007).

Fluoxetine oxidation occurred on much longer time-scales during ozonation of each wastewater than for TRI, thus permitting a direct monitoring of FLX loss (Figure 7-3).

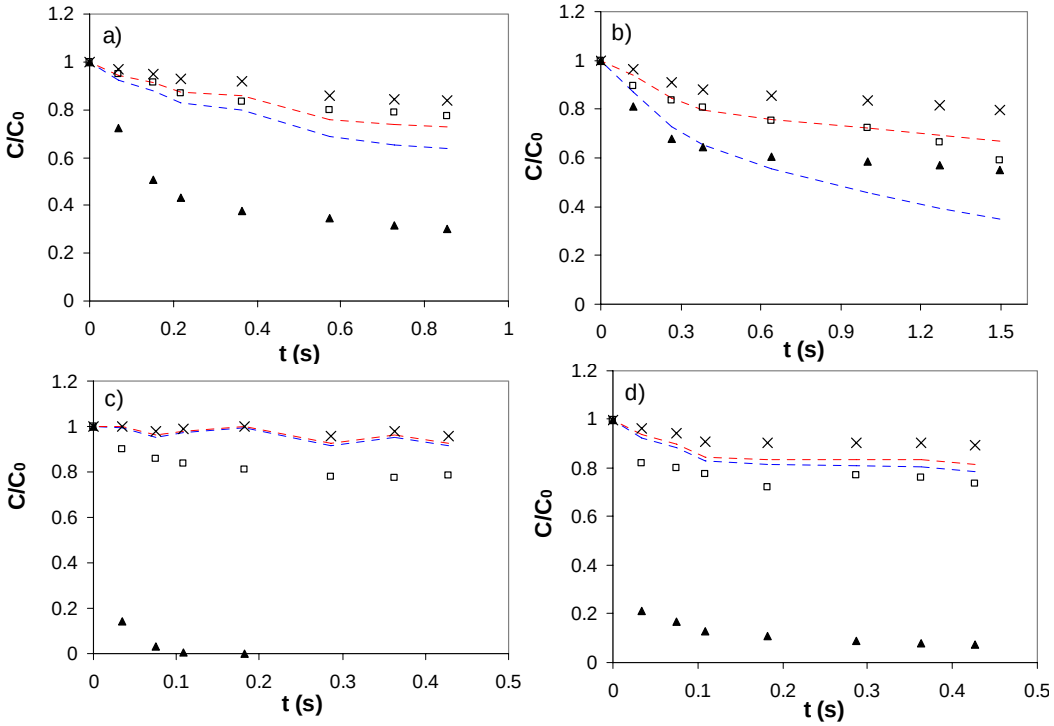


Figure 7-3. Oxidation of FLX during PS and FS wastewater ozonation at applied O_3 doses of a) 2.5 and b) 5 mg/L for the first and c) 5 and d) 10 mg/L for the second. (▲) O_3; (□) measured concentrations of FLX; (×) *p*CBA. FLX oxidation modelled at pH 4 (---) and at that of the corresponding effluent (---).

In this case, O_3 and •OH exposures were determined to allow calculation of expected FLX transformation, according to Equation 7-9. The apparent second-order rate constants for the reactions of FLX and *p*CBA with •OH that have been considered for the modelling were 9×10^9 and 5×10^9 $M^{-1}s^{-1}$, respectively (Lam et al., 2005; Neta and Dorfman, 1968), whereas $k_{app,O3}$ values have been calculated according to Equation 7-4. Since the wastewaters were used without buffering, pH could not be considered as constant, but it was known to vary between that of the ozone stock solution (pH 4) and that of the considered wastewater (Table 7-2). Data regarding measured ozone, *p*CBA and FLX depletion have been included in

Figure 7-3, where also a comparison between experimental data and model calculations has been included.

As shown in Figure 7-3 (a-b), the relatively low DOC content and high alkalinity of the PS effluent (Table 7-2) led to stabilization of O_3 (Elovitz et al., 2000), whereas O_3 was quickly consumed in the FS effluent, which contained a comparably higher DOC concentration and much lower alkalinity (Figure 7-3c-d). In fact, in the PS effluent, more than 30% of the initial O_3 dose applied was still present at the end of each experiment, whereas in the FS effluent, more than 80% of O_3 reacted prior to 35 ms. Model calculations at pH 4 fitted very satisfactory measured FLX concentrations during PS wastewater ozonation, whereas model predictions were somewhat less accurate for the experiments performed with FS effluents, especially at the lower ozone dose applied. In this latter case FLX losses not attributable to reaction with ozone seemed to be responsible for the observed discrepancies.

The relative contribution of •OH to FLX oxidation according to Equation 7-10 ($f_{\bullet OH}$) was in the range 0.7-1, indicating that direct reactions with ozone exert little influence on this process.

In general, the contributions of •OH to total pollutant oxidation were higher for FLX than for TRI, due primarily to the 4-5 orders of magnitude lower apparent, second-order rate constant for direct O_3-FLX reactions, as compared to O_3-TRI reactions, at the corresponding pH range of wastewater ozonation. For TRI, comparably better removal rates were observed during ozonation of the PS effluent. However, this was not the case for FLX, where similar transformations were observed in both wastewaters. This difference could be explained by the fact that TRI transformation was dominated by direct reactions with O_3, whereas FLX transformation was dominated by •OH oxidation. The lifetime of O_3 in the wastewater matrixes, which is largely controlled by the content of dissolved organic matter, measured as DOC, was therefore a crucial factor with regard to TRI oxidation efficiency. In contrast, FLX was oxidized predominantly by •OH, so •OH scavenging rate of the water matrix was of critical importance. Because •OH scavenging rates were relatively similar in the PS and FS effluents, only a small difference in FLX oxidation efficiencies was observed for the two waters.

7.4. Conclusions

Second-order rate constants were determined for the reactions of TRI and FLX with O_3. The deprotonated species of each compound were found to be highly reactive toward O_3, with specific rate constants of 5.1×10^8 $M^{-1}s^{-1}$ and 1.1×10^6 $M^{-1}s^{-1}$ for TRI and FLX, respectively. However, due to the large difference in TRI's and FLX's pK_a values, TRI was much faster reacting than FLX at circumneutral pH, with $t_{1/2,TRI}$ = 0.4 ms, compared to $t_{1/2,FLX}$ = 17 s, at pH 7, for an applied ozone concentration of 2 mg/L.

Kinetic parameters indicated that O_3 reacted with TRI by direct electrophilic attack of the latter's phenol ring, while O_3 reacted with FLX by electrophilic attack of the latter's neutral secondary amine.

Experiments with the secondary effluents of two wastewater treatment plants with different water quality parameters showed that rate constants determined in pure waters could be successfully applied to characterize TRI and FLX oxidation during wastewater ozonation. Generally, the contributions of •OH to FLX oxidation were higher than for TRI, due primarily to FLX's lower reactivity toward O_3. Therefore, the rate of •OH scavenging by the water matrix played an important role in FLX oxidation efficiency.

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General conclusions

This Thesis aims at contributing to the broadening of the current state of knowledge concerning the occurrence and behaviour of Pharmaceuticals and Personal Care Products (PPCPs) during municipal wastewater treatment, including primary, secondary and post-treatments. The main conclusions drawn from this work are summarised in the following paragraphs.

Comparing **municipal** and **hospital wastewaters**, three main differences have been observed: i) Hospital effluents are in general stronger contaminated regarding conventional pollutants (TS, TSS, COD, etc.) as well as PPCP concentrations; ii) Characteristics of hospital effluents vary more strongly between samplings; iii) Water consumption per bed in hospitals is 6-8 times higher than in common households.

The highest concentrations of PPCPs in raw sewage (> 1 µg/L) were measured for the anti-inflammatory drugs ibuprofen (IBP) and naproxen (NPX), followed by the two fragrances, galaxolide (HHCB) and tonalide (AHTN), whose concentrations were in the range 0.2-1.2 µg/L. The concentration of the natural estrogens estrone (E1) and estriol (E3) was almost one order of magnitude below that of fragrances and even lower in the case of the third natural estrogen, 17β-estradiol (E2). The rest of PPCPs included in the monitoring of municipal wastewater (celestolide (ADBI), diclofenac (DCF), carbamazepine (CBZ), diazepam (DZP), 17α-ethinylestradiol (EE2), fluoxetine (FLX) and citalopram (CTL)), were not detected or could not be quantified in any of the samples considered.

Concentrations of PPCPs in the effluent from the STP could be used to evaluate the potential risk derived from its discharge into the receiving river, where the conclusion, under worst-case assumption, was that CBZ, EE2, E1 and E2 could exert a potential adverse effect on aquatic organisms.

Transformation of PPCPs during a **conventional denitrifying/nitrifying activated sludge treatment process** has been correlated with the physico-chemical properties of the compounds, as follows:

- √ The three compounds with low K_d (sorption coefficient) and low k_{biol} (biological transformation rate constant), namely CBZ, DZP and DCF, were not significantly removed during the treatment.

- √ In the case of FLX, NPX and IBP high removal (>80%) of the parent compound has been observed, which was associated to the good biodegradability of the compounds, since sorption of these compounds is negligible.

√ Natural estrogens (E1+E2) have also been very efficiently removed (>90%), confirming their fast biodegradation, additionally favoured by a slight sorption capacity.

√ On the contrary, musk fragrances (HHCB, AHTN and ADBI) are efficiently transformed inside the pilot plant, presumably due to their enhanced retention inside the reactor by means of lipophilic interactions with the sludge (absorption), thus promoting their transformation despite their very low biological degradation constants.

√ In the case of EE2 and roxithromycin (ROX), less than 40% of the influent concentration remains in the final effluent of the pilot plant, which can be again partially attributed to their enhanced retention inside the pilot plant due to their medium sorption capacity.

√ For the rest of PPCPs, CTL, sulfamethoxazole (SMX), trimethoprim (TMP) and erythromicyn (ERY), the transformation degree in the pilot plant was at least 40%, according to their moderate sorption and biodegradation potential.

The influence of **redox conditions** on the removal of PPCPs could be analysed by means of mass balance calculations applied to the denitrifying/nitrifying pilot plant, as well as from experimental data measured in two lab-scale reactors, one working at nitrifying aerobic conditions and the other in an anoxic denitrifying environment.

√ FLX, natural estrogens (E1+E2) and musk fragrances (HHCB, AHTN and ADBI) are highly biodegradable under aerobic and anoxic conditions.

√ NPX, EE2, ROX and ERY are highly biodegradable under aerobic conditions but persistent in the anoxic reactor.

√ CTL is moderately biodegradable under aerobic and anoxic conditions.

√ CBZ and DZP are resistant to biological transformation.

√ SMX is moderately transformed only under aerobic conditions.

√ IBP is efficiently transformed in aerobic conditions, while removal under anoxic environments depends on biomass adaptation.

The influence of some **operational parameters** such as temperature, Sludge Retention Time (SRT) and the concentration, adaptation and type of sludge on the biological processes was evaluated.

√ The positive effect of increasing SRT from below 20 d to more than 50 d has been observed for some compounds, including SMX, FLX, CTL, NPX, EE2 and natural estrogens, although the increase in the removal efficiency of these PPCPs was in general low (max. 25%).

√ Similarly, warmer temperatures (from ~17ºC to ~22ºC) led to higher transformation efficiencies for some PPCPs (CTL, FLX, E1+E2, SMX and ERY) with a maximum increase in the removal of 32%.

√ By far, the sludge type, concentration and/or adaptation were the parameters that affected the removal of PPCPs to the highest degree:
i) The development of a specific nitrifying biota lead to a very important removal of DCF (>70%), which on the contrary showed to be very persistent in the pilot plant, indicating that the type of sludge can influence the fate of some PPCPs. A similar effect was observed for IBP in the anoxic biodegradation reactor, where transformation efficiencies increased gradually with time from below 16% up to 75%.
ii) A clear correlation between sludge concentration in the reactor and removal efficiency has been observed for NPX in the pilot plant, as well as for DCF in the nitrifying biodegradation reactor.
iii) Acclimation of microorganisms to the presence of PPCPs seemed to be responsible for the 70% increase in the removal of NPX in the pilot plant.

Coagulation-flocculation and **flotation** can be used as primary treatment in conventional STPs, but also in order to pre-treat specific streams with significantly lower flows than municipal wastewater, but containing higher PPCP concentrations, such as hospital effluents. When these two technologies are applied separately, generally flotation leads to slightly worse results compared to coagulation regarding both, TSS and PPCPs removal, although if they are sequentially applied (coagulation+flotation), the overall efficiency of the process can be somewhat improved.

Concerning PPCPs, the compounds iopromide (IPM), CBZ and DZP could generally not be eliminated from the liquid phase, whereas very high removal efficiencies (>90%) have been measured for the three fragrances (HHCB, AHTN and ADBI). For NPX, IBP and DCF the decrease in concentration was in between the previous substances (~50%). In the case of antibiotics, negative removals have been measured for macrolides (ROX and ERY) and TMP, whereas SMX concentrations were not significantly altered during the process.

The two main mechanisms known to be responsible for sorption of PPCPs onto suspended solids were absorption and adsorption. The first, based on lipophilic interactions, was mainly responsible for the removal of fragrances, where better results were obtained in streams with higher fat content, whereas the second,

based on electrostatic interactions, was the driving force for the removal of ionic compounds (e.g. cationic species of CBZ).

In order to remove persistent PPCPs in conventional sewage treatment or whose discharge into the aquatic environment raises special concern even at very low concentrations, a post-treatment step needs to be implemented. **Oxidation with ozone** could be one possibility, which has been further studied for two PPCPs, FLX and triclosan (TRI). The deprotonated species of each compound were found to be highly reactive toward O_3, although, due to the large difference in TRI's and FLX's pK_a values, TRI was much faster reacting than FLX at circumneutral pH ($t_{1/2,TRI}$ = 0.4 ms; $t_{1/2,FLX}$ = 17 s, at pH 7 and $[O_3]_0$ 2 mg/L).

Kinetic parameters indicated that O_3 reacted with TRI by direct electrophilic attack of the latter's phenol ring, while O_3 reacted with FLX by electrophilic attack of the latter's neutral secondary amine.

Experiments with the secondary effluents of two wastewater treatment plants with different water quality parameters showed that rate constants determined in pure waters could be successfully applied to characterize TRI and FLX oxidation during wastewater ozonation. Generally, the contributions of •OH to FLX oxidation were higher than for TRI, due primarily to FLX's lower reactivity toward O_3. Therefore, the rate of •OH scavenging by the water matrix played an important role in FLX oxidation efficiency.

Conclusiones generales

Esta Tesis contribuye a mejorar y ampliar el conocimiento actual relativo a la presencia y al comportamiento de compuestos farmacéuticos y de cuidado personal (PPCPs) en el tratamiento de aguas residuales municipales, incluyendo procesos primarios, secundarios y post-tratamientos. Las conclusiones principales de este trabajo se resumen en los siguientes párrafos.

Comparando las **aguas residuales municipales y hospitalarias**, se han observado tres diferencias principales: i) Los efluentes de hospital presentan, en general, una mayor carga contaminante tanto en lo que respecta a parámetros convencionales (ST, SST, DQO, etc.) como a las concentraciones de PPCPs; ii) Las características de los efluentes de hospital presentan una variabilidad mayor comparando los distintos muestreos; iii) El consumo de agua por cama en hospitales es de 6-8 veces más alto que el consumo doméstico medio.

Las concentraciones más elevadas de PPCPs en aguas residuales brutas (> 1 µg/L) se midieron para los anti-inflamatorios ibuprofeno (IBP) y naproxeno (NPX), seguidos de las dos fragancias, galaxolide (HHCB) y tonalide (AHTN), cuyas concentraciones estaban en el rango de 0.2-1.2 µg/L. Los estrógenos naturales, estrona (E1) y estriol (E3), se detectaron en concentraciones de aproximadamente una orden de magnitud inferior a las de fragancias e incluso menores para el tercer estrógeno natural, 17β-estradiol (E2). El resto de PPCPs incluidos en el muestreo de aguas residuales municipales (celestolide (ADBI), diclofenaco (DCF), carbamazepina (CBZ), diazepam (DZP), 17α-etinilestradiol (EE2), fluoxetina (FLX) y citalopram (CTL)), no se han detectado o no pudieron ser cuantificados en ninguna de las muestras analizadas.

Las concentraciones de PPCPs en los efluentes de la estación depuradora permitieron evaluar el riesgo potencial derivado de su descarga al río receptor. La conclusión, asumiendo el caso más desfavorable, fue que CBZ, EE2, E1 y E2 podrían ejercer un efecto adverso en los organismos acuáticos que habitan en esos ríos.

La transformación de PPCPs durante un **proceso de desnitrificación/nitrificación de lodos activos convencional** se ha correlacionado con las propiedades físico-químicas de los compuestos como sigue:

- √ Los tres compuestos con K_d (coeficiente de adsorción) y k_{biol} (constante de degradación biológica) bajos, como la CBZ, el DZP y el DCF, no se eliminaron significativamente durante el tratamiento.

- √ En el caso de la FLX, del NPX y del IBP se han medido elevados porcentajes de eliminación (> 80%), que se asociaron a la alta biodegradabilidad de los

compuestos, ya que su adsorción sobre el lodo puede despreciarse.

√ Los estrógenos naturales (E1 +E2) también se han eliminado de manera muy eficaz (> 90%), confirmando su rápida biodegradación, favorecida adicionalmente por una cierta tendencia a la adsorción.

√ Por el contrario, las fragancias policíclicas (HHCB, AHTN y ADBI) se han transformado eficazmente en la planta piloto, probablemente porque las interacciones lipofílicas con el lodo (absorción) han realzado su retención en el reactor, promoviendo de este modo su transformación, a pesar de presentar constantes de degradación biológicas muy bajas.

√ En el caso del EE2 y de la roxitromicina (ROX), la concentración de compuesto en el efluente final de la planta piloto está por debajo del 40% de la concentración en el influente. Esto puede deberse una vez más a la realzada retención de esto compuestos en la planta piloto como consecuencia de su adsorción sobre el lodo.

√ Para el resto de PPCPs (CTL, sulfametoxazol (SMX), trimetoprim (TMP) y eritromicina (ERY)) el grado de transformación en la planta piloto fue superior al 40%, en consonancia con su moderado potencial de adsorción y de biodegradación.

La influencia de las **condiciones de oxidación/reducción (redox)** sobre la eliminación de los PPCPs pudo evaluarse en base a balances de materia aplicados a la planta piloto de desnitrificación/nitrificación y también a partir de datos experimentales medidos en dos reactores a escala de laboratorio, funcionando en condiciones aerobias nitrificantes y anóxicas desnitrificantes.

√ La FLX, los estrógenos naturales (E1+E2) y las fragancias policíclicas (HHCB, AHTN y ADBI) son altamente biodegradables bajo condiciones aerobias y anóxicas.

√ Los compuestos NPX, EE2, ROX y ERY son altamente biodegradables bajo condiciones aerobias, aunque persistentes en el reactor anóxico.

√ El CTL es moderadamente biodegradable en condiciones aerobias y anóxicas.

√ La CBZ y el DZP son resistentes a la transformación biológica.

√ El SMX sólo se transformó de forma moderada en condiciones aerobias.

√ El IBP se eliminó de forma eficaz en condiciones aerobias, mientras que su degradación en ambientes anóxicos depende del grado de adaptación de la biomasa.

Se ha evaluado la influencia de algunos **parámetros de operación** sobre los procesos biológicos, tales como la temperatura, el Tiempo de Retención Celular (TRC) y la concentración, la adaptación y el tipo de fango.

- √ Para algunos compuestos, como el SMX, la FLX, el CTL, el NPX, el EE2 y los estrógenos naturales, operar con TRC superiores a 50 d ha tenido un efecto positivo sobre la eficacia de eliminación en comparación con los resultados obtenidos al trabajar con TRC inferiores a 20 d, si bien esta mejora fue en general moderada (máx. 25 %).

- √ De manera similar, operar con temperaturas más elevadas (~22ºC en lugar de ~17ºC) condujo a eficiencias de transformación superiores para algunos PPCPs (CTL, FLX, E1+E2, SMX y ERY) con un incremento máximo en la eficacia de un 32%.

- √ Los parámetros que mayor influencia ejercieron sobre la eliminación de PPCPs fueron el tipo de fango, su concentración y/o su grado de adaptación:
  i) El desarrollo de una biomasa nitrificante específica permitió alcanzar unos porcentajes de eliminación de DCF muy importantes (> 70%), a pesar de ser un compuesto con una gran resistencia a la degradación según los resultados obtenidos en la planta piloto. Este hecho reveló que el tipo de fango puede influenciar el comportamiento de algunos PPCPs. Un efecto similar se ha observado para el IBP en el reactor de biodegradación anóxica, donde las eficiencias de transformación han ido aumentando con el tiempo desde valores inferiores al 16% hasta alcanzar un máximo del 75%.
  ii) Para el NPX en la planta piloto y para el DCF en el reactor de biodegradación nitrificante, se ha observado una clara correlación entre la concentración de lodos en el reactor y la eficacia de eliminación alcanzada para estos compuestos.
  iii) La aclimatación de los microorganismos a la presencia de PPCPs en el agua residual pudo ser responsable del incremento del 70 % en la eliminación de NPX en la planta piloto.

La coagulación-floculación y la flotación pueden utilizarse como tratamiento primario en estaciones depuradoras convencionales, o también para realizar un pre-tratamiento de corrientes específicas con caudales inferiores al de las aguas residuales municipales, aunque con concentraciones de PPCPs más elevadas, como ocurre por ejemplo con los efluentes de hospital. Cuando estas dos tecnologías se aplican por separado, generalmente la flotación da lugar a eliminaciones más bajas de SST y de PPCPs comparadas con las obtenidas por coagulación. Sin embargo, cuando estos procesos se aplican secuencialmente (coagulación+flotación), se logra mejorar la eficiencia global del proceso.

La conclusión general a la que se llega en cuanto a eliminación de PPCPs es que algunos compuestos como el iopromide (IPM), la CBZ y el DZP no se eliminan de la fase líquida, mientras que para otras sustancias como las fragancias (HHCB, AHTN y ADBI) se alcanzan eficacias de eliminación muy elevadas (> 90%). Entre estos dos extremos se sitúan los resultados obtenidos para NPX, IBP y DCF con eficacias de eliminación de ~50%. Para los antibióticos el pre-tratamiento no ha resultado ser una técnica de depuración eficaz, ya que la concentración de SMX no se vio afectada por el proceso y para los demás antibióticos (ROX, ERY y TMP) ésta incluso ha sido mayor en el efluente que en el influente.

Los dos mecanismos principales responsables de la interacción de los PPCPs con los sólidos en suspensión fueron la absorción y la adsorción. El primero, que se basa en interacciones de tipo lipofílico, jugó un papel importante en la eliminación de fragancias, para las cuales se obtuvieron los mejores resultados en el tratamiento de corrientes con mayor contenido en grasas. El segundo mecanismo, basado en interacciones electrostáticas, fue la fuerza impulsora para la eliminación de compuestos iónicos (por ejemplo la forma catiónica de la CBZ).

Para completar la eliminación de los PPCPs más persistentes en el tratamiento convencional de aguas residuales o de aquellos cuya descarga a los cauces de agua naturales preocupa de manera especial incluso a concentraciones muy bajas, es necesario instalar una etapa de post-tratamiento. La oxidación con ozono podría ser una posibilidad, opción que ha sido evaluada para dos PPCPs, la FLX y el triclosan (TRI). Las especies desprotonadas de cada compuesto han resultado ser altamente reactivas con O_3, aunque, debido a la gran diferencia de pKa del TRI y de la FLX, el TRI ha reaccionado mucho más rápidamente que la FLX a pH neutro ($t_{1/2,\ TRI}$ = 0.4 ms; $t_{1/2,\ FLX}$ = 17 s, a pH 7 y con $[O_3]_0$ 2 mg/L).

A partir del análisis de estos parámetros cinéticos se concluye que el O_3 reaccionó con el TRI y con la FLX por ataque electrofílico directo a su anillo fenólico, y grupo amino, respectivamente.

A partir de los experimentos realizados con los efluentes secundarios de dos plantas de tratamiento de aguas residuales que presentaban parámetros de calidad de agua diferentes, se dedujo que las constantes cinéticas determinadas en agua destilada pueden extrapolarse con éxito a la ozonización de TRI y de FLX en aguas residuales. Generalmente, los radicales •OH contribuyeron en mayor medida a la oxidación de FLX que a la de TRI, debido principalmente a la menor reactividad de la FLX con O_3. Por ello, la competencia por los radicales •OH ejercida por la matriz del agua residual resultó ser especialmente importante para la oxidación de FLX.

Conclusións xerais

Esta Tese contribúe a mellorar e ampliar o coñecemento actual relativo á presenza e ao comportamento de compostos farmacéuticos e de coidado persoal (PPCPs) no tratamento de augas residuais municipais, incluíndo procesos primarios, secundarios e post-tratamentos. As conclusións principais deste traballo resúmense nos seguintes parágrafos.

Comparando as **augas residuais municipais e hospitalarias**, observáronse tres diferenzas principais: i) Os efluentes de hospital presentan, en xeral, unha maior carga contaminante tanto no que respecta a parámetros convencionais (ST, SST, DQO, etc.) como ás concentracións de PPCPs; ii) As características dos efluentes de hospital presentan unha variabilidade maior comparando as distintas mostraxes; iii) O consumo de auga por cama en hospitais é de 6-8 veces máis alto que o consumo doméstico medio.

As concentracións máis elevadas de PPCPs en augas residuais brutas (> 1 µg/L) medíronse para os anti-inflamatorios ibuprofeno (IBP) e naproxeno (NPX), seguidos das dúas fragrancias, galaxolide (HHCB) e tonalide (AHTN), cuxas concentracións estaban no rango de 0.2-1.2 µg/L. Os estróxenos naturais, estrona (E1) e estriol (E3), detectáronse en concentracións de aproximadamente unha orde de magnitude inferior ás de fragrancias e ata menores para o terceiro estróxeno natural, 17β-estradiol (E2). O resto de PPCPs incluídos na mostraxe de augas residuais municipais (celestolide (ADBI), diclofenaco (DCF), carbamazepina (CBZ), diazepam (DZP), 17α-etinilestradiol (EE2), fluoxetina (FLX) e citalopram (CTL)), non se detectaron ou non puideron ser cuantificados en ningunha das mostras analizadas.

As concentracións de PPCPs nos efluentes da estación depuradora puideron utilizarse para avaliar o risco potencial derivado da súa descarga ao río receptor. A conclusión, asumindo o caso máis desfavorable, foi que CBZ, EE2, E1 e E2 poderían exercer un efecto adverso nos organismos acuáticos que habitan neses ríos.

A transformación de PPCPs durante un **proceso de desnitrificación/nitrificación** de lodos activos convencional relacionouse coas propiedades físico-químicas dos compostos, como segue:

- √ Os tres compostos con K_d (coeficiente de adsorción) e k_{biol} (constante de degradación biolóxica) baixos, como a CBZ, o DZP e o DCF, non se eliminaron significativamente durante o tratamento.
- √ No caso da FLX, do NPX e do IBP medíronse elevadas porcentaxes de

eliminación (> 80%), que se asociaron á alta biodegradabilidade dos compostos, xa que a súa adsorción sobre o lodo pode desprezarse.

√ Os estróxenos naturais (E1+E2) tamén se eliminaron de xeito moi eficaz (> 90%), confirmando a súa rápida biodegradación, favorecida adicionalmente por unha certa tendencia á adsorción.

√ Pola contra, as fragrancias policíclicas (HHCB, AHTN e ADBI) transformáronse eficazmente na planta piloto, probablemente porque as interaccións lipofílicas co lodo (absorción) realzaron a súa retención no reactor, promovendo deste xeito a súa transformación, a pesar de presentar constantes de degradación biolóxicas moi baixas.

√ No caso do EE2 e da roxitromicina (ROX), a concentración de composto no efluente final da planta piloto está por baixo do 40% da concentración no influente. Isto pode deberse unha vez máis á realzada retención deste compostos na planta piloto como consecuencia da súa adsorción sobre o lodo.

√ Para o resto de PPCPs (CTL, sulfametoxazol (SMX), trimetoprim (TMP) e eritromicina (ERY)) o grao de transformación na planta piloto foi superior ao 40%, en consonancia co seu moderado potencial de adsorción e de biodegradación.

A influencia das **condicións de oxidación/redución (redox)** sobre a eliminación dos PPCPs puido avaliarse en base a balances de materia aplicados á planta piloto de desnitrificación/nitrificación e tamén a partir de datos experimentais medidos en dous reactores a escala de laboratorio, funcionando en condicións aerobias nitrificantes e anóxicas desnitrificantes.

√ A FLX, os estróxenos naturais (E1+E2) e as fragrancias policíclicas (HHCB, AHTN e ADBI) son altamente biodegradables baixo condicións aerobias e anóxicas.

√ Os compostos NPX, EE2, ROX e ERY son altamente biodegradables baixo condicións aerobias, aínda que persistentes no reactor anóxico.

√ O CTL é moderadamente biodegradable en condicións aerobias e anóxicas.

√ A CBZ e o DZP son resistentes á transformación biolóxica.

√ O SMX só se transformou de forma moderada en condicións aerobias.

- √ O IBP eliminouse de forma eficaz en condicións aerobias, mentres que a súa degradación en ambientes anóxicos depende do grao de adaptación da biomasa.

Avaliouse a influencia dalgúns **parámetros de operación** sobre os procesos biolóxicos, tales como a temperatura, o Tempo de Retención Celular (TRC) e a concentración, a adaptación e o tipo de lodo.

- √ Para algúns compostos, como o SMX, a FLX, o CTL, o NPX, o EE2 e os estróxenos naturais, operar con TRC superiores a 50 d tivo un efecto positivo sobre a eficacia de eliminación en comparación cos resultados obtidos ao traballar con TRC inferiores a 20 d, aínda que esta mellora foi en xeral moderada (máx. 25 %).

- √ De xeito similar, operar con temperaturas máis elevadas (~22ºC en lugar de ~17ºC) conduciu a eficiencias de transformación superiores para algúns PPCPs (CTL, FLX, E1+E2, SMX e ERY) cun incremento máximo na eficacia dun 32%.

- √ Os parámetros que maior influencia exerceron sobre a eliminación de PPCPs foron o tipo de lodo, a súa concentración e/ou o seu grao de adaptación:
  i) O desenvolvemento dunha biomasa nitrificante específica permitiu alcanzar unhas porcentaxes de eliminación de DCF moi importantes (> 70%), a pesar de ser un composto cunha gran resistencia á degradación segundo os resultados obtidos na planta piloto. Este feito revelou que o tipo de lodo pode influenciar o comportamento dalgúns PPCPs. Un efecto similar observouse para o IBP no reactor de biodegradación anóxica, onde as eficiencias de transformación foron aumentando co tempo desde valores inferiores ao 16% ata alcanzar un máximo do 75%.
  ii) Para o NPX na planta piloto e para o DCF no reactor de biodegradación nitrificante, observouse unha clara correlación entre a concentración de lodos no reactor e a eficacia de eliminación alcanzada para estes compostos.
  iii) A aclimatación dos microorganismos á presenza de PPCPs na auga residual puido ser responsable do incremento do 70% na eliminación de NPX na planta piloto.

A **coagulación-floculación e a flotación** poden utilizarse como tratamento primario en estacións depuradoras convencionais, pero tamén para realizar un pre-

tratamento de correntes específicas con caudais inferiores ao das augas residuais municipais, pero con concentracións de PPCPs máis elevadas, como ocorre por exemplo cos efluentes de hospital. Cando estas dúas tecnoloxías se aplican por separado, xeralmente a flotación dá lugar a eliminacións máis baixas de SST e de PPCPs comparadas coas obtidas por coagulación. Con todo, cando estes procesos se aplican secuencialmente (coagulación+flotación), lógrase mellorar a eficiencia global do proceso.

A conclusión xeral á que se chega en canto a eliminación de PPCPs é que algúns compostos como o iopromide (IPM), a CBZ e o DZP non se eliminan da fase líquida, mentres que para outras sustancias como as fragrancias (HHCB, AHTN e ADBI) alcánzanse eficacias de eliminación moi elevadas (> 90%). Entre estes dous extremos sitúanse os resultados obtidos para NPX, IBP e DCF con eficacias de eliminación de ~50%. Para os antibióticos o pre-tratamento non resultou ser unha técnica de depuración eficaz, xa que a concentración de SMX non se viu afectada polo proceso e para os demais antibióticos (ROX, ERY e TMP) esta ata foi maior no efluente que no influente.

Os dous mecanismos principais responsables da interacción dos PPCPs cos sólidos en suspensión foron a absorción e a adsorción. O primeiro, que se basea en interaccións de tipo lipofílico, xogou un papel importante na eliminación de fragrancias, para as cales se obtiveron os mellores resultados no tratamento de correntes con maior contido en graxas. O segundo mecanismo, baseado en interaccións electrostáticas, foi a forza impulsora para a eliminación de compostos iónicos (por exemplo a forma catiónica da CBZ).

Para completar a eliminación dos PPCPs máis persistentes no tratamento convencional de augas residuais ou daqueles cuxa descarga ás canles de auga naturais preocupa de xeito especial ata a concentracións moi baixas, é necesario instalar unha etapa de post-tratamento. A **oxidación con ozono** podería ser unha posibilidade, opción que foi avaliada para dous PPCPs, a FLX e o triclosan (TRI). As especies desprotonadas de cada composto resultaron ser altamente reactivas con O_3, aínda que, debido á gran diferenza de pKa do TRI e da FLX, o TRI reaccionou moito máis rapidamente que a FLX a pH neutro ($t_{1/2,\ TRI}$ = 0.4 ms; $t_{1/2,\ FLX}$ = 17 s, a pH 7 e con $[O_3]_0$ 2 mg/L).

A partir da análise destes parámetros cinéticos conclúese que o O_3 reaccionou co TRI e coa FLX por ataque electrofílico directo o seu anel fenólico e grupo amino, respectivamente.

A partir dos experimentos realizados cos efluentes secundarios de dúas plantas de tratamento de augas residuais que presentaban parámetros de calidade da auga diferentes, deduciuse que as constantes cinéticas determinadas en auga destilada

poden extrapolarse con éxito á ozonización de TRI e de FLX en augas residuais. Xeralmente, os radicais •OH contribuíron en maior medida á oxidación de FLX que á de TRI, debido principalmente á menor reactividade da FLX con O_3. Por conseguinte, a competencia polos radicais •OH exercida pola matriz da auga residual resultou ser especialmente importante para a oxidación de FLX.