UNIVERSIDAD DE SANTIAGO DE COMPOSTELA

Departamento de Ingeniería Química

Combining submerged membrane technology with anaerobic and aerobic wastewater treatment

Memoria presentada por

Alberto Sánchez Sánchez

Para optar al grado de Doctor por la

Universidad de Santiago de Compostela

UNIVERSIDAD DE SANTIAGO DE COMPOSTELA

Departamento de Ingeniería Química

Juan Manuel Garrido Fernández, Profesor Titular del Departamento de Ingeniería Química de la Universidad de Santiago de Compostela,

Informa:

Que la memoria titulada "Combining submerged membrane technology with anaerobic and aerobic wastewater treatment", que para optar al grado de Doctor de Ingeniería Química, Programa de Doctorado en Ingeniería Química y Ambiental, presenta don Alberto Sánchez Sánchez, ha sido realizada bajo mi inmediata dirección en el Departamento de Ingeniería Química de la Universidad de Santiago de Compostela.

Y para que así conste, firma el presente informe en Santiago de Compostela a 19 de marzo de 2013.

Juan Manuel Garrido Fernández

Agradecimientos

A lo largo de estas líneas me gustaría expresar mi gratitud a todas las personas que han hecho posible la realización de esta tesis, así como resaltar desde un punto de vista humano lo que ha supuesto este proceso para mí. El primer lugar de estos agradecimientos tiene que estar reservado a mi tutor Juan Garrido por ser una inagotable fuente de aprendizaje para mí como mentor, pero sobre todo por haberse excedido en sus funciones y haberse convertido en algo más, en un amigo, que me ha apoyado siempre y que ha sabido sacar lo mejor de mí.

Por supuesto, quiero agradecer a todos y cada uno de los profesores por su importantísima labor en la mejora continua de este grupo, a Juan Lema por la dirección del mismo, a los técnicos por su inestimable apoyo y a todos mis compañeros por su esfuerzo, ayuda y respeto mutuo. Especialmente me gustaría mencionar a Moncho Pampín por codirigir parte de mi trabajo, pero sobre todo porque fue con la primera persona que me puse en contacto para entrar al grupo y todo lo que recibí por su parte fueron facilidades y ayuda. También me gustaría hacer una mención especial a Dagmara y así poder agradecerle su importante aportación a esta tesis. Ha sido un auténtico placer poder trabajar contigo codo con codo. En general me gustaría dar la enhorabuena a todos los miembros (incluidos los que ya no están y los que vendrán) del grupo por contribuir a formar un ambiente de trabajo tan agradable y productivo. Me siento muy afortunado de pertenecer a este grupo humano y agradecido por haber tenido la posibilidad de doctorarme en el mismo.

Un pequeña pero importante parte de mi periodo de formación como doctor tuvo lugar en el grupo de Ingeniería Química y de Procesos perteneciente a la Universidad Técnica de Berlín. Por ello, quiero mostrar mi agradecimiento al profesor Matthias Kraume por darme la oportunidad de realizar esta estancia de investigación y muy especialmente a mi amigo Christoph Brand, por ser un perfecto anfitrión, por compartir, bromas, ideas, preocupaciones y trabajo.

Haciendo balance de los últimos siete años me doy cuenta de que la decisión de cursar los estudios de doctorado en el grupo de ingeniería ambiental y bioprocesos de la USC ha sido con total seguridad la mejor decisión que he tomado a lo largo de mi vida, tanto en el plano profesional como en el plano personal. El único aspecto negativo ha sido la gran distancia que me separa de mi familia y amigos en Malpartida de Plasencia. A mis

padres Macario y Marisa, les agradezco su apoyo incondicional y su confianza ciega en mí; a mis hermanos Nuria, Marta y Eduardo, su paciencia y cariño; y a mis queridos sobrinos Alejandro y María, solo les pido disculpas por haberme convertido en “el tío gallego”. Este gallego de adopción nunca estará suficientemente agradecido a Javier, Puri y Pablo por haberme tratado como a un hijo y un hermano desde el primer momento en que me conocieron.

Por último que decirte a ti, Andrea, la causa de todo, mi compañera de viaje, la persona más importante de mi vida, gracias por darme la calma, gracias por tu amor y por tu amistad, gracias por el pasado y por el futuro, gracias por tantas cosas, pero sobre todo gracias por compartir conmigo el tesoro más precioso: Teo y Olivia. A ellos, mi vida, muchísimas gracias por ser como sois y por paralizar mi mundo con cada sonrisa, llanto, palabra o sueño.

Table of contents

Objetivos y resumen

Esta tesis se encuadra en el marco de la depuración de aguas residuales tanto urbanas como industriales. Legislaciones cada vez más restrictivas dan lugar a la necesidad de desarrollar sistemas compactos y eficientes para la eliminación tanto de materia orgánica como de nutrientes. La aplicación de los procesos de filtración de membranas al tratamiento de aguas residuales se origina a finales de los años 60, mediante el uso de módulos de membrana tubulares, usados en procesos de filtración industrial, acoplados externamente a reactores biológicos. Sin embargo durante unos 20 años su uso se limitó al tratamiento de aguas residuales industriales, ya que los altos costes energéticos y de operación hacían inviable su aplicación a la depuración de aguas residuales urbanas. Esta situación cambió a principio de la década de los 90, cuando se desarrollaron módulos de membranas de filtración sumergibles que se pueden introducir directamente en el reactor biológico de lodos activos. Estas membranas (de placa plana y fibra hueca) son más baratas y se aplican en sustitución del clásico proceso de sedimentación secundaria, dando lugar al biorreactor de membranas (BRM). La combinación de la tecnología de filtración con membranas de baja presión y los procesos biológicos para el tratamiento de aguas residuales ha evolucionado dando lugar a diferentes configuraciones y aplicaciones como los reactores de membrana anaerobios o los biorreactores de membranas híbridos de biopelículas. Estos sistemas, con diferentes configuraciones son empleados para la eliminación tanto de materia orgánica como de nutrientes de las aguas residuales industriales o urbanas. Además, la tecnología de membranas sumergidas está siendo también aplicada en la filtración terciaria de efluentes secundarios.

El proceso de filtración terciaria, especialmente filtración en profundidad, ha sido tradicionalmente empleado para la eliminación de sólidos en suspensión de los efluentes secundarios. También son de utilidad para la eliminación de materia particulada y coloidal de los efluentes secundarios decantados, para así incrementar la efectividad de una posible etapa de desinfección ultravioleta o con ozono y garantizar la producción de un agua tratada de gran calidad. Sin embargo, en los últimos años, el uso de sistemas de filtración terciaria con membranas se está convirtiendo en una práctica común. Las membranas de filtración terciaria de baja presión han probado su efectividad para

satisfacer los cada vez más exigentes estándares de calidad tanto para descarga directa como para la reutilización del agua tratada. El uso de sistemas de filtración terciaria con membranas podría ser una elección acertada para la eliminación de sólidos en suspension y microorganismos del agua tratada, pero esta tecnología no permite el tratamiento de contaminantes disueltos (sales y microcontaminates), que deben ser eliminados mediante el uso de otras tecnologías (como la adsorción sobre carbón activo o la ósmosis inversa). Además, la filtración terciaria con membranas esta siendo cada vez más empleada en detrimento de la filtración en profundidad como pre-tratamiento al proceso de ósmosis inversa. En comparación con la filtración en profundidad, el tratamiento con membranas produce un agua con una mejor calidad. Este hecho es de especial consideración cuando el agua tratada quiera ser reutilizada o descargada en un área sensible.

En términos generales, las membranas sumergidas requieren unos costes iniciales y de aireación superiores a los necesarios en la configuración externa. Por contra, los costes de operación y los asociados al consumo energético de las bombas son inferiores, debido a los menores flujos aplicados y a la menor frecuencia de limpiezas químicas. Por este motivo, en el caso del tratamiento de aguas urbanas, la selección entre las configuraciones sumergida y externa parece de algún modo decantada a favor de la primera de ellas. De hecho el uso de membranas sumergidas en aplicaciones municipales representa la práctica totalidad de la superficie de membrana instalada en Europa en la última década. Aunque hoy en día la mayor parte de las aplicaciones comerciales se basan en la configuración de membranas sumergidas debido al menor coste asociado a ellas, la configuración externa sigue siendo comúnmente aplicada en determinados usos industriales y de filtración terciaria.

Las principales ventajas derivadas del empleo de membranas sumergidas combinadas con los diferentes tratamientos biológicos son el elevado control que se obtiene en la edad del fango del reactor biológico, la alta estabilidad del proceso frente a variaciones de carga y temperatura y la obtención de un efluente de alta calidad, susceptible de ser reutilizado. Además, el uso de las membranas permite la retención y por lo tanto el desarrollo de poblaciones de microorganismos con una velocidad de crecimiento extremadamente baja, susceptibles de ser lavados de los sistemas biológicos donde operan, como por ejemplo las bacterias desnitrificantes metanótrofas recientemente descubiertas.

Por el contrario, uno de los principales inconvenientes que tiene la operación de reactores de membrana es el ensuciamiento de la membrana, que disminuye la

permeabilidad de la membrana, limita el flujo y acorta su vida útil, incrementando los costes asociados a este proceso. El ensuciamiento se produce por la deposición sobre la superficie de la membrana o los poros de la misma de compuestos orgánicos e inorgánicos que se adsorben o precipitan en la misma. El ensuciamiento orgánico está ocasionado por compuestos orgánicos de naturaleza coloidal y soluble, así como por el mismo fango. Para evitar el ensuciamiento de las membranas sumergidas se utilizan diversas técnicas de limpieza física o química. El ensuciamiento reversible puede ser contrarrestado mediante medidas físicas como son los periodos de contralavado y/o relajación y el burbujeo de aire (o biogás en ambiente anaerobio) sobre la superficie de la membrana, mientras que el ensuciamiento irreversible es aquel que solo puede ser eliminado mediante una limpieza con reactivos químicos. Por último, el ensuciamiento irrecuperable hace referencia a aquel que no puede ser contrarrestado usando estrategias de limpieza ni físicas ni químicas.

En base a lo anteriormente citado, en la presente tesis se ha estudiado la aplicabilidad de la tecnología de filtración con membranas sumergidas a diferentes sistemas aerobios y anaerobios de tratamiento de aguas residuales tanto industriales como urbanas. El uso de membranas sumergidas para el tratamiento terciario de diferentes efluentes secundarios procedentes de reactores secuenciales discontinuos con biomasas granular y floculenta fue investigado en el Capítulo 3. Posteriormente, se ha estudiado el uso de un biorreactor de membranas en combinación con un reactor metanogénico tipo UASB formando un único sistema integrado o como post-tratamiento del efluente tratado anaeróbicamente (Capítulo 4), prestando especial atención a las posibles causas de ensuciamiento (Capítulo 5) y a la posibilidad de eliminar nitrógeno utilizando el metano disuelto presente en el efluente anaerobio como fuente de carbono (Capítulo 6). Finalmente, fue estudiada la operación de un biorreactor anaerobio de membranas con elevada concentración de biomasa para el tratamiento de aguas residuales industriales, prestando especial atención al ensuciamiento de la membrana y a su posible minimización a través de la adición de carbono activo en polvo (Capítulo 7).

A continuación se detallaran los contenidos de cada uno de los capítulos de la presente tesis.

En el Capítulo 1, se presenta una revisión bibliográfica actualizada de los estudios realizados hasta la fecha sobre la tecnología de membranas sumergidas y su combinación con diferentes sistemas de tratamiento de aguas residuales tanto urbanas como industriales. Se presenta también información relativa a los tipos de membranas comúnmente empleadas en este tipo de aplicaciones así como su introducción y su actual

situación en el mercado. Adicionalmente se hace especial hincapié en la principal desventaja de la operación con membranas, el ensuciamiento de las mismas, identificando los principales tipos, sus causas, posibles indicadores y las medidas necesarias para su minimización.

En el Capítulo 2, se desarrollan los materiales y métodos utilizados en los experimentos realizados a lo largo de la mayor parte de los capítulos posteriores.

En el Capítulo 3, los efluentes de diferentes reactores secuenciales discontinuos con biomasa granular y floculenta fueron tratados con un sistema de filtración terciaria con membranas, permitiendo la completa eliminación de sólidos en suspensión.

Las eficacias globales de eliminación de demanda química de oxígeno (DQO) estuvieron normalmente por encima del 85% en ambos sistemas. Debido a la continua aireación aplicada en las cámaras de filtración terciaria para la minimización del ensuciamiento en la membrana y aportar el oxígeno necesario a la biomasa lavada de los reactores secuenciales discontinuos, dichas cámaras se comportaron como una etapa de tratamiento biológico adicional, causando variaciones en las concentraciones de materia orgánica y nitrógeno. Los módulos de membrana fueron operados con altas concentraciones de biomasa (entre 0.3 y 6.8 $g \cdot L^{-1}$), comparado con los valores típicos referenciados para filtración terciaria, a raíz de las estrategias de operación empleadas. La operación de ambos sistemas (granular y floculento) fue comparada para determinar la influencia del estado de agregación de la biomasa sobre la misma. Ninguna diferencia significativa fue observada entre ambos sistemas en términos de capacidad de tratamiento y permeabilidad de las membranas. Valores de permeabilidad entre 160 y 75 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ con un flujo de operación de 10 $L \cdot m^{-2} \cdot h^{-1}$ fueron observados en ambos sistemas. Además, estos resultados fueron mejores que otros obtenidos previamente por nuestro grupo de investigación usando la misma membrana en un BRM para el tratamiento de agua residual urbana. Los resultados experimentales indicaron que la presencia o no de sólidos suspendidos en el agua residual a tratar afecto mas significativamente el rendimiento de los sistemas que la morfología de la biomasa. La incorporación de agua residual libre de sólidos en suspensión durante uno de los de periodos de operación empeoró significativamente el funcionamiento de las membranas de filtración terciaria en ambos sistemas, disminuyendo su permeabilidad hasta un 40%. Además, otros factores como la nitrificación, la presencia de productos microbianos solubles y la concentración de carbono orgánico disuelto parecieron jugar una función importante en la operación de la membrana de filtración terciaria. Este estudio confirmó la importancia de la fracción de carbohidratos de los productos microbianos solubles como

uno de los parámetros más importantes relacionado con el ensuciamiento de la membrana. Además, la fracción coloidal de las sustancias biopoliméricas (cBPC) fue introducida como posible indicador del ensuciamiento de una membrana debido a la relación observada entre este parámetro y la permeabilidad de la membrana, especialmente a bajas velocidades de carga orgánica (VCO).

En el Capítulo 4 se propuso la combinación de un BRM aerobio y un reactor anaerobio UASB para el tratamiento de aguas residuales de baja carga a temperatura ambiente. El BRM consistió en una etapa aeróbica con biomasa en suspensión y formando biopelículas sobre soportes plásticos y de otra etapa a parte donde se ubicó el módulo de membranas. Ambas tecnologías fueron operadas conjuntamente como un único sistema integrado o como un reactor UASB seguido de un post-tratamiento en un BRM cuando la recirculación entre ellos fue eliminada. Esta combinación puede resultar especialmente interesante para el tratamiento de aguas residuales urbanas o industriales en países de clima cálido.

Las VCO aplicadas variaron entre 0.7 y 3.1 $kgDQO \cdot m^{-3} \cdot d^{-1}$ y las eliminación de DQO estuvo por encima del 95% durante la mayor parte de la operación, de la cual entre un 40 y un 80% tuvo lugar en el reactor anaerobio. Producción de biogás con un contenido en metano alrededor del 80% fue observada durante toda la operación. La producción de biogás fue de aproximadamente 0.15 $m^3_{metano} \cdot kgDQO_{eliminada}^{-1}$ durante los cuatro periodos de operación estudiados. En cuanto a la producción de biomasa, varió entre 0.09 y 0.12 $gSSV \cdot gDQO^{-1}$, lo que es mucho menor que los valores típicos referenciados para BRM aerobios (0.25 - 0.61 $gSSV \cdot gDQO^{-1}$) y cercanos a aquellos observados para el tratamiento anaeróbico, entre 0.11 y 0.14 $gSSV \cdot gDQO^{-1}$. Además, la producción de lodo observada durante los periodos en los que se aplicó recirculación entre el BRM y el reactor UASB (0.09 $gSSV \cdot gDQO^{-1}$) fue mucho menor que en aquellos periodos en los que la recirculación estuvo apagada (0.09 $gSSV \cdot gDQO^{-1}$). Este hecho indicó que una fracción de biomasa generada durante la etapa aerobia en el BRM fue digerida en el reactor UASB, disminuyendo la producción global.

Adicionalmente, el sistema propuesto hizo factible la manipulación de la conversión de nitrógeno a amoníaco y/o nitrato, lo que pudo resultar especialmente interesante para la reutilización del agua tratada en diferentes aplicaciones industriales o para regadío en agricultura. Aunque la eliminación de nitrógeno fue promovida durante parte de la operación gracias a la aplicación de ciclos anóxicos en la primera cámara del BRM, ningún efecto fue observado.

Respecto a la operación de la membrana, permeabilidades alrededor de 150 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ con flujos de operación de 12-15 $L \cdot m^{-2} \cdot h^{-1}$ fueron obtenidas. El mejor rendimiento en la operación de la membrana tuvo lugar cuando la recirculación entre el BRM y el reactor anaerobio UASB estuvo apagada. Las altas eliminaciones de DQO que tuvieron lugar en el reactor anaerobio, especialmente cuando se operó a temperaturas más altas, causaron un déficit de la materia orgánica biodegradable suministrada al BRM. Esta baja VCO aplicada a las etapas aeróbicas (BRM) tuvo un impacto significativo en la concentración de biomasa. Esta concentración de biomasa en la cámara de membrana varió entre 0.5 y 4.0 $g \cdot L^{-1}$, valores más bajos que aquellos típicamente recomendados. Estas bajas concentraciones, causaron la falta de protección de la membrana otorgada por la torta de lodo que se forma sobre ella, llevando a un ensuciamiento irreversible de la misma por la oclusión de sus poros con sustancias biopoliméricas solubles y coloidales. Las velocidades de ensuciamiento observadas fueron un 60% mayor cuando las concentraciones de biomasa fueron más bajas. Por lo tanto, el aporte de una mínima VCO a las etapas aeróbicas (BRM) sería necesario para mantener una concentración de biomasa adecuada y controlar el ensuciamiento de la membrana.

En este sentido, el sistema propuesto podría ser modificado para alimentar una pequeña fracción del agua residual directamente a la etapa aeróbica, para asegurar un suministro mínimo de materia orgánica biodegradable, y así mantener una relación de alimento/microorganismo por encima del valor mínimo típicamente recomendado (0.1 $gDQO \cdot gSSV^{-1} \cdot d^{-1}$).

En el Capítulo 5, se estudió el impacto de la etapa metanogénica sobre el ensuciamiento de la membrana en el sistema propuesto en el Capítulo 4. Flujos de operación entre 11 y 18 $L \cdot m^{-2} \cdot h^{-1}$ y permeabilidades entre 100 y 250 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ fueron observadas. La recirculación de biomasa aeróbica a la etapa anaeróbica llevó al aumento en la concentración de cBPC en el BRM, empeorando el rendimiento de la membrana. Esta misma tendencia fue observada cuando la recirculación entre el BRM y el reactor UASB estuvo apagada pero lodo procedente de una planta de tratamiento de aguas residuales municipales fue externamente alimentado al reactor anaerobio. Experimentos en discontinuo demostraron que la hidrólisis de la biomasa aerobia (sustrato complejo) en condiciones anaerobias provocaron una liberación de sustancias biopoliméricas, aumentando la concentración de todos los indicadores de ensuciamiento estudiados.

Las concentraciones de la fracción de carbohidratos de los productos microbianos solubles, la fracción coloidal de los BPC, y las partículas exopoliméricas transparentes (TEP) fueron estudiadas como posibles indicadores de ensuciamiento de la membrana en

el sistema propuesto, encontrándose una fuerte correlación entre la concentración de cBPC y TEP y la velocidad de ensuciamiento de la membrana, especialmente a concentraciones de biomasa inferiores a 4 $g \cdot L^{-1}$.

La concentración de biomasa fue un parámetro clave debido a su papel protector de la membrana contra el ensuciamiento provocado por las sustancias biopoliméricas solubles y coloidales. Dependiendo de la concentración de biomasa en la cámara de membrana, la presencia de biopolímeros empeoró el rendimiento de la membrana en mayor o menor grado. La velocidad de ensuciamiento resultó ser 3 veces más alta cuando la concentración de biomasa disminuyó de 8 a 2 $g \cdot L^{-1}$, operando con concentraciones similares de biopolímeros en la cámara de membrana. Además, la presencia del soporte plástico en la etapa aeróbica se mostró como un aspecto importante para la mejora del rendimiento de la membrana, fomentando la disminución de la concentración de los indicadores de ensuciamiento estudiados. La observación microscópica mostró una cantidad grande de protozoos ciliados en la biopelícula. Hipotéticamente, la ausencia de estos organismos filtrantes causó el aumento de la concentración de biopolímeros coloidales.

En el Capítulo 6, el mismo sistema empleado en los Capítulos 4 y 5 fue utilizado para estudiar la posible eliminación de nitrógeno en el mismo. El efluente del reactor UASB fue post-tratado en un BRM dotado de una primera cámara anóxica con la finalidad de poder utilizar el metano disuelto como fuente de carbono en el proceso de desnitrificación.

La presencia de metano disuelto, especialmente a bajas temperatura, representa un problema medioambiental importante en términos de emisiones de efecto invernadero de las aguas residuales tratadas en reactores metanogénicos. El metano tiene un potencial de calentamiento global 25 veces más alto que el dióxido de carbono. Para aguas poco cargadas, el metano disuelto puede representar hasta 50% del metano producido. El metano disuelto es fácilmente desorbido de los efluentes, especialmente si estos son directamente descargados o post-tratados en reactores aerobios. Por ello, el uso de tecnología anaeróbica aumenta las emisiones de gases de efecto invernadero asociados con tratamiento de aguas residuales.

Por tanto, el uso de este metano disuelto como fuente de carbono para la desnitrificación biológica propuesta en este capítulo puede ser una alternativa para reducir tanto las emisiones de gases de efecto invernadero como el contenido de nitrógeno del agua residual tratada. Hasta un 60% de eliminación de nitrógeno y un 95% de consumo

de metano fueron observados durante la operación. La eliminación del metano disuelto presente en el efluente del reactor anaerobio llevó a un empeoramiento en la eliminación de nitrógeno. Experimentos discontinuos confirmaron la presencia de microorganismos capaces de desnitrificar utilizando el metano presente como fuente de carbono. El proceso de desnitrificación pareció ser llevada a cabo por un consorcio de bacterias aerobias y anaerobias oxidantes de metano aeróbico y bacterias heterotróficas, que utilizó los productos de oxidación del metano como fuente de carbono para desnitrificar. Sin embargo, la velocidad de oxidación de metano fue mucho mayor que la predicha teóricamente según la estequiometria del proceso de desnitrificación con metano, tanto en condiciones microaerobias como anaerobias. La relación de recirculación interna en el BRM (entre las cámaras aerobia y anóxica) y la presencia o ausencia de metano disuelto fueron revelados como los parámetros claves en el desarrollo del proceso de desnitrificación. El porcentaje de eliminación de metano disminuyó del 60% al 27% cuando el metano disuelto fue desorbido del efluente del reactor UASB. Por otra parte, a altas relaciones de recirculación, la oxidación anaerobia de metano pareció ser inhibida, disminuyendo la velocidad de consumo de metano más de un 50%. Esta inhibición fue debida a la entrada de oxígeno a la cámara anóxica. Este hecho confirmó los resultados obtenidos en Capítulo 4, cuándo la aplicación de ciclos de aerobia/anoxia no estimularon el proceso de desnitrificación.

La posible influencia del proceso de desnitrificación con metano en la operación de la membrana también fue estudiada, mostrándose un aumento significativo en la concentración de biopolímeros coloidales cuando el proceso de desnitrificación se vio afectado por la eliminación del metano disuelto del efluente anaerobio. Este efecto es similar al que se observa cuando el proceso de nitrificación es afectado.

En el Capítulo 7 se estudió la operación de un biorreactor anaerobio de membranas de tanque agitado para el tratamiento de aguas residuales industriales procedentes del proceso de extracción de aceites esenciales del romero.

La complejidad y baja biodegradabilidad de esta agua residual llevó a una operación con una elevada concentración de biomasa en el reactor. En este sentido, la relación entre la concentración de biomasa y el rendimiento de la membrana no ha sido extensamente investigada y la información con respecto a la operación de BRM anaerobios operados a altas concentraciones de biomasa es muy limitada. Los flujos alcanzados durante este estudio variaron entre 1 y 2.5 $L \cdot m^{-2} \cdot h^{-1}$, trabajando con concentraciones de biomasa entre 38 y 61 $g \cdot L^{-1}$. A pesar de que estos valores son similares a los obtenidos en otros trabajos con BRM anaerobios operados con

concentraciones de biomasa por encima de 30 $g \cdot L^{-1}$, la posibilidad de mejorar el rendimiento de la membrana mediante la adición de carbón activo en polvo fue también evaluada. Experimentos en discontinuo con diferentes tipos de carbón activo fueron llevados a cabo, y una dosificación óptima de 1.5 $g \cdot L^{-1}$ fue determinada.

El sistema operó establemente sin control de alcalinidad con un tiempo de retención hidráulico de hasta 4 días para una concentración de DQO en la alimentación de 8 $g \cdot L^{-1}$ resultando en una VCO de entre 2 y 3 $kgDQO \cdot m^{-3} \cdot d^{-1}$ y logrando eficacias de eliminación de DQO de hasta el 60%. No obstante, la concentración de ácidos grasos volátiles fue extremadamente alta durante toda la operación, indicando alguna clase de inhibición del proceso metanogénico, probablemente relacionada con las propiedades antibacterianas de los extractos del romero. Este hecho podría tener un efecto nocivo sobre el proceso biológico anaeróbico, causando la destabilization de las poblaciones microbianas y llevando a la acumulación de ácidos grasos volátiles y la acidificación del reactor. El control de la alcalinidad mediante la adición en continuo de $NaHCO_3$ fue una medida clave para la mejora de las eficacias de eliminación de DQO hasta el 70%, trabajando con VCO de hasta 5.0 $kgDQO \cdot m^{-3} \cdot d^{-1}$. Una producción de biogás de 0.3 $m^3_{metano} \cdot kgDQO_{eliminada}^{-1}$ con una concentración de metano de aproximadamente 60% fue observada.

Adicionalmente, las concentraciones de los típicos indicadores de ensuciamiento para BRM aerobios previamente mencionados (cBPC y TEP) fueron medidas durante la operación. Además, las propiedades de filtrabilidad del lodo fueron monitorizadas y analizadas en profundidad con el objeto de examinar la posible mejora de las mismas tras la adición de carbón activo en el reactor. Tanto las concentraciones de las sustancias biopoliméricas como las resistencias a la filtración del lodo medidas fueron extremadamente elevadas y la adición de carbón activo no contribuyó a la mejora de ellas. El ensuciamiento de la membrana estuvo gobernado por las condiciones hidrodinámicas derivadas del alto contenido de sólidos en el reactor. Ya que la alta concentración de biomasa no mejoró sustancialmente la eliminación de materia orgánica, una disminución de la misma a valores por debajo de los 20 $g \cdot L^{-1}$ podría llevar al aumento del flujo de permeado, especialmente al añadir carbón activo en estas nuevas condiciones como es sugerido en la bibliografía.

Con los trabajos realizados en esta tesis se ha conseguido aportar una información relevante para la operación de sistemas de filtración con membranas sumergidas y su aplicación en combinación con otros sistemas anaerobios y aerobios de tratamiento de aguas residuales.

Obxectivos e resumo

Esta tese encádrase no marco da depuración de augas residuais tanto urbanas coma industriais. Lexislacións cada vez máis restritivas dan lugar á necesidade de desenvolver sistemas compactos e eficientes para a eliminación tanto de materia orgánica coma de nutrientes. A aplicación dos procesos de filtración de membranas ao tratamento de augas residuais orixínase a finais dos anos 60, mediante o uso de módulos de membrana tubulares, usados en procesos de filtración industrial, adaptados externamente a reactores biolóxicos. Non obstante durante uns 20 anos o seu uso limitouse ao tratamento de augas residuais industriais, xa que os altos custos enerxéticos e de operación facían inviable a súa aplicación á depuración de augas residuais urbanas. Esta situación cambiou a principio da década dos 90, cando se desenvolveron módulos de membranas de filtración somerxibles que se poden introducir directamente no reactor biolóxico de lamas activas. Estas membranas (de placa plana e fibra oca) son máis baratas e aplícanse en substitución do clásico proceso de sedimentación secundaria, dando lugar ao biorreactor de membranas (BRM). A combinación da tecnoloxía de filtración con membranas de baixa presión e os procesos biolóxicos para o tratamento de augas residuais evolucionou dando lugar a diferentes configuracións e aplicacións como os reactores de membrana anaerobios ou os biorreactores de membranas híbridos de biopelículas. Estes sistemas, con diferentes configuracións son empregados para a eliminación tanto de materia orgánica coma de nutrientes das augas residuais industriais ou urbanas. Ademáis, a tecnoloxía de membranas somerxidas está sendo tamén aplicada na filtración terciaria de efluentes secundarios.

O proceso de filtración terciaria, especialmente filtración en profundidade, foi tradicionalmente empregado para a eliminación de sólidos en suspensión dos efluentes secundarios. Tamén son de utilidade para a eliminación de material particulado e coloidal dos efluentes secundarios decantados, para así incrementar a efectividade dunha posible etapa de desinfección ultravioleta ou con ozono e garantir a produción da agua tratada de gran calidade. Non obstante, nos últimos anos, o uso de sistemas de filtración terciaria con membranas estase a converter nunha práctica común. As membranas de filtración terciaria de baixa presión probaron a súa efectividade para satisfacer os cada vez máis esixentes estándares de calidade tanto para descarga directa coma para a reutilización da

auga tratada. O uso de sistemas de filtración terciaria con membranas podería ser unha elección axeitada para a eliminación de sólidos en suspensión e microorganismos da agua tratada, pero esta tecnoloxía non permite o tratamento de contaminantes disoltos (sales e microcontaminates), que deben ser eliminados mediante o uso de outras tecnoloxías (como a adsorción sobre carbón activo ou a ósmose inversa). Ademáis, a filtración terciaria con membranas esta sendo cada vez máis empregada en detrimento da filtración en profundidade como pre-tratamento ao proceso de ósmose inversa. En comparación coa filtración en profundidade, o tratamento con membranas produce unha auga cunha mellor calidade. Este feito é de especial consideración cando a auga tratada queira ser reutilizada ou descargada nunha área sensible.

En termos xerais, as membranas somerxidas requiren uns custos iniciais e de aireación superiores aos necesarios na configuración externa. En contraste, os custos de operación e os asociados ao consumo enerxético das bombas son inferiores, debido aos menores fluxos aplicados e á menor frecuencia de limpezas químicas. Por este motivo, no caso do tratamento de augas urbanas, a selección entre as configuracións somerxidas e externa parece dalgún modo decantada a favor da primeira delas. De feito, o uso de membranas somerxidas en aplicacións municipais representa a práctica totalidade da superficie de membrana instalada en Europa na última década. Aínda que hoxe en día, a maior parte das aplicacións comerciais baséanse na configuración de membranas somerxidas debido ao menor custo asociado a elas, a configuración externa segue sendo comunmente empregada en determinadas usos industriais e de filtración terciaria,

As principais vantaxes derivadas do emprego de membranas somerxidas combinadas cos diferentes tratamentos biolóxicos son o elevado control que se obtén na idade do lama do reactor biolóxico, a alta estabilidade do proceso fronte a variacións de carga e temperatura e a obtención dun efluente de alta calidade, susceptible de ser reutilizado. Ademais, o uso das membranas permite a retención e polo tanto o desenvolvemento de poboacións de microorganismos cunha velocidade de crecemento extremadamente baixa, susceptibles de ser lavados dos sistemas biolóxicos onde operan, como por exemplo as bacterias desnitrificantes metanótrofas recentemente descubertas.

Pola contra, un dos principais inconvenientes que ten a operación de reactores de membrana é o ensuciamento da membrana, que diminúe a permeabilidade da membrana, limita o fluxo e acurta a súa vida útil, incrementando os custos asociados a este proceso. O ensuciamento prodúcese pola deposición sobre a superficie da membrana ou os poros desta de compostos orgánicos e inorgánicos que se absorben ou precipitan nesta. O ensuciamento orgánico está ocasionado por compostos orgánicos de natureza coloidal e

soluble, así como pola mesma lama. Para evitar o ensuciamento das membranas somerxidas utilízanse diversas técnicas de limpeza física ou química. O ensuciamento reversible pode ser contrarrestado mediante medidas físicas como son os períodos de contralavado e/ou relaxación e o burbullo de aire (ou biogás en ambiente anaerobio) sobre a superficie da membrana, mentres que o ensuciamento irreversible é aquel que só pode ser eliminado mediante unha limpeza con reactivos químicos. Por último, o ensuzamento irrecuperable fai referencia a aquel que non pode ser contrarrestado empregando estratexias de limpeza nin físicas nin químicas.

Sobre a base do anteriormente citado, na presente tese estudouse a aplicabilidade da tecnoloxía de filtración con membranas somerxidas a diferentes sistemas aerobios e anaerobios de tratamento de augas residuais tanto industriais coma urbanas. O uso de membranas somerxidas para o tratamento terciario de diferentes efluentes secundarios procedentes de reactores secuenciais descontinuos con biomasas granular e floculenta foi estudado no Capítulo 3. Posteriormente, investigouse o uso dun biorreactor de membrana en combinación cun reactor metanogénico tipo UASB formando un único sistema integrado ou como post-tratamento do efluente tratado anaeróbicamente (Capítulo 4), prestando especial atención ás posibles causas de ensuciamento (Capítulo 5) e á posibilidade de eliminar nitróxeno utilizando o metano disolto presente no efluente anaerobio como fonte de carbono (Capítulo 6). Finalmente, foi estudada a operación dun biorreactor anaerobio de membranas con elevada concentración de biomasa para o tratamento de augas residuais industriais, prestando especial atención ao ensuciamento da membrana e á súa posible minimización a través da adición de carbono activo en po (Capítulo 7).

A continuación detallásense os contidos de cada un dos capítulos da presente tese.

No Capítulo 1, preséntase unha revisión bibliográfica actualizada dos estudos realizados ata a data sobre a tecnoloxía de membranas somerxidas e a súa combinación con diferentes sistemas de tratamento de augas residuais tanto urbanas coma industriais. Preséntase tamén información relativa aos tipos de membranas comunmente empregadas neste tipo de aplicacións así como a súa introdución e a súa actual situación no mercado. Adicionalmente faise especial fincapé na principal desvantaxe da operación con membranas, o ensuciamento destas, identificando os principais tipos, as súas causas, posibles indicadores e as medidas necesarias para a súa minimización.

No Capítulo 2, desenvólvense os materiais e métodos empregados nos experimentos realizados ao longo da maior parte dos capítulos posteriores.

No Capítulo 3, os efluentes de diferentes reactores secuenciais descontinuos con biomasa granular e floculenta foron tratados cun sistema de filtración terciaria con membranas, permitindo a completa eliminación de sólidos en suspensión.

As eficacias globais de eliminación de demanda química de osíxeno (DQO) estiveron normalmente por enriba do 85% en ámbolos dous sistemas. Debido á continua aireación aplicada nas cámaras de filtración terciaria para a minimización do ensuciamento na membrana e achegar o osíxeno necesario á biomasa lavada dos reactores secuenciais descontinuos, as devanditas cámaras comportáronse como unha etapa de tratamento biolóxico adicional, causando variacións nas concentracións de materia orgánica e nitróxeno. Os módulos de membrana foron operados con altas concentracións de biomasa (entre 0.3 e 6.8 $g \cdot L^{-1}$), comparado cos valores típicos referenciados para filtración terciaria, a raíz das estratexias de operación empregadas. A operación de ámbolos dous sistemas (granular e floculento) foi comparada para determinar a influenza do estado de agregación da biomasa sobre esta. Ningunha diferenza significativa foi observada entre ámbolos dous sistemas en termos de capacidade de tratamento e permeabilidade das membranas. Valores de permeabilidade entre 160 e 75 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ cun fluxo de operación de 10 $L \cdot m^{-2} \cdot h^{-1}$ foron observados en ambos sistemas. Ademáis, estes resultados foron mellores que outros obtidos previamente polo noso grupo de investigación usando a mesma membrana nun BRM para o tratamento de auga residual urbana. Os resultados experimentais indicaron que a presenza ou non de sólidos suspendidos na auga residual a tratar afectou mais significativamente ao rendemento dos sistemas que á morfoloxía da biomasa. A incorporación de auga residual libre de sólidos en suspensión durante un dos de períodos de operación empeorou significativamente o funcionamento das membranas de filtración terciaria en ámbolos dous sistemas, diminuíndo a súa permeabilidade ata un 40%. Ademais, outros factores como a nitrificación, a presenza de produtos microbianos solubles e a concentración de carbono orgánico disolto pareceron xogar unha función importante na operación da membrana de filtración terciaria. Este estudo confirmou a importancia da fracción de carbohidratos dos produtos microbianos solubles como un dos parámetros máis importantes relacionado co ensuciamento da membrana. Ademais, a fracción coloidal das substancias biopoliméricas (cBPC) foi introducida como posible indicador do ensuciamento dunha membrana debido á relación observada entre este parámetro e a permeabilidade da membrana, especialmente a baixas velocidades de carga orgánica (VCO).

No Capítulo 4 propúxose a combinación dun BRM aerobio e un reactor anaerobio UASB para o tratamento de augas residuais de baixa carga a temperatura ambiente. O BRM consistiu nunha etapa aeróbica con biomasa en suspensión e formando biopelículas sobre soportes plásticos e doutra etapa a parte onde se situou o módulo de membranas. Ámbalas dúas tecnoloxías foron operadas conxuntamente como un único sistema integrado ou como un reactor UASB seguido dun post-tratamento nun BRM cando a recirculación entre eles foi eliminada. Esta combinación pode resultar especialmente interesante para o tratamento de augas residuais urbanas ou industriais en países de clima cálido.

As VCO aplicadas variaron entre 0.7 e 3.1 $kgDQO \cdot m^{-3} \cdot d^{-1}$ e a eliminación de DQO estivo por enriba do 95% durante a maior parte da operación, da cal entre un 40 e un 80% tivo lugar no reactor anaerobio. A produción de biogás cun contido en metano arredor do 80% foi observada durante toda a operación. A produción de biogás foi de aproximadamente 0.15 $m^3_{metano} \cdot kgDQO_{eliminada}^{-1}$ durante os catro períodos de operación estudados. En canto á produción de biomasa, variou entre 0.09 e 0.12 $gSSV \cdot gDQO^{-1}$, o que é moito menor que os valores típicos referenciados para BRM aerobios (0.25 -0.61 $gSSV \cdot gDQO^{-1}$) e próximos a aqueles observados para o tratamento anaeróbico, entre 0.11 e 0.14 $gSSV \cdot gDQO^{-1}$. Ademáis, a produción de lama observada durante os periodos nos que se aplicou recirculación entre o BRM e o reactor UASB (0.09 $gSSV \cdot gDQO^{-1}$) foi moito menor que naqueles periodos nos que a recirculación estivo apagada (0.09 $gSSV \cdot gDQO^{-1}$). Este feito indicou que unha fracción de biomasa xerada durante a etapa aerobia no BRM foi dixerida nun reactor UASB, disminuíndo a produción global.

Adicionalmente, o sistema proposto fixo factible a manipulación da conversión de nitróxeno a amoníaco e/ou nitrato, o que resultou especialmente interesante para a reutilización da auga tratada en diferentes aplicacións industriais ou en agricultura. Aínda, a eliminación de nitróxeno foi promovida durante parte da operación grazas á aplicación de ciclos anóxicos na primeira cámara do BRM, que ningún efecto foi observado.

Con respecto á operación da membrana, foron obtidas permeabilidades ao redor de 150 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ con fluxos de operación de 12-15 $L \cdot m^{-2} \cdot h^{-1}$. O mellor rendemento na operación da membrana tivo lugar cando a recirculación entre o BRM e o reactor anaerobio UASB estivo apagada. As altas eliminacións de DQO que tiveron lugar no reactor anaerobio, especialmente cando se operou a temperaturas máis altas, causaron un déficit da materia orgánica biodegradable subministrada ao BRM. Esta baixa VCO aplicada ás etapas aeróbicas (BRM) tivo un impacto significativo na concentración de biomasa. Esta concentración de biomasa na cámara de membrana variou entre 0.5 e 4.0

$g \cdot L^{-1}$, valores máis baixos que aqueles tipicamente recomendados. Estas baixas concentracións, causaron a falta de protección da membrana outorgada pola torta de lama que se forma sobre ela, levando a un ensuciamento irreversible desta pola oclusión dos seus poros con substancias biopoliméricas solubles e coloidais. As velocidades de ensuciamento observadas foron un 60% maior cando as concentracións de biomasa foron máis baixas. Polo tanto, a achega dunha mínima VCO ás etapas aeróbicas (BRM) sería necesaria para manter unha concentración de biomasa axeitada e controlar o ensuciamento da membrana. Neste sentido, o sistema proposto podería ser modificado para alimentar unha pequena fracción da auga residual directamente á etapa aeróbica, para asegurar unha subministración mínima de materia orgánica biodegradable, e así manter unha relación de alimento/microorganismo por enriba do valor mínimo tipicamente recomendado (0.1 $gDQO \cdot gSSV^{-1} \cdot d^{-1}$).

No Capítulo 5, estudouse o impacto da etapa metanoxénica sobre o ensuciamento da membrana no sistema proposto no Capítulo 4. Observáronse fluxos de operación entre 11 e 18 $L \cdot m^{-2} \cdot h^{-1}$ e permeabilidades entre 100 e 250 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$. A recirculación de biomasa aeróbica á etapa anaeróbica levou ao aumento na concentración de cBPC no BRM, empeorando o rendemento da membrana.

Esta mesma tendencia foi observada cando a recirculación entre o BRM e o reactor UASB estivo apagada pero a lama procedente dunha planta de tratamento de augas residuais municipais foi externamente alimentada ao reactor anaerobio. Experimentos en descontinuo demostraron que a hidrólise da biomasa aerobia (substrato complexo) en condicións anaerobias provocaron unha liberación de substancias biopoliméricas, aumentando a concentración de tódolos indicadores de ensuciamento estudados.

As concentracións da fracción de carbohidratos dos produtos microbianos solubles, a fracción coloidal dos BPC, e as partículas exopoliméricas transparentes (TEP) foron estudadas como posibles indicadores de ensuciamento da membrana no sistema proposto, encontrándose unha forte correlación entre a concentración de cBPC e TEP e a velocidade de ensuciamento da membrana, especialmente a concentracións de biomasa inferiores a 4 $g \cdot L^{-1}$.

A concentración de biomasa foi un parámetro clave debido ao seu papel protector da membrana contra o ensuciamento provocado polas substancias biopoliméricas solubles e coloidais. Dependendo da concentración de biomasa na cámara de membrana, a presenza de biopolímeros empeorou o rendemento da membrana en maior ou menor grao. A velocidade de ensuciamento resultou ser 3 veces máis alta cando a concentración

de biomasa diminuíu de 8 a 2 $g \cdot L^{-1}$, operando con concentracións similares de biopolímeros na cámara de membrana. Ademais, a presenza do soporte plástico na etapa aeróbica mostrouse como un aspecto importante para a mellora do rendemento da membrana, fomentando a diminución da concentración dos indicadores de ensuciamento estudados. A observación microscópica mostrou unha cantidade grande de protozoos ciliados na biopelícula. Hipoteticamente, a ausencia destes organismos filtrantes causou o aumento da concentración de biopolímeros coloidais.

No Capítulo 6, o mesmo sistema empregado nos Capítulos 4 e 5 foi utilizado para estudar a posible eliminación de nitróxeno neste. O efluente do reactor UASB foi post-tratado nun BRM dotado dunha primeira cámara anóxica coa finalidade de poder utilizar o metano disolto como fonte de carbono no proceso de desnitrificación.

A presenza de metano disolto, especialmente a baixas temperaturas, representa un problema ambiental importante en termos de emisións de efecto invernadoiro das augas residuais tratadas en reactores metanoxénicos. O metano ten un potencial de aquecemento global 25 veces máis alto que o dióxido de carbono. Para augas pouco cargadas, o metano disolto pode representar ata 50% do metano producido. O metano disolto é doadamente desorbido dos efluentes, especialmente se estes son directamente descargados ou post-tratados en reactores aerobios. Por iso o uso de tecnoloxía anaeróbica aumenta as emisións de gases de efecto invernadoiro asociadas con tratamento de augas residuais.

Polo tanto, o uso deste metano disolto como fonte de carbono para a desnitrificación biolóxica proposta neste capítulo pode ser unha alternativa para reducir tanto as emisións de gases de efecto invernadoiro como o contido de nitróxeno da auga residual tratada. Ata un 60% de eliminación de nitróxeno e un 95% de consumo de metano foron observados durante a operación. A eliminación do metano disolto presente no efluente do reactor anaerobio levou a un empeoramento na eliminación de nitróxeno. Experimentos descontinuos confirmaron a presenza de microorganismos capaces de desnitrificar utilizando o metano presente como fonte de carbono. O proceso de desnitrificación pareceu ser levado a cabo por un consorcio de bacterias aerobias e anaerobias oxidantes de metano aeróbico e bacterias heterótrofas, que utilizou os produtos de oxidación do metano como fonte de carbono para desnitrificar. Sen embargo, a velocidade de oxidación de metano foi moito maior que a predita teóricamente segundo a estequiometria do proceso de desnitrificación con metano, tanto en condicións microaerobias como anaerobias. A relación de recirculación interna no BRM (entre as cámaras aerobia e anóxica) e a presenza ou ausencia de metano disolto foron revelados como os

parámetros claves no desenvolvemento do proceso de desnitrificación. A porcentaxe de eliminación de metano diminuíu do 60% ao 27% cando o metano disolto foi desorbido do efluente do reactor UASB. Por outra parte, a altas relacións de recirculación, a oxidación anaerobia de metano pareceu ser inhibida, diminuíndo a velocidade de consumo de metano máis dun 50%. Esta inhibición foi debida á entrada de osíxeno á cámara anóxica. Este feito confirmou os resultados obtidos en Capítulo 4, cando a aplicación de ciclos de aerobia/anoxia non estimularon o proceso de desnitrificación.

A posible influencia do proceso de desnitrificación con metano na operación da membrana tamén foi estudada, amosándose un aumento significativo na concentración de biopolímeros coloidais cando o proceso de desnitrificación se viu afectado pola eliminación do metano disolto do efluente anaerobio. Este feito é similar ao que se observa cando o proceso de nitrificación é afectado.

No Capítulo 7 foi estudada a operación dun biorreactor anaerobio de membranas de tanque axitado para o tratamento de augas residuais industriais procedentes do proceso de extracción de aceites esenciais do romeiro. A complexidade e baixa biodegradabilidade desta auga residual levou a unha operación cunha elevada concentración de biomasa no reactor. Neste sentido, a relación entre a concentración de biomasa e o rendemento da membrana non foi extensamente investigada e a información con respecto á operación de BRM anaerobios operados a altas concentracións de biomasa é moi limitada. Os fluxos alcanzados durante este estudo variaron entre 1 e 2.5 $L \cdot m^{-2} \cdot h^{-1}$, traballando con concentracións de biomasa entre 38 e 61 $g \cdot L^{-1}$. A pesar de que estes valores son similares aos obtidos noutros traballos con BRM anaerobios operados con concentracións de biomasa por enriba de 30 $g \cdot L^{-1}$, a posibilidade de mellorar o rendemento da membrana mediante a adición de carbón activo en po foi tamén avaliada. Experimentos en descontinuo con diferentes tipos de carbón activo foron levados a cabo, e unha dosificación óptima de 1.5 $g \cdot L^{-1}$ foi determinada.

O sistema operou establemente sen control de alcalinidade cun tempo de retención hidráulico de ata 4 días para unha concentración de DQO na alimentación de 8 $g \cdot L^{-1}$ resultando nunha VCO de entre 2 e 3 $kgDQO \cdot m^{-3} \cdot d^{-1}$ e logrando eficacias de eliminación de DQO de ata o 60%. Non obstante, a concentración de ácidos graxos volátiles foi extremadamente alta durante toda a operación, indicando algunha clase de inhibición do proceso metanoxénico, probablemente relacionada coas propiedades antibacterianas do extractos de romeu. Este feito podería ter un efecto nocivo sobre o proceso biolóxico anaeróbico, causando a desestabilización das poboacións microbianas e levando á acumulación de ácidos graxos volátiles e á acidificación do reactor. O control da

alcalinidade mediante a adición en continuo de $NaHCO_3$ foi unha medida clave para a mellora das eficacias de eliminación de DQO ata o 70%, traballando con VCO de ata 5.0 $kgDQO \cdot m^{-3} \cdot d^{-1}$. Observouse unha produción de biogás de 0.3 $m^3_{metano} \cdot kgDQO_{eliminada}^{-1}$ cunha concentración de metano de aproximadamente 60%.

Adicionalmente, al longo da operación medíronse as concentracións dos típicos indicadores de ensuciamento para BRM aerobios previamente mencionados (cBPC e TEP). Ademais, as propiedades de filtrabilidade da lama foron monitorizadas e analizadas en profundidade co obxecto de examinar a posible mellora destas trala adición de carbón activo no reactor. Tanto as concentracións das substancias biopoliméricas coma as resistencias á filtración da lama medidas foron extremadamente elevadas e a adición de carbón activo non contribuíu á mellora delas. O ensuciamento da membrana estivo gobernado polas condicións hidrodinámicas derivadas do alto contido de sólidos no reactor. Xa que a alta concentración de biomasa non mellorou substancialmente a eliminación de materia orgánica, unha diminución desta a valores por debaixo dos 20 $g \cdot L^{-1}$ podería levar ao aumento do fluxo de permeado, especialmente ao engadir carbón activo nestas novas condicións como é suxerido na bibliografía.

Cos traballos realizados nesta tese conseguiuse achegar unha información relevante para a operación de sistemas de filtración con membranas somerxidas e a súa aplicación en combinación con outros sistemas anaerobios e aerobios de tratamento de augas residuais.

Objectives and summary

This thesis is framed in the field of industrial and municipal wastewater treatment. Increasingly strict legislations lead to the need of developing compact and efficient systems for the removal of both organic matter and nutrients. The application of membrane filtration processes to wastewater treatment was originated in the late sixties, through the use of tubular membrane modules in external (side-stream) configuration with biological reactors for the treatment of industrial wastewaters. However during the next 20 years the use of membranes was limited to the treatment of industrial wastewaters, since the high energy and operational costs made unfeasible its application for the treatment of municipal wastewaters. This situation changed in the early nineties, when submerged membrane modules were developed. These modules can be directly placed in the mixed liquor of the biological reactor and the membranes (flat sheet and hollow fiber), which are cheaper, are applied in replacement of secondary settlers, resulting in the so-called membrane bioreactor (MBR). The combination of low-pressure membrane filtration technology with biological processes for the treatment wastewaters has evolved, resulting in different configurations and applications such as, anaerobic membrane bioreactors (AnMBR) or hybrid biofilm membrane bioreactors. These systems, with different configurations are employed for the removal of organic matter and nutrients in both industrial and municipal wastewaters. Moreover, submerged membrane technology is also being applied for tertiary filtration of secondary effluents.

Tertiary filtration, especially depth filtration, has been traditionally used to remove suspended solids from secondary treated waters. They can also be used to remove particulate and colloidal matter from settled secondary effluents, which increases the effectiveness of disinfection with either ultraviolet radiation or ozone and guarantees the production of higher quality reclaimed water. However, in recent years, the use of tertiary membrane filtration systems is becoming more common. Low-pressure tertiary membranes have been proved to meet increasingly stringent standards for discharge or reuse. The use of TMF could be the right choice for removing suspended solids or microorganism of the treated water, but this technology is unable to manage dissolved pollutant (salts and micropollutants) that should be treated using other technologies (adsorption using activated carbon, reverse osmosis). Moreover, TMF is being more and

more used instead depth filtration as pre-treatment step for the reverse osmosis process. Compared to depth filtration, tertiary membrane treatments produce water of better quality. This should be taken into account when water will be reused or discharged into sensitive areas.

In general, submerged MBR require higher aeration and initial investment costs, with respect to side-stream membrane configurations. In contrast, pumping and operating costs are lower, requiring lower operating flows and cleaning frequencies. Thus, in the case of sewage treatment, the selection between submerged and external configurations for aerobic MBRs seems somehow settled, in favour of submerged MBRs. In fact, submerged membrane systems in municipal applications, represent in practice the totality of the installed membrane surface in Europe during the last decade. Although, nowadays most of the commercial applications are based on the submerged configuration, due to lower associated energy requirements, external configuration is still commonly used for certain industrial applications as well as for tertiary filtration treatment.

The main advantages of the employment of submerged membranes in combination with biological wastewater treatments are the total control of sludge retention time, the high stability of the process against peak loads and temperature and the high quality of the obtained effluent, which enable water reuse. Besides, the use of the membranes allows the complete retention and development of extremely slow-growth bacteria, such as newly discovered denitrifying methanotrophs, avoiding its wash-out from the biological systems.

On the contrary, membrane fouling is one of the main drawbacks associated with the application of membrane technology for wastewater treatment. Fouling decreases the permeability of a membrane, limits flux and shortens the life of membrane modules, thus increasing both the capital and the operating costs of filtration systems. Membrane fouling is caused by the deposition of organic and inorganic substances on the membrane surface or within the pores. Organic fouling is mainly caused by colloidal and soluble organic matter as well as by the sludge itself, which forms the so-called sludge cake layer.

Different strategies can be adopted in order to minimize membrane fouling. Reversible fouling can be counteracted by physical means such as backwashing or relaxation and air scouring (biogas in the case of AnMBR), whereas irreversible fouling can only be removed by chemical cleaning. Finally, irrecoverable fouling refers to the phenomena which cannot be recovered using either physical or chemical cleaning strategies.

On the basis of all the aforementioned, in the present thesis applicability of submerged membrane technology to different anaerobic and aerobic systems, for the treatment of municipal and industrial wastewaters, was studied. The use of submerged membranes for the tertiary treatment of different secondary effluents from sequential batch reactors with granular and flocculent biomasses was studied in Chapter 3. Later, the combination of an MBR with an anaerobic UASB reactor into one single integrated system or as a post-treatment of the anaerobically treated effluent was investigated (Chapter 4), paying special attention to the possible causes responsible for membrane fouling (Chapter 5) and to the feasibility of nitrogen removal by using the dissolved methane present in the anaerobic effluent as carbon source for denitrification (Chapter 6). Finally, the operation of an AnMBR with high biomass concentration for the treatment of industrial herbal extraction wastewater was studied, paying special attention to membrane fouling and to its possible minimization through the addition of powdered activated carbon (PAC) (Chapter 7).

The main content of each chapter of the present thesis will be detailed in the following sections.

In Chapter 1 an actualized literature review about the studies performed up to date in the field of submerged membranes and its combination with different wastewater treatment systems is presented. Information regarding membrane types and configurations commonly used in wastewater treatment applications as well as its introduction and current status in the market is also presented. In addition, special attention is paid to the knowledge of the membrane fouling phenomena, its causes, possible indicators and strategies for its minimization.

In Chapter 2, the material and methods used during the different experiments performed along most of the experimental chapters are described.

In Chapter 3, effluents from a flocculent biomass SBR (F-SBR) and a granular biomass SBR (G-SBR) were treated in tertiary membrane filtration chambers to remove suspended solids. Overall COD removal efficiencies were normally above 85% in both of systems. Since the tertiary filtration chambers were continuously aerated to reduce membrane fouling and to provide oxygen to the washed-out biomass, these chambers acted as a biological polishing stage and caused variations in the COD and nitrogen concentrations. In this sense, the tertiary membrane modules were operated with high biomass concentrations (between 0.3-6.8 $g \cdot L^{-1}$), compared with typical values reported for tertiary membrane filtration, as a result of the operating strategies of the filtration systems. The performances of the operating systems were compared to determine the influence of

the state of the biomass on the filtration process. No significant differences were observed between the two tertiary filtration systems in terms of capacity and permeability. Permeability values between 160 and 75 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ were observed in the two tertiary membrane filtration systems at an operating flux of 10 $L \cdot m^{-2} \cdot h^{-1}$. Moreover, these results were better than those obtained previously by our research group, using this membrane in a MBR treating sewage. The experimental results indicated that the presence of suspended solids in the influent affected more significantly membrane performance than the morphology of the aggregated biomass. The incorporation of wastewater free of suspended solids during one of the operating periods significantly worsened operation of the tertiary membrane filtration systems, decreasing permeability by up to 40 percent in both systems. Additionally, other factors such as nitrification, the presence of soluble microbial products and the concentration of dissolved organic carbon seem to play an important role in tertiary membrane filtration. This study confirmed the importance of the carbohydrate fraction of SMP as one of the most important parameters related to membrane fouling. Moreover, the colloidal fraction of biopolymer clusters is introduced as a possible fouling indicator. A certain trend between cBPC concentration and permeability, especially at a constant OLR, was observed.

In Chapter 4, the combination of UASB reactor and aerobic MBR process for the treatment of low-strength wastewaters at ambient temperature was proposed. The aerobic MBR consisted in an aerobic stage with biomass growing both on suspended carriers and in suspension and a separate chamber with a membrane filtration module. Both technologies were operated combined into one single system trough the continuous internal recirculation from the aerobic MBR to the methanogenic UASB or as a UASB reactor followed by an MBR post-treatment when the recirculation was turned off. The combination of anaerobic treatment with an aerobic MBR as a polishing step is an alternative to treat some industrial wastewater and/or urban wastewaters generated in warm climate countries. Applied OLR varied between 0.7 and 3.1 $kgCOD \cdot m^{-3} \cdot d^{-1}$ and COD removal was above 95 % during most of the operation, of which in between 40 and 80% was removed in the UASB reactor. Biogas production with methane content around 80% was observed. Biogas production yield was around 0.15 $m^3_{methane} \cdot kgCOD_{eliminated}^{-1}$ during the four experimental periods. The overall biomass yield varied between 0.09 and 0.12 $gMLVSS \cdot gCOD^{-1}$, which are values much lower than the typical values determined for aerobic MBRs (0.25 - 0.61 $gMLVSS \cdot gCOD^{-1}$) and close to those observed for the anaerobic treatment of wastewaters, that are in the range between 0.11 and 0.14 $gMLVSS \cdot gCOD^{-1}$. Moreover, biomass yield observed during periods in which recirculation

from the MBR to the UASB was applied, 0.09 was much lower than those of 0.12 determined in periods in which this recirculation was turned off. This indicated that a fraction of sludge generated during the aerobic MBR stage was digested in the UASB system, decreasing biomass yield.

In addition, the proposed system made feasible to manipulate nitrogen conversion to ammonia and/or nitrate, which might be of especial interest for reuse the treated wastewater in some industrial or agriculture applications. Although nitrogen removal was promoted during one operational period through the application of anoxic cycles in the first stage of the MBR, any effect was observed.

Regarding membrane operation, permeabilities around 150 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ were achieved, operating with fluxes of 12-15 $L \cdot m^{-2} \cdot h^{-1}$. A better membrane performance was observed when recirculation between MBR and UASB reactors was turned off. The high COD removals achieved in the UASB reactor, especially when it operated at high ambient temperatures, caused a diminution of the biodegradable COD supplied to the aerobic stages. This low OLR applied to the aerobic stages (MBR) had a great impact on MLVSS concentration. MLVSS concentration in the membrane chamber varied between 0.5 and 4 $g \cdot L^{-1}$, which were lower values than those typically recommended. At lower biomass concentrations, the lack of protection by the cake layer led to an irreversible membrane fouling caused by pore clogging of soluble and colloidal biopolymers and the fouling rate increased more than a 60 %. Therefore, the supply of a minimum OLR in the aerobic stage was shown to be of prime importance in order to maintain MLVSS, and hence to control membrane fouling. In this sense, the proposed system could be modified in order to allow the feeding of a small fraction of the raw influent directly into the aerobic stage, in order to assure a minimum biodegradable COD supply, and thus maintain food to microorganism ratio (F/M) above the minimum value typically recommended (0.1 $gCOD \cdot gMLVSS^{-1} \cdot d^{-1}$) .

In Chapter 5 the impact of the methanogenic stage on membrane fouling in the system proposed in chapter 4 was studied. Operating fluxes of 11-18 $L \cdot m^{-2} \cdot h^{-1}$ and permeabilities of 100-250 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ were reported. The recirculation of aerobic biomass to the anaerobic stage led to the increase of colloidal BPC concentration in the membrane chamber and the worsening of membrane performance. The same trend was observed when recirculation was turned off and external sludge from a municipal WWTP was fed to the UASB reactor. Batch experiments demonstrated that the hydrolysis of aerobic biomass (complex substrate) in anaerobic conditions led to a release of biopolymers, and hence an increase in the concentration of all the fouling indicators studied.

Carbohydrate fraction of soluble microbial products, biopolymer clusters (BPC) and transparent exopolymer particles (TEP) concentrations were studied as possible fouling indicators for this system. A strong correlation between both colloidal fraction of BPC (cBPC) and TEP with membrane fouling rate was observed, especially at MLVSS lower than 4 $g \cdot L^{-1}$. MLVSS concentration was shown to be an important parameter in order to protect the membrane against the fouling provoked by soluble and colloidal biopolymers. Depending on biomass concentration in membrane chamber, the presence of biopolymers worsened membrane performance. Fouling rate was three times higher when biomass concentration decreased from 8 to 2 $g \cdot L^{-1}$, with similar concentrations of biopolymers present. Moreover, the presence of plastic support in the aerobic stage was shown to improve membrane performance, decreasing the concentrations of the studied fouling indicators. Microscopic observation showed a great amount of attached ciliated protozoa in the biofilm. Hypothetically, the absence of these filtering organisms caused the increase of colloidal biopolymer concentration.

In Chapter 6, the same set-up employed in Chapters 4 and 5 was used in order to study nitrogen removal. The effluent of the UASB reactor was post-treated in an MBR with a first anoxic chamber in order to use dissolved methane as carbon source for denitrification. The presence of dissolved methane, especially at low temperature, represents an important environmental problem in terms of greenhouse gas (GHG) emissions of wastewaters treated using methanogenic bioreactors. Methane has a global warming potential 25 times higher than carbon dioxide. For low strength wastewaters, dissolved methane might account up to 50% of the produced methane. The dissolved methane is easily desorbed from the effluents, especially if these are either released in the environment or post-treated using aerobic bioreactors. Thus the use of anaerobic technology increases GHG emissions associated with wastewater treatment.

Therefore, the use of this dissolved methane as a carbon source for biological denitrification proposed in this chapter may be an alternative to reduce both GHG emissions and nitrogen content of the treated wastewater. Up to 60% and 95% nitrogen removal and methane consumption were observed, respectively. The stripping of the dissolved methane present in the UASB effluent led to a worsening of nitrogen removal in the MBR system. Batch experiments confirmed the presence of microorganisms capable of denitrifying using the dissolved methane as carbon source. Denitrification seems to be carried out by a consortium of aerobic and anaerobic methane oxidizing bacteria and heterotrophic bacteria that used the oxidation products as carbon source for denitrification. Nevertheless, methane oxidation rate was much higher than that theoretically predicted

considering the stoichiometry of denitrification with methane, either in microaerobic or anaerobic conditions. Recirculation ratio between the anoxic and aerobic chambers of the MBR system, and either the presence or absence of dissolved methane were shown as the main important parameters governing the denitrification process. Nitrogen removal decreased from 60 to 27% when dissolved methane was removed from the UASB effluent. At higher recirculation ratios the anaerobic oxidation pathway seemed to be inhibited, decreasing methane oxidation rate more than a 50%. This inhibition was associated to the higher oxygen input to the anoxic chamber. This fact confirmed the results obtained in Chapter 4, when the application of aerobic/anoxic cycles did not stimulate denitrification process.

The influence of denitrification with methane on membrane performance was also studied, showing a remarkable increase on biopolymer concentration when denitrification activity was affected by the removal of dissolved methane from the UASB effluent. This effect is similar to that observed when nitrification is affected.

In Chapter 7 a completely stirred tank anaerobic membrane bioreactor (AnMBR) was operated for the treatment of an herbal extraction wastewater. The complexity and low biodegradability of this industrial wastewater led to the operation of the bioreactor at high mixed liquor total solids (MLTS) concentrations. The exact relationship between MLTS concentration and the steady-state permeate flux in an AnMBR has not been extensively investigated and the information regarding AnMBR operation at high MLTS concentration is very limited. The fluxes achieved in the studied AnMBR ranged between 1 and 2.5 $L \cdot m^{-2} \cdot h^{-1}$, working with MLTS between 38 and 61 $g \cdot L^{-1}$. Although these values were similar to those obtained in other AnMBR treating industrial wastewaters with submerged membrane modules at MLTS above 30 $g \cdot L^{-1}$, the possibility of improving membrane performance by adding powdered activated carbon (PAC) was also evaluated. Batch and fed-batch experiments with different activated carbons were performed and an optimum dosage of 1.5 $g \cdot L^{-1}$ was determined.

Stable operation of the system was maintained applying HRT below 4 d, at a feed concentration of 8 $g \cdot L^{-1}$ resulting in an OLR of 2.0-3.0 $kgCOD \cdot m^{-3} \cdot d^{-1}$ without controlling alkalinity reaching COD removal efficiencies up to 60%. Nevertheless volatile fatty acid (VFA) concentration was extremely high during the operation, indicating some kind of inhibition of the methanogenic process, probably related with the antibacterial activity of rosemary extracts. This fact might have a harmful effect on anaerobic biological process, causing destabilization of the microbial populations leading to VFA accumulation that can acidify the reactor, and therefore inhibit methanogenic microorganisms. The control of

alkalinity through the continous addition of NaHCO3 was shown to be essential in order to improve COD removal efficiencies up to 70 % working with OLR up to 5.0 $kgCOD \cdot m^{-3} \cdot d^{-1}$. A methane yield around 0.3 $m^3_{methane} \cdot kgCOD_{eliminated}^{-1}$ with a methane concentration of approximately 60% was observed.

Furthermore, typical fouling indicator concentrations recently studied during the operation of aerobic membrane bioreactors MBR, such as biopolymer cluster (BPC) and transparent exopolymer (TEP), were measured during the operation. Moreover, the filterability properties of the sludge were also determined during the operation in order to examine if the addition of PAC could improve the resistance to filtration of the mixed liquor. The concentrations of the fouling indicators measured during this studying were extremely high, as well as specific resistance to filtration and the addition of PAC to the AnMBR did not improve anyone of them. Membrane fouling was governed by the hydrodynamics derived from the high MLTS concentration. Since this high MLTS concentration did not improve organic matter removal, a diminution below 20 $g \cdot L^{-1}$ could enhance membrane fluxes, especially when PAC would be added into the reactor, as suggested by literature.

With the work performed in this thesis, important information for the operation of submerged membrane technology and its application combined with anaerobic and aerobic wastewater treatments was obtained.

Chapter 1

Introduction

Summary

In this chapter are detailed the scope and the motivations of this thesis and a broad background regarding submerged membrane filtration technology is provided. Membrane technology has expanded at a remarkable rate over the pass twenty years, and nowadays enhances a multi-billion dollar industry. This work has been focused on the treatment of both urban and industrial wastewaters with different technologies, all of them combined with submerged membranes.

The two main applications for low-pressure membranes in the field of wastewater treatment are: tertiary membrane filtration (TMF) of a secondary effluent and direct treatment in a membrane bioreactor (MBR). Although there are many similarities between both treatment processes, it is of a core importance the knowledge of their differences in order to determine the best alternative for a given application.

A general background of low-pressure membrane technology and their two main applications: MBR and TMF, is given in this chapter as an introduction of the present thesis.

1.1. Membranes in wastewater treatment

A membrane is a thin sheet of material capable of separating substances based on their physical and chemical properties when applying a driving force through it (AWWA, 1998). Membranes, a porous material, use the filtration as separation mechanism, being the pressure difference between the two phases separated by the membrane the driving force of the process.

For wastewater treatment applications, the five key membrane separation processes in which water forms the permeate product are microfiltration (MF), ultrafiltration (UF), electrodialisis (ED), nanofiltration (NF) and reverse osmosis (RO). Neglecting ED and depending on the separation mechanism, the membranes can be classified as porous membranes and non porous membranes. MF, UF and the coarser end of NF are considered as porous membranes, and its degree of selectivity depends on membrane pore size. Nevertheless, NF and RO osmosis are considered as non porous membranes since the degree of selectivity not only depends on pore size but also on others factors such as diffusion and solubility. Therefore, the coarsest membrane is associated with MF and can reject particulate matter, by size exclusion mechanism, whereas the most selective membrane is associated with reverse osmosis (RO) and can reject singly charged (i.e. monovalent) ions, such as sodium (Na^+) and chloride (Cl^-) (figure 1.1). MF, UF and NF are considered as low-pressure membranes (up to 7 bar) whereas RO is considered as a high-pressure membrane process (up to 70 bar) (Rushton et al., 1996).

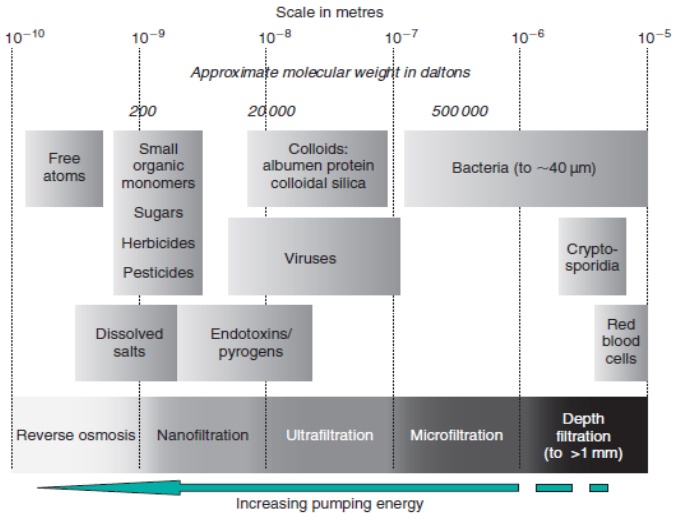

Figure 1.1. Membrane separation processes overview (Judd and Jefferson, 2003)

The combination of membrane filtration technology with biological processes for wastewater treatment is the origin of membrane bioreactors (MBR) and also tertiary membrane filtration (TMF), which are the two major applications for low-pressure membranes within wastewater treatment (Gallagher et al., 2008).

The ability to attach a physical process of filtration in conjunction with a biological wastewater treatment allows combining the individual advantages of each one of the techniques, giving also a synergy between the two technologies, resulting in clear improvements in the overall treatment process. First, biological treatment can remove not only most of the organic matter but nutrients working in the proper configuration. Moreover, filtration process allows to obtain a permeate of excellent quality, with negligible amounts of suspended solids and organic matter, and the absence of microorganisms, viruses and fecal coliforms. In general, the permeate obtained not only meets the current discharge limits, it encourages reuse applications such as irrigation, heating or cooling water or for cleaning purposes. Moreover, the use of membrane technology (MF or UF) has been shown to be the ideal pre-treatment for RO in water reclamation and even seawater desalination (Côté et al., 2005).

1.2. Membrane configuration and characteristics

Membrane geometry and the way it is mounted and oriented in relation to the flow of water, is crucial in determining the overall process performance. There are mainly three configurations that are currently used to manufacture membranes for MBR or TMF applications (figure 1.2). These configurations are based on both flat and cylindrical geometries and include hollow fiber, flat sheet, and tubular. It is very common to have multiple membrane elements to form a multi-tubular module (MT), a flat sheet module (FS) or a hollow fiber module (HF).

The production of permeate in HF or FS membranes occurs from outside to inside by aspiration (being the shell-side in contact with the mixed liquor), while in tubular membranes the filtration takes place from inside to outside by introducing the mixed liquor along the lumen of the membrane, (being the shell-side in contact with the permeate) (figure 1.3).

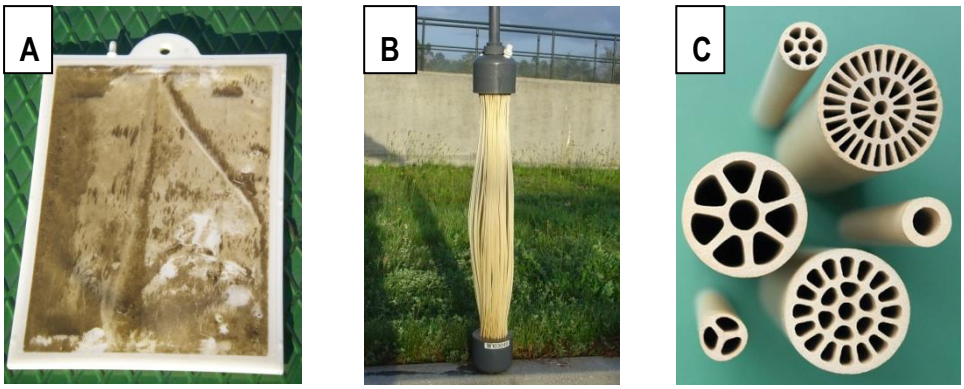

Figure 1.2. Pictures of Flat sheet (A), hollow fiber (B) and tubular (C) membranes.

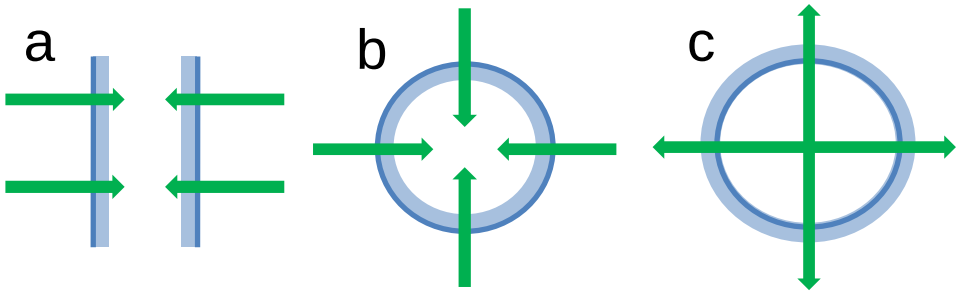

Figure 1.3. Schematics showing flow through membrane configured as: flat sheet (a), hollow fiber (b) and tubular (c).

The main considerations that have to be taken into account with respect a membrane module are (Judd, 2011):

- a high membrane area to module bulk volume ratio,
- a high degree of turbulence for mass transfer promotion on the feed side,
- a low energy expenditure per unit product water volume,
- a low cost per unit of membrane area,
- a design that facilitates cleaning and modularization.

Membranes can be also classified attending to the material they are constructed with in organic polymeric membranes, ceramic membranes and metal membranes, although the use of metal membranes in MBR is rare due to their high cost.

The membranes should have adequate mechanical and chemical resistance. They also should be resistant to fouling and membrane cleaning procedures. Therefore membranes have to bear variations of temperature, pH and/or concentrations of chemicals applied during chemical cleaning. In this sense should be noted that ceramic membranes have a much higher chemical resistance than organic. The main materials used for the manufacturing of organic membranes are polyvinylidene difluoride (PVDF), polyethylsulfone (PES), polyethylene (PE) or polypropylene (PP). These organic polymers have a hydrophobic nature and therefore can be easily fouled. That is why the manufacturers apply a hydrophilic treatment to the external surface (that is going to be in contact with the mixed liquor) by chemical oxidation, organic chemical reactions or plasma treatment in order to alleviate membrane fouling. This hydrophilic treatment and method used to manufacture the membrane module is information classified by most membrane manufacturers.

1.3. Membrane bioreactor (MBR)

The membrane bioreactor (MBR) may be considered as one of the most significant advances in wastewater treatment technologies performed in the last two decades. Compared with traditional biological treatment systems (activated sludge reactor, rotating biological contactor, trickling filter and submerged biofilter), they produce a high quality treated effluent in a much smaller space. Generically an MBR can be defined as an conventional activated sludge (CAS) reactor in which the secondary sedimentation stage has been replaced by a filtration stage using microfiltration (MF) or ultrafiltration (UF) membranes with pore sizes ranging from the 0.01 y 2.0 μm to produce an effluent free of suspended solids and microorganisms, thereby allowing complete control of solids retention time (SRT). Apart of uncoupling hydraulic retention time (HRT) and SRT, another advantage is that MBR can work with much higher concentrations of biomass. In addition to the intensification of biological treatment, this lead to more compact systems without secondary clarifiers and higher capacity for water treatment. This is a key advantage when a treatment plant needs to increase its treatment capacity (nuclei of rapidly growing population) but there is no way for the expansion in size. Finally, in the case of urban wastewater, MBR work with low F/ M ratios, resulting in a lower sludge production (Sun et al., 2007), than typically observed in conventional aerobic systems.

The simplest definition of MBR, as a CAS in which the secondary settler has been replaced by the membranes, would imply only the removal of organic matter. Moreover, as in activated sludge systems, MBRs can be operated under many different configurations,

incorporating anaerobic and/or anoxic compartments in order to enable simultaneous biological nutrients removal.

Despite the advantages mentioned before, MBR technology increases operating costs with respect to activated sludge reactors, due to a higher consumption of electricity and the need to replace the membrane modules, so their use is justified only under the following circumstances:

1) Use in areas with high environmental sensitivity, where the legislation force to discharge treated wastewater with a low content of chemical or biological contaminants.

2) Use in areas of water scarcity, where is necessary to reuse treated water.

3) Wastewater treatment plants (WWTPs) with space limitations, preventing the use of other purification technologies. Under this heading would be included the expansion of treatment capacity of conventional WWTPs in operation, on which a field expansion is not possible or desirable.

4) Treatment of complex industrial wastewaters in which the use of another treatment technology is not effective or reliable and biological treatment of industrial wastewater in areas with strong seasonal component.

1.3.1. Background

MBR technology emerged in 1969 when the company Dorr-Oliver replaced the secondary clarifier in a CAS system by a tangential flow UF membrane. In this study, performed by Smith et al. (1969), the reactor mixed liquor was pumped to the membrane in order to separate the treated water from the sludge, resulting in a treated effluent or permeate and a concentrated sludge stream that was returned to the reactor. This type of system is called the sidestream membrane MBR (figure 1.5A). The main problems in these initial steps were related with the heavy fouling and the rupture of the filters. All MBRs implanted between 1969 and the end of the eighties were based on the sidestream configuration and nowadays are mainly applied for the treatment of landfill leachates or wastewater generated by industries, ships and anaerobic digesters.

In this configuration, tangential flow tubular modules placed in vertical are commonly used (flat sheet modules can also be used) to which the sludge is pumped from the bottom at higher speeds, inducing turbulent flow in order to prevent radial gradients. The high pressures justify the high energy consumption, estimated at between 3 and 5 $kWh \cdot m^{-3}$ of purified water, which limits the use of sidestream MBR for the purification of large volumes

of water. Energy savings can be achieved by injecting air into the base of the vertical membrane modules, obtaining an airlift effect and avoiding the use of a pump (reaching 1.2 kWh·m^{-3} of purified water).

The first systems developed were small-scale and industrial scale applications, treating small volumes of wastewater streams with high organic loads. In any case, operational costs remained high, with special emphasis on the modules and the power consumption, limiting the competitiveness of the technology compared to conventional processes.

In the eighties started the development of filtration membranes on a larger scale, especially on three fronts: North America, Japan and Europe. Many types of membranes were then developed specifically for the food industry. Nevertheless, the ease with which the modules rupture occurred generated distrust and uncertainty.

In the early nineties, the membrane modules were optimized, developing new models more robust and reliable. The Japanese government launched an ambitious R&D project which led to the most important technological and industrial advance of the MBR process, with the development of submerged membrane modules, resulting in the submerged MBR membrane (figure 1.5B). In these systems the membrane module is submerged in the aeration tank, in contact with the mixed liquor. Therefore it was possible to suppress the pump that was used to drive the sludge and replace it for another pump that suck the filtered effluent or permeate from the membrane module. Thus there was a significant reduction in investment and operation costs due to the reduction and simplification of equipment and energy saving was needed to pump the sludge. Energy consumption associated with water treatment by submerged MBR is between 0.55-1.5 kWh·m^{-3} depending on configuration and membrane technology (Judd, 2011) and is higher than that observed in well operated CAS reactors (0.38 to 0.48 kWh·m^{-3}, Evans and Laughton, 1994). Similarly, the costs and the operational problems decreased, emerging new markets as well as pharmaceutical and food industries.

In most of the first submerged MBR membrane modules were installed in the same tank where the influent was received. However there is a tendency today to remove the membrane from the influent inlet using an additional chamber to immerse membrane modules. This external submerged MBR configuration (figure 1.5C) significantly reduces membrane fouling.

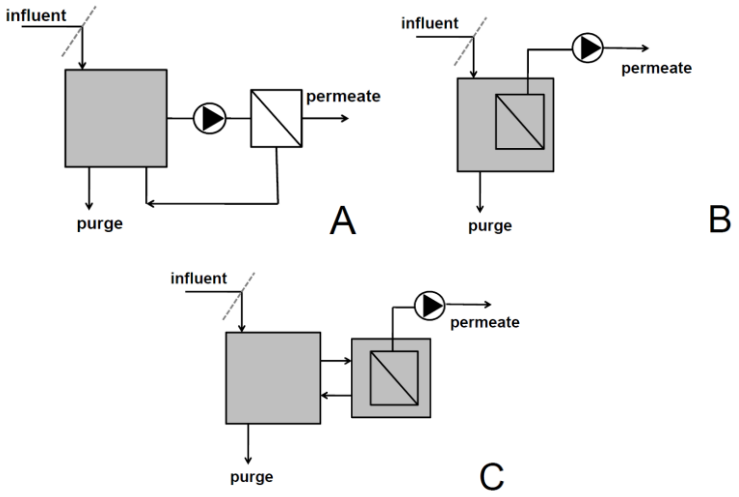

Figure 1.5. MBR configurations: (A) Sidestream; (B) Submerged; and (C) External submerged.

This cheaper and less energy consumption system allowed jumping into the urban wastewater treatment in the late nineties. The first full-scale MBR plant for domestic wastewater treatment has been installed in the UK in 1998, and features a capacity of 1900 $m^3 \cdot d^{-1}$, in Porlock. Since then, the range of capacities and applications developed significantly. By 2006, more than 100 municipal MBR plants with a capacity larger than 500 person equivalent were in operation in Europe only. Today, several thousand MBRs have been commissioned worldwide (table 1.1).

Table 1.1. Some of the largest MBR plants

Location	Country	Capacity ($m^3 \cdot d^{-1}$)	MBR technology	Year
Al Ansab	Oman	220000	Kubota	2012
Guangzhou	China	100000	Memstar	2010
Sao Paulo	Brazil	86400	Koch (PURON)	2011
Beijing	China	78000	Siemens	2008
Sabadell	Spain	55000	Kubota	2009
S. Pedro del pinatar	Spain	48000	GE Zenon	2007
Nordkanal	Germany	45000	GE Zenon	2004

In Europe and Asia more research in the area of urban wastewater treatment than in industrial was carried out while in Northamerica the situation was reversed, because in Europe and Asia there was more space restrictions to expand conventional treatment plants, making membrane technology very attractive for the treatment of wastewater with high flow and low organic loads, such as urban (Lesjean et al., 2004).

1.3.2. Market

Over the past twenty years, research has been focused mainly to determine the feasibility of MBR technology, and the search for methods to improve the process. As a result, MBRs are increasingly becoming the technology of choice for water and wastewater applications where the above criteria apply, as is evident by the substantial increase in the membrane bioreactor market over recent years - MBRs are now implemented in more than 200 countries and global market growth rates of between 11.5% and 13% are regularly reported in market analysis reports, the MBR industry market value being estimated as worth $500 million by 2013 (Judd, 2011).

In figure 1.6 can be observed the evolution of the MBR implementation in Europe during the last two decades for the treatment of both industrial and municipal wastewaters. Although some industrial applications were the first niche for MBR technology, the progressive reduction of operational costs has made this technology competitive for municipal wastewater treatment during the last decade.

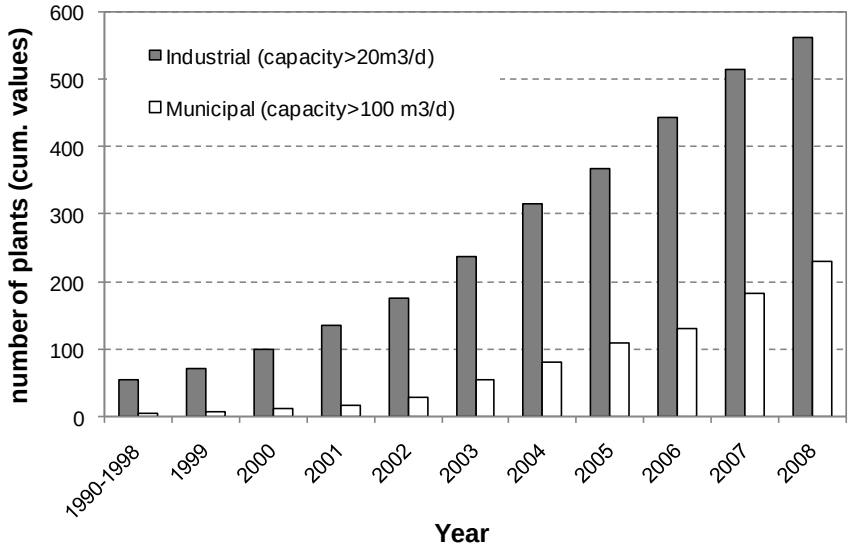

Figure 1.6. Number of MBR in Europe (Huisjes et al., 2009).

Table 1.2. Main suppliers of submerged MBR technology (adapted from Santos et al., 2010 and www.thembrsite.com).

FS Supplier	Country	HF Supplier	Country
A3	Germany	Asahi Kasei	Japan
Alfa Laval	Sweden	Beijing Origin Water	China/Taiwan
Anua	USA	Canpure	Canada
Brightwater	S. Ireland	Ecologix	China/Taiwan
Colloide	N. Ireland	ECONITY	Korea
Ecologix	Canada	ENE	Korea
Huber	Germany	GE Zenon	USA
Hyflux	Singapore	Hainan Litree	China/Taiwan
LG	Korea	Hangzhou H-Filtration	China/Taiwan
Kubota	Japan	Koch Memb. Syst (PURON)	USA
Martin Systems	Germany	Memstar	Singapore
Microdyn-Nadir	Germany	Micronet Porous Fibers	Spain
Pure Envitech	Korea	Mitsubishi Rayon	Japan
Shanghai Megavision	China/Taiwan	Philos	Korea
Shangai SINAP	China/Taiwan	SENUO Filtration	China/Taiwan
Suzhou Vina	China/Taiwan	Shanghai Dehong	China/Taiwan
Toray	Japan	Siemens	Germany
Weise	Germany	Sumitono	Japan
		Superstring	China/Taiwan
		Suzhou Vina	China/Taiwan
		Tianjin Motimo	China/Taiwan
		Zena SRO	Czech Rep.

Submerged MBR system in municipal applications, represent the 99% of the installed membrane surface in Europe in the period 2002–05. On the other hand, side-stream configuration is commonly used in industrial applications. In general, submerged MBR require higher initial investment costs, and aeration with respect to side-stream membrane configurations. In contrast, pumping costs are lower and operating, requiring lower operating flows and cleaning frequencies (Stephenson et al., 2000). The selection between submerged and side-stream configurations for aerobic MBRs seems somehow settled, in favour of submerged MBRs. In fact, nowadays, most of the commercial applications are based on the submerged configuration, due to lower associated energy requirements (Judd, 2011).

Today there are approaching 60 MBR membrane module products available and the number of technology suppliers continues to expand. On table 1.2 can be observed the manufacturers of submerged membrane technology, both flat sheet and hollow fiber.

1.4. Tertiary membrane filtration

Tertiary filtration, especially depth filtration, has been traditionally used to remove suspended solids from secondary treated waters. They can also be used to remove particulate and colloidal matter from settled secondary effluents, which increases the effectiveness of disinfection with either ultraviolet radiation or ozone for reuse applications (Tchobanoglous et al., 1998; Lubello et al., 2003). However, in recent years, the use of tertiary membrane filtration systems is becoming more common.

For tertiary filtration applications, MF and UF membrane systems can be divided in two different types: submerged, when the membranes are submerged in a feed water tank where permeate is sucked (via vacuum) into the inside of the membrane (dead-end filtration); and pressurized (or contained), when the membranes are housed in modules where pressurized feed water is forced through the fiber and permeate is collect on the outside (cross-filtration). In turn, FS or HF membranes can be used in submerged systems whereas contained systems are composed by HF or MT ones (Li et al. 2008). Submerged membrane modules have gained popularity, especially for low solids feeds, because of the lower costs associated and the acceptance of modest fluxes and low transmembrane pressures (TMP).

Low-pressure tertiary membranes have been proved to meet increasingly stringent standards for discharge or reuse. In fact, more than 78% of wastewater reclamation plants used low-pressure membranes as a pretreatment for the reverse osmosis process

(Burbano et al., 2007). Thus, compared to depth filtration, tertiary MF or UF membrane treatments produce water of better microbiological quality that is also free of suspended solids. This should be taken into account when water is reused or discharged into sensitive areas.

Operational permeabilities of 160-250 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ typically reported for TMF are similar to those obtained during the operation of MBRs. Nevertheless, the fluxes obtained in TMF, between 25 and 70 $L \cdot m^{-2} \cdot h^{-1}$ are much higher that fluxes reported in submerged MBR, around 20-30 $L \cdot m^{-2} \cdot h^{-1}$ (Judd, 2002; Wen et al., 2004).

While recommended mixed liquor total suspended solids (MLTSS) concentrations in MBRs range between 6 and 15 $g \cdot L^{-1}$ (Rosenberger et al., 2006; Judd, 2011), membranes treating secondary effluents are normally operated at MLTSS concentration in the range of $mg \cdot L^{-1}$ (Citulsky et al., 2009).Therefore different fouling mechanisms and operational strategies can be expected for the membrane filtration of secondary effluents. Nevertheless it could be also possible to operate a TMF system like an MBR by accumulating MLTSS washed out with the secondary effluent (Sánchez et al., 2011) (in present work).

1.5. Membrane fouling and its control

Fouling is the main drawback associated with the application of membrane technology for wastewater treatment (Kimura et al., 2005; Meng et al., 2009; Drews, 2010). Fouling decreases the permeability of a membrane, limits flux and shortens the life of membrane modules, thus increasing both the capital and the operating costs of filtration systems.

Membrane fouling is the result of complex phenomena that are not yet completely understood and can be defined as the undesirable deposition of microorganisms, colloids, organic and inorganic precipitates, solutes and cell debris on membrane surface or within its pores. This phenomenon restricts the application of MBR technology by limiting membrane flux and increasing TMP.

Membrane fouling in MBRs can be attributed to both membrane pore clogging and sludge cake deposition on membranes which is usually the predominant fouling component (Lee et al., 2001). Membrane fouling occurs due to the following mechanisms: (1) adsorption of solutes or colloids within/on membranes; (2) deposition of sludge flocs onto the membrane surface; (3) formation of a cake layer on the membrane surface; (4) detachment of foulants attributed mainly to shear forces; (5) the spatial and temporal

changes of the foulant composition during the long-term operation (e.g., the change of bacteria community and biopolymer components in the cake layer) (Meng et al., 2009).

Membrane fouling is a very complex phenomenon very difficult to understand. Figure 1.7 summarizes the main factors that can affect it and the possible relationships between them.

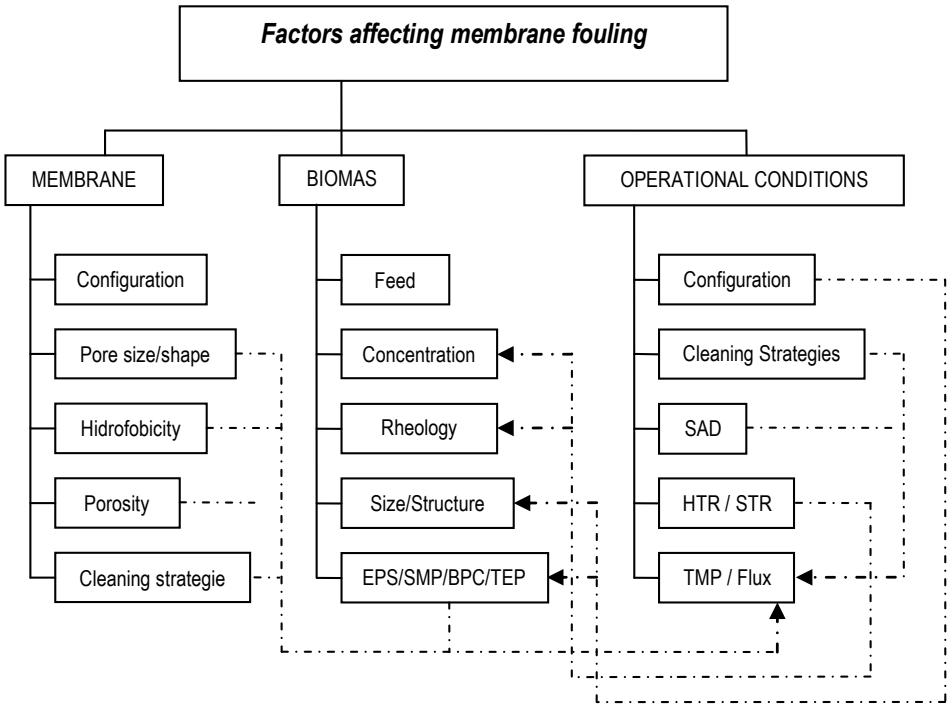

Figure 1.7. Main factors affecting membrane fouling in the MBR process. (adapted from Chang et al., 2002)

Membrane fouling can be classified in reversible, irreversible and irrecoverable (Drews, 2010). Reversible fouling occurs due to external deposition of material (cake filtration) and can be removed by physical means such as air scouring backwashing or relaxation, irreversible fouling refers to fouling which can only be removed by chemical cleaning and irrecoverable fouling can not be removed by any cleaning and occurs over long periods.

Membrane operation should be controlled in order to prevent or delay its fouling. Thus, physical and/or chemical cleaning strategies are commonly used, taking into

account the recommendation of manufacturers. Regarding physical strategies, membrane fouling can be typically limited by three different ways:

- Air scouring: Coarse bubble aeration produces turbulence over the membrane surface, which facilitates detachment of the biomass cake deposited on it. Generally, all submerged module manufacturers recommend the use of air scouring systems. Typical values of specific air demand per unit of membrane surface (SAD_m) range between 0.3 and 0.8 $Nm^3 \cdot h^{-1} \cdot m^{-2}$ (Judd, 2011).

- Relaxation periods: With the use of relaxation periods is interrupted the flow of permeate, encouraging diffusive back transport of foulants away from the membrane surface under a concentration gradient, which together with the air scouring assists the detachment of the biomass cake and the polymers accumulated over the membrane surface. Relaxation is typically applied for 1-2 min every 8-15 min of operation, both for flat sheet and hollow fiber systems.

- Backwashing: Produced permeate is cyclically pumped towards the membrane module, in order to detach the biomass cake and the polymers accumulated over the membrane surface. For hollow fiber systems, backwashing, if employed, is usually applied at fluxes of around 2-3 times the operating flux and usually supplements rather than displaces relaxation. This strategy was firstly used for HF membranes but nowadays, some FS providers such as Alfa Laval or Microdyn-Nadir have developed FS membranes that could be backwashed without risk to the integrity of the membrane. In the case of TMF applications, filtration cycles of 15-60 min (Metcalf & Eddie, 2003; Pearce, 2010; Zheng et al., 2011) with backwashing periods of 30-100 s (Zheng et al., 2011) are values typically used.

Physical cleaning is supplemented with chemical cleaning to remove irreversible fouling. There are two main chemical cleaning strategies that may vary depending if the membrane is used for MBR (Judd, 2011) or TMF (Zheng et al., 2011) applications:

- Intensive (or recovery) chemical cleaning. Intensive chemical cleaning is carried out with a combination of sodium hypochlorite for removing organic matter, and organic acid (either citric or oxalic) for removing inorganic scalants, and can be performed either *in situ* ("cleaning in place" or CIP) or *ex situ.* Intensive cleaning is generally carried out when further filtration is no longer sustainable because of an elevated TMP (once or twice a year). Recovery cleaning employs rather higher reagent concentrations of 0.2-0.3 % NaOCl, coupled with 0.2-0.3 % citric acid or 0.5-1 % oxalic acid. Reagents concentrations used for intensive chemical cleanings in TMF applications are even higher, being typically

of 2% NaOH and 0.5% citric acid. This kind of cleaning is normally carried out every 1-2 months.

- Maintenance chemical cleaning. Maintenance cleaning is conducted *in situ* and is used to maintain membrane permeability and helps reduce the frequency of intensive cleaning. Maintenance cleaning is normally carried out moderate reagent concentrations of 200-500 $mg \cdot L^{-1}$ NaOCl and a frequency of 1-2 weeks or days for MBRs or TMF applications, respectively. Alternatively, a low concentration of chemical cleaning agent can be added to the backwash water to produce a "chemically enhanced backwashing" (CEB) on a daily basis. In the case of TMF applications, CEB is commonly used with a frequency of 1-2 hours and moderate reagent concentrations of 20-200 $mg \cdot L^{-1}$ NaOCl (Zheng et al., 2011).

Apart from the measures of physical or chemical cleaning, a second strategy can be applied to limit the consequences of membrane fouling, which is to reduce its causes. This strategy is based on modifying the physical and chemical nature of the membrane, the composition of the wastewater and the characteristics of mixed liquor

The characteristics of the mixed liquor are the factor on which a major research effort has been made regarding membrane fouling.

Membrane fouling in MBRs has been largely attributed to extracellular polymeric substances (EPS) (Chang and Lee, 1998; Cho and Fane, 2002; Nagaoka et al., 1996; Nagaoka et al., 1998; Rosenberger and Kraume, 2002) and soluble microbial products (SMP) (Chang et al., 2002; Liu et al., 2005; Evenblij and van der Graaf, 2004; Ji and Zhou, 2006) althought SMP have been reported to have a greater impact on membrane fouling as will be mentioned later.

EPS consist of insoluble materials (sheaths, capsular polymers, condensed gel, loosely bound polymers and attached organic material) secreted by the cell, shed from the cell surface or generated by cell lysis (Jang et al., 2005). On the other hand SMP are defined as the pool of organic compounds that are released into solution from substrate metabolism (usually with biomass growth) and biomass decay (Barker and Stuckey, 1999). The EPS or SMP are constituted by proteins, polysaccharides, nucleic acids, lipids, and humic acids.

A number of different studies have indicated a direct relationship between the carbohydrate level in SMP fraction and MBR membrane fouling (Lesjean et al., 2005; Le-Clech et al., 2005; Rosenberger et al., 2005). The hydrophilic nature of carbohydrate may explain the apparently higher fouling propensity of this fraction over that of proteins, given

that proteins are more generally hydrophobic than carbohydrates. Strong interaction between the hydrophilic membrane generally used in MBRs and hydrophilic organic compounds may be the cause of the initial fouling observed in MBR systems. On the other hand, correlation of MBR membrane fouling with SMP protein has not been widely reported. Humic matter may not significantly contribute to fouling due to the generally lower MW of these materials (Drews et al., 2005).

Recently, the terms biopolymers or biopolymeric clusters (BPC) have also come into use (Sun et al., 2008; Wang et al., 2008). BPC are defined as a new pool of organic substances in the MBR sludge mixture that are a solute independent of the biomass and are much larger than SMP in the sludge suspension. The BPC content in the MBR was estimated by calculating the difference in TOC concentration between the AS supernatant and the effluent. Therefore is a reliable easy to measure method compared with protein or carbohydrate determination.

Another group which until recently had only been studied in the formation of biofilms in seawater environments (Pasow, 2002) are the so-called transparent exopolymer particles (TEP), an acidic fraction of polysaccharides. These compounds consist mainly of exopolysaccharides of a sticky nature, a characteristic which makes them a group of interesting substances in processes like sedimentation, flocculation and membrane fouling. TEP concentration is easy to measure and does not involve the use of sulfuric acid as in the case of carbohydrate concentration (Dubois et al., 1956). TEP concentration has been monitored for the first time in sludge filtrate by de la Torre et al. (2008) highlighting the potential of this parameter as a fouling indicator for MBR systems.

By definition, all these groups of compounds are produced and excreted by microorganisms and depending on the applied assays, these groups are not distinct but overlap (figure 1.8). Unfortunately, the location of the fouling relevant fraction is still unknown, so are the conditions that shift it to different locations (Drews, 2010).

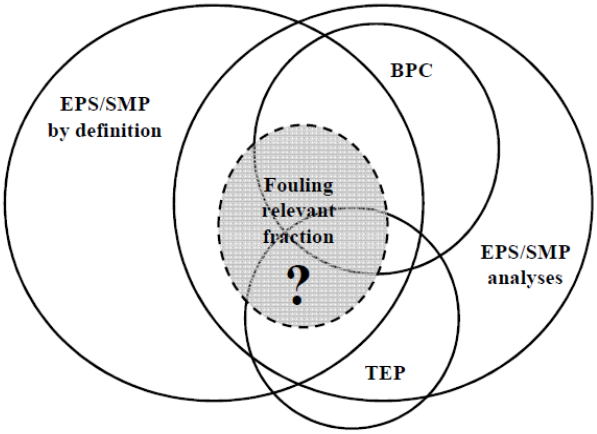

Figure 1.8. Posible relation between polymer fractions (Drews, 2010)

The characteristics of mixed liquor are directly related with the characteristics of the wastewater and the operating conditions applied to the biomass. Some parameters, such as SRT and F/M ratio should be controlled in order to limit membrane fouling. SRT between 15 and 50 d (Meng et al., 2009) and are recommended in MBRs. Regarding F/M it is important to prevent higher ratios but also to assure a minimum F/M relationship in order to guarantee a proper development of the biomass. Typical values for aerobic MBR treating municipal wastewaters are in the range of 0.1-0.3 $kgCOD \cdot kgMLVSS^{-1} \cdot d^{-1}$ (Brepols, 2006; Judd, 2011).

Another common rules applied to MBR operation are to operate with a flux away from the critical flux, to avoid working with high TMP, to maintain the membrane modules aerated and avoid operating with too low dissolved oxygen concentrations in order to prevent the proliferation of filamentous bacteria or sludge deflocculation.

A relatively recent concept in fouling minimization is the use of coagulants/flocculants such as aluminium sulphate, ferric chloride, poly-aluminum chloride or poly-ferric sulphate (Ji et al., 2008; Zhang et al., 2008; Teli et al., 2012). The addition or adsorbents such as powdered activated carbon (PAC) and granular activated carbon (GAC) have been also reported to improve membrane filtration performance through the adsorption of biopolymer foulants and the increase on sludge particle size (Hu and Stuckey, 2007; Akram and Stuckey, 2008; Remy et al., 2010) . Moreover, in the case of AnMBR, the addition of PAC could be also beneficial in case of shock load event since volatile fatty acids would be adsorbed.

A more recent advance in this field has been the study of the so-called flux enhancers (Koseoglu et al., 2008; Iversen et al., 2008; Guo et al., 2008). Membrane flux enhancers (MFE) are a modified cationic polymer capable of reducing membrane fouling. Moreover, aeration energy savings in the range of 20-60% can be achieved due to an enhanced flux and a slightly better oxygen transfer (Yoon and Collins, 2006; Iversen et al., 2009).

In general, all the additives mentioned above may be especially useful to handle the peak flow conditions that could take place in a real wastewater treatment plant.

1.6. Other submerged membrane technologies for wastewater treatment

There are other different membrane technologies that are being developed or applied for wastewater treatment:

- Anaerobic membrane bioreactors (AnMBR) in which methanogenic biomass is used for treating the wastewater
- Biofilm MBR in which the growth of biofilms in carrier particles is promoted.
- Hybrid suspended biomass-biofilms membrane bioreactors, in which both biofilms and suspended biomass grows in the bioreactor.
- Methanogenic-aerobic MBRs, which are a combination of a first methanogenic stage and a second stage in which the remaining COD fraction is treated aerobically in a MBR.

1.6.1. Anaerobic membrane bioreactor (AnMBR)

Anaerobic treatment of domestic wastewater can be very interesting and cost-effective in countries were the priority in discharge control is in removal of organic pollutants. Anaerobic biomass has very low biomass yield, which eliminates one of the crucial disadvantages of aerobic treatment. However, at low temperatures, which would be the case for domestic wastewater treatment, the growing rate of these microorganisms, and thus the capacity for degrading organic compounds diminish. For this reason, it is important to avoid any loss of anaerobic biomass with the treated water. The anaerobic MBR (AnMBR) is a combination of an anaerobic reactor coupled with the membrane unit. According to how the membrane is integrated with the bioreactor, two MBR processes configurations can be identified: submerged and side-stream AnMBR.

Side-stream MBRs involve much higher energy requirements, due to higher operational transmembrane pressures (TMP) and the elevated volumetric flow required to achieve the desired cross-flow velocity. However, side-stream reactors have the advantage that the cleaning operation of membrane modules can be performed more easily in comparison with submerged technology, since membrane extraction from the reactor is needed in the later case. Submerged MBRs involve lower energy needs, but they operate at lower permeate fluxes, since they provide lower levels of membrane surface shear. The latter means higher membrane surface requirements.

The AnMBR has the advantages of aeration-energy savings, possible biogas recovery, and lower sludge production, resulting in competitive capital and operating costs. However, negligible or no total nitrogen or phosphorus removal can be expected from an anaerobic MBR process.

Anaerobic processes are often operated at mesophilic (35 °C) and thermophilic (55 °C) temperatures. However, for wastewaters with a low organic content (e.g., municipal wastewater), the methane production cannot cover the heating requirement and operation would be better under ambient temperatures (An et al., 2009).

Hu and Stuckey (2006) achieved 90% soluble COD removal efficiency at a 3 h HRT with an inlet concentration of 460 $mg \cdot L^{-1}$, using two AnMBR with both, flat sheet and hollow fibber modules. Ho and Sung (2010) investigated the performance of a cross-flow AnMBR treating synthetic municipal wastewater. They achieved more than 95% COD removal, with permeate concentration lower than 40 $mg \cdot L^{-1}$. This demonstrates that the AnMBR can treat low-strength wastewater with similar treatment performance as aerobic MBRs.

One of the main drawbacks of using AnMBR is related with membrane fouling and the maximum operating flux that can be achieved. Instead of using air, part of the biogas obtained in AnMBR can be recirculated in order to alleviate membrane fouling. Most of the authors working with AnMBR reported fluxes in the range of 5-15 $L \cdot m^{-2} \cdot h^{-1}$ at temperatures above 30 °C (Trzcinski and Stuckey, 2009). Jeison and van Lier (2006) obtained critical flux values in the range 16-23 $L \cdot m^{-2} \cdot h^{-1}$ under thermophilic (30 °C), and 5-21 $L \cdot m^{-2} \cdot h^{-1}$ under mesophilic (55 °C) conditions. In the case of domestic wastewater treated at ambient temperatures, operating fluxes are significantly lower. Robles et al. (2013) reported fluxes between 9 and 13 $L \cdot m^{-2} \cdot h^{-1}$ treating municipal wastewaters at temperatures between 15 and 33 ºC. Lew et al. (2009) reported 11.25 $L \cdot m^{-2} \cdot h^{-1}$ at 25 °C, while Wen et al. (1999), operating a laboratory scale anaerobic bioreactor coupled with a membrane filtration worked with flux of 5 $L \cdot m^{-2} \cdot h^{-1}$. Similar results were obtained by Ho and Sung

(2010), who operated with flux set on 5 $L \cdot m^{-2} \cdot h^{-1}$ and the temperature of 15 and 20 °C. Applicable fluxes reported for the treatment of industrial wastewaters in mesophilic submerged AnMBR are generally lower, ranging between 2 and 5 $L \cdot m^{-2} \cdot h^{-1}$ (Skouteris et al., 2012), especially at high biomass concentration (Van Zyl et al., 2008; Spagni et al., 2010). Moreover, Spagni et al. (2010) demonstrated that the applicable fluxes obtained in AnMBR working with high biomass concentration ranged between 2 and 5 $L \cdot m^{-2} \cdot h^{-1}$ depending strongly on operational conditions and rapid membrane fouling was usually observed. Therefore, the fluxes obtained in AnMBR are lower than those observed in aerobic MBR, that are in the range between 20 and 30 $L \cdot m^{-2} \cdot h^{-1}$ (Judd, 2002; Wen et al., 2004).

1.6.2. Biofilm membrane bioreactor (BF-MBR)

Biofilm bioreactors are systems where the growth of the biomass develops over a plastic material. The placement of packing material in the aeration tank of the activated-sludge process dates back to the 1940s with the Hays and Griffith processes. At the beginning of the 70s and extending into the 1980s, a new class of aerobic attached growth process became established alternatives for biological wastewater treatment. These are upflow and downflow packed-bed reactors and fluidized-bed reactors that do not use secondary clarification. Therefore, the most important advantage of this kind of processes is their small footprint. Other advantages are:

- Increased treatment capacity
- Greater process stability
- Reduced sludge production
- Enhanced sludge settleability
- No increase in operation and maintenance costs.

Actually, several synthetic plastic packing materials have been developed for use in activated sludge systems. These packing materials may be suspended in the activated-sludge mixed liquor or fixed in the aeration tank. Although efficient in removing soluble organic matter, biofilm reactors designed as trickling filters or submerged filters using granular media are prone to clogging when the wastewater contains high loads of particulate matter. Consequently, there is a limit to the loading rate that can be applied to such processes, often needing a pretreatment step for particle removal prior to the biofilm unit. The moving-bed-biofilm reactor (MBBR) is an alternative process design which

utilizes the advantages of a biofilm reactor and which at the same time can handle high loads of particles.

The Moving Bed Bioreactor (MBBR) consists in an activated-sludge system where the biomass is attached to the carriers suspended in the mixed liquor. These carriers have a great internal surface in order to improve the optimal contact of liquid, oxygen and biomass. It is in the internal surface where the development of the biofilm takes place. This technology addresses some of the most important challenges of the Water and Wastewater industry, such as the upgrading of existing treatment plants and tight nutrient discharge limits (Frost & Sullivan, 2009).

From the combination of this technology with the technology of membrane bioreactors is born today a new system called Biofilm Membrane Bioreactor (BF-MBR) also called moving bed membrane bioreactor (MBMBR) (Leikness and Ødegaard, 2007) (figure 1.9). This system would present an alternative to the activated sludge MBR by combining a biofilm reactor with membrane separation of the suspended solids (BF-MBR), which may reduce the effect of membrane fouling by high biomass concentrations. The membrane module in this kind of systems is located in a separate chamber from that containing the carriers in order to avoid possible damages in the membrane.

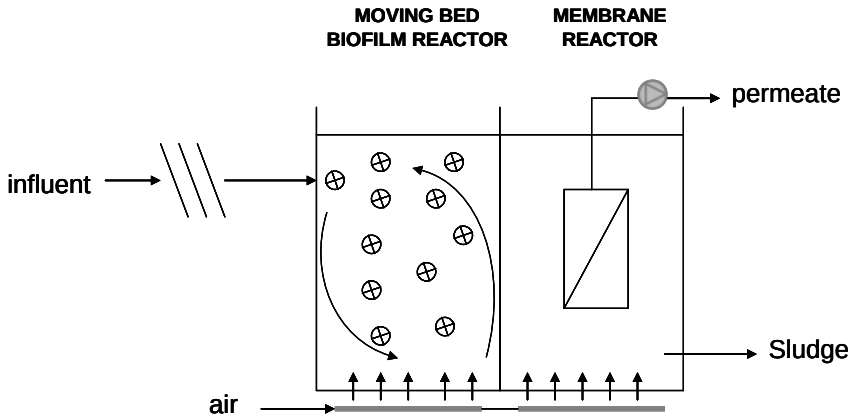

Figure 1.9. Biofilm Membrane Bioreactor (Leikness and Ødegaard, 2007).

BF-MBR process has the potential of operating with volumetric loading rates of 2-8 kg COD·m^{-3}·d^{-1}, higher than those observed in typical MBRs of 1-3 kg COD·m^{-3}·d^{-1}, and HRTs up to 4 h. Sustainable process operation with membrane fluxes around 50 L·m^{-2}·h^{-1} can be achieved in BF-MBR (Leikness and Ødegaard, 2007), which are much higher than

fluxes typically reported, between 20 and 30 $L \cdot m^{-2} \cdot h^{-1}$, in aerobic membrane bioreactors operating with similar membrane modules (Judd, 2002; Wen et al., 2004).

Moreover, The BF-MBR is an alternative strategy to reduce the effect of membrane fouling by high biomass concentrations, particularly under low loading rates.

Many studies have pointed out that the main foulants for membrane fouling are soluble organic polymers such as soluble microbial products (SMP) and extracellular polymers (EPS) (Rosenberger et al., 2006). Attached biomass, such as biofilm can adsorb these soluble organic polymers from the liquid, and therefore can decrease their effect on membrane fouling (Li and Yang, 2007). This tends to explain the reason of the TMP decrease under the assistance of the biomass on the suspended carriers reported in some studies (Leikness and Ødegaard, 2007; Liu et al., 2010).

Nevertheless, it was reported by Yang et al. (2009b) a rate of membrane fouling in MBMBR three times higher than conventional MBR, with higher carbohydrate and protein concentrations. Comparison of composing of suspended solids indicated that the overgrowth of filamentous bacteria resulted in a thick and compact cake layer in MBMBR, affecting negatively to membrane filtration. Therefore, further studies are still needed in order to know best the implications and mechanisms of this technology regarding membrane fouling.

1.6.3. Hybrid biofilm-suspended biomass membrane bioreactors

Hybrid reactors are those systems in which suspended and attached biomass grow in the same system. These systems can achieve high biomass concentrations and solid retention time in comparison with conventional activated sludge systems. Hybrid systems have been successfully used to upgrade the nitrifying capacity in conventional activated sludge (Christensson and Welander, 2004). These systems have been constructed using fixed carriers (Watanabe et al., 1994), suspended support (Christensson and Welander, 2004), and even clay and powders materials. Recently membrane technology has been successfully tested to improve hybrid systems efficiency (Oyanedel et al., 2003; Artiga et al., 2005). Hybrid biofilm-suspended biomass systems have been widely studied and their advantages, in comparison with conventional activated sludge, are well known. A drawback of the above mentioned hybrid systems are those which can arise from the application of high organic load rates, as well as from the physicochemical features of the waste water, which can negatively affect the settleability properties of the sludge which is generated in the biological systems and can therefore negatively affect the separation, by

means of settlers, of solids from the treated water. Most of said hybrid systems use settlers due to which, for certain applications and under certain conditions, their efficiency can be affected by an incorrect separation of the solids from the treated water.

The development of hybrid biofilm-suspended biomass was carried out at the University of Santiago de Compostela during the first's years of 2000's (European Patent 148427 B1, 2002). This system could be considered a combination of both, the typical suspended biomass MBR with and the BF-MBR system, but it was developed several years before than BF-MBR. One of the most important features of the hybrid MBR technology is that biofilm growth take place in the aerobic chamber. Moreover, the growth of nitrifiers in hybrid MBR systems is promoted in the biofilm while heterotrophs are maintained in suspension. Therefore, besides the advantages of high biomass concentration due to the high specific surface area for biofilm growth, the introduction of carriers provides a suitable environment for both aerobic and anoxic microorganisms within the same ecosystem (Watanabe et al., 1992). This can make it possible that simultaneous nitrification-denitrification can occur in the continuously aerated bioreactor. As a result, the total nitrogen removal can be enhanced in this class of processes, as reported by Artiga et al. (2005), Yang et al. (2009a) and Mtinch et al. (2000). The use of membrane filtration units in the hybrid reactor makes it possible to obtain an effluent with low levels of solids in suspension, which would comply with the most demanding dumping requirements of this pollutant, significantly decreases the dumping of microorganisms with the effluent (including pathogens and other health vectors); furthermore, it is suitable for dumping close to marine culture areas or fish hatcheries and collection areas for collecting waters used for irrigation or for producing drinking water.

1.6.4. Methanogenic-aerobic MBRs.

Some authors have tried to overcome the main disadvantage of AnMBR (membrane fouling) by combining methanogenic technology with aerobic MBR technology. Thus, not only membrane fouling would be minimized but also lower biomass production yield, energy savings, high quality effluent and the possibility of nitrogen removal could be achieved. There are various types of configurations combining methanogenesis, aerobic processes and MBRs.

An et al. (2008) and Buntner et al. (2010) (in present work) proposed a combined system consisting of an up-flow anaerobic sludge blanket (UASB) reactor and an aerobic membrane bioreactor (MBR) for the treatment of low-strenght wastewaters. In the case of An et al. (2008) the system operated at 28-30 °C and the MBR sludge was recirculated

into the UASB with a ratio of 50-800%. Nitrogen removal was promoted by recirculating the $N\text{-}NO_X$, produced by nitrification in the MBR, to the UASB. On the other hand, Buntner et al. (2010) operated at ambient temperatures and the MBR sludge was recirculated to the UASB with a ratio of 7.5-15% from a first chamber of the MBR, with biomass growing onto plastic support and in suspension. Nitrogen was not removed using this configuration in the case of Buntner et al. (2010). The results obtained in both of systems were similar with applied fluxes between 12 and 15 $L \cdot m^{-2} \cdot h^{-1}$, and a stable COD removal efficiency of above 98.0%.

Phattaranawik and Leiknes (2010) proposed a hybrid vertical anaerobic sludge–aerated biofilm reactor (HyVAB) coupled with external submerged membrane filtration for municipal wastewater treatment. The HyVAB featured an upper chamber of aerobic biofilm, a lower chamber of anaerobic activated sludge, and a roof-shaped separator located between the chambers, to prevent diffusion of dissolved oxygen to the anaerobic chamber. The lower chamber was used for anaerobic digestion of aerobic sludge waste. Lower COD removal (above 80%) than those obtained by An et al. (2008) and Buntner et al. (2010) were reported. Nevertheless, the applied fluxes were considerably higher (up to 23 $L \cdot m^{-2} \cdot h^{-1}$). Although very low nitrogen removal was observed, denitrification process might be enhanced by recirculating part of the effluent (from the aerobic biofilm chamber or from the membrane chamber) to the anaerobic chamber.

Finally, Zhang et al. (2005) proposed a staged anaerobic and aerobic membrane bioreactor with the anaerobic zone in the bottom part and the membrane module submerged in the aerobic zone, in the upper part of the reactor. Some porcelain carriers were installed in order to prevent the blockade of the orifice between the two parts of the reactor. COD removal efficiencies above 97 % and fluxes in the range of 5-14 $L \cdot m^{-2} \cdot h^{-1}$ were achieved. The anaerobic digestion of COD produced a great amount of methane, which passed to the aerobic zone of the reactor with the wastewater anaerobically digested. The denitrification using methane as carbon source could be integrated with anaerobic methanogensis through the special structure of the reactor used in this work. All ammonium was nitrified and more than 84% of $N\text{-}NO_X$ was then denitrified.

Denitrification using methane as carbon source was also stimulated in the system proposed by Buntner et al. (2010) with some modifications such as the implementation of an anoxic chamber by the elimination of aeration in the first chamber of the MBR and the elimination of the recirculation between the MBR and the UASB. This configuration favoured denitrification process using the dissolved methane present in the effluent of the

UASB, achieving total nitrogen removal percentages up to 60% and removing up to 95% of dissolved methane.

In this sense, it is important to underline that the presence of dissolved methane, especially at low temperature, represents an important environmental problem in terms of greenhouse gas (GHG) emissions of wastewaters treated using methanogenic bioreactors. For low strength wastewaters, dissolved methane might account up to 50% of the produced methane. The dissolved methane is easily desorbed from the effluents, especially if these are either released in the environment or post-treated using aerobic bioreactors.

All the examples mentioned before demonstrate that integration of anaerobic processes (in particular methanogenesis) with simultaneous nitrification and denitrification is possible. Moreover, application of MBR, a very low COD concentration and the level of nutrients in the effluent (e.g. in the case of water reuse in agriculture, nitrogen elimination could be not necessary) allows obtaining a high quality, re-usable effluent, which might conduce to significant water savings.

On the other hand, biogas rich with methane can be produced, depending on the wastewater treated. Relatively high membrane fluxes could be obtained, being higher than those applied in the case of AnMBR, and similar to aerobic MBRs.

1.7. References

Akram, A., Stuckey, D.C. 2008. Flux and performance improvement in a submerged anaerobic membrane bioreactor (SAMBR) using powdered activated carbon (PAC). *Process Biochemistry* **43**, 93-102.

An, Y., Yang, F., Chua, H. C., Wong, F. S., Wu, B. 2008. The integration of methanogenesis with shortcut nitrification and denitrification in a combined UASB with MBR. *Bioresource Technology* **99**, 3714–3720.

An, Y., Wang, Z., Wu, Z., Yang, D., Zhou, Q. 2009. Characterization of membrane foulants in an anaerobic non-woven fabric membrane bioreactor for municipal wastewater treatment, *Chemical Engineering Journal* **155**, 709–715.

Artiga, P., Oyanedel, V., Garrido, J.M., Méndez, R. 2005. An innovative biofilm-suspended biomass hybrid membrane bioreactor for wastewater treatment, *Desalination* **179**, 171-179.

AWWA-Lyonaisse des eaux. 1998. Tratamiento del agua por procesos de membrana. Ed. McGraw-Hill, Madrid, Spain.

Barker DJ, Stuckey DC. 1999. A review of soluble microbial products (SMP) in wastewater treatment systems. *Water Research* **33**, 3063–82.

Brepols, C. 2011. Operating large scale membrane bioreactors for municipal wastewater treatment, 1st ed., *IWA publishing*, London.

Buntner, D., Sánchez, A., Garrido, J.M. 2011. Three stages MBR (methanogenic, aerobic biofilm and membrane filtration) for the treatment of low-strength wastewaters. *Water Science and Technology* **64**(2), 397-402.

Burbano, A. A.,. Adham, S. S., Pearce, W.R. 2007. The state of full-scale RO/NF desalination – Results from a worldwide survey. *Journal AWWA* **99**(4), 116-127.

Cho, B.D., Fane, A.G. 2002. Fouling transients in nominally sub-critical flux operation of a membrane bioreactor. *Journal of Membrane Science* **209**, 391–403.

Chang, I.-S., Lee, C.-H. 1998. Membrane filtration characteristics in membrane-coupled activated sludge system -- the effect of physiological states of activated sludge on membrane fouling. *Desalination* **120**, 221-233.

Chang, I.-S., Le Clech, P., Jefferson, B., Judd, S. 2002 Membrane fouling in membrane bioreactors for wastewater treatment. *Journal of Environmental Engineering* **128**, 1018–1029.

Christensson, M., Welander, T. 2004. Treatment of municipal wastewater in a hybrid process using a new suspended carrier with large surface area. *Water Science and Technology*, **49**(11-12), 207–214.

Citulsky, J.A., Farahbakhsh, K., Kent, F.C. 2009. Effect of total suspended solids loading on short-term fouling in the treatment of secondary effluent by an immersed ultrafiltration pilot system. *Water Environmental Research* **81**(12), 2427-2436.

Côté, P., Siverns, S., Monti, S. 2005. Comparison of Membrane-based Solutions for Water Reclamation and Desalination. *Desalination* **182**, 51–257.

de la Torre, T., Lesjean, B., Drews, A., Kraume, M. 2008. Monitoring of transparent exopolymer particles (TEP) in a membrane bioreactor (MBR) and correlation with other fouling indicators. *Water Science and Technology* **58**(10), 1903-1910.

Drews, A., Vocks, M., Iversen, V., Lesjean, B. Kraume, M. 2005. Influence of unsteady membrane bioreactor operation on eps formation and filtration resistance, Proceedings of International Congress on Membranes and Membrane Processes (ICOM), Seoul, Korea.

Drews, A. 2010. Membrane fouling in membrane bioreactors—Characterisation, contradictions, cause and cures. *Journal of Membrane Science* **363**, 1–28.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith F. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**, 350–356.

European Patent 148427 B1. Hybrid Biological Membrane Reactor for the Treatment of Urban and Industrial Waste Water. Inventors: Oyanedel V., Garrido J.M., Méndez R.. Propietor: University of Santiago de Compostela, Spain. Priority 1 February 2002.

Evans, B., Laughton, P. (1994) Emerging trends in electrical usage at Canadian (Ontario) municipal wastewater treatment facilities and strategies for improving energy efficiency. *Water Science and Technology* **30**(4), 17-23.

Evenblij, H., van der Graaf, J.H. 2004. Occurrence of EPS in activated sludge from a membrane bioreactor treating municipal wastewater. *Water Science and Technology* **50**(12), 293-300.

Frost & Sullivan. 2009. Moving Bed Bioreactor Systems Market Predicted to Grow Five-fold by 2015, *in press*.

Gallagher, P., Nguyen, A., Zha, F. 2008. Wastewater reuse via MBR and tertiary membrane filtration—case studies, Proceedings of the Water Environment Federation Membrane Technology Conference and Exposition, January 27–30, 2008, Atlanta, USA.

Guo, W.S., Vigneswaran, S., Ngo, H.H., Kandasamy, J., Yoon, S., 2008. The role of a membrane performance enhancer in a membrane bioreactor: a comparison with other submerged membrane hybrid systems. *Desalination* **231**(1–3), 305–313.

Ho, J., Sung, S. 2010. Methanogenic activities in anaerobic membrane bioreactors (AnMBR) treating synthetic municipal wastewater. *Bioresource Technology* **101**(7), 2191-2196.

Hu, A.Y., Stuckey, D.C. 2006. Treatment of dilute wastewaters using a novel submerged anaerobic membrane bioreactor. *Journal of Environmental Engineering* **132**, 190-198.

Huisjes, E., Colombel, K., Lesjean, B. 2009. The European MBR market: Specifities and future trends. Final MBR-Network workshop "Salient outcomes of the European R&D projects on MBR technology" 31 March – 1 April 2009 Berlin, Germany.

Iversen, V., Mohaupt, J., Drews, A., Kraume, M., Lesjean, B., 2008. Side effects of flux enhancing chemicals in membrane bioreactors (MBRs): study on their biological toxicity and their residual fouling propensity. *Water Science and Technology* **57**(1), 117–123.

Iversen, V., Koseoglu, H., Yigit, N.O., Drews, A., Kitis, M., Lesjean, B., Kraume, M., 2008. Impacts of membrane flux enhancers on activated sludge respiration and nutrient removal in MBRs. *Water Research* **43**, 822-830.

Jang, N., Ren, X., Choi, K., Kim, I.S. 2005. Comparison of membrane biofouling in nitrification and denitrification for the membrane bio-reactor (MBR), Proceedings of IWA, Aspire, Singapore.

Jeison, D., van Lier, J.B. 2006. Cake layer formation in anaerobic submerged membrane bioreactors (AnSMBR) for wastewater treatment. *Journal of Membrane Science* **284**, 227–236.

Ji, L., Zhou, J. 2006. Influence of aeration on microbial polymers and membrane fouling in submerged membrane bioreactors. *Journal of Membrane Science* **276,** 168– 177.

Ji, J., Qiu, J., Wong, F.-s., Li, Y., 2008. Enhancement of filterability in MBR achieved by improvement of supernatant and floc characteristics via filter aids addition. *Water Research* **42**(14), 3611-3622.

Judd, S.J. 2002. Submerged membrane bioreactors: flat plate or hollow fibre? *Filtration and Separation* **39**(5), 30-31.

Judd, S., Jefferson, B. 2003. Membranes for Industrial Wastewater Recovery and Re-Use. Elsevier Ltd. Oxford, UK.

Judd, S.J. 2011. The MBR Book: Principles and Applications of Membrane Bioreactors for Water and Wastewater Treatment, 2nd ed., Elsevier Science Ltd, Oxford.

Kimura, K., Yamato, N., Yamamura, H., Watanare, Y. 2005. Membrane fouling in pilot-scale membrane bioreactors (MBRs) treating municipal wastewater. *Environmental Science Technology* **39**, 6293.

Koseoglu, H., Yigit, N.O., Iversen, V., Drews, A., Kitis, M., Lesjean, B., Kraume, M., 2008. Effects of several different flux enhancing chemicals on filterability and fouling reduction of membrane bioreactor (MBR) mixed liquors. *Journal of Membrane Science* **320**(1–2), 57–64.

Le-Clech, P., Jefferson, B. Judd, S.J. 2005. A comparison of submerged and sidestream tubular membrane bioreactor configurations. *Desalination* **173**, 113–122.

Lee, J., Ahn, W.-Y., Lee, C.-H. 2001 Comparison of the filtration characteristics between attached and suspended growth microorganisms in submerged membrane bioreactor. *Water Research* **35**, 2435–2445.

Leiknes, T., Ødegaard, H. 2007. The development of a biofilm membrane bioreactor, *Desalination,* **202**, 135-143.

Lesjean, B., Rosenberger, S., Schrotter, J.C. 2004 Membrane aided biological wastewater treatment - an overview of applied systems. *Membrane Technology*, August 2004, 5–10.

Lesjean, B., Rosenberger, S., Laabs, C., Jekel, M., Gnirss, R., Amy, G. 2005. Correlation between membrane fouling and soluble/colloidal organic substances in membrane bioreactors for municipal wastewater treatment, *Water Science and Technology* **51**, 1-8.

Lew, B., Tarreb, S., Beliavski, M., Dosoretzb, C., Green, M. 2009. Anaerobic membrane bioreactor (AnMBR) for domestic wastewater treatment. *Desalination* **243**, 251–257.

Li, X.Y., Yang, S.F. 2007. Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. *Water Research* **41**, 1022–1030.

Li, N.N., Fane, A.G., Winston Ho, W.S., Matsuura, T. 2008. Advanced membrane technology. John Wiley and sons Inc. publications, New Jersey, USA.

Liu, Q.S., Liu, Y., Tay, S.T.L., Show, K.Y., Ivanov, V., Benjamin, M., Tay, J.H. 2005. Startup of pilot-scale aerobic granular sludge reactor by stored granules. *Environmental Technology* **26***(12),* 1363-1369.

Liu, Q., Wang, X.C., Liu, Y., Yuan, H., Du, Y. 2010. Performance of a hybrid membrane bioreactor in municipal wastewater treatment, *Desalination* **258**, 143-147.

Lubello, C., Gori, R., de Bernardinis, A.M., Simonelli, G. 2003. Ultrafiltration as tertiary treatment for industrial reuse. *Water Science and Technology: Water supply* **3**, 161-168.

Meng, F., Chae, S.R., Drews, A., Kraume, M., Shin, H.S., Yang, F. 2009. Recent advances in membrane bioreactors (MBRs): Membrane fouling and membrane material. *Water Research* **43**, 1489-1512.

Metcalf & Eddy. 2003. Wastewater Engineering: Treatment and Reuse, 4th ed., McGraw-Hill, New York, USA.

Mtinch, E.V., Ban, K., Watts, S., Keller, J. 2000. Suspended carrier technology allows upgrading high rate activated sludge plants for nitrogen removal via process intensification. *Water Science and Technology* **41**(4–5), 5–12.

Nagaoka, H., Ueda, S., Miya, A., 1996. Influence of bacterial extracellular polymers on the membrane separation activated sludge process. *Water Science and Technology* **34**(9), 165–172.

Nagaoka, H., Yamanishi, S., Miya, A. 1998. Modeling of biofouling by extracellular polymers in a membrane separation activated sludge system. *Water Science and Technology* **38**(4–5), 497–504.

Oyanedel, V., Garrido, J.M., Lema, J.M., Méndez R. 2003. A membrane assisted hybrid bioreactor for the post treatment of an anaerobic effluent from a fish canning factory. *Water Science and Technology* **48**, 301-309.

Passow, U. 2002. Transparent exopolymer particles (TEP) in aquatic environments. *Progress in oceanography* **55**, 287-333.

Pearce, G. 2010. Membrane system design, in: M. Wilf, The Guidebook to Membrane Technology for Wastewater Reclamation, *Desalination Publications*, Hopkinton (MA), USA, 187-245.

Phattaranawik, J., Leiknes, T. 2010. Study of Hybrid Vertical Anaerobic Sludge-Aerobic Biofilm Membrane Bioreactor for Wastewater Treatment. *Water Environment Research* **82**(3), 273-280.

Remy, M., Potier, V., Temmink, H., Rulkems, W. 2009. Why low powdered activated carbon addition reduces membrane fouling in MBRs. *Water Research* **44**, 861-867.

Robles, A., Ruano, M.V., Ribes, J., Ferrer, J. 2013. Factors that affect the permeability of comercial hollow-fibre membranes in a submerged anaerobic MBR (HF-SAnMBR) system. *Water Research* **47**, 1277-1288.

Rosenberger, S., Kraume, M. 2002. Filterability of activated sludge in membrane bioreactors. *Desalination* **146**(1-3), 373-379.

Rosenberger, S., Evenblij, H., te Poele, S., Wintgens, T., Laabs, C. 2005. The importance of liquid phase analyses to understand fouling in membrane assisted activated sludge processes -six case studies of different European research groups. *Journal of Membrane Science* **263**, 113–126.

Rosenberger, S., Laabs, C., Lesjean, B., Gnirss, R., Amy, G., Jekel, M., Schrotter, J.-C. 2006. Impact of colloidal and soluble organic material on membrane performance in membrane bioreactors for municipal wastewater treatment. *Water Research* **40**, 710 – 720.

Rushton, A., Ward, A.S., Holdich, R.G. 1996. Solid-Liquid filtration and separation technology. 1st ed., VCH, Weinheim, Gemany.

Sánchez, A., Garrido, J.M., Méndez, R. 2011. Tertiary membrane filtration of an industrial wastewater using granular or flocculent biomass sequencing batch reactors. *Journal of Membrane Science* **382**, 316-322.

Santos, A., Ma, W., Judd, S.J. 2011. Membrane bioreactors: Two decades of research and implementation. *Desalination* **273**(1), 148-154.

Skouteris, G., Hermosilla, D., López, P., Negro, C., Blanco, A. 2012. Anaerobic membrane bioreactors for wastewater treatment: A review. *Chemical Engineering Journal* **198–199**(0), 138–148.

Smith, C. W., Di Gregorio, D., Taleott, R. M. 1969. The use of ultrafiltration membrane for activated sludge separation. In: Proceeding of the 24th Annual Purdue Industrial Waste Conference. Purdue University, West Lafayette, Indiana. 1300-1310.

Spagni, A., Casu, S., Crispino, N.A., Farina, R., Mattioli, D. 2010. Filterability in a submerged anaerobic membrane bioreactor. *Desalination* **250**, 787-792.

Stephenson, T., Judd, S., Jefferson, B., Brindle, K. 2000. Membrane Bioreactors for Wastewater Treatment, IWA Publishing, London.

Sun, D.D., Khora, S.L., Haya, C.T., Leckieb, J.O. 2007. Impact of prolonged sludge retention time on the performance of a submerged membrane bioreactor. *Desalination* **208**, 101–112.

Sun, F.-Y., Wang, X.-M., Li, X.-Y. 2008. Visualisation and characterisation of biopolymer clusters in a submerged membrane bioreactor. *Journal of Membrane Science* **325**, 691–697.

Tchobanoglous, G., Darby, J., Bourgeous, K., McArdle, J., Genest, P., Tylla, M. 1998. Ultrafiltration as an advanced tertiary treatment process for municipal wastewater. *Desalination* **119**, 315-322.

Teli, A., Antonelli, M., Bonomo, L., Malpei, F. 2012. MBR fouling control and permeate quality enhancement by polyaluminium chloride dosage: a case study. *Water Science and Technology* **66**(6), 1289-95.

The MBR site. *http://www.thembrsite.com.*

Trzcinski, A.P., Stuckey, D.C. 2009. Anaerobic digestion of the organic fraction of municipal solid waste in a two-stage membrane process. *Water Science and Technology* **60**(8), 1965-1978.

Van Zyl, P. J., Wentzel, M. C., Ekama ,G. A., Riedel, K. J. 2008. Design and start-up of a high rate anaerobic membrane bioreactor for the treatment of a low pH, high strength,dissolved organic waste water. *Water Science and Technology* **57**(2), 291-296.

Wang, X.-M., Li, X.-Y. 2008. Accumulation of biopolymer clusters in a submerged membrane bioreactor and its effect on membrane fouling. *Water Research* **42**, 855-862.

Watanabe, Y., Masuda, S., Ishiguro, M., 1992. Simultaneous nitrification and denitrification in micro-aerobic biofilms. *Water Science and Technology* **26**(3-4), 511–522.

Watanabe, S., Bang, D.Y., Itoh, K., Matsui, K. 1994. Nitrogen removal from wastewater by bioreactorwith partially and fully submerged rotating biofilm, *Water Science and Technology* **29**, 431-438.

Wen, X., Bu, Q., Huang, X. 2004. Study on fouling characteristic of axial hollow fibers cross-flow microfiltration under different flux operations, in: *Proceedings of the Water Environment-Membrane Technology Conference, Seoul, Korea*.

Yang, S., Yang, F., Fu, Z., Lei, R. 2009a. Comparison between a moving bed membrane bioreactor and a conventional membrane bioreactor on organic carbon and nitrogen removal. *Bioresource technology* **100**, 2369-2374.

Yang, S., Yang, F., Fu, Z., Lei, R. 2009b. Comparison between a moving bed membrane bioreactor and a conventional membrane bioreactor on membrane fouling. *Bioresource technology* **100**, 6655-6657.

Yoon, S.-H., Collins, J.H. 2006. A novel flux enhancing method for membrane bioreactor (MBR) process using polymer. *Desalination* **191**, 52-61.

Zhang, D.J., Lu, P.L., Long, T.R., Verstraete, W. 2005. The integration of methanogensis with simultaneous nitrification and denitrification in a membrane bioreactor. *Process Biochemistry* **40**(2), 541-547.

Zhang, H.-F., Sun, B.-S., Zhao, X.-H., Gao, Z.-H., 2008c. Effect of ferric chloride on fouling in membrane bioreactor. *Separation and Purification Technology* **63**(2), 341-347.

Zheng, X., Ernst, M., Jekel, M. 2011. Stabilizing the performance of ultrafiltration in filtering tertiary effluent – Technical choices and economical comparisons. *Journal of Membrane Science* **366**, 82-91.

Chapter 2

Material and methods

Summary

In this chapter, the analytical methods used in this work are described. It comprises the conventional parameters used for the wastewater (organic matter, nitrogen and phosphorous compounds, pH, dissolved oxygen, solids and carbon compounds concentrations) and the biomass characterisation.

Most of the conventional parameters measured in both the liquid and the solid phase such as chemical oxigen demand (COD), nitrite, nitrate, phosphate and mixed liquor (total and volatile) suspended solid (MLTSS and MLVSS) concentrations were determined following Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WPCF, 1998). Nevertheless, all the procedures are described in detail throughout this chapter.

Especial attention is given to the methodology related with the performance of the membrane. Conventional parameters such as flux or permeability are described, but also theoretical explanation of the resistance to filtration determination. Moreover, methodology related with the determination of different biopolymers concentration, measured as soluble microbial products (SMP), extracellular polymeric substances (EPS), transparent exopolymer particles (TEP) and biopolymer clusters (BPC), is described in detail.

The specific analytical methods used in a single part of the work are described in the corresponding chapters, as well as the corresponding experimental set-up.

2.1. Liquid phase

In this section, the methods used for the determination of the conventional parameters of wastewater and sludge are described. For soluble fraction analysis, the samples were previously filtered using nitrocellulose membrane filters (HA, Millipore) with a pore size of 0.45 μm in order to remove suspended solids.

2.1.1. Carbon compounds

2.1.1.1. Chemical oxygen demand (COD)

The chemical oxygen demand (COD) is defined as the amount of a specified oxidant that reacts with the sample under controlled conditions. The quantity oxidant consumed is expressed in terms of its oxygen equivalence. Because of its unique chemical properties, the dichromate ion is the specified oxidant. A catalyst (silver sulphate) in acid medium is used to improve the oxidation of some organic compounds. After digestion, the remaining unreduced $K_2Cr_2O_7$ is titrated with ferrous ammonium sulphate to determine the amount of $K_2Cr_2O_7$ consumed, being the amount of oxidable matter calculated in terms of oxygen equivalent. Both ortganic and inorganic components of a sample are subject to oxidation, but in most cases the organic content predominates and is of the greater interest (APHA-AWWA-WPCF, 1998). The total and soluble chemical oxygen demand (COD_t and COD_s) were determined following the method described by Soto et al. (1989), which is a modification from the method 5220C of the Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WPCF, 1985). The difference between total and soluble COD is that COD_t is determined using the raw sample, while for COD_s determination, the sample is previously filtered through nitrocellulose membrane filters (HA, Millipore) with a pore size of 0.45 μm.

Reagents preparation

a) Standard potassium dichromate digestion solution: 10.216 g of $K_2Cr_2O_7$ and 33 g of $HgSO_4$ are dissolved in 500 mL of distilled water. Then, 167 mL of concentrated H_2SO_4 are added. The solution is cooled to room temperature and, finally, diluted to 1000 mL. A dilution 1:2 of this solution was used for COD concentration determination below 100 $mg \cdot L^{-1}$.

b) Sulphuric acid reagent: 10.7 g of Ag_2SO_4 are added to 1 L of concentrated H_2SO_4. The solution is used after 2 days of preparation.

c) Ferroin indicator solution: 1.485 g of $C_{18}H_8N_2 \cdot H_2O$ (phenanthroline monohydrate) and 0.695 g of $SO_4Fe \cdot 7H_2O$ are dissolved in 100 mL of distilled water.

d) Standard potassium dichromate solution 0.05 N. 1.226 g of $K_2Cr_2O_7$, previously dried at 105ºC for 2 hours, are dissolved in 500 mL of distilled water.

e) Standard ferrous ammonium sulphate titrant (FAS) 0.035 N: 13.72 g of $Fe(NH)_4(SO)_2 \cdot 6H_2O$ are dissolved in distilled water. Then, 20 mL of concentrated H_2SO_4 are added and, finally, the solution is cooled and diluted to 1000 mL. A FAS solution concentration of 0.016 N was used for COD concentration determination below 100 $mg \cdot L^{-1}$.

Determination procedure

This procedure is applicable to samples with COD concentrations between 90-900 $mg \cdot L^{-1}$. COD values of 100 $mg \cdot L^{-1}$ or less can be determined by using a more dilute dichromate digestion solution and a more dilute FAS titrant. Place 2.5 mL of sample in 10-mL Pyrex tubes. Add 1.5 mL of digestion solution and 3.5 mL of sulphuric acid reagent slowly on the wall of the tube slightly inclined (to avoid mixing). A blank sample using distilled water is prepared in the same way. This blank acts as “reference”, representing the COD of the distilled water. After being sealed with Teflon and tightly capped, the tubes are finally mixed and placed in the block digester (HACH 16500-100) preheated to 150ºC. The duration of the digestion period is 2 h.

After digestion, the tubes are cooled to room temperature. Then, the content of the tubes is transferred to a beaker and, once added 1-2 drops of ferroin indicator, the solution is titrated under rapid stirring with standard FAS. The FAS solution is standardised daily as follows: Put 5 mL of distilled water into a small beaker. Add 3.5 mL of sulphuric acid reagent. Cool to room temperature and add 5 mL of standard potassium dichromate solution (0.05 N). Add 1-2 drops of ferroin indicator and titrate with FAS titrant. The end-point is a sharp colour change from blue-green to reddish brown. Molarity of FAS solution is calculated with the following equation 2.1:

$$M_{fas} = \frac{5 \cdot 0.05}{V_{fas}} \qquad \text{eq. 2.1}$$

where:

M_{fas}: molarity of FAS (mol·L^{-1}), and

V_{fas}: volume of FAS consumed in the titration (mL).

The COD is calculated with the following equation 2.2:

$$COD = \frac{(A-B) \cdot 8000 \cdot M_{fas}}{V} \quad \text{eq. 2.2}$$

where:

COD: chemical oxygen demand (mg O_2·L^{-1}),

A: mL of FAS consumed by the blank,

B: mL of FAS consumed by the sample,

M_{fas}: molarity of FAS (mol·L^{-1}), and

8000: milliequivalent weight of oxygen x 1000 mL·L^{-1}.

V: mL of sample

Interferences

The most common interferent is the chloride ion. Chloride reacts with silver ion to precipitate silver chloride, and thus inhibits the catalytic activity of silver. Bromide and Iodide can interfere similarly.

2.1.1.2. Total dissolved carbon (TDC), dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC)

The organic carbon in water and wastewater is composed of a variety of organic compounds in different oxidation states. Some of this carbon compounds can be oxidised further by biological or chemical processes and the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) may be used to characterise these fractions. Total organic carbon (TOC) is a more convenient and direct expression of total organic content than COD, but does not provide the same information. Unlike 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 COD (APHA-AWWA-WPCF, 1998). 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. In this case, the DOC

concentration was measured since the equipment employed only could analyze filtered samples. DOC concentration was determined by a Shimadzu analyzer (TOC-5000) as the difference between TDC and DIC concentrations. The instrument is connected to an automated sampler (Shimadzu, ASI-5000-S). The TDC concentrations are 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 DIC concentrations are 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 $mL \cdot min^{-1}$. A curve comprising 4 calibration points in the range of 0 to 1 $gC \cdot L^{-1}$, using potassium phthalate as standard for TDC and a mixture of sodium carbonate and bicarbonate ($Na_2CO_3/NaHCO_3$, 3:4 w/w) for DIC, is used for the quantification (figure 2.1). The detection limit of the equipment is 2 $mg \cdot L^{-1}$.

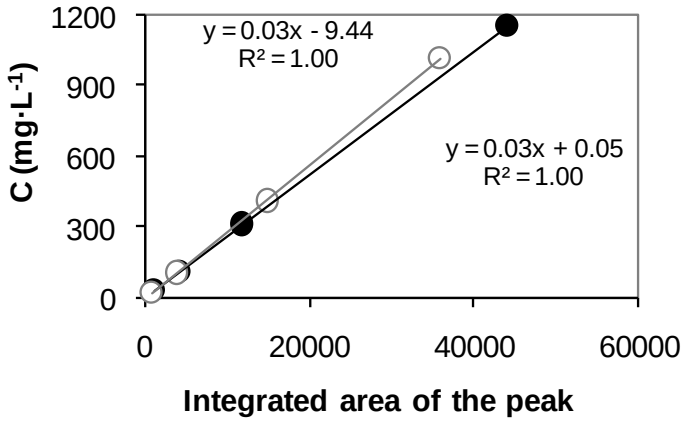

Figure 2.1. Example of a calibration curve to determine TDC (●) and DIC (○) concentrations.

2.1.1.3. Volatile fatty acids (VFA)

Volatile fatty acids (VFA) are fatty acids with a carbon chain of six carbons or fewer, such as acetic, propionic, i-butyric, n-butyric, i-valeric and n-valeric, which are intermediate products of the anaerobic digestion. The measurement of VFA concentration is commonly used as a control test for anaerobic digestion since a VFA accumulation reflects a kinetic disequilibrium between the acids producers and the acids consumers (Switzembaum et al., 1990) and it is an indicator of process destabilization.

VFA are determined by gas chromatography (HP, 5890A) equipped with a flame ionization detector (FID) and an automatic injector (HP, 7673A). The determination is performed in a glass column (3 m long and 2 mm of internal diameter) filled with chromosorb WAW (mesh 100/120) impregnated with NPGA (25%) and H_3PO_4 (2%). The column, injector and detector temperatures are 105, 260 and 280°C, respectively. Gas N_2, previously saturated with formic acid before entering into the injector, is used as carrier gas with a flow of 24 mL·min^{-1}. Air and H_2 are used as auxiliary gases with flows of 400 and 30 mL·min^{-1}, respectively. VFA, after being separated in the column according to their molecular weights, are burnt in a H_2-air flame and finally measured in the FID at 280°C. The quantification of the sample is made with a 6-8 point calibration curve for each acid in the range of 0-1 g·L^{-1}, using pivalic acid as internal standard (figure 2.2). The detection limit of the equipment is 20 mg·L^{-1}.

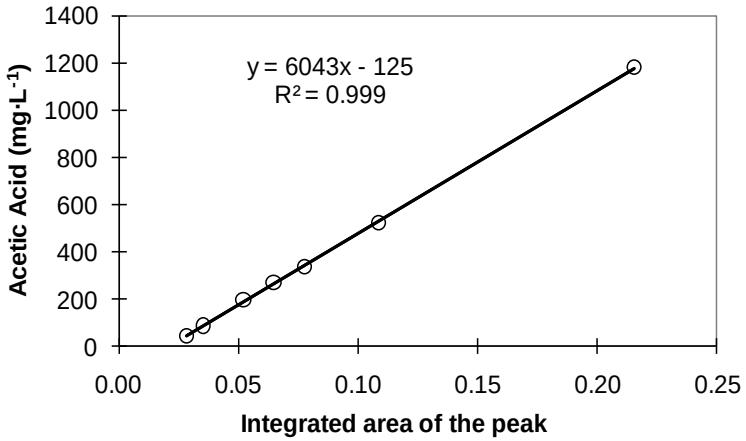

Figure 2.2. Example of a calibration curve for the acetic acid.

2.1.2. Nitrogen compounds

2.1.2.1. Ammonium

The two major factors that influence the ammonia determination method are the concentration and the presence of interferences, such as chlorine. For the determination of low ammonia concentration without the presence of interferents, a colorimetric method (Wheatherburn, 1967) is used. This method base on the reaction of NH_3 with HClO and phenol, forming a strong-blue compound (indophenol) which can be colorimetrically determined using a spectrophotometer (Cecil CE 7200) at 635 nm.

Reagents preparation

a) Solution 1: Phenol-nitroprusside: 15 g of phenol and 0.05 g of sodium nitroprusside are added to 250 mL of buffer solution. The buffer solution was prepared adding 30 g of $Na_3PO_4 \cdot 12\ H_2O$, 30 g $Na_3C_6H_5O_7 \cdot 2H_2O$ and 3 g EDTA per litre, 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.

Determination procedure

Place 2.5 mL of sample (diluted if necessary to get a maximum concentration of 1 mg NH_4^+-N·L^{-1}) and add, 1.0 and 1.5 mL of solution 1 and 2, respectively. After waiting 45 min at room temperature, the concentration of NH_4^+-N is measured in a spectrophotometer at 635 nm. The quantification is done with a 5-7 points calibration curve in the range of 0-1 mg NH_4^+-N·L^{-1}, using NH_4Cl as standard (figure 2.3). Free ammonia was calculated according to the method by Anthonisen et al. (1976).

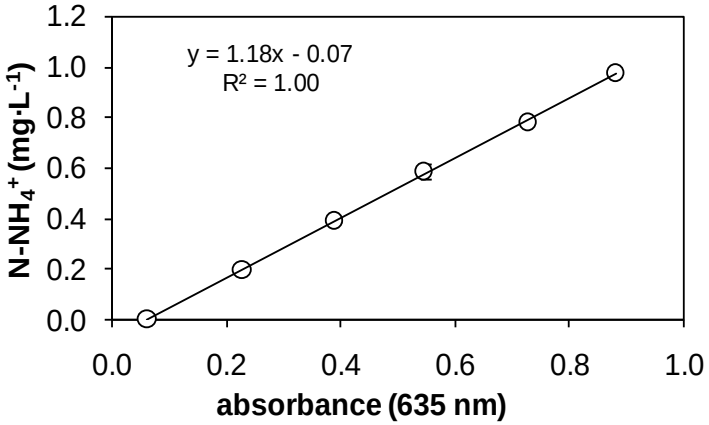

Figure 2.3. Example of a calibration curve for ammonium concentration determination.

Interferences

Residual chlorine reacts with ammonia and should be removed by sample pre-treatment. The determination should be promptly made on fresh samples in order to avoid bacterial conversions of NH_4^+. At least filtration of the samples should be done immediately after collection.

2.1.2.2. 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, 1998).

Nitrite is determined through the formation of a reddish purple azo dye produced at pH 2.0 to 2.5 by coupling diazotized sulphanilamide with N-(1-napththyl)-ethylenediamine dihydrochloride (NED dihydrochloride). The applicable range of the method for spectrophotometric measurements is 10 to 1000 µgN-NO_2^-·L^{-1}.

Reagents preparation

a) Sulphanilamide: 10 g of sulphanilamide are dissolved in 100 mL of concentrated HCl and 600 mL of distilled water. After cooling, the volume is filled up to 1 L with distilled water.

b) NED: 0.5 g of NED is dissolved in 500 mL of distilled water.

Determination procedure

To 5 mL of sample (diluted if necessary to fit the concentration range of the method), 0.1 mL of each solution (sulphanilamide and NED) are added. After waiting 20 min for colour stabilisation, the sample is measured in a spectrophotometer (Cecil CE 7200) at 543 nm. The quantification is done with 6-8 points calibration curve in the range of 0-0.25 mg N-NO_2^-·L^{-1}, using $NaNO_2$ as standard (figure 2.4).

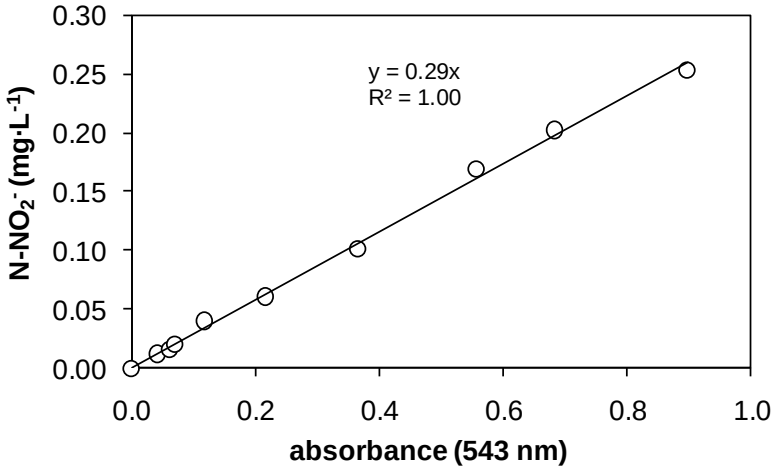

Figure 2.4. Example of a calibration curve for nitrite concentration determination.

Interferences

Chemical incompatibility makes it unlikely that NO_2^-, free chlorine and nitrogen trichloride (NCl_3) will coexist. NCl_3 imparts a false red colour when colour reagent is added. The following ions interfere because of precipitation under test conditions and should be absent: Sb^{3+}, Au^{3+}, Bi^{3+}, Fe^{3+}, Pb^{2+}, Hg^{2+}, Ag^+, chloroplatine and metavanadate. Moreover, cupric ion may cause low results by catalizing decomposition of the diazonium salt.

The determination should be promptly made on fresh samples in order to avoid bacterial conversions of NO_2^-. At least filtration of the samples should be done immediately after collection.

2.1.2.3. 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, 1998).

Measurement of UV absorption at 220 nm enables rapid determination of NO_3^- ions. Because dissolved organic matter also may absorb at 220 nm and NO_3^- does not absorb at 275 nm, a second measurement at 275 nm is used to correct the NO_3^- value. If correction value is more than 10% of the reading at 220 nm, this method should not be used.

Determination procedure

Place 5 mL of sample (diluted if necessary to get a maximum concentration of N-NO_3^- of 2.5 mg·L^{-1}) and add 0.1 mL of HCl 1N. Afterwards, the absorbance at 220 and 275 nm is measured in a spectrophotometer (Cecil CE 7200) with quartz or matched silica cells of 1 cm or longer light path. The absorbance related to nitrate is obtained by subtracting two times the absorbance reading at 275 nm from the reading at 220 nm according to equation 2.3. The quantification is done with a 6-8 points calibration curve in the range of 0-17.5 mg N-NO_3^- ·L^{-1}, using KNO_3 as standard (figure 2.5).

$$mgN - NO_3^- \cdot L^{-1} = a \cdot (A_{220nm} - 2 \cdot A_{275nm}) + b \qquad \text{eq. 2.3}$$

where A_{220nm} and A_{275nm} are the absorbances at 220 and 275 nm, respectively, a is the slope of the calibration curve and b is the intercept.

Interferences

Dissolved organic matter, surfactants, NO_2^- and Cr^{6+} interfere with NO_3^- determination. Moreover, various inorganic ions such as chlorite and chlorate may

interfere. The determination should be promptly made on fresh samples in order to avoid bacterial conversions of NO_2^-. At least filtration of the samples should be done immediately after collection. For longer storage of unchlorinated samples (more than two days), preserve with 2 mL conc. $H_2SO_4 \cdot L^{-1}$ and store at 4 ºC. When sample is preserved with acid, NO_3^- and NO_2^- cannot be determined as single species.

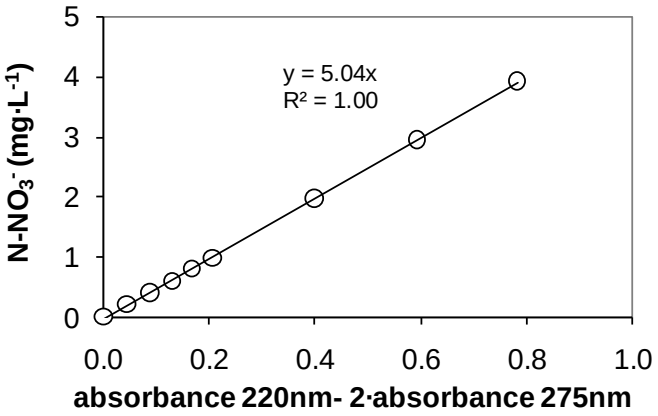

Figure 2.5. Example of a calibration curve for nitrate concentration determination.

2.1.2.4. Dissolved total nitrogen (DTN), dissolved organic nitrogen (DON) and dissolved inorganic nitrogen (DIN)

DTN was determined in a total organic nitrogen analyzer (Rosemount-Dohrmann DN-1900) equipped with a quimioluminiscence detector with two channels. One channel determines the DTN, by oxidation at high temperature, and the other determines the DIN, by a chemical reduction. DON is determined as the difference between DTN and DIN.

All the nitrogen present in the water is catalytically oxidised to nitrous oxide (NO). The process for DTN determination occurs in two steps. The first step is a catalytic (Cu as catalyst) oxidation 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 DIN 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 releases a proton, from which the determination of DTN and DIN is carried out by quimioluminiscence, using a multiplicator tube. The instrument is calibrated with a certified standard solution (KNO_3, 20 mg $N \cdot L^{-1}$) using a response factor method.

2.1.3. Phosphorus compounds

2.1.3.1. Orthophosphates

Orthophosphate concentration in wastewater is determined following the method 4500-P-E described in Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WPCF, 1998).

Ammonium molybdate and antimony potassium tartrate react with orthophosphate in acid medium to form phosphomolybdic heteropolyacid. This compound is reduced by ascorbic acid into molybdate blue.

Reagents preparation

Reagent A: Sulphuric acid 5N.

Reagent B: Solution of antimony potassium tartrate. 1.3715 g of $K(SbO)C_4H_4O_6 \cdot 0.5H_2O$ are dissolved in 500 mL of distilled water. This solution must be kept in a bottle with glass top in order to be preserved.

Reagent C: Solution of ammonium molybdate. 20 g of $(NH_4)6Mo_7O_{24} \cdot 4H_2O$ are dissolved in 500 mL of distilled water. This solution must be kept in a bottle with glass top in order to be preserved.

Reagent D: Ascorbic acid 0.01M. This solution is stable for one week.

Combined reagent: To prepare 100 mL of the combined reagent, the reagents A to D are mixed according to the following volumes: 50 mL of reagent A, 5 mL of reagent B, 15 mL of reagent C and 30 mL of reagent D. The mixture must be stirred after the addition of each reagent, following the mentioned order. This combined reagent is stable for 4 hours.

Determination procedure

A sample of 5 mL is taken and one drop of phenolphthalein indicator solution (0.5-1 g phenolphthalein in 1 L of ethanol at 80% concentration) is added. If red color appears, reagent A (H_2SO_4 5N) is added (drops) until the red color disappears. Then, 0.8 mL of the combined reagent is added and the mixture is stirred with a vortex stirrer. After 10 minutes but before 30 minutes, the absorbance at 880 nm is measured with a spectrophotometer Cecil CE 7200. The quantification is done with a 6-8 points calibration curve in the range of 0-1 mg $P\text{-}PO_4^{3-} \cdot L^{-1}$, using KH_2PO_4 as standard (figure 2.6).

Interferences

Concentrations of arsenates as low as 0.1 $mg \cdot L^{-1}$ react with the molybdate reagent to produce a blue color similar to that formed with phosphate. Hexavalent chromium and NO_2^- interfere to give results about 3% low at concentrations of 1 $mg \cdot L^{-1}$ and 10 to 15% low at 10 $mg \cdot L^{-1}$. Filtration of the samples should be done immediately after collection.

2.1.3.2. Total phosphorus

Because phosphorus may occur in combination with organic matter, in order to analyze the soluble total phosphorus, the sample is digested to hydrolyze the polyphosphates to orthophosphate and then this latter compound can be measured with the previously described colorimetric method.

A sample of 50 mL is taken and one drop of phenolphthalein indicator solution is added. If red color appears, some drops of reagent A (H_2SO_4 5N) are slowly added until the red color disappears. Then, 1 mL of H_2SO_4 solution (300 mL of concentrated H_2SO_4 diluted to 1 L with distilled water) and 0.4 g of solid $(NH_4)_2S_2O_8$ are added. The mixture is gently boiled in an electric heater during 30-40 min in order to have a final volume about 10 mL. Organo-phosphorous compounds like AMP may need up to 1.5-2 h to be completely digested. The mixture is cooled and diluted to 30 mL with distilled water. A drop of phenolphthalein indicator solution is added and the mixture is neutralized with NaOH 1N till pale pink color is obtained. Then the phosphorus concentration is determined with the colorimetric method previously described for orthophosphate

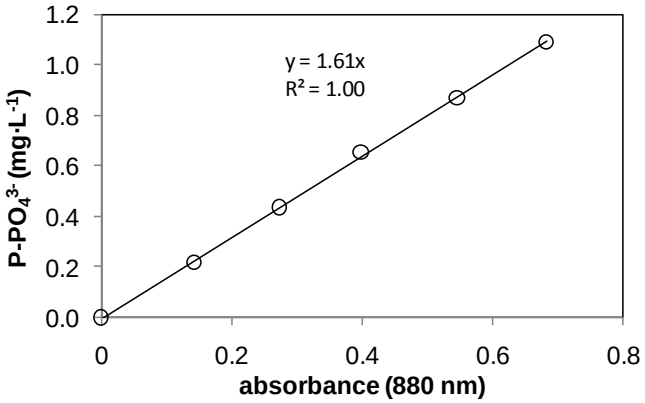

Figure 2.6. Example of a calibration curve for orthophosphate concentration determination.

2.1.4. Other control parameters

2.1.4.1. pH

The pH is one of the key parameters measured in wastewater treatment systems, since its control is important to maintain the biological activity of the microorganisms involved in the treatment process. The pH measurements were performed with different electrodes (Crison Instruments, S.A., 52-03). The sensibility of the instrument is ± 1 mV, corresponding to 0.01 pH units. The electrodes are calibrated at room temperature with two standard buffer solutions of pH 7.02 and 4.00.

2.1.4.2. Dissolved oxygen

Different dissolved oxygen probes (AQUALITYC, model OXI-921 and WTW, model OXY-3401) connected to a meter (M-Design Instruments TM-3659) was used to control DO concentration in the reactor.

2.1.4.3. Temperature

The oxygen probes previously mentioned were equipped with a thermo par that allowed to measure temperature.

2.1.4.4. Alkalinity and alkalinity ratio

Alkalinity of water is its acids-neutralization capacity it is the sum of all the titratable bases and its value may vary significantly with the end-point pH used. Alkalinity is significant in many uses and treatments of natural waters and wastewaters. Because the alkalinity of many surface waters is primarily a function of carbonate, bicarbonate and hydroxide content, it is taken as an indication of the concentration of these constituents. The measured values may include contributions from borates, phosphates, silicates, or other bases. Alkalinity measurements are used in the interpretation and control of water and wastewater treatment process, such as anaerobic digestion. A typical symptom of the abnormal operation of an anaerobic reactor is the increase of the organic acids concentration, which occurs when their production exceeds their consumption.

Total alkalinity (TA) can be considered, approximately, as a sum of the alkalinity due to the presence of bicarbonate and volatile fatty acids (VFA), expressed as $mg \cdot L^{-1}$ equivalent of $CaCO_3$. Partial alkalinity (PA), measured by the titration till pH 5.75, corresponds to the alkalinity of bicarbonate (Jenkins et al., 1983), while the intermediate

alkalinity (IA), which is the difference between TA (titration till pH 4.3) and PA, represents – in an approximate form – the alkalinity due to the VFA concentration (Ripley et al., 1986).

Various authors established that the relation between IA and TA is an adequate parameter of the anaerobic digestion process, and should not exceed the value of 0.3 (Ripley et al., 1986; Switzembaum et al., 1990; Soto et al., 1993; Wentzel et al., 1994) to avoid the accumulation of the VFA in the system.

Determination of the alkalinity is performed according to the method 2320 of APHA-AWWA-WPCF (1998) and consists of the titration of the centrifuged or filtrated sample with H_2SO_4 (with titrated normality) at two points of pH: 5.75 (which corresponds to the partial alkalinity) and 4.30 (which corresponds to the total alkalinity).

Values of the alkalinity are expressed as mg $CaCO_3 \cdot L^{-1}$ and are calculated as follows (APHA-AWWA-WPCF, 1998):

$$PA = A \cdot N \cdot 50000/V \qquad \text{eq. 2.4}$$

$$TA = B \cdot N \cdot 50000/V \qquad \text{eq. 2.5}$$

being:

V: volume of the sample (25 mL)

N: normality of H_2SO_4

A: volume of H_2SO_4 (mL) necessary to reach pH 5.75

B: volume de H_2SO_4 (mL) necessary to reach pH 4.3

2.2. Solid phase: Sludge characterisation

2.2.1. Mixed liquor total suspended solids (MLTSS) and mixed liquor volatile suspended solids (MLVSS) concentration

Solids present in water can be organic or inorganic. Mixed liquor total solids (MLTS) is the term applied to the material residue left in the vessel after evaporation of a sample and its subsequent drying in an oven at a defined temperature. MLTS includes mixed liquor total suspended solids (MLTSS), the portion of MLTS retained by a filter, and dissolved solids, the portion that passes thorough the filter. Mixed liquor volatile solids (MLVS) and mixed liquor volatile suspended solids (MLVSS) are the fraction of MLTS and MLTSS, respectively that are loss on ignition at a specified temperature. The determination of MLVSS concentration is especially useful in the control of wastewater treatment plant

operation because it offers a rough approximation of the amount of organic matter present in the solid fraction of wastewater, activated sludge or industrial wastes. MLTS and MLTSS are determined following the methods 2540B and 2540D described in Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WPCF, 1998), whereas MLVS and MLVSS are determined following the method 2540E.

Determination procedure

MLTS are determined weighing a selected (in order to yield a residue between 2.5 and 200 mg) well-mixed sample volume in a previously clean (heated to 103-105ºC for 2 h) porcelain dish after being evaporated at 103-105ºC until constant weight. The increase in weight over that of the empty dish represents the total solids in the initial volume of sample.

For the determination of MLTSS, a selected (in order to yield a residue between 2.5 and 200 mg) well-mixed sample volume is filtered through a weighed glassfiber filter (Whatman, GF/C, 4.7 cm of diameter, 1.2 µm of pore size) and the residue retained on the filter is dried to a constant weight (2h) at 103-105ºC. The increase in weight of the filter represents the total suspended solids.

To determine the volatile solids (MLVS or MLVSS), the residue from method 2540B and 2540D, respectively, is burnt to constant weight at 550ºC during half an hour. The weight lost during ignition corresponds to the volatile solids.

Interferences

Highly mineralized water with a significant concentration of calcium, magnesium, chloride and/or sulphate may be hygroscopic and requires prolonged drying, proper desiccation and rapid weighing. Some inorganic salts such as hydroxides, carbonates or ammonium salts are decomposed and volatilised at 550 ºC and therefore can give a higher value for the volatile content in the sample.

2.2.2. Biofilm concentration

Biomass concentration in the biofilm attached to the plastic support was also determined. Ten plastic supports were sonicated for 10 min in 100 mL of permeate at 65% of amplitude using a probe sonicator (UP200s, Dr. Hielscher). MLTSS and MLVSS were determined in the resulting mixed liquor according to the methodology previously detailed, and this concentration was referred to the surface of the plastic support.

2.2.3. Sludge volumetric index

The sludge volumetric index (SVI) determination is defined in the Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WPCF, 1998) as the volume in mL occupied by 1 g of a suspension after 30 min settling.

2.2.4. Sludge settling rate

The sludge settling rate (SSR) was determined by measuring the velocity of sedimentation of the mixed liquor (1 $g \cdot L^{-1}$) in a 100 mL measuring cylinder.

2.3. Gas phase

To measure biogas composition a gas chromatograph HP 5890 Series II with the column of Porapack Q 80/100 2m x 1/8" (SUPELCO) is used. 1 mL of well-mixed sample should be injected through the septum at the following conditions: oven temperature (column) at 35 °C; injector and the detector temperature at 110 °C. The obtained peaks correspond to the percentage of the N_2, CH_4, CO_2 and H_2S content in the sample. Biogas production was measured using a Milli GasCounter MGC-10 (Ritter, Germany), which basically consists in a tilting body inside a container with a special packing liquid. The entrance of gas bubbles led the tilting body to change its position. Each change is counted with a magnet and a counter and the internal calibration give the gas flow in the display.

2.4. Membrane performance

2.4.1. Flux and permeability

Membrane flux can be calculated as:

$$J = \frac{Q}{A} \qquad \text{eq. 2.6}$$

where:

J: flux expressed in $L \cdot m^{-2} \cdot h^{-1}$,

Q: flow expressed in $L \cdot h^{-1}$,

A: membrane area expressed in m^2.

Therefore, permeability can be calculated as:

$$P = \frac{J}{TMP} \quad \text{eq. 2.7}$$

where:

P: permeability expressed in $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$,

TMP: transmembrane pressure in bar.

2.4.2. Critical flux

The critical flux hypothesis is that on start-up there exists a flux below which a decline of flux with time does not occur; above it fouling is observed. This flux is the critical flux and its value depends on the hydrodynamics and probably other variables. The critical flux was determined according to the modified flux-step method proposed by van der Marel et al. (2009). The criterion employed was that the increment of TMP with respect to time was higher than 10 $Pa \cdot min^{-1}$ (Le-Clech et al., 2003).

2.4.3 Filterability

The specific resistance to filtration of a sludge sample was determined by a dead-end filterability test. The test was conducted at 25ºC in a 180 mL stirred cell (Model 8200, Amicon) using a 0.45 µm flat-sheet PVDF membrane filter of (HVLP 09050, Millipore) (Chapter 3) or in a 200 mL pressurized cylinder (Model Sartorius SM 16249) using a 0.2 µm flat-sheet cellulose acetate membrane filter (12587-47-N Sartorius) (Chapter 7). The stirred cell and the cylinder were filled with 180 mL of the sample liquor and a constant pressure was applied by pressurized nitrogen. The production of filtrate under pressure was continuously recorded by an electric balance (Sartorius BP 1200) that was connected to a computer.

The resistance-in-series model was applied to evaluate the filtration characteristics.

$$J = \frac{\Delta P}{\eta \cdot R_t} \quad \text{eq. 2.8}$$

$$R_t = R_m + R_c + R_{pb} \quad \text{eq. 2.9}$$

Where J is the permeation flux [$m^3 \cdot m^{-2} \cdot s^{-1}$], ΔP is the TMP [Pa], η is the dinamic viscosity of the permeate [$Pa \cdot s^{-1}$]; R_t is the total resistance [m^{-1}]; R_m is the intrinsic membrane resistance [m^{-1}]; R_c is the cake resistance formed by the cake layer deposited over the membrane surface [m^{-1}]; and the pore blocking resistance, R_{pb}, is the resistance caused by solute adsorption into the membrane pores and walls [m^{-1}]. Each resistance value can be obtained through the equations 2.10, 2.11 and 2.12:

$$R_m = \frac{\Delta P}{\eta \cdot J_m} \quad \text{eq. 2.10}$$

$$R_{pb} = \frac{\Delta P}{\eta \cdot J_{pb}} - R_m \quad \text{eq. 2.11}$$

$$R_c = \frac{\Delta P}{\eta \cdot J} - (R_m + R_{pb}) \quad \text{eq. 2.12}$$

The experimental procedure to determine each resistance value was as follows: (a) R_m was estimated by measuring the permeate flux of tap water; (b) R_t was evaluated by the flux of biomass microfiltration; (c) the membrane surface was then flushed with tap water and cleaned with a sponge to remove the cake layer. After that, the tap water flux was measured again to obtain the resistance of R_m + R_{pb}. From steps (a)–(c), R_t, R_m, R_{pb} and R_c could be calculated. The resistance of the colloidal fraction of the cake was also determined using a new filter according to equation 2.13:

$$R_{col} = \frac{\Delta P}{\eta \cdot J_{col}} - R_m \quad \text{eq. 2.13}$$

where J_{col} *is* the flux of the supernanatant after centrifugation of biomass at 4000 g during 10 min.

Using the Carman-Kozeny equation to calculate the pressure drop of a fluid flowing through a packed bed of solids in laminar flow and taking into account that the filtration takes place at constant pressure, the specific resistance to filtration (SRF) (α, m·kg^{-1}) can be calculated after linearization according equation 2.14:

$$\alpha = \frac{2 \cdot A^2 \cdot P \cdot b}{\eta \cdot w} \quad \text{eq. 2.14}$$

where P is the pressure applied [Pa], A the filtration area [m^2], w the total suspended solids [kg·m^{-3}], η is the dinamic viscosity of filtrate [Pa·s] and b is the time-to-filtration ratio [s·m^{-6}], which is the slope of the curve that is obtained by plotting the time of filtration to the volume of filtrate ratio (t/V) versus the filtrate volume (V). From the conventional constant pressure filtration equation, a plot of t/V vs. V is expected to yield a linear relationship for the entire filtration data. The linearity of t/V vs. V plot is observed only when the value of V (or time) or the cake thickness is sufficiently large.

2.4.4. Extracellular polymeric substances (EPS) and soluble microbial products (SMP)

To determine the concentration of extracellular polymeric substances (EPS), which mainly consist of polysaccharides and proteins, the sample must be analysed according to the protocol proposed by the members of AMEDEUS & EUROMBRA during the meeting which took place in Berlin, 1 of June 2006. The method of extraction consists of a modification of the method used by Zhang et al. (1999). The sample of 200 mL of biomass is centrifuged at 5000 rpm during 20 minutes. The supernatant is removed. Its content in carbohydrates and proteins is analyzed in order to obtain SMP concentration. Next, 200 mL of deionised water is added to the remaining biomass and carefully shaken (manually) and the sample is placed in the oven at 80 °C, during 10 minutes. The tubes, still warm, are centrifuged at 5000 rpm during 20 minutes. The supernatant is filtered with the fiberglass filter. Its content in carbohydrates and proteins is analyzed in order to obtain EPS concentration.

2.4.4.1. Carbohydrates

Carbohydrate concentration was analysed using a modified phenol–sulphuric acid method proposed by Dubois et al. (1956).

Reagents preparation

The following reagents are necessary in order to carry out the procedure:

Reagent A: Phenol solution 5 % (v/v)

Reagent B: Sulphuric acid (97 %)

Determination procedure

A sample of 1.0 mL is thoroughly mixed with 1.0 mL of reagent A and left for 10 minutes at room temperature. Then 5.0 mL of reagent B are added rapidly (in stream) and left for 5 minutes at room temperature for cooling. The test tube is then mixed again. After 25 minutes, the absorbance at 490 nm is measured with a spectrophotometer Cecil CE 7200. A blank with reagents must be also measured as a reference. The quantification is done with 6-8 points calibration curve in the range of 0-100 $mg \cdot L^{-1}$, using D-glucose monohydrate (figure 2.7).

Interferences

Nitrate and nitrite interferences over carbohydrate concentration have been reported by Drews et al. (2008). The cuantification of this interference is given by equation 2.15:

$$C_{CH} = C_{CH,measured} - 0.099 \cdot C_{N-NO3} - 1.9 \cdot C_{N-NO2} \quad \text{eq. 2.15}$$

where C_{CH} is the real concentration of carbohydrates in mg·L^{-1}, $C_{CH,measured}$ is the measured concentration of carbohydrates in mg·L^{-1}, and $C_{N\text{-}NO3\text{-}}$ and $C_{N\text{-}NO2\text{-}}$ are the measured concentrations of nitrogen as nitrate and nitrite in mg·L^{-1}, respectively.

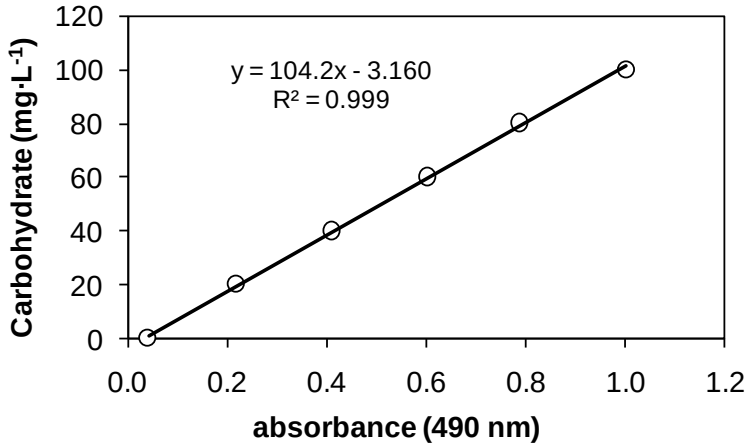

Figure 2.7. Calibration curve for carbohydrate concentration determination.

2.4.4.2. Proteins

Determination of proteins was done according a modified method based on Lowry et al. (1951) and Frølund et al. (1996). First the proteins are pretreated with copper ion in alkali solution, and then the aromatic aminoacids in the treated sample reduce the phosphomolybdatephosphotungnstic acid present in the Folin reagent.

Reagents preparation

The following reagents are necessary in order to carry out the procedure:

Reagent A: Solution of sodium hydroxide (NaOH) 143 mM and sodium bicarbonate (Na_2CO_3) 270 mM.

Reagent B: Solution of cupric sulfate ($CuSO_4$) 57 mM

Reagent C: Solution of sodium tartrate ($Na_2C_4H_4O_6$) 124 mM

Reagent D: Mixture of reagents A, B, C in ratio of 100:1:1. Reagent D has to be done freshly.

Reagent E: Solution of Folin-Ciocalteu-reagent (1:2 in deionised water)

Determination procedure

A sample of 1.5 mL is rapidly mixed with 2.1 mL of reagent D and left for 10 minutes at room temperature. Then 0.3 mL of reagent E are added rapidly and mixed. After 45 minutes, the absorbance at 750 nm is measured with a spectrophotometer Cecil CE 7200. A blank with reagents must be also measured as a reference. The quantification is done with 6-8 points calibration curve in the range of 0-250 $mg \cdot L^{-1}$, using protein standard bovine serum albumin (BSA) (figure 2.8).

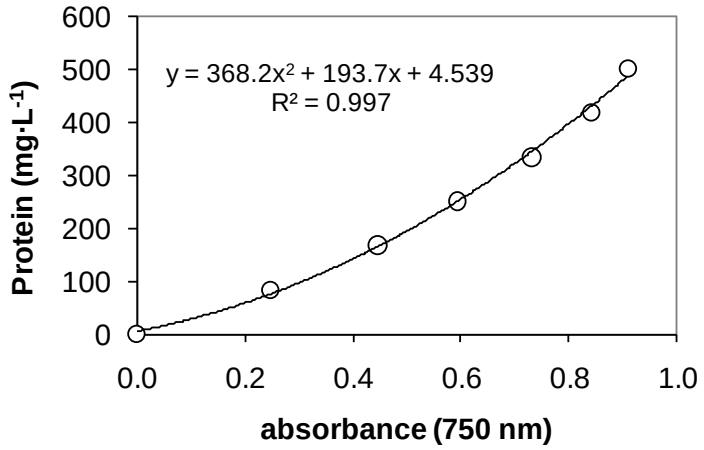

Figure 2.8. Calibration curve for carbohydrate concentration determination.

2.4.4.3. Biopolymer clusters (BPC)

A pool of biopolymer clusters (BPC) ranging from 2.5 to 60 µm in size was identified in the liquid phase of the MBR sludge and in the cake sludge on the membrane surface. According to the CLSM examination, BPC are free and independent organic solutes that are different from biomass flocs and EPS and much larger than SMP (Sun et al., 2008). Concentration of total dissolved organic carbon (tDOC) was measured with a Shimadzu analyser (TOC-5000). The difference in tDOC concentration between the sludge mixture after filtration through a 0.45 µm nitrocellulose membrane filter (HA, Millipore) and the permeate was assigned to the colloidal fraction of BPC in the liquid phase of the sludge mixture suspension.

2.4.4.4. Transparent exopolymer particles (TEP)

The analysis method used for the determination of the TEP concentrations (de la Torre et al., 2008) is based on the protocol developed for TEP quantification in sea water (Arruda et al., 2004). The former consists of mixing 5 mL of prefiltered sample with 0.5 mL of 0.055% (m/v) alcian blue solution and 4.5mL of 0.2 mol·L^{-1} acetate buffer solution (pH 4) in a flask. The flask is then stirred for 1 min and then centrifuged (Centrifuge MR23i Jouan GmbH, Germany) at 15300 rpm for 10 min. TEP react with the alcian blue solution yielding a low solubility dye–TEP complex. The concentration of the alcian blue in excess is determined by reading the absorbance at 602 nm (UV-vis spectrophotometer, Analytic Jena, Germany). The quantification is done with 6-8 points calibration curve in the range of 0-250 mg·L^{-1}, using xanthan gum (XG) (figure 2.9). The results expressed in mg·L^{-1} xanthan gum equivalent.

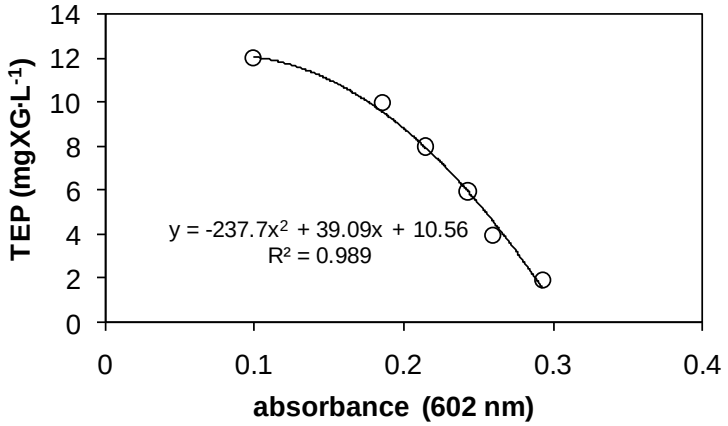

Figure 2.9. Calibration curve for TEP concentration determination.

2.5. Membrane cleaning procedures

The membrane cleaning procedures performed were either a physical washing with tap water, or a chemical (maintenance or intensive) cleaning (when necessary).

Maintenance Cleaning

The maintenance cleaning could be performed inside the reactor and the procedure was the following:

1) physical cleaning by rinsing with tap water, and

2) backwashing with chlorinated water (250-500 ppm Cl_2:1) for 1 h.

Intensive Chemical Cleaning

Chemical cleaning was performed outside the membrane chamber only when permeability value was below 50 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$, approximately. The cleaning procedure was:

1) physical cleaning by rinsing with tap water,

2) Submerging the membrane in chlorinated water (2000 ppm Cl_2:1) for 2 h, and

3) Backwashing with chlorinated water (2000 ppm Cl_2:1) for 1 h.

2.6. References

AMEDEUS & EUROMBRA project proceedings, meeting of Berlin, 1 of June 2006.

Anthonisen, A., Loehr, R., Prakasan, T., Srineth, E. 1976. Inhibition of nitrification by ammonia and nitrous acid. *Journal Water Pollution Control Federation* **48**, 835-852.

APHA-AWWA-WPCF. 1985. Standard Methods for the Examination of Water and Wastewater, 16th ed., American Public Health Association, Washington, USA.

APHA-AWWA-WPCF. 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, USA.

Arruda Fatibello, S.H.S, Henriques Vieira, A.A., Fatibello-Filho, O. 2004. A rapid spectrophotometric method for the determination of transparent exopolymer particles (TEP) in freshwater. *Talanta* **62**, 81–85

de la Torre, T., Lesjean, B., Drews, A., Kraume, M. 2008. Monitoring of transparent exopolymer particles (TEP) in a membrane bioreactor (MBR) and correlation with other fouling indicators. *Water Science and Technology* **58** (10), 1903-1910.

Drews, A., Vocks, M., Bracklow, U., Iversen, V., Kraume, M. 2008. Does fouling in MBRs depend on SMP? *Desalination* **231**, 141–149.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Analytical chemistry* **28**, 350-356.

Frølund, B., Palmgrem, R., Keiding, K., Nielsen, P.H. 1996. Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Water research* **30**(8), 1749-1758.

Le-Clech, P., Jefferson, B., Chang, I.S., Judd, S. 2003. Critical flux determination by the flux-step method in a submerged membrane bioreactor. *Journal of Membrane Science* **227**, 81-93.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J. 1951. Protein measurements with the folin phenol reagent. *The Journal of Biological Chemistry* **193**(1), 265- 275.

Jenkins, S.R., Morgan, J.M., Sawyer, C.L. 1983. Measuring anaerobic sludge digestion and growth by simple alkalimetric titration. *Journal of the Water Pollution Control Federation* **55**, 448-453.

Ripley, L.E., Boyle, W.C., Converse, J.C. 1986. Improved alkalimetric monitoring for anaerobic digestion of high-strength wastes. *Journal of the Water Pollution Control Federation* **58**(5), 406-411.

Soto, M., Méndez, R., Lema, J. 1993. Methanogenic and non-methanogenic activity tests. Theoretical basis and experimental set-up. *Water Research* **27**(8), 1361-1376.

Sun F.-Y., Wang X.-M., Li, X.-Y. (2008). Visualisation and characterisation of biopolymer clusters in a submerged membrane bioreactor. *Journal of Membrane Science* **325**, 691–697

Switzembaum, M., Giraldo-Gomez, E., Hickey, R.F. 1990. Monitoring of the anaerobic treatment technology. *Bioresource Technology* **53**, 255–262.

van der Marel P., Zwijnenburg A., Kemperman A., Wessling M., Temmink H., van der Meer W. (2009). An improved flux-step method to determine the critical flux and the critical flux for irreversibility in a membrane bioreactor. *Journal of Membrane Science*, **332**, 24-29.

Wentzel, M.C., Moosbrugger, R.E., Sam-Soon, P., Ekama, G.A., Marais, G.R. 1994. Tentative guidelines for waste selection, process design, operation and control of upflow anaerobic sludge bed reactors. *Water Science and Technology* **30**(12), 31-42.

Wheatherburn, M.W., 1967. Phenol-hypochlorite reaction for determination of ammonia. *Analytical chemistry* **28**, 971-974

Zhang, X., Bishop, P.L., Kinkle, B.K. 1999. Comparison of extraction methods for quantifying extracellular polymers in biofilms. *Water Science and Technology* **39**(7), 211-218.

Chapter 3

Tertiary membrane filtration of an industrial wastewater using granular and flocculent biomass SBRs

Summary

Sequencing batch reactors (SBR) are widely used for wastewater treatment. The use of granular biomass in SBR allows higher organic loading rates (OLR). Nevertheless, the main disadvantage of these reactors, with regard to flocculent biomass SBRs, is the presence of suspended solids in the effluent. Therefore, a suitable post-treatment process may be required to fulfil local requirements for the amount of total suspended solids (TSS) in the effluent, which can be accomplished using membrane filtration units.

In this study, effluents from a flocculent biomass SBR (F-SBR) and a granular biomass SBR (G-SBR) were treated in tertiary membrane filtration (TMF) chambers to remove suspended solids. The performances of the operating systems were compared in order to determine the influence of the aggregation state of the biomass on the filtration process. No significant differences were observed between the two TMF systems in terms of capacity and permeability. Tertiary filtration of the effluent from the G-SBR was similar to that of the F-SBR system. The incorporation of wastewater free of suspended solids during one of the operating periods significantly worsened operation of the TMF systems; permeability decreased by up to 40 percent in both systems. Additionally, other factors such as nitrification, the presence of soluble microbial products and the concentration of dissolved organic carbon seem to play an important role in TMF.

Parts of this chapter have been published as:

Sánchez, A., Garrido, J.M., Méndez, R. 2010. A comparative study of tertiary membrane filtration of industrial wastewater treated in a granular and a flocculent sludge SBR. *Desalination* 250, 810-814.

Sánchez, A., Garrido, J.M.., Méndez, R. 2011. Tertiary membrane filtration of an industrial wastewater using granular or flocculent biomass sequencing batch reactors. *Journal of Membrane Science* 382, 316-322.

3.1. Introduction

In recent years, granular sludge has been proposed as an alternative for high capacity SBR wastewater treatment systems. G-SBR systems can be operated at higher OLR than F-SBR systems. Arrojo et al. (2004) reported organic and nitrogen loading rates of 7 $gCOD \cdot L^{-1} \cdot d^{-1}$ and 0.7 $gN \cdot L^{-1} \cdot d^{-1}$, respectively, in a G-SBR. The OLR values recommended for flocculent SBRs in the literature range from 0.5 to 2.0 $gCOD \cdot L^{-1} \cdot d^{-1}$ (Beun et al., 1999).

The basis of granulation is the continuous selection of sludge particles that occur inside a reactor. The part of the biomass that does not settle quickly enough is washed out with the effluent (Beun et al., 1999). However, a main drawback of G-SBR systems is the presence of suspended solids that are washed out with the treated effluent. Therefore, a suitable post-treatment process may be required to fulfil local requirements for the amount of total suspended solids (TSS) in the effluent, which can be accomplished using membrane filtration units, depth filters, surface filters or external settlers (Arrojo et al., 2004).

Tertiary filtration, especially depth filtration, has been used to remove suspended solids from secondary treated waters. However, in recent years, the use of tertiary membrane filtration (TMF) systems is becoming more common. Low-pressure tertiary membranes have been proved to meet stringent standards. Membranes are a physical barrier to suspended solids that are larger than the membrane pore size. Micro- and ultrafiltration membranes (MF/UF) have been used since the early 1990s for drinking water production. They can also be used to remove particulate and colloidal matter from settled secondary effluents, which increases the effectiveness of disinfection with either ultraviolet radiation or ozone for reuse applications (Tchobanoglous et al., 1998; Lubello et al., 2003). Thus, compared to depth filtration, tertiary MF or UF membrane treatments produce water of better microbiological quality that is also free of suspended solids. This should be taken into account when water will be reused or discharged into sensible areas.

Fouling is the main drawback associated with the application of membrane technology for wastewater treatment (Kimura et al., 2005; Meng et al., 2009; Drews, 2010). Fouling decreases the permeability of a membrane, limits flux and shortens the life of membrane modules, thus increasing both the capital and the operating costs of filtration systems. Membrane fouling is the result of complex phenomena that are not yet completely understood.

The fluxes obtained with submerged UF membranes in the tertiary filtration of secondary wastewaters are normally below 70 $L \cdot m^{-2} \cdot h^{-1}$, with a backwashing interval of 15-60 min (Metcalf & Eddie, 2003; Pearce, 2010; Zheng et al., 2011). Operational permeabilities of 160-250 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ and fluxes of 25-50 $L \cdot m^{-2} \cdot h^{-1}$ are typical values for pilot units that treat secondary effluents during filtration cycles of 22-27 min. Shortening the backwashing interval and decreasing the flux can slow fouling development (Zheng et al., 2011).

Some studies (Lee et al., 2003; Lesjean et al., 2005; Meng et al., 2009; Drews, 2010) have quantified the fouling caused by each sludge fraction (suspended solids, colloids and solutes) and shown that colloids are of prime importance in this process. This concept is based on the fact that granular biomass produces less membrane fouling than flocculent biomass in secondary membrane bioreactors (MBR) (Li et al., 2005).

3.2. Objectives

The main objective of this study was to compare the effluents from laboratory-scale G-SBR and F-SBR systems to determine if effluents with granulated suspended solids cause a different fouling pattern in TMF systems. One additional objective of this study was to investigate the role of particulate COD in the efficiencies of the SBRs and the performance of tertiary filtration membranes.

3.3. Material and methods

3.3.1. Experimental set-up and operating strategy

3.3.1.1. Operating system

Two identical operating systems consisting of an SBR coupled in series with a tertiary filtration chamber (figure 3.1) were operated in parallel. The volumes of the SBRs and filtration chambers were 1.5 L and 1.0 L, respectively. In the first stage, wastewater was treated in the SBR. The SBR effluent then moved into the filtration chamber where suspended solids were removed with an MF membrane module.

The submerged membrane modules were constructed with MF polyvinylidene fluoride (PVDF) hollow-fibre membranes, with a pore size of 0.1 μm and a membrane area of 0.027 m^2 (figure 3.2). The membrane module was prepared in the laboratory using a hollow-fibre membrane manufactured by a Spanish company (Porous Fibers). The number and length of the fibres in each module was similar, and clean water permeability was the

same in both modules; tap water at 20 ºC produced a value of approximately 300 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$. The membranes were operated in cycles, with 7 minutes of permeation and 0.5 minutes of backwashing (10 $L \cdot m^{-2} \cdot h^{-1}$). The tertiary filtration chambers were aerated to minimise membrane fouling. Aeration rate applied was 24 $L \cdot h^{-1}$, which correspond to a specific air demand (SAD_m) of 0.89 $Nm^3 \cdot m^{-2} \cdot h^{-1}$.

Operation of the system was controlled by a PLC Siemens S7-200 connected to a computer. Transmembrane pressure (TMP) data were measured with an electronic pressure sensor IFM Efector500 PN-2009 with an analogue 4-20 mA output and collected on the PC via an analogue PLC module Siemens EM 235 and a data acquisition program.

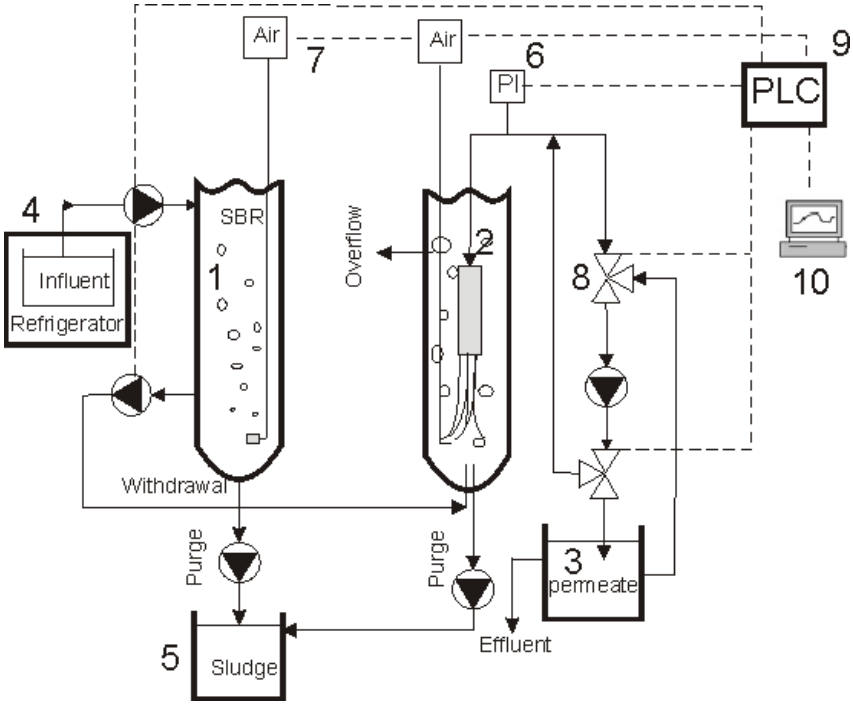

Figure 3.1. Schematic diagram of the laboratory-scale system consisting of a sequencing batch reactor and a tertiary filtration module coupled in series**.** (1) SBR; (2) TMF chamber; (3) Permeate; (4) Influent; (5) Sludge purge; (6) Pressure sensor; (7) Aeration; (8) Valve; (9) PLC; (10) Computer.

Figure 3.2. Membrane module.

3.3.1.2. Cleaning strategy

Physical cleaning of the membrane module was performed by rinsing with tap water. Chemically enhanced backwashing was not used during the experiments. Chemical recovery cleaning was conducted when TMP in the membranes was higher than 30 kPa or permeability value was below 50 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$. The cleaning procedure was:

- Soak in chlored water (2000 ppm Cl_2:l) for 2 h.
- Backwashing with chlored water (2000 ppm Cl_2:1) for 1 h.

3.3.1.3. Influent and operating strategy

Industrial wastewater from a fish freezing industry was treated during the study. The raw wastewater contained 50-300 $mg \cdot L^{-1}$ ammonia and 1000-6300 $mg \cdot L^{-1}$ total COD. Total phosphorous ranged between 70 and 190 $mg \cdot L^{-1}$ of which 60-70% was orthophosphate. Soluble COD represented around 85% total COD. Lots of the industrial wastewater were taken and diluted with tap water to obtain the desired OLR and COD values in the influent. The soluble COD values varied from 100 to 1100 $mg \cdot L^{-1}$. The dissolved total nitrogen (DTN) concentration ranged from 20 to 180 $mg \cdot L^{-1}$, and the total phosphorus concentration ranged from 20 to 110 $mg \cdot L^{-1}$.

An exchange volume ratio of 50 % and a cycle length of 3 hours were fixed as operating parameters. The two SBR were operated with constant and similar HRTs and SRTs during all the operation. The lengths of the feeding, reaction, settling and withdrawal

phases were varied (table 3.1) to promote the growth of either granular or flocculent sludge in each SBR reactor.

Table 3.1. Lengths of the phases during a cycle.

	Phase					
Reactor	**Feeding**	**Reaction**	**Settling**	**Withdrawal**	**Dead**	**Total**
G-SBR (min)	3 (aerobic)	147	1	3	26	180
F-SBR (min)	60 (anoxic)	90	20	10	0	180

The study lasted for 349 days that could be divided into five different experimental periods during which the applied OLR or the concentration of particulate COD was modified.

Period I (from day 0 until day 114). The objective of this period was to develop either granular or flocculent sludge with good settling properties prior to coupling the filtration membrane modules to the SBRs. Both SBR were operated without tertiary filtration membranes in order to achieve a correct development of granular biomass. A SBR cycle time of 3 hours, an exchange volume ratio of 50 % and an OLR of 2 $kgCOD \cdot m^{-3} \cdot d^{-1}$ were fixed like operation parameters in both systems.

Period II (from day 115 until day 231). During this period, both SBRs received a similar OLR of approximately 2 $kgCOD \cdot m^{-3} \cdot d^{-1}$. The objective was to investigate TMF, with similar operating conditions in both reactors. Later, during periods III, IV and V, the applied OLR in the G-SBR was higher than that of the flocculent reactor, and the same average HRT was maintained in both systems by diluting the wastewater fed to the F-SBR with tap water.

Period III (from day 232 until day 253). The OLR applied to the G-SBR was maintained at approximately 3 $kgCOD \cdot m^{-3} \cdot d^{-1}$. This value was greater than the rate of 1.5 $kgCOD \cdot m^{-3} \cdot d^{-1}$ applied to the F-SBR.

Period IV (from day 254 until day 324). Wastewater free of suspended solids was fed to both systems. The wastewater was prepared by first removing particulate matter in a centrifugation step and then filtering the supernatant with 0.45-µm flat-sheet PVDF

membrane filters. The objective of this period was to investigate the effect of suspended solids on TMF in both systems.

Period V (from day 325 until day 349). The feeding of diluted wastewater with suspended solids was restarted on day 325 and continued till the end of operation.

3.3.2. Analytical methods

Samples of the influents and effluents from both SBRs and the permeates from the filtration chambers were taken. COD, ammonia, nitrite, nitrate, phosphate, mixed liquor total and volatile suspended solids (MLTSS and MLVSS) concentrations and sludge volume index (SVI) were analysed according to Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WPCF, 1998). Dissolved organic carbon (DOC) and dissolved total nitrogen (DTN) were determined with a Shimadzu TOC-500 analyser, and a DN 1900 analyser (Rosemount-Dohrmann).

Samples were taken at the end of the withdrawal period, when the filtration chambers had achieved their maximum operating volumes, a result of the entrance of secondary treated effluent from the SBRs.

Dissolved oxygen concentrations in the reactors and filtration chambers were determined with an Oxi 340 WTW oxygen selective electrode, and pH was determined with a U-455 Ingold electrode connected to a Crison 506 pH-meter. Conductivity and temperature were monitored with a CM 35 conductivimeter.

Soluble microbial products (SMP) were extracted by centrifuging the biomass for 5 min at 5000 rpm (Heraeus, Labofuge 200) and filtering the supernatant through 0.2-mm glass fibre filters (GF 52, Schleicher and Schuell). Extracellular polymeric substances (EPS) were extracted according to the heating procedure (Zhang et al., 1999). Protein and carbohydrate concentrations were determined by visible absorbance on a spectrophotometer (Cecil CE 7200), using bovine serum albumin (Sigma) and glucose standards (Merck), respectively. Carbohydrate and protein concentrations were determined according to the methods of Dubois et al. (1956) and Lowry et al. (1951), respectively. The difference in DOC concentration between the sludge mixture after filtration through a 0.45 μm nitrocellulose membrane filters (HA, Millipore) and the permeate was assigned to the colloidal fraction of biopolymer clusters (cBPC) in the liquid phase of the sludge mixture suspension (Sun et al., 2008). The critical flux was determined according to the modified flux-step method proposed by van der Marel et al. (2009). The criterion employed was that the increment of transmembrane pressure (TMP) with respect

to time was higher than 10 Pa·min^{-1} (Le-Clech et al., 2003). Fouling rate was calculated as the TMP increase (Pa·min^{-1}) experimented during twelve hours, maintaining the flux constant.

Particle size distributions in the filtration chamber and in the effluents were determined by laser diffraction (Mastersizer HYDRO 2000MU, Malvern Inst.). This method is based on the fact that the diffraction angle is inversely proportional to particle size.

The specific resistance to filtration of a sludge sample was determined by a dead-end filterability test. The test was conducted at 25ºC in a 180 mL stirred cell (Model 8200, Amicon) using a 0.45 µm flat-sheet PVDF membrane filter of (HVLP 09050, Millipore). Using the Carman-Kozeny equation to calculate the pressure drop of a fluid flowing through a packed bed of solids in laminar flow and taking into account that the filtration takes place at constant pressure, the specific resistance to filtration (SRF) (α, m·kg^{-1}) can be calculated after linearization.

Further information regarding analytical methods is provided in Chapter 2.

3.4. Results and discussion

3.4.1. SBRs and tertiary filtration system performance

Figure 3.3 shows the evolutions of the applied OLR and the HRT, referred to the reactor volume, during the five experimental periods. The HRTs were similar in both reactors for the entire operation. Moreover, during periods I and II (from day 0 until day 231), both units were operated with similar COD concentrations in the feed water and therefore, similar OLR rates were applied.

The OLR for both SBRs was approximately 2 kgCOD·m^{-3}·d^{-1} prior to the beginning of tertiary filtration in order to develop the different biomasses. At the end of period I (day 111), the OLR was increased from 2 to 4 kgCOD·m^{-3}·d^{-1} to achieve better granular biomass properties (figure 3.3). Nevertheless, this increase worsened the settling properties of the F-SBR sludge in terms of the SVI and the sludge settling rate (SSR). This led to a partial wash out of the biomass in this reactor. For this reason, on day 133, the OLR was decreased to approximately 2.5 kgCOD·m^{-3}·d^{-1} in both reactors and maintained at this level until the end of period II.

During periods III, IV and V, the applied OLR was higher in the G-SBR than in the F-SBR; the HRTs were constant in both systems. G-SBRs are typically operated at higher OLR (2.5-15 kgCOD·m^{-3}·d^{-1} (Moy et al., 2002; Liu et al., 2003)) than F-SBRs (0.5-2.0

kgCOD·m^{-3}·d^{-1} (Beun et al., 1999)). The wastewater fed to the F-SBR had a lower COD concentration (between 100 and 400 mg·L^{-1}) than that fed to the G-SBR (between 400 and 800 mg·L^{-1}). The objective of periods III, IV and V was to compare the behaviour of the two SBR at their recommended OLR and of the respective TMF chambers.

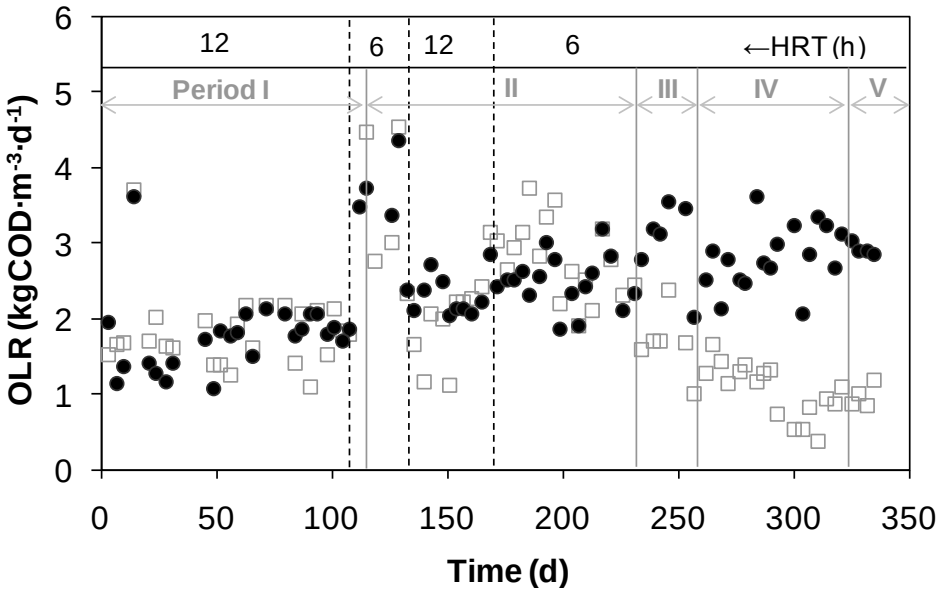

Figure 3.3. Evolution of the applied OLR in the (□) F-SBR and (●) G-SBR systems during the four experimental periods.

Figure 3.4 depicts the evolution of the overall COD removal percentage in both systems. During period I this overall COD removal percentage was referred to the soluble COD in the effluent, since the absence of the TMF stage led to the wash-out of part of the biomass from the system and hence to low total COD removal percentages. As can be observed on figure 3.4, the COD removal efficiency was around 90 % for both systems during periods I and II. The presence of either flocculent or granular biomass in SBR systems with tertiary filtration chambers did not affect COD removal efficiency, in terms of soluble COD. After period II, the COD removal efficiency in the F-SBR was lower than that in the G-SBR because of the lower COD concentration in the feed water of the F-SBR during periods III, IV and V. COD removal efficiencies in the flocculent system ranged between 66 and 97 %, whereas for the granular system, these efficiencies varied between 89 and 96%.

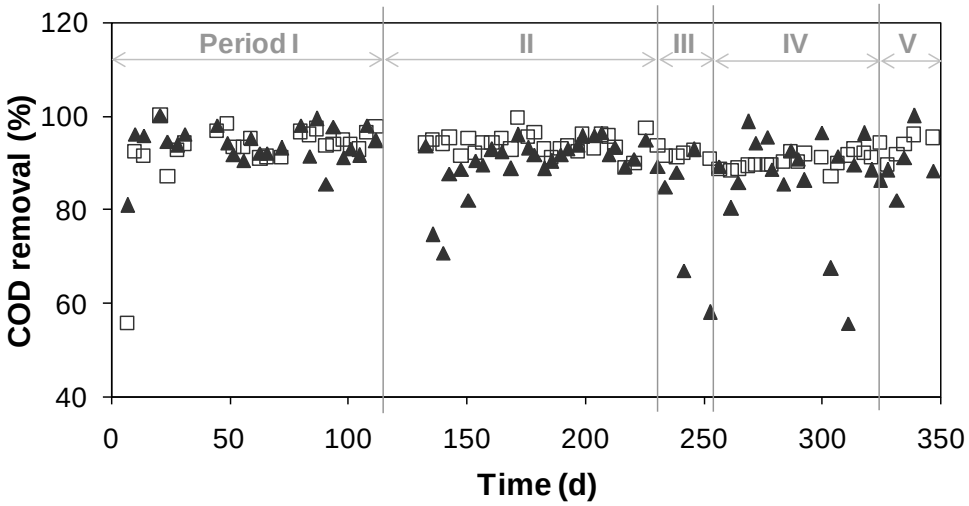

Figure 3.4. Overall COD removal efficiency for the F-SBR (▲) and G-SBR (□) systems.

The evolution of soluble COD in the effluent of the biological reactors is depicted in figure 3.5a. In figure 3.5b, the evolution of soluble COD in the permeates of the two filtration chambers is shown. The ratio between soluble and total COD in the fed wastewater was 0.81 ± 0.09 $g \cdot g^{-1}$, except during period IV when the wastewater fed to the system was filtered. The behaviour of both SBRs was similar during period I, with soluble COD concentrations ranging between 10 and 80 $mg \cdot L^{-1}$. The increase on the soluble COD concentration of both SBRs observed at the beginning of period II was due to higher OLR applied between days 111 and 133 (figure 3.3).

During period II, similar soluble COD concentrations were observed in the effluents of the SBRs and in the permeates of both tertiary filtration chambers. The COD concentration in the SBR effluents gradually decreased from 140 $mg \cdot L^{-1}$ to 20 $mg \cdot L^{-1}$ (figure 3.5a). A similar trend was observed for the filtration chamber permeates. During periods III, IV and V, the COD concentration in the permeate of the granular system was higher than that observed in the flocculent system because the former system was fed with water with a higher COD concentration. It was also observed that COD in the permeate was higher than the SBR effluent for the G-SBR system. A possible reason of such behaviour is presented in section 3.4.2.

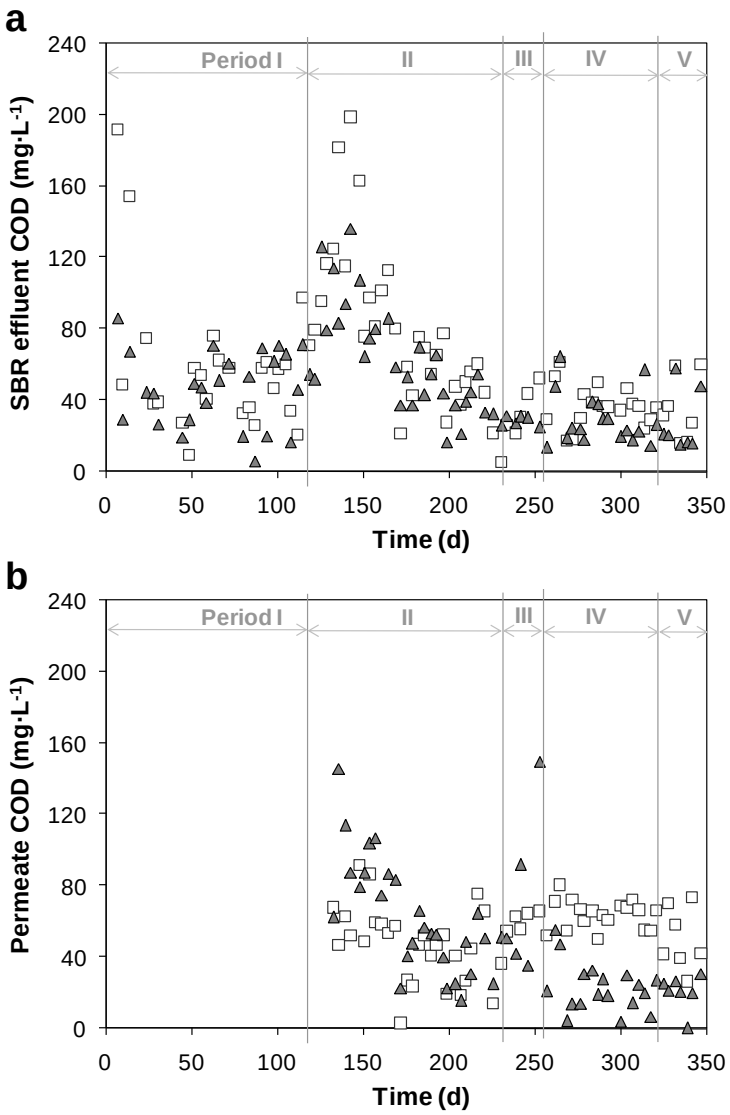

Figure 3.5. Soluble COD concentration in the effluents from both SBRs (a) and in the filtration chamber permeates (b) of either the F-SBR (▲) or the G-SBR (□) systems.

As mentioned in section 3.2, one additional objective of this study was to investigate the role of particulate COD in the efficiencies of the SBRs and the tertiary filtration chambers. The presence (periods I, II, III and V) or absence (period IV) of particulate matter did not affect the COD removal efficiencies of the SBRs or the filtration chambers.

No significant nitrification was observed in either SBR. During period I, nitrification was initiated in the F-SBR, but the dissolved oxygen limitation and the OLR increase that took place on day 111 interrupted this biological process. Nevertheless, ammonia was oxidised to either nitrite or nitrate in both tertiary filtration chambers, from day 215 onwards (figure 3.6).

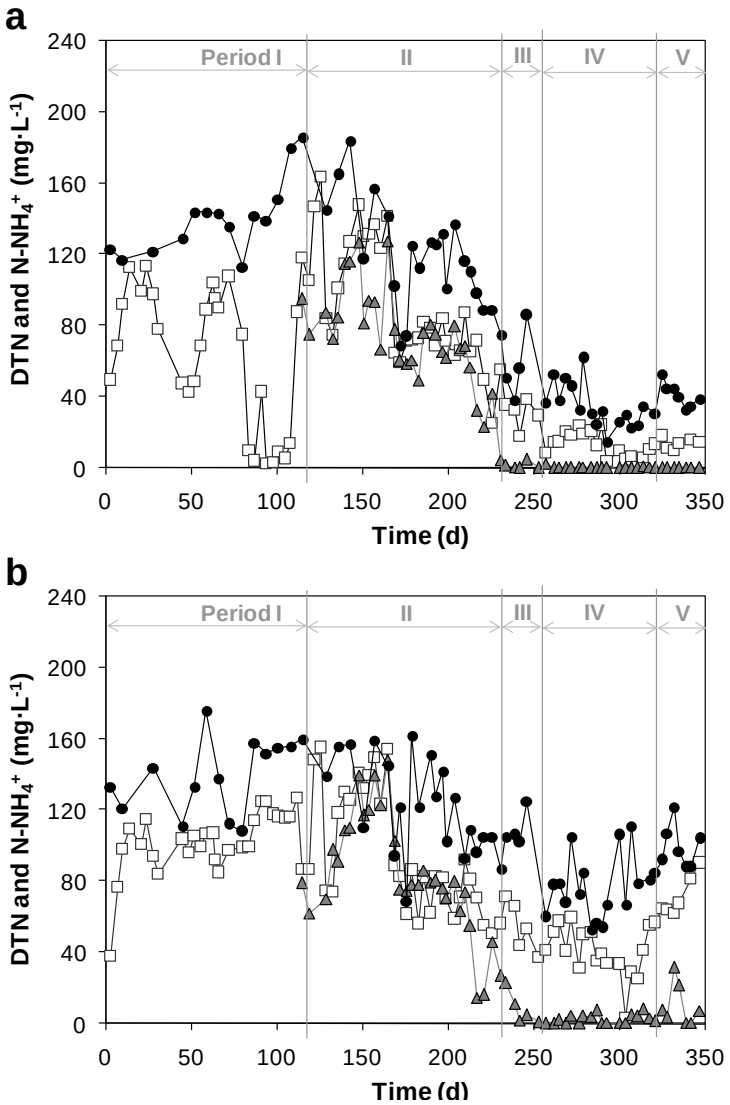

Figure 3.6. Dissolved total nitrogen in the feeding (•) and ammonia concentration in the SBR effluent (□) and the filtration chamber permeate (▲) in the F-SBR (a) and G-SBR (b) systems.

The tertiary filtration chambers were continuously aerated to reduce membrane fouling and to provide oxygen to the suspended, washed-out biomass. Thus, the tertiary membrane filtration chambers acted as a biological polishing stage and caused variations in the COD and nitrogen concentrations (figures 3.5 and 3.6).

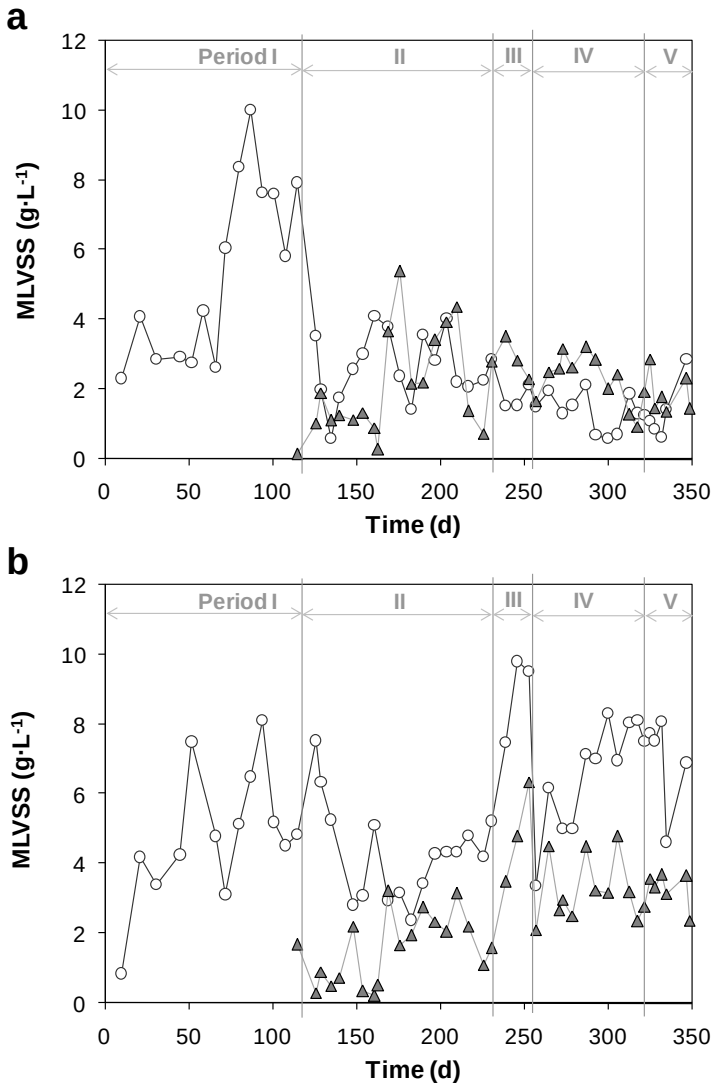

Figure 3.7. MLVSS concentration in the SBR (○) and TMF chambers (▲) of the F-SBR (a) and G-SBR (b) systems.

Regarding biomass concentration, it ranged between 0.5 - 5.0 and 3.0 - 10.0 $g \cdot L^{-1}$ in the flocculent and granular SBRs, respectively. The tertiary membrane modules were

operated with high MLTSS concentrations compared with typical values reported for TMF (Citulsky et al., 2009), in the range of mg·L^{-1}. MLVSS concentrations in the tertiary filtration chambers were between 0.3 - 6.0 and 0.3 - 6.8 g·L^{-1} (figure 3.7) as a result of the operating strategies of the filtration systems. The TMF chambers were punctually purged in order to maintain similar MLVSS and SRT values (between 20 and 30 d).

As expected, the sludge settling properties were significantly different for the two systems. The differences between the settling properties of the biomasses developed in both reactors were remarkable. In the F-SBR, the SVI values were between 50 and 300 ml·g^{-1}, while in the G-SBR, the SVI values ranged from 30 to 100 ml·g^{-1}. Moreover, the SSR were 0.7 m·h^{-1} and 9.7 m·h^{-1} for the flocculent and granular sludge, respectively.

Microscopic observation shows remarkable differences not only between biomass in both reactors but also between biomass into filtration chambers. As can be observed in figure 3.8, microbiological aggregates in granular system are substantially bigger.

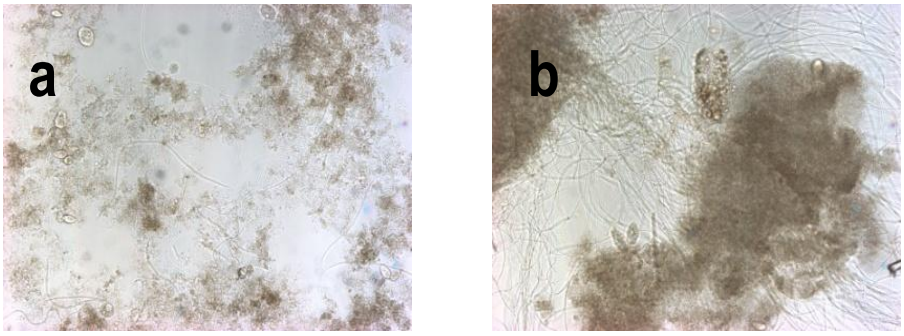

Figure 3.8. Microscopy observation of flocculent (a) and granular (b) biomass in TMF chambers.

3.4.2. Tertiary membrane filtration

3.4.2.1. Membrane performances

One of the main objectives of this study was to determine if the effluent from a granular biomass bioreactor caused less membrane fouling than that from a flocculent bioreactor. Nevertheless, permeability values between 160 and 75 L·m^{-2}·h^{-1}·bar^{-1} were observed in the two TMF systems at a flux of 10 L·m^{-2}·h^{-1}, indicating that TMF of both effluents produced similar results. Moreover, the permeability evolutions of the two membrane modules were similar (figure 3.9). For both membranes, the maximum permeability value was achieved after performing either a physical or a chemical cleaning

procedure. Permeability gradually decreased until the subsequent cleaning. These results are different from those reported by Li et al. (2005), who compared two submerged MBRs with flocculent and granular sludge. Nevertheless, in that study, the granular sludge was cultivated from anaerobic granular sludge, and the bioreactors were inoculated directly.

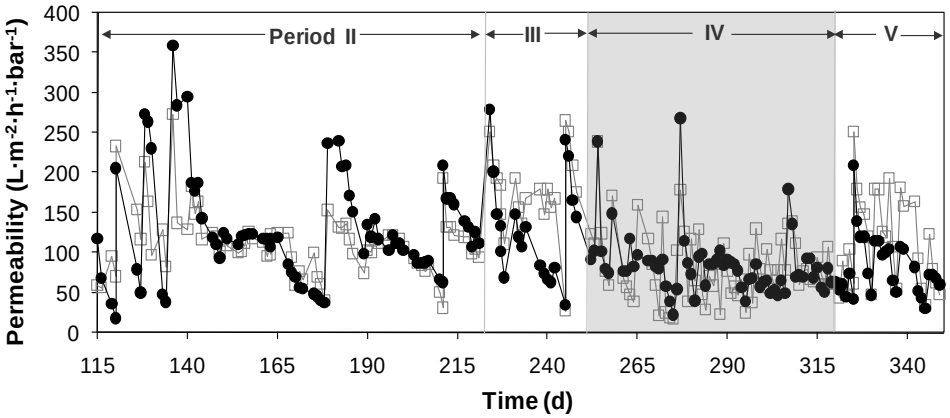

Figure 3.9. Permeability evolution in the F-SBR (□) and G-SBR (●) systems. The grey area represents period IV, during which the feed was filtered. The observed peaks in permeability correspond to those days on which chemical recoveries were performed.

The permeability values obtained during tertiary filtration were significantly better than those obtained previously in a pilot-scale MBR treating municipal wastewater with the same fibre. The permeabilities in the MBR varied between 50 and 70 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ (Artiga et al., 2006). TMP behaviour was similar for both membranes.

The objective of period IV was to assess the effect of particulate COD on the permeability of both filtration systems and on SBR performance. During this period, the fed wastewater was firstly centrifuged, and the supernatant was filtered later to remove particulate matter in the influent. During this study, it was assumed that the permeability of the systems fed with wastewaters with low MLTSS values, such as primary treated wastewaters, would be higher than that of systems fed with raw pre-treated water. Nevertheless, the permeability values in both systems during period IV were lower than those observed during periods II, III and V. Permeability was below 100 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ during this period (grey area in figure 3.9). Membrane fouling in both systems was clearly more severe during this period because suspended solids were removed from the influent. MLTSS concentration in the wastewater ranged from 0.05 to 0.20 $g \cdot L^{-1}$, depending on the COD concentration. MLVSS represented 75 % of the MLTSS.

3.4.2.2. Influence of nitrification on membrane performance

In addition to the presence of suspended solids in the fed wastewater, nitrification also affected TMF. The performance of both membranes was better when nitrification did not occur than when full ammonia oxidation took place (approximately 30 mgN-NO_x·L^{-1}) (figures 3.10.1 and 3.10.3, respectively), although no significant differences were observed depending on biomass aggregation state. However, when ammonia was partially oxidised (approximately 10 mgN-NOx·L^{-1}) in the TMF chambers, the membrane in the granular system performed better than the one in the flocculent system, reaching 50% lower pressures when operated at the critical flux (figure 3.10.2). The effects of ammonia nitrification variations on membrane behaviour have been previously reported for secondary MBR systems (Drews et al., 2007).

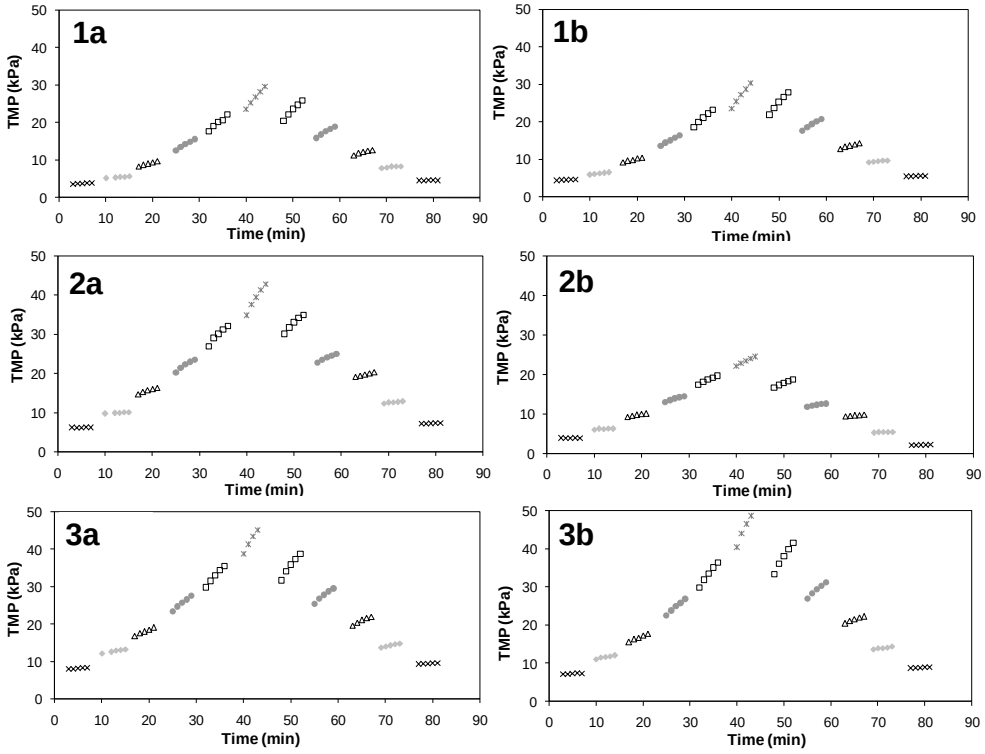

Figure 3.10. Critical flux test for flocculent (a) and granular (b) systems in conditions of no nitrification (1), moderate nitrification (2) and full nitrification (3). (×) 8.9 L·m^{-2}·h^{-1}, (◆) 14.5 L·m^{-2}·h^{-1}, (△) 19.0 L·m^{-2}·h^{-1}, (●) 24.5 L·m^{-2}·h^{-1}, (□) 30.1 L·m^{-2}·h^{-1} and (∗) 34.6 L·m^{-2}·h^{-1}.

These tests were carried out with biomass concentrations between 1 and 2 g·L^{-1} in both of TMF chambers. It was reported (Judd, 2004) that at low biomass concentrations,

membrane fouling would be dominated by solutes and colloids, that cause obstruction of the pores, while at higher concentrations, it would form a kind of cake on the membrane that to some extent protect it. This fact was observed during the performance but nitrification process seemed to be a more important parameter regarding membrane performance.

3.4.2.3. Influence of biopolymers on membrane performance

Several authors have reported EPS and, especially SMP, as the most significant biological factor responsible for membrane fouling (Chang and Lee, 1998; Nagaoka, 1999; Drews, 2010). The EPS concentrations in the liquor of the two TMF chambers were similar (40-100 mg·gMLVSS^{-1}). Nevertheless, the SMP concentrations (25-75 mg·gMLVSS^{-1}) in the TMF chamber of the granular system were significantly higher than those (10-40 mg·gMLVSS^{-1}) in the chamber of the flocculent system. The same trend was observed regarding the carbohydrate fraction of SMP (figure 3.11), which has been widely considered as the most important parameter regarding membrane fouling (Rosenberger et al., 2006; Drews et al., 2008; Wu and Huang, 2009) due to its hydrophilic properties (Liu and Fang, 2003) (figure 3.11). It is thought that the nature of this hydrophilic fraction in addition to the range of subcolloidal particle size (1000 to 10000 Da) (Huang et al., 2000) promotes pore clogging of the filter cake as well as the formation of sticky hydrogels on membrane surface (Harscoat et al., 1999; Frank and Belfort, 2003).

Tay et al. (2001) showed that aerobic granular sludge excretes more SMP than conventional bioflocs and biofilms. These smaller compounds are formed during the hydrolysis of MLVSS, which increases the COD concentration of the permeate. This fact could explain the higher soluble COD values in the permeate of this system (average 60 mg·L^{-1}) compared to the effluent of the biological reactor (average 40 mg·L^{-1}) (figures 3.5a and 3.5b). According to studies of Ahmed et al. (2007) and Massé et al. (2006), a high cellular retention time can cause a decrease in the EPS concentration, in systems where the relationship F/M is low, because microorganisms entering endogenous phase can use these products as a substrate, although initial use would be SMP, by being more available. This may be one reason why the concentration of SMP is usually lower than that of associated with biomass (EPS). Membranes location in the annexed filtration chambers where the supernatants were collected after each cycle made that organic matter concentration was very low, so that by increasing the biomass concentration of them by its washing of reactors, the low F/M could lead to the use of these SMP as feed.

Dissolved oxygen provides an oxygen source for the mineralisation of SMP, and nitrate also seems to influence SMP elimination. Elevated carbohydrate rejection and high carbohydrate concentrations of up to 150 mg·L^{-1} were reported by Drews et al. (2008) during a period of low nitrification activity. However, fouling was simultaneously low, which could indicate that under these conditions, SMP were too large to cause internal fouling and formed a loose cake instead (Drews et al., 2007). In spite of being tertiary filtration systems EPS and SMP concentrations are more similar than those reported by MBR studies. Other researchers (Tchobanoglous et al., 1998; Coté et al., 2004; Citulsky et al., 2009) have worked in tertiary filtration systems with low biomass concentrations (mg·L^{-1}). In this work, biomass concentration at filtration chambers is usually above 2 g·L^{-1}, being this values more appropriated for MBR operation. Therefore, this TMF chambers behaved like secondary MBRs.

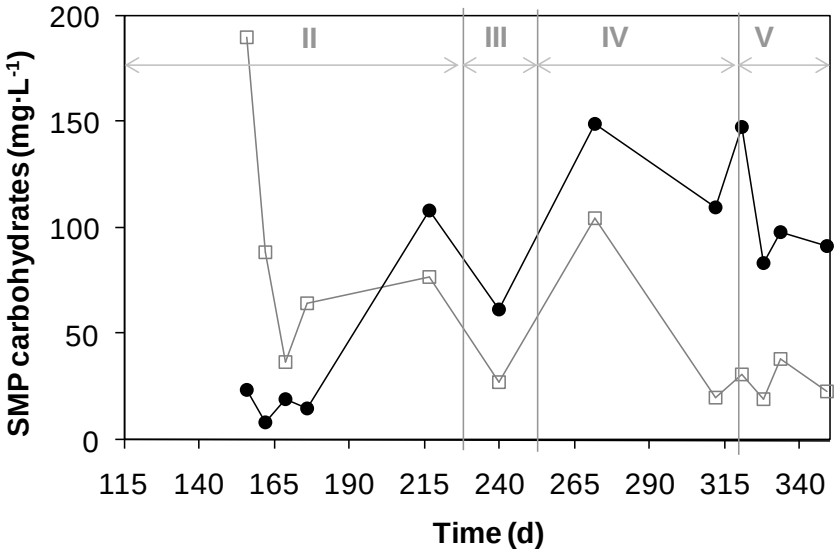

Figure 3.11. Carbohydrate SMP concentration in the F-SBR (□) and G-SBR (●) filtration chambers.

Recent studies have introduced a more general approach to the biopolymers responsible for membrane fouling by defining biopolymer cluster (BPC) as an important factor in the formation of the sludge fouling layer on the membrane surface and the increase of fouling potential (Wang and Li, 2008; Sun et al., 2008). BPC have been defined as a special form of organic materials formed by the affinity clustering of free EPS or SMP in the sludge cake on the membrane surface (Wang and Li, 2008). Figure 3.12 shows the average permeability and standard deviation values versus the colloidal fraction of BPC

(cBPC) concentrations during three different periods. As previously indicated, the lowest permeabilities were measured in period IV for both systems, corresponding to the period during which the supply of particulate COD was interrupted. The cBPC concentrations in the filtration chamber of the flocculent system during periods IV and V were lower than those observed in the granular one, which seemed to influence the permeability of the membrane located in the flocculent system. The influence of cBPC concentration on permeability was analysed by comparing periods II, IV and V in the filtration chamber of the granular system. As can be observed on figure 3.12b, the highest permeabilities were obtained when the cBPC concentration was low and viceversa. Considering the OLR applied in flocculent system during periods II, IV and V, this effect was also observed; the OLR was considerably lower during periods IV and V. Average values were calculated for the days on which the cBPC concentration was measured, and these values were complemented with their standard deviations.

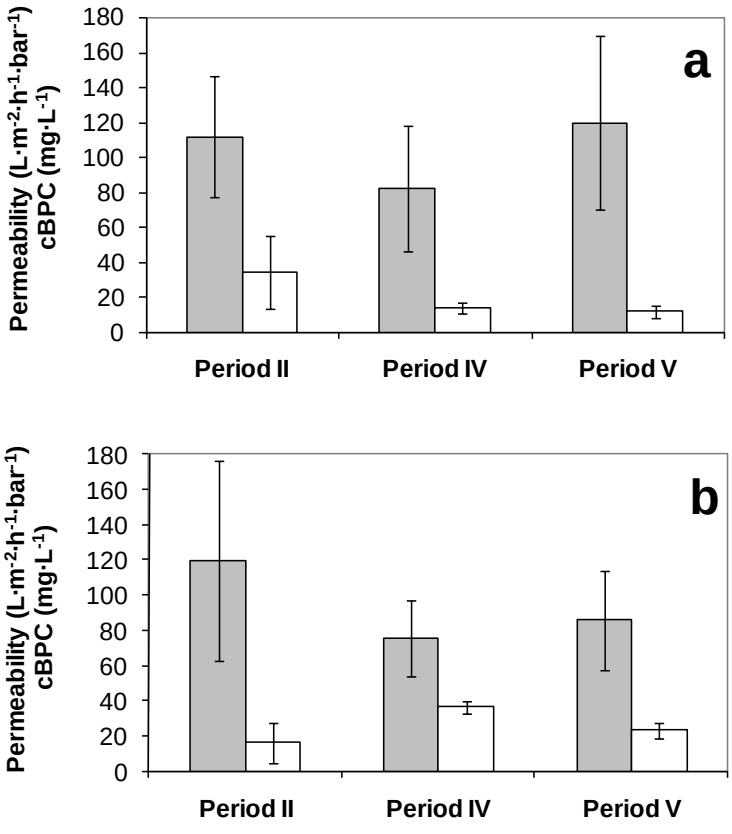

Figure 3.12. Relationship between permeability (■) and cBPC (□) in the F-SBR (a) and G-SBR (b) filtration chambers.

Therefore, the cBPC concentration in the TMF chamber might be representative of membrane fouling when the OLR was held constant (figure 3.12b). Conversely, this parameter was not indicative of membrane fouling in the flocculent system because the OLR decreased during operation (figure 3.12a). Thus, the cBPC concentration might be related to membrane permeability only when the OLR was held constant.

3.4.3. Specific cake resistance and particle size distribution

The particle size distributions of the solids in the chambers indicated that the particles in the flocculent system filtration chamber were smaller than those in the chamber of the granular system (figure 3.13). The mean particle size in the flocculent and granular filtration chambers were 80 and 250 μm, respectively. Moreover, 50 % of the particles in the granular filtration chamber were larger than 1000 μm.

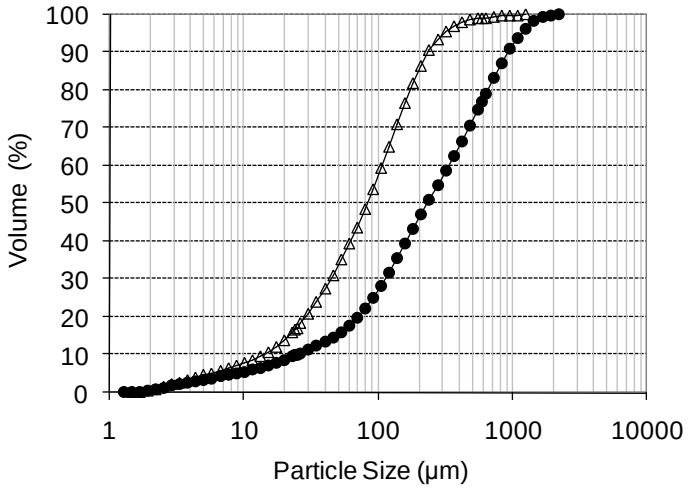

Figure 3.13. Cumulative volume percentage distributions for flocculent (△) and granular (1 mm sifted) (●) sludges sampled on operating day 210 (period III).

The sludge filterability test produced similar results for both TMF chamber liquors. As shown in table 3.2, the specific resistance to filtration (SRF) values were generally lower for the granular liquor. The SRF values ($1 \cdot 10^{11}$-$1 \cdot 10^{13}$ m·kg^{-1}) are similar to those obtained by Pollice et al. (2008) ($1 \cdot 10^{11}$-$1 \cdot 10^{13}$ m·kg^{-1}) and to the typical values observed for CAS (Metcalf & Eddie, 2003) ($1 \cdot 10^{13}$-$1 \cdot 10^{14}$ m·kg^{-1}). However, they were markedly lower than previously reported SRF values for MBRs of approximately $1 \cdot 10^{15}$ m·kg^{-1} (Cicek et al., 1999). The SRF values were similar for the liquors from both membrane filtration chambers. Moreover, similar SRF values were observed in period I but not in period IV,

during which the fed wastewater was free of solids. Theoretical and experimental studies have shown that fine particles cause severe membrane fouling (Bai and Leow, 2002). Smaller aggregates increase the resistance of the cake formed, and the critical flux is reached at lower values (Belfort and Davis, 1994). However, despite observed differences in the particle size distributions, this phenomenon was not observed, as the SRF values were similar for both systems. This result likely indicated that other factors, such as the presence of cBPC, could impact TMF more significantly than the size distribution.

Table 3.2. SRF of liquors from the TMF chambers of F-SBR and G-SBR systems

Day	Period	Floc SRF ($m \cdot kg^{-1}$)	Granular SRF ($m \cdot kg^{-1}$)
184	II	$1.5 \cdot 10^{13}$	$7.0 \cdot 10^{12}$
218	II	$8.6 \cdot 10^{12}$	$1.3 \cdot 10^{13}$
226	II	$5.1 \cdot 10^{11}$	$4.7 \cdot 10^{11}$
247	III	$1.4 \cdot 10^{13}$	$4.4 \cdot 10^{12}$
270	IV	$8.4 \cdot 10^{13}$	$8.4 \cdot 10^{12}$
304	IV	$5.8 \cdot 10^{12}$	$4.0 \cdot 10^{12}$

3.5. Conclusions

- Flocculent and granular SBR provided optimal organic matter removal, with values of 90 % in both systems. With respect to tertiary filtration operation both systems behaved as secondary bioreactors, eliminating part of the COD and nitrifying the ammonium proceeding from the reactors.
- The permeability, transmembrane pressure and critical flux of both membranes were similar. Thus, no significant operational differences between the G-SBR and the F-SBR were observed. The experimental results indicate that the presence of suspended solids in the influent and the nitrification process more significantly affected membrane performance than the morphology of the aggregated biomass.
- The positive effects of suspended solids in wastewater from the fish freezing industry on membrane performance were demonstrated, and permeability was improved. Because SRF was not affected by the absence of suspended solids, the lower observed permeabilities could have been a result of changes in the soluble matrix, but the exact mechanism is unknown.
- This study confirmed the importance of the carbohydrate fraction of SMP as one of the most important parameters related to membrane fouling, and the observed trend is

identical to that of the cBPC concentration. A certain trend between cBPC concentration and permeability, especially at a constant OLR, was observed. Therefore, organic carbon is suggested as an indicator of membrane fouling.

- To obtain the most appropriate tertiary filtration system, future investigations should focus on more specific aspects to identify accurately the differences between the systems and to determine the impact of suspended solids in the fed solution.

3.6. References

Ahmed, Z., Cho, J., Lim, B.R., Song, K.G., Ahn, K.H. 2007. Effects of sludge retention time on membrane fouling and microbial community structure in a membrane bioreactor. *Journal of Membrane Science* **287,** 211-218.

APHA-AWWA-WPCF. 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, USA.

Arrojo, B., Mosquera-Corral, A., Garrido, J.M., Méndez, R. 2004. Aerobic granulation with industrial wastewater in sequencing batch reactors. *Water Research* **38,** 3389-3399.

Artiga, P., García-Torrielo, G., Garrido, J.M., Méndez, R. 2006. Membrane bioreactor: An advanced technology for the treatment and reuse of waste waters. *Tecnología del agua* **26,** 54-60.

Bai, R., Leow, H.F. 2002. Microfiltration of activated sludge wastewater - the effect of system operation parameters. *Separation and Purification Technology* **29,** 189-198.

Belfort, G., Davis, R.H. 1994. The behavior of suspensions and macromolecular solutions in crossflow microfiltration. *Journal of Membrane Science* **96,** 1-58.

Beun, J.J., Hendriks, A., van Loosdrecht, M.C.M., Morgenroth, E., Wilderer, P.A., Heijnen, J.J. 1999. Aerobic granulation in a sequencing batch reactor. *Water Research* **33,** 2283-2290.

Chang, I.-S., Lee, C.-H. 1998. Membrane filtration characteristics in membrane-coupled activated sludge system -- the effect of physiological states of activated sludge on membrane fouling. *Desalination* **120,** 221-233

Chu, H.P., Li, X.Y. 2005. Membrane fouling in a membrane bioreactor (MBR): sludge cake formation and fouling characteristics. *Biotechnology Bioenengineering* **90,** 323–331.

Cicek, N., Franco, J.P., Suidan, M.T., Urbain, V., Manem J. 1999. Characterization and comparison of a membrane bioreactor and a conventional activated-sludge system in the treatment of wastewater containing high-molecular weight compounds. *Water Environmental Research* **71,** 64–70.

Citulsky, J.A., Farahbakhsh, K., Kent, F.C. 2009. Effect of total suspended solids loading on short-term fouling in the treatment of secondary effluent by an immersed ultrafiltration pilot system. *Water Environmental Research* **81**(12), 2427-2436.

Côté, P., Masini, M., Mourato, D. 2004. Comparison of membrane options for water reuse and reclamation. *Desalination* **167,** 1–11.

Drews, A., Mante, J., Iversen, V., Vocks, M., Lesjean, B., Kraume, M. 2007. Impact of ambient conditions on SMP elimination and rejection in MBR. *Water Research* **41,** 3850–3858.

Drews, A., Vocks, M., Bracklow, U., Iversen, V., Kraume, M. 2008. Does fouling in MBRs depend on SMP?. *Desalination* **231,** 141–149

Drews, A. 2010. Membrane fouling in membrane bioreactors – Characterisation, contradictions, cause and cures. *Journal of Membrane Science* **363,** 1-28

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith F. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28,** 350–356.

Huang, X., Liu, R., Qian, Y. Behaviour of soluble microbial products in membrane bioreactor. 2000. *Process Biochemistry* ***36*****,** 401-406.

Frank, B. P., Belfort, G. 2003. Polysaccharides and sticky membrane surfaces: critical ionic effects. *Journal of Membrane Science* **212,** 205-212.

Harscoat, C., Jaffrin, M. Y., Bouzerar, R., Courtois, J. 1999. Influence of fermentation conditions and microfiltration processes on membrane fouling during recovery of glucuronane polysaccharides from fermentation broths. *Biotechnology and Bioengineering* **65,** 500–511.

Judd, S.J. 2004. A review of fouling of membrane bioreactors in sewage treatment. *Water Science Technology* **49,** 229–235.

Kimura, K., Yamato, N., Yamamura, H., Watanare, Y. 2005. Membrane fouling in pilot-scale membrane bioreactors (MBRs) treating municipal wastewater. *Environmental Science Technology* **39,** 6293.

Lee, W., Kang, S., Shin, H.S. 2003. Sludge characteristics and their contribution to microfiltration in submerged membrane bioreactors. *Journal of Membrane Science* **216,** 217-227.

Lesjean, B., Rosenberger, S., Laabs, C., Jekel, M., Gnirss, R., Amy G. 2005. Correlation between membrane fouling and soluble/colloidal organic substances in membrane bioreactors for municipal wastewater treatment, *Water Science and Technology* **51,** 1-8.

Li, X.F., Gao, F.S., Hua, Z.Z., Du, G.C., Chen, J. 2005. Treatment of synthetic wastewater by a novel MBR with granular sludge developed for controlling membrane fouling. *Separation and Purification Technology* **46,** 19–25.

Liu, Q.S., Tay, J.H., Liu, Y. 2003. Substrate concentration-independent aerobic granulation in sequential aerobic sludge blanket reactor. *Environmental Technology* **24,** 1235 – 1243.

Liu, Y., Fang, H.H.P. 2003. Influences of extracellular polymeric substances (EPS) on flocculation, settling, and dewatering of activated sludge. *Critical reviews in environmental science and technology* **33,** 237–273.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. 1951. Protein measurement with the Folin Phenol Reagent. *Journal of Biological Chemistry* **193,** 265–275.

Lubello, C., Gori, R., de Bernardinis, A.M., Simonelli, G. 2003. Ultrafiltration as tertiary treatment for industrial reuse. *Water Science and Technology: Water supply* **3,** 161-168.

Massé, A. Spérandio, M., Cabassud, C. 2006. Comparison of sludge characteristics and performance of a submerged membrane bioreactor and an activated sludge process at high solids retention time. *Water Research* **40,** 2405–2415

Meng, F., Chae, S.R., Drews, A., Kraume, M., Shin, H.S., Yang, F. 2009. Recent advances in membrane bioreactors (MBRs): Membrane fouling and membrane material. *Water Research* **43,** 1489-1512

Metcalf & Eddy. 2003. Wastewater Engineering: Treatment and Reuse**,** fourth ed., McGraw-Hill, New York.

Moy, B.Y.-P., Tay, J.-H., Toh, S.-K., Liu, Y., Tay S.T.-L. 2002. High organic loading influences the physical characteristics of aerobic sludge granules. *Letters in applied microbiology* **34,** 407-412.

Nagaoka H. 1999. Nitrogen removal by submerged membrane separation activated sludge process. *Water Science and Technology* **39**, 107-114

Pearce, G. 2010. Membrane system design, in: M. Wilf, The Guidebook to Membrane Technology for Wastewater Reclamation, *Desalination Publications*, Hopkinton (MA), USA, 187-245.

Pollice, A., Giordano, C., Laera, G., Saturno, D., Mininni, G. 2008. Physical characteristics of the sludge in a complet retention membrane bioreactor. *Water Research* **41,** 1832-1840.

Rosenberger, S., Laabs, C., Lesjean, B., Gnirss, R., Amy, G., Jekel, M., Schrotter, J.-C. 2006. Impact of colloidal and soluble organic material on membrane performance in membrane bioreactors for municipal wastewater treatment. *Water Research* **40,** 710–720.

Tay, J.H., Liu, Q.S., Liu, Y. 2001. The role of cellular polysaccharides in the formation and stability of aerobic granules. *Letters in applied microbiology* **33,** 222–226.

Tchobanoglous, G., Darby, J., Bourgeous, K., McArdle, J., Genest, P., Tylla, M. 1998. Ultrafiltration as an advanced tertiary treatment process for municipal wastewater. *Desalination* **119,** 315-322.

Wang, X.-M., Li, X.-Y. 2008. Accumulation of biopolymers clusters in a submerged membrane bioreactor and its effect on membrane fouling. *Water Research* **42,** 855-862.

Wu, J., Huang, X. 2009. Effect of mixed liquor properties on fouling propensity in membrane bioreactors. *Journal of Membrane Science* **342,** 88–96.

Zhang, X., Bishop, P.L., Kinkle, B.K. 1999. Comparison of extraction methods for quantifying extracellular polymers in biofilms. *Water Science and Technology* **39,** 211-218.

Zheng, X., Ernst, M., Jekel, M. 2011. Stabilizing the performance of ultrafiltration in filtering tertiary effluent – Technical choices and economical comparisons. *Journal of Membrane Science* **366,** 82-91.

Chapter 4

Combining UASB and MBR for the treatment of low-strength wastewaters at ambient temperature

Summary

In this chapter, the combination of upflow anaerobic sludge blanket (UASB) reactor and aerobic MBR process for the treatment of low-strength wastewaters at ambient temperature was proposed. Both technologies were operated combined into one single system through the continuous internal recirculation from the aerobic MBR to the methanogenic UASB or as a UASB reactor followed by an MBR post-treatment when the recirculation was turned off. The objective of the methanogenic UASB reactor was to diminish COD of the raw wastewater, producing a biogas rich in methane, and to decrease the sludge production. The aerobic MBR consisted in an aerobic stage with biomass growing both on suspended carriers and in suspension and a separate chamber with a membrane filtration module. In the MBR, the remaining soluble biodegradable COD was oxidized and a high quality effluent was obtained. Moreover, nitrogen removal was stimulated during one operational period through the application of anoxic cycles in the first stage of the MBR, but any effect was observed. Applied OLR varied between 0.7 and 3.1 $kgCOD \cdot m^{-3} \cdot d^{-1}$ and COD removal was above 95 % during most of the operation, of which in between 40 and 80% was removed in the UASB reactor. Biogas production with methane content around 80% was observed. Regarding membrane operation, permeabilities around 150 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ were achieved, operating with fluxes of 12-15 $L \cdot m^{-2} \cdot h^{-1}$. A better membrane performance was observed during period II, when recirculation between MBR and UASB reactor was off.

Parts of this chapter have been published or submitted for publication as:

Buntner, D., Sánchez, A., Garrido, J.M. 2011. Three stages MBR (methanogenic, aerobic biofilm and membrane filtration) for the treatment of low-strength wastewaters. *Water Science and Technology* 64(2), 397-402.

Buntner, D., Sánchez A., Garrido, J. M. 2013. Feasibility of combined UASB and MBR system in dairy wastewater treatment at ambient temperatures. Submitted to *Journal of Chemical Engineering*.

4.1. Introduction

The application of anaerobic processes for treating low-strength wastewaters has received high attention in recent years. One of the reasons is that it may guarantee the process sustainability with regard to the use of the aerobic processes due to the lower energy consumption, generation of a biogas with a high methane content and diminution of biomass production. Among all these technologies, the upflow anaerobic sludge blanket (UASB) reactor has been the most relevant due to its simplicity and compactness.

UASB reactors have been proposed and applied to the treatment of various industrial wastewaters and even domestic wastewater in warm or tropical regions of the planet where the residual water has a temperature greater than 20 °C throughout the year (Seghezzo et al., 1998; Leitao et al., 2006). Its use has become popular in countries like India, Pakistan, China, Colombia, Brazil, Indonesia and Egypt. On the contrary, the use of UASB reactor for the treatment of urban wastewater in countries with cold or temperate climates is not feasible due to a combination of interrelated factors such as low cellular productivity and activity of microorganisms at these temperatures. Biomass losses in the final effluent might of UASB reactor may be compensated by the increased activity of the sludge at higher temperature (hot countries), but in countries with temperate or cold wastewater it results into a vicious circle in which the loss of biomass avoids to increase capacity and the diminution of capacity prevents to produce the biomass that is required to compensate the losses observed.

Over the last decade, the adaptation of membranes coupled with anaerobic biological processes has made anaerobic membrane bioreactors (AnMBR) a promising alternative to conventional wastewater treatment. Moreover, the use of filtration membranes allows avoiding the observed biomass losses, and could make wastewater treatment feasible even at lower temperatures (Judd, 2011). However, the main drawback of using AnMBR is related with membrane fouling and the maximum operating flux that can be achieved. Feasible flux has a strong influence on both the capital and operation costs of the process. Most of the authors working with AnMBR reported fluxes in the range of 5-15 $L \cdot m^{-2} \cdot h^{-1}$ at temperatures above 30 °C (Zhang et al., 2005; Saddoud et al., 2007; Trzcinski and Stuckey, 2009). Jeison and van Lier (2006) obtained critical flux values in the range 16-23 $L \cdot m^{-2} \cdot h^{-1}$ under mesophilic (30 °C), and 5-21 $L \cdot m^{-2} \cdot h^{-1}$ under thermophilic (55 °C) conditions. In the case of domestic wastewater treated at ambient temperatures, operating fluxes are significantly lower. Robles et al. (2013) reported fluxes between 9 and 13 $L \cdot m^{-2} \cdot h^{-1}$ treating municipal wastewaters at temperatures between 15 and 33 ºC. Lew et

al. (2009) reported 11.25 $L \cdot m^{-2} \cdot h^{-1}$ at 25 °C, while Wen et al. (1999), operating a laboratory scale anaerobic bioreactor coupled with a membrane filtration worked with flux of 5 $L \cdot m^{-2} \cdot h^{-1}$. Similar results were obtained by Ho and Sung (2010), who operated with flux set on 5 $L \cdot m^{-2} \cdot h^{-1}$ at a temperature of 15-20 °C. Moreover, Spagni et al. (2010) demonstrated that the applicable fluxes obtained in AnMBR ranged between 2 and 5 $L \cdot m^{-2} \cdot h^{-1}$ depending strongly on operational conditions and rapid membrane fouling was usually observed. Therefore, the fluxes obtained in AnMBR are lower than those observed in aerobic MBR, normally between 20 and 30 $L \cdot m^{-2} \cdot h^{-1}$ (Judd, 2002; Wen et al., 2004).

On the other hand, anaerobic effluents might require additional treatment due to the presence of residual biodegradable organic matter. Although different technologies has been operated as an UASB post-treatment (Chong et al., 2012), aerobic MBRs have been receiving increasing applications as a post-treatment unit due to their capability of producing high-quality effluents, free of pathogens and suspended solids.

4.2. Objectives

In this study, the combination of an UASB reactor and an MBR, in two different configurations (as a single system and as an MBR after a methanogenic pre-treatment), for the treatment of low-strength wastewater at ambient temperatures was investigated. The objective was to diminish the COD, producing biogas rich with methane, to diminish overall sludge production, and to obtain high quality effluent due to the implementation of membrane filtration. A complementary objective was the removal of nitrogen through the application of anoxic cycles in the first chamber of the MBR. Moreover, the hypothesis that membrane module, aerated in the membrane chamber, could be operated at higher fluxes than those reported for AnMBR, and closer to those obtained in aerobic MBR treating raw wastewater, was checked.

4.3. Material and methods

4.3.1. Experimental set-up and operating strategy

4.3.1.1. Operating system

A 176 L bioreactor (figure 4.1) was operated at ambient temperature (17.5-21.0 ºC). A 120 L volume UASB system was used for the first methanogenic stage. The effluent of the UASB reactor was led to the aerobic chamber (36 L), with biomass growing onto plastic support and in suspension. 18.5 L (50% of the effective volume) of Kaldnes K3

support were added in this chamber. Finally, the membrane filtration was carried out in a 20 L aerobic chamber, where a membrane module Zenon ZW10 with a surface area of 0.9 m^2 was employed. This module consisted of PVDF hollow-fibre membrane, with a pore size of 0.04 µm. The membrane was operated in cycles of 7.5 min with a permeation period of 7 min and a backwashing period of 0.5 min. The membrane filtration chamber was aerated in order to minimize membrane fouling. The specific air demand (SAD_m) applied was 0.7 $Nm^3 \cdot m^{-2} \cdot h^{-1}$. An internal recirculation between membrane filtration and biofilm aerobic chambers was implemented in the MBR (R=1). The operation of the system was controlled by a PLC (Siemens S7-200) connected to a computer. Trans-membrane pressure (TMP) data was measured with an analogue pressure sensor (Efector500 PN-2009) and collected in the PC via an analogue PLC module Siemens EM 235.

The UASB reactor was seeded with 50 L of anaerobic biomass (27 $g \cdot L^{-1}$) from the internal circulation anaerobic reactor of a brewery industry located in Galicia (Spain), whereas 5 L of biomass from a MBR pilot plant treating urban wastewater was employed as an aerobic biomass inoculum.

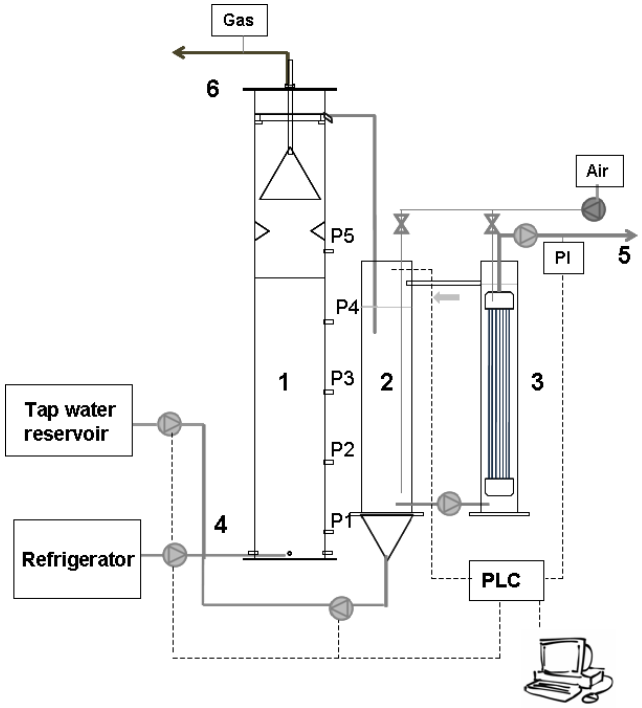

Figure 4.1. Schematic diagram of the system. (1) UASB reactor, (2) Biofilm aerobic chamber, (3) Membrane chamber, (4) Feeding and recirculation, (5) Permeate (backwashing), (6) Biogas. P1, P2, P3, P4 and P5 refer to the sampling ports.

4.3.1.2. Influent and operating strategy

The study was performed during 364 days and the operation could be divided in four different periods:

Period I: Days 0 to 175. During this period continuous recirculation between the biofilm aerobic chamber and the UASB reactor was implemented (R=0.15). With respect to the internal recirculation in the MBR (between the membrane filtration chamber and the aerobic biofilm one), a constant recirculation ratio of 1 was applied during the four experimental periods. During the start-up, permeate flux was fixed at 11 $L \cdot m^{-2} \cdot h^{-1}$ and was varied between 12 and 19 $L \cdot m^{-2} \cdot h^{-1}$ after day 30 till the end of the operation. The UASB reactor was fed using synthetic wastewater composed of diluted skimmed milk, $NaHCO_3$ and trace elements. Feeding COD concentration was 641.2±219.0 $mg \cdot L^{-1}$.The system was purged from day 58 on, in order to maintain sludge retention time (SRT) in the aerobic and filtration chambers below 30 d. These purges took place from the sampling port P5 (figure 4.1) in the UASB reactor and from membrane filtration chamber due to the accumulation of biomass.

Period II: Days 176 to 260. During this period, recirculation between the biofilm aerobic chamber and the methanogenic reactor was turned off. Therefore in this period the system could be considered as a UASB reactor followed by an MBR post-treatment. Feeding COD concentration was 738.6±125.1 $mg \cdot L^{-1}$.

Period III: Days 261 to 315. During this period, recirculation between the biofilm aerobic chamber of the MBR and the methanogenic reactor was turned on, converting the two reactors connected in series into one again. Recirculation ratio was the same than during period I (R=0.15) and feeding COD concentration was 810.6±209.5 $mg \cdot L^{-1}$.

Period IV: Days 316 to 364. During this period, the feasibility of nitrogen removal in the system was studied. For this purpose, anoxic cycles in the biofilm aerobic chamber were implemented, with on/off aeration periods of 30/20 min. Moreover, recirculation ratio between the biofilm aerobic chamber of the MBR and the methanogenic reactor was diminished from 0.15 to 0.075 on day 325. COD concentration fed to the system during this period was 707.2±176.4 $mg \cdot L^{-1}$.

Regarding membrane maintenance, two different membrane cleaning procedures were performed. An in-situ maintenance cleaning was performed every two weeks by backwashing with NaHClO solution (250 ppm Cl_2) for 1 h. Recovery cleaning was performed outside the reactor only when permeability value was below 50 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$.

This cleaning was performed by soaking the membrane module in NaHClO solution (2000 ppm Cl_2) for 2 h and backwashing with NaHClO (2000 ppm Cl_2) for 1 h. Prior to both chemical cleanings, a physical rinsing with tap water was performed.

4.3.2. Analytical methods

Temperature, pH, alkalinity and the concentrations of dissolved oxygen, mixed liquor volatile suspended solids (MLVSS), total and soluble chemical oxygen demand (COD), ammonium, nitrite, nitrate, phosphate and total phosphorous were determined according to the Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WPCF, 1998). Volatile fatty acids (VFA) (i-butyric, n-butyric, i-valeric and n-valeric) were analyzed by gas chromatography (HP, 5890A) equipped with a flame ionization detector (HP, 7673A). Biomass concentration in the biofilm was measured detaching the biomass of ten carriers in 200 mL of permeate with a sonicator (Dr. Hielscher, UP200s) at 100 μm of amplitude during 30 min. MLVSS concentration was then determined and referred to support surface. The carbohydrate concentration was determined following the method of Dubois et al. (1956).

Biogas production was measured using a Milli GasCounter MGC-10 (Ritter, Germany) and its composition was measured in a gas chromatograph HP 5890 Series II with the column of Porapack Q 80/100 2m x 1/8" (SUPELCO). Soluble microbial products (SMP) were determined by centrifuging the biomass for 20 min at 5000 rpm (Heraeus, Labofuge 200).

With respect to the membrane operation, trans-membrane pressure and permeability were measured continuously. The critical flux was determined according to the modified flux-step method proposed by van der Marel et al. (2009). The criterion employed was that the increment of transmembrane pressure (TMP) with respect to time was higher than 10 $Pa \cdot min^{-1}$ (Le-Clech et al., 2003). Fouling rate was calculated by measuring the observed TMP drop ($Pa \cdot min^{-1}$) experimented during twelve hours, while constant flux was maintained.

Further information regarding analytical methods is provided in Chapter 2.

4.4. Results and discussion

4.4.1. System performance

4.4.1.1. General results

The system was operated at ambient temperature, and wastewater temperatures changed with seasons (21.0-17.5 °C). The pH of the effluent from UASB was around 6.7. Aerobic chamber and permeate pH varied from 6.7 to 7.7 and from 7.0 to 8.2, respectively, depending on the system performance.

Despite operating in psychrophilic conditions volatile fatty acids (VFA) concentration in the UASB effluent was below minimum detection limit of the method used (20 $mg \cdot L^{-1}$) during the whole experimentation. Methane reached more than 70% of the biogas composition during the whole operation. Biogas production in the UASB chamber was detected during the four experimental periods, with an average production rate of 46.3±9.5 $L \cdot d^{-1}$, depending on OLR applied and temperature. Biogas production yield was around 0.15 $m^3_{methane} \cdot kgCOD_{eliminated}^{-1}$. The upflow velocity in the UASB reactor was around 0.15 $m \cdot h^{-1}$, below maximum value of 1 $m \cdot h^{-1}$ typically recommended for sewage treatment in conventional UASB systems (van Haandel and Lettinga, 1994).

4.4.1.2. Organic matter removal

Figure 4.2 depicts the evolution of the COD concentration and OLR applied to the UASB and the MBR reactor as well as the total COD removal percentage and the COD removal percentage achieved in the UASB reactor. As can be observed on figure 4.2b, the overall COD removal was normally above 97.5 % during the four operational periods. The most part of the organic matter removal took place in the UASB reactor, with percentages above 80 %, except from days 75 to 90, when some micropollutants degradation experiments were carried out. The addition of methanol in the feeding in order to dissolve this micropollutants led to a drastic increase on COD concentration in the feeding (figure 4.2c) and provoked a slight shock load in the UASB reactor, decreasing COD removal percentage in this reactor (figure 4.2b) and increasing OLR applied to the MBR (figure 4.2a). With the exception of these days in period I, COD concentration in the permeate was always lower than 15 $mg \cdot L^{-1}$. Differences between soluble COD concentration in the membrane chamber and the COD concentration in the permeate were observed during the most part of the operation, indicating that the membrane retained a fraction of colloidal matter present in the mixed liquor.

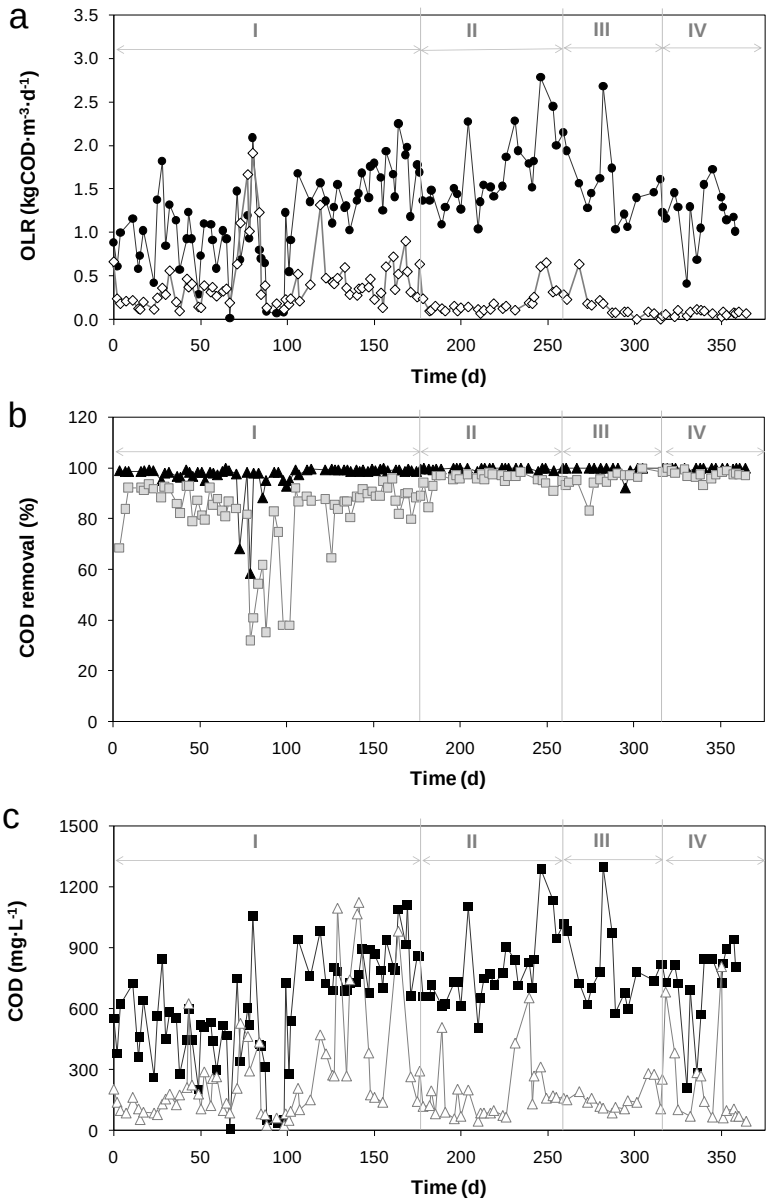

Figure 4.2. a) Evolution of OLR applied to the UASB (●) and the MBR (◇); b) COD removal percentages in the UASB (■) and the entire system (▲); c) total COD concentration in the feeding (■) and the UASB effluent (△) during periods I, II, III and IV.

As can be observed on figure 4.2a, the applied organic loading rate was very variable during the first operational weeks. During the first 114 days the synthetic

wastewater was stored at environmental temperature and the milk used was subjected to rapid degradation in the feeding tank. The coagulation of the milk, reflected in a lower soluble COD/total COD ratio (around 0.75), provoked lower COD removal percentages in the UASB reactor (81.4±15.4%). Nevertheless, the system showed robustness and COD removal was not affected by these fluctuations, reaching 96.8±3.2%. From day 114 on, the total COD fed did not change so much except punctual days (figure 4.2c) as the synthetic wastewater was cooled and the soluble COD/total COD ratio increased up to 0.88.

The OLR applied during period I varied between 0.7 and 2.3 kgCOD·m^{-3}·d^{-1}, with an HRT in the range of 9-17 h for the UASB and 13-21 h for the entire system, whereas the OLR applied to the MBR was 0.43±0.36 kgCOD·m^{-3}·d^{-1} referred to the soluble COD. COD removal percentages in the UASB reactor (figure 4.2.b) were also referred to the soluble COD concentration in the UASB effluent. It should be taken into account that the total COD concentration in the effluent of the UASB reactor (figure 4.2c) was mainly due to biomass wash-out, especially during periods I, III and IV, when recirculation between biofilm aerobic chamber and methanogenic reactor was turned on. The recirculated biomass was accumulated in the UASB reactor before being washing out again towards the aerobic biofilm chamber. That is the reason why at the beginning of period I and during period III the total COD in the effluent of the UASB reactor was not so high.

During period II, the recirculation between the first chamber of the MBR and the UASB reactor was turned off, but this fact did not affected significantly to organic matter removal. The OLR applied during period II varied between 1.0 and 3.1 kgCOD·m^{-3}·d^{-1}, with an HRT in the range of 9-17 h for the UASB and 11-18 h for the entire system. Overall COD removal did not change significantly with respect to period I, but during this period the 95.0±3.1% of COD removal took place in the UASB reactor (figure 4.2b). The absence of suspended biomass recirculated from the aerobic biofilm chamber led to an improvement of methanogenic activity. In fact, methane composition during this period was higher than that observed during period I, reaching values above 80%. As a consequence, OLR applied to the MBR was lower (0.22±0.17 kgCOD·m^{-3}·d^{-1}) (figure 4.2a). The increase in average temperature from 19 ºC in period I to 21 ºC in period II also played an important role in the improvement of UASB efficiency. The total COD concentration peaks observed during period II (figure 4.2c) were consequence of two punctual anaerobic biomass wash out from the UASB reactor due to operational problems.

During periods III and IV, both reactors were operated again like one single integrated system as occurred in period I. The main difference was that during period III,

the recirculation ratio between the MBR and the UASB reactor was maintained in 0.15 whereas in period IV this ratio was decreased to 0.075. Moreover, during period IV anoxic cycles were implemented in the first chamber of the MBR in order to stimulate nitrogen removal (section 4.4.1.3). Nevertheless, COD removal percentages in the UASB and the MBR did not change with respect to period II.

The removals achieved in the UASB reactor were similar than that obtained by other authors treating low-strength wastewaters at ambient temperatures. Lettinga et al. (1983) achieved 60-80% COD removal, with OLR of 1.6 $kgCOD \cdot m^{-3} \cdot d^{-1}$ at 21 °C, whereas de Man et al. (1986) achieved COD removal efficiency between 45 and 60%, working with an UASB reactor treating similar OLR at 10-18 °C. Nevertheless, the combination of the UASB with the MBR proposed in this work, led to much higher organic matter removal rates. Moreover, due to the presence of the membrane, a high quality effluent and a total retention of the solids at ambient temperatures were guaranteed.

4.4.1.3. Nitrification and nitrogen removal

Although this system was originally designed to produce nutrient rich wastewater, free of microbial indicators that could be reused in agriculture, the feasibility of nitrogen removal was studied by the implementation of anoxic cycles in the first chamber of the MBR during period IV.

All the ammonia present in the UASB effluent (between 25 and 35 $mg \cdot L^{-1}$) was produced as the effect of protein hydrolysis in the methanogenic reactor. It should be taken into account that the dissolved total nitrogen (DTN) in the influent of the UASB reactor was 19.6±8.0 $mg \cdot L^{-1}$.

During period I only partial nitrification was observed, with $N\text{-}NH_4^+$ and $N\text{-}NO_x$ concentrations in the permeate of 21.5±17.0 and 3.5±3.5 $mg \cdot L^{-1}$, respectively (figure 4.3). Since the recirculation ratio in this period was 0.15, with the average flow of 287 $L \cdot d^{-1}$, the aparent SRT of suspended biomass in the MBR was around 1.3 d, which is not sufficient to maintain stable nitrification. All the ammonia was oxidized probably by the nitrifying biomass in biofilm growing on plastic support present in the biofilm aerobic chamber. One of the advantages of the use of plastic support for the formation of biofilms was the complete retention of these biofilms in the first chamber of the MBR. Nevertheless the slow growth of microorganisms in the biofilm led to its incomplete development during period I.

During period II, total nitrification was observed. $N\text{-}NH_4^+$ and $N\text{-}NO_x$ concentrations in the permeate were 4.7±4.2 and 25.6±13.6 $mg \cdot L^{-1}$ (figure 4.3), respectively. During this

period the system operation was stable. The low C/N ratio and the absence of recirculation led to a stable ammonia oxidation and nitrifying bacteria could grow both in suspension and in the form of biofilm. This fact explains why most of the DTN in permeate was present as $N\text{-}NO_X$, while the $N\text{-}NH_4^+$ was very low (figure 4.3). As can be observed on figure 4.3 the $N\text{-}NO_x$ in the permeate concentrations were slightly higher than expected taking into account the ammonia present in the UASB effluent. This fact was observed during periods II, III and IV and could indicate that during these periods additional ammonia, produced by the hydrolysis of particulate fraction of COD, was oxidized.

During period III the recirculation between the first chamber of the MBR and the UASB reactor was turned on. As occurred during period I, the low aparent SRT of suspended biomass in the MBR (1.6 d) could affect nitrification process as a consequence of the gradual wash-out of suspended nitrifying bacteria with recycled sludge. Nevertheless, during period III, the biofilm was well developed, with concentrations around 45 $gMLVSS \cdot m^{-2}$, and allowed to maintain nitrification process achieved during period II (figure 4.3).

From day 316 (beginning of period IV) onwards, anoxic cycles (30 min aeration/20 min no aeration) were implemented in the first (aerobic biofilm) chamber of the MBR in order to stimulate nitrogen removal in the system. However, the introduction of the anoxic cycles caused a sharp decrease on DO concentration and thus nitrification was strongly affected due to the competition between heterotrophs and nitrifiers for the oxygen. Although DO was normally above 4 $mg \cdot L^{-1}$ in both chambers of the MBR, the application of anoxic cycles led to a decrease in the DO concentration in the first chamber to values below 2 $mg \cdot L^{-1}$ (during aeration period of the cycle). The diminution of the biomass concentration in the aerobic biofilm chamber (as effect of sludge recirculation) also had a negative influence on nitrification.

As can be observed on figure 4.3, the proposed system made feasible to manipulate nitrogen conversion to ammonia and/or nitrate. This characteristic might be used for certain applications of the effluent treated. Since membrane bioreactor encourages water reuse applicability, the treated wastewater could be suitable for agriculture, heating or cooling water or for cleaning purposes, depending on the quality standards.

In the case of agriculture, the most beneficial nutrient for plants is nitrogen. Nevertheless, at very high concentrations (over $30 mgTN \cdot L^{-1}$) it can overstimulate plant growth, causing problems such as lodging and excessive foliar growth and also delay maturity or result in poor crop quality. Both the concentration and forms of nitrogen (nitrate

and ammonium) need to be considered in irrigation water (Lazarova and Bahri, 2004). As an example in the case of treated wastewater reuse for cooling systems, the beneficial of ammonia for the control of biological as makeup water with monochloramine has been recently reported by Chien et al. (2012). The biocide monochloramine would be formed in situ through the reaction of free chlorine and ammonia in the incoming water to the cooling system. Thus, it could be important to avoid nitrification. It should be taken into consideration the great amounts of water required in thermoelectric power plants. For instance, the freshwater withdrawal for thermoelectric power plant cooling exceeds withdrawals for agricultural irrigation in the United States (Chien et al., 2012).

Nitrogen removal was not observed during period IV (figure 4.3), when anoxic cycles were implemented in order to stimulate denitrification process. Nevertheless, nitrogen removal feasibility in the system was analyzed in further experiments. The reason of such behaviour will be explained there (Chapter 6) and is related with the imput of oxygen associated with aeration in the aerobic/anoxic biofilm chamber.

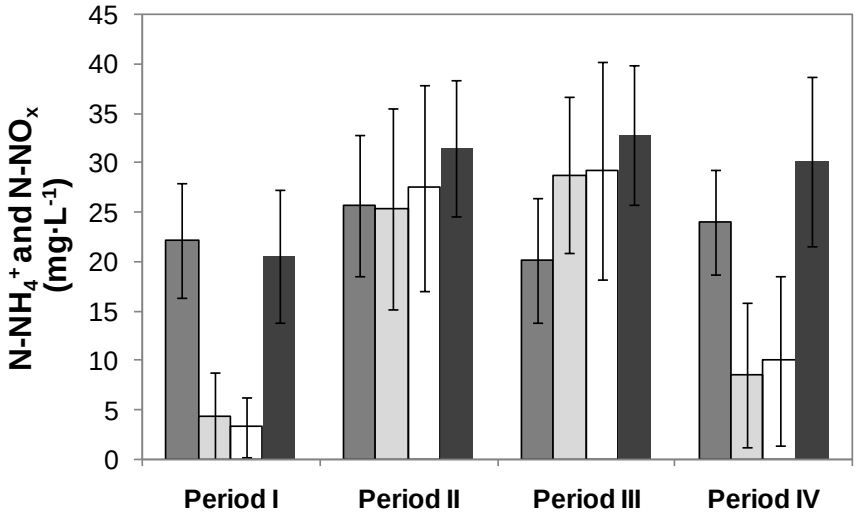

Figure 4.3. N-NH4+ concentration in the UASB effluent (■); DTN concentration in the permeate (■); N-NOx- concentration in the first chamber of the MBR (■) and the permeate (□) during periods I, II, III and IV.

4.4.2. Biomass

The retention of anaerobic biomass was almost complete during the four experimental periods. During periods I, III and IV anaerobic granules washed-out were

returned to the UASB reactor through the recirculation. During period II, the small amounts of anaerobic granules washed-out from the UASB reactor were accumulated in the MBR and eventually purged from the system. Nevertheless, the MLVSS concentration in the bottom part of the UASB reactor was maintained at 30-35 $g \cdot L^{-1}$ and the growth of seed granules were observed during the four experimental periods.

Suspended biomass concentration in aerobic chamber was 0.7±0.5, 1.6±0.6, 0.4±0.2 and 0.5±0.2 $gMLVSS \cdot L^{-1}$ during periods I, II, III and IV respectively. On the other hand, biofilm growing on the support carrier in aerobic chamber was well developed within the experiment. A progressive development of the biofilm was observed. Thus, biomass concentration in the biofilm was around 45 $gMLVSS \cdot m^{-2}$ from period III onwards, which was equivalent to MLVSS concentration of approximately 6 $g \cdot L^{-1}$.

With respect to the membrane filtration chamber, MLVSS concentration ranged between 0.5 and 4.0 $g \cdot L^{-1}$ (table 4.1). Although the recommended MLVSS concentration for MBR operation are normally between 5 and 12 $g \cdot L^{-1}$ (Rosenberger et al., 2005; Judd, 2011), the low OLR applied to the MBR as a consequence of the methanogenic pre-treatment led to a slow development of the biomass. Moreover during periods I, III and IV, MLVSS concentration was even lower than in period II (table 4.1) due to the recirculation of biomass to the UASB reactor. The food to microorganism (F/M) ratio, referred to soluble COD, applied to the MBR was very low during the four studied periods. F/M ratio were 0.011, 0.025, 0.027 and 0.042 $kgCOD \cdot kgMLVSS^{-1} \cdot d^{-1}$ during periods I, II, III and IV respectively. Typical values previously reported for aerobic MBR treating municipal wastewaters are in the range of 0.1-0.3 $kgCOD \cdot kgMLVSS^{-1} \cdot d^{-1}$ (Brepols, 2006; Judd, 2011).

The employment of internal recirculation from aerobic biofilm chamber of the MBR to the UASB allowed avoiding the anaerobic biomass losses as well as diminishing biomass production, since part of the excess aerobic sludge was hydrolyzed in the methanogenic reactor. The overall biomass yield was 0.09 and 0.12 $gMLVSS \cdot gCOD^{-1}$ for periods I, III and IV and period II, respectively. Both values were much lower than the typical values determined for aerobic MBRs (0.25 – 0.61 $gMLVSS \cdot gCOD^{-1}$) (Judd, 2011), and close to those observed for the anaerobic treatment of wastewaters, that are in the range between 0.11 and 0.14 $gMLVSS \cdot gCOD^{-1}$ (van Haandel and Lettinga, 1994).

The average sludge retention time (SRT) was above 100 d for the whole system. The overall aerobic SRT (referred to the MBR) was around 15 d for period II, which is a typical value for aerobic MBR (Judd, 2011). During periods I, III and IV (with suspended biomass

recirculation from the MBR to the UASB system) it was difficult to define a real SRT, since a fraction of aerobic biomass was continually recirculated between the MBR and UASB reactors. Nevertheless, the amount of aerobic biomass purged from the system was similar than that purged during period II (without recirculation).

4.4.3. Membrane performance

The main parameters regarding membrane performance are presented in table 4.1. The flux was maintained between 12 and 15 $L \cdot m^{-2} \cdot h^{-1}$ during most of the operational periods, being more variable on period I due to the higher fouling rate observed during this period (figure 4.4b). During periods III and especially IV, the lower fluxes values were obtained due to the extremely low MLVSS concentrations in the membrane filtration chamber (table 4.1). Only in the period II, without recirculation between MBR and UASB reactor and a higher MLVSS concentration in membrane filtration chamber, stable operation at 19 $L \cdot m^{-2} \cdot h^{-1}$ was achieved. The flux achieved was higher than those observed in AnMBR, with values between 5 and 10 $L \cdot m^{-2} \cdot h^{-1}$ (Spagni et al., 2010; Zhang et al., 2007; Lew et al., 2009; Ho and Sung, 2010), but lower than those typically reported in aerobic MBR operating with similar membrane modules, being between 20 and 25 $L \cdot m^{-2} \cdot h^{-1}$ (Judd, 2002; Wen et al., 2004). On the other hand, the observed fluxes were much lower than those referred by Leikness et al. (2007), who worked with a biofilm membrane bioreactor with a first moving bed bioreactor (MBBR) followed by a filtration chamber connected in series and obtained fluxes of 50 $L \cdot m^{-2} \cdot h^{-1}$.

Table 4.1. Parameters related with membrane performance.

Parameter	Unit	Period I	Period II	Period III	Period IV
MLVSS[1]	$g \cdot L^{-1}$	1.3±0.7	3.3±0.7	0.8±0.3	0.9±0.4
Flux	$L \cdot m^{-2} \cdot h^{-1}$	13.3±1.8	15.5±2.2	13.3 ± 2.8	11.0±1.5
Permeability	$L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$	153±68	189±32	170 ± 42	148±47
Fouling rate	$Pa \cdot min^{-1}$	1.7±0.9	0.5±0.4	0.8±0.3	0.9±0.6
SMP_C	$mg \cdot L^{-1}$	37.6±17.3	10.4±3.1	14.6±2.0	14.1±5.0
Recirculation	-	yes (0.15)	no	yes (0.15)	yes (0.075)

[1]In the membrane filtration chamber

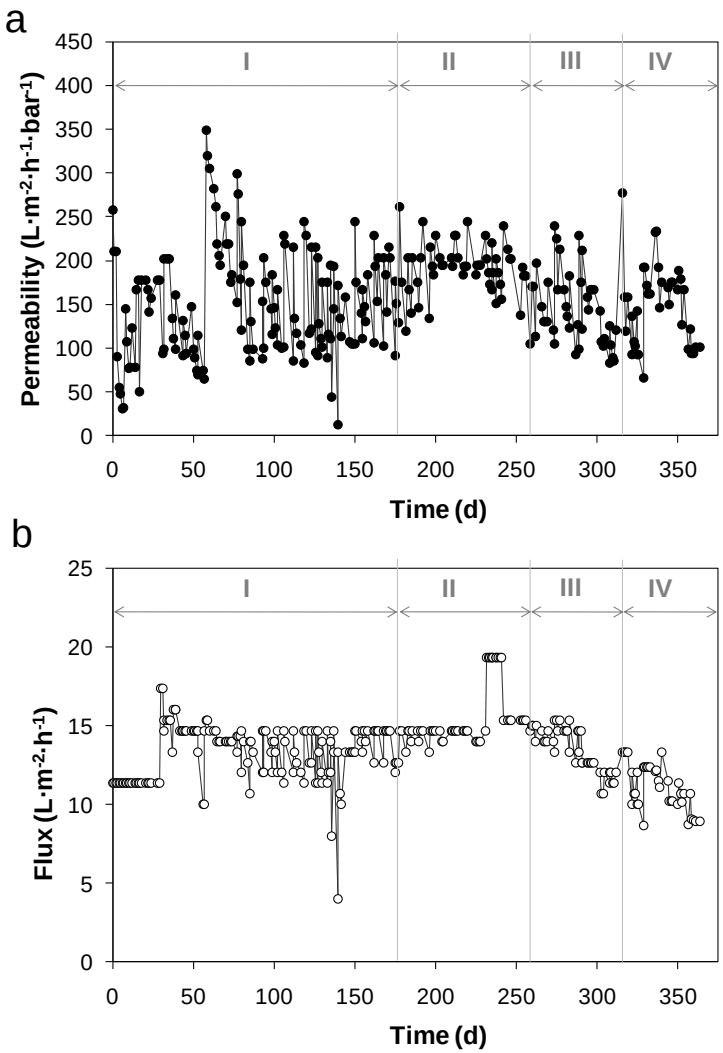

Figure 4.4. a) Evolution of permeability (●) and b) flux (○) during periods I, II, III and IV.

Membrane critical flux did not varied significantly during periods I, III and IV increasing from 19 $L \cdot m^{-2} \cdot h^{-1}$ during the first period to 20 $L \cdot m^{-2} \cdot h^{-1}$ in periods III and IV. Nevertheless, measured critical flux during period II increased up to 24 $L \cdot m^{-2} \cdot h^{-1}$. The flux applied during the operation was always below the critical flux, thus it was expected that reversible fouling was predominant. However, this effect was only observed during period II, when permeability was almost fully recovered when a physical cleaning with tap water was carried out. On the other hand, two intensive chemical cleanings were performed on

days 57 (period I) and 316 (end of period III), probably due to the low MLVSS concentration in the membrane chamber that did not prevent membrane pore blocking (Drews, 2010). Maintenance chemical cleanings were performed fortnightly, except on period II. Chemical reagents were not necessary during period II.

Permeabilities between 100 and 200 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ were normally observed during the two operational periods (figure 4.4a). These values were slightly better than those observed during the operation of similar membrane modules, (Judd, 2002; Bouhabila et al., 2001), and also were higher than permeabilities observed in AnMBR (Spagni et al., 2010; Zhang et al., 2007). The reason of such behaviour is still unclear, but it is considered that aerobic biomass, both in suspension and in biofilm retained or degraded some foulants generated in the methanogenic reactor. This fact made feasible to achieve the observed permeabilities. The highest permeability values and the lowest fouling rates were observed in period II (table 4.1). Rapid permeability drops were observed during period I, whereas fouling rate was almost negligible during period II (figure 4.4a). In fact, as mentioned before, several maintenance cleanings and one recovery cleaning on day 57 were performed during period I. Although the possible causes responsible for membrane fouling will be analyzed in deep in Chapter 5, the worse membrane performance observed during period I, coincided with a higher concentration of SMP carbohydrate fraction (SMPc) and a lower MLVSS concentration in membrane filtration chamber (table 4.1). During periods III and IV, SMPc was not so high than in period I, probably due to the higher F/M ratio, but the lower MLVSS concentration led to a strong membrane fouling and lower operational fluxes, especially in period IV.

The membrane filtration chamber in the MBR of the proposed system worked with MLVSS concentrations much lower than those recommended in the literature (Judd, 2011). In figure 4.5 are represented to different TMP profiles corresponding to days on which MLVSS concentration was very low (around 0.5 $gMLVSS \cdot L^{-1}$) and moderate (around 3.0 $gMLVSS \cdot L^{-1}$). The other operational parameters such as SRT, SAD_m SMP_c concentration or flux were similar. As can be observed, at higher biomass concentrations the TMP profile is typical of cake layer formation fouling (Drews, 2010), which is easily removed by mechanical cleaning. On the other hand, at lower biomass concentrations TMP profile changed significantly, indicating that the lack of protection by the cake layer led to an irreversible membrane fouling provoked by pore clogging of soluble and colloidal biopolymers. In fact this kind of fouling is more harmful since it can be only removed by chemical cleaning. The fouling rate increased more than a 60 % from one scenario to another, being MLVSS the only different parameter.

The low OLR applied to the MBR had a great impact on MLVSS concentration. As mentioned before, temperature also played an important role in the membrane performance. COD removal efficiency in the methanogenic reactor increased with temperature, causing a diminution of the biodegradable COD supplied to the aerobic MBR, and, as a consequence, leading to a lower MLVSS. The beginning of period II coincided with the beginning of the springtime. Therefore, higher temperatures observed from period II onwards provoked an improvement of COD removal in the methanogenic reactor and hence, a decrease in the OLR applied to the MBR (figure 4.2a). Therefore, the supply of a minimum OLR in the MBR was shown to be of prime importance in order to maintain MLVSS, and hence to control membrane fouling rate. In this sense, the proposed system could be modified in order to allow the feeding of a small fraction of the raw influent directly into the aerobic biofilm chamber, in order to assure a minimum biodegradable COD supply, and thus maintain F/M ratio above 0.1 kgCOD·kgMLVSS^{-1}·d^{-1} (Brepols, 2006; Judd, 2011), especially when operating at higher temperatures.

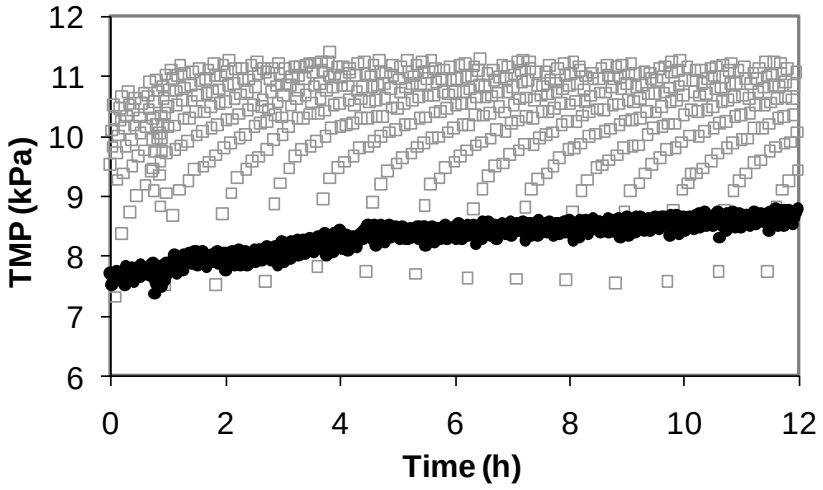

Figure 4.5. TMP profiles after a physical cleaning at 3.0 gMLVSS·L^{-1} (□) and 0.5 gMLVSS·L^{-1} (●) during operating days 145 and 319, respectively.

4.5. Conclusions

- The combination of UASB and aerobic MBR technologies in one single system or as a post-treatment, presented a good performance for the treatment of low-strength wastewaters at ambient temperatures. Both proposed configurations presented an

excellent COD removal performance. On average, the permeate COD was less than 15 $mg \cdot L^{-1}$ with COD removals above 95%.

- The absence of suspended solids, the very low COD concentration and the level of nutrients in the effluent allow reusing purified wastewater (e.g. in agriculture).

- The application of anoxic cycles in the first chamber of the MBR did not estimulate denitrification process as a consequence of the worsening on nitrification capacity.

- The proposed system showed flexibility to convert total nitrogen to NH_4^+ and/or NO_3^-.

- Biogas production was detected during the whole operating period, with average methane content of 75-80%. Due to effective retention of biomass by the UASB reactor and membrane module, sludge concentration in the anaerobic bioreactor could be kept at high values, reaching more than 30 $g \cdot L^{-1}$. Moreover, granular sludge growth was observed.

- The employment of internal recirculation from aerobic biofilm chamber of the MBR to the UASB allowed diminishing biomass production, to values similar than those observed for the anaerobic treatment.

- The membrane was operated at ambient temperature with fluxes of 15 $L \cdot m^{-2} \cdot h^{-1}$, lower than those achieved in aerobic MBRs treating municipal wastewater, but higher than fluxes obtained in AnMBR.

- The only difference between the two proposed configurations was the better membrane performance observed during period II, when recirculation was turned off and the system could be considered as a UASB reactor with an aerobic MBR post-treatment.

- In this kind of configurations is important to assure a minimum F/M ratio in the MBR in order to reach a suitable MLVSS concentration for membrane operation.

4.6. References

APHA-AWWA-WPCF. 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, USA.

Bouhabila, H., Ben Aïm, R., Buisson, H. 2001. Fouling characterisation in membrane bioreactors. *Separation and Purification Technology* **22-23**, 123-132.

Brepols, C. 2011. Operating large scale membrane bioreactors for municipal wastewater treatment, 1st ed., IWA publishing, London, UK.

Chien, S.-H., Chowdhury, I., Hsieh, M.-K., Li, H., Dzombak, D.A., Vidic, R.D. 2012. Control of biological growth in recirculating cooling systems used treated secondary effluent as makeup water with monochloramine. *Water Research* **46**, 6508-.6518.

Chong, S., Sen, T.K., Kayaalp, A., Ang, H.M. 2012. The performance enhancements of upflow anaerobic sludge blanket (UASB) reactors for domestic sludge treatment – A State-of-the-art review. Water Research **46**, 3434-3470.

de Man, A.W.A., Grin, P.C., Roersma, R.E., Grolle, K.C.F., Lettinga, G. 1986. Anaerobic treatment of municipal wastewater at low temperatures. In: Anaerobic treatment. A grown-up technology Conference papers (Aquatech '86), Amsterdam, 451–466.

Drews, A. 2010. Membrane fouling in membrane bioreactors – Characterisation, contradictions, cause and cures. *Journal Membrane Science* **363,** 1-28

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith F. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28,** 350–356.

Ho, J., Sung, S. 2010. Methanogenic activities in anaerobic membrane bioreactors (AnMBR) treating synthetic municipal wastewater. *Bioresource Technology* **101**(7), 2191-2196.

Jeison, D., van Lier, J.B. 2006. Cake layer formation in anaerobic submerged membrane bioreactors (AnSMBR) for wastewater treatment. *Journal of Membrane Science* **284**, 227–236.

Judd, S.J. 2002. Submerged membrane bioreactors: flat plate or hollow fibre? *Filtration and Separation* **39**(5), 30-31.

Judd, S.J. 2011. The MBR Book: Principles and Applications of Membrane Bioreactors for Water and Wastewater Treatment, 2nd ed., Elsevier Science Ltd, Oxford.

Lazarova, V., Bahri, A. 2004. Water reuse for irrigation: agriculture, landscapes, and turf grass. CRC press, Boca Raton, USA.

Le-Clech, P., Jefferson, B., Chang, I.S., Judd, S.J. 2003. Critical flux determination by the flux-step method in a submerged membrane bioreactor. *Journal of Membrane Science* **227**, 81-93.

Leiknes, T., Odegaard, H. 2007. The development of a biofilm membrane bioreactor. *Desalination* **202**, 135-143.

Leitao, R.C., van Haandel, A.C., Zeeman, G., Lettinga, G. 2006. The effects of operational and environmental variations on anaerobic wastewater treatment systems: A review. *Bioresource technology* **97**, 1105-1118.

Lettinga, G., Hobma, S.W., Hulshoff Pol, L.W., de Zeeuw, W., de Jong, P., Grin P., Roersma, R. 1983. Design operation and economy of anaerobic treatment. *Water Science and Technology* **15**, 177–195.

Lew, B., Tarreb, S., Beliavski, M., Dosoretzb, C., Green, M. 2009. Anaerobic membrane bioreactor (AnMBR) for domestic wastewater treatment. *Desalination* **243**, 251–257.

Robles, A., Ruano, M.V., Ribes, J., Ferrer, J. 2013. Factors that affect the permeability of comercial hollow-fibre membranes in a submerged anaerobic MBR (HF-SAnMBR) system. *Water Research* **47**, 1277-1288.

Rosenberger, S., Evenblij, H., te Poele, S., Wintgens, T., Laabs, C. 2005. The importance of liquid phase analyses to understand fouling in membrane assisted activated sludge processes -six case studies of different European research groups. *Journal of Membrane Science* **263**, 113–126.

Saddoud, A., Ellouze, M., Dhouib, A., Sayadi, S. 2007. Anaerobic membrane bioreactor treatment of domestic wastewater in Tunisia. *Desalination* **207**, 205–215.

Seghezzo, L., Zeeman, G., van Lier, J.B., Hamelers, H.V.M., Lettinga, G. 1998. A review: the anaerobic treatment of sewage in UASB and EGSB reactors. *Bioresource and Technology* **65**, 175–190.

Spagni, A., Casu, S., Crispino, N.A., Farina, R., Mattioli, D. 2010. Filterability in a submerged anaerobic membrane bioreactor, *Desalination* **250**, 787-792.

Trzcinski, A.P., Stuckey, D.C. 2009. Anaerobic digestion of the organic fraction of municipal solid waste in a two-stage membrane process. *Water Science and Technology* **60**(8), 1965-1978.

van der Marel, P., Zwijnenburg, A., Kemperman, A., Wessling, M., Temmink, H., van der Meer, W. 2009. An improved flux-step method to determine the critical flux and the critical flux for irreversibility in a membrane bioreactor. *Journal of Membrane Science* **332**, 24-29.

van Haandel, A.C., Lettinga, G. 1994. Anaerobic sewage treatment: a practical guide for regions with a hot climate, Chichester (England), J. Wiley & Sons Ltd.

Wen, C., Huang, X., Quian, Y. 1999. Domestic wastewater treatment using an anaerobic bioreactor coupled with membrane filtration. *Process Biochemistry* **35**, 335-340.

Wen, X., Bu, Q., Huang, X. 2004. Study on fouling characteristic of axial hollow fibers cross-flow microfiltration under different flux operations, in: *Proceedings of the Water Environment-Membrane Technology Conference, Seoul, Korea*.

Zhang, D.J., Lu, P.L., Long, T.R., Verstraete, W. 2005. The integration of methanogenesis with simultaneous nitrification and denitrification in a membrane bioreactor. *Process Biochemistry* **40 (2)**, 541-547.

Zhang, J., Padmasiri, S.I., Fitch, M., Norddhal, B., Raskin, L., Morgenroth, E. 2007. Influence of cleaning frequency and membrane history on fouling in an anaerobic membrane bioreactor. *Desalination* **207**, 153-166.

Chapter 5

Impact of methanogenic pre-treatment on the performance of an aerobic MBR system

Summary

The combination of anaerobic treatment with an aerobic MBR as a polishing step is an alternative to treat some industrial wastewater and/or urban wastewaters generated in warm climate countries. In this chapter a pilot-scale UASB reactor and an aerobic MBR as a polishing step were operated. The impact of the methanogenic stage on membrane fouling was studied. Operating fluxes of 11-18 $L \cdot m^{-2} \cdot h^{-1}$ and permeabilities of 100-250 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ were reported. It was demonstrated that the recirculation of aerobic biomass to the anaerobic stage provoked a release of biopolymers due to the hydrolysis of aerobic biomass in these conditions. Depending on biomass concentration in the membrane filtration chamber, the presence of biopolymers worsened membrane performance. Fouling rate was three times higher when biomass concentration decreased from 8 to 2 $g \cdot L^{-1}$, with similar concentrations of biopolymers present. Moreover, the presence of plastic support in the aerobic biofilm chamber was shown to improve membrane performance, decreasing the concentrations of the studied fouling indicators.

Carbohydrate fraction of soluble microbial products (SMP_C), colloidal biopolymer clusters (cBPC) and transparent exopolymer particles (TEP) concentrations were studied as possible fouling indicators for this system. A strong correlation between both cBPC and TEP concentrations with membrane fouling rate was observed.

Parts of this chapter have been published as:

Sánchez, A., Buntner D., Garrido, J. M. 2013. Impact of methanogenic pre-treatment on the performance of an aerobic MBR system. *Water Research* 47(3), 1229–1236.

5.1. Introduction

Anaerobic methanogenic technology is widely used, especially in warm climate regions, for treating low strength wastewaters at ambient temperature. Nevertheless, despite the advantages of anaerobic treatment, the final wastewater quality would not be high enough for a direct discharge to a watercourse. Anaerobic biological treatment systems are typically not effective in removing residual levels of soluble and colloidal organic contaminants (Berubé et al., 2006). Other concerns regarding the use of this technology, especially in temperate climate regions, are related with biomass loss in the effluent. These problems related with anaerobic treatment have been overcome in the last decade by coupling a membrane to the bioreactor. However, one of the main drawbacks of using anaerobic membrane bioreactors (AnMBR) is related with membrane fouling and the maximum flux that can be achieved. Flux has a strong influence on both the capital and operation costs of the process. For submerged membranes, most of the authors working with AnMBR reported fluxes in the range of 5-15 $L \cdot m^{-2} \cdot h^{-1}$ at temperatures above 30 °C (Zhang et al., 2005; Trzcinski and Stuckey, 2009). Jeison and van Lier (2006) obtained critical flux values in the range 16-23 $L \cdot m^{-2} \cdot h^{-1}$ under thermophilic (30 °C), and 5-21 $L \cdot m^{-2} \cdot h^{-1}$ under mesophilic (55 ºC) conditions. In the case of domestic wastewater treated at ambient temperatures, fluxes are significantly lower. Robles et al. (2013) reported fluxes between 9 and 13 $L \cdot m^{-2} \cdot h^{-1}$ treating municipal wastewaters at temperatures between 15 and 33 ºC. Lew et al. (2009) reported 11.25 $L \cdot m^{-2} \cdot h^{-1}$ at 25 ºC, while Wen et al. (1999), operating a laboratory scale anaerobic bioreactor coupled with a membrane filtration worked with flux of 5 $L \cdot m^{-2} \cdot h^{-1}$. Similar results were obtained by Ho and Sung (2010), who operated with flux set on 5 $L \cdot m^{-2} \cdot h^{-1}$ and the temperature of 15-20 ºC. Moreover, Spagni et al. (2010) demonstrated that the applicable fluxes obtained in AnMBR ranged between 2 and 5 $L \cdot m^{-2} \cdot h^{-1}$ depending strongly on operational conditions and rapid membrane fouling was usually observed. Therefore, the fluxes obtained in AnMBR are lower than those observed in aerobic MBR, typically being in the range between 20 and 30 $L \cdot m^{-2} \cdot h^{-1}$ (Judd, 2002; Wen et al., 2004).

Methanogenic reactors may be operated as a pre-treatment step, followed by an aerobic MBR system, for the treatment at ambient temperatures of domestic and industrial wastewaters (He et al., 2003; Buntner et al., 2011; Kushwaha et al., 2011). Additionally, the combination of both technologies might be an alternative to overcome problems related with the operation of AnMBR (fouling) and aerobic MBR (high energy consumption and sludge production). The energy gained from the anaerobic plant can be equivalent to that consumed by the aerobic step (BREF, 2006). The treated wastewater could be suitable for

reuse both in agriculture and as heating or cooling water or for cleaning purposes, depending on the quality standards.

The methanogenic treatment could affect results obtained in aerobic MBRs. Partial degradation products coming from the treatment of complex substrates in the methanogenic stage might have a negative impact on membrane performance. Wilén et al. (2000) observed that a complex substrate as activated sludge flocs deflocculated under anaerobic conditions. The deflocculated particles were mainly bacteria and floc fragments, although some soluble polymeric substances were also released. Therefore, the hydrolysis of complex substrates such as aerobic sludge (for instance coming from the recirculation between aerobic and methanogenic stages) could lead to a release of biopolymers, affecting membrane fouling.

The fraction of biopolymers most frequently mentioned in relation with membrane fouling is the group of soluble microbial products (SMP). This group contains soluble and colloidal biopolymers, mostly carbohydrates (SMPc) and proteins (SMPp) (Drews, 2010). SMPc has been widely considered as the most important parameter regarding membrane fouling (Rosenberger et al., 2006; Drews, 2010). Nevertheless, recent studies have introduced a more general approach to the biopolymers responsible for membrane fouling by defining biopolymer cluster (BPC) and transparent exopolymer particles (TEP) as important factors in the formation of the sludge fouling layer on the membrane surface and the increase of fouling potential (Sun et al., 2008; de la Torre et al., 2008). BPC have been defined as a pool of non-filterable organic matter in the liquid phase of the MBR sludge mixture much larger than SMP (Sun et al., 2008) whereas TEP are very sticky particles that exhibit the characteristics of gels, and consist predominantly of acidic polysaccharides (Passow, 2002). Depending on the applied assays, these groups are not distinct but overlap (Drews, 2010).

Different post-treatment systems for UASB effluents have been widely studied, among them aerobic MBRs being one of the most recent and not yet understood possibilities (Chong et al., 2012). Therefore, it is of prime importance to identify and understand the causes responsible for membrane fouling in these systems.

5.2. Objectives

The objective of this work was to study membrane fouling in an aerobic MBR after a methanogenic pre-treatment of low-strength wastewater at ambient temperatures.

5.3. Material and methods

5.3.1. Experimental set-up and operating strategy

A 176 L bioreactor (figure 5.1) was operated at ambient temperature (17-23 ºC). A 120 L volume UASB system was used for the first methanogenic stage. The effluent of the UASB reactor was led to an aerobic biofilm chamber (36 L), with biomass growing onto plastic support and in suspension. 18.5 L (50% of the effective volume) of Kaldnes K3 support were added in this chamber. Finally, the membrane filtration was carried out in a 20 L aerobic chamber, where a membrane module Zenon ZW10 with a surface area of 0.9 m^2 was employed. This module consisted of PVDF hollow-fibre membrane, with a pore size of 0.04 µm. The membrane was operated in cycles of 7.5 min with a permeation period of 7 min and a backwashing period of 0.5 min. The filtration chamber was aerated in order to minimize membrane fouling. The specific air demand (SAD_m) applied to the membrane was 0.7 $Nm^3 \cdot m^{-2} \cdot h^{-1}$. An internal recirculation between filtration and aerobic stages was implemented in the MBR (R=1). The operation of the system was controlled by a PLC (Siemens S7-200) connected to a computer. Trans-membrane Pressure (TMP) data was measured with an analogue pressure sensor (Efector500 PN-2009) and collected in the PC via an analogue PLC module Siemens EM 235.

Table 5.1. Main operational conditions of the bioreactor during the different periods.

Period	days	Feeding	Recirculation[1]	Ratio	Support[2]
I	0-175	Synthetic	yes	0.15	yes
II	176-260	Synthetic	no	-	yes
III	261-540	Synthetic	yes	0.15-0.075	yes
IV	541-569	Synthetic	no	-	yes
V	570-635	Synthetic	no	-	no
VI	636-680	Synthetic	yes	0.075	yes
VII	700-875	Synthetic + aerobic sludge[3]	no (fed)	-	yes

[1] Sludge recirculation between aerobic biofilm chamber and UASB reactor

[2] Plastic carriers Kaldnes K3 in the aerobic biofilm chamber

[3] Aerobic sludge was fed between days 769 and 841

The impact of different variables (recycle ratio between aerobic and methanogenic stages, presence of biofilm in the aerobic chamber and mixed liquor volatile suspended solids (MLVSS) concentration in the membrane filtration chamber) was studied during seven operational periods (table 5.1).

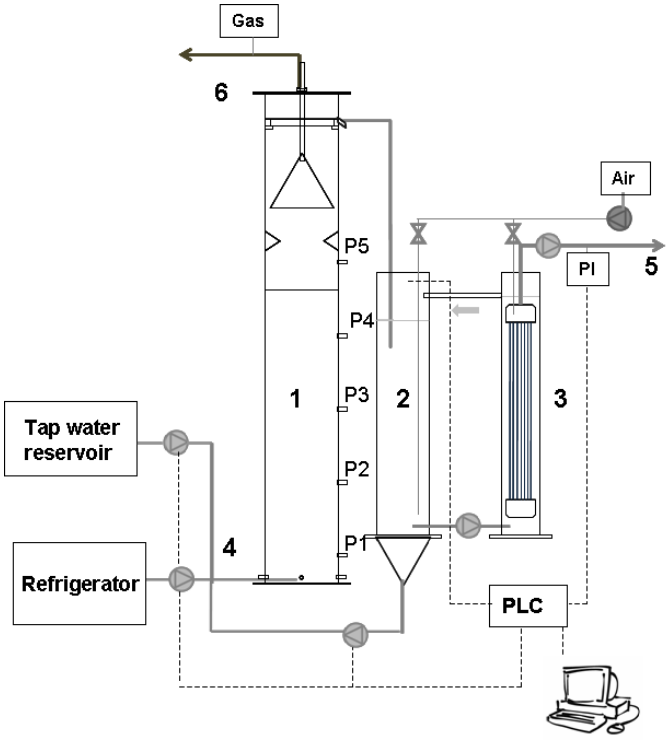

Figure 5.1. Schematic diagram of the bioreactor. **(**1) UASB methanogenic reactor; (2) Aerobic biofilm chamber; (3) Membrane filtration chamber; (4) Feeding and recirculation; (5) Permeate (backwashing); (6) Biogas outlet. P1, P2, P3, P4 and P5 refer to the sampling ports.

The reactor was fed using synthetic wastewater composed of diluted skimmed milk, $NaHCO_3$ and trace elements. COD concentration in the feeding was increased step-wisely from 500 to 2000 $mg \cdot L^{-1}$ until the period III and maintained between 2000-2500 $mg \cdot L^{-1}$ until the end of the operation.

During period VII, the impact of external MLVSS addition was studied. Aerobic biomass from a municipal wastewater treatment plant (WWTP) with a MLVSS concentration around 7 $g \cdot L^{-1}$ was fed batch-wise during 2 hours each 12 hours of operation. The total amount of biomass dosed was around 21 $gMLVSS \cdot d^{-1}$ which

represented an increment of 25% in total COD fed to the system. The dosage of the aerobic biomass into the UASB reactor only took place between days 769 and 841. Between days 805 and 841, MLVSS concentration in the membrane filtration chamber was manipulated from 8 to 2 $g \cdot L^{-1}$ in order to check its influence on membrane fouling. Three different MLVSS concentrations were checked in three steps of twelve days of duration each.

5.3.2. Analytical methods

Temperature, pH, alkalinity and the concentrations of dissolved oxygen, MLVSS, total and soluble chemical oxygen demands (COD) were determined according to the Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WPCF, 1998). Biogas production was measured using a Milli GasCounter MGC-10 (Ritter, Germany) and its composition was measured in a gas chromatograph HP 5890 Series II with the column of Porapack Q 80/100 2m x 1/8" (SUPELCO). Concentration of dissolved organic carbon (DOC) was measured with a Shimadzu analyser (TOC-5000). Soluble microbial products (SMP) were determined by centrifuging the biomass for 20 min at 5000 rpm (Heraeus, Labofuge 200). Carbohydrate and protein concentration were determined following the methods of Dubois et al. (1956) and Lowry et al. (1951), respectively.

The difference in DOC concentration between the sludge mixture after filtration through a 0.45 μm nitrocellulose membrane filters (HA, Millipore) and the permeate was assigned to the colloidal fraction of biopolymer clusters (cBPC) in the liquid phase of the sludge mixture suspension (Sun et al., 2008). The analysis method used for the determination of the transparent exopolymer particle (TEP) concentration is based on the protocol developed for TEP quantification in sea water by Arruda et al. (2004), using the modifications proposed by de la Torre et al. (2008). The critical flux was determined according to the modified flux-step method proposed by van der Marel et al. (2009). The criterion employed was that the increment of transmembrane pressure (TMP) with respect to time was higher than 10 $Pa \cdot min^{-1}$ (Le-Clech et al., 2003). Fouling rate was calculated by measuring the observed TMP drop ($Pa \cdot min^{-1}$) experimented during twelve hours, while constant flux was maintained.

Further information regarding analytical methods is provided in Chapter 2.

5.3.3. Batch experiments to study biomass hydrolysis

Batch experiments under aerobic and anaerobic conditions were carried out by mixing 1.5 L of anaerobic biomass and 1.0 L of aerobic biomass. Both experiments were performed in parallel, with a soft magnetic stirring, during eight hours. Samples were taken each 1-2 hours and filtered through 0.45 µm nitrocellulose membrane filters (HA, Millipore). DOC concentration was measured with a Shimadzu analyser (TOC-5000). Aerobic biomass either from the aerobic stage of the system (periods I to VI) and from an activated sludge system of a municipal WWTP (period VII) were used. The anaerobic biomass was taken from the UASB stage, where MLVSS concentration typically ranged between 28 and 35 $g \cdot L^{-1}$.

The batch experiment performed in anaerobic conditions with biomass from the aerobic stage of the system was repeated in order to measure not only DOC but also the concentration of SMPp, TEP and SMPc. These batch experiments were performed in duplicate during the different operational periods (periods III, IV, VI and VII).

5.4. Results and discussion

5.4.1. System performance

Total COD removal above 95% was achieved during the experimentation, of which more than 75% took place in the methanogenic stage. Regarding soluble COD, above 95% was removed in the UASB reactor. Stable nitrification, with the complete oxidation of approximately 30 mg NH_4-$N \cdot L^{-1}$, was achieved. The overall biomass yield calculated for the entire system was 0.14 $gMLVSS \cdot gCOD^{-1}$. The system was able to produce up to 130 $L \cdot d^{-1}$ of biogas, which corresponds to a maximum biogas yield of 0.260 $m^3_{methane} \cdot kgCOD_{eliminated}^{-1}$. During most of the experimentation, the overall aerobic sludge retention time (SRT) was between 12 and 30 d referred to the aerobic biofilm and membrane filtration chambers.

More detailed information regarding the operation of this system was given by Buntner et al. (2011) and also in Chapter 4.

5.4.2. Membrane performance

The flux was maintained between 11 and 18 $L \cdot m^{-2} \cdot h^{-1}$ during most of the operation. In general, the flux achieved was higher than those between 5 and 10 $L \cdot m^{-2} \cdot h^{-1}$ observed in anaerobic membrane bioreactors (Spagni et al., 2010; Zhang et al., 2007; Lew et al., 2009; Ho and Sung, 2010), but lower than those typically reported in aerobic MBRs

operating with similar membrane modules, being between 20 and 25 $L \cdot m^{-2} \cdot h^{-1}$ (Judd, 2002; Wen et al., 2004). Only in the period II, without recirculation between aerobic biofilm chamber and UASB reactor and MLVSS concentration in membrane filtration chamber of 3.3 ± 0.7 $g \cdot L^{-1}$, stable operation at 18 $L \cdot m^{-2} \cdot h^{-1}$ was achieved. Permeabilities between 100 and 250 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ were normally observed during the operation (figure 5.2). These values were slightly better than those observed during the operation of similar submerged membrane modules (Judd, 2002, Wen et al., 2004), and higher than permeabilities observed in AnMBR (Spagni et al. 2010; Zhang et al., 2007).

In figure 5.2 the evolution of membrane permeability and colloidal BPC concentration is depicted. During the periods I to III the impact of feeding the methanogenic reactor with suspended sludge of the aerobic biofilm chamber was studied. In this sense, biomass from the aerobic biofilm chamber was recirculated to the UASB reactor. The lowest values of colloidal BPC concentration (cBPC) and the highest stable permeabilities were obtained in the period II, when recirculation was turned off (figure 5.2a, table 5.2). Moreover, higher fluxes were also applied during the period II (table 5.2).

One of the advantages of the studied MBR configuration is the possible recovery of washed out anaerobic biomass from the biofilm aerobic chamber. This might avoid the loss of capacity of the methanogenic system, especially when operated at lower temperatures. However, altogether with the anaerobic sludge, aerobic biomass was also recirculated to the methanogenic stage. Recirculation ratio (R) was diminished in the period III from 0.15 to 0.075, in order to study the impact of recirculation. As a result, cBPC concentration was lower in the period III than in the period I (table 5.2). Hydrolysis of complex substrates might be the limiting step of methanogenic process, especially at ambient temperatures (van Haandel and Lettinga, 1994). Thus, cBPC increase might be caused by the partial degradation of aerobic MLVSS recycled to the UASB reactor.

The impact of plastic support in the aerobic stage was studied from the period IV to the period VI. The small carrier elements were used to promote the growth of an aerobic biofilm that regardless of the recirculation ratio used, was maintained in the aerobic biofilm chamber. This plastic support was removed during the period V, in order to seek how biofilm affects the system performance (period without recirculation). This led to a remarkable increase of cBPC concentration and a worsening of membrane performance (table 5.2, figure 5.2b).

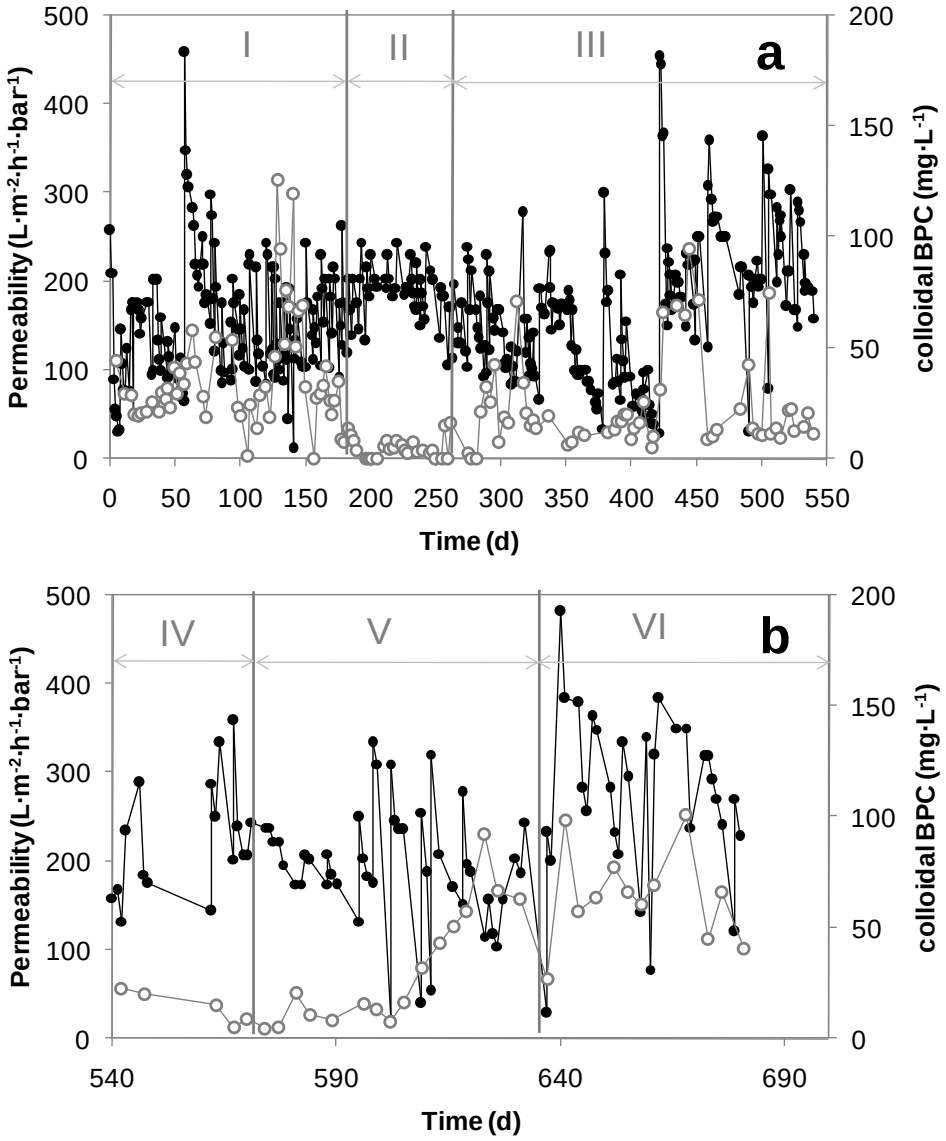

Figure 5.2. Permeability (●) and cBPC concentration (○) during periods I-III (a) and periods IV-VI (b).

The observed increase of cBPC concentration did not take place at the point when the plastic support was removed, but after more than 20 operating days (figure 5.2b). cBPC levels were similar to those observed for periods I and III, during which biomass was recirculated from the aerobic biofilm chamber to the UASB reactor. The reason of such

behaviour might be related with a shift of the microbial community caused by the removal of the carrier. Microscopic observation showed a great amount of attached ciliated protozoa in the biofilm. Hypothetically, the absence of these filtering organisms caused the increase of colloidal biopolymer concentration. Although digestion of detrital colloids by protozoans is not fully understood, assimilation of some forms of colloidal exopolymers by protozoans has been reported (Sherr, 1988). Thus, the use of a carrier would be beneficial for promoting development of filtering protozoa, diminishing cBPC levels and enhancing the membrane performance. The beneficial effects of carriers on membrane fouling were also reported previously by other authors in an MBR system with both suspended biomass and biofilms (Liu et al., 2010).

In the period VI, when plastic support was returned to the aerobic biofilm chamber, the recirculation was turned on again. The positive effect of the plastic support was counteracted by the products of partial hydrolysis of aerobic sludge that occurred in the methanogenic stage. That was the reason why cBPC concentration did not diminish. A clear drop in permeability, decreasing from 400 to 50 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$, took place during the first 20 days of period VI. Rapid permeability drops were also observed during periods I, III and also during period V (period without support).

Table 5.2. Main results in the membrane filtration chamber during periods I-VI.

Period	days	cBPC ($mg \cdot L^{-1}$)	MLVSS ($g \cdot L^{-1}$)	Flux ($L \cdot m^{-2} \cdot h^{-1}$)	Permeability ($L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$)
I*	0 - 175	39.7 ± 24.2	1.3 ± 0.7	13.3 ± 1.8	153 ± 68
II	176 - 260	4.9 ± 4.2	3.3 ± 0.7	15.5 ± 2.0	189 ± 32
III*	261 - 540	22.9 ± 20.7	2.6 ± 2.0	12.6 ± 2.6	169 ± 78
IV	541 - 569	12.2 ± 6.7	4.0 ± 1.5	13.2 ± 1.3	229 ± 69
V	570 - 635	31.3 ± 26.2	4.8 ± 2.0	14.5 ± 2.6	193 ± 70
VI*	636 - 680	67.7 ± 18.9	2.7 ± 1.5	14.9 ± 1.9	285 ± 85

* Chemical recovery cleaning that took place during this period

The applied food to microorganism (F/M) ratio, or SRT might influence the membrane performance of the MBR. F/M referred to soluble COD, and applied to the aerobic and filtration stages was very low during the seven operational periods. The lowest values were observed during periods I and V (0.011 $kgCOD \cdot kgMLVSS^{-1} \cdot d^{-1}$) whereas the

highest value corresponded to period III (0.036 kgCOD·kgMLVSS^{-1}·d^{-1}). During periods II, IV, VI and VII the F/M was around 0.025 kgCOD·kgMLVSS^{-1}·d^{-1}. Regarding SRT, the values calculated in periods II, IV, V and VII, during which aerobic MLVSS were not recycled to the UASB system, were very similar (between 12 and 16 d). In periods I, III and VI (with suspended biomass recirculation from the MBR to the UASB system) it was difficult to define a SRT, since a fraction of aerobic biomass was continually recirculated between the UASB and MBR systems. Nevertheless, the amount of aerobic biomass purged from the system was similar to that in periods without recirculation. Thus, variations of SRT or F/M could be discharged to be the main cause of the observed MBR behaviour.

Recovery cleanings were performed in periods I and III where recirculation was used and also at the beginning of period VI, as a consequence of severe permeability loss at the end of period V. This confirmed the impact of plastic support and hydrolysis of aerobic biomass in the methanogenic stage over membrane fouling. Moreover, membrane critical flux was 20.2 ± 2.8 L·m^{-2}·h^{-1} during the periods I, III, V and VI. . The highest critical flux values were obtained during the periods II and IV, reaching 28.0 L·m^{-2}·h^{-1}, with no aerobic sludge recycling and the presence of the plastic support in the aerobic biofilm chamber.

5.4.3. Fouling indicators

The carbohydrate fraction of soluble microbial products (SMPc), transparent exopolymers (TEP) and biopolymer clusters (BPC) has been reported as possible fouling indicators (Rosenberger et al., 2006; Drews, 2010; Sun et al., 2008; de la Torre et al., 2008). In this study, these parameters were measured in order to establish a relationship with fouling rate and membrane performance. As can be observed in figure 5.3, certain linear relationships between these indicators and fouling rate can be established. The higher was the concentration of each one of these parameters, the higher was the fouling rate. A linear correlation between SMPc and fouling rate has been reported previously by some authors (Rosenberger et al., 2006) but not in the case of cBPC and TEP.

BPC have been defined as a pool of non-filterable organic matter in the liquid phase of the MBR sludge mixture much larger than SMP, being an important factor in the formation of the sludge fouling layer on the membrane surface and responsible for the increase of fouling potential (Sun et al., 2008). TEP are very sticky particles that exhibit the characteristics of gels, and consist predominantly of acidic polysaccharides (Passow, 2002). TEP has been recently reported as a useful tool for MBR investigation that may help understanding the complex phenomenon of membrane fouling.

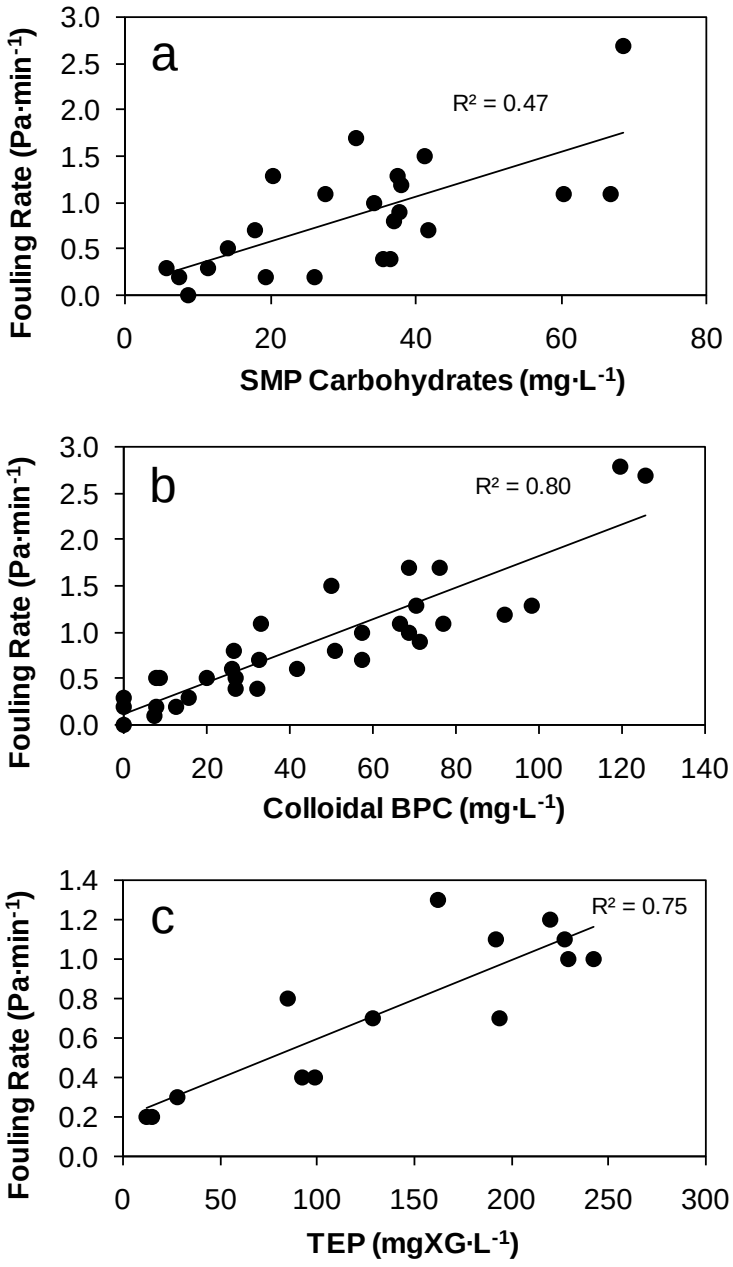

Figure 5.3. Relationship between fouling rate and SMP_C (a), cBPC (b) and TEP (c) concentration (●) during periods II, III, IV, V and VI. TEP was only measured on periods IV, V and VI.

SMP_C has been often cited as one of the main factors affecting MBR fouling (Le-Clech et al., 2006). Nevertheless, the results obtained in this study suggest a better correlation of colloidal BPC (cBPC) and TEP with fouling rate than that obtained with SMP_C. Both cBPC and TEP determinations are easy to perform, and are not so laborious processes as SMP_C. Nevertheless, from these two methods cBPC could be more reliable, as it depends only on DOC measurements. As can be observed in figure 5.2 and table 5.2, stable operation in terms of permeability and lower membrane fouling was achieved in the period II, coinciding with the lower values of the cBPC concentration. On the other hand, cBPC concentration increase led to a severe membrane fouling and hence rapid decrease of permeability.

5.4.4. Batch hydrolysis assays

The relationship between cBPC concentration and membrane fouling might be attributed to the entrance of hydrolysis products of aerobic biomass recirculated to the methanogenic stage. It does not mean that recirculation is the only factor affecting fouling, since biomass concentration in membrane filtration chamber can also influence the fouling mechanisms and therefore membrane performance. Two parallel batch tests were carried out in order to verify the hydrolysis of flocculent aerobic biomass recirculated to the UASB reactor could lead to a release of polymeric substances and hence, an increase in colloidal BPC concentration and membrane fouling. One of the tests was performed under anaerobic conditions while the other was performed in an aerobic environment. As can be observed in figure 5.4, DOC and hence cBPC concentration increased remarkably with time under anaerobic conditions while the same tendency was not observed for the aerobic environment. The results suggest that the hydrolysis of suspended aerobic biomass in anaerobic conditions could lead to a release of biopolymers as a result of deflocculating process. Additionally, the experiment was repeated using aerobic biomass from a WWTP and the results obtained were similar (figure 5.4b).

It was reported by Wilén et al. (2000) that activated sludge flocs deflocculated under anaerobic conditions, and they deflocculated more the longer the anaerobic period was. The deflocculated particles were mainly bacteria and floc fragments, although some soluble EPS were also released.

All the parameters typically reported as responsible for membrane fouling (Rosenberger et al., 2006; Sun et al., 2008; de la Torre et al., 2008; Drews, 2010) were monitored during the anaerobic hydrolysis of aerobic biomass. As can be observed in figure 5.5, the anaerobic digestion at ambient temperature of aerobic biomass led to an

increase of SMP_C, SMP_P, TEP and cBPC concentrations, which confirms the negative impact of aerobic sludge hydrolysis on fouling properties.

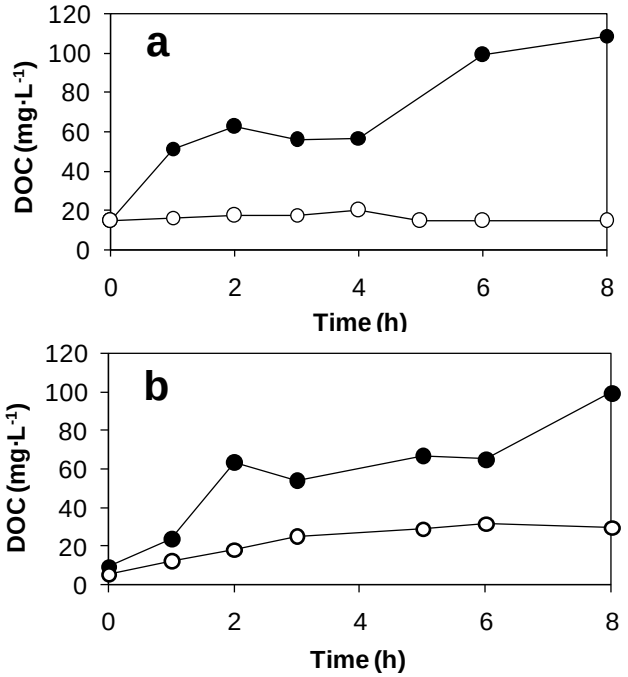

Figure 5.4. DOC concentration of the batch test performed in anaerobic (●) and aerobic (O) conditions using aerobic biomass from the aerobic stage of the system (a) and aerobic biomass from a municipal WWTP (b).

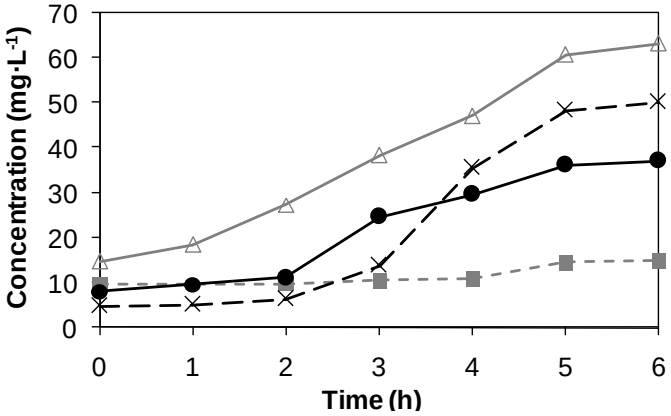

Figure 5.5. Concentration of proteins (△), TEP (×), DOC (●) and carbohydrates (■) during the batch digestion of aerobic biomass by anaerobic biomass in anaerobic conditions.

5.4.5. Effect of external MLVSS source on membrane fouling

In order to study the impact of biopolymers release in the anaerobic digester, sludge from a municipal WWTP was fed to the UASB reactor in the period VII. As shown in figure 5.6, colloidal BPC concentration increased remarkably when the addition of sludge started, confirming that the release of biopolymers took place due to the hydrolysis of this complex substrate in anaerobic conditions. Nevertheless, the same impact over membrane performance that the one reported on periods I to VI was not observed. It has to be taken into account that at the beginning of the periods I, III and VI (in which recirculation was turned on), the effect of recirculation not only led to an increase of cBPC concentration but also a rapid decrease of MLVSS concentration in both aerobic biofilm and membrane filtration chambers (0.5-2.0 $g \cdot L^{-1}$). Therefore, the membrane fouling observed could be explained as a combined effect of both factors. The assumption is that with a high MLVSS concentration, the membrane would be protected and the influence of the biopolymers released during the hydrolysis of aerobic biomass could be lower.

After stopping the addition of external sludge on day 841, cBPC concentration remained in high values due to the slow degradation of this complex substrate accumulated in the UASB reactor (figure 5.6).

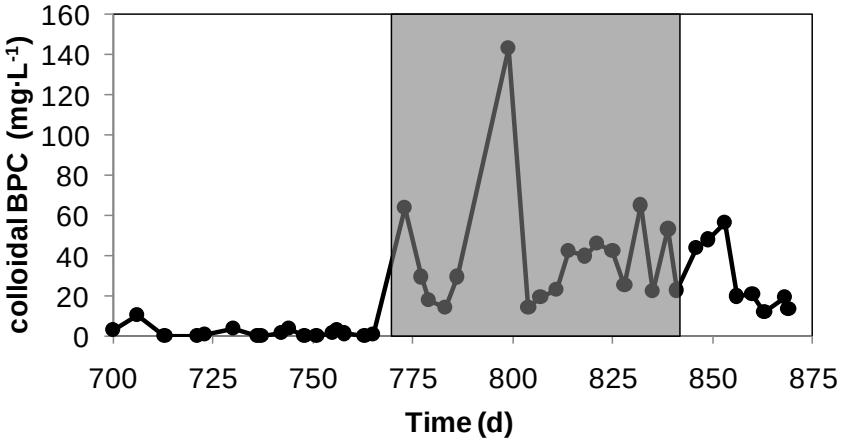

Figure 5.6. Evolution of cBPC concentration during period VI. In grey the period in which external sludge from a WWTP was fed to the system.

During the days in which the external addition of sludge to the methanogenic stage took place, MLVSS concentration in the membrane filtration chamber was manipulated in order to check the influence of this parameter on membrane fouling. The results presented

in table 5.3 showed that higher MLVSS concentration led to lower observed fouling rate and higher critical flux and permeability. A detailed fouling trend has been described by Rosenberger et al. (2005), where an increase in MLTSS reduced fouling at low MLTSS levels (<6 $g \cdot L^{-1}$) whilst exacerbating fouling at MLTSS concentrations above 15 $g \cdot L^{-1}$. The level of MLTSS did not appear to have a significant effect on membrane fouling between 8 and 12 $g \cdot L^{-1}$. In another study about the impact of MLTSS concentration, it was concluded that hydrodynamics (more than MLTSS concentration) controlled the critical flux at MLTSS levels above 5 $g \cdot L^{-1}$ (Judd, 2011).

Operation below recommended values led to an increase in the membrane fouling rate in steps 2 and 3. Fouling rate was normalized (FR/J) to avoid the influence of flux. As can be observed in table 3 the membrane performance in terms of permeability and critical flux was better in step 3 than in step 2. This is probably related with the lower values of cBPC concentration measured during step 3. During steps 2 and 3 the MLVSS concentration was very low and the membrane was exposed to soluble and colloidal biopolymers. These results confirmed the influence of cBPC concentration on membrane performance observed in the first six periods.

Table 5.3. Influence of MLVSS concentration on cBPC and membrane fouling during period VII.

Step	days	MLVSS ($g \cdot L^{-1}$)	cBPC ($mg \cdot L^{-1}$)	FR/J ($kPa \cdot m^{-1}$)	Critical flux ($L \cdot m^{-2} \cdot h^{-1}$)	Permeability ($L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$)
1	805-817	7.6±0.4	28.3±12.5	0.80±0.14	26.5	225 ± 23
2	817-829	3.9±1.0	43.7±14.3	3.20±1.66	20.0	163 ± 24
3	830-841	2.1±0.2	32.8±17.7	2.98±1.23	22.4	184 ± 43

5.5. Conclusions

- TEP and colloidal BPC concentration presented a strong relationship with fouling rate. Nevertheless, cBPC concentration was recommended as fouling indicator due to its simplicity and reliability.

- The feeding to the methanogenic stage of a complex substrate as aerobic sludge had a negative impact on membrane performance.

- Batch experiments demonstrated that the hydrolysis of aerobic biomass in anaerobic conditions led to a release of biopolymers, and hence an increase in TEP, colloidal BPC, SMP carbohydrate and SMP protein concentration.

- It was demonstrated that the presence of plastic support positively influence membrane performance.

- As expected, MLVSS concentration was shown to be an important parameter in order to protect the membrane against the fouling provoked by soluble and colloidal biopolymers.

5.6. References

Arruda Fatibello, S.H.S., Henriques Vieira, A.A., Fatibello-Filho, O. 2004. A rapid spectrophotometric method for the determination of transparent exopolymer particles (TEP) in freshwater. *Talanta* **62**, 81–85

APHA-AWWA-WPCF. 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, USA.

Berubé, P.R., Hall, E.R., Sutton, P.M. 2006. Parameters Governing Permeate Flux in an Anaerobic Membrane Bioreactor Treating Low-Strength Municipal Wastewaters: A Literature Review. *Water Environmental Research* **78**, 887-897.

BREF 2006, Integrated pollution prevention and control, reference document on best available techniques in the food, drink and milk industries.

Buntner, D., Sánchez, A., Garrido, J.M. 2011. Three stages MBR (methanogenic, aerobic biofilm and membrane filtration) for the treatment of low-strength wastewaters. *Water Science and Technology* **64**(2), 397-402.

Chong, S., Sen, T.K., Kayaalp, A., Ang, H.M. 2012. The performance enhancements of upflow anaerobic sludge blanket (UASB) reactors for domestic sludge treatment – A State-of-the-art review. *Water Research* **46**, 3434-3470.

de la Torre, T., Lesjean, B., Drews, A., Kraume, M. 2008. Monitoring of transparent exopolymer particles (TEP) in a membrane bioreactor (MBR) and correlation with other fouling indicators. *Water Science and Technology* **58**(10), 1903-1910.

Drews, A. 2010. Membrane fouling in membrane bioreactors—Characterisation, contradictions, cause and cures. *Journal of Membrane Science* **363**, 1–28

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Analytical chemistry* **28**, 350-356.

He, S.-B., Wang, B.-Z., Wang, L., Jiang, Y.-F., Zhang, L.-Q. 2003. A novel approach to treat combined domestic wastewater and excess sludge in MBR. *Journal of Environmental Science* **15**(5), 674–679.

Ho, J., Sung, S. 2010. Methanogenic activities in anaerobic membrane bioreactors (AnMBR) treating synthetic municipal wastewater. *Bioresource Technology* **101**(7), 2191-2196.

Jeison, D., van Lier, J.B. 2006. Cake layer formation in anaerobic submerged membrane bioreactors (AnSMBR) for wastewater treatment. *Journal of Membrane Science* **284**, 227–236.

Judd, S.J. 2002. Submerged membrane bioreactors: flat plate or hollow fibre? *Filtration and Separation* **39**(5), 30-31.

Judd, S.J. 2011. The MBR Book: Principles and Applications of Membrane Bioreactors for Water and Wastewater Treatment, 2nd Ed, Elsevier Science Ltd, Oxford.

Kushwaha, J.P., Srivastava, V.C., Mall, I.D. 2011. An overview of various technologies for the treatment of dairy wastewaters. *Critical Reviews in Food Science and Nutrition* **51**, 442-452.

Le-Clech, P., Jefferson, B., Chang, I.S., Judd, S.J. 2003. Critical flux determination by the flux-step method in a submerged membrane bioreactor, *Journal of Membrane Science* **227**, 81-93.

Le-Clech, P., Chen V., Fane, T.A.G. 2006. Fouling in membrane bioreactors used in wastewater treatment. *Journal of Membrane Science* **284**, 17-53.

Lew, B., Tarreb, S., Beliavski, M., Dosoretzb, C., Green, M. 2009. Anaerobic membrane bioreactor (AnMBR) for domestic wastewater treatment. *Desalination* **243**, 251–257.

Liu, Q., Wang, X.C., Liu, Y., Yuan, H., Du, Y. 2010. Performance of a hybrid membrane bioreactor in municipal wastewater treatment. *Desalination* **258**, 143-147.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J. 1951. Protein measurements with the folin phenol reagent. *The Journal of Biological Chemistry* **193**(1), 265- 275.

Passow, U. 2002. Transparent exopolymer particles (TEP) in aquatic environments. *Progress in oceanography* **55**, 287-333.

Robles, A., Ruano, M.V., Ribes, J., Ferrer, J. 2013. Factors that affect the permeability of comercial hollow-fibre membranes in a submerged anaerobic MBR (HF-SAnMBR) system. *Water Research* **47**, 1277-1288.

Rosenberger, S., Evenblij, H., te Poele, S., Wintgens, T., Laabs, C. 2005. The importance of liquid phase analyses to understand fouling in membrane assisted activated sludge processes -six case studies of different European research groups. *Journal of Membrane Science* **263**, 113–126.

Rosenberger, S., Laabs, C., Lesjean, B., Gnirss, R., Amy, G., Jekel, M., Schrotter, J.-C. 2006. Impact of colloidal and soluble organic material on membrane performance in membrane bioreactors for municipal wastewater treatment. *Water Research* **40**, 710–720.

Sherr, E.B. 1988. Direct use of high molecular weight polysaccharides by heterotrophic flagellates. *Nature* **335**, 348-351.

Spagni, A., Casu, S., Crispino, N.A., Farina, R., Mattioli. D. 2010. Filterability in a submerged anaerobic membrane bioreactor. *Desalination* **250**, 787-792.

Sun, F.-Y., Wang, X.-M., Li, X.-Y. 2008. Visualisation and characterisation of biopolymer clusters in a submerged membrane bioreactor. *Journal of Membrane Science* **325**, 691–697.

Trzcinski, A.P., Stuckey, D.C. 2009. Anaerobic digestion of the organic fraction of municipal solid waste in a two-stage membrane process. *Water Science and Technology* **60**(8), 1965-1978.

van der Marel, P., Zwijnenburg, A., Kemperman, A., Wessling, M., Temmink, H., van der Meer, W. 2009. An improved flux-step method to determine the critical flux and the critical flux for irreversibility in a membrane bioreactor. *Journal of Membrane Science* **332**, 24-29.

van Haandel, A.C., Lettinga, G. 1994. Anaerobic sewage treatment: a practical guide for regions with a hot climate, Chichester (England), J. Wiley & Sons Ltd.

Wen, C., Huang, X.; Quian, Y. 1999. Domestic wastewater treatment using an anaerobic bioreactor coupled with membrane filtration. *Process Biochemistry* **35**, 335–340.

Wen, X., Bu, Q., Huang, X. 2004. Study on fouling characteristic of axial hollow fibers cross-flow microfiltration under different flux operations, in: Proceedings of the Water Environment-Membrane Technology Conference, Seoul, Korea.

Wilén, B.-M., Keiding, K., Nielsen, P.H. 2000. Anaerobic deflocculation and aerobic reflocculation of activated sludge. *Water Research* **34**(16), 3933-3942

Zhang, D.J., Lu, P.L., Long, T.R., Verstraete, W. 2005. The integration of methanogenesis with simultaneous nitrification and denitrification in a membrane bioreactor. *Process Biochemistry* **40**(2), 541-547.

Zhang, J., Padmasiri, S.I., Fitch, M., Norddhal, B., Raskin, L., Morgenroth, E. 2007. Influence of cleaning frequency and membrane history on fouling in an anaerobic membrane bioreactor. *Desalination* **207**, 153-166.

Chapter 6

Denitrification with dissolved methane in an MBR after a methanogenic pre-treatment at ambient temperature

Summary

The presence of dissolved methane, especially at low temperature, represents an important environmental problem in terms of greenhouse gas (GHG) emissions of wastewaters treated using methanogenic bioreactors. Methane has a global warming potential 25 times higher than carbon dioxide. For low strength wastewaters, dissolved methane might account up to 50% of the produced methane. The dissolved methane is easily desorbed from the effluents, especially if these are either released in the environment or post-treated using aerobic bioreactors, increasing GHG emissions.

The use of this dissolved methane as a carbon source for biological denitrification has been proposed as an alternative to reduce both GHGs emissions and nitrogen content of the treated wastewater. In this study the effluent of a UASB reactor was post-treated in an MBR with a first anoxic chamber in order to use dissolved methane as carbon source for denitrification. Up to 60% and 95% nitrogen removal and methane consumption were observed, respectively. The stripping of the dissolved methane present in the UASB effluent led to a worsening of nitrogen removal in the MBR system. Batch experiments confirmed the presence of microorganisms capable of denitrifying using the dissolved methane as carbon source. Recirculation ratio between the anoxic and aerobic chambers of the MBR system, and either the presence of dissolved methane were shown as the main important parameters governing the denitrification process. The influence of denitrification with methane on membrane performance was also studied, showing a remarkable increase on biopolymer concentration when denitrification activity was affected by the removal of dissolved methane from the UASB effluent.

Parts of this chapter have been submitted for publication as:

Sánchez, A., Rodríguez-Hernández, L., Buntner, D., Esteban-García, A.L., Tejero, I., Garrido, J. M. 2013. Denitrification coupled with methane oxidation in a membrane bioreactor after a methanogenic pre-treatment. Submitted to *Water Research*.

6.1. Introduction

Anaerobic treatment processes have been widely applied to various types of wastewaters because of advantages such as lower energy consumption, energy recovery as methane, and less excess sludge production compared with conventional aerobic treatment systems. Anaerobic technology is widely used in temperate and warm climate countries for the treatment of municipal wastewaters. Nevertheless, anaerobic treatment produces methane, a greenhouse gas (GHG) with a warming potential 25 times higher than that of carbon dioxide. A fraction of the methane generated is present in the effluent. Dissolved methane can be estimated considering that effluents are, at least, in equilibrium with the biogas formed by using the Henry´s law. Thus, methane concentrations in the UASB effluent between 13.4 and 20.8 $mg \cdot L^{-1}$ may be expected operating at 17-25 ºC, with 60-80% methane composition in the biogas at operating pressure of 1 atm. For low strength wastewaters, such as municipal wastewater, treated in anaerobic reactors dissolved methane might account up to 50% of the produced methane (Noyola et al., 2006). Moreover, Souza et al. (2011) indicated methane losses accounting for 36-41% of methane produced in two pilot scale anaerobic reactors.

Methane may be emmited to the atmosphere by stripping, if the effluents are either aerobically post-treated or discharged in the environment without further post-treatment, increasing the environmental impact of anaerobic wastewater treatment due to GHG emissions. Cakir and Stenstrom (2005) analyzed GHG emissions associated to anaerobic municipal wastewater treatment. These authors confirmed that the presence of dissolved methane in the effluent strongly increases GHG emissions, if it is released to the environment.

Different strategies could be followed in order to reduce methane emissions. There are several studies of aerobic biological methane oxidation using gas biofilters to reduce methane emissions from sanitary landfills or manure storage facilities, and reduction of methane concentrations in coal mine (Park et al., 2009; Melse and van der Werf, 2005). Hatamoto et al. (2010) used an encapsulated down-flow hanging sponge reactor as a post-treatment to biologically oxidize dissolved methane in an anaerobically treated wastewater effluent. They achieved up to 550 $mgCH_4 \cdot L^{-1} \cdot d^{-1}$ removal.

Methane present in the effluents of methanogenic bioreactors may be used also as an inexpensive electron donor for denitrification. Even in those locations in which nitrogen removal is not considered as an environmental concern, this process might be a way to reduce GHG emissions after anaerobic wastewater treatment.

From the microbiological point of view, biological methane oxidation coupled to denitrification proceeds via two different pathways (Modin et al., 2007):

1) Aerobic, where methane oxidation is driven by a wide group of bacteria, methanotrophs, which utilize methane as sole carbon source and energy source. Partial oxidation products may be further consumed by denitrifying microorganisms (Hanson and Hanson, 1996; Rhee and Fuhs, 1978; Mechsner and Hamer, 1985). The theoretical stoichiometry of the process is given by equation 6.1:

$$5CH_4 + 5O_2 + 4NO_3^- + 4H^+ \rightarrow 2N_2 + 12H_2O + 5CO_2 \quad \text{eq. 6.1}$$

Until recently, the process of aerobic methane oxidation coupled to denitrification was the only one observed in systems in which methane was the sole carbon source (Modin et al., 2007).

2) Anaerobic, where oxidation of methane coupled to denitrification is carried out by a consortium of microorganisms, or by a newly discovered denitrifying methanotroph. In the first case, the consortium may be composed by a syntrophic association of anaerobic methanogenic archaea (ANME) and sulphate-reducing bacteria (SRB) (Boetius et al., 2000; Knittel and Boetius, 2009). Another possible consortium is that formed by an achaeal partner and bacteria belonging to NC10 phylum (Raghoebarsing et al., 2006; Ettwig et al., 2008). In the second case, the process is carried out by a newly discovered denitrifying methanotroph belonging to NC10 phylum (Wu, 2012).

The stoichiometry of anaerobic methane oxidation coupled to denitrification is independent from the microorganisms involved, and it is given by equation 2:

$$5CH_4 + 8NO_3^- + 8H^+ \rightarrow 5CO_2 + 4N_2 + 14H_2O \quad \text{eq. 6.2}$$

Most of the studies on denitrification coupled to methane oxidation have been performed using batch assays (Thalasso et al., 1997; Lee et al., 2001; Khin and Annachhatre, 2004; Islas-Lima et al., 2004). Other studies involving continuous reactors (Rajapakse and Scutt, 1999; Kampman et al., 2012) have also proved the feasibility of the process. Nevertheless, those studies focused on the use of methane gas as the carbon source for denitrification. Such use of methane has a negative consequence, the reduction of the amount of biogas that could be used as energy source. As far as we are concerned, the use of dissolved methane present in anaerobic effluents as carbon source for denitrification was proposed theoretically by Kampman et al. (2012), but has not been studied. This alternative would allow reducing GHG emissions and it might be potentially used for denitrification.

Membrane bioreactors (MBR) might be the suitable technology as a post-treatment for an anaerobic digester effluent. Methanogenic reactors have been operated as a pre-treatment step, followed by an aerobic MBR system, for the treatment at environmental temperatures of domestic and industrial wastewaters (He et al., 2003; Sánchez et al., 2013). Despite the higher energy consumption referred for this kind of systems, the use of membranes would produce a high quality effluent, suitable for reuse.

In this sense, an MBR for promoting the removal of nitrogen and dissolved methane should consist of a first anoxic chamber, in order to limit methane emissions and promote denitrification coupled to methane oxidation. Moreover, given that the presence of nitrate in the effluents of methanogenic reactors is negligible (van Haandel and Lettinga, 1994), nitrate should be recycled from an aerobic chamber in which ammonia is nitrified. The use of MBR systems could be a good choice to enhance denitrification coupled to methane oxidation as result of the high sludge concentration of these systems, typically between 8-12 $gMLVSS \cdot L^{-1}$ for submerged MBR systems (Judd, 2011). Denitrification coupled to methane oxidation is characterised by its low specific denitrification activity. Different authors, using batch assays, found activities in between 15 and 90 $mgN \cdot gMLVSS^{-1} \cdot d^{-1}$ at temperatures around 20-25 ºC (Lee et al., 2001; Khin and Annachhatre, 2004). These values are much lower than 250 $mgN \cdot gMLVSS^{-1} \cdot d^{-1}$ referred for denitrification with readily biodegradable organic matter under similar conditions (Henze et al., 2002). The presence of biofilms in the anoxic tank would be beneficial for microbial diversity (Shen et al., 2013), assuring that part of the biomass would remain always under anoxic conditions and increasing the effective biomass concentration in this chamber. Moreover, the installation of a membrane in the aerobic compartment would allow complete microorganisms retention in the system. Kampman et al. (2012) estimated that around 50 % of produced biomass was washed out from a sequencing batch reactor in which the growth of the newly discovered anaerobic denitrifying methanotrophic biomass, was promoted.

6.2. Objectives

The main objective of this research was to study denitrification coupled to methane oxidation in an MBR using the dissolved methane present in the effluent of an anaerobic UASB system.

6.3. Material and methods

6.3.1. Experimental set-up and operating strategy

A schematic diagram of the experimental set-up can be observed on figure 6.1. A 120 L volume UASB system was used for the first methanogenic stage. The effluent of the UASB reactor was led to an MBR reactor composed by two chambers: a first chamber (36 L), with biomass growing onto plastic support and in suspension, and a second aerobic membrane filtration chamber (20 L volume). 18.5 L (50% of the effective volume) of support (Kaldnes® K3) were added in the first MBR chamber. An internal recirculation (R) from the filtration chamber to the first chamber was used to return suspended solids and nitrate to this chamber. A membrane ultrafiltration module Zenon ZW10 with a surface area of 0.9 m^2 was employed in the filtration chamber. This module consisted of PVDF hollow-fibre membrane, with a pore size of 0.04 μm. The membrane was operated in cycles of 7.5 min with a permeation period of 7 min and a backwashing period of 0.5 min. The filtration chamber was aerated in order to minimize membrane fouling and promote ammonia oxidation. The specific air demand (SAD_m) applied to the membrane was 0.7 $Nm^3 \cdot m^{-2} \cdot h^{-1}$. The operation of the system was controlled by a PLC (Siemens S7-200) connected to a computer. Trans-membrane pressure (TMP) data was measured with an analogue pressure sensor (Efector500 PN-2009) and collected in the PC via an analogue PLC module Siemens EM 235.

The reactor was operated at ambient temperature (19-21.5 ºC) and fed using synthetic wastewater composed of diluted skimmed milk, $NaHCO_3$ and trace elements. COD concentration in the feeding was varied between 800 and 1300 $mg \cdot L^{-1}$.

The impact of recirculation ratio (R) and the presence of methane in the UASB effluent were studied during six different periods (table 6.1). The first chamber of the MBR system was aerated during a first experimental period (period I) in order to establish a base base scenario for the emissions of dissolved total nitrogen and methane. Afterwards it was maintained under anoxic conditions in order to promote denitrification (periods II, III, IV, V and VI). During periods II, III, V and VI the impact of the internal recirculation ratio on nitrogen removal in the MBR system was investigated. During period IV, methane present in the effluent from the UASB reactor was stripped off by aerating this stream before entering in the MBR. The main objective of this period was to determine denitrification caused by the remaining biodegradable COD fraction of this stream.

Table 6.1. Main operational conditions of the bioreactor during the different periods.

Period	days	Environment[1]	R[2]	CH_4 stripping[3]
I	0-84	Aerobic	1.0	no
II	85-120	Anoxic	3.0[4]	no
III	121-150	Anoxic	1.0	no
IV	151-169	Anoxic	1.0	yes
V	170-198	Anoxic	0.5-1.0	no
VI	199-233	Anoxic	1.5-2.0	no

[1] In the first MBR chamber

[2] Internal recirculation ratio in the MBR

[3] Methane was stripped off from UASB effluent before entering the first MBR chamber

[4] From days 85 to 91 the recirculation rate was fixed at R=1

6.3.2. Analytical methods

Temperature, pH, alkalinity and the concentrations of dissolved oxygen, mixed liquor volatile suspended solids (MLVSS), total and soluble chemical oxygen demand (COD), nitrite, nitrate and ammonia were determined according to the Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WPCF, 1998). Dissolved total nitrogen (DTN) was determined with a DN 1900 analyser (Rosemount, Dohrmann). DTN was referred to the sum of the measured nitrogen ions and soluble organic nitrogen. Volatile fatty acids (VFA) (i-butyric, n-butyric, i-valeric and n-valeric) were analyzed by gas chromatography (HP, 5890A) equipped with a flame ionization detector (HP, 7673A). Biogas production was measured using a Milli GasCounter MGC-10 (Ritter) and its composition was measured in a gas chromatograph HP 5890 Series II with the column of Porapack Q 80/100 2m x 1/8" (SUPELCO).

Remaining methane dissolved in the liquid phase was estimated by Henry´s law. Methane is characterized by a Henry constant of $1.5 \cdot 10^{-3}$ mol$\cdot$ L^{-1} $\cdot atm^{-1}$ at 25ºC (Sander, 1999). 300 mL of sample was hand-shaked in a 500 mL Erlenmeyer. After three minutes of shaking gas phase was analyzed in the gas chromatograph.

The difference in DOC concentration between the sludge mixture after filtration through a 0.45 µm nitrocellulose membrane filters (HA, Millipore) and the permeate was assigned to the colloidal fraction of biopolymer clusters (cBPC) in the liquid phase of the

sludge mixture suspension (Sun et al., 2008). The critical flux was determined according to the modified flux-step method proposed by van der Marel et al. (2009). The criterion employed was that the increment of transmembrane pressure (TMP) with respect to time was higher than 10 $Pa \cdot min^{-1}$ (Le-Clech et al., 2003).

Further information regarding analythical methods is provided in Chapter 2.

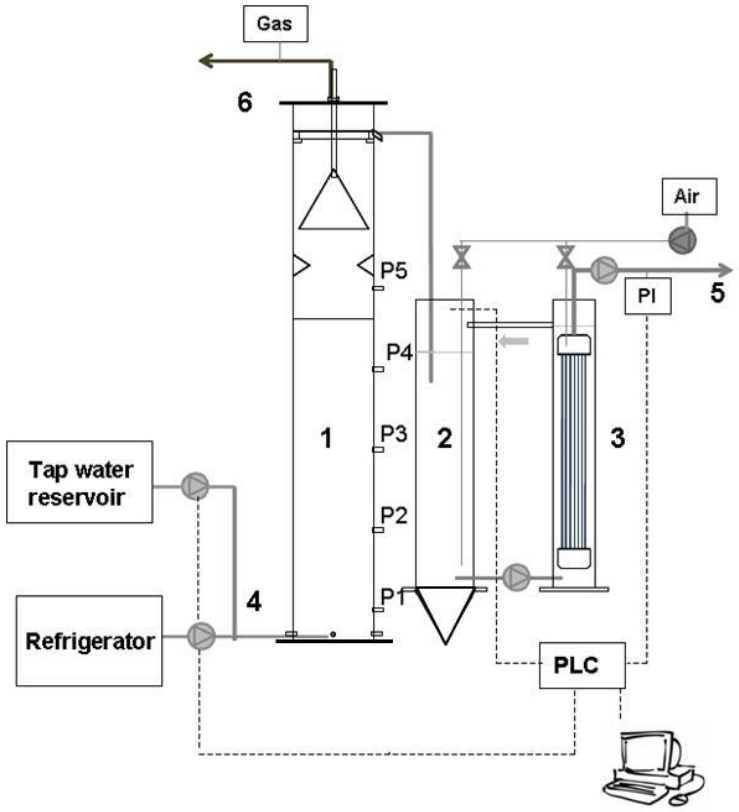

Figure 6.1. Schematic diagram of the bioreactor. (1) UASB methanogenic pre-treatment; (2) First MBR chamber; (3) Membrane filtration chamber; (4) Feeding; (5) Permeate (backwashing); (6) Biogas. P1, P2, P3, P4 and P5 refer to the sampling ports. Grey arrow represents the internal MBR recirculation between the membrane filtration and first chambers.

6.3.3. Denitrification batch experiments

Two different batch denitrification assays using methane and/or acetate as electron donor were performed using 500 ml flasks. In one of them the flasks were filled with 400

mL of suspended biomass (2 gMLVSS·L^{-1}) and 20 plastic carriers Kaldnes K3 (40% of apparent volume). In the other the four bottles were filled with 50 plastic carriers Kaldnes K3 and 400 mL of phosphate buffer (KH_2PO_4: 0.143 g·L^{-1}; K_2HPO_4: 0.740 g·L^{-1}).

Both biofilm and suspended biomass were taken from the anoxic chamber of the reactor, settled for at least 12 h and washed three times with phosphate buffer in order to assure the absence of organic matter or nitrogen. The absence of any soluble carbon source in the supernatant was confirmed by COD measurement. After inoculation, the flasks were flushed for 5 min using nitrogen or methane depending on the conditions (table 6.2), to guarantee anaerobic atmosphere.

5 mL of $NaC_2H_3O_2 \cdot 3H_2O$ 0.9M were spiked as a carbon source in the corresponding flasks (table 6.2). 1 mL of KNO_3 0.86M was spiked to each bottle at the beginning of the experiment.

The flasks were incubated at 25 ºC and stirred in a shaker at 150 rpm during five hours. 5 mL liquid samples were taken each hour with a syringe through a septum and filtered through 0.45 µm nitrocellulose membrane filters (HA, Millipore). All control assays were performed in duplicate. These batch experiments were carried out during period VI and thus the biomass conditions were specific from that period (table 6.1).

Table 6.2. Denitrification batch experiments conditions

Flask	Headspace	Carbon Source
Blank	N_2	None
Methane	CH_4	CH_4
Acetate	N_2	Acetate
Methane + Acetate	CH_4	Acetate + CH_4

6.3.4. Determination of methane and oxygen transfer in the first MBR chamber

The methane emissions to the environment in the first (anoxic) chamber were estimated by closing the headspace with parafilm (Pechiney Plastic Packaging, USA) and monitoring the methane build-up in this headspace during 3 hours. Samples of 1 mL were taken in duplicate each 30 minutes and its composition was measured in a gas chromatograph HP 5890 Series II with the column of Porapack Q 80/100 2m x 1/8" (SUPELCO). The flow of methane desorbed was calculated, according to equation 6.3, by

performing a mass balance to the headspace of the first chamber. It should be taken into account that there is no generation or output of methane in the headspace and the accumulation of methane is only due to its desorption from the bulk liquid.

$$m_{CH4} = v\left(\frac{dC_{CH4}}{dt}\right) \quad \text{eq. 6.3}$$

Where m_{CH4} is the mass flow of methane that is desorbed in the first chamber [mg·d^{-1}], v is the headspace volume of this chamber [L] (v=5L), C_{CH4} is the concentration of methane in the headspace [mg·L^{-1}] and t is the time [d^{-1}].

Desorbed methane mass flow (m_{CH4}) might be calculated by plotting the methane concentration in the headspace versus time as the slope of the linear representation. Desorbed methane flow can be expressed according to equation 6.4 as:

$$m_{CH4} = \mathrm{k_L a_{CH4}} \cdot (\mathrm{C} - \mathrm{C}^*) \cdot \mathrm{V} \quad \text{eq. 6.4}$$

where C is the dissolved methane concentration in the bulk liquid of the first chamber [mg·L^{-1}], C* is the methane concentration in equilibrium with air (considered as zero) [mg·L^{-1}], V is the volume of the first chamber [L] and $k_L a_{CH4}$ is the mass transfer coefficient for methane [d^{-1}].

From the penetration film theory (van´t Riet and Traper, 1991) the ratio of k_L of two different substances is equal to the ratio of their diffusion coefficients. Therefore, $k_L a$ for the oxygen ($k_L a_{O2}$) can be also calculated in our system. This value was used to estimate the amount of oxygen transferred from the surface air to the first (anoxic) chamber according to equation 6.5:

$$\mathrm{k_L a_{O2}} = \mathrm{k_L a_{CH4}} \cdot \left(\frac{\mathrm{D_{O2}}}{\mathrm{D_{CH4}}}\right)^{0.5} \quad \text{eq.6. 5}$$

where D_{O2} and D_{CH4} are the diffusive coefficients for oxygen and methane [cm^2·s^{-1}], respectively.

6.3.5. Mass balances in the first MBR chamber

Considering this chamber as a continuous stirred tank reactor (CSTR), mass balances were performed in order to determine denitrification, methane and oxygen apparent specific consumption rates as well as CH_4:O_2 molar ratio when anoxic conditions were implemented (from period II onwards). Assuming steady state, mass balances were performed to individual components according to equation 6.6:

$$Q_{IN,i} \cdot C_{0i} - Q_{OUT} \cdot C_i = V \cdot r_i + K_L a_i \cdot (C_i - C_i^*) \cdot V \quad \text{eq. 6.6}$$

where subindex i correspond to each component (nitrogen anions, oxygen and methane) of the mass balance, $Q_{IN,i}$ is the input flow [$L \cdot d^{-1}$], C_{0i} is the input concentration [$mg \cdot L^{-1}$], $Q_{OUT,}$ is the output flow from the first (anoxic) chamber [$L \cdot d^{-1}$], C_i is the output concentration [$mg \cdot L^{-1}$], V is the volume of the first (anoxic) chamber [L], r_i is the volumetric reaction rate [$mg \cdot L^{-1} \cdot d^{-1}$], C_i^* is the concentration of either methane or oxygen in equilibrium with air [$mg \cdot L^{-1}$] and k_La_i is the mass transfer coefficient for either methane or oxygen. The last term of equation 6 was not taken into account for nitrogen ions mass balance.

Operational data was grouped depending on the recirculation ratio. Average values of dissolved methane concentration (input and output), dissolved oxygen concentration (input and output) and nitrogen anions concentration (input and output) were calculated from experimental data for each one of the recirculation scenarios. Dissolved methane input was due to the UASB effluent whereas nitrogen anions and dissolved oxygen entered the first chamber through the recirculation from the aerobic membrane filtration chamber. Desorbed methane from the first chamber and oxygen input due to oxygen transferred from the surface air to this chamber were calculated according to section 2.4.

6.4. Results and discussion

6.4.1. General results

The system was operated at ambient temperature, and wastewater temperatures changed with seasons (21.5 – 19.0 °C). Despite operating in psychrophilic conditions volatile fatty acids (VFA) concentration in the UASB effluent was below minimum detection limit of the method used (20 $mg \cdot L^{-1}$) during the six experimental periods. Biogas production in the UASB reactor was detected during the six experimental periods, with an average production rate of 50.9±10.8 $L \cdot d^{-1}$, depending on OLR applied. Biogas production yield was around 0.15 $m^3_{methane} \cdot kgCOD_{eliminated}^{-1}$. Methane reached more than 70% of the biogas composition during the whole operation. Methane in the biogas corresponded approximately to the 75% of the total methane produced. Therefore, up to 25 % of the methane produced in the anaerobic reactor would be dissolved in the effluent, which confirmed the values reported by previous studies (Noyola et al., 2006; Souza et al. 2011).

The system treated an average of 280 $L \cdot d^{-1}$ of wastewater with a total COD concentration in the feeding varying between 800 and 1300 $mg \cdot L^{-1}$. The concentration of total COD in the permeate of the MBR was normally lower than 20 $mg \cdot L^{-1}$. Therefore COD removals achieved in the system were above 97.5%. The average organic loading rate (OLR) applied to the UASB reactor was between 1.7 and 2.8 $kgCOD \cdot m^{-3} \cdot d^{-1}$. With respect

to the MBR, the OLR applied was between 0.1 and 0.9 kgCOD·m^{-3}·d^{-1}, depending on the suspended solids washed-out from the UASB reactor.

Regarding MLVSS, the concentrations in the UASB reactor, the first MBR chamber and the membrane filtration chamber ranged between 28-35 g·L^{-1}, 2-5 g·L^{-1} and 4-8 g·L^{-1}, respectively. Biomass concentration in the biofilm was around 45 gMLVSS·m^{-2}, which was equivalent to an MLVSS concentration of approximately 6 g·L^{-1}. Sludge retention time (SRT), referred to the MBR, was maintained between 15 and 30 d during the whole operation. Anaerobic biomass was not purged from the UASB reactor during the study. Food to microorganism (F/M) ratio applied to the MBR was around 0.03 gCOD·gMLVSS^{-1}·d^{-1}, referred to non-methane soluble COD. Dissolved oxygen concentration in the anoxic compartment ranged between 0.1 and 0.3 mg·L^{-1}.

6.4.2. Influence of dissolved methane on denitrification

The remaining non-methane biodegradable COD and dissolved methane in the effluents from the UASB can be used as carbon source for denitrification. Soluble COD in the UASB effluent during the experiments was very low, 57±34 mg·L^{-1}. Moreover, VFAs in the UASB effluent were monitored during the six experimental periods, being its concentration below minimum detection limit of the method used (20 mg·L^{-1}). Dissolved methane in the influent to the MBR was normally between 19 and 25 mg·L^{-1}, except on period IV, when methane was stripped off and its concentration decreased to values between 3 and 8 mg·L^{-1}.

Most of the total nitrogen in the effluent of the UASB reactor was present as soluble ammonia (35.7±7.9 mg·L^{-1}). Ammonia was fully nitrified during period I, during which the first MBR chamber was maintained under aerobic conditions. Dissolved total nitrogen (DTN) in the permeate was similar to the ammonia concentration fed to the MBR system (figure 6.2). Therefore, no nitrogen removal took place during this period. Moreover, after the first experimental days of period I, most of the ammonia oxidation to nitrate took place in the first chamber (figure 6.2b). During the first operating days of period II, complete denitrification of nitrate was observed in the first (anoxic) chamber. Later on, the concentration of this compound increased. Significant nitrogen removal was also observed during periods III, IV, V and VI, during which the first MBR chamber was operated under anoxic conditions. This caused a remarkable diminution of DTN in the permeate (figure 6.2a). Up to 60% nitrogen removal was observed during periods II, III and VI.

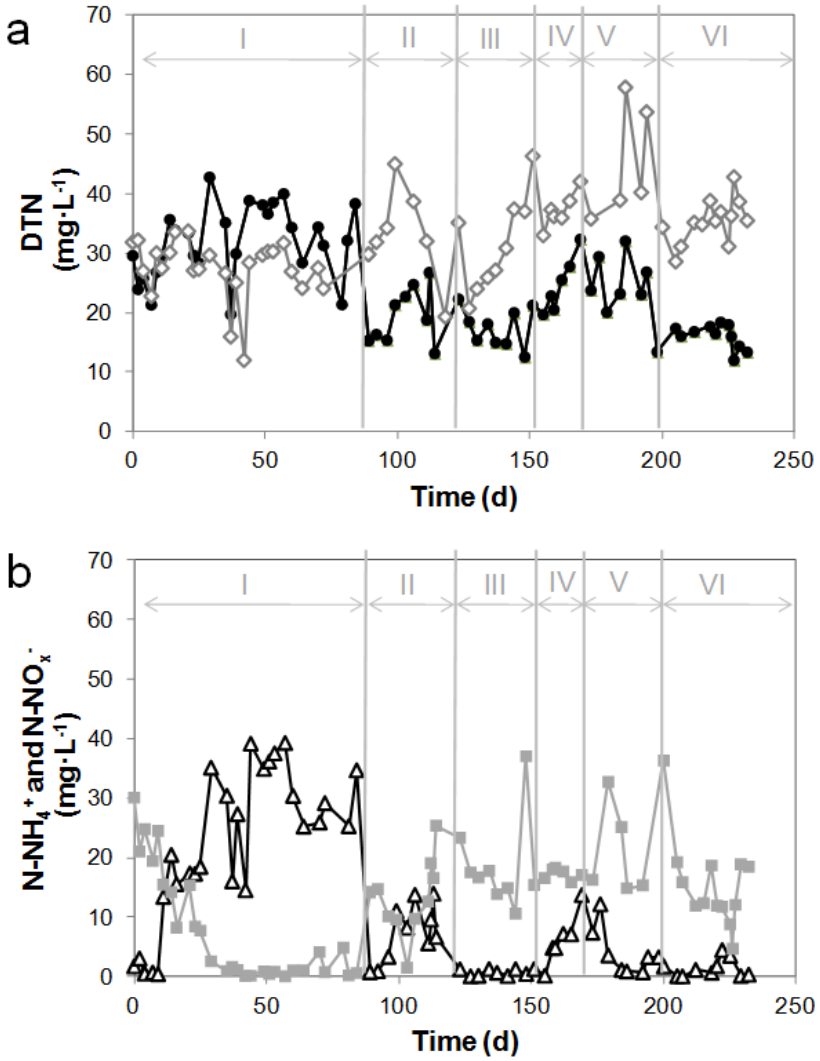

Figure 6.2. Evolution of (a) DTN concentration in the UASB effluent (◇) and in the permeate (●); (b) N-NH_4^+ (■) and N-NO_x^- concentration (Δ) in the first MBR chamber during the six experimental periods.

Denitrification could proceed using either dissolved methane, or remaining biodegradable COD at the effluent of the UASB system. Thus, during period IV, methane was stripped off from the UASB effluent in order to estimate the fraction of nitrogen removed due to the remaining biodegradable COD. This caused a gradual increase of DTN concentration in the permeate (figure 6.3). Soluble COD in the UASB effluent during

that period ranged between 21 and 27 $mg \cdot L^{-1}$. Figure 6.2 shows that $N\text{-}NO_x^-$ concentration was almost zero in the first (anoxic) chamber. Thus, denitrification was limited by nitrate availability. Nevertheless, during period IV, the absence of dissolved methane led to a progressive increase of nitrate in the first (anoxic) chamber, indicating that the limiting factor in this period was the carbon source (figures 6.2b and 6.3). From the data obtained at the end of period IV (figure 6.3), a constant nitrogen removal rate of 73 $mgN \cdot L^{-1} \cdot d^{-1}$ was obtained, whereas, considering the end of period III and the beginning of period IV, this nitrogen removal rate was around 164 $mgN \cdot L^{-1} \cdot d^{-1}$. The difference between both nitrogen removal rates could be probably due to dissolved methane. From the 60% nitrogen removal observed, DTN removal percentage due to the oxidation of methane could account, at least, up to 33 %. Thus, the nitrogen removal percentage due to other processes at the end of period IV was, at most, 27 %. It should be taken into account that some dissolved methane was still remaining in the UASB effluent (3-8 $mg \cdot L^{-1}$). 50% of this methane was oxidized in the anoxic compartment during this period. When stripping of methane was stopped on period V, nitrogen removal increased again to the previous values observed during period III, up to 60 %, confirming the relevant role of methane in denitrification.

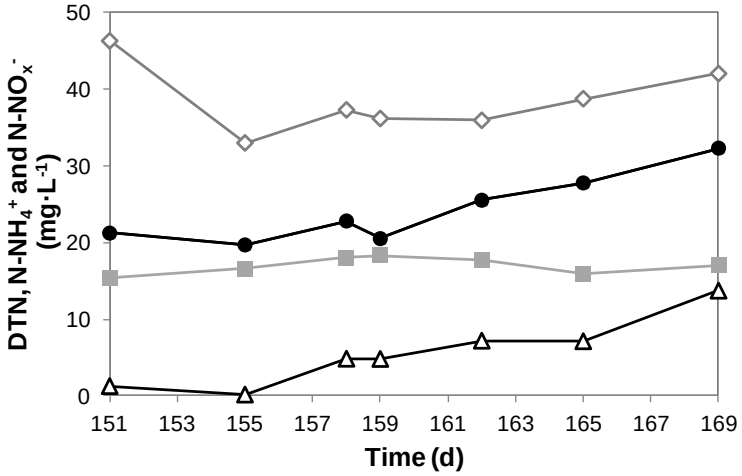

Figure 6.3. Evolution of DTN concentration in the UASB effluent (◇) and the permeate (●) and $N\text{-}NH_4^+$ (■) and $N\text{-}NO_x^-$ concentration (Δ) in the first MBR (anoxic) chamber during period IV.

The results presented show that denitrification, using methane as a carbon source is effectively possible and feasible. Soluble COD concentration in the UASB effluent was used for conventional heterotrophic denitrification. Nevertheless this low COD

concentration promoted the use of dissolved methane as a complementary carbon source to denitrify. However, heterotrophic denitrification was probably not the only process responsible for nitrogen removal. According to figure 6.2b, ammonia was also removed with no build-up of nitrate at least during periods II, III and VI, in which a reduction of ammonia concentration was observed in the first (anoxic) chamber. The removal of ammonia in anoxic conditions can be only explained by means of anammox process.

When dissolved methane desorption was implemented, nitrogen removal did not decrease instantly but progressively, maintaining certain denitrification capacity (figure 6.3). This fact was probably related to a mechanism involving either endogenous respiration or biomass accumulation products (period IV). Thus, the impact of methane depletion increased with time, causing the increase of nitrate accumulation in the effluent. The same effect was observed when methane desorption was stopped (period V). The process did not recover instantly and only after a few days at R=0.5 the previous observed nitrogen removal rates were achieved.

6.4.3. Influence of internal recirculation in MBR on denitrification

Recirculation ratio (R) between the aerobic membrane filtration and the first MBR chamber in the MBR also played a crucial role in DTN removal as well as in methane emissions to the environment, as depicted in figure 6.4. Methane is a gas that may be easily desorbed from the liquid phase by aeration. During the first period (no anoxic environment), the 100% of the dissolved methane present in the UASB effluent was stripped off in the first MBR chamber, due to the aeration. When anoxic conditions were implemented during periods II, III, V and VI, a fraction of this dissolved methane was oxidized. Dissolved methane concentration in the first MBR chamber ranged between 1 and 7 $mg \cdot L^{-1}$ during periods II, III, V and VI, whereas these values were between 0.6 and 1.3 $mg \cdot L^{-1}$ when methane was stripped off from the UASB effluent in period IV. Neglecting period IV, the methane volumetric loading rate to the anoxic compartment was between 150 and 190 $mgCH_4 \cdot L^{-1} \cdot d^{-1}$. The methane volumetric removal rate observed was in between 50 and 160 $mgCH_4 \cdot L^{-1} \cdot d^{-1}$.

As can be observed on figure 6.4, the lower the recirculation ratio (R) was, the lower methane emissions were. The remaining methane that was not oxidized in the first (anoxic) chamber was desorbed in the membrane filtration chamber, which was continuously aerated. The volumetric mass transfer coefficient for methane was calculated (0.79 d^{-1}) and used for the estimation of methane emissions to the environment in the first (anoxic) chamber was also measured, representing only a 1.5% of the total dissolved

methane present in the UASB effluent. Therefore, the best results in terms of nitrogen removal and lower methane emissions were obtained operating with lower R values (between 0.5 and 1).

Mass balances of methane, nitrogen (as nitrate and nitrite) and oxygen in the first (anoxic) chamber were performed in order to try to clarify the methane oxidation and denitrification mechanism at different recirculation ratios (table 6.3). Apparent specific denitrification rates at different recirculation ratios were similar, with a maximum value at R=1 (table 6.3). Regarding methane consumption rates, the values obtained were similar at R=0.5 and R=1 (40.1 and 43.7 $mgCH_4 \cdot gMLVSS^{-1} \cdot d^{-1}$, respectively) but significantly lower at R=2 (15.9 $mgCH_4 \cdot gMLVSS^{-1} \cdot d^{-1}$). As observed in figure 6.4, the higher was the recirculation ratio the lower was the methane consumption, provoking the higher methane emissions. In this sense, Daelman et al. (2012) observed that no methane oxidation took place in the anoxic part of a plug flow reactor from a conventional WWTP operating at a recirculation ratio of 3. The higher recirculation rates were applied during period II (R=3), when a progressive decrease on nitrogen removal was observed (figures 6.2 and 6.3). This nitrogen removal decrease took place from day 91 on, when recirculation rate increased from 1 (R applied from days 85 to 91) to 3. A remarkable raise on $N\text{-}NO_x^-$ concentration in the first (anoxic) chamber occurred when R was increased to 3, similar than that observed when methane was desorbed from UASB effluent during period IV (figure 6.2b).

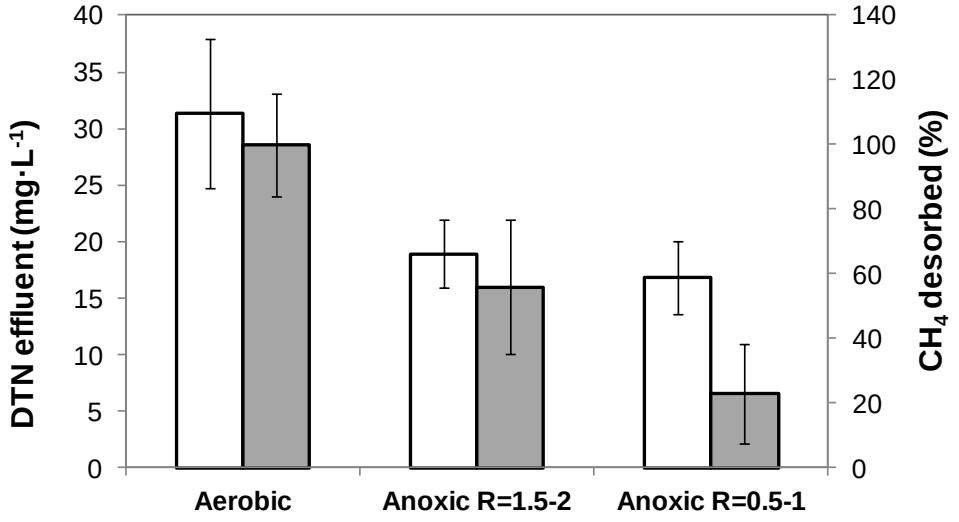

Figure 6.4. Percentage of methane desorbed (■) and DTN concentration in the effluent (□) during three different operational scenarios.

Volumetric mass transfer coefficient for oxygen was also calculated (0.90 d^{-1}) in order to determine the oxygen transferred from the environment to the first MBR chamber. This value was not negligible, representing 48, 25 and 12% of the oxygen transferred with the recirculation from the aerobic membrane filtration chamber at R of 0.5, 1.0 and 2.0 respectively.

Table 6.3. Average denitrification, methane and oxygen apparent specific consumption rates and $N\text{-}NO_x$:$C\text{-}CH_4$ and CH_4:O_2 molar ratio in the first (anoxic) chamber

R	$mgN \cdot gMLVSS^{-1} \cdot d^{-1}$	$mgCH_4 \cdot gMLVSS^{-1} \cdot d^{-1}$	$mgO_2 \cdot gMLVSS^{-1} \cdot d^{-1}$	mol NO_3^-/mol CH_4	mol CH_4/mol O_2
0.5	14.3	40.1	4.9	0.5	16.4
1.0	22.5	43.7	10.9	0.6	8.0
2.0	16.8	15.9	13.0	1.2	2.4

The experimental molar ratio between the oxidized methane and the oxygen consumed was from 2.4 to 16.5, which is much higher than theoretical molar relationship 1:1 according to the stoichiometry of the aerobic pathway (equation 6.1), suggesting a combination of both, aerobic and anaerobic oxidation of methane. It should be taken into account the importance of anaerobic oxidation of methane, especially at lower recirculation rates. The increase in recirculation ratio from the aerated membrane filtration chamber increased the amount of oxygen entering the first (anoxic) chamber and probably caused the observed sharp decrease of methane oxidation rate. The oxidation of methane seemed to be inhibited. Other authors have reported the complex role of oxygen in the process, observing and increase of specific denitrification rate with oxygen up to a maximum, but decreasing again after it (Thalasso et al., 1997). Moreover, as reported by Waki et al. (2009), the removal of nitrogen in the presence of methane and oxygen is a complex process that might occur through some different mechanisms such as aerobic and anaerobic methane oxidation coupled to denitrification or even anammox. This could be a reason for the observed impact of recirculation, or oxygen, on methane oxidation rate during the present study. In this sense, the high dissolved oxygen concentrations in the

first chamber of the MBR as a consequence of the aerobic/anoxic cycles applied, equivalent to the oxygen introduced at very high recirculation ratios, would explain the absence of nitrogen removal observed in Chapter 4.

Regarding the experimental molar ratio between the removed nitrogen and the oxidized methane, it varied between 0.5 and 1.2 depending on recirculation ratio, which is much lower than the theoretical molar relationship 8:5 according to the stoichiometry of the anaerobic pathway (equation 6.2). Assuming that the 20% of the nitrogen was removed using remaining biodegradable COD, as stated before, these molar ratios would be even lower. With respect to the theoretical molar relationship 4:5 given by the stoichiometry of the aerobic pathway, only the experimental ratio determined at R=2 was similar. Nevertheless, the higher molar ratio observed at R=2 was caused by the sharp decrease of the methane oxidation rate. Therefore, these results pointed out that other processes might be responsible for the consumption of the methane in addition to denitrification.

The findings of this research could be extrapolated for reducing GHG and nutrient emissions of wastewaters treated anaerobically, especially for low-strength wastewaters in (semi)tropical countries (van Lier, 2008). Souza et al. (2011) quantified the dissolved methane present in different UASB effluents treating domestic wastewater at ambient temperature in Brazil. These effluents were 30 to 60 % oversaturated with methane, reaching concentrations up to 22 $mg \cdot L^{-1}$. These values were very similar than those obtained in this work (average of 23 $mg \cdot L^{-1}$). Around 50 $mg \cdot L^{-1}$ of total nitrogen can be expected in an anaerobically treated municipal wastewater effluent (van Haandel and Lettinga, 1994). According with the results of the present study and considering equation 6.1, it could be expected, at least, a 16 $mg \cdot L^{-1}$ of total nitrogen removal for this kind of wastewater. Moreover, this nitrogen removal could be increased up to 32 $mg \cdot L^{-1}$ according to equation 6.2 and neglecting other denitrification processes.

6.4.4. Batch experiments to study denitrification mechanism

Different batch experiments were carried out in order to determine the main denitrification mechanism in our system. The results presented before show that denitrification, using methane as a complementary carbon source in the presence of the oxygen recirculated, was possible. Nevertheless, the denitrification mechanism might be complex, involving different pathways (Modin et al., 2007). Batch experiments were performed in anaerobic conditions in order to prove if anaerobic methane oxidation coupled to denitrification was feasible. On the other hand, it was also studied if the denitrification might occur preferably in the biofilm than in suspended biomass as a

consequence of the continuous permanence or not under anoxic conditions. It should be taken into account the continuous recirculation of suspended biomass that took place between aerobic and anoxic conditions.

Batch experiments (figure 6.5) showed higher denitrification rates for the flasks fed with acetate, independently of the presence of methane (57.1±19.1 mgN·gMLVSS^{-1}·d^{-1}). Nevertheless some activity (28.2±11.2 mgN·gMLVSS^{-1}·d^{-1}) was also observed in the flask containing methane as sole carbon source with respect to the blank (20.0±14.3 mgN·gMLVSS^{-1}·d^{-1}). Maximum rates observed with acetate were only three times higher than those ones corresponding to endogenous denitrification. Similar denitrification rates were observed for the suspended and the biofilm biomass. Moreover, these denitrification rates were much lower than those typically reported at 20 ºC, using acetate as carbon source, being around 250 mgN·gMLVSS^{-1}·d^{-1} (Henze et al., 2002). Apparent specific denitrification rates observed during the continuous operation of the system were lower than observed in batch experiments, around 30 mgN·gMLVSS^{-1}·d^{-1}. Furthermore, results regarding the obtained specific denitrification rates were of the same order of magnitude than those of 15 and 90 mgN·gMLVSS^{-1}·d^{-} referred by other authors (Lee et al., 2001; Khin and Annachhatre, 2004). Thus, the use of MBR systems, with relatively high biomass concentration, could be a good choice for increasing the reactor's capacity.

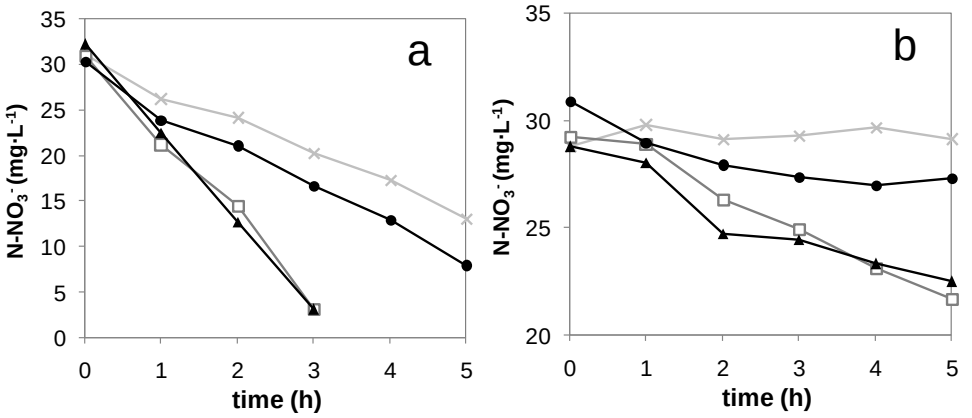

Figure 6.5. Batch denitrification assays with the presence of both suspended and biofilm biomass (a) and only biofilm biomass (b) as inocula. Carbon sources employed were: blank test (×), acetate (□), methane (●) and methane and acetate (▲).

Fluorescent in situ hybridization (FISH) analyses were used in order to determine microbial populations. Detailed information of these results can be found in the thesis of Buntner (2013). Abundant methanotrophs type I were found in both suspended and biofilm

biomass. Anaerobic methanogenic bacteria (ANME), which are capable to carry out reversed methanogenesis and convert methane into acetic acid/acetate, (Knittel and Boetius, 2009; Valentine and Reeburgh, 2000), were also found. It could be another possible explanation for methane oxidation observed in the reactor even though the oxygen molar ratio was always lower than the one given by stoichiometry of the aerobic methane oxidation pathway (eq. 6.1; table 6.3).

Moreover, FISH analyses of the biofilms indicated the abundance of large clusters of anammox bacteria. Therefore, as previously reported by Waki et al. (2009), nitrogen removal in the presence of CH_4 and O_2 seemed to be a complex mixture of methanotrophic, denitrifying, ammonia-oxidizing and anammox processes.

6.4.5. Membrane performance

Membrane critical flux did not varied significantly during the six experimental periods, with an average value of 20.8±2.0 $L \cdot m^{-2} \cdot h^{-1}$. The flux applied (14.5 ± 1.0 $L \cdot m^{-2} \cdot h^{-1}$) was below the critical flux, thus it was expected that reversible fouling was predominant. In fact, it was observed during all the operation time that permeability was almost fully recovered when a physical cleaning with tap water was carried out. Only two maintenance chemical cleanings were performed during the operation, at the beginning of periods III and V.

As can be observed in figure 6.6, permeabilities normally ranged between 150 and 230 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$. Although the fluxes obtained were lower than those typically reported in aerobic MBRs operating with similar membrane modules, being between 20 and 25 $L \cdot m^{-2} \cdot h^{-1}$, observed permeability values were similar (Judd, 2002; Wen et al., 2004).

As recommended in Chapter 5, colloidal BPC concentration was measured in order to establish a possible relationship between the operational conditions of the system and the membrane fouling. A slight increase on cBPC concentration was observed at the beginning of period II, when the environment in first chamber of the MBR was changed from aerobic to anoxic. As a consequence of the high MLVSS concentrations maintained in the MBR, between 4 and 8 $g \cdot L^{-1}$, it was not observed a significant impact of cBPC concentration on membrane performance.

Nevertheless, the remarkable increase observed during period IV, when methane was desorbed from the UASB effluent (figure 6.6), was accomplished by a significant drop on permeability, being necessary a maintenance chemical cleaning. cBPC concentration did not decrease instantly when dissolved methane desorption was stopped but a progressive diminution was observed during periods V and VI.

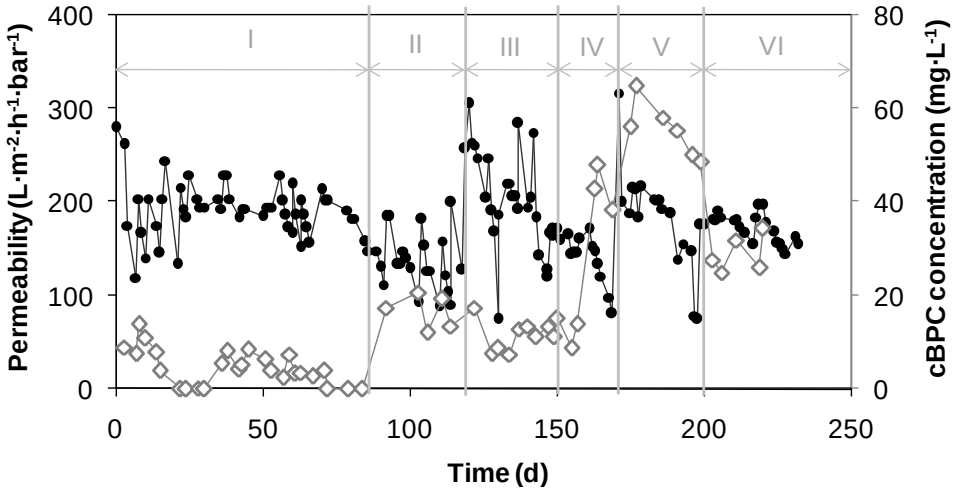

Figure 6.6. Evolution of permeability (●) and colloidal BPC concentration (◇) in the MBR.

It is widely accepted that stress conditions induces the production and release of polymeric substances. Therefore, the change in the conditions at the beginning of periods II and IV would explain the increase on cBPC concentration. The desorption of dissolved methane on period VI might impact indirectly on cBPC concentration through the loss of denitrification activity (section 6.4.2). In fact, althoug membrane fouling in denitrification MBRs has not been extensively characterized, the results obtained in period VI were in accordance with those reported by Paetkau and Cicek (2011), who studied nitrogen removal in an MBR and reported that the highest TEP concentrations took place during an unstable denitrification period.

Despite the flux limitations, the application of membrane technology was of core importance in the studied system. Membrane cut-off could be the solution to problems related with the wash-out of extremely slow-growing bacteria, such as denitrifying methanotrophs (Kampman et al., 2012), and avoid the loss of methanogenic bacteria that reaches the MBR from the UASB reactor.

6.5. Conclusions

- Denitrification using methane as a carbon source was proved to be feasible in a system with a UASB pre-treatment followed by an aerobic MBR with a previous anoxic

chamber.

- Although remaining biodegradable COD might be also used as a carbon source, the presence of dissolved methane in the UASB effluent was shown to be essential for the removal of nitrogen in the anoxic chamber.

- Internal recirculation in the MBR was also an important parameter governing this sensitive process. Higher nitrogen removal and lower methane emissions were reported at lower recirculation ratios (between 0.5 and 1).

- Denitrification seemed to be carried out by a consortium of aerobic and anaerobic methane oxidizing bacteria, anammox and heterotrophic bacteria. At higher recirculation ratios the anaerobic oxidation pathway seemed to be inhibited, decreasing methane oxidation rate.

- Batch experiments confirmed that anaerobic methane oxidation coupled to denitrification was feasible.

- Denitrification process seemed to influence membrane performance. The highest cBPC concentrations and the lowest permeabilities were observed when denitrification activity dimished due to the desorption of dissolved methane from the UASB effluent.

6.6. References

APHA-AWWA-WPCF. 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, USA.

Boetius, A., Ravenschlag, K., Schubert, C.J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jorgensen, B.B., Witte, U., Pfannkuche, O. 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* **407**, 623–626.

Buntner, D. 2013. Combined UASB-MBR system for the treatment of low-strength wastewaters. PhD Thesis, University of Santiago de Compostela, Santiago de Compostela, Spain.

Cakir, F.Y., Stenstrom, M.K. 2005. Greenhouse gas production: A comparison between aerobic and anaerobic wastewater treatment technology. *Water Research* **39**, 4197-4203.

Daelman, M.R.J., van Voorthuizen, E.M, van Dongen, U.G.J.M., Volcke, E.I.P., van Loosdrecht, M.C.M. 2012. Methane emissions during municipal wastewater treatment. *Water Research* **46**, 3657-3670.

Ettwig, K. F., Shima, S., van de Pas-Schoonen, K. T., Kahnt, J., Medema, M. H., op den Camp, H. J. M., Jetten, M. S. M., Strous M. 2008. Denitrifying bacteria anaerobically oxidize methane in the absence of Archaea. *Environmental Microbiology* **10**(11), 3164–3173.

Hanson, R.S., Hanson, T.E. 1996. Methanotrophic bacteria. *Microbiology and Molecular Biology Reviews* **60**(2), 439-471.

Hatamoto, M., Yamamoto, H., Kindaichi, T., Ozaki, N., Ohasji, A. 2010. Biological oxidation of dissolved methane in effluents from anaerobic reactors using a down-flow hanging sponge reactor. *Water Research* **44**(5), 1409-1418.

He, S.-B., Wang, B.-Z., Wang, L., Jiang, Y.-F., Zhang, L.-Q. 2003. A novel approach to treat combined domestic wastewater and excess sludge in MBR. *Journal of Environmental Science* **15**(5), 674–679.

Henze, M., Harremoës, P., Jansen, J.I.C., Arvin, E. 2002. Wastewater treatment: biological and chemical processes, 3rd ed*., Springer*, Berlin.

Islas-Lima, S., Thalasso, F., Gómez-Hernández J. 2004. Evidence of anoxic methane oxidation coupled to denitrification. *Water Research* **38**(1), 13–16.

Judd, S.J. 2002. Submerged membrane bioreactors: flat plate or hollow fibre? *Filtration and Separation* **39**(5), 30-31.

Judd, S.J. 2011. The MBR Book: Principles and Applications of Membrane Bioreactors for Water and Wastewater Treatment, 2nd Ed, Elsevier Science Ltd, Oxford.

Kampman, C., Hendrickx, T.L., Luesken, F.A., van Alen, T.A., Op den Camp, H.J., Jetten, M.S., Zeeman, G., Buisman, C.J., Temmink, H. 2012. Enrichment of denitrifying methanotrophic bacteria for application after direct low-temperature anaerobic sewage treatment. *Journal of Hazardous Materials* **227–228**, 164–171.

Khin, T., Annachhatre, A.P. 2004. Nitrogen removal in a fluidized bed bioreactor by using mixed culture under oxygen-limited conditions. *Water Science and Technology* **50**(6), 313-320.

Knittel, K., Boetius, A. 2009. The anaerobic oxidation of methane - progress with an unknown process. *Annual Reviews of Microbiology* **63**, 311–34.

Lee, H.-J., Bae, J.-H., Choo, K.-M. 2001. Simultaneous nitrification and denitrification in a mixed methanotrophic culture. *Biotechnology Letters* **23**, 935-941.

Luesken, F.A., Sanchez, J., van Alen, T.A., Sanabria, J., Op den Camp, H.J.M., Jetten, M.S.M., Kartal, B. 2011. Simultaneous nitrite-dependent anaerobic methane and ammonium oxidation processes. Applied and Environmental Microbiology **77**(19), 6802–6807.

Melse, R.W., van der Werf, A.W. 2005. Biofiltration for mitigation of methane emission from animal husbandry. *Environmental Science and Technology* **39**(14), 5460–5468.

Mechsner K., Hamer G. 1985. Denitrification by methanotrophic/methylotrophic bacterial association in aquatic environment. In Denitrification in the Nitrogen Cycle (Edited by H. L. Golterman), 257-272. Plenum Publ. Corp., New York

Modin, O., Fukushi, K., Yamamoto, K. 2007. Denitrification with methane as external carbon source. *Water Research* **41**(12), 2726–2738.

Noyola, A., Morgan-Sagastume, J.M., López-Hernández, J.E. 2006. Treatment of biogas produced in anaerobic reactors for domestic wastewater: odor control and energy/resource recovery. *Reviews in Environmental Science and Biotechnology* **5**, 93-114.

Paetkau, M., Cicek, N. 2011. Comparison of nitrogen removal and sludge characteristics between a conventional and a simultaneous nitrification-denitrification membrane bioreactor. *Desalination* **283**, 165-168.

Park, S., Lee, C.-H., Ryu, C.-R., Sung, K. 2009. Biofiltration for reducing methane emissions from modern sanitary landfills at the low methane generation stage. *Water, Air, and Soil Pollution* **196**(1), 19–27.

Raghoebarsing, A.A., Pol, A., van de Pas-Schoonen, K.T., Smolders, A.J.P., Ettwig, K.F., Rijpstra, I.C., Schouten, S., Sinninghe Damste, J.S., Op den Camp, H.J.M., Jetten, M.S.M., Strous, M. 2006. A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature* **440**, 918–921.

Rajapakse, J.P., Scutt, J. E. 1999. Denitrification with natural gas and various new growth media. *Water Research* **33**(18), 3723-3734.

Rhee G. and Fuhs G. (1978) Wastewater denitrification with one-carbon compounds as energy source. *Journal of the Water Pollution Control Federation* **50**(9), 2111-2119.

Sánchez, A., Buntner, D., Garrido, J.M. 2013. Impact of methanogenic pre-treatment on the performance of an aerobic MBR system. *Water Research* **47**, 1229-1236.

Sander, R. 1999. Compilation of Henry's Law Constants for Inorganic and Organic Species of Potential Importance in Environmental Chemistry, http://www.rolf-sander.net/henry/.

Shen, Z., Zhou, Y., Hu, J., Wang, J. 2013. Denitrification performance and microbial diversity in a packed-bed bioreactor using biodegradable polymer as carbon source and biofilm support. *Journal of Hazardous Materials* **250-251**, 431-438.

Souza, C.L., Chernicharo, C.A.L., Aquino, S.F. 2011. Quantification of dissolved methane in UASB reactors treating domestic wastewater under different operating conditions. *Water Science and Technology* **64**(11), 2259-2265.

Thalasso, F., Vallecillo, A., García-Encina, P., Fernández-Polanco, F. 1997. The use of methane as a sole carbon source for wastewater denitrification. *Water Research* **31**(1), 55–60.

Valentine, D.L., Reeburgh, W.S. 2000. New perspectives on anaerobic methane oxidation. *Environmental Microbiology* **2**(5), 477-484.

van Haandel, A. C., Lettinga, G. 1994. Anaerobic Sewage Treatment – A Practical Guide for Regions with a Hot Climate. John Wiley & Sons Ltd, Chichester, UK.

van Lier, J.B. 2008. High-rate anaerobic wastewater treatment: diversifying from end-of-the-pipe treatment to resource-oriented conversion techniques. *Water Science and Technology* **57**(8), 1137-1149.

van't Riet, K., Tramper, J. 1991. Basic Bioreactor Design. Marcel Dekker Inc., New York-Basel.

Waki, M., Yasuda, T., Yokoyama, H., Hanajima, D., Ogino, A., Suzuki, K., Yamagishi, T., Suwa, Y., Tanaka, Y. 2009. Nitrogen removal by co-occurring methane oxidation, denitrification, aerobic ammonium oxidation, and anammox. *Applied Microbiology and Biotechnology* **84**(5), 977-985.

Wen, X., Bu, Q., Huang, X. 2004. Study on fouling characteristic of axial hollow fibers cross-flow microfiltration under different flux operations, in: Proceedings of the Water Environment-Membrane Technology Conference, Seoul, Korea.

Wu, Ming L. 2012. Cell biology and biochemistry of the denitrifying methanotroph “Candidatus Methylomirabilis oxyfera”. Radboud University Nijmegen (The Netherlands), ISBN: 978-94-6191-408-8.

Chapter 7

Membrane fouling in an AnMBR treating industrial wastewater at high total solids concentration

Summary

In this chapter an anaerobic membrane bioreactor (AnMBR) was operated for the treatment of an herbal extraction wastewater. The complexity and low biodegradability of this industrial wastewater led to the operation of the bioreactor at high mixed liquor total solids (MLTS) concentrations. The fluxes achieved ranged between 1 and 2.5 $L \cdot m^{-2} \cdot h^{-1}$, working with MLTS between 38 and 61 $g \cdot L^{-1}$. These values were similar to those obtained in other AnMBR treating industrial wastewaters with submerged membrane modules at MLTS above 30 $g \cdot L^{-1}$. Nevertheless, the information regarding membrane performance of AnMBR operated at high MLTS concentration is limited. Thus, the possibility of improving membrane performance by adding powdered activated carbon (PAC) was evaluated.

Furthermore typical fouling indicator concentrations recently studied during the operation of aerobic MBRs, such as biopolymer cluster (BPC) and transparent exopolymer particles (TEP), were measured during the operation. Moreover, the filterability properties of the sludge were determined during the operation in order to examine if the addition of PAC could improve the resistance to filtration of the mixed liquor. The concentrations of the fouling indicators measured during this studying were extremely high, as well as specific resistance to filtration and the addition of PAC to the AnMBR did not improve anyone of them. Membrane fouling was governed by the hydrodynamics derived from the high MLTS concentration. Since this high MLTS concentration did not improve organic matter removal, a diminution below 20 $g \cdot L^{-1}$ could enhance membrane fluxes, especially when PAC would be added into the reactor, as suggested by literature.

This research was carried out in the chair of Chemical & Process Engineering at the **Technical University of Berlin**. Parts of this chapter will be published as:

Brand, C., Sánchez, A., Chlaida, M., Kraume, M. 2013. Membrane fouling in an AnMBR treating industrial wastewater at high total solids concentration. In preparation.

Brand, C., Sánchez, A., Chlaida, M., Kraume, M. 2013. Acidification in an Anaerobic Membrane Bioreactor treating Herbal Extraction Wastewater. In preparation

7.1. Introduction

Submerged membrane bioreactors (MBR) represent an attractive technological solution which has been widely applied for the aerobic and/or anaerobic treatment of industrial and municipal wastewater (Kang et al., 2002; Rosenberger et al., 2002; He et al., 2005; Sridang et al., 2008; Kanai et al., 2010; Buntner et al., 2010). The major drawback to this technology is the fouling of the membrane, especially in anaerobic MBR (AnMBR). Considering that the physical, chemical and biological characteristics of the mixed liquor in aerobic and anaerobic systems differ significantly, the importance of the fouling mechanisms impacting might be very different. Feasible flux has a strong influence on both the capital and operating costs of the process. Most of the authors working with submerged AnMBR reported fluxes in the range of 5-10 $L \cdot m^{-2} \cdot h^{-1}$ at temperatures above 30 °C treating municipal wastewaters (Saddoud et al., 2007; Trzcinski and Stuckey, 2009; Skouteris et al., 2012). Applicable fluxes reported for the treatment of industrial wastewaters in mesophilic submerged AnMBR are generally lower, ranging between 2 and 5 $L \cdot m^{-2} \cdot h^{-1}$ (Van Zyl et al., 2008; Spagni et al., 2010; Skouteris et al., 2012). Spagni et al. (2010) demonstrated that the applicable fluxes obtained in AnMBR depend strongly on operational conditions and rapid membrane fouling was usually observed.

The causes responsible for membrane fouling in aerobic MBR have been widely studied whereas a limited amount of research has focused on the parameters that limits permeate flux in AnMBR, specially treating industrial wastewaters. Anyway, mixed liquor total solids (MLTS) concentration is considered as one of the most important parameters for MBR (Jeison and van Lier, 2006), especially for AnMBR since high MLTS are required due to low growth rates of anaerobic bacteria. Moreover, some industrial wastewaters are very difficult to degrade due to its complex matrix, the presence of particulate slowly biodegradable matter and/or inorganic substances; and hence a high biomass concentration could improve the volumetric biological capacity of the reactor. The exact relationship between MLTS concentration and the steady-state permeate flux in an AnMBR has not been extensively investigated (Berubé et al., 2006), and the information regarding AnMBR operation at high MLTS concentration is very limited. Stuckey and Hu (2003) observed that the TMP required to maintain a constant permeate flux in an AnMBR treating a synthetic wastewater at an MLTS concentration of 35 $g \cdot L^{-1}$ was more than twice as high as required at an MLTS concentration of 7 $g \cdot L^{-1}$. Kitamura et al. (1996) observed a similar behaviour treating industrial wastewater in an AnMBR. This is in accordance with Ho and Sung (2009), who reported that high MLTS concentrations in an AnMBR lead to a sudden, rapid fouling. Nevertheless there is limited information regarding membrane

fouling in a submerged AnMBR at high MLTS concentration (above 30 $g \cdot L^{-1}$) (Jeison and van Lier, 2006; Van Zyl et al., 2008; Spagni et al., 2010).

Membrane fouling in aerobic MBR has been related with different fractions of biopolymers, commonly used as fouling indicators. The fraction most frequently mentioned in relation with membrane fouling is the group of soluble microbial products (SMP). Nevertheless, recent studies have introduced a more general approach to the biopolymers responsible for membrane fouling by defining biopolymer clusters (BPC) and transparent exopolymer particles (TEP) as important factors in the formation of the sludge fouling layer on the membrane surface and the increase of fouling potential (Sun et al., 2008; de la Torre et al., 2008; Sánchez et al., 2013). BPC have been defined as a pool of non-filterable organic matter in the liquid phase of the MBR sludge mixture much larger than SMP (Sun et al., 2008) whereas TEP are very sticky particles that exhibit the characteristics of gels, and consist predominantly of acidic polysaccharides (Passow, 2002). Depending on the applied assays, these groups are not distinct but overlap (Drews, 2010).

The addition of powdered activated carbon (PAC) to improve membrane performance has been extensively studied in membrane filtration of potable water and in aerobic MBR. Such studies have also been conducted on AnMBR (Park et al., 1999; Akram and Stuckey, 2008). PAC has been used not only to enhance permeate flux in AnMBR (Park et al., 1999), but also for improved COD and volatile fatty acids (VFA) removal during shock loading. Flux enhancement has been attributed to the scouring effect of the PAC on the membrane surface (Park et al., 1999) and to the PAC adsorption of dissolved/colloidal material from the mixed liquor (Fang et al., 2006; Ng et al., 2008). However the agglomeration of colloids to form larger and stronger particles and therefore a higher shear resistance and lower release of foulants was demonstrated to be the most likely explanation (Choo and Lee, 1996; Li et al., 2005; Hu and Stuckey, 2007). On the other hand, the interaction with other important parameters as MLTS concentration, cross-flow velocity or wastewater is still unclear.

PAC dosages between 1 and 5 $g \cdot L^{-1}$ have been reported for AnMBR. Park et al. (1999) found that membrane fouling decreased continuously with increasing PAC doses up to 5 $g \cdot L^{-1}$. Nevertheless, Akram and Stuckey (2008) found an optimum concentration of 1.67 $g \cdot L^{-1}$ of PAC, decreasing permeate flux when adding 3.4 $g \cdot L^{-1}$ of PAC. Hu and Stuckey (2007) added 1.7 $g \cdot L^{-1}$ of either PAC or granular activated carbon (GAC) to study its effect on membrane performance. Nevertheless, reports in the literature suggested that

GAC did not adsorb VFA, whereas PAC did (Barker and Stuckey, 1999; Akram and Stuckey, 2008).

7.2. Objectives

In this study, an AnMBR was operated to treat industrial herbal extraction wastewater at high MLTS concentration. The aims of this study were to evaluate membrane fouling in AnMBR through typical fouling indicators used in aerobic MBR and to assess the addition of PAC to an AnMBR treating wastewater from an herbal extraction industry at high MLTS concentration in order to increase permeate flux. PAC addition was also tested as a useful tool during shock loading.

7.3. Materials and methods

7.3.1. Experimental setup

The continous stirred tank reactor (CSTR) (figure 7.1) had a volume of 23 L and was operated under anaerobic mesophilic conditions (36 °C) during 198 days with an organic loading rate (OLR) ranging between 2 and 4 $kgCOD \cdot m^{-3} \cdot d^{-1}$. At a feed COD concentration of 8000 $mg \cdot L^{-1}$ this resulted in a HRT between 2 and 4 d. The separation of the biomass was assured by a flat sheet ultrafiltration membrane, manufactured by A3 water solutions GmbH (Germany), with a pore size of 0.1 µm made of polyethersulfone. The total membrane area of the used immersed membrane was 0.27 m². The membrane was operated in cycles of 10 min, with 8 min of filtration and 2 min of relaxation. Moreover, membrane fouling was minimized by sparging the membrane with biogas.The average specific gas demand per membrane area (SGD_m) was 0.41 $Nm^3 \cdot h^{-1} \cdot m^{-2}$, which is around 60% of the recommended value of the full scale module. The biogas is taken from the head space of the reactor and is recycled to the reactor by a compressor. Furthermore a gas-lift loop mixing is achieved. Membrane module was replaced by a new one on day 76 due to the high fouling observed.

Prior to this operation, the reactor was operated during 125 days at an OLR of 2 $kgCOD \cdot m^{-3} \cdot d^{-1}$.The reactor was inoculated with anaerobic digestion sludge of a municipal wastewater treatment plant with a concentration of approximately 20 $g \cdot L^{-1}$.

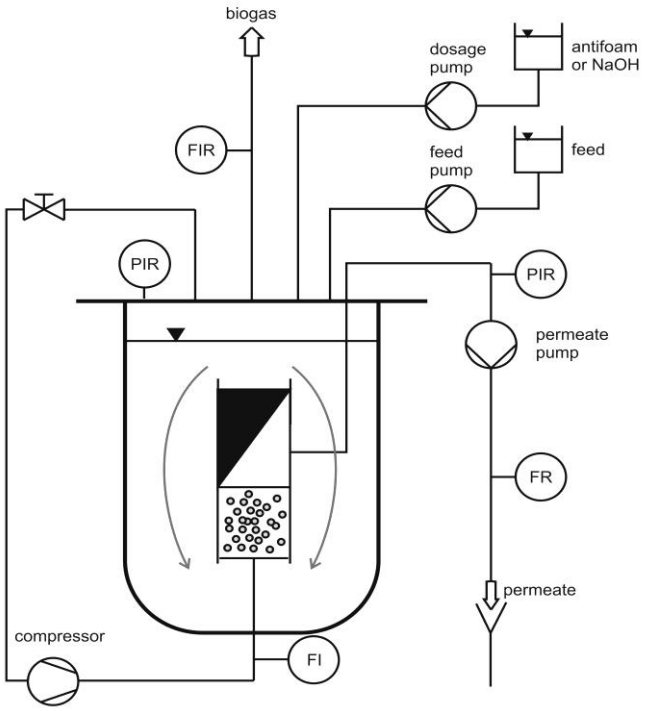

Figure 7.1. Scheme of the AnMBR reactor set-up.

7.3.2. Synthetic wastewater

The wastewater was prepared by using an aqueous extraction of rosemary (*Rosmarinus officinalis*), as rosemary is one of the main raw materials in the industrial process. The rosemary was boiled for 3 hours in water (1 kg rosemary per 10 L water). The produced concentrate had a COD of 20 to 22 $g \cdot L^{-1}$ and is diluted to COD concentration of 8 to 10 $g \cdot L^{-1}$ for the AnMBR.

7.3.3. Powder activated carbon

Commercial PAC CX1 (CECA, France) was used in this study. PAC was manufactured pine wood charcoal chemically activated with phosphoric acid. The selected PAC is specially employed for decolourization of liquids and fatty acids elimination in agro-food and chemical industries. The main characteristics of PAC are given in table 7.1.

Table 7.1. Characteristics of PAC

Parameter	Unit	PAC CX1
Mean particle size	µm	30
Specific surface	$m^2 \cdot g^{-1}$	1000
Methylene blue number	$mL \cdot 100g^{-1}$	11
Iodine number	$cg \cdot g^{-1}$	100

7.3.4. Analytical methods

Mixed liquor volatile total solids (MLVTS) and MLTS were determined according to the German standard DIN 38409-1 using a porcelain melting pot. Concentration of chemical oxygen demand (COD), total nitrogen (TN), total phosphorous, organic acids (OA) and total organic carbon (TOC) were measured with selective Hach-Lange tests. Total alkalinity (TA), partial alkalinity (PA) and intermediate alkalinity (IA) were determined following the Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WPCF, 1998). Concentration of methane, CO_2 and nitrogen were measured using a gas chromatograph (SRI-Instruments).

With respect to the membrane operation, transmembrane pressure (TMP) and permeability were measured continuously. The difference in TOC concentration between the mixed liquor after filtration through a 0.45 µm nitrocellulose membrane filters (HA, Millipore) and the permeate was assigned to the colloidal fraction of biopolymer clusters (cBPC) in the liquid phase of the sludge suspension (Sun et al., 2008). The analysis method used for the determination of the transparent exopolymer particles (TEP) concentration (de la Torre et al., 2008) is based on the protocol developed for TEP quantification in sea water (Arruda et al., 2004). The critical flux was determined according to the modified flux-step method proposed by van der Marel et al. (2009). The criterion employed was that the increment of transmembrane pressure (TMP) with respect to time was higher than 10 $Pa \cdot min^{-1}$ (Le-Clech et al., 2003).

The specific resistance to filtration of a sludge sample was determined by a dead-end filterability test. The test was conducted at 25ºC in a 200-mL pressurized cylinder (Model Sartorius SM 16249) using a 0.2 µm flat-sheet cellulose acetate membrane filter with a diameter of 47 mm (12587-47-N Sartorius). Using the Carman-Kozeny equation to calculate the pressure drop of a fluid flowing through a packed bed of solids in laminar flow

and taking into account that the filtration takes place at constant pressure, the specific resistance to filtration (SRF) (α, $m \cdot kg^{-1}$) can be calculated after linearization.

Further information regarding analytical methods is provided in Chapter 2.

7.3.5. Batch and fed-batch experiments

Batch experiments using the permeate of the AnMBR were carried out in order to determine the adsorption capacity of the different PAC and GAC. With respect to the fed-batch experiments, the objective was to determine the optimum dose of the selected PAC in similar operational conditions than those applied during the operation of the AnMBR.

Both, batch and fed-batch experiments were carried out at 36 ºC in closed 250 mL closed bottles with magnetic stirring. The PAC was rinsed several times with deionized water to remove inorganic ashes, then dried at 105ºC and always stored in desiccators before use. 10 mL samples were taken with a syringe and filtered through 0.45 µm filter in order to determine TEP and BPC concentration. Batch experiments were performed using permeate from the AnMBR whereas in fed-batch experiments were carried out with sludge from the AnMBR. In the case of fed batch experiments, dilutions were performed with deionized water at 36 ºC in order to avoid biomass stress and bottles were fed every day with 10 mL of industrial herbal extraction wastewater (raw wastewater fed to the AnMBR).

7.4. Results and discussion

7.4.1. System performance

Stable operation of the system was maintained applying HRT below 4 d, at a feed concentration of 8 $g \cdot L^{-1}$ resulting in an OLR of 2.0 - 3.0 $kgCOD \cdot m^{-3}d^{-1}$ (figure 7.2c) without controlling alkalinity during the first 84 operational days. It is important to point out that the AnMBR was operated during 125 days before the present study. At the beginning of the operation, COD removal efficiency was very low (around 25 %) due to the dramatic accumulation of organic acids and the absence of alkalinity in the reactor (figure 7.2a and 7.2b). Therefore, it was necessary to add $NaHCO_3$ and to stop the feeding (day 10) during five days in order to recover the methanogenic process. As can be observed on figure 7.2b, the pH increased from 6.5 to 7.2 with this strategy, but only after a few days OA concentration decreased from 5 to 3 $g \cdot L^{-1}$. Once the COD removal percentages increased above 60%, it was tried to check the stability of the process by decreasing HRT. The reduction of the HRT from 4 d to 2 d in one step and the subsequent increase of OLR resulted in a drop of COD elimination to 46% on day 52, due to a slight increase on

organic acids (OA) concentration identified by an alkalinity measurement. Subsequently the HRT was increased to 3 d, $NaHCO_3$ was added in order to increase pH and the reactor reached 60% of COD elimination again. As can be observed on figure 7.2c, the organic removal rate (ORR) gradually increased from day 15 till day 52. After a slight decrease due to the punctual rise of OLR, ORR increased again when HRT was decreased from 4 to 3 d (figure 7.2c). After another decrease of HRT to 2.5 d on day 72, COD removal percentage decreased again to values lower than 40%, increasing alkalinity ratio (figure 7.2a). The cause of the ORR diminution was the OA accumulation and the subsequent drop of pH. Therefore bicarbonate was added and the HRT was increased again to 4d to stabilize the system. In addition to the punctual dosing of $NaHCO_3$, it was also necessary to stop the feeding during 48 hours in order to recover the process. Therefore, acidification events that took place on days 52 and 72 showed an OLR threshold value around 4 $kgCOD \cdot m^{-3}d^{-1}$.

From day 84 onwards alkalinity was continuously controlled through $NaHCO_3$ addition in the feeding. The COD elimination was around 60%, reaching maximum values above 70% (figure 7.2a) and pH values and OA concentrations were controlled (figure 7.2b). Furthermore, OLR was increased during the last operational days, showing stable operation at 4 $kgCOD \cdot m^{-3}d^{-1}$. Nevertheless, OA concentration remained above 3 $g \cdot L^{-1}$, indicating a possible inhibition of methane formation in the anaerobic digester (Kroeker et al., 1979). During stable operation the methane yield was 0.27 to 0.32 $m^3_{methane} \cdot kgCOD_{eliminated}^{-1}$ with a methane concentration of approximately 60%.

In figure 7.2b is depicted the evolution of organic acids (OA) concentration and the pH in the AnMBR during the operation. OA concentration measurement was representative of all fatty acids and it was given in acetic acid equivalents ($mg \cdot L^{-1}$). As can be observed in figure 7.2b OA concentration was extremely high during the operation, indicating some kind of inhibition of the methanogenic process. Although there is no information in the literature regarding the anaerobic treatment of this kind of wastewaters, rosemary is widely known by its antibacterial activity (Bousbia et al., 2009). This fact might have a harmful effect on anaerobic biological process, causing destabilization of the microbial populations leading to VFA accumulation that can acidify the reactor, and therefore inhibit methanogenic microorganisms.

No sludge purge, except for sampling purposes, took place during the operation of the AnMBR. MLTS increased constantly from 38 to 61 $g \cdot L^{-1}$. Nevertheless, only a slight improvement of COD removal efficiency was observed, as a consequence of such increase. COD removal variations were more related with the acidification of the reactor when OLR was increased and to the addition of alkalinity. Even during stable operation

with alkalinity control, the lowest COD concentrations measured in the permeate were above 2 g·L^{-1}. Therefore, an aerobic post-treatment would be required in order to obtain an effluent suitable for indirect discharge (threshold value is 1 g·L^{-1}).

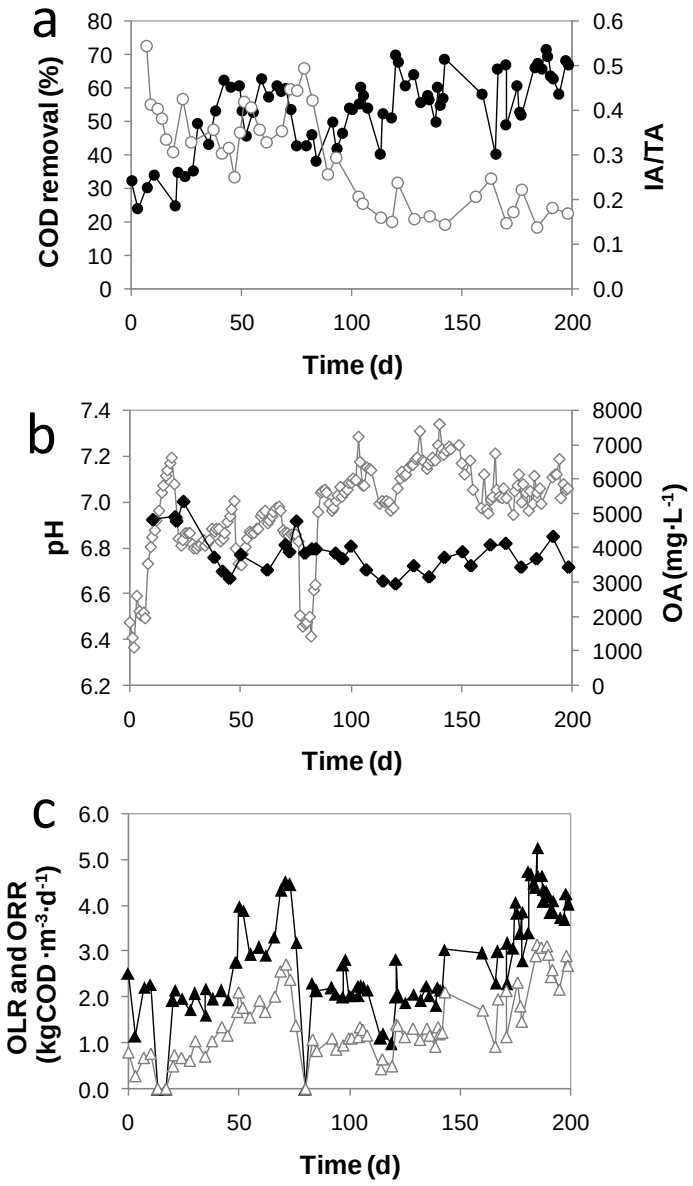

Figure 7.2. a) COD removal efficiency (●) and alkalinity ratio (□); b) Organic acids (OA) concentration (●) and pH (◇); c) OLR and ORR in the in the AnMBR treating industrial herbal extraction wastewater.

7.4.2. Fed-batch experiments to study the optimum PAC dosage in the AnMBR

Different PAC and GAC were tested (data not shown) in order to select the best option. Contrary to the affirmation stated in the introduction supported by some authors (Barker and Stuckey, 1999; Akram and Stuckey, 2008), GAC did exhibit good OA adsorption rates, similar than those obtained with the PAC tested.

In figure 7.3 can be observed the adsorption isotherm of the selected PAC. This was the best of the PAC and GAC tested in terms of adsorption capacity. The adsorption capacity of the PAC was determined by adding different PAC doses to a certain volume of permeate and measuring the residual dissolved organic carbon concentration (DOC) after 24 h. The selected PAC presented a good adsorption capacity, in the range of those reported for the adsorption of organic compounds in activated carbon used in this kind of applications (BREF, 2003).

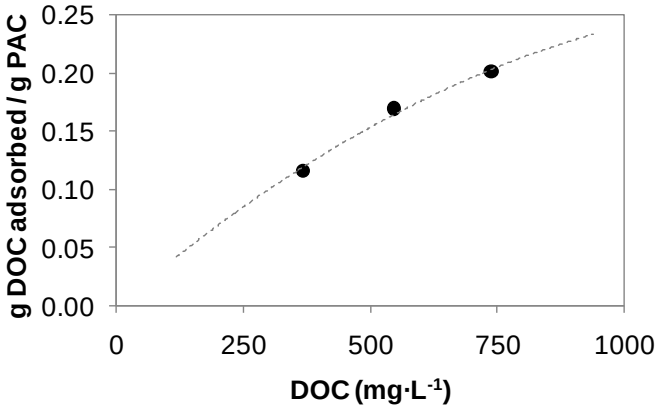

Figure 7.3. DOC Adsorption isotherm of the selected PAC.

The first experiments with PAC were performed using the permeate from the AnMBR in order to determine the adsorption capacity of possible foulants such as BPC or TEP as well as the adsorption capacity of organic acids in case of shock load of the reactor. As can be observed in figure 7.4, all the studied parameters presented a very good removal with PAC dosages of 1.5 $g \cdot L^{-1}$. It has to be taken into account that the adsorption of COD was not the objective of PAC dosage, but the improvement of filterability and the possible mitigation of VFAs accumulation during a shock load event. Therefore, the dosage of higher amounts of PAC than 1.5 $g \cdot L^{-1}$ was not justified considering the adsorption of BPC, TEP and OA. In figure 7.4b the accumulation of organic acids in the flask without PAC can

be observed. This result confirmed the OA accumulation observed during continuous operation of the AnMBR and the subsequent worsening of COD removal efficiency.

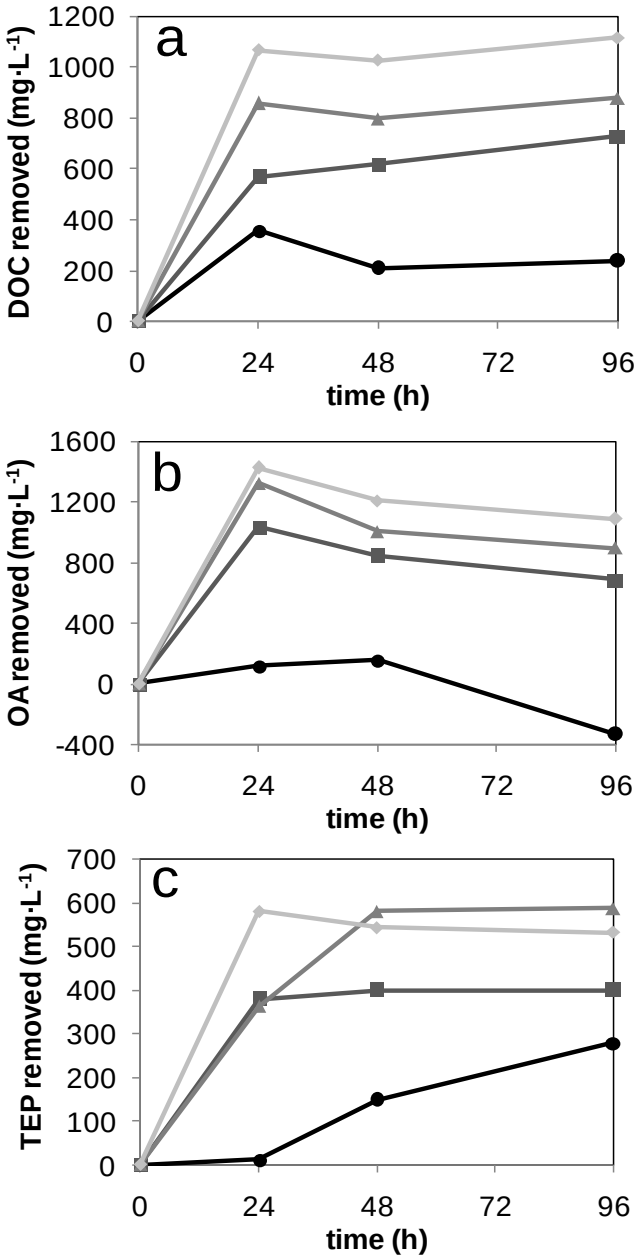

Figure 7.4. Evolution of DOC (a), Organic acid (b) and TEP (c) concentration during fed-batch experiments without PAC (●), 1.5 g·L^{-1} (■), 3.0 g·L^{-1} (▲) and 6.0 g·L^{-1} (◆) of PAC.

Filterability test were carried out in order to determine the influence of the different PAC dosages on the resistance to filtration of the different fractions studied (cake, pore blocking and colloidal). These tests were performed 24 hours after the beginning of the experiments. The fraction most influenced by PAC dosage was the colloidal one. In figure 7.5 can be observed that the resistance to filtration of colloidal fraction diminished with the addition of PAC. Nevertheless the amount of PAC seemed not to influence this diminution. Therefore an optimum PAC dose of 1.5 $g \cdot L^{-1}$ was determined. This value was similar to that reported by Akram and Stuckey (2008), who found an optimum concentration of 1.67 $g \cdot L^{-1}$ of PAC, but operating the AnMBR with a MLTS concentration below 20 $g \cdot L^{-1}$.

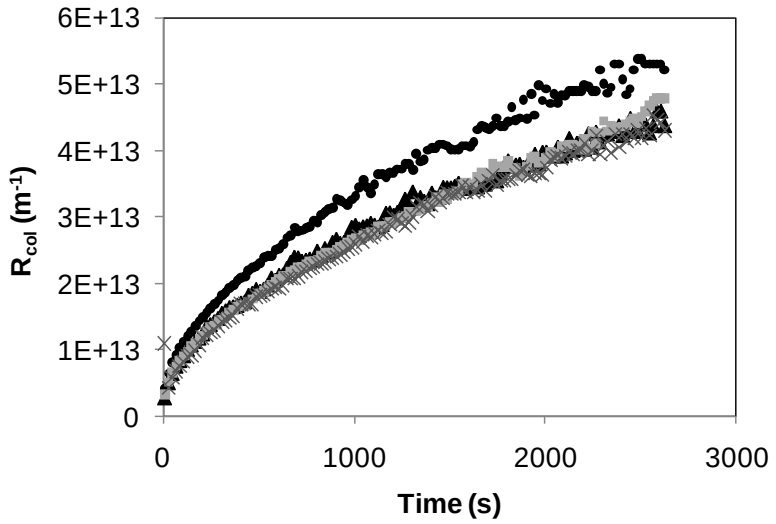

Figure 7.5. Colloidal resistance without PAC (●), with 1.5 $g \cdot L^{-1}$ (▲), 3.0 $g \cdot L^{-1}$ (■) and 6.0 $g \cdot L^{-1}$ (X) of PAC.

Filterability experiments were carried out again with the optimum PAC dosage of 1.5 $g \cdot L^{-1}$ in order to confirm the results obtained before and to evaluate the influence of PAC in the specific resistance to filtration (SRF) of the sludge. These batch experiments were performed after 24 hours, without feeding the bottles. As can be observed on figures 7.6 and 7.7, the addition of PAC in to the sludge led to a slight diminution of SRF and a remarkable decrease of the resistance to filtration of the colloidal fraction.

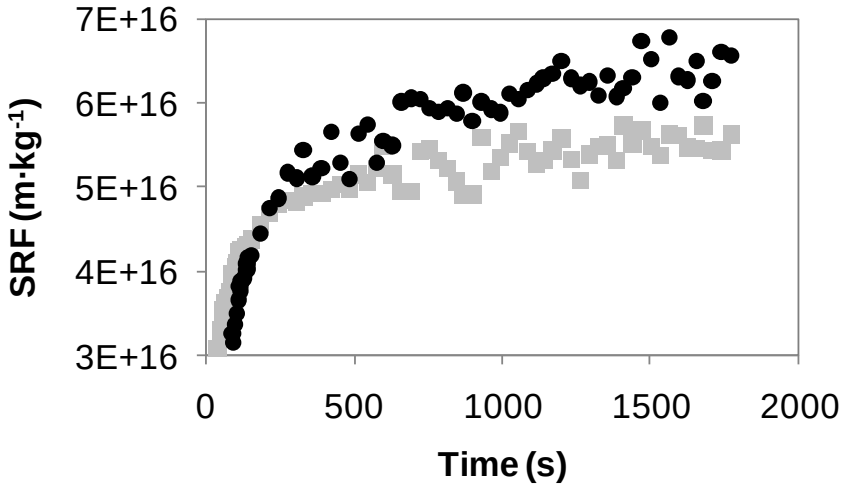

Figure 7.6. Evolution of SRF without PAC (●) and with 1.5 g·L^{-1} of PAC (■).

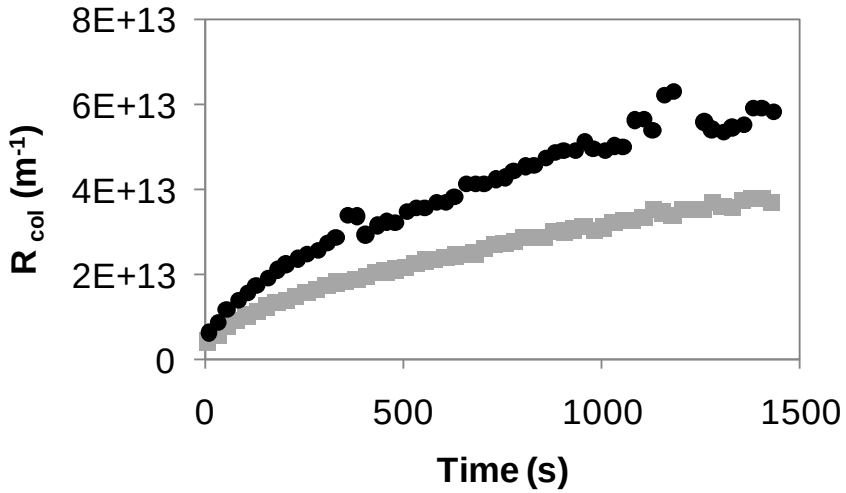

Figure 7.7. Resistance of colloidal fraction without PAC (●) and with 1.5 g·L^{-1} of PAC (■).

In this work, a novel approach to filterability experiments is proposed. Dead-end filtration studies of activated sludge (Sørensen and Sørensen, 1997) and studies of membrane bioreactor sludge have indicated that sludge is highly compressible (Bugge et al., 2012). Nevertheless, the MBR-cake-fouling models found in the literature often use constant values for the specific resistance to filtration (Li and Wang, 2006), neglecting compressibility. This assumption would cause significant errors in analyzing filtration data, especially at high solids concentration. Therefore, fouling layer compressibility should be considered in order to fully understand changes in filterability during operation.

In this study it was observed that either resistance or specific resistance to filtration (SRF) were variable with time due to the compressibility of the cake formed over the membrane surface (figures 7.5, 7.6 and 7.7).

7.4.3. Membrane performance and influence of PAC addition in the AnMBR

Low membrane fluxes, between 1.1 and 2.5 $L \cdot m^{-2} \cdot h^{-1}$ were obtained during the operation. Maximum critical flux measured was 3.6 $L \cdot m^{-2} \cdot h^{-1}$. These fluxes were lower than typical fluxes obtained in submerged AnMBR at temperatures above 30 ºC (Skouteris et al., 2012). Neverthtless, among them, only Jeison and van Lier (2006), Van Zyl et al. (2008) and Spagni et al. (2010) operated at high MLTS (above 40 $g \cdot L^{-1}$). In fact, the fluxes obtained by Spagni et al. (2010) at MLTS concentration of 53 $g \cdot L^{-1}$ were similar to that obtained in this study (around 2 $L \cdot m^{-2} \cdot h^{-1}$). Jeison and van Lier (2006) studied the influence of MLTS concentration on critical flux, reporting a decrease on this parameter from 21 to 5 $L \cdot m^{-2} \cdot h^{-1}$ when MLTS increased from 25 to 50 $g \cdot L^{-1}$. Regarding permeability, values around 100 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ were obtained which were similar than that obtained by Robles et al. (2013) operating an AnMBR at a MLTS concentration of 25 $g \cdot L^{-1}$. Due to the low permeabilities observed, the membrane module was replaced by a new one on day 76. Nevertheless, permeabilities obtained with the new module were similar to that achieved before.

The carbohydrate fraction of soluble microbial products (SMP_C) has been widely considered as the most important parameter regarding membrane fouling (Rosenberger et al., 2006; Drews, 2010). Nevertheless, recent studies have introduced a more general approach to the biopolymers responsible for membrane fouling such as BPC and TEP (Sun et al., 2008; de la Torre et al., 2008; Sánchez et al., 2013). In this study, the applicability of these parameters as possible fouling indicators in an AnMBR treating industrial wastewater at high MLTS concentration was evaluated.

The supernatant TOC of the mixed liquor was always significantly higher than the effluent TOC, indicating significant retention of organic matter by the membrane filtration and cake layers. The same phenomenon was observed in both submerged MBR and AnMBR (Wang and Li, 2008; Hu and Stuckey 2006; and Lin et al., 2009). Wang and Li (2008) suggested that a group of organic substance classified as BPC exerted a significant influence on filtration resistance, and measured them as the difference in TOC concentration between the supernatant of the mixed liquor and the effluent. On the other hand Lin et al. (2009) used COD instead TOC to determine BPC content. In this study, total and colloidal BPC concentration was monitored using TOC measurements. The

concentrations obtained were extremely higher than that observed in aerobic MBRs (Wang and Li, 2008, Sánchez et al., 2013) but also much higher than reported in AnMBR treating industrial wastewaters (Lin et al., 2009). Moreover, Wang et al. (2007) reported that BPC in the sludge cake was much higher than that in the bulk sludge, suggesting that the accumulation of BPC in the sludge liquor would facilitate the formation of the sludge cake layer on the membrane surface. Thus, high BPC concentration observed during the operation (figure 7.8) would be expected to form denser cake layers, and thus cause serious fouling (Lin et al., 2009).

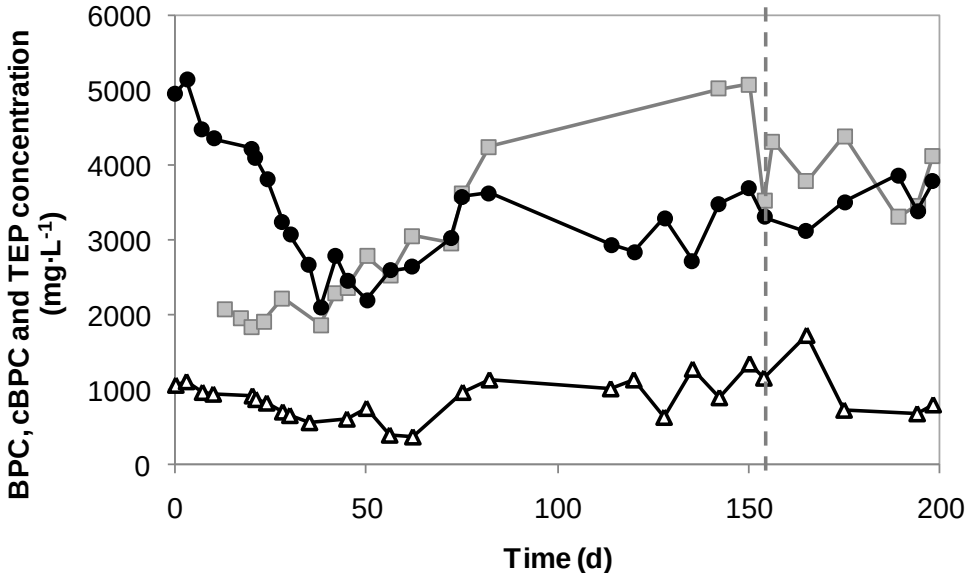

Figure 7.8. Evolution of BPC (●), colloidal BPC (Δ) and TEP (■). Dashed line represents the day when PAC addition in the reactor took place.

Regarding TEP concentration, the values measured during the operation (between 2000 and 5000 $mg \cdot L^{-1}$) were also much higher than those previously reported in submerged aerobic MBRs (de la Torre et al., 2008; Sánchez et al., 2013), which are normally below 250 $mg \cdot L^{-1}$. No information about the concentration of TEP in AnMBR was found in the literature.

The measured parameters showed that membrane fouling was extremely high in our AnMBR. As a consequence, 1.5 $g \cdot L^{-1}$ of PAC were added to the bioreactor on day 152 in order to study its influence on membrane performance. The evolution of BPC and TEP concentration was studied after the addition of PAC. As observed in figure 7.8, the addition of 1.5 $g \cdot L^{-1}$ PAC in the reactor did not exhibit any significant improvement on BPC and

cBPC concentrations. Regarding TEP, although it seemed to control the progressive accumulation observed until day 150, its concentration remained at extremely high levels, never reported before.

The evolution of sludge filterability properties was also monitored before and after the PAC addition. On figure 7.9 can be observed the evolution of SRF and cake and colloidal fraction resistances. Although it was demonstrated that these parameters did not remain constant (figures 7.6 and 7.7), it was necessary to calculate punctual values in order to follow its evolution. In this sense, filterability data corresponding to the first phase of formation of the cake were discarded and only the data corresponding to the moment on which the cake was consistently formed were taken into account (from 1000s of filtration onwards). Contrary to observed in other studies (Choo et al., 2000; Hu and Stuckey, 2007; Akram and Stuckey, 2008), the addition of PAC to the AnMBR on day 152 did not exhibit a significant effect on membrane performance. Although SRF punctually decreased after the addition of PAC, cake and colloidal fraction resistances were not affected, and even increased slightly (figure 7.9). As occurred with TEP and BPC concentration, SRF values obtained were much higher than those typically reported for anaerobic sludge (Metcalf & Eddy, 2003). For instance, Cho et al. (2000) observed a decrease on SRF after the addition of PAC reaching values of $9.9 \cdot 10^{15}$ $m \cdot kg^{-1}$, which was twice smaller than that of the cake without PAC. These values represented only the 15 % of typical SRF values obtained during the present study. Regarding cake resistances, the values obtained were 10-fold higher than those reported by Robles et al. (2013) in a submerged AnMBR treating municipal wastewater at MLTS concentrations up to 25 $g \cdot L^{-1}$.

Moreover, critical flux was also determined before and after the addition of PAC. Any significant improvement was observed with respect to critical flux, remaining between 3.0 and 3.6 $L \cdot m^{-2} \cdot h^{-1}$. This might indicate that the amount of activated carbon used in this study was probably less than optimum, and thus the flux could probably be further improved by adding more PAC to the reactors as suggested by Park et al., 1999. Nevertheless, Akram and Stuckey (2008) found the worsening of membrane performance fluxes with an increase in PAC addition. Others factors such as solution chemistry (Braghetta et al., 1997) and type and concentration of dissolved organic compounds (Chang and Lee, 1998) also influenced the role of PAC in flux improvement.

It is recognized that the colloidal material is mainly responsible for fouling in an AnMBR (Choo and Lee, 1996, 1998). Lower diffusion rates of the colloidal particles result into a slower transport back into the bulk solution than coarser ones (Choo and Lee, 1998), which means they tend to accumulate at the membrane surface and form a dense cake

layer. Moreover, their size can clog membrane pores, particularly in the case of microfiltration membranes.

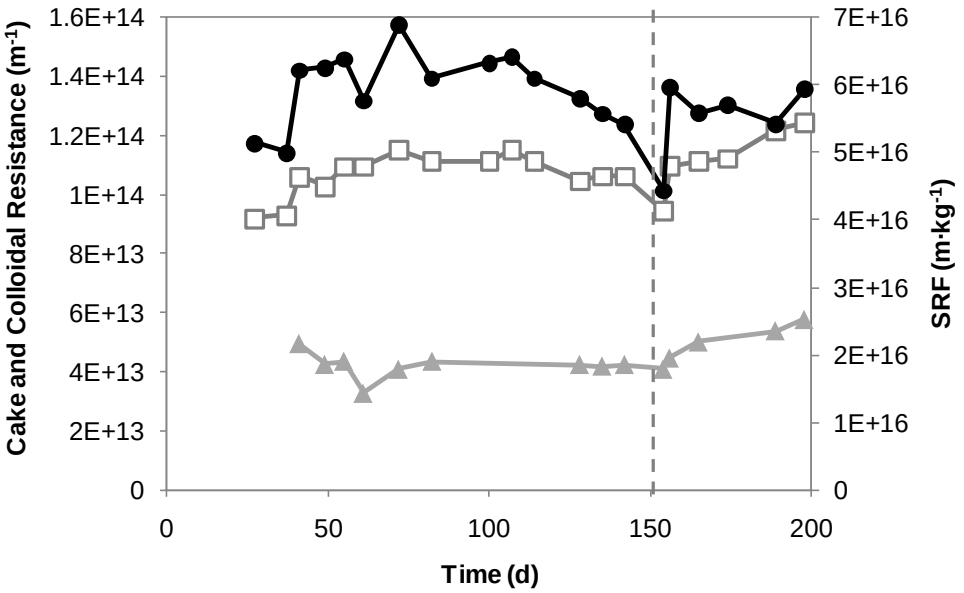

Figure 7.9. Evolution of specific resistance to filtration (●) and resistances of cake (□) and colloidal fractions (▲). Dashed line represents the day when PAC addition in the reactor took place.

The resistance of the colloidal fraction in the mixed liquor was always around 40% of the cake resistance. Therefore, according to the results obtained in batch experiments (figure 7.7), a certain improvement on membrane performance might be expected when PAC was added to the AnMBR mixed liquor. Choo and Lee (1996) suggested that the addition of an adsorbent or a coagulant could enhance permeate flux by agglomerating the fine colloids, present in the mixed liquor, forming larger particles that have a lower tendency to foul membranes. Although this effect was observed during batch experiments, it did not occur when the PAC was added to the AnMBR, and colloidal fraction resistance was not positively affected. The main hypothesis of such behavior was related with the presence of a compact sticky layer over the membrane surface observed when the membrane module was replaced on day 76. The module was submerged in water and the permeability before and after removing the cake by rinsing with tap water (no chemical cleaning) was evaluated. It was observed that the permeability before removing the cake was around the 10% of the permeability after doing it (350 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$). Considering that

measured permeability of the new membrane module was 500 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$, it can be assumed that the main membrane fouling mechanism was cake layer formation. This cake layer acted like a shield, protecting the membrane from internal pore blocking. Therefore, the high MLTS concentration led to the formation of a dense sticky cake layer that clogged the membrane, and the SGD_m applied, which was lower than the recommended value of the full scale module, did not help to alleviate its effect.

Sludge properties, including biopolymer concentration, are the core parameters in governing sludge cake formation and membrane fouling in submerged AnMBR systems (Lin et al., 2009). The characteristics of the mixed liquor in an AnMBR are expected to vary significantly based on the type of wastewater being treated (Kataoka et al., 1992). In addition, inorganic fouling should not be underestimated when treating complex wastewaters such as industrial herbal extraction wastewater since Inorganic species can interact with biopolymers in the reactor and enhance the mechanical stability of the fouling layer (Lin et al., 2009). The high COD concentration in the effluent led to increase MLTS concentration in order to achieve higher removal capacities. However, COD removal was only slightly improved due to the poor anaerobic biodegradability of the wastewater but membrane performance was seriously limited.

The existence of a threshold value (30 $g \cdot L^{-1}$) above which the MLTS concentration has a negative influence on membrane filtration has been reported (Yamamoto et al. 1994; Lubbecke et al., 1995; Hong et al., 2002). In accordance with this studies, Robles et al. (2013) recently established a MLTS critical value between 28 and 31.5 $g \cdot L^{-1}$ for an AnMBR treating municipal wastewater. Operation above this critical value would lead to lower fluxes and shorter membrane lifespan. Therefore, the MLTS concentrations, between 38 and 61 $g \cdot L^{-1}$, achieved during the operation led to the severe fouling of the membrane and the futility of PAC addition.

In order to achieve higher membrane fluxes it would be recommended to diminish MLTS concentration below 25 $g \cdot L^{-1}$. In this scenario, PAC addition might influence sludge filterability and enhance membrane performance.

Nevertheless, with the proposed treatment, COD values were much higher that the allowed threshold value for direct or indirect discharge. Therefore an aerobic treatment step has to be established. A combination of another high strength anaerobic system such as an upflow anaerobic sludge blanket reactor (UASB) with an aerobic MBR would achieve higher COD removals, allowing to apply higher membrane fluxes as reported in Chapter 5 (Sánchez et al., 2013).

7.5. Conclusions

- COD removal efficiency did not exhibit a significant improvement by increasing MLTS concentration, probably due to the complexity of industrial herbal extraction wastewaters. Maximun COD removals of 70 % were achieved only when alkalinity was stabilized through sodium bicarbonate addition.
- Membrane fouling was seriously affected by the high MLTS concentration. The formation of a dense cake layer that clogged the membrane governed fouling mechanisms.
- All the fouling parameters studies such as SRF, BPC, cBPC and TEP concentrations were extremely high, compared with those previously reported. The applicability of cBPC and TEP concentration as a fouling indicator in AnMBR treating industrial wastewater was not as reliable as in aerobic MBRs due to its high values.
- PAC addition into the reactor was evaluated as a possible way to reduce membrane fouling, nevertheless no significant effect was observed regarding foulants concentration and sludge filterability.
- PAC addition would be beneficial for the system at lower MLTS concentrations, and even would be useful during shock load events as observed in fed-batch experiments.
- The combination of an UASB reactor with an aerobic MBR would be more appropriate in order to enhance membrane flux and perhaps improve COD removal efficiency.

7.6. References

Akram, A., Stuckey, D.C. 2008. Flux and performance improvement in a submerged anaerobic membrane bioreactor (SAMBR) using powdered activated carbon (PAC). *Process Biochemistry* **43**, 93-102.

APHA-AWWA-WPCF. 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, USA.

Arruda Fatibello, S.H.S., Henriques Vieira, A.A., Fatibello-Filho, O. 2004. A rapid spectrophotometric method for the determination of transparent exopolymer particles (TEP) in freshwater. *Talanta* **62**, 81–85.

Barker DJ, Stuckey DC. 1999. A review of soluble microbial products (SMP) in wastewater treatment systems. *Water Research* **33**, 3063–82.

Berubé, P.R., Hall, E.R., Sutton, P.M. 2006. Parameters Governing Permeate Flux in an Anaerobic Membrane Bioreactor Treating Low-Strength Municipal Wastewaters: A Literature Review. *Water Environmental Research* **78**, 887-897.

Bousbia, N., Vian, M.A., Ferhat, M.A., Petitcolas, E., Meklati, E.I., Chemat, F. 2009. Comparison of two isolation methods for essential oil from rosemary leaves: Hydrodistillation and microwave hydrodiffusion and gravity. *Food chemistry* **114**(1), 355-362.

Braghetta A, DiGiano FA, Ball WP. 1997. Nanofiltration of natural organic matter: pH and ionic strength effects. *Journal of Environmental Engineering* **123**, 628–41.

BREF 2003, Integrated Pollution Prevention and Control, Reference Document on Common Waste Water and Waste Gas Treatment/Management System in Chemical Sector.

Bugge, T.V., Jørgensen, M.K., Christensen, M.L., Keiding, K. 2012. Modeling cake buildup under TMP-step filtration in a membrane bioreactor: Cake compressibility is significant. *Water Research* **46**, 4330-4338

Buntner, D., Sánchez, A., Garrido, J.M. 2011. Three stages MBR (methanogenic, aerobic biofilm and membrane filtration) for the treatment of low-strength wastewaters. *Water Science and Technology* **64**(2), 397-402.

Chang, I-S., Lee, C.-H. 1998. Membrane filtration characteristics in membrane coupled activated sludge system—the effect of physiological states of activated sludge on membrane fouling. *Desalination* **120**, 221–33.

Choo, K.H., Lee, C.H. 1996. Effect of anaerobic digestion broth composition on membrane permeability. *Water Science and Technology* **34**, 173–179.

Choo, K.H., Lee, C.H. 1998. Hydrodynamic Behaviour of Anaerobic Biosolids During Crossflow Filtration in the Membrane Anaerobic Bioreactor. *Water Research* **32**, 3387.

Choo, K. H.; Kang, I. J.; Yoon, S. H.; Park, H.; Kim, J. H.; Adlya, S.; Lee, C. H. 2000. Approaches to Membrane Fouling Control in Anaerobic Membrane Bioreactors. *Water Science and Technology* **41**(10–11), 363.

de la Torre, T., Lesjean, B., Drews, A., Kraume, M. 2008. Monitoring of transparent exopolymer particles (TEP) in a membrane bioreactor (MBR) and correlation with other fouling indicators. *Water Science and Technology* **58**(10), 1903-1910.

Drews, A. 2010. Membrane fouling in membrane bioreactors—Characterisation, contradictions, cause and cures. *Journal of Membrane Science* **363**, 1–28.

Fang, H.H.P., Shi, X., Zhang, T. 2006. Effect of activated carbon on fouling of activated sludge filtration. *Desalination* **189**, 193–199.

He, Y., Xu, P., Li, C., Zhang, B., 2005. High-concentration food wastewater treatment by an anaerobic membrane bioreactor. *Water Research* **39**, 4110-4118.

Ho, J., Sung, S. 2010. Methanogenic activities in anaerobic membrane bioreactors (AnMBR) treating synthetic municipal wastewater. *Bioresource Technology* **101**(7), 2191-2196.

Hong, S.P., Bae, T.H., Tak, T.M., Hong, S., Randall, A. 2002. Fouling control in activated sludge submerged hollow fiber membrane bioreactors. *Desalination* **143**, 219–228.

Hu, Y., Stuckey, D.C. 2007. Activated carbon addition to a submerged anaerobic membrane bioreactor: effect on performance, transmembrane pressure, and flux. *Journal of Environmental Engineering* **133**, 73–80.

Jeison, D., van Lier, J.B. 2006. Cake layer formation in anaerobic submerged membrane bioreactors (AnSMBR) for wastewater treatment. *Journal of Membrane Science* **284**, 227-236.

Kanai, M., Ferre, V., Wakahara, S., Yamamoto, T., Moro, M. 2010. A novel combination of methane fermentation and MBR - Kubota submerged anaerobic membrane bioreactor process. *Desalination* **250**, 964-967.

Kang, I.J., Yoon, S.H., Lee, C.H. 2002. Comparison of the filtration characteristics of organic and inorganic membranes in a membrane-coupled anaerobic bioreactor. *Water Research* **36**, 1803-1813.

Kataoka, N., Tokiwa, Y., Tanaka, Y., Fujiki, K., Taroda, H., Takeda, K. 1992. Examination of Bacterial Characteristics of Anaerobic Membrane Bioreactors in Three Pilot-Scale Plants for Treating Low-Strength Wastewater by Application of the Colony-Forming-Curve Analysis. *Applied and Environmental Microbiology* **58**, 2751.

Kitamura, Y., Maekawa, T., Tagawa, A., Hayashi, H., Farrell-Poe, K. L. 1996. Treatment of Strong Organic, Nitrogenous Wastewater by an Anaerobic Contact Process Incorporating Ultrafiltration. *Applied Engineering in Agriculture* **12**, 709.

Kroeker, E. J., Schulte, D. D., Sparling, A. B., Lapp, H. M. 1979**.** Anaerobic treatment process stability. *Journal of the Water Pollution Control Federation* **51**, 718-727.

Le-Clech, P., Jefferson, B., Chang, I.S., Judd, S.J. 2003. Critical flux determination by the flux-step method in a submerged membrane bioreactor. *Journal of Membrane Science* **227**, 81-93.

Li, Y.-Z., He, Y.-L., Liu, Y.-H., Yang, S.-C., Zhang, G.-J. 2005. Comparison of the filtration characteristics between biological powdered activated carbon sludge and activated sludge in submerged membrane bioreactors. *Desalination* **174**, 305–314.

Li, X., Wang, X. 2006. Modelling of membrane fouling in a submerged membrane bioreactor. *Journal of Membrane Science* **278**, 151-161.

Lin, H.J., Xie, K., Mahendran, B., Bagley, D.M., Leung, K.T., Liss, S.N., Liao, B.Q. 2009. Sludge properties and their effects on membrane fouling in submerged anaerobic membrane bioreactors (AnMBRs). *Water Research* **43**, 3827-3837.

Lubbecke, A., Vogelpohl, A., Dewjanin, W. 1995. Wastewater treatment in a biological high-performance system with high biomass concentration. *Water Research* **29**, 793.

Metcalf & Eddy, Wastewater Engineering: Treatment and Reuse, 4th ed., McGraw-Hill, New York, 2003.

Ng, C.A., Sun, D., Fane, A.G. 2008. Operation of membrane bioreactor with powdered activated carbon addition. *Separation Science and Technology* **41**, 1447–1466.

Park, H., Choo, K., Lee, C. 1999. Flux enhancement with powdered activated carbon addition in the membrane anaerobic bioreactor. *Separation Science and Technology* **34**(14), 2781-2792.

Passow, U. 2002. Transparent exopolymer particles (TEP) in aquatic environments. *Progress in oceanography* **55**, 287-333.

Robles, A., Ruano, M.V., Ribes, J., Ferrer, J. 2013. Factors that affect the permeability of comercial hollow-fibre membranes in a submerged anaerobic MBR (HF-SAnMBR) system. *Water Research* **47**, 1277-1288.

Rosenberger, S., Krüger, U., Witzig, R., Manz, W., Szewzyk, U., Kraume, M. 2002. Performance of a bioreactor with submerged membranes for aerobic treatment of municipal waste water. *Water Research* **36**, 413–420.

Rosenberger, S., Laabs, C., Lesjean, B., Gnirss, R., Amy, G., Jekel, M., Schrotter, J.-C. 2006. Impact of colloidal and soluble organic material on membrane performance in membrane bioreactors for municipal wastewater treatment. *Water Research* **40**, 710– 720.

Saddoud, A., Ellouze, M., Dhouib, A., Sayadi, S. 2007. Anaerobic membrane bioreactor treatment of domestic wastewater in Tunisia. *Desalination* **207**, 205–215.

Sánchez, A., Buntner, D., Garrido, J.M. 2013. Impact of methanogenic pre-treatment on the performance of an aerobic MBR system. *Water Research* **47**, 1229-1236.

Skouteris, G., Hermosilla, D., López, P., Negro, C., Blanco, A. 2012. Anaerobic membrane bioreactors for wastewater treatment: A review. *Chemical Engineering Journal* **198–199**(0), 138–148.

Sørensen, B.L., Sørensen, P.B. 1997. Structure compression in cake filtration. *Journal of Environmental Engineering* **123**, 345-353.

Spagni, A., Casu, S., Crispino, N.A., Farina, R., Mattioli., D. 2010. Filterability in a submerged anaerobic membrane bioreactor. *Desalination* **250**, 787-792.

Sridang, P.C., Pottier, A., Wisniewski, C., Grasmick, A. 2008. Performance and microbial surveying in submerged membrane bioreactor for seafood processing wastewater treatment. *Journal of Membrane Science* **317**, 43–49.

Stuckey, D.C., Hu, A. 2003. The submerged anaerobic membrane bioreactor (SAMBR): an intensification of anaerobic wastewater treatment. Presented at the International Water Association Leading Edge Conference on Drinking Water and Wastewater Treatment Technologies, Noordwijk, The Netherlands.

Sun, F.-Y., Wang, X.-M., Li, X.-Y. 2008. Visualisation and characterisation of biopolymer clusters in a submerged membrane bioreactor. *Journal of Membrane Science* **325**, 691–697.

Trzcinski, A.P., Stuckey, D.C. 2009. Anaerobic digestion of the organic fraction of municipal solid waste in a two-stage membrane process. *Water Science and Technology* **60**(8), 1965-1978.

van der Marel, P., Zwijnenburg, A., Kemperman, A., Wessling, M., Temmink, H., van der Meer, W. 2009. An improved flux-step method to determine the critical flux and the critical flux for irreversibility in a membrane bioreactor. *Journal of Membrane Science* **332**, 24-29.

Van Zyl, P. J., Wentzel, M. C., Ekama ,G. A., Riedel, K. J. 2008. Design and start-up of a high rate anaerobic membrane bioreactor for the treatment of a low pH, high strength,dissolved organic waste water. *Water Science and Technology* **57**(2), 291-296.

Wang, X.M., Li, X.Y., Huang, X. 2007. Membrane fouling in a submerged membrane bioreactor (SMBR): characterisation of the sludge cake and its high filtration resistance. *Separation and Purification Technology* **52**, 439-445.

Wang, X.-M., Li, X.-Y. 2008. Accumulation of biopolymer clusters in a submerged membrane bioreactor and its effect on membrane fouling. *Water Research* **42**, 855-862.

Yamamoto, K., Hissa, M., Mahmood, T., Mutsuo, T. 1994. Direct solid liquid separation using hollow fiber membrane in an activated sludge aeration tank. *Water Science and Technology* **30**, 21–27.

Conclusiones

Las principales conclusiones de esta investigación, que se centra en la combinación de la tecnología de membranas sumergidas con tratamientos biológicos aeróbicos y anaeróbicos, se presentan a continuación.

1. Membranas sumergidas en sistemas terciarios de filtración con membranas

De los resultados obtenidos, se puede concluir que la tecnología de membranas sumergidas es una buena elección, para la obtención de un efluente con una alta calidad y libre de sólidos en suspensión, después de un tratamiento biológico en reactores secuanciales discontinuos con biomasa granular y floculenta. La operación de las unidades de filtración terciaria con una concentración de biomasa mayor que la normalmente recomendada para este tipo de sistemas hizo que se comportaran como biorreactores secundarios, eliminando parte de la DQO y nitrificando el amonio procedente de los reactores secuenciales discontinuos. El efecto negativo de esta operación fueron los menores flujos de permeado obtenidos. Finalmente, el estado de agregación de la biomasa no tuvo ninguna influencia en el funcionamiento de los sistemas de filtración terciarios, y otros parámetros, tales como la nitrificación o la presencia de sólidos suspendidos en el agua residual bruta mostraron un impacto más relevante en funcionamiento de la membrana.

2. Combinación de biorreactor de membrana (BRM) con reactor UASB

La combinación de un BRM aeróbico con un reactor UASB anaeróbico, en un único sistema integrado o como post-tratamiento, demostró ser una buena solución para el tratamiento de aguas residuales de baja carga a temperatura ambiente, produciendo un efluente de alta calidad, biogás rico en metano y disminuyendo la producción de lodos. Además, el sistema combinado mostró flexibilidad para convertir el nitrógeno total a amoníaco y / o nitrato, lo que resulta especialmente interesante para su empleo en la reutilización del agua tratada, dependiendo de la aplicación y los estándares de calidad. La hidrólisis de la biomasa en suspensión recirculada desde el BRM al reator UASB provocó la liberación de sustancias biopoliméricas, empeorando el rendimiento de la membrana. Por lo tanto, sería interesante desarrollar estrategias con el fin de eliminar la materia coloidal resultante de la digestión anaerobia de sustratos complejos. En este

sentido, el uso de una etapa con organismos superiores tales como protozoos podría ser una alternativa interesante. La presencia de soporte de plástico durante este estudio promovió la presencia de protozoos e influenció la concentración de sustancias biopolímericas coloidal.

Por otra parte, la concentración de biomasa resultó ser un parámetro importante para la protección de la membrana contra el ensuciamiento provocado por biopolímeros solubles y coloidales. Por lo tanto, para un óptimo rendimiento de la membrana después de un pre-tratamiento metanogénico de sustratos complejos, debe garantizarse una relación alimento/microorganismos mínima en el BRM, a fin de alcanzar una concentración de biomasa adecuada para el funcionamiento de membrana, especialmente cuando se opere a altas temperaturas. En este sentido, una posible alternativa sería la modificación del sistema propuesto con el fin de permitir la alimentación de una pequeña fracción del influente directamente en la etapa aeróbica.

3. Viabilidad de la desnitrificación con metano en un BRM después de un pre-tratamiento metanogénico.

La combinación propuesta consistente en un BRM como post-tratamiento de un reactor metanogénico hizo factible la eliminación de nitrógeno. El uso de una cámara anóxica previa en el MBR, con biomasa creciendo tanto en suspensión como en forma de biopelícula, promovió el uso del metano disuelto presente en el efluente del reactor metanogénico como fuente de carbono para la desnitrificación. Esta desnitrificación pareció ser llevada a cabo por un consorcio de bacterias aerobias y anaerobias oxidantes de metano y bacterias heterotróficas que utilizaron los productos de oxidación como fuente de carbono para la desnitrificación. Otros procesos como la desnitrificación heterótrofa convencional y la oxidación anaerobia de amonio también contribuyeron a la de eliminación de nitrógeno global. La recirculación interna entre las cámaras aeróbica y anóxica del BRM fue un parámetro clave ya que la entrada de oxígeno en la cámara anóxica, asociada a altas tasas de recirculación, pareció inhibir la oxidación anaerobia de metano, disminuyendo la tasa de oxidación de metano. Además, la disminución en la actividad desnitrificante observada cuando el metano disuelto se eliminó del efluente UASB, condujo a un aumento notable de la concentración de sustancias biopoliméricas que influyó negativamente en el rendimiento de membrana.

El potencial uso de la tecnología BRM como un post-tratamiento de los reactores anaeróbicos de tratamiento de aguas residuales de baja carga podría ser especialmente interesante en países (semi)tropicales, donde el uso de la tecnología anaerobia para el

tratamiento de estas aguas es generalizado. El presente estudio demuestra que sería factible eliminar nitrógeno en todas las instalaciones ya construidas simplemente instalando un BRM dotado de una cámara anóxica previa y utilizando el metano disuelto presente en el efluente anaerobio como fuente de carbono para la desnitrificación. Podrían lograrse eliminaciones teóricas de nitrógeno de hasta 32 $mg \cdot L^{-1}$ para aguas residuales urbanas tratada anaeróbicamente a temperatura ambiente, sin tener en cuenta la presencia de materia orgánica biodegradable remanente en estos efluentes. Por lo tanto, futuras investigaciónes en este campo deberán ser desarrolladas con el fin de optimizar el proceso y alcanzar valores de eliminación de nitrógeno próximos al máximo teórico.

4. Biorreactor anaerobio de membrana

Un biorreactor de membrana sumergido anaeróbico fue operado a alta concentración de biomasa para el tratamiento de las aguas residuales industriales procedentes de la producción de extractos herbales. El control de la alcalinidad aumentó la eficiencia de eliminación de materia orgánica, lo que permitió el funcionamiento a altas velocidades de carga orgánica. La operación a altas concentraciónes biomasa no mejoró el tratamiento biológico y por el contrario, el rendimiento de la membrana resultó seriamente afectado como consecuencia de la formación de una densa torta que obstruyó la membrana. Este fenómeno contituyó el principal mecanismo de ensuciamiento de la membrana. Todos los parámetros de ensuciamiento estudios tales como la resistencia a la filtración, y las concentraciones de biopolímeros coloidales (BPC) y partículas exopoliméricas transparentes (TEP), fueron muy elevados, y la adición de carbón activado en polvo en el reactor no mostró ningún efecto beneficioso sobre los mismos. Por lo tanto, se recomienda la operación de estos sistemas a una concentración de biomasa inferior (por debajo de 20 $g \cdot L^{-1}$) para el tratamiento de este tipo de aguas residuales. Sin embargo, a partir de los resultados obtenidos, se puede concluir que la combinación de un reactor UASB con un BRM aerobio sería más apropiada con el fin de mejorar el flujo de membrana y quizás también mejorar la eficiencia de eliminación de materia orgánica.

5. Indicadores de ensuciamiento

A lo largo del presente estudio, la medición de diferentes indicadores de ensuciamiento, tales como la fracción de carbohidratos de los productos microbianos solubles, partículas transparentes exopoliméricas o sustancias biopoliméricas coloidales, se midieron con el fin de establecer una relación con ensuciamiento de la membrana. Entre ellos, la determinación de los BPC coloidales mostró una mejor correlación con el

ensuciamiento de la membrana, y su empleo es especialmente recomendado debido a su simplicidad y fiabilidad. Sin embargo, la aplicabilidad de este parámetro como un indicador de ensuciamiento en BRM anaerobios para el tratamiento de aguas residuales industriales no resulta tan fiable como en el caso de los BRM aeróbicos, como consecuencia de sus elevados valores.

6. Aplicabilidad y perspectivas futuras

La tecnología de filtración de membranas sumergidas confiere robustez a los sistemas biológicos estudiados en este trabajo, mejorando su rendimiento y produciendo un efluente de alta calidad, libre de sólidos en suspensión. Por lo tanto, dependiendo de los estándares de calidad, el uso de membranas sumergidas es especialmente recomiendo para una amplia gama de aplicaciones de reutilización como la agricultura, los sistemas de refrigeración o limpieza. Además, la posibilidad de la eliminación de nitrógeno de los efluentes de digestores anaerobios ya construidos en un BRM dotado de una cámara anóxica anterior, confirma el uso de la tecnología de membranas como una elección interesante de cara a futuras aplicaciones e investigaciones en el campo de los tratamientos de aguas residuales biológicas.

Conclusións

As principais conclusións desta investigación, que se centra na combinación da tecnoloxía de membranas somerxidas con tratamentos biolóxicos aeróbicos e anaeróbicos, preséntanse a continuación.

1. Membranas somerxidas en sistemas terciarios de filtración con membranas

Dos resultados obtidos, pódese concluír que a tecnoloxía de membranas mergulladas é unha boa elección, para a obtención dun efluente cunha alta calidade e libre de sólidos en suspensión, despois dun tratamento biolóxico en reactores secuanciais discontinuos con biomasa granular e floculenta. A operación das unidades de filtración terciaria cunha concentración de biomasa maior que a normalmente recomendada para este tipo de sistemas fixo que se comportasen como biorreactores secundarios, eliminando parte da DQO e nitrificando o amonio procedente dos reactores secuenciais discontinuos. O efecto negativo desta operación foron os menores fluxos de permeado obtidos. Finalmente, o estado de agregación da biomasa non tivo ningunha influencia no funcionamento dos sistemas de filtración terciarios, e outros parámetros, tales como a nitrificación ou a presenza de sólidos suspendidos na auga residual bruta mostraron un impacto máis relevante no funcionamento da membrana.

2. Combinación de biorreactor de membrana (BRM) con reactor UASB

A combinación dun BRM aeróbico cun reactor UASB anaeróbico, nun único sistema integrado ou como post-tratamento, demostrou ser unha boa solución para o tratamento de augas residuais de baixa carga a temperatura ambiente, producindo un efluente de alta calidade, biogás rico en metano e diminuíndo a produción de lamas. Ademais, o sistema combinado mostrou flexibilidade para converter o nitróxeno total a amoníaco e / ou nitrato, o que resulta especialmente interesante para o seu emprego na reutilización da auga tratada, dependendo da aplicación e os estándares de calidade. A hidrólise da biomasa en suspensión recirculada dende o BRM ao reactor UASB provocou a liberación de substancias biopoliméricas, empeorando o rendemento da membrana. Polo tanto, sería interesante desenvolver estratexias co fin de eliminar a materia coloidal resultante da dixestión anaerobia de substratos complexos. Neste sentido, o uso dunha etapa con organismos superiores tales como protozoos podería ser unha alternativa interesante. A

presenza de soporte de plástico durante este estudo promoveu a presenza de protozoos e influenciou a concentración de substancias biopolímericas coloidais. Por outra parte, a concentración de biomasa resultou ser un parámetro importante para a protección da membrana contra o ensuzamento provocado por biopolímeros solubles e coloidais. Polo tanto, para un óptimo rendemento da membrana despois dun pre-tratamento metanoxénico de substratos complexos, debe garantirse unha relación alimento/microorganismos mínima no BRM, co fin de acadar unha concentración de biomasa axeitada para o funcionamento de membrana, especialmente cando se opere a altas temperaturas. Neste sentido, unha posible alternativa sería a modificación do sistema proposto co fin de permitir a alimentación dunha pequena fracción do influente directamente na etapa aeróbica.

3. Viabilidade da desnitrificación con metano nun BRM despois dun pre-tratamento metanoxénico.

A combinación proposta consistente nun BRM como post-tratamento dun reactor metanoxénico fixo factible a eliminación de nitróxeno. O uso dunha cámara anóxica previa no MBR, con biomasa crecendo tanto en suspensión como en forma de biopelícula, promoveu o uso do metano disolto presente no efluente do reactor metanoxénico como fonte de carbono para a desnitrificación. Esta desnitrificación pareceu ser levada a cabo por un consorcio de bacterias aerobias e anaerobias oxidantes de metano e bacterias heterotróficas que utilizaron os produtos de oxidación como fonte de carbono para a desnitrificación. Outros procesos como a desnitrificación heterótrofa convencional e a oxidación anaerobia de amonio tamén contribuíron á eliminación global de nitróxeno. A recirculación interna entre as cámaras aeróbica e anóxica do BRM foi un parámetro clave xa que a entrada de osíxeno na cámara anóxica, asociada a altas taxas de recirculación, pareceu inhibir a oxidación anaerobia de metano, diminuíndo a taxa de oxidación de metano. Ademais, a diminución na actividade desnitrificante observada cando o metano disolto se eliminou do efluente UASB, conduciu a un aumento notable da concentración de substancias biopoliméricas que influíu negativamente no rendemento de membrana.

O potencial uso da tecnoloxía BRM como un post-tratamento dos reactores anaeróbicos de tratamento de augas residuais de baixa carga podería ser especialmente interesante en países (semi)tropicales, onde o uso da tecnoloxía anaerobia para o tratamento destas augas é xeneralizado. O presente estudo demostra que sería factible eliminar nitróxeno en todas as instalacións xa construídas simplemente instalando un BRM dotado dunha cámara anóxica previa e utilizando o metano disolto presente no efluente anaerobio como fonte de carbono para a desnitrificación. Poderían lograrse

eliminacións teóricas de nitróxeno de ata 32 $mg \cdot L^{-1}$ para augas residuais urbanas tratadas anaeróbicamente a temperatura ambiente, sen ter en conta a presenza de materia orgánica biodegradable remanente nestes efluentes. Polo tanto, futuras investigacións neste eido deberán ser desenvolvidas co fin de optimizar o proceso e acadar valores de eliminación de nitróxeno próximos ao máximo teórico.

4. Biorreactor anaerobio de membrana

Un biorreactor de membrana mergullado anaeróbico foi operado a alta concentración de biomasa para o tratamento das augas residuais industriais procedentes da producción de extractos herbales. O control da alcalinidade aumentou a eficiencia de eliminación de materia orgánica, o que permitiu o funcionamento a altas velocidades de carga orgánica. A operación a altas concentracións de biomasa non mellorou o tratamento biolóxico e pola contra, o rendemento da membrana resultou seriamente afectado como consecuencia da formación dunha densa torta que obstruíu a membrana. Este fenómeno contituíu o principal mecanismo de ensuzamento da membrana. Tódolos parámetros de ensuzamento estudados tales como a resistencia á filtración, e as concentracións de biopolímeros coloidais (BPC) e partículas exopoliméricas transparentes (TEP), foron moi elevados, e a adición de carbón activado en po no reactor non mostrou ningún efecto beneficioso sobre estes. Polo tanto, recoméndase a operación destes sistemas a unha concentración de biomasa inferior (por debaixo de 20 $g \cdot L^{-1}$) para o tratamento deste tipo de augas residuais. Non obstante, a partires dos resultados obtidos, pódese concluír que a combinación dun reactor UASB cun BRM aerobio sería máis axeitada co fin de mellorar o fluxo de membrana e quizáis tamén mellorar a eficiencia de eliminación de materia orgánica.

5. Indicadores de ensuzamento

Ao longo do presente estudo, a medición de diferentes indicadores de ensuzamento, tales como a fracción de carbohidratos dos produtos microbianos solubles, partículas transparentes exopoliméricas ou substancias biopoliméricas coloidais foron medidas co fin de establecer unha relación con ensuzamento da membrana. Entre eles, a determinación dos BPC coloidais mostrou unha mellor correlación co ensuzamento da membrana, o seu emprego é especialmente recomendado debido á súa simplicidade e fiabilidade. Non obstante, a aplicabilidade deste parámetro como un indicador de ensuzamento en BRM anaerobios para o tratamento de augas residuais industriais non resulta tan fiable como no caso dos BRM aeróbicos, como consecuencia dos seus elevados valores.

6. Aplicabilidade e perspectivas futuras

A tecnoloxía de filtración de membranas mergulladas confire robustez aos sistemas biolóxicos estudados neste traballo, mellorando o seu rendemento e producindo un efluente de alta calidade, libre de sólidos en suspensión. Polo tanto, dependendo dos estándares de calidade, o uso de membranas mergulladas é especialmente recomendado para unha ampla gama de aplicacións de reutilización como a agricultura, os sistemas de refrixeración ou limpeza. Ademais, a posibilidade da eliminación de nitróxeno dos efluentes de dixestores anaerobios xa construídos nun BRM dotado dunha cámara anóxica anterior, confirma o uso da tecnoloxía de membranas como unha elección interesante de cara a futuras aplicacións e investigacións no campo dos tratamentos de augas residuais biolóxicas

Conclusions

The main conclusions of this research, which is focused on the combination of submerged membrane technology with anaerobic and aerobic biological treatments, are now presented.

1. Submerged membranes in tertiary membrane filtration systems

From the results obtained it can be conclude that submerged membrane technology is a good choice in order to obtain an effluent with a high quality and free of suspended solids after a biological treatment in sequencing batch reactors with granular and flocculent biomass. The operation of the tertiary filtration units with a higher biomass concentration that that typically recommended for this kind of systems made them to behave as secondary bioreactors, eliminating part of the COD and nitrifying the ammonium proceeding from the reactors. The negative effect was the lower membrane fluxes achieved. Finally, the aggregation state of the biomass did not make any difference in the operation of the tertiary filtration systems and other parameters such as nitrification or the presence of suspended solids in the raw wastewater showed a more relevant impact in membrane performance.

2. Membrane bioreactor combined with UASB reactor

The combination of an aerobic MBR with an anaerobic UASB reactor, into one single integrated system or as post-treatment, was shown to be a good solution for the treatment of low-strength wastewaters at ambient temperature, producing a high quality effluent, producing biogas rich with methane and diminishing sludge production yield. Moreover, the combined system showed flexibility to convert total nitrogen to ammonia and/or nitrate, with is really interesting for its use in water reuse depending on the application and quality standards. The hydrolysis of suspended biomass recirculated from the MBR to the UASB provoked the release of biopolymeric substances that worsened membrane performance. Therefore, it would be interesting to develop strategies in order to remove the colloidal matter resulting from the anaerobic digestion of complex substrates. In this sense, the use of a stage with superior organisms such as protozoa could be an interesting alternative. The presence of plastic support during this study promoted the presence of protozoa and influenced colloidal biopolymer concentration.

Moreover, biomass concentration was an important parameter in order to protect the membrane against the fouling provoked by soluble and colloidal biopolymers. Therefore, for a better membrane performance after a methanogenic pre-treatment of complex substrates, a minimum F/M ratio in the MBR should be assured in order to reach a suitable biomass concentration for membrane operation, especially when operating at higher temperatures. In this sense, a possible alternative would be the modification of the proposed system in order to allow the feeding of a small fraction of the raw influent directly into the aerobic stage

3. Feasibility of methane denitrification in an MBR after a methanogenic pre-treatment.

The proposed combination of MBR technology as a post-treatment of a methanogenic reactor made feasible the removal of nitrogen. The use of a previous anoxic chamber, with biomass growing both in suspension and biofilm, in the MBR, promoted the use of the dissolved methane present in the effluent of the methanogenic reactor as a carbon source for denitrification. Denitrification was carried out by a consortium of aerobic and anaerobic methane oxidizing bacteria and heterotrophic bacteria that used the oxidation products as carbon source for denitrification. Other processes such as conventional heterothrophic denitrification or anaerobic ammonia oxidation (anammox) also contributed to the global elimination of nitrogen. The internal recirculation between the aerobic and anoxic chamber of the MBR was showed to be a key parameter since the input of oxygen in the anoxic chamber seemed to inhibit the anaerobic oxidation pathway at high recirculation rates, decreasing methane oxidation rate. Moreover the diminution on denitrification activity observed when dissolved methane was removed from the UASB effluent, led to a remarkable increase on biopolymer concentration that influenced negatively membrane performance.

The potential application of MBR technology as a post-treatment of anaerobic reactors treating low-strength wastewaters could be especially interesting in (semi)tropical countries, where the use of anaerobic technology for these applications is generalized. The present study demonstrate that it would be feasible to remove nitrogen in all the facilities already constructed installing an MBR with a previous anoxic chamber and using the dissolved methane present in the effluent as carbon source for denitrification. Theoretical nitrogen removals up to 32 $mg \cdot L^{-1}$ could be achieved from domestic wastewater treated anaerobically at ambient temperature, neglecting the presence of remaining biodegradable COD in these effluents. Therefore, further research in this field

should be developed in order to optimize the process and approach to the theoretical maximum nitrogen removal.

4. Anaerobic membrane bioreactor

A submerged anaerobic membrane bioreactor (AnMBR) was operated at high biomass concentration for the treatment of industrial herbal extraction wastewater. Alkalinity control enhanced organic matter removal efficiency, allowing the operation at higher organic loading rates (OLR). The operation at high mixed liquor total solids (MLTS) concentration did not improve biological treatment and seriously affected membrane performance, forming of a dense cake layer that clogged the membrane. This phenomenon was the main fouling mechanism. All the fouling parameters studies such as SRF, BPC and TEP concentrations were extremely high, and powdered activated carbon (PAC) addition in the reactor did not exhibit beneficial effects on them. Therefore, it would be recommended to operate at lower biomass concentration (below 20 $g \cdot L^{-1}$) for the treatment of this wastewater in an AnMBR. Nevertheless, from the results obtained it can be concluded that the combination of an UASB reactor with an aerobic MBR would be more appropriate in order to enhance membrane flux and perhaps improve organic matter removal efficiency.

5. Fouling indicators

Along the present research, the measurement of different fouling indicators such as carbohydrate fraction of soluble microbial products, transparent exopolymer particles or colloidal biopolymer cluster were measured in order to establish a relationship with membrane fouling. Among them, the determination of colloidal biopolymer clusters showed a better correlation with membrane fouling, and was especially recommended due to its simplicity and reliability. Nevertheless, the applicability this parameter as a fouling indicator in AnMBR treating industrial wastewater was not as reliable as in aerobic MBRs due to its high values.

6. Applicability and future perspectives

Submerged membrane filtration technology conferred robustness to the biological systems studied in this work, enhancing their performances and producing a high quality effluent, free of suspended solids. Therefore, depending on quality standards, the use of submerged membranes would be especially recommended for a wide range of reuse applications such as agriculture, cooling systems or for cleaning purposes. Moreover, the possibility of nitrogen removal from the effluents of anaerobic digesters already constructed in an MBR with a previous anoxic chamber, confirms the use of membrane

technology and interesting choice for future investigations and applications in the field of biological wastewater treatments.

List of symbols

1. Acronyms

AnMBR	Anaerobic Membrane Bioreactor	
ANME	Anaerobic Methanogenic bacteria	
BPC	Biopolymer Clusters	$mg \cdot L^{-1}$
BF-MBR	Biofilm Membrane Bioreactor	
CAS	Conventional Activated Sludge	
cBPC	Colloidal fraction of Biopolymer Clusters	$mg \cdot L^{-1}$
CEB	Chemical Enhanced Backwashing	
CIP	Clean-In-Place	
COD	Chemical Oxygen Demand	$mg \cdot L^{-1}$
CSTR	Continuous Stirred Tank Reactor	
DIC	Dissolved Inorganic Carbon	$mg \cdot L^{-1}$
DIN	Dissolved Inorganic Nitrogen	$mg \cdot L^{-1}$
DOC	Dissolved Organic Carbon	$mg \cdot L^{-1}$
DON	Dissolved Organic Nitrogen	$mg \cdot L^{-1}$
DTN	Dissolved Total Nitrogen	$mg \cdot L^{-1}$
ED	Electrodiálisis	
EPS	Extracellular Polymeric Substances	
FS	Flat Sheet	
F-SBR	Flocculent Sequencing Batch Reactor	
GAC	Granular Activated Carbon	
GHG	Greenhouse Gas	
G-SBR	Granular Sequencing Batch Reactor	

HF	Hollow Fiber	
HRT	Hidraulic Retention Time	
HyVAB	Hybrid Vertical Anaerobic Sludge–Aerated Biofilm Reactor	
IA	Intermediate Alkalinity	$mgCaCO_3 \cdot L^{-1}$
MBR	Membrane Bioreactor	
MBBR	Moving Bed Biofilm Reactor	
MBMBR	Moving Bed Membrane Bioreactor	
MF	Microfiltration	
MFE	Membrane Flux Enhancer	
MLTSS	Mixed Liquor Total Suspended Solids	$g \cdot L^{-1}$
MLVSS	Mixed Liquor Volatile Suspended Solids	$g \cdot L^{-1}$
MT	Multi-Tubular	
NF	Nanofiltración	
OA	Organic Acids	$g \cdot L^{-1}$
OLR	Organic Loading Rate	$kgCOD \cdot m^{-3} \cdot d^{-1}$
ORR	Organic Removal Rate	$kgCOD \cdot m^{-3} \cdot d^{-1}$
PA	Partial Alkalinity	$mgCaCO_3 \cdot L^{-1}$
PAC	Powdered Activated Carbon	
PE	Polyethylene	
PES	Polyethylsulfone	
PLC	Programmable Logic Controller	
PP	Polypropylene	
PVDF	Polyvinylidene difluoride	
RO	Reverse Osmosis	
SAD	Specific Air Demand	$Nm^3 \cdot m^{-2} \cdot h^{-1}$
SGD	Specific Gas Demand	$Nm^3 \cdot m^{-2} \cdot h^{-1}$

SBR	Sequencing Batch Reactor	
SMP	Soluble Microbial Products	
SMPc	Carbohydrate fraction of SMP	$mg \cdot L^{-1}$
SMPp	Protein fraction of SMP	$mg \cdot L^{-1}$
SRF	Sludge Resistance to Filtration	$m \cdot kg^{-1}$
SRT	Sludge Retention Time	d
SSR	Sludge Settling Rate	$m \cdot h^{-1}$
SVI	Sludge Volume Index	$mL \cdot g^{-1}$
TA	Total Alkalinity	$mgCaCO_3 \cdot L^{-1}$
TEP	Transparent Exopolymer Particles	$mgXG \cdot L^{-1}$
TDC	Total Dissolved Carbon	$mg \cdot L^{-1}$
TMF	Tertiary Membrane Filtration	
TMP	Transmembrane Pressure	kPa
TN	Total Nitrogen	$mg \cdot L^{-1}$
TOC	Total Organic Carbon	$mg \cdot L^{-1}$
TSS	Total Suspended Solids	$g \cdot L^{-1}$
UASB	Upload Anaerobic Sludge Blanket	
UF	Ultra filtration	
VFA	Volatile Fatty Acids	$mg \cdot L^{-1}$
WWTP	Waste Water Treatment Plant	
XG	Xanthan Gum	

2. Symbols

D	Diffusive coefficients	$cm^2 \cdot s^{-1}$
F/ M	Food to Microorganism ratio	$kgCOD \cdot kgMLVSS^{-1} \cdot d^{-1}$

FR/J	Normalized Fouling Rate/Permeability ratio	$kPa \cdot m^{-1}$
k_La	Volumetric mass transfer coefficient	d^{-1}
R_c	Cake resistance to filtration	m^{-1}
R_{col}	Colloidal resistance to filtration	m^{-1}
R_m	Membrane resistance to filtration	m^{-1}
R_{pb}	Pore blocking resistance to filtration	m^{-1}
R_t	Total resistance to filtration	m^{-1}
r	Volumetric reaction rate	$mg \cdot L^{-1} \cdot d^{-1}$
m	mass flow	$mg \cdot d^{-1}$

List of publications

1. Pending patents

Alberto Sánchez Sánchez, Dagmara Buntner, Juan Manuel Lema Rodicio and Juan Manuel Garrido Fernández. 2013. Sistema Integrado de reactor anaerobio metanogénico y biorreactor de membranas para la eliminación de materia orgánica y nitrógeno en aguas residuales. Application: P201330118

2. Journal publications

2.1. International Journal Publications

Sánchez, A., Garrido, J.M., Méndez, R. 2010. A comparative study of tertiary membrane filtration of industrial wastewater treated in a granular and a flocculent sludge SBR. Desalination 250, 810-814.

Buntner, D., **Sánchez, A.,** Garrido, J.M. 2011. Three stages MBR (methanogenic, aerobic biofilm and membrane filtration) for the treatment of low-strength wastewaters. *Water Science and Technology* 64(2), 397-402.

Sánchez, A., Garrido, J.M.., Méndez, R. 2011. Tertiary membrane filtration of an industrial wastewater using granular or flocculent biomass sequencing batch reactors. Journal of Membrane Science 382, 316-322.

Sánchez, A., Buntner D., Garrido, J. M. 2013. Impact of methanogenic pre-treatment on the performance of an aerobic MBR system. *Water Research* 47(3), 1229–1236.

2.2. Articles in preparation

Brand, C., **Sánchez, A.,** Kraume, M. 2013. Membrane fouling in an AnMBR treating industrial wastewater at high total solids concentration. In preparation.

Brand, C., **Sánchez, A.**, Chlaida, M., Kraume, M. 2013. Acidification in an Anaerobic Membrane Bioreactor treating Herbal Extraction Wastewater. In preparation

Buntner, D., **Sánchez A.,** Garrido, J. M. 2013. Feasibility of combined UASB and MBR system in dairy wastewater treatment at ambient temperatures. Submitted to *Journal of Chemical Engineering*.

Crutchik, D., **Sánchez, A.,** Garrido, J. M. 2013. Simulation of multiple phosphate precipitates in a saline industrial wastewater. Submitted to *Desalination.*

Iglesias, A., **Sánchez, A.**, Fernández, R., Solis, D, Garrido, J.M. 2013. Operation of a Flat Sheet MBR with primary wastewater. Submitted to *Separation and Purification technology.*

Sánchez, A., Rodríguez-Hernández, L., Buntner, D., Esteban-García, A.L., Tejero, I., Garrido, J. M. 2013. Denitrification coupled with methane oxidation in a membrane bioreactor after a methanogenic pre-treatment. Submitted to *Water Research*.

3. Book Chapters

Sánchez, A., Barros, S., Méndez, R., Garrido, J.M. 2009. Phosphorous removal from an industrial wastewater by struvite crystallization into an airlift reactor. International conference on nutrient recovery from wastewater streams. ISBN: 1843392321. IWA publishing, 89 – 99.

Sánchez, A., Garrido, J.M. 2009. Capítulo X: Biorreactores de membrana. *Tecnologías Avanzadas para el Tratamiento de Aguas Residuales* ISBN: 978-84-692-5028-0

Buntner, D., **Sánchez, A.,** Garrido, J.M., Comas, J., Rodríguez-Roda, I. 2010. Capítulo VI: Hybrid and other membrane bioreactor Technologies. *Innovative Technologies for Urban Wastewater*. ISBN: 13-978-84-693-3992-3

4. Contribution to congress

Sánchez, A., Barros, S., Méndez, R., Garrido, J.M. Phosphorus removal from an industrial wastewater by struvite crystallization into an airlift reactor. 10th International Chemical and Biological Engineering Conference. Braga (Portugal), 2008.

Sánchez, A., Garrido, J.M., Méndez, R. A comparative study of wastewater treatment from a fish canning factory, in a granular and a flocculent sludge SBR. 3rd International

meeting on Environmental Biotechnology and Bioengineering (3IMEBE). Palma de Mallorca (Spain), 2008

Sánchez, A., Garrido, J.M., Méndez, R. A Comparative study of tertiary membrane filtration of industrial wastewater treated in a granule and a flocculent sludge SBR. Conference on Membranes in Drinking Water Production and Wastewater Treatment. Toulouse (France), 2008

Sánchez, A., Barros, S., Méndez, R., Garrido, J.M. Phosphorous removal from an industrial wastewater by struvite crystallization into an airlift reactor. IWA International conference on nutrient recovery from wastewater streams. Vancouver (Canada), 2009.

Sánchez, A., Garrido, J.M., Méndez, R. Tertiary membrane filtration of a wastewater treated in a granular and a flocculent sludge SBR. 8th Conference Water and Membranes. Aachen (Germany), 2009.

Buntner, D., **Sánchez, A.**, Iglesias, A., Garrido, J.M. Three stages membrane biological reactor for industrial and urban wastewaters treatment – towards wastewater reuse. 7th ANQUE International Congress. Oviedo (Spain), 2010.

Buntner, D., **Sánchez, A.**, Garrido, J.M. Three stages MBR (methanogenic, aerobic biofilm and membrane filtration) for the treatment of municipal wastewaters. 8th Conference Water and Membranes.Trondheim (Norwey), 2010

Buntner, D., **Sánchez, A.**, Garrido, J.M. Membrane Fouling in a Hybrid Three Stages Anaerobic/Aerobic MBR. 6th IWA Specialist Conference on Membrane Technology for Water and Wastewater Treatment. Aachen (Germany), 2011

Iglesias, A., **Sánchez, A.**, Fernández, R., Solis, D, Garrido, J.M. 2013. Operation of a flat sheet MBR: Could be the BOD of permeate an indicator of cake layer formation? 6th IWA Specialist Conference on Membrane Technology for Water and Wastewater Treatment. Aachen (Germany), 2011.

Sánchez, A., Buntner, D., Garrido, J.M. Membrane Fouling in a Hybrid Three Stages Anaerobic/Aerobic MBR. IWA Specialist Conference on Ecotechnologes for Wastewater Treatment. (EcoSTP). Santiago de Compostela (Spain), 2012

Buntner, D., **Sánchez, A.**, Pinto, V., Garrido, J.M. Effect of methanogenic stage on membrane fouling in an aerobic membrane filtration chamber. IWA Specialist

Conference on Ecotechnologes for Wastewater Treatment. (EcoSTP). Santiago de Compostela (Spain), 2012.

Brand, C., **Sánchez, A.**, Chlaida, M., Kraume, M. Industrial herbal extraction wastewater treatment using an anaerobic membrane bioreactor . IWA Specialist Conference on Ecotechnologes for Wastewater Treatment. (EcoSTP). Santiago de Compostela (Spain), 2012.

Sánchez, A., Rodríguez-Hernández, L., Buntner, D., Garrido, J.M. Denitrification of wastewater in an MBR system using dissolved methane from a methanogenic pre-treatment. Anaerobic Digestion 2013 conference. (AD13). Santiago de Compostela (Spain), 2013.