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DPTO. DE CIENCIAS Y TÉCNICAS DEL AGUA Y DEL MEDIO AMBIENTE
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UNIVERSIDAD DE CANTABRIA

TESIS DOCTORAL

REACTORES HÍBRIDOS CON MEMBRANAS PARA EL TRATAMIENTO DE AGUAS RESIDUALES URBANAS: APLICACIONES PARA LA ELIMINACIÓN DE CARBONO Y NITRÓGENO

HYBRID MEMBRANE BIOREACTORS FOR URBAN WASTEWATER TREATMENT: APPLICATIONS FOR CARBON AND NITROGEN REMOVAL

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Resumen

Los procesos convencionales de fangos activos y de biopelícula se han aplicado extensamente y han demostrado su eficacia para la eliminación de carbono y nutrientes de las aguas residuales. No obstante, hoy en día se requieren nuevas tecnologías capaces de adaptarse a normativas cada vez más estrictas y a la creciente escasez de recursos de agua dulce.

Los reactores biológicos con membranas (RBM), en los cuales el proceso de fangos activos se integra con la separación sólido/líquido a través de membranas de filtración, es una de las nuevas tecnologías capaces de satisfacer estas necesidades. El RBM híbrido es el resultado de la combinación de un RBM con un reactor biopelícula, donde la biodegradación se lleva a cabo por ambas biomasas, en suspensión y en biopelícula. La adición de un medio soporte al RBM, se ha propuesto principalmente con el objetivo de superar algunas de las limitaciones de los RBM, como la eliminación de nutrientes y el ensuciamiento de membranas.

Teniendo en cuenta este contexto, en la presente tesis doctoral se investiga la aplicación de sistemas híbridos RBM para el tratamiento de las aguas residuales. Este trabajo se basa en la experimentación de diferentes configuraciones a escala de bancada y piloto, especialmente construidas, desarrolladas y modificadas con el objetivo de ampliar el conocimiento sobre el efecto de los soportes biopelícula en estos sistemas.

Como punto de partida, en el **capítulo 1** se incluye una revisión actualizada de la literatura en relación a los procesos biopelícula, la tecnología RBM y su combinación (RBM híbridos).

En el **capítulo 2** se describen los materiales y métodos utilizados a lo largo de las diferentes experimentaciones.

En la primera configuración propuesta (**capítulo 3**) se desarrolló a escala de bancada un nuevo RBM híbrido de configuración vertical, muy compacta y con requisitos optimizados de aireación. El reactor estuvo situado en la planta de tratamiento de aguas residuales municipales de Santander (España). Se alimentó con agua residual pre-tratada para realizar una evaluación previa de su funcionamiento como sistema alternativo a otros RBM convencionales. En este trabajo se empleó un medio soporte fijo tipo esponja ubicado por encima de la unidad de filtración, no observándose signos de sobrecarga durante toda la

experimentación. El caudal de aireación, localizado debajo de la unidad de filtración, fue suficiente para la limpieza de la membrana, la oxigenación de ambas biomasas (biopelícula y en suspensión) y la mezcla del líquido en el reactor. A pesar de la gran variabilidad de la carga aplicada (valores medios de entre 1,1 y 2,7 kg DQO m^{-3} d^{-1}) se lograron altas eficiencias de eliminación de materia orgánica, con valores superiores al 90% en DQO y 96% en DBO_5. Además, el nuevo RBM híbrido mostró un buen rendimiento en la eliminación de nitrógeno total (NT) a través de la nitrificación y desnitrificación simultánea, a pesar de aplicarle una aireación continua. En el afluente se alcanzó una concentración media de 7,3 mg NT L^{-1}. Esta configuración fue capaz de obtener un afluente con calidad suficiente para reutilización en términos de contaminación bacteriológica, nitrógeno, materia orgánica, sólidos suspendidos y turbidez. Además, la recirculación pareció jugar un papel importante en el comportamiento del RBM híbrido. Con una recirculación del 300% se mejoró ligeramente la eficiencia de eliminación de carbono orgánico (99% vs. 96% in DBO_5) y notablemente la eliminación de nitrógeno (98% vs. 91% en amonio; 80% vs. 69% en NT).

Estos resultados son mejores que los obtenidos en otros RBM híbridos y similares a los valores alcanzados usando RBM más complejos, con tanques anóxicos adicionales, aireación intermitente o deflectores internos.

Una vez probado el nuevo RBM híbrido a escala de bancada, se construyó, arrancó y evaluó un RBM híbrido vertical original a escala piloto, para estudiar su viabilidad como tratamiento descentralizado (**capítulo 4**). Situado en la planta de tratamiento de aguas residuales municipales de Santander, la planta piloto demostrativa disponía de una unidad de pre-tratamiento (tamizado fino y desarenador) previo al reactor y se alimentó con agua residual bruta no decantada. El RBM híbrido consistió en un tanque de aireación de acero inoxidable con membranas de microfiltración sumergidas y un medio soporte fijo para el crecimiento de la biopelícula. A diferencia del capítulo 3, el medio soporte fijo empleado (llamado BLAS) fue de fabricación propia basado en un diseño específico desarrollado previamente por el Grupo de Ingeniería Ambiental de la Universidad de Cantabria.

La aplicación del RBM híbrido a escala piloto demostró ser técnicamente viable ya que fue capaz de tratar las aguas residuales municipales sin necesidad de decantación primaria, lo cual le confiere la alta compacidad que requieren los tratamientos descentralizados. Durante la experimentación, la carga orgánica aplicada osciló entre 0,36 y 1,76 kg DQO m^{-3} d^{-1}. Sin embargo, el sistema mantuvo buenos rendimientos generales. Las eliminaciones alcanzadas de materia orgánica en términos de DQO y DBO_5 fueron del 84% y 98%, respectivamente.

La eliminación de nitrógeno amoniacal fue del 97% y la de nitrógeno total del 75% (utilizando un único reactor), lo cual se atribuyó a la ocurrencia de nitrificación y desnitrificación simultáneas (SND). Las características medias del efluente obtenido fueron DQO < 55 mg L^{-1}, SS < 4 mg L^{-1}, NT < 10 mg L^{-1} y turbidez < 2 NTU, cumpliendo con los estándares para descarga en zonas sensibles y para la reutilización.

En el **capítulo 5**, el objetivo fue comparar el comportamiento de la configuración RBM híbrida estudiado en el capítulo 4, con un RBM convencional. Para este propósito, se añadió otro reactor idéntico en diseño al RBM híbrido pero sin la adición del lecho fijo. Ambas plantas piloto fueron caracterizadas y funcionaron en paralelo. Se llevó a cabo un análisis estadístico para verificar si existían diferencias significativas entre los resultados obtenidos en ambos sistemas. Un estudio de trazadores mostró para ambos reactores un comportamiento hidrodinámico similar, con mezcla completa. En cuanto al coeficiente de transferencia de oxígeno, K_La (a 20 °C), se observó una mejora en el RBM hibrido con respecto al RBM convencional (33,9 h^{-1} vs. 18,3 h^{-1}), lo que se atribuyó al mayor tiempo de retención de las burbujas dentro del reactor de lecho fijo.

Ambos RBM mostraron buena eficiencia de eliminación en materia orgánica y amonio, aunque la calidad del efluente fue mejor en el RBM híbrido. Las eficiencias de eliminación de DQO, DBO_5 y N-NH_4^+ en el RBM híbrido fueron del 84, 98 y 97%, respectivamente, en comparación con 80, 96 y 93% en el RBM convencional. En el caso de la eliminación de la materia orgánica, la ligera mejora se atribuyó a la mayor concentración o actividad de la biopelícula. La mejor nitrificación se debe principalmente a la mayor resistencia a las puntas contaminantes en los sistemas híbridos en comparación con los fangos activos. El RBM híbrido también obtuvo mucha mejor eliminación de NT en comparación con el RBM convencional (promedio de 75 vs. 38% en el RBM convencional), lo que se explicó por la existencia de nitrificación y desnitrificación simultánea (SND) en este reactor. Los rendimientos medios de eliminación de fosfatos fueron de 42% en el RBM híbrido y de 37% en el RBM convencional, sin encontrarse diferencias estadísticas significativas. La eliminación del fósforo por PAOs parece ser insignificante debido a la baja concentración afluente de fosfatos y a los largos tiempos de retención celular (TRC ≈ 47 – 80 d). En consecuencia, la eliminación de fósforo sería debida principalmente a la asimilación por los microorganismos. En cuanto a las características del fango, el examen microscópico de los mismos reveló que la comunidad microbiana en el RBM híbrido era más rica que en el RBM convencional. Además, el fango del RBM híbrido tenía mejor filtrabilidad en comparación con el RBM convencional

(promedio de $1{,}28 \cdot 10^{12}$ and $5{,}70 \cdot 10^{12}$ m kg^{-1}, respectivamente) y mejor decantación (IVF con valores medios de 52 y 174 mL g^{-1}, respectivamente).

El principal problema asociado a la aplicación de la tecnología de membranas para el tratamiento de aguas residuales es el ensuciamiento de la membrana. Por lo tanto, resultó de gran interés para este trabajo comparar la tasa de ensuciamiento y algunos indicadores del mismo entre ambas configuraciones RBM. Para ello, se emplearon como indicadores las concentraciones de sólidos en suspensión del licor mezcla (SSLM) y los biopolímeros coloidales (cBPC). Para el rango de operación probado en este trabajo (hasta 6 g L^{-1}), no se encontró correlación entre la concentración de SSLM y la tasa de ensuciamiento. Con respecto a las concentraciones de cBPC, éstas fueron más altas y más variables en el RBM convencional comparado con el RBM híbrido, lo que también correspondió con una tasa de ensuciamiento más fuerte y variable. Este resultado sugiere que existe una cierta relación entre la tasa de ensuciamiento y la concentración de cBPC. En este sentido, la menor concentración de cBPC en el fango del RBM híbrido, probablemente debido a su retención por la biopelícula, pudo ser parcialmente responsable de la diferencia en el ensuciamiento. Como conclusión, todas las mejoras observadas en el RBM híbrido fueron atribuidas al crecimiento híbrido que se consigue cuando la biopelícula y la biomasa suspendida crecen simultáneamente.

La presencia de metano disuelto, especialmente a bajas temperaturas, representa un importante problema ambiental en términos de emisiones de gases de efecto invernadero (GEI) en las aguas residuales tratadas con biorreactores metanogénicos. El metano tiene un potencial de calentamiento global de 25. Una alternativa para reducir las emisiones de gases de efecto invernadero y el contenido de nitrógeno de las aguas residuales tratadas es el uso del metano disuelto como una fuente de carbono para la desnitrificación biológica, pero su viabilidad no se ha estudiado todavía en detalle. En el **capítulo 6** de este trabajo, se propone un sistema RBM híbrido como post-tratamiento para biorreactores metanogénicos. El efluente de un reactor anaerobio de flujo ascendente (UASB) fue post-tratado en un RBM de dos compartimentos. El primer compartimento fue un reactor de lecho móvil anóxico (con soportes K3) cuyo propósito era utilizar el metano disuelto como fuente de carbono para la desnitrificación. El segundo compartimento fue un reactor aerobio de filtración con membranas. El sistema propuesto alcanzó hasta un 60% de consumo de metano y un 95% de eliminación de nitrógeno. El ratio de recirculación entre el compartimento aeróbico y el anóxico, y la concentración de metano disuelto, se mostraron como parámetros importantes que gobiernan este proceso. Los ratios de recirculación

más bajos estudiados (entre 0,5 y 1) mostraron la mayor eliminación de nitrógeno y las emisiones de metano más bajos. La eliminación de nitrógeno se redujo de 60% a 27% cuando se procedió a la desorción del metano disuelto del efluente del UASB. Así, el porcentaje de eliminación de nitrógeno procedente de la oxidación de metano pudo suponer hasta el 33% del total. Además, los ensayos en batch y los análisis de FISH indicaron la presencia de microorganismos capaces de desnitrificar usando metano disuelto como fuente de carbono, tanto en condiciones aeróbicas como anaeróbicas. Parece que la desnitrificación la llevan a cabo un consorcio de bacterias oxidantes de metano aerobias y anaerobias, anammox y bacterias heterotróficas.

También se estudió la influencia de la desnitrificación con metano en el rendimiento de la membrana. Se observaron las mayores concentraciones de cBPC y las permeabilidades más bajas cuando disminuyó la actividad de desnitrificación.

El RBM híbrido propuesto parece ser una tecnología adecuada para el post-tratamiento de los reactores UASB. La presencia de biopelícula favoreció el desarrollo de una amplia variedad de poblaciones de microorganismos, lo que podría ser ventajoso para el crecimiento de aquellos implicados en el proceso de desnitrificación. Además, el uso de membranas permite una retención completa de bacterias de crecimiento lento que participan en la eliminación de nitrógeno y metano.

En conclusión, a partir del trabajo realizado en esta tesis se obtuvo información, a escala de bancada y piloto, importante para el funcionamiento de los reactores biológicos híbridos con membranas. Estos reactores han demostrado ser una tecnología atractiva para la eliminación de carbono y nitrógeno de las aguas residuales urbanas.

Summary

Conventional activated sludge and biofilm processes have been widely applied and they have established their efficiency for carbon and nutrient removal from wastewaters. However, new technologies capable of adapting to more strict normative and increasing scarcity of fresh water resources are required nowadays. In some cases the effluent of these technologies must reach an excellent quality suitable for the reuse of the wastewater.

The membrane bioreactor (MBR), in which activated sludge process is integrated with solid/liquid separation through membrane filtration, is one of the new technologies capable of meeting these needs. The hybrid MBR results from the combination of an MBR with a biofilm reactor, being biodegradation carried out by both suspended and attached biomasses. The addition of biofilm support to MBRs, has been mainly proposed with the goal of overcoming some limitations of the MBR regarding nutrients removal and membrane fouling.

Taking into account this context, in this doctoral thesis the application of hybrid MBR systems for the treatment of municipal wastewaters, was researched. The present work is experimentally based on bench and pilot-scale configurations specially built, developed and modified with the aim of extending the understanding of the effect of biofilm support in these systems.

As a starting point of this work, an actualized literature review about biofilm processes, MBR technology and its combination (hybrid MBR), is presented in **chapter 1**.

In **chapter 2** the materials and methods used along the different experiments are described.

In the first configuration proposed (**chapter 3**) a new hybrid MBR, consisting of a vertical configuration very compact and with optimized requirements for aeration, was developed at bench-scale. Placed in the municipal waste water treatment plant of Santander (Spain), this configuration was fed with pre-treated raw wastewater to pre-evaluate its performance as an alternative system to other conventional MBRs. In this work, a sponge fixed bed above the filtration unit was employed as support medium and no signs of overloading were observed during the whole experimentation. The aeration flow, located under the membrane unit,

was sufficient for membrane cleaning, oxygenation of the two biomasses (biofilm and suspended) and the mixing of the bulk liquid in the reactor. In spite of the great variability of the applied load (average 1.1 and 2.7 kg COD m^{-3} d^{-1}) high efficiencies of organic matter removal were achieved, with values above 90% in COD and 96% in BOD_5. In addition, the new HMBR showed good performance in total nitrogen removal through simultaneous nitrification and denitrification (SND), regardless of the continuous aeration applied. Remarkably, an average concentration of 7.3 mg TN L^{-1} in the effluent was achieved. This configuration was capable of obtaining an effluent with quality for reuse in terms of bacterial contamination, nitrogen, organic matter, suspended solids and turbidity. Additionally, the recirculation rate seems to play an important role in the behavior of this hybrid MBR. A recirculation rate of 300% improved slightly organic carbon removal efficiencies (99% vs. 96% in BOD_5) and notably nitrogen removal (98% vs. 91% in NH_4^+-N; 80% vs. 69% in TN).

These results are better than those obtained in other HMBRs and similar to the values reached using more complex MBRs with extra anoxic tanks, intermittent aeration or internal deflectors.

Once the new HMBR configuration had been tested at bench-scale, an original vertical HMBR was built, started up and evaluated at pilot-scale to study its feasibility as decentralized treatment (**Chapter 4**). Located in the municipal waste water treatment plant of Santander, the demonstrative pilot-plant had a pre-treatment unit (fine screen and grit removal) before the reactor, and was fed with raw unsettled wastewater. The HMBR consisted in a stainless steel aeration tank with submerged microfiltration membrane, in which a fixed support media for the biofilm attachment also takes place. Unlike in chapter 3, the fixed biofilm support media (called BLAS) implemented in the reactor, was self-produced based on the specific design previously developed by the Group of Environmental Engineering of the University of Cantabria.

The application of the HMBR at pilot-scale proved to be technically feasible since it was able to treat municipal wastewater without need of primary settling thus awarding high compactness as required to decentralized treatments. During experimentation, applied organic loading rate ranged between 0.36 and 1.76 kg COD m^{-3} d^{-1}. Nevertheless, the system maintained good overall performances. Organic matter removal for COD and BOD_5 were 84% and 98%, respectively.

Ammonium removal was 97% and total nitrogen 75% (in one single reactor), which was attributed to simultaneous nitrification and denitrification (SND). The average characteristics of the effluent achieved

were COD < 55 mg L^{-1}, SS < 4 mg L^{-1}, TN < 10 mg L^{-1} and turbidity < 2 NTU, meeting the standards for discharge in sensitive areas as well as for reuse.

In **chapter 5**, the goal was to compare the performance of the hybrid membrane bioreactor configuration studied in chapter 4 with a conventional membrane bioreactor (CMBR). For this purpose, other reactor identical in design to the HMBR but without the addition of the fixed bed was added. Both pilot plants were characterized and operated in parallel. Statistical analysis was performed to verify if there were significant differences between the results obtained in both systems. A tracer study showed similar hydrodynamic behavior with optimum mixing for both reactors. An improvement in the oxygen transfer coefficient K_La (at 20 °C) in the HMBR with respect to the CMBR was observed (33.9 h^{-1} vs. 18.3 h^{-1}), being attributed to extended bubble retention time within fixed bed reactors.

Both MBRs showed good removal efficiencies of organic matter and ammonia, but the effluent quality was better with the HMBR. The removal efficiencies of COD, BOD_5 and NH_4^+-N with the HMBR were 84, 98 and 97%, respectively, as compared to 80, 96 and 93% with the CMBR. In the case of organic matter removal this slight improvement was attributed to the higher concentration or activity of the attached biomass. The greater resistance to shock loading in hybrid systems compared to activated sludge would be the main reason for better nitrification. The HMBR also exhibited far better TN removal compared to the CMBR (average 75 vs. 38%, in the CMBR), which was attributed to simultaneous nitrification and denitrification (SND) in this reactor. The average PO_4^{3-} removal efficiencies were 42% in the HMBR and 37% in the CMBR, not being statistically different. Phosphorus removal by PAOs appears to be negligible because of the low influent PO_4^{3-} concentrations and the long sludge retention times (SRT≈ 47 -80 d). Accordingly, the phosphorus removal would be mainly due to assimilation by the microorganisms. Regarding sludge characteristics, the microscopic examination of the sludge in both MBRs revealed that the microbial community in the HMBR was richer than that in the CMBR. Furthermore, the HMBR sludge had better filterability compared to the CMBR (average $1.28 \cdot 10^{12}$ and $5.70 \cdot 10^{12}$ m kg^{-1}, respectively) and settleability (with SVI average values of 52 and 174 mL g^{-1}, respectively).

The main drawback associated with the application of membrane technology for wastewater treatment is the membrane fouling. Therefore, the comparison of the fouling rate and some fouling indicators between both MBRs, was of great interest in this work. To do so, the MLSS and the colloidal biopolymer clusters (cBPC) concentrations were employed as indicators. The results indicated that the

HMBR exhibited a notably lower membrane fouling rate (43% decrease) than the CMBR. For the range of operation tested in this work (up to 6 g L^{-1}), no correlation was found between MLSS concentration and fouling rate. With respect to cBPC concentrations, they were higher and more variable in the CMBR than in the HMBR which also corresponded to a faster and more variable fouling rate. This result suggested a certain relationship between fouling rate and cBPC concentration. In this sense, the lower concentration of cBPC in the HMBR sludge, probably due to their retention by the biofilm, could be partially responsible for this difference in fouling. Finally, all the improvements with the HMBR were attributable to the hybrid growth achieved when biofilm and suspended biomass grew simultaneously.

The presence of dissolved methane, especially at low temperature, represents an important environmental concern in terms of greenhouse gas (GHG) emissions of wastewater treated using methanogenic bioreactors. Methane has a global warming potential of 25. An alternative to reduce both greenhouse gas emissions and nitrogen content of the treated wastewater is the use of the dissolved methane as a carbon source for biological denitrification, but its feasibility had not been studied yet. In **chapter 6** of this work, a hybrid MBR system is proposed as a post-treatment of methanogenic bioreactors. The effluent of an upflow anaerobic sludge blanket (UASB) reactor was post-treated in a two-compartment membrane bioreactor (MBR). The first compartment was an anoxic moving-bed reactor (with K3 carriers) intended to use dissolved methane as carbon source for denitrification, while the second compartment was an aerobic membrane filtration reactor. Up to 60% and 95% nitrogen removal and methane consumption were observed, respectively. The recirculation rate between the aerobic and the anoxic compartments and the concentration of dissolved methane were shown as the important parameters governing the process. The lower recirculation ratios studied (between 0.5 and 1) showed the higher nitrogen removal and the lower methane emissions. Nitrogen removal decreased from 60 to 27% when dissolved methane was removed (stripped off) from the UASB effluent. Thus, the percentage nitrogen removal coming from the oxidation of methane could account, up to 33%. In addition, batch experiments and fluorescence in situ hybridization (FISH) analysis indicated the presence of microorganisms capable of denitrifying using the dissolved methane as a carbon source, both aerobically and anaerobically. Denitrification seems to be carried out by a consortium of aerobic and anaerobic methane oxidizing bacteria, anammox and heterotrophic bacteria.

The influence of denitrification with methane on membrane performance was also studied. The highest cBPC concentrations and the lowest permeabilities were observed when denitrification activity diminished.

The HMBR proposed seems to be a suitable technology for the post-treatment of UASB reactors. The biofilm presence favoured the development of a wide variety of populations of microorganisms, which could be advantageous for the growth of those implicated in the denitrification process. In addition, the use of membranes allows for a complete retention of the slow growing bacteria involved in methane and nitrogen removal.

In conclusion, from the work performed in this thesis important information for the operation of hybrid membrane bioreactors at bench and pilot-scale was obtained. These reactors have proved to be an attractive technology for carbon and nitrogen removal in urban wastewaters.

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List of acronyms and symbols

16S	Specific region in rRNA genes	-
23S	Specific region in rRNA genes	-
A	Filtration area	m^2
aBF-MBR	Assisted Biofilm Membrane Bioreactor	-
Anammox	Anaerobic Ammonium Oxidation	-
AnMBR	Anaerobic Membrane Bioreactor	-
ANME	Anaerobic Methanogenic bacteria	-
APHA	American Public Health Association	-
AS	Activated Sludge	-
AWWA	American Water Works Association	-
b	Time to filtration ratio	$s\ m^{-6}$
BF	Biofilter	-
BF-MBR	Biofilm Membrane Bioreactor	-
BOD	Biochemical Oxygen Demand	$mg\ L^{-1}$
BPC	Biopolymer Clusters	$mg\ L^{-1}$
C	Cytosine	-
CAS	Conventional Activated Sludge	-
cBPC	Colloidal fraction of Biopolymer Clusters	$mg\ L^{-1}$
CIP	Cleaning In Place	-
CLSM	Confocal Laser Scanning Microscopy	-
CMBR	Conventional Membrane Bioreactor	-
COD	Chemical Oxygen Demand	$mg\ L^{-1}$
COD_s	Soluble Chemical Oxygen Demand	$mg\ L^{-1}$
COD_t	Total Chemical Oxygen Demand	$mg\ L^{-1}$
COP	Cleaning Out of Place	-
CST	Capillary Suction Time	-
D	Diffusive coefficients	$cm^2\ s^{-1}$
DAPI	4,6-DiAmidino-2-Phenylindole	-
DIC	Dissolved Inorganic carbon	$mg\ L^{-1}$
DIN	Dissolved Inorganic Nitrogen	$mg\ L^{-1}$
DNA	Deoxyribo-Nucleic Acid	-
DO	Dissolved Oxygen	$mg\ L^{-1}$
DOC	Dissolved Organic Carbon	$mg\ L^{-1}$
DON	Dissolved Organic Nitrogen	$mg\ L^{-1}$
DTN	Dissolved Total Nitrogen	$mg\ L^{-1}$

EBPR	Enhanced Biological Phosphorus Removal	-
EPA	US Environmental Protection Agency	-
EPS	Extracellular Polymeric Substances	mg L^{-1}
F	Formamide	%
F/M	Food to Microorganism ratio	Kg COD Kg $MLVSS^{-1}$ d^{-1}
FAS	Ferrous Ammonium Sulphate	-
FID	Flame Ionization Detector	-
FISH	Fluorescent In Situ Hybridization	-
FS	Flat Sheet	-
FT	Filter Test	-
GHG	Greenhouse Gas	-
HF	Hollow Fiber	-
HMBR	Hybrid Membrane Bioreactor	**-**
HRT	Hydraulic Retention Time	h
IC	Ion Chromatography	-
IFAS	Integrate Fixed Activated Sludge	-
IWA	International Water Association	-
J	Flux	L m^{-2} h^{-1}
$K_L a$	Mass transfer coefficient	d^{-1}
LMH	Litres per m^2 per hour	-
m	Mass flow	mg d^{-1}
MBBR	Moving Bed Biofilm Reactor	-
MBMBR	Moving Bed Membrane Bioreactor	-
MBR	Membrane Biological Reactor	-
MF	Microfiltration	-
MI	Morril Index	-
MLSS	Mixed Liquor Total Suspended Solids	mg L^{-1}
MLVSS	Mixed Liquor Volatile Suspended Solids	mg L^{-1}
MT	Multi-Tubular	-
N	Nitrogen	-
NDIR	Non-dispersive infrared analyzer	-
NF	Nanofiltration	-
NH_4^+-N	Ammonia nitrogen concentration	mg L^{-1}
NO	Nitrous oxide	mg L^{-1}
NO_2-N	Nitrite nitrogen concentration	mg L^{-1}
NO_3-N	Nitrate nitrogen concentration	mg L^{-1}
NO_x-N	Nitrite and nitrate nitrogen concentration	mg L^{-1}
NPGA	Neopentylgiycol adipate	-
OLR	Organic Loading Rate	Kg COD m^{-3} d^{-1}

ORR	Organic Removal Rate	Kg COD m^{-3} d^{-1}
OTR	Oxygen Transfer Rate	
P	Permeability	L m^{-2} h^{-1} bar^{-1}
P	Phosphorus	-
p	Statistical significance	-
PAO	Polyphosphate Accumulating Organisms	-
pBF-MBR	Pure Biofilm Membrane Bioreactor	-
PBS	Phosphate Buffer Solution	-
PE	Polyethylene	-
PES	Polyethylsulphon	-
PLC	Programmable Logic Controller	-
PO_4^+-P	Phosphate concentration	mg L^{-1}
PP	Polypropylene	-
PVC	polyvinylchloride	-
PVDF	Polyvinylidene difluoride	-
R	Recirculation Ratio	-
r	Volumetric reaction rate	mg L^{-1} d^{-1}
RBC	Rotating Biofilm Contactor	-
R_c	Cake resistance	m^{-1}
RE	Removal Efficiencies	%
R_m	Initial membrane resistance	m^{-1}
RO	Reverse Osmosis	-
R_{pb}	Pore blocking	m^{-1}
rRNA	Ribosomal Ribo-Nucleic Acid	-
R_t	Total Resistance	m^{-1}
SA	Specific Area	-
SAA	Specific Anammox Activity	-
SAD_m	Specific Air Demand per membrane area	Nm^3 m^{-2} h^{-1}
SAD_p	Specific Air Demand per permeate volume	Nm^3m^{-3}permeate
SBR	Sequencing Batch Reactor	-
SFBBR	Submerged Fixed Bed Biofilm Reactor	-
SFI	Sludge Filtration Index	s % TSS^{-1}
SMP	Soluble Microbial Products	-
SND	Simultaneous Nitrification-Denitrification	-
SRB	Sulphate-Reducing Bacteria	-
SRF	Specific Resistance to Filtration	m kg^{-1}
SRT	Solids Retention Time	d
SS	Suspended Solids	mg L^{-1}
SVI	Sludge Volumetric Index	mL g^{-1}

TDC	Total Dissolved Carbon	mg L^{-1}
TEP	Transparent Exopolymer Particles	mg XG L^{-1}
TF	Trickling Filter	-
TIC	Total Inorganic Carbon	mg L^{-1}
TKN	Total Kjeldhal Nitrogen	mg L^{-1}
TMF	Tertiary Membrane Filtration	-
TMP	Transmembrane Pressure	bar
TN	Total Nitrogen	mg L^{-1}
TOC	Total Organic Carbon	mg L^{-1}
TSS	Total Suspended Solids	g L^{-1}
UASB	Upflow Anaerobic Sludge Blanket	-
UF	Ultrafiltration	-
V	Filtrate volume	L
VFA	Volatile Fatty Acids	mg L^{-1}
VSS	Volatile Suspended Solids	g L^{-1}
W	Weight of dry solids per volume of filtrate	kg m^{-3}
WHO	World Health Organization	-
WWTP	Waste Water Treatment Plant	-
XG	Xanthan Gum	-

α	Specific resistance to filtration	m kg^{-1}
μ	Viscosity	kg m^{-1} s^{-1}

Chapter 0

Introduction

0.1 BACKGROUND

Municipal and industrial wastewater treatment industry has experienced significant growth in the last decade. This growth has been boosted by strict legalization and an increasing scarcity of fresh water resources. For instance, in Europe, and in particular in Spain, large zones have been designated as sensitive areas, requiring better quality effluents. Growing municipalities must solve their problems with the capacity of their wastewater treatment plants, which become too small for the actual population. In addition, existing plants are often not able to fulfill nutrients removal requirements when space is limited.

The activated sludge processes have been widely applied for carbon, nitrogen and phosphorus removal in medium and large cities due to the simplicity of its design and operation; however they present two important limitations.

On the one hand the total biomass concentration in the mixed liquor in suspension must not exceed around 4 TSS g L^{-1} in order to avoid solids overflowing from the secondary settler. This leads to relatively high hydraulic retention times (around HRT 10 – 15 hours), especially for nutrient removal. Consequently, this process requires a large footprint. On the other hand, filamentous bulking episodes are usually present, which cause severe problems in the performance of the system (Henze et al., 2008).

New biological processes, which are able to overcome these limitations, have emerged in the last years. They include:

- Membrane bioreactor (MBR) processes.
- Innovative biofilm technologies (with either fixed or moving support).

The MBR technology, which comprises activated sludge process and membrane technology, has gained recognition due to several advantages over conventional technologies, such as high quality effluent, small footprint, lower sludge production, controlled biomass separation and improved nutrient removal. The major obstacle for the general application of MBRs technology is the rapid decrease of membrane permeability, known as membrane fouling. The membrane fouling has been widely reviewed by researches including Le-Clech et al., (2006), Drews, (2010) and Judd, (2011).

On the other hand the biofilm process, in which the biomass grows on support media, has been successfully used in water and wastewater treatment for over a century. It offers several advantages over activated sludge, such as operational

simplicity, higher biomass activity due to accumulation of highly specialized microorganisms, better oxygen transfer and high resistance to overloading/toxic compounds (Sombatsompop et al., 2006).

Many research groups are searching for new alternatives to overcome the limitations of conventional MBR processes. In this context, a new technology named hybrid membrane bioreactors (HMBR) emerges with the intention to combine the advantages of MBR and biofilm process. In HMBR, suspended and attached biomasses grow simultaneously in the same system.

As Ivanovic and colleagues reflected in his review (Ivanovic and Leiknes, 2012), the internationally available publications in the field of biofilm MBR processes prove that this subject has not received significant attention yet, in comparison with the amount of research done about conventional MBR.

Previous work has shown enhanced nutrient removal and, in general, improved membrane performances in biofilm MBR with respect to conventional MBR. These results are encouraging, but there is a need for a deeper investigation through different process configurations and operational conditions, in order to understand the potential capabilities of the hybrid MBR process.

This Doctoral Thesis arises in order to contribute to generate new knowledge within this field. This work is the result of experimental research of new systems for advanced wastewater treatment based on combining a biofilm reactor and membrane separation technology.

0.2 OBJETIVES OF THE STUDY

Most researches on hybrid MBR are based on a purely scientific approach, often with synthetic wastewater at small-scale or lab-scale. Although this approach is necessary to develop fundamental knowledge, it is difficult to extrapolate these results at full-scale installations.

In this work the experiments were carried out in bench-scale and pilot-scale in order to provide a complementary knowledge to small-scale research. Once we move from small-scale to full-scale many unknowns show up and therefore pure scientific research can hardly be carried out. The results obtained in pilot-scale are of great importance to give response to the problems that are encountered in full-scale installations.

In this context, the objectives of this study are:

1. Development, construction at bench-scale and performance evaluation of a new hybrid MBR, compact and with optimized requirements for aeration.
2. Construction and evaluation at pilot-scale of the proposed hybrid MBR, based on the findings at bench-scale. Study of its feasibility for decentralized treatment.
3. Comparison in parallel and at pilot-scale of the proposed hybrid MBR with a conventional MBR focused on carbon and nutrient removal, membrane fouling and sludge properties.
4. Evaluation at bench-scale of a hybrid MBR for the post-treatment of methanogenic bioreactors.

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Chapter 1

State of the art[1]

[1] Part of section 1.3 of this chapter has been published as:

I. Tejero, A.L. Esteban-García, L. De Florio, R. Díez Montero, A. Lobo, **L. Rodríguez-Hernández** (2012). *Tecnologías de biopelícula innovadoras para la depuración de aguas residuales: veinticinco años de investigación del Grupo de Ingeniería Ambiental de la Universidad de Cantabria*. Ingeniería Civil 168, 61-73.

OUTLINE

SUMMARY

In this chapter, starting with a brief summary of the requirements for water reuse, an overview of the research literature on biofilm processes and membrane technologies is provided. Firstly, the fundamentals and the main types of biofilm processes are described, focusing on hybrid processes. These processes combine biofilm and suspended biomasses, whereby they are an attractive alternative to overcome the limitations of conventional processes. On the other hand, this chapter also reviews MBR technology fundamentals, drawbacks and advantages over conventional activated sludge systems (AS). Among the factors affecting MBR operation, special interest in membrane fouling is shown. Finally, different types of non-conventional MBR reactors are described, with emphasis on hybrid MBR (HMBR) systems, and a summary of the main literature on HMBR technology is presented.

1.1 INTRODUCTION

Biological treatment processes are based on the use of active biomass or set of organisms, in charge of carrying out the removal of contaminants present in wastewater. The biomass dispersed in suspension within the bulk liquid has been called activated sludge (AS) process and the biomass attached to a support media has been called biofilm (BF) process.

Biofilm technology was historically the first to be spread and applied. Nonetheless, since the 1950s, activated sludge technology gained more and more popularity due to the supposed operation simplicity and higher quality of the effluent. Thus, the AS processes have been widely employed in urban wastewater treatment plants for organic matter, nitrogen and phosphorus removal.

Recently, new development pushed forward the biofilm technology again. Innovative biofilm technologies, with either fixed or suspended support, which are able to overcome some of the limitations of the AS (Water Environment Federation, 2010), have emerged in the last years. In this way, these novel biofilm technologies are able to fulfill the increasingly stringent requirements demanded nowadays.

1.2 WASTEWATER REUSE REGULATIONS

At the present time, in response to the problems of water scarcity, there is a trend towards more stringent laws to protect against water pollution. This fact has led to a major growth in the treatment infrastructure and a lot of work in the option of reusing this treated wastewater as a new source of water, safe and stable over time.

In Spain, as in the rest of the European Union countries, when Community Directive 91/271/EEC concerning urban wastewater came into effect (transposed to Spanish Law via Act 11/1995 and Royal Decree 509/1996), there was a considerable increase in the construction of treatment plants and the obtaining of good quality treated effluent (Iglesias and Ortega de Miguel, 2008).

Regarding Spanish legislation, the adoption of the Royal Decree 1620/2007 has represented an important advance in the consolidation and standardization of water reuse practices.

In general, the regulations in Spain and Europe are based on World Health Organization (WHO) and the Program for Pollution Monitoring and Research reuse guidelines (World Health Organization, 2006; MED POL, 2005) instead of American regulations (US EPA, 2004; State of California, 2005).

The Royal Decree 1620/2007 defined both the responsibilities of the Public Administrations and those of concession holders and end users, establishing i) permitted uses and quality criteria, ii) the minimum frequency of sampling, iii) the benchmark for analytical methods and iv) the conformity criteria.

Concretely, this Royal Decree classified the use of reclaimed water according to the quality criteria and differentiates 14 uses under five main headings: 1) Urban, 2) Agricultural Irrigation, 3) Industrial, 4) Recreational and 5) Environmental. Minimum acceptable limits are established for each type of use. Further, the reuse of treated wastewater is forbidden for the following purposes: a) human consumption, except in situations of declared disasters; b) specific uses of the food industry; c) use in hospital installations and other similar uses; d) breeding of filtering mollusks in aquaculture; e) recreational use as swimming waters; f) use in fountains and ornamental waters in public spaces or inside public buildings and g) any other use that the Health Authorities may deem to be a hazard to human health.

Consequently, the current problems of water scarcity and the implementation of these new regulations in Spain, which impose rigorous quality requirements for

reclaimed water, have made it necessary to adapt an important part of existing reuse systems.

According to Iglesias et al., (2010) 40% of the total volume reused in Spain has to be adapted to quality criteria in the coming years. This scenario is going to promote suitable reclaimed water treatment processes.

The processes most widespread in Spain, to meet any application for water reuse, are a physical-chemical treatment followed by sand filtration together with disinfection treatments. These technologies achieve great removal of measureable constituents, but in response to dissolved solids, pathogenic organisms, or trace constituents, membrane processes are now being used to avoid possible environmental or health risks (Iglesias et al., 2010) .

Therefore, taking into account the need to increase water availability and to contribute to improve the treatment trains applied, this Thesis develops innovative membrane configurations that may be employed for this purpose.

1.3 BIOFILM TECHNOLOGY

1.3.1 Fundamentals of biofilm systems

The microorganisms charged of contaminants removal in wastewater, can be dispersed in suspension within the bulk liquid (AS processes) or attached to a support media (BF processes).

Most microorganisms (predominantly bacteria), can colonize the surface of an inert support, becoming attached or immobilized and forming biofilms or biomass aggregates.

Thus, a biofilm is an aggregate of microorganisms in which cells adhere to each other on a surface. These adherent cells are frequently embedded within a self-produced Extracellular Polymeric Substance (EPS) (Hence 2008). The biofilm expands because of the bacteria growth, using substrates present in the bulk liquid, reaching a density concentration and thickness that depends on the balance between growth and detachment due to shear forces in the bulk liquid.

Basic metabolic processes of both activated sludge and biofilm systems are the same. The main differences are based on the way of retaining biomass in the reactors and transport processes of the substrates.

In activated sludge systems the separation of suspended biomass and effluent through settling is necessary, and a recirculation is used to return the biomass to the reactor in order to operate with certain MLVSS concentration (3-4 g L^{-1}).

In biofilm systems, the active biomass is largely retained so there is no need to recirculate any displaced biomass back to the reactor in order to maintain a sufficient density of microorganisms, as is the case in the activated sludge process. This system is thus clearly advantageous in that a settler is not required.

On the other hand, the suspended biomass is composed of small biological flocs and thus substrates are theoretically available for every cell. Thus, biochemical reactions taking place in the bulk liquid are performed by this biomass without mass transfer limitation. In contrast, in the biofilm process substrates (oxygen and dissolved and particulate compounds) must cross through a layer of stagnant liquid adjacent to the biofilm (boundary layer) and then be transported through the biofilm to the place where they are used. Whereby, the overall rate of substrate removal in a biofilm process depends on the mass transfer velocity and the substrate concentrations in the biofilm. Biofilm thickness is determined by attachment and detachment phenomena from bulk liquid to biofilm and from biofilm to bulk liquid, respectively.

Another consequence of these transport phenomena is that different environments may coexist in the same biofilm, offering the additional advantage of integrated simultaneous nitrification and denitrification (SND). The physical fundament for SND within biofilm is dissolved oxygen (DO) concentration gradients from the bulk liquid into the biofilm as a result of diffusion limitations. The aerobic bulk liquid provides an oxidizing environment in the outer part of the biofilm where BOD is removed and ammonia is nitrified (see Figure 1-1). The nitrite and nitrate produced during nitrification diffuse to the inner parts of the biofilm where an anoxic micro-zone harbors heterotrophic denitrifiers that convert them into nitrogen gas.

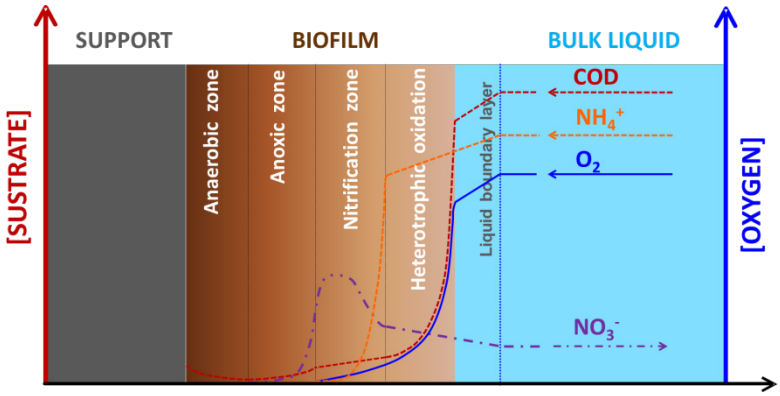

Figure 1-1 Profile of substrates and oxygen in thick conventional biofilms.

Nowadays there are a wide variety of treatment systems based on biofilm processes with different characteristics. These treatment systems are generally referred to as fixed-growth or biofilm reactors (Schlegel and Koeser, 2007). In general, compared to AS processes, biofilm reactors offer a multitude of advantages: closer to natural biofilm systems (prevents washout of biomass), high biomass loading per unit reactor volume, higher mean cell retention time, operational ease of solids-liquid separation, surface biodegradation (facilitates resistance to shock loadings), higher biodegradation rates (higher active biomass), extensive microbial diversity, stable gene pool and enhanced rates of genetic transfer, and greater efficiency to degrade recalcitrant (Verma et al., 2006).

The disadvantages include the possibility of overloading (either due to an insufficient pre-treatment or excessive growth of biofilm), higher difficulty in achieving a homogeneous mixture of liquid and greater complexity for modeling, and therefore control, of the process (Water Environment Federation, 2010).

1.3.2 Typology of biofilm processes

There is a wide variety of biofilm processes which have been applied to the treatment of wastewater. Conventionally, trickling filters (TFs), rotating biological contactors (RBCs) and sand filters, among others, have been used in biological wastewater treatment for several decades. Although the capital and operation costs are low, they still have several disadvantages like regular maintenance, odor problems, and temperature sensitivity failure at low temperatures (Verma et al., 2006).

According to Schlegel and Koeser, (2007) biofilm systems can be classified into three main groups based on the mobility of the growth media, as shown in Figure 1-2. In addition, the combined systems, which use a combination of AS and fixed biofilm, are also referred to as hybrid processes (see section 1.3.2.3).

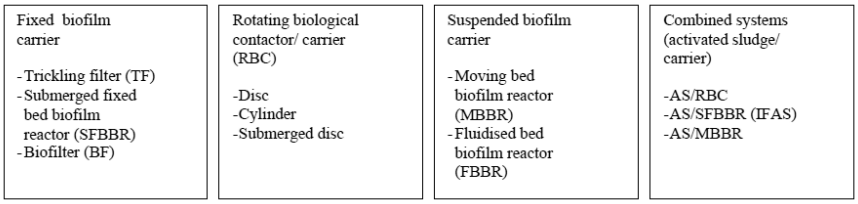

Figure 1-2 Biofilm reactor systems for wastewater treatment (source: Schlegel and Koeser, 2007).

1.3.2.1 Biofilm systems with fixed support: SFBBR

Different biofilm reactors are commercially used for the treatment of municipal as well as industrial wastewater. One of the most recent biofilm systems was commercially introduced in Europa in the last two decades and it is knows as submerged fixed bed biofilm reactor (SFBBR). Since then, the use of this process has expanded and its reliability has been established. In SFBBRs the influent flow of wastewater passes through the media, over which the biofilm growth is favored and the bio-oxidation is performed. Thus, the wastewater is treated by contact with the biofilm submerged in the reactor. Due to the design of the support, with large voids and spaces to prevent clogging, the solid retention on it is minimal; therefore, the SFBBR do not act as filtering media and a subsequent final sedimentation is required. Because of the high activity in the biofilm, high dissolved oxygen concentrations of about 4 mg L^{-1} must be maintained in the bulk liquid in order to keep the biofilm sufficiently aerobic. In addition, the injected air serves to mix the bulk liquid and allow contact of the biofilm with the wastewater. The sludge production of this system is lower than in conventional activated sludge and has also good settling properties (Schlegel and Koeser, 2007). Other operational and infrastructure advantages are that wastewater treatment plants with SFBBRs are easy to operate, require minimal pre-treatment and do not need much supervision. As a result of the adaptation of the biofilm attached to the submersed fixed bed, the treatment of hardly degradable industrial effluents may also be possible. Thus, the SFBBR system is applied especially for small municipal wastewater treatment plants and pre-treatment of industrial sewage.

To avoid clogging, which is the most critical aspect of this process, an intensive periodic flushing cycle of the fixed bed modules with air is recommendable in order to slough away the excessive biomass (Schlegel and Koeser, 2007).

Nowadays, plastic media are almost exclusively used as support or carrier for the biofilm in SFBBRs. Normally, they consist of moulded polyethylene (PE), polypropylene (PP) or polyvinylchloride (PVC), but woven polyester fibers or stripes of PVC films are employed as well.

Special importance have the durability, specific surface area and percent void space of the media. Greater surface permits a larger biomass per unit volume, while greater void space allows for a higher oxygen and mass transfer to the biofilm and reduces the clogging risks of the channels of the support media by excessive biofilm growth. Therefore, specific surface area from 100 to 300 m^2 m^{-3} have proven viable (Water Environment Federation, 2010; Schlegel and Koeser, 2007).

Among the marketed fixed beds Bioweb, Biosource (Wabag), Expo-Net's Bio-Blok and Accu-web of Brentwood Industries can be mentioned (see Figure 1-3 a, b). The support medium named BLAS (Figure 1-3 c) is a patent of the University of Cantabria. It consists of a three-dimensional biofilm carrier made of flat nets or meshes overlapping one another, with a fixed opening and separation between one another. The main objective of the support geometry is to provide sufficient surface for the support of the biofilm biomass without giving rise to elevate hydraulic head loss and clogging phenomena occurrence, therefore depending on the type of biofilm intended to develop in the reactor, the opening among the net voids is set to more than double the biofilm thickness (Tejero and Santamaría, 2000).

Figure 1-3 Fixed bed support media used in SFBBR: (a) Ropes or nets AccuWeb Brentwood Industries, (a) EXPO-NET's BIO-BLOK® and (c) BLAS®.

1.3.2.2 Biofilm systems with suspended moving support

The first initiatives of suspended biofilm systems used sponges (like Captor and Linpor processes), which added a filling fraction of 20 – 30 % of the reactor volume and can remain in suspension when they are subjected to adequate mixing in aerated or agitated tanks. They use a specific surface area of 200 to 500 m^2 m^{-3} and have a density of approximately 0.95 g cm^{-3}.

At present, the most successful technologies use plastic carriers with very diverse geometric configurations and sizes (specific area (SA): 500 – 1000 m^2 m^{-3}) and they use filling fraction of up to 60%.

Due to intense movement and shear forces involved, the carriers are self-washed automatically. This detached biomass (excess sludge with 300 – 500 mg TSS L^{-1}) must be separated in a secondary settler.

The moving bed biofilm reactor (MBBR) is the most widespread configuration of the biofilm systems with suspended moving support. They use biofilm support medium with a density close to water so that it can be kept in suspension with minimum mixing energy provided by aeration or mechanical mixing (Odegaard, 2006). These suspended support media are manufactured in shapes which can be retained in the reactor by screens or wire wedges.

Advantages of the MBBR process over the conventional AS process include better oxygen transfer, shorter hydraulic residence time (HRT), higher organic loading rates, higher nitrification rates and a larger surface for mass transfer (Sombatsompop et al., 2006). Unfortunately, in these systems the production of filamentous bacteria and poorly settling biomass often hinder solid separation in secondary settler operations. According to Odegaard, (2000) settleability of biosolids remains the largest challenge in MBBR design.

MBBRs can be operated without or with sludge recycles. When the system is operated without biomass recycle (Figure 1-4a) the MBBR biomass retention in the system is limited to biofilms retained on the support medium, while a system with biomass recycle retains both attached and suspended biomass. The later type of system is discussed further in the next section (Figure 1-4b).

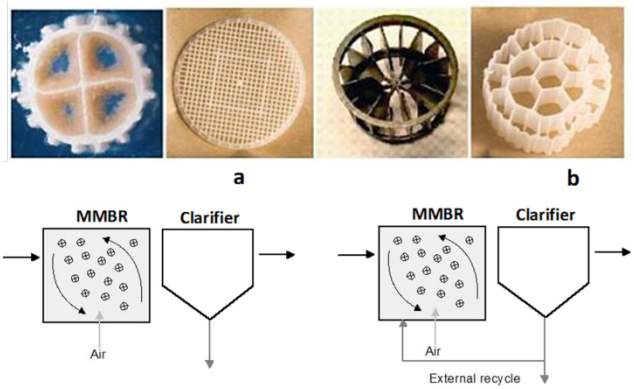

Figure 1-4 Plastic media used in MBBR that can be configured without (a) or with recycle (b) of suspended biomass (source of images: Henze et al., 2008).

1.3.2.3 Hybrid processes (suspended – biofilm biomass)

In general, the word hybrid can describe any type of treatment process that combines the features of several different technologies. Nevertheless, the focus of this thesis on hybrid processes is in the introduction of biofilm support medium to enhance the performance of activated sludge systems. If external recycling from the secondary setter is employed, biomass in suspension (flocs) can increase to values around 3 – 4 g L^{-1} (Larrea and Albizuri, 2009). In such cases, biomasses in suspension and in the biofilm coexist, interact and compete for the carbon substrate and this configuration is referred to as hybrid systems or combined systems. Among them, the best known is the so-called integrated fixed film activated sludge system (IFAS).

The IFAS process has been typically considered as an upgrade option in existing treatment plants since it can be applied to almost any type of process flow schematic and reactor configuration. It has been used mainly in the aerobic zones of treatment processes to enhance organic matter removal and nitrification. Depending on the type of media, IFAS has also been applied to anoxic zones to enhance denitrification (Water Environment Federation, 2010).

Sometimes the IFAS process is confused with the MBBR process, because both processes use the same type of media. However, normally the MBBR does not incorporate a return activated sludge and thus is a pure fixed-film process. When MBBR is operated with biomass recycle, then it can also be considered a hybrid process (see Figure 1-4b).

Some of the general advantages and disadvantages of hybrid process are described below (Water Environment Federation, 2010):

Advantages

- Ability to phase in additional capacity or improve performance by adding more media;
- Additional biomass for treatment without increasing the solids loading on final clarifiers;
- Higher rate treatment processes possible, thus allowing greater treatment in a smaller space;
- Improved settling characteristics (reduced sludge volumetric index, SVI);
- Reduced sludge production;
- Simultaneous nitrification and denitrification and
- Improved recovery from process upsets.

Disadvantages

- Potential for odor (when tank dewatered);
- Additional operating appurtenances;
- Need to relocate media and
- Increased head loss associated with media retention screens.

Finally, the scope of research in the area of biofilm processes continues to expand beyond the traditional TF, biofilter (BF) or RBC into biofilm measurement and characterization methods, growth and modeling, new biofilm growth media and innovative bioreactors. In this sense, biofilm processes will continue to have relevance in the treatment of wastewater as technological advances evolve, such as membrane bioreactors and their hybrids. Removal of nutrients, xenobiotics, pharmaceuticals, among others, may open up new applications of biofilm systems.

1.4 MEMBRANE BIOREACTOR TECHNOLOGY

1.4.1 Introduction

Membrane bioreactors (MBRs) can be considered as the most important advancement in wastewater treatment technologies performed in the last decade, compared to conventional systems of biological treatment. The

Membrane bioreactor (MBR) is the combination of an activated sludge process with a solid-liquid separation by membrane filtration step in replacement of the usual sedimentations step to retain the biomass and separate the treated water.

The MBR technology combines the unit operations of aeration, secondary clarification and filtration into a single process (Figure 1-5), producing a high quality effluent, suitable for any discharge and most reuse applications, while greatly reducing space requirements and under stringent norms.

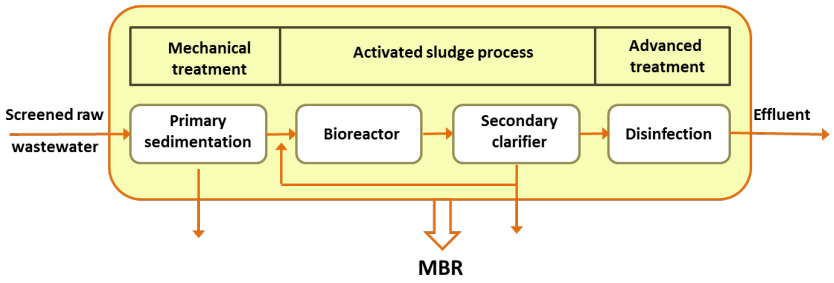

Figure 1-5 Flow diagram of a CAS process and a MBR

As a result, MBRs technology can operate with long solids retention times (SRT) to keep high biomass concentrations and therefore reduce sludge production and minimize reactor volumes. The effluent obtained through membrane separation is usually referred to as permeate. The advantage of membrane techniques include continuous separation, easy combination with other existing techniques, easy up-scaling, and no additives used. However, an excessively high biomass concentration could cause a rise in sludge viscosity affecting the energy requirements for pumping, oxygen supply of the microorganisms and performance of membrane (Drews et al., 2005). Consequently the MBRs operate in a MLSS range of 6 – 15 g L^{-1} (Rosenberger et al., 2006; Judd, 2011), with an optimum value related to the specific installations and also to biomass characteristics.

1.4.2 Historical perspective

The MBR process was introduced to the market in the late 1960s, when ultrafiltration (UF) and microfiltration (MF) membranes were available. The original process was invented by Dorr-Olivier as an application for ship-board sewage treatment and combined the use of an activated sludge bioreactor with a cross-flow membrane filtration loop. First system was based on polymeric flat-sheet membranes. The original process was very expensive,

mainly due to high cost of membranes, low economic value of the product (tertiary effluent) and the rapid loss of performance due to membrane fouling. To reduce fouling, the activated sludge was pumped at high cross-flow velocity, with a significant energy consumption of 10 kWh m^{-3} of produced permeate (Le-Clech et al., 2006). The high cost of the first generation MBRs, restricted its application in niche areas with special needs such as isolated trailer parks and sky resorts. These MBRs design, with the separation device located external to the reactor and high transmembrane pressure (TMP) to maintain filtration, is named side-stream MBR (Figure 1-6a).

The breakthrough for the MBR technology happened in 1989 when Yamamoto and co-workers presented a new MBR where membranes were directly immersed into the bioreactor (Yamamoto et al., 1989) (Figure 1-6b). In the new generation of MBRs, the static pressure, caused by the activated sludge liquid on top of the membranes, contributed to the extraction of permeate. This design allows suppressing the impeller pump of the sludge which is replaced by suction pump to extract the filtrate or permeate effluent from the membrane module. The submerged configuration uses air to produce mixing, to provide oxygen to the biomass and to control fouling. Therefore, this new design was much cheaper than the earlier configuration. Investment and operating costs can be significantly reduced due to the reduction and simplification of equipment and energy (0.55 – 1.5 kWh m^{-3}) compared to the side-stream MBRs (3 – 5 kWh m^{-3}). Further, the side-steam can work with higher fluxes and has greater hydrodynamic control, but generally provides lower permeability (Judd, 2011). Nowadays, the side-stream is used in leachates treatment and industrial wastewater, while the submerged configuration is usually preferred for domestic wastewater treatment. In the first submerged MBR, the membrane modules were placed in the same biological compartment where influent is introduced, but nowadays the tendency is to locate them in a separate compartment. This external submerged MBR configuration (Figure 1-6c) reduces significantly membrane fouling. The new MBR design, together with decreasing membrane costs, stimulated and exponentially increased MBR application since the mid-1990s (Judd, 2011).

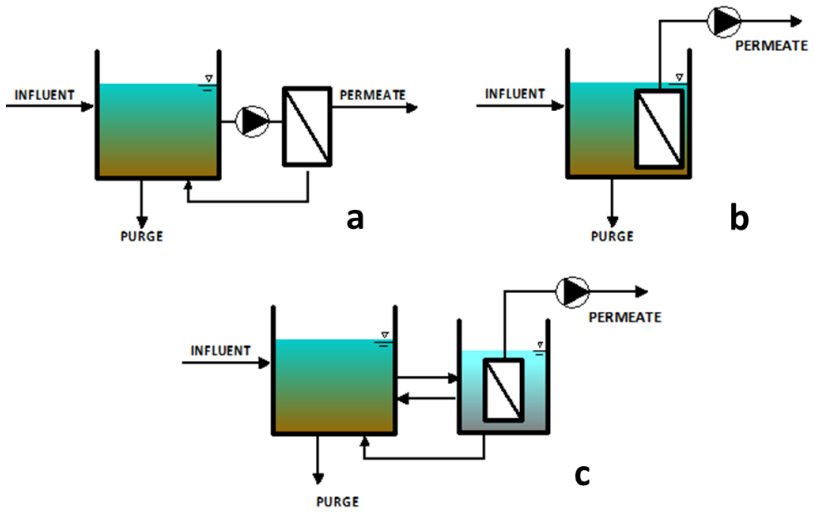

Figure 1-6 MBR process configurations: (a) External (side-stream); (b) Submerged; (c) external submerged.

1.4.3 Worldwide research and implementation on the market

As a result of technical innovations, the MBR process has become an attractive alternative for the treatment and reuse of municipal and industrial wastewater, as evidenced by its accelerated and faster growth compared to others advanced wastewater treatment systems.

The first full-scale MBR plant for domestic wastewater treatment was installed in Porlock (UK) in 1998, and features a capacity of 1900 m^3 d^{-1}. Since then, the range of capacities and applications developed significantly. In 2006, more than 100 municipal MBR plants with capacity larger than 500 person equivalents were in operation in Europe only. Today, several thousand MBRs have been commissioned worldwide.

At global scale, larger MBR are concentrated in Middle East countries, China and USA, where water reuse for irrigation is commonly carried out. It is also evident that MBR installations are increasing in size year after year. Both in Europe and Asia more research in the sector of urban water treatment than in industrial has been conducted, while in North America the opposite is true. The reason is that in Europe and Asia there is 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).

MBR systems have been implemented in more than 200 countries and global growth rates between 11.6 and 12.7% are routinely reported. The market value of the MBR industry in 2011 was $746 mill and is expected to be $2052 mill in 2017 (Marketsandmarkets, 2012).

The evolution of the MBR implementation in Europe during the last decades (industrial and municipal wastewater) can be observed in Figure 1-7.

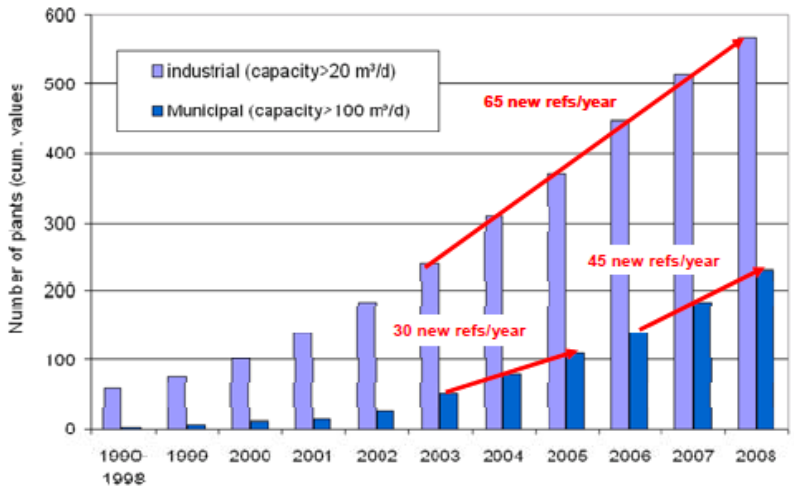

Figure 1-7 Evolution of MBR market in Europa (source: Huisjes et al., 2009).

Observed differences in trends between municipal and industrial systems exhibit that the MBR technology is especially competitive in the industrial market. The industrial market is considered mature and stable where the inversion costs of the MBR plants and conventional treatments are similar. However, in the municipal market a further growth is expected, especially if energy costs are reduced, which nowadays are 30 to 50% higher in MBR plants than in conventional ones. In this sense, if that difference were reduced, MBR technology could become the referral process for the treatment of urban wastewater (Huisjes et al., 2009).

1.4.4 Barriers and opportunities for MBRs technology

Currently, two significant components in operation costs of the MBRs are the replacement of membranes and the energy consumption, both aspects related directly to membrane fouling. In addition, it is estimated that an important part of the energy demand of submerged MBRs (30 – 50%) is associated to the aeration

to avoid membrane fouling (Judd, 2006). Thus, their cost is the most significant barrier limiting the wider application of MBRs. Nevertheless, there are several drivers which mitigate this factor. Likely the most important is the increasingly stringent legislation related to freshwater preservation and pollution removal, affecting both domestic and industrial wastewater discharge. This fact together with the introduction of state or regional incentives to encourage the development of new technologies and recycling has driven development to more sophisticated technologies in the water sector. When used with domestic wastewater, MBR process can produce effluent of high quality enough to be discharged to coastal or to surface waterways or to be reclaimed for urban irrigation. Other advantages of MBRs over conventional systems include small footprint, easy retrofit and upgrade of old WWTP into MBRs. Thus, even without regional legislation, water resourcing problems can provide sufficient motivation for water reuse. Both investment and operating cost of the MBR systems, main barriers limiting their application, have decreased dramatically over the past 20 years. Finally, confidence and acceptance of the MBR technology is growing as a consequence of their increase in number and maturity.

1.4.5 Fundamentals of membrane processes

1.4.5.1 Pore size, materials and internal structure of membranes

Membrane filtration is defined as a pressure-or vacuum-driven separation process in which a membrane acts as barrier allowing some physical or chemical components to pass more readily through it than others (perm-selective). The degree of selectivity depends on the membrane pore size. According to Metcalf and Eddy, (2003), the pressure-driven membranes can be classified in the following operations according to the nominal size of the separation achieved (Figure 1-8):

- Microfiltration (UF): separation of particulate or suspended material range in size from 0.1 to 10 µm;
- Ultrafiltration (UF): separation of virus and colloids in the 0.01 to 0.1 µm range;
- Nanofiltration (NF): separation of small molecules and viruses with a pore size of 0.001 to 0.01 µm through a combination of charge rejection, solubility – diffusion and sieving through micropores;
- Reverse Osmosis (RO): separation of singly charged ions (0.001 µm) by differing solubility and diffusion rates of water and solutes in water.

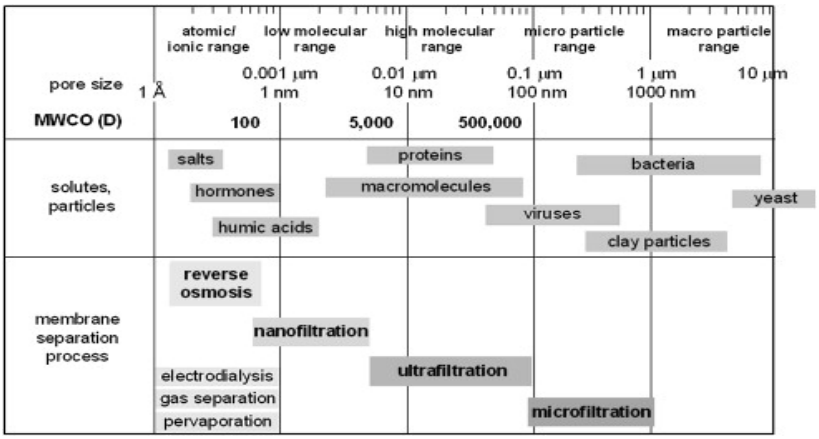

Fig. 1-8 Membrane separation processes overview (source: Peter-Varbanets et al., 2009)

The permeate is the solution that passes through the membrane, while the rejected fraction is commonly called retentate.

There are mainly two different types of membranes according to the material used:

- Organic polymeric membranes
- Ceramic membranes

There also exist metallic membrane filters but they are not relevant, having very specific applications not related to MBRs.

All membrane materials must have a desirable chemical and physical resistance, fouling resistance and resistance to extremes of temperature, pH and/or oxidant concentrations that normally arise when the membrane is chemically cleaned (see section 1.4.6.2). In this sense, ceramic membranes have higher chemical resistance than organic membranes.

Although any polymer can be used for the production of organic membranes, the most widely used materials are polyvinylidene difluoride (PVDF), polyethylsulphone (PES), polyethylene (PE) and polypropylene (PP). The organic polymeric materials are hydrophobic, and it is known that hydrophobic membranes are more prone to fouling than hydrophilic ones due to the fact that most interactions between the membrane and the foulants (on mixed liquor) are of hydrophobic nature. The base material is treated to obtain a hydrophilic surface through chemical oxidation, organic chemical reaction, plasma treatment or grafting. With these techniques the fouling is limited. This modification

process together with the method for assembling membrane modules (i.e. the configuration of the membrane) are proprietary information of the suppliers.

In symmetric membranes no changes in chemical composition or physical structure of the membrane are observed. Asymmetric membranes have a uniform chemical composition but with different pore size distribution along the depth of the membrane. Mixed membranes have layers of different composition and pore size.

The internal structure of the membranes used in MBRs is composed by a thin surface layer which provide suitable permeation and by a thicker porous layer which provide mechanical stability.

1.4.5.2 Membrane configurations

The configuration of the membrane, for instance, the 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 three principal configurations permitting turbulence promotion and an efficient cleaning strategy with regards to MBR process (Figure 1-9):

- Flat sheet (FS)
- Hollow Fiber (HF)
- Multi-tubular (MT)

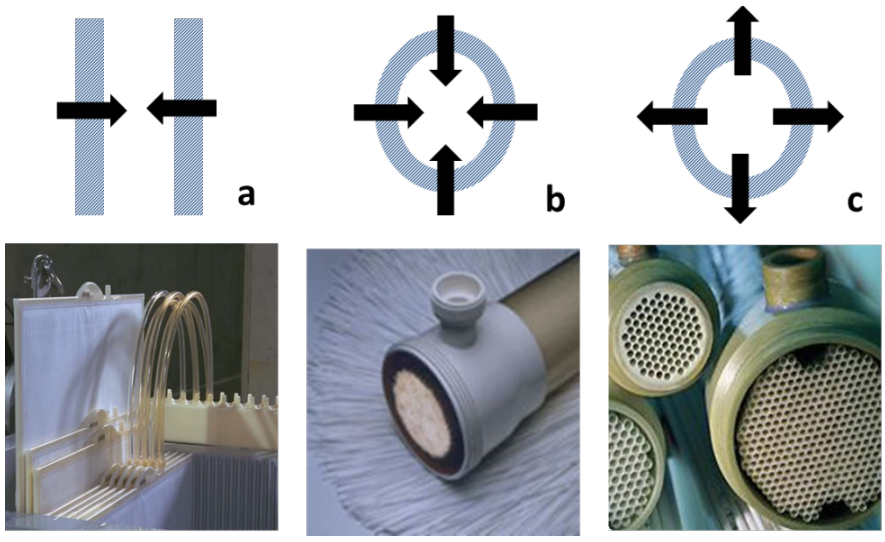

Figure 1-9 Schematics flow through membrane and pictures of (a) FS, (b) HF and (c) MT.

According to Judd, (2008), FS and HF membranes are placed inside the bioreactor in submerged MBRs, whereas MT membranes are placed outside in side-stream MBRs. HF membranes are cheaper, but require more frequent cleaning. FS membranes are 20 – 25% more expensive than hollow fiber but tend to run at higher permeability and are simpler to operate (Judd, 2005).

Moreover, with regards to filtration mode, in MT configuration is referred cross-flow, meaning that, for a single passage of activated sludge across the membrane, only a fraction is converted into permeate. Whereas to the filtration mode in FS and HF configurations, is dead-end operation mode, where all the activated sludge reaching the membrane is converted into permeate.

Therefore, the main considerations expected of a membrane module in this sense are (Judd, 2011):

- High membrane surface per unit of volume occupied by module;

- To promote a high turbulence which favors the permeate;

- To minimize energy consumption by unit volume of permeate;

- To reduce costs per unit membrane surface;

- Modular design which favors capacity expansions.

1.4.5.3 The key parameters of membrane filtration

The main parameters of any membrane process are the following:

Flux (J): quantity of liquid phase passing through a unit are of membrane per unit of time, in SI units m^3 m^{-2} s^{-1}, but more commonly expressed as liters per m^2 per hour (L m^{-2} h^{-1}).

$$J = \frac{TMP}{\mu_T \cdot R} \qquad \text{Eq. 1-1}$$

Transmembrane pressure (TMP): It is defined as the existing pressure drop (difference) between the membrane pressure at the sludge side and the pressure at the permeate side it is the driving force of the biomass separation process (bar).

μ_T: is the dynamic viscosity of the permeate at temperature T

Resistance (R): It is inversely related to permeability and to the fluid viscosity. It includes the membrane resistance, the resistance of the cake layer or biofilm

(reversible fouling) and the resistance due to pore blocking or adsorption (irreversible fouling). Hence:

Since flux and TMP are interrelated (Eq. 1-1), membrane filtration processes can be executed in constant flux or in constant pressure operation. However, for conventional pressure-driven water filtration, it is usual to fix the value of the flux and then determine the appropriate value for the TMP (Judd, 2011).

In order to take into account the temperature effect on viscosity Eq. 1-2 and 1-3 (Rosenberger et al., 2006) are considered:

$$\mu_T = \mu_{20} \cdot f_T \qquad \text{Eq. 1-2}$$

$$f_T = e^{-0.0239 \cdot (T-20)} \qquad \text{Eq. 1-3}$$

Permeability (P): It is calculated as permeate flux per unit of TMP and is usually given as L m^{-2} h^{-1} bar^{-1}. Hence:

$$P = \frac{J}{TMP} \qquad \text{Eq. 1-4}$$

It is an important parameter to measure the effect of fouling during filtration. The permeability can be also expressed as follows:

$$P = \frac{1}{\mu_{20} \cdot f_T \cdot R} = \frac{1}{\mu_{20} \cdot e^{-0.0239 \cdot (T-20)} \cdot R} \qquad \text{Eq. 1-5}$$

As stated in Eq. 1-5, the permeability *P* is correlated to the total hydraulic resistance R. Since fouling implies an increase in *R*, *P* is an indicator of the effect of fouling on the filtration process. Permeability also depends on permeate temperature T. Thus, the common approach for comparing hydraulic performances obtained at different temperatures is to normalize the operating flux at a reference temperature. The permeability at a temperature of 20 °C (P_{20}) is therefore introduced and calculated as the product of *P* and the factor f_t (Eq. 1-6), hence:

$$P_{20} = P \cdot f_T \qquad \text{Eq. 1-6}$$

Considering Eq. 1-5, P_{20} can be also expressed as follows:

$$P_{20} = \frac{1}{\mu_{20} \cdot R} \qquad \text{Eq. 1-7}$$

In conclusion, equation 1-7 states that P_{20} is function of R and μ_{20} (permeate viscosity) only. The latter parameter is considered a constant value, usually

equivalent to the water viscosity at 20 °C, that is 0.001 Pa s^{-1}. Consequently, P_{20} is an excellent parameter for measuring fouling effects over time, as its variations depend on R increase.

Specific aeration demand (SAD): It is the air flow necessary for membrane physical cleaning. It can be expressed as the air flow per membrane unit area (SAD_m m^3 m^{-2} h^{-1}) or as the air flow per permeate volume unit (SAD_p m^3air m^{-3}permeate).

1.4.6 Fouling in MBRs

1.4.6.1 Fouling mechanisms

The definitions and subdivisions of fouling vary according to the author. In this Thesis, membrane fouling refers to various phenomena related to the rejection of solids and their accumulation (deposition and/or adsorption) at the membrane surface (Judd, 2011). It is a major obstacle that hinders faster commercialization of MBRs because as a result of all these phenomena, an increase of TMP or a reduction of permeate flux occurs. This reduction leads to larger required membrane surfaces, higher applied pressures or crossflow velocities/shear rates which both result in higher energy expenditure, or frequent cleanings of the fouled membranes (Drews, 2010).

As shown in Figure 1-10, membrane fouling in MBRs can be attributed to membrane pore clogging and sludge cake deposition on membranes, which is normally the predominant fouling component (Lee et al., 2001).

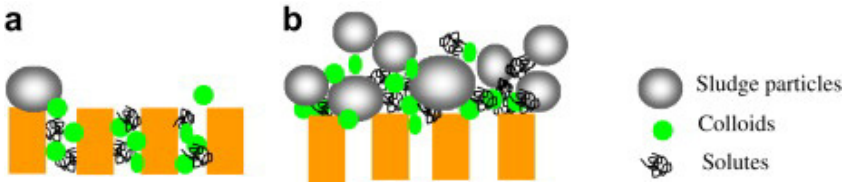

Figure 1-10 Membrane fouling process in MBRs: (a) pore blocking and (b) cake layer (source: Meng et al., 2009).

The factors effecting membrane fouling can be classified into four groups: membrane materials, biomass characteristics, feedwater characteristics, and operating conditions. The complex interactions between these aspects complicate the understanding of membrane fouling. For a given MBR process, the fouling behavior is directly determined by sludge characteristics and hydrodynamic conditions. But, operating conditions (i.e., SRT, HRT and food to microorganisms

ratio (F/M)) and feedwater have indirect actions on membrane fouling by modifying sludge characteristics (Meng et al., 2009).

In general, the increase of TMP due to gradual fouling of membranes occurs in three stages (Meng et al., 2009): 1) start-up with clean membrane and initial short-term rapid rise in TMP; 2) long-term weak rise in TMP; 3) sharp increase in dTMP/dt, also known as TMP jump.

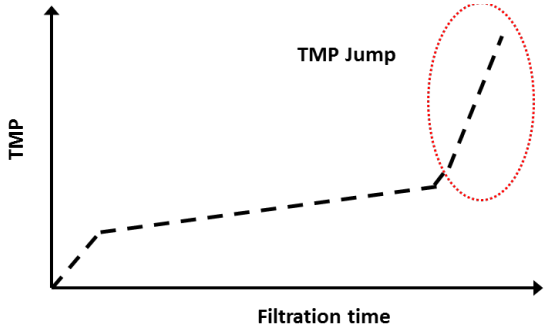

Figure 1-11 Displayed the generally evolution of TMP and the occurrence of TMP jump in a MBR.

Figure 1-11 shows the schematic illustration of the occurrence of TMP jump. The TMP jump is believed to be the consequence of severe membrane fouling. Cho et al., (2005) attributed the TMP jump to the changes in the local flux due to fouling eventually causing local fluxes to be higher than the critical flux.

From a mechanistic point of view, membrane fouling occurs due to the following mechanisms: (a) adsorption of solutes or colloids within/on membranes; (b) deposition of sludge flocs onto the membrane surface; (c) formation of a cake layer on the membrane surface; (d) detachment of foulants attributed mainly to shear forces; (e) the spatial and temporal changes of the foulant composition during the long-term operation (Meng et al., 2009).

From the viewpoint of fouling components, three main types of foulants can be differentiated (Mulder, 1996):

- Particulates: also named biofouling refers to the deposition, growth and metabolism of bacteria cells or flocs on the membrane. For a low pressure membrane such as MF and UF for treating wastewater, biofouling is a major problem because most foulants (microbial flocs) in MBRs are much larger than the membrane pore size.
- Organic precipitates: refers to the deposition of biopolymers (i.e., proteins and polysaccharides) on the membranes. Due to their small size, the

biopolymers can be deposited onto the membranes more readily because of the permeate flow, but they have lower back transport velocity due to lift forces in comparison to large particles (e.g., colloids and sludge flocs).

- Inorganic precipitate (metal hydroxides, calcium salts, etc.): changes in the environmental conditions (pH, solute/ion strength) are due to microorganisms actions in MBR which can form precipitates. Gelatinous precipitates (such as hydrated complex of calcium phosphate and citrate, etc.) can easily foul membranes.

Obviously, the MBR operation should be performed so as to prevent or delay the membrane fouling. To do so, the operation of the membrane modules includes physical and chemical cleaning strategies following the protocols given by manufactures.

1.4.6.2 Fouling control

There are three types of fouling: removable, irremovable and irreversible (Meng et al., 2009). The removable or reversible fouling is caused by loosely attached biomass and can be easily eliminated by implementation of physical cleaning (e.g., aeration bubbles during relaxation periods or backwashing). The irremovable fouling is caused by pore blocking and strongly attached foulants during filtration and needs chemical cleaning to be eliminated. In general, removable fouling is attributed to the formation of cake layer, and the irremovable fouling is attributed to pore blocking. The irreversible fouling is a permanent fouling which cannot be removed by any approaches.

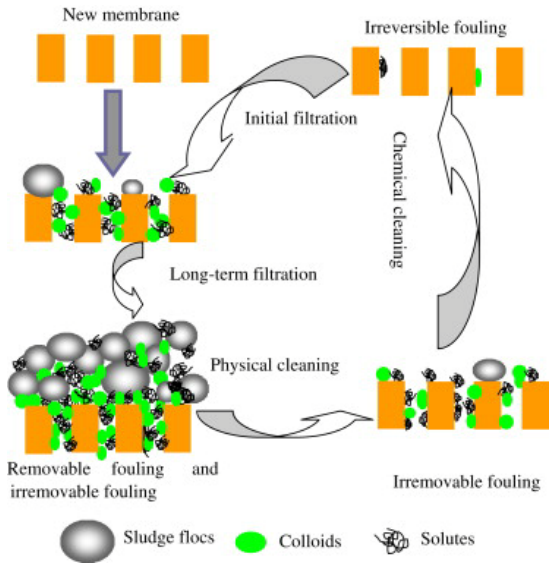

Figure 1-12 Schematic illustration of the formation and removal of removable and irremovable fouling in MBRs (source: Meng et al., 2009).

Generally, the control of fouling and clogging in practice is limited to five main strategies viable for full-scale operating MBR (Judd, 2011).

- Applying appropriate pre-treatment to the feed of water (e.g., precipitation of sparingly soluble ions or other problematic compounds that may foul the membrane);
- Employing appropriate physical or chemical cleaning protocols;
- Reducing of applied flux (in order to operate under sub-critical conditions);
- Increasing the aeration;
- Biochemically or chemically modifying the mixed liquor.

With regard to **physical cleaning**, it is normally achieved either by backwashing or relaxation periods. Back flushing consists on reversing the flow, and relaxation is simply ceasing permeation whilst continuing to scour the membrane with air bubbles. The physical cleaning is employed to eliminate the removable fouling. Both duration and frequency are key cleaning parameters since they determine process downtime and are conditioned by the recommendations of the manufacturer of the membrane modules.

Physical cleaning is supplemented with **chemical cleaning**. As inorganic fouling can result in severe irremovable fouling, chemical cleaning is more effective than physical cleaning in the removal of inorganic precipitation. Two strategies are used: maintenance and intensive chemical cleaning. Both can be carried out with organic acid (citric or oxalic for removing inorganic scalants) or sodium hypochlorite (for removing organic matter).

- The maintenance chemical cleaning is performed in situ ("cleaning in place" or CIP) and is used to maintain membrane permeability and minimize the frequency of intensive cleaning. Normally, the CIP is conducted at intervals of several weeks and low concentration of chemical cleaning agent can be added to the backflush water to produce a chemically enhanced backflush.
- Intensive chemical cleaning is carried out using high chemical concentration and can be performed either in situ (CIP) or ex situ ("cleaning out of place" or COP). Intensive cleaning is generally conducted when further filtration is no longer sustainable because of an elevated TMP (once or twice a year).

On the other hand, reducing the flux always reduces fouling. Operating with a flux below the critical flux prevents high TMP. However, obviously this strategy directly impacts on capital cost through a greater demand of membrane area.

The rise in the **aeration rate** increases the critical flux up to some threshold value. However, this strategy is normally prohibitively expensive. Much attention has been focused on commercial development of efficient and effective aeration systems to reduce the specific aeration demand. In this respect, developing methods of ensuring homogeneity of air distribution will advance both fouling and clogging control.

Mixed liquor characteristics are due to a combination of multiple factors, which are the final product of the characteristics of the feed water and the operating conditions of the bioreactor. In this sense, the feed water characteristics can be changed with difficulty, whereby the most common strategy to limit the membrane fouling is to act on operation variables, e.g. TMP, aeration type, F/M, etc.

Some operation parameters, such as SRT and F/M ratio should be controlled in order to limit membrane fouling. SRT between 20 and 50 d is recommended and higher F/M ratios lead to higher fouling rates (Meng et al., 2009). Typical values

for F/M in aerobic MBR treating municipal wastewater are in the range of 0.1 - 0.3 kg COD kg $MLVSS^{-1}$ d^{-1} (Judd, 2011).

The characteristics of the mixed liquor are the factor on which a major research effort has been made regarding membrane fouling. Several attempts have been made to correlate membrane fouling with biomass concentration (Judd, 2006; Chang et al., 2002; Le-Clech et al., 2003), floc size, sludge rheology (Chang et al., 2002) and the concentration of suspended extracellular polymeric substances (EPS) (Rosenberger and Kraume, 2002) and soluble microbial products (SMP) (Chang et al., 2002; Ji and Zhou, 2006).

Since one of the advantages of MBR is the possibility of increasing MLSS concentration, this was one of the first parameters to be investigated. The absence of a clear correlation between MLSS concentration and fouling parameters leads to the conclusion that MLSS alone is a poor indicator of biomass fouling propensity (Jefferson et al., 2004). One possible explanation for apparently contradictory results in the literature is that the effect of MLSS concentration on filtration resistance varies according to the applied MLSS range in MBR operation (Lousada-Ferreira et al., 2010); whereby, it should not be excluded it as a fouling parameter.

The study of the constituents of the mixed liquor and their respective contribution to fouling was undertaken by Wisniewski et al., (2000) and Bouhabila et al., (2001) among others. According to Bouhabila et al., (2001), colloids are responsible for 50% of the fouling.

All studies apply different means of fractionation, resulting in different and contradictory results. Furthermore, the filtration cells used to determine filterability or specific resistance of the different fractions are operated under totally different conditions to real ones in an MBR. However, the general observed trend is that soluble constituents are involved in membrane fouling for at least 50%.

A closer investigation of this fraction made clear that the so-called extracellular polymeric substance (EPS) form the most ‘dangerous’ part of the soluble fraction (Rosenberger and Kraume, 2002). EPS consist of insoluble materials (sheaths, capsular polymers, condensed gel, loosely bound polymers and attached organic materials) secreted by the cell, shed from the cell surface or generated by cell lysis. Moreover, SMP are defined as soluble cellular components released during substrate metabolism (usually with biomass growth) and cell lysis (Jang et al., 2007). The EPS and SMP are constituted by proteins, polysaccharides, nucleic acids, lipids, and humic acids. Some authors report an influence of SRT and F/M in the EPS production. Others suggest that ionic strength and substrate conditions

are involved in this process. There seems to be an optimum SRT value for which SMP production is minimum. The same holds for organic loading rate; minimum SMP production was observed at 0.3 - 1.2 g COD g $MLSS^{-1}$ d^{-1}. Increasing MLSS concentration leads to higher SMP concentrations (Barker and Stuckey, 1999).

Recently, the terms biopolymers or biopolymeric clusters (BPC) have also come into use (Wang and Li, 2008). BPC are defined as a new pool of organic substances in the MBR sludge mixture that are solute independent of the biomass and are much larger than SMP in the sludge suspension. Normally, the BPC content in the MBR is estimated by calculating the difference in TOC concentration between the AS supernatant and the effluent (Sun et al., 2008), but organic matter can also be measured in terms of COD (Lin et al., 2009). Therefore is a reliable easy to measure method compared with proteins, polysaccharides or nucleic acids.

Another group, which until recently had only been studied in the formation of biofilms in sea water environments, are the so-called transparent exopolymer particles (TEP) (Drews, 2010). 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.

All these groups of compounds are produced and excreted by microorganisms but what is analyzed as EPS, SMP, BPC or TEP by commonly agreed on methods is not necessarily of microbial origin but can also be terrestrial or manmade (Drews, 2010). In addition, Drews, (2010) also stresses that these groups are not distinct but may overlap (depending on the assays), such as shown in Figure 1-13. Nowadays, the location of the fouling relevant fraction is still unknown.

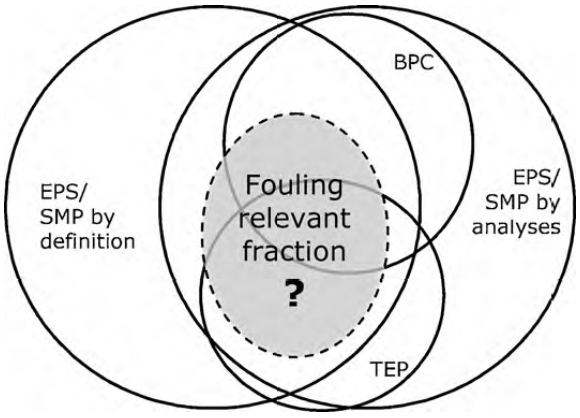

Fig 1-13 Possible relation between different polymer fractions (source: Drews, 2010).

Recently, studies have shown that a modicum of fouling control can be attained through the addition of chemicals (coagulant/flocculants, adsorbent agents and membrane performance enhancers) (Zhang et al., 2008; Teli et al., 2012), which may be especially useful in real wastewater treatment plants.

1.5 HYBRID AND OTHER MBR TECHNOLOGY

In an attempt to improve the elimination capacity even further, enhance the operation or expand the uses, several combinations of MBRs with waste water technologies other than the CAS process, have been proposed. Thus, in the literature is common to find the term hybrid MBR in a broad sense, namely for an MBR combined with any other technology.

Examples of such technologies:

- Hybrid biofilm – suspended biomass MBR (HMBR), in which both suspended and biofilm biomass grow in the reactor (Oyanedel et al., 2005).
- Biofilm MBR (BF-MBR) in which the growth of biomass develops on a plastic material (Leiknes and Odegaard, 2007).
- Dual configuration Activated Sludge – MBR Technology, in which a secondary settler and a filtration membrane are used in parallel for promoting the separation of the treated water (Amadeus report 2009).
- Anaerobic membrane bioreactor (AnMBR), which is a combination of an anaerobic reactor coupled with a membrane unit (Liao et al., 2006).
- Methanogenic – MBR, that are a combination of a first methanogenic stage and a second stage in which the remaining organic matter fraction is treated in a MBR (Sánchez et al., 2013).

Nevertheless, it is important to note that the term hybrid MBR is mostly applied when suspended sludge and biofilm coexist in the MBR reactor, being this terminology adopted for this Doctoral Thesis.

The implementation of biofilm processes in MBR systems by addition of support media for biofilm growth is covered in the following section.

1.5.1 Biofilm Membrane Bioreactors (BF-MBR)

Biofilm technologies are systems where the growth of biomass develops on a support media and their main advantages were collected in section 1.3.

The moving bed biofilm reactor (MBBR) 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). These systems are operated similarly to the activated sludge process with the addition of freely moving carrier media (Odegaard, 2006). Thus, many are the advantages of the MBBR process over the conventional activated sludge (CAS) (section 1.3.2.2).

Recently proposed, from the combination of MBBR technology with the membrane bioreactors technology arises a new system called Moving Bed Membrane Bioreactor (MBMBR) or Biofilm Membrane Bioreactor (BF-MBR). This system would present an alternative to the activated sludge MBR, since, the BF-MBR aims to partially mitigate the aforementioned fouling and the settleability concerns with MBBRs. The membrane module in this kind of systems is located in a separate chamber from the containing the carriers in order to avoid possible damages in the membrane. Originally introduced by Leiknes and Odegaard, (2007), the BF-MBR or MBMBR process has shown good treatment efficiencies with production of high quality effluent. Typical schematic for BF-MBR process is presented in Figure 1-14.

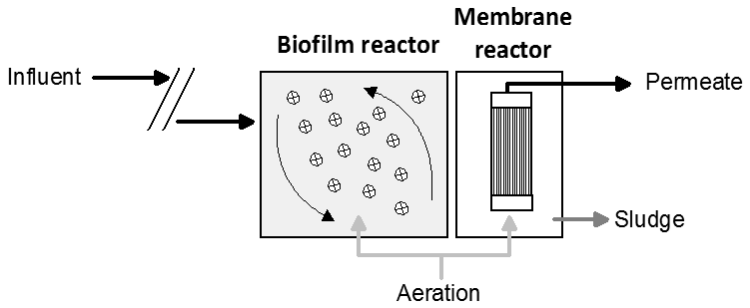

Figure 1-14 Biofilm Membrane Bioreactor (BF-MBR) (adapted from source: Leiknes and Odegaard, 2007) .

Comparing with other membrane bioreactor, BF-MBR could operate at 10 – 15 times higher volumetric loading and at 10 – 30 times shorter HRT (Sombatsompop et al., 2006). In addition, Leiknes and Odegaard, (2007) achieved in a BF-MBR fluxes around 50 L m^{-2} h^{-1}, which are much higher than fluxes typically reported in conventional MBR (20 – 25 L m^{-2} h^{-1}) operating with similar membrane modules (Judd, 2002).

Within this BF-MBR technology, Ivanovic and colleagues differentiated between pure BF-MBR (pBF-MBR) and assisted BF-MBR (aBF-MBR) (Ivanovic and Leiknes,

2012). In pBF-MBR biodegradation is exclusively carried out by attached biomass and the activity of suspended biomass is neglected due to very low concentrations and low biologically active MLSS in the reactor (Ivanovic and Leiknes, 2012; Phattaranawik and Leiknes, 2009). Whilst in aBF-MBR biodegradation is carried out by both biomasses (Jamal Khan et al., 2011; Liu et al., 2010). In addition, the aBF-MBR has been defined as a hybrid MBR by some authors.

As noted in section 1.4.4, successful application of MBR technology in full-scale wastewater treatment is hindered by the costly and energy intensive cleaning processes required for the removal of the aforementioned foulants.

Many researches have pointed out that the applicability of BF-MBR is an alternative to reduce the effect of membrane fouling (Leiknes and Odegaard, 2007; Liu et al., 2010). Some studies have also reported the ability of the attached biofilm to adsorb organic foulants generated from biological processes from the liquid (Liu et al., 2010; Wang et al., 2010). This tends to explain the reason why the TMP decreases under the assistance of the biomass on the suspended carriers (Liu et al., 2010). Contradictory results have been presented in terms of major foulants production and the potential reduction of fouling rates in parallel conventional MBR and BF-MBR operations (Yang et al., 2009a). Therefore, further studies are still needed in order to better know the implications and mechanisms of this technology regarding membrane fouling.

1.5.2 Hybrid (suspended – biofilm biomass) membrane bioreactors

Hybrid (or assisted biofilm) MBRs are obtained by adding biofilm support in a conventional MBR process. The motives for using biofilm supports include the reduction of the negative effects of high suspended solids, lower membrane fouling or improved nutrient removal (Ivanovic et al., 2012). In other cases, HMBR arised form adding membrane filtration to hybrid systems (Oyanedel et al., 2003). They observed a decrease in sludge settleability in hybrid systems with high organic loading rates, and thus replaced settlers with membranes for biomass separation, overcoming this limitation (Figure 1-15).

During the last decade, the University of Cantabria and the University of Santiago de Compostela have been developing hybrid MBR systems (Garrido et al., 2002; Tejero and Cuevas, 2005), which have been designed using fixed support, suspended moving support and others configurations. Therefore, these systems could be considered a combination of both, the typical suspended biomass MBR

and the BF-MBR system, but these were developed several years before BF-MBR were reported.

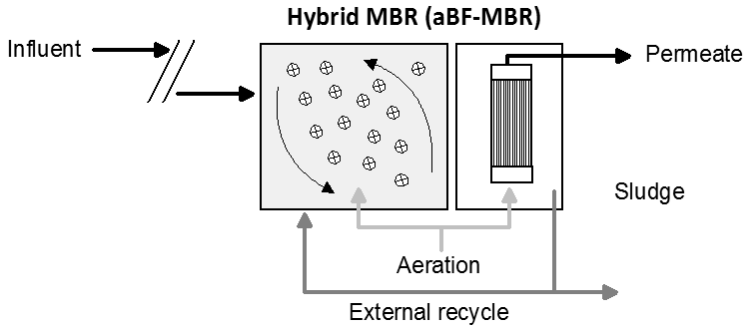

Figure 1-15 Hybrid MBR (aBF-MBR)

One of the most important features of the Hybrid MBR technology is that a fraction of total biomass grows on support media. Moreover, due to those biomasses (suspension and biofilm) competition for the substrate, the growth of nitrifiers in HMBR systems is promoted in the biofilm while heterothops are maintained in suspension. Besides the advantages of high biomass concentration due to the high specific surface area for biofilm growth, the immobilized microbial cells offer an additional advantage in the continuously aerated bioreactor: the occurrence of simultaneous nitrification and denitrification (SND). Denitrification can take place in the inner parts of the biofilm where anoxic conditions exist, as indicated in section 1.3.1.

In conclusion HMBR systems have also several advantages in comparison with conventional activated sludge systems like:

- Small footprint;
- High biomass concentrations;
- High solids retention time;
- Increased treatment capacity;
- Reduced sludge production;
- Enhanced sludge settleability.

With regard to conventional MBR, the use of support media provides advantages that include (Ivanovic and Leiknes, 2012):

- Improved nutrient removal;
- Reduction of a negative effect of suspended solids;
- Improved filterability and in some cases decreased membrane fouling.

The basis of the research presented in this Thesis, is to provide a more thorough understanding of hybrid MBR technologies. A summary of the current state of the literature as well as the contributions from this Thesis is presented in Table 1-1.

Table 1-1 Summary of literature on hybrid MBR

Hybrid MBR Research	Wastewater Type	Support Type	Reference
Development of a membrane-assisted hybrid bioreactor for ammonia and COD removal in wastewaters	Synthetic: varying COD (COD < 450 mg L^{-1}) (OLR < 3.6 kg COD m^{-3} d^{-1})	Moving: (granular particles)	(Oyanedel et al., 2005)
Comparison of the filtration characteristics between attached and suspended growth microorganisms in submerged membrane bioreactor	Synthetic (COD: 250 mg L^{-1}) (OLR: 0.75 kg COD m^{-3} d^{-1})	Fixed: looped cord media (BioMatrix Technologies Inc., USA)	(Lee et al., 2001)
Factors affecting filtration characteristics in membrane coupled moving bed biofilm reactor	Synthetic (COD: 1000 mg L^{-1}) (OLR: 2.4 kg COD m^{-3} d^{-1})	Moving: polyurethane cubes coated with activated carbon	(Lee et al., 2006)
Membrane Fouling Control of Hybrid Membrane Bioreactor: effect of Extracellular Polymeric Substances	Municipal after a sand settler and a coarse screen (COD: 240-846 mg L^{-1})	Moving: Kaldnes K3, AnoxKaldnes	(Wang et al., 2010)
Performance of a hybrid membrane bioreactor in municipal wastewater treatment	Municipal (COD: 243-846 mg L^{-1})	Moving: Kaldnes K3, AnoxKaldnes	(Liu et al., 2010)
Performance of suspended and attached growth MBR systems in treating high strength synthetic wastewater	Synthetic (COD: 1000 mg L^{-1}) (OLR: 3 kg COD m^{-3} d^{-1})	Moving: polyurethane sponge, Unifoam from United Foam Industries	(Jamal Khan et al., 2011)
Evaluation of biofouling phenomenon in suspended and attached growth membrane bioreactor systems	Synthetic (COD: 500 mg L^{-1})	Moving: cylindrical polypropylene hollow rings	(Sombatsompop et al., 2006)
Biomass characteristics of two types of submerged membrane bioreactors for nitrogen removal from wastewater	Synthetic (nonfat dry milk powder) (COD: 600 mg L^{-1})	Moving: AqWise biomass carrier, Siemens	(Liang et al., 2010)
Comparison between a moving bed membrane bioreactor and a conventional membrane bioreactor on organic carbon and nitrogen removal	Synthetic (COD: 400-900 mg L^{-1}) (OLR: 0.8-1.6 kg COD m^{-3} d^{-1})	Moving: new kind of nonwovens carriers	(Yang et al., 2009c)

Hybrid MBR Research	Wastewater Type	Support Type	Reference
Comparison between a moving bed membrane bioreactor and a conventional membrane bioreactor on membrane fouling	Synthetic (COD: 800 mg L^{-1})	Moving: new kind of nonwovens carriers	(Yang et al., 2009a)
Mechanism of membrane fouling control by suspended carriers in a submerged membrane bioreactor	Synthetic	Moving: suspended carrier, cylindrical rigid body	(Huang et al., 2008)
Effect of a suspended carrier on membrane fouling in a submerged membrane bioreactor	Activated sludge from a pilot-scale SMBR treating municipal wastewater	Moving: suspended carrier cylindrical	(Wei et al., 2006)
Membrane fouling control in a submerged membrane bioreactor with porous, flexible suspended carriers	Terephthalic acid wastewater discharged (COD: 1560 mg L^{-1})	Moving: flexible suspended carrier	(Yang et al., 2006)
Filtration characteristics of activated sludge in hybrid membrane bioreactor with porous suspended carriers (HMBR)	Synthetic (COD: 250-1470 mg L^{-1}); Industrial (COD: 760-1760 mg L^{-1})	Moving: flexible suspended carrier	(Yang et al., 2009b)
Roles of sponge sizes and membrane types in a single stage sponge-submerged membrane bioreactor for improving nutrient removal from wastewater for reuse	Synthetic (COD: 340-390 mg L^{-1})	Moving: polyester urethane sponge	(Guo et al., 2009)
Influence of biofilm carriers on membrane fouling propensity in moving biofilm membrane bioreactor	Synthetic (OLR: 3 kg COD m^{-3} d^{-1})	Moving: Polyurethane sponge	(Jamal Khan et al., 2012)
Comparative kinetic study between moving bed biofilm reactor-membrane bioreactor and membrane bioreactor systems and their influence on organic matter and nutrients removal	Municipal (COD < 80 mg L^{-1})	Moving: Kaldnes K1, AnoxKaldnes	(Leyva-Díaz et al., 2013)
Impact of methanogenic pretreatment on the performance of an aerobic MBR system	Synthetic of diluted skimmed milk (COD: 500-2500 mg L^{-1})	Moving: Kaldnes K3, AnoxKaldnes	(Sánchez et al., 2013)
New process for alleviation of membrane fouling of modified hybrid MBR system for advanced domestic wastewater treatment	Municipal (COD: 500 mg L^{-1})	Fixed: synthetic fibrous fabric carrier	(Shuo et al., 2008)

Hybrid MBR Research	Wastewater Type	Support Type	Reference
Influence of activated sludge characteristics on membrane fouling in a hybrid membrane bioreactor	Municipal after a sand settler and a coarse screen (COD: 240-846 mg L^{-1})	Moving: Kaldnes K3, AnoxKaldnes	(Wang et al., 2012)
Evaluation of a hybrid vertical membrane bioreactor (HVMBR) for wastewater treatment	Municipal after coarse and fine screen, grit and grease removal (COD: 118-1873 mg L^{-1}) (OLR: 1.1-2.6 kg COD m^{-3} d^{-1})	Fixed: sponge	(Rodríguez-Hernández et al., 2012) (chapter 3)
Hybrid membrane bioreactor application for decentralized treatment and reuse	Municipal after coarse and fine screen, grit and grease removal (average COD: 400 mg L^{-1}) (OLR: 0.36-1.71 kg COD m^{-3} d^{-1})	Fixed: BLAS®	(Rodríguez-Hernández et al., 2013) (chapter 4)
Comparison between a fixed bed hybrid membrane bioreactor and a conventional membrane bioreactor for municipal wastewater treatment: a pilot-scale study	Municipal after coarse and fine screen, grit and grease removal (average COD: 400 mg L^{-1}) (OLR: 0.36-3.87 kg COD m^{-3} d^{-1})	Fixed: BLAS®	(Rodríguez-Hernández et al., 2013) (Rodríguez-Hernández et al., 2014) (chapter 5)

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Chapter 2

Materials & methods

OUTLINE

SUMMARY

In this chapter, detailed information about the materials and methods used in this Thesis are explained, including:

- Conventional chemical parameters used to characterize the liquid phase, such as COD, BOD_5, forms of nitrogen, phosphates, etc.
- Parameters to characterize the solid and gaseous phases, such as total and volatile suspended solids (TSS and VSS), biomass composition, methane concentration in gas, etc.
- Techniques for the characterization of the biomass present in the different stages of the experimental setup, and other microbiological determinations, for instance Fluorescent In Situ Hybridization (FISH) applied to biofilm and suspended biomass.
- Methodology applied for membrane filtration control and monitoring.

The specific methods and corresponding experimental set-up employed in a single part of the work are described in the corresponding chapters.

EXPERIMENTAL SET-UPS

The experimental study of this research Thesis was carried out in three configurations. The work presented in chapter 3 was conducted in a bench-scale pilot plant. Chapters 4 and 5 refer to semi-industrial pilot plants, located at the University of Cantabria (UC, Cantabria, Spain). Finally, the experimentation presented in chapters 6 was conducted in a bench-scale pilot plant at the University of Santiago de Compostela (USC, Galicia, Spain) during a pre-doctoral stage.

The features of the pilot plants used in every part of the work are described in the corresponding chapters.

2.1 LIQUID PHASE ANALYTICAL METHODS

Different methods were employed during the experimental period for the determination of the conventional parameters of wastewater and sludge. For soluble fraction analysis, samples were previously filtered with a pore size of 0.45 µm in order to remove suspended solids.

2.1.1 Carbon compounds

2.1.1.1 Chemical Organic Demand (COD)

The Chemical Oxygen Demand (COD) is defined as the amount of oxygen required to oxidise the organic matter present in a liquid sample (in this case wastewater) using a strong chemical oxidant (potassium dichromate) in an acid medium.

The total and soluble Chemical Oxygen Demand (COD_t and COD_s) were determined following the method 5220C of the Standard Methods for the Examination of Water and Wastewater (APHA, 1998). The difference between total COD and soluble (filtrated) COD is that COD_t is determined using the raw sample, while for COD_s determination, the sample is previously filtered through filters (nitrocellulose-fiber Whatman, GCF or similar) with a pore size of 0.45 µm.

Silver sulphate is used as catalyst 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.

Reagents:

$K_2Cr_2O_7$ digestion solution: 4.913 g of $K_2Cr_2O_7$ (previously dried at 103 °C for 2 hours) are dissolved in 500 mL of distilled water. Then, 167 mL of concentrated H_2SO_4 are added. Once cooled, the solution is stirred and 33.3 g of $HgSO_4$, dissolved in 500 mL of distilled water, are added. The solution is cooled to room temperature and, finally, diluted to 1000 mL.

H_2SO_4 + Ag_2SO_4 reagent: 10.12 g of Ag_2SO_4 are added to 1 L of concentrated H_2SO_4. The solution must stand 2 days to dissolve before use.

Ferroin indicator solution: 1.485 g of $C_{18}H_8N_2 \cdot H_2O$ (phenanthroline monohydrate) and 0.695 g of $FeSO_4 7H_2O$ are dissolved in 100 mL of distilled water.

Standard ferrous ammonium sulphate titrant (FAS): 39.2 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.

FAS factor calculation:

Every sample (2.5 mL) is placed in a Pyrex tube of 10 mL (six samples). 1.5 mL of digestion solution and 3.5 mL of sulphuric acid reagent are added in each tube. After being tightly capped, the tubes are mixed completely. Then, the content of each tube is transferred to a beaker and, once 1-2 drops of ferroin indicator are added, the solution is titrated under rapid stirring with FAS titrant. The *end-point is* a sharp color change from blue-green to reddish brown. FAS factor is calculated with the following Equation 2-1:

$$Factor\ FAS = Factor\ FAS \frac{480}{V_{fas}} \quad \text{Eq. 2-1}$$

Where:

V_{fas} : average value of FAS volume consumed in each titration (mL).

Procedure:

This procedure is applicable to samples with COD concentrations between 40 – 400 mg L^{-1}. 2.5 mL of sample are placed in 10 mL Pyrex tubes 1.5 mL of digestion solution ($K_2Cr_2O_7$) and 3.5 mL of sulphuric acid reagent are added 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 tightly capped, the tubes are finally mixed completely and placed in the digester preheated to 150 °C. The duration of the digestion period is 2 h. After digestion, the tubes are cooled to room temperature. Then, the content of each tube 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 end-point is a sharp colour change from blue-green to reddish-brown. The COD is calculated with Equation 2-2:

$$COD = (A - B) \cdot F \quad \text{Eq. 2-2}$$

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;

F: factor FAS.

2.1.1.2 Biological Oxygen Demand (BOD)

Biological oxygen demand (BOD) was carried out using respirometric BOD Oxitop® method, based also on Standard Methods 5210D (APHA, 1998). BOD measurements can be used to evaluate the impact of biodegradable substances in waters and wastewater by measuring the quality of water and treatment results in wastewater. Further, BOD instrumentation measurements are used in the planning and design of wastewater treatment facilities.

In Oxitop® method, which is especially designed to determine the BOD of wastewater, Oxygen consumption by microorganisms is determined by the decrease of gas pressure in a closed bottle, being the CO_2 generated absorbed by NaOH. The measurements are fully automated and the instrument calculates biological oxygen demand (BOD) in mg L^{-1}. The sample volume regulates the amount of oxygen available for a complete BOD. BOD of up to 4,000 mg L^{-1} can be measured using different volumes.

2.1.1.3 Total Dissolved Carbon (TDC), Dissolved Organic Carbon (DOC) and Dissolved Inorganic Carbon (DIC)

The organic carbon in water and wastewater may include a variety of organic compounds in different oxidation states. Some of this carbon compounds can be oxidized further by chemical or biological processes and the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) may be used to characterize these fractions. Total Organic Carbon (TOC) is a more convenient and direct expression of total organic content than either BOD or COD, but does not provide the same kind of information. Unlike BOD or COD, TOC is independent of the oxidation state of the organic matter and does not measure other organically bound elements, such as nitrogen and hydrogen, and inorganics that can contribute to the oxygen demand measured by BOD and COD (APHA, 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.

Since the equipment employed could exclusively analyze filtered samples, only dissolved carbon was measured. DOC concentration was determined by a Shimadzu analyzer (TOC-5000) as the difference between the Total Dissolved Carbon (TDC) and the Dissolved Inorganic Carbon (DIC). The instrument was connected to an automated sampler (TOC-V CHS Shimadzu, ASI-5000-S). The TDC

was determined from the amount of CO_2 produced during the combustion of the sample at 680 °C, using platinum immobilized over alumina spheres as catalyst.

The DIC concentration was 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 was optically measured with a non-dispersive infrared analyzer (NDIR) after being cooled and dried. High purity air was used as carrier gas with a flow of 150 mL min^{-1}. A curve comprising 4 calibration points in the range from 0 to 1 g C L^{-1}, using potassium phthalate as standard for TDC and a mixture of sodium carbonate and bicarbonate for DIC, was used for the quantification. The detection limit of the equipment was 2 mg L^{-1}.

2.1.1.4 Volatile Fatty Acids (VFA)

Volatile Fatty Acids (VFA), acetic, propionic, i-butyric, n-butyric, i-valeric and n-valeric, are intermediate products of the anaerobic digestion. A VFA accumulation reflects a kinetic disequilibrium between the acids producers and the acids consumers (Switzenbaum et al., 1990) and it is an indicator of process destabilization.

VFA were determined by gas chromatography (HP, 5890A) equipped with a Flame Ionization Detector (FID) and an automatic injector (HP, 7673A). The determination was performed in a glass column (3 m long and 2 mm of internal diameter) filled with Chromosorb WAW (mesh 100/120) impregnated with neopentylgiycol adipate (NPGA) (25%) and H_3PO_4 (2%). The column, injector and detector temperatures were 105, 260 and 280 °C, respectively. Gas N_2, previously saturated with formic acid before entering into the injector, was the carrier gas with a flow of 24 mL min^{-1}. Air and H_2 were 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, were burnt in a H_2-air flame and finally measured in the FID at 280 °C. The quantification of the sample was 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. The detection limit of the equipment was 20 mg L^{-1}.

2.1.2 Nitrogen

In waters and wastewaters, the forms of nitrogen of greatest interest are, in order of decreasing oxidation state, nitrate, nitrite, ammonia and organic nitrogen. All these forms, as well as nitrogen gas (N_2), are biochemically interconvertible and they are the components of the nitrogen cycle.

2.1.2.1 Total Nitrogen (TN)

Total nitrogen (TN) was determined as the sum of ammonium nitrogen (NH_4^+-N), organic nitrogen (N_{org}-N), nitrites (NO_2^--N) and nitrates (NO_3^--N) concentrations as mg L^{-1}. Every compound was analysed independently as described below.

2.1.2.2 Ammonium (NH_4^+-N)

Ammonium (NH_4^+-N) can be analysed in different ways. During the experimental period two methods were used: selective electrode (chapters 3, 4 and 5) and colorimetric method (chapter 6).

a) Determination of ammonium by selective electrode

The ammonium ion-selective electrode has a solid-state PVC polymer matrix membrane which is designed for the detection of ammonium ions (NH_4^+) in aqueous solutions.

Materials:

Ion-selective electrode, Thermo Orion Model 95-12

Orion 720 Aplus computer analyser.

Reagents:

Standard solution: 1000 ppm of NH_3-N

Buffer solution: (ISAB) or NaOH 10N

Procedure:

Before use, the electrodes must be calibrated by measuring a series of known standard solutions (e.g., 1, 10 and 20 ppm), made by serial dilution of the 1000 ppm standard solution. Following the General Operating Instructions, these standard solutions are measured and the curve calibration is prepared.

To measure a sample, the electrode is directly immersed in a beaker with approximately 50 to 100 mL of sample. The electrode must be washed and dried between each sample, to avoid cross contamination. To permit the electrode signal to reach a stable value, sufficient time after immersion must be allowed (2 or 3 minutes) before taking a reading. For better accuracy, frequent recalibration is recommended. The results will be displayed as ppm and mol L^{-1}.

b) Determination of Ammonia, method Bower, Holm-Hansen

Total ammonia-nitrogen (NH_4^+-N) was determined spectrophotometrically by this method through the production of indophenol blue by the reaction of ammonia with salicylate and hypochlorite, in the presence of sodium nitroprusside (Bower and Holm-Hansen, 1980). This method is safer than the phenol-hypochlorite method, 4500-NH3F (APHA, 1998), because phenol is not used, but it may not be suitable for field determinations because of photosensitivity. The characteristic colours produced by increasing concentrations of ammonia make the assay useful for the direct, visual estimation of ammonia in culture systems.

Reagents:

a) Reactive A: 0.28 g L^{-1} sodium nitroprusside and 440 g L^{-1} sodium salicylate.

b) Reactive B: 18.5 g L^{-1} NaOH and 120 g L^{-1} sodium citrate.

c) Reactive C: Standard commercial solution of sodium hypochlorite.

d) Reactive D: 7 part of B and 1 part of C (stable for 1 hour).

Procedure:

120 µL of reactive A and 200 µL of reactive D are added to 1 mL of sample (diluted if necessary). Then, each sample is stored protected from light for more than 2 hours but less than 3 hours. Then, it is measured at 640 nm and compare with a calibration curve.

2.1.2.3 Total Kjeldahl Nitrogen (TKN)

Total Kjeldahl Nitrogen method determines nitrogen in the tri-negative state or the sum of organic nitrogen (N_{org}-N) and ammonia nitrogen (NH_4^+-N). Having the ammonia nitrogen concentration, organic nitrogen can be determined. TKN was analysed by adapting method 4500-Norg B of Standard Methods (APHA, 1998).

In the presence of H_2SO_4, potassium sulphate (K_2SO_4) and cupric sulphate ($CuSO_4$) catalyst organic nitrogen is converted to ammonium. Free ammonia is also converted to ammonium. After digestion, alkalization and distillation using boric acid or sulphuric acid as absorbent solution, ammonia in the distillate can be determined colorimetrically, by ammonia-selective electrode, or by titration with a standard mineral acid. The result is presented as mg TKN-N L^{-1}.

2.1.2.4 Organic Nitrogen (Norg)

Organic nitrogen can be calculated as the difference between Total Kjeldahl Nitrogen (TKN) and ammonium nitrogen (NH_4^+-N). The result is presented as mg N_{org}-N L^{-1}.

2.1.2.5 Nitrites (NO_2^--N) and nitrates (NO_3^--N)

Nitrites (NO_2^--N) and nitrates (NO_3^--N) were analysed in different ways, depending on the experimental part of the research. Two methods were used: ion Chromatography (IC) and colorimetry.

a) Determination by Ion Chromatography

Ion chromatography with chemical suppression of eluent conductivity is a method for the determination of common anions such as chloride, bromide, fluoride, nitrate, nitrite, phosphate and sulphate. Nitrates, nitrites and phosphates were analysed following method 4110 B from Standard Methods (APHA, 1998).

Procedure:

A water sample is injected into a stream of carbonate-bicarbonate eluent and passed through a series of ion exchangers. The anions of interest are separated on the basis of their relative affinities for a low capacity, strongly basic anion exchanger (guard and separator columns). The separated anions are directed through a hollow fiber cation exchanger membrane (fibre suppressor) or micromembrane suppressor bathed in continuously flowing strongly acid solution (regenerant solution). In the suppressor the separated anions are converted to their highly conductive acid forms and the carbonate-bicarbonate eluent is converted to weakly conductive carbonic acid. The separated anions and their acids forms are measured by conductivity. They are identified on the basis of retention time as compared to standards. Quantitation is performed by measurement of peak area or peak height.

Ion Chromatography: Agilent and Metrohm

The samples were injected automatically through a compact autosampler and using a loop of 20 µL. The total time for every sample is around 30 minutes.

All the samples were filtered at 0.2 µm before their introduction in the autosampler.

b) Determination by Colorimetry

Nitrite and nitrate concentration in wastewater were determined following the method 4500-NO_2^--B and 4500-NO_3^--B described in Standard Methods for the Examination of Water and Wastewater (APHA, 1998).

Nitrite (NO_2^--N)

Nitrite was determined through the formation of a reddish purple azo dye produced at pH 2.0 – 2.5 by coupling diazotized sulphanilamide with N-(1-napththyl)-ethylenediaminedihydrochloride (NED dihydrochloride). This method is suitable for concentration of 0.01 to 1 mg NO_2^--N L^{-1}.

Reagents:

Solution 1: 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.

Solution 2: NED. 0.5 g of NED is dissolved in 500 mL of distilled water.

Procedure:

0.1 mL of each solution (sulphanilamide and NED) are added to 5 mL of sample (diluted if necessary to have a maximum concentration of 0.30 mg NO_2^--N L^{-1}), previously filtrated through 0.45 µm filter. After 20 min, the intensity of color of the sample is measured in a spectrophotometer (Cecil CE 7200) at 543 nm. The quantification is done with a 5-7 points calibration curve in the range of 0 – 0.30 mg NO_2^--N L^{-1}, using $NaNO_2$ as standard.

Nitrate (NO_3^--N)

Measurement of UV absorption at 220 nm allowed the rapid determination of NO_3^- ions. Since dissolved organic matter may also absorb at 220 nm and NO_3^- does not absorb at 275 nm, a second measurement at 275 nm was used to correct the NO_3^-value. Acidification with 1*N* HCl was designed to prevent interference from hydroxide or carbonate concentrations up to 1000 mg $CaCO_3$ L^{-1}. Chloride had no effect on determination.

Procedure:

A volume of 5 mL of sample (diluted if necessary to get a maximum concentration of NO_3^--N of 2.5 mg L^{-1}) is taken. Then, 0.1 mL of HCl 1N is added to each sample.

Afterwards, the absorbance at 220 and 275 nm is measured in a spectrophotometer (Cecil CE 7200). The absorbance related to nitrate is obtained by subtracting twice the absorbance reading at 275 nm from the reading at 220 nm. The quantification is done with a 6-8 points calibration curve in the range of 0-17.5 mg NO_3^--N L^{-1}, using KNO_3 as standard.

$$mg\ NO_3^- - N\ L^{-1} = a \cdot (A_{220nm} - 2 \cdot A_{275nm}) + b \qquad \text{Eq. 2-3}$$

Where:

A_{220nm}: absorbance at 220 nm;

A_{275nm}: absorbance at 275 nm;

a: slope of the calibration curve;

b: intercept of the calibration curve.

2.2.2.6 Dissolved Total Nitrogen (DTN) 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 Dissolved Total Nitrogen (DTN), by oxidation at high temperature, and the other determines the Dissolved Inorganic Nitrogen (DIN), by a chemical reduction. In addition, Dissolved Organic Nitrogen (DON) was determined as the difference between DTN and DIN.

All the nitrogen present in the water was catalytically oxidised to nitrous oxide (NO). The process for DTN determination occurs in two steps. The first step was 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 was the chemical reduction of residual NO_2 with H_2SO_4 at 80 °C and catalyzed by $VaCl_3$. For the DIN determination, only the second step (chemical reduction) was used. The NO obtained in the two steps was 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 TN and IN was carried out by quimioluminiscence, using a multiplicator tube. The instrument was calibrated with a certified standard solution (KNO_3, 20 mg N L^{-1}) using a response factor method.

2.1.3 Phosphate (PO_4^{3}-P) determination

Phosphates were analysed in different ways, depending the experimental part of the research. Two methods were used: ion chromatography and colorimety.

a) Determination by Ion Chromatography

See section 2.1.2.5.

b) Determination by Colorimetry

The method was based on the absorbance measurement at the radiation of 880 nm Method 4500-PE of Standard Methods (APHA, 1998). The minimum concentration that can be detected with this method is 0.01 mg PO_4^{3}-P L^{-1}.

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:

Reagent A: Sulphuric acid 5N. 70 mL of concentrated H_2SO_4 are dissolved in 500 mL of distilled water.

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. 1.76 g of ascorbic acid is dissolved in 100 mL of distilled water. This solution is stable for one week and should be kept at 4 °C.

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;

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.

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 (drop by drop) 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, absorbance at 880 nm is measured with a spectrophotometer Cecil CE 7200 and results are given by comparison with a calibration curve, made with commercial solution of phosphate (1000 mg L^{-1}). A blank with reagents must also be measured as a reference.

2.2 SOLID PHASE ANALYTICAL METHODS

Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS)

Total or Mixed Liquor Suspended Solids (TSS, MLSS) and Volatile or Mixed Liquor Volatile Suspended Solids (VSS, MLVSS) are solids that refer to matter suspended or dissolved in water or wastewater. Both parameters were measured according to the analytical methods 2540D and 2450E of Standard Methods (APHA, 1998).

Procedure:

For MLSS determination, a well-mixed sample is filtered through a weight standard glass-fiber filter disk (Whatman, GF/C 47 mm of diameter, 1.2 µm of pore size or other filter that gives demonstrably equivalent results) and the residue retained in the filter is dried for two hours to a constant weight at 103-105°C. The weight of the filter and the dried residue is determined and used to calculate the TSS in mg L^{-1}.

$$TSS = (A - B) \cdot 1000/V \qquad \text{Eq. 2-4}$$

Where:

TSS: total suspended solids (mg L^{-1});

A: weight of the filter + dried residue (mg);

B: weight of the filter (mg);

V: sample volume (mL).

VSS is determined by the combustion of the MLSS filter in a furnace at a temperature of 550 °C for one hour. The remaining solids (weighted after cooling first in air and after in desiccator) represent the fixed total, dissolved, or suspended solids, while the weight lost on ignition corresponds to the volatile solids. This determination offers a rough approximation of the amount of organic matter present in the solid fraction of wastewater, activated sludge and industrial wastes.

VSS concentration is calculated as follows:

$$VSS = (A - B) \cdot 1000/V \quad \text{Eq. 2-5}$$

$$FS = (B - C) \cdot 1000/V \quad \text{Eq. 2-6}$$

Where:

VSS: volatile suspended solids (mg L^{-1});

FS: fixed solids (mg L^{-1});

A: weight of residue + filter before ignition (mg);

B: weight of residue + filter after ignition (mg);

C: weight of filter (mg).

2.3 GASEOUS PHASE ANALYTICAL METHODS

The analyses described in this section were only conducted in the bench-scale pilot plant at the University of Santiago de Compostela (see chapter 6).

2.3.1 Biogas production

Biogas production was measured by Ritter MILLIGASCOUNTER® Type MGC-10 (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 was counted with a magnet and a counter and the internal calibration give the gas flow in the display (Figures 2-1 and 2-2).

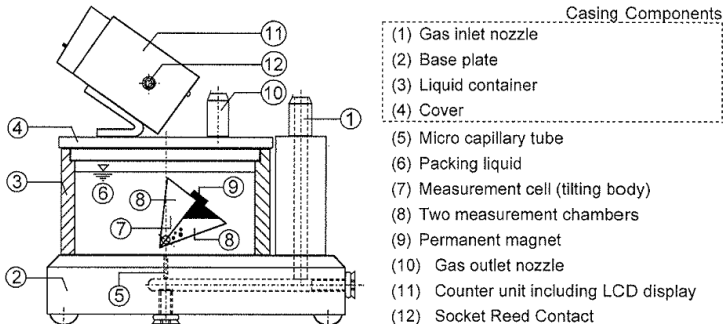

Figure 2-1 Ritter MilliGascounter® and its components

Figure 2-2 A picture of the Ritter MilliGascounter®

2.3.2 Biogas composition

A gas chromatograph HP 5890 Series II with the column of Porapack Q 80/100 2m x 1/8" (SUPELCO) was used to measure biogas composition. 1 mL of well-mixed sample should be injected through the septum at the following conditions: oven temperature (column) set on 35 °C; injector and the detector temperature set on 110 °C. The obtained peaks corresponded to the percentage of the N_2, CH_4, CO_2 and H_2S content in the sample.

2.3.3 Dissolved methane

Dissolved methane content in the liquid phase was estimated by Henry´s law. Methane is characterized by a Henry constant of $1.5 \cdot 10^{-3}$ mol L^{-1} atm^{-1} at 25 °C (Sander, 1999). 300 mL of sample was hand-shaked in a 500 mL Erlenmeyer for three minutes. Then, gas phase was analyzed in the gas chromatograph indicated in the previous epigraph.

2.4 BIOMASS CHARACTERIZATION

The biomass characterization present in the different stages of the experimental setup was performed by means of parameters such as sedimentability and filterability, and by electronic microscopy. Also, the identification of different populations present in the biomass samples was conducted by Fluorescent In Situ Hybridization (FISH). The FISH technique was applied to biofilm and suspended biomass (chapter 6).

Specific analytical techniques and batch experiments conducted in a single part of the work are described in the corresponding chapter.

2.4.1 Sludge volumetric index (SVI)

The Sludge Volume Index (SVI, mL g^{-1}), an index of sludge settling propensity representing the volume occupied by 1 g of MLSS after 30 min of static settling, was evaluated according to 2710D Standard Methods (APHA, 1998). The 30 min settled sludge volume (V_{30}, mL L^{-1}) was determined using a 1 L glass cylinder and SVI was then calculated, as follows:

$$SVI = \left(\frac{V_{30}}{MLSS}\right) \cdot 1000 \qquad \text{Eq. 2-7}$$

Where:

MLSS: mixed liquor suspended solids concentration (mg L^{-1}) of the sludge sample.

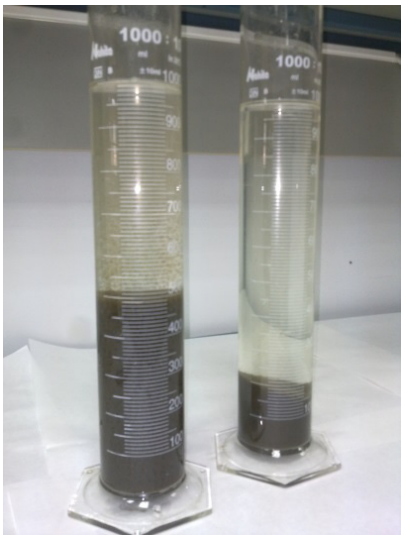

Figure 2-3 A picture of the SVI test

2.4.2 Sludge filterability

Biological processes generate excess biomass, i.e., sludge, which normally must be dewatered before disposal. One of the parameters that have been used widely as an indicator of biomass dewaterability/filterability is the specific resistance to filtration (SRF). It is a measure of the resistance of the filter cake to the transport of filtrate through the cake.

The simplest theoretical model that describes the membrane fouling, the resistance-in-series model, was applied to evaluate the filtration characteristics (specifically total resistance to filtration and its individual fractions). The total resistance (R_t) (Eq. 2-8) is the result of the sum of its fractions (Eq. 2-9)

$$R_t = \left(\frac{\Delta P}{\mu J}\right) - R_m \qquad \text{Eq. 2-8}$$

$$R_t = R_m + R_c + R_{pb} \qquad \text{Eq. 2-9}$$

Where:

J: the instantaneous permeate flux;

ΔP: the transmembrane pressure;

μ: the permeate viscosity (water viscosity, 20 °C);

Rt: the total hydraulic resistance;

R_m: the initial membrane resistance (m^{-1});

R_c: the cake resistance formed by the cake layer deposited over the membrane surface;

R_{pb}: the pore blocking resistance caused by adsorption of dissolved matter and/or colloidal pore blockage within the membrane and walls.

Each resistance value can be obtained through Eq. 2-10 to 2-12.

$$R_m = \frac{\Delta P}{\mu J_m} \quad \text{Eq. 2-10}$$

$$R_{pb} = \frac{\Delta P}{\mu J_{pb}} \quad \text{Eq. 2-11}$$

$$R_m = \frac{\Delta P}{\mu J_m} - (R_m - R_{pb}) \quad \text{Eq. 2-12}$$

Where:

J_m: the flux obtained with deionized water;

J_{pb}: the flux obtained with deionized water after removing the cake layer;

J is the flux with the mixed liquor.

J_m, J_{pb} and J are the flux values determined experimentally.

The mixed liquor was then centrifuged in order to determine the resistance of the colloidal fraction of the cake Eq. 2-13.

$$R_{col} = \frac{\Delta P}{\mu J_{col}} - R_m \quad \text{Eq. 2-13}$$

Where:

J_{col}: the flux obtained with the supernatant after centrifugation at 4000 g during 10 min.

Considering the Carman-Kozeny equation (Eq. 2-14) to calculate the pressure drop of a fluid flowing through a packed bed of solids in laminar flow:

$$\frac{\Delta P}{L} = \frac{150(1-\varepsilon)^2 \mu v}{\varepsilon^3 {d_p}^2} \quad \text{Eq. 2-14}$$

$$v = \frac{1}{A}\frac{dV}{dt} = \frac{\varepsilon^3}{k \cdot \mu \cdot {S_o}^2 (1-\varepsilon)^2} \cdot \frac{\Delta P}{L} \quad \text{Eq. 2-15}$$

$$\frac{1}{A}\frac{dV}{dt} = \frac{\Delta P}{R_c + R_f} \quad \text{Eq. 2-16}$$

Where:

ΔP: the pressure drop;

L: the total height of the bed;

μ: the viscosity of the fluid;

v: the superficial velocity;

k: the hydraulic conductivity or coefficient of permeability;

A: the cross sectional area;

V: the volume of filtrate;

S_o: the specific surface;

ε: the porosity of the bed;

d_p: the diameter of the related spherical particle;

R_c: the resistance to filtration of the cake;

R_f: the resistance to filtration of the filter.

$$w = \frac{s \cdot \rho}{1 - m \cdot s} \qquad \text{Eq. 2-17}$$

$$\alpha = \frac{k \cdot {S_o}^2 \cdot (1-\varepsilon)}{\rho_s \cdot \varepsilon^3} \qquad \text{Eq. 2-18}$$

$$\frac{dV}{dt} = \frac{A^2 \cdot \Delta P}{\mu \cdot \alpha \cdot w \cdot (V - V_e)} \qquad \text{Eq. 2-19}$$

Where:

w: the total suspended solid concentration;

s: the fraction of solids in the sludge;

m: the mass ratio between wet and dry cake;

α: specific resistance to filtration of the cake;

ρ: density of the fluid;

V_e: the equivalent volume of liquid that gives the same resistance to filtration than the filter medium (m^3).

Taking into account that the filtration takes place at constant pressure and carrying out the linearization of the expression:

$$\frac{t}{V} = \frac{k_1}{2} \cdot V + k_2 \qquad \text{Eq. 2-20}$$

Where,

$$k_1 = \frac{\mu \cdot \alpha \cdot w}{\Delta P \cdot A^2} \qquad \text{Eq. 2-21}$$

$$k_2 = k_1 \cdot V_e \qquad \text{Eq. 2-22}$$

Therefore, the specific resistance to filtration (α, m kg^{-1}) can be calculated by:

$$\alpha = \frac{2 \cdot A^2 \cdot P \cdot b}{\mu \cdot w} \qquad \text{Eq. 2-23}$$

Where:

P: the pressure applied (Pa);

A: the filtration area (m^2);

w: the total suspended solids (kg m^{-3});

μ: the viscosity of filtrate (Pa·s);

b: the time-to-filtration ratio(s m^{-6});

V: the filtrate volume.

Thus, *b* 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*).

Buchner funnel test

One of the most commonly tests to obtain the SRF is a dead-end filterability test according to standard (UNE EN 14701-2, 2006), known as the Buchner funnel test. The filtering was performed using a 9 cm diameter Whatman nº 1 filter paper at an applied vacuum pressure of 51 kPa. The volume of filtrate (200 mL) collected was recorded as a function of time.

Figure 2-4 A picture of the Buchner funnel test and detail of filter paper

2.4.3 Sludge Filtration Index (SFI)

Other indicator of the sludge filterability employed during this work was the Sludge Filtration Index (SFI) accordingly to the procedure developed by Thiemig, (2012). This indicator can be obtained by the following expression:

$$\mathrm{SFI(s/\%TSS)} = \frac{\Delta t}{\mathrm{TSS}} \qquad \text{Eq. 2-24}$$

This new method is simple to be conducted on site with commonly used equipment and produces reliable and reproducible data describing the membrane filtration relevant sludge properties. Others methods more complex such as Capillary Suction Time (CST) and Filter Test (FT) use a very small sludge amount which affects significantly the variation and quality of results. In this method, a larger sludge sample (500 mL) is used. A detailed description of the method development and validation can be obtained in Thiemig, (2012).

The protocol and set-up (Figure 2-5) were the following:

- Take 1 L of sludge sample of the MBR shortly before measurement.
- Insert the filter paper (Macherey-Nagel MN 85/70 into the funnel (Ø 150 mm).
- Place the empty 250 mL measuring cylinder under the funnel.

- Clamp blade agitator at a height of 0.5 cm over the filter paper. Activate the drive and set to 40 rpm (according to a usual cross-flow velocity).
- Mix the sludge sample well and temper a 500 mL sample in a water bath to 20 °C.
- Measure the amount of suspended solids of the remaining sludge sample.
- Quickly pour the 500 mL sample into the filter.
- Activate the stopwatch when filter volume reaches 100 mL mark.
- Stop the watch when filter volume reaches then 150 mL mark.
- The specific value of the Sludge Filtration Index (SFI) is calculated from the measured timeΔt (s) with respect to the concentration of suspended solids SS (%) as indicated in Equation 2-24.

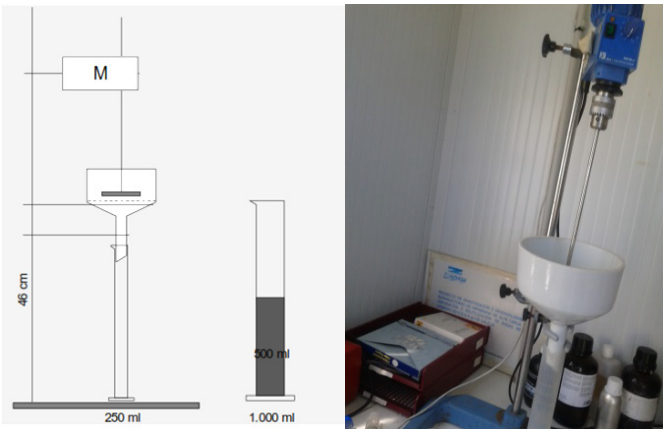

Figure 2-5 Scheme and picture of method for measuring the sludge filtration index SFI.

2.5 MICROBIOLOGICAL DETERMINATIONS

2.5.1 Microbial Indicators

Total and fecal coliforms were used as indicator of water microbiological pollution in this Thesis. To determinate the amount of both microorganisms the membrane filter technique was employed.

This method consists of filtering a certain amount of water with a membrane filter (0.45 µm pore) which is able to retain coliform bacteria. After filtration, retained

coliforms remain disposed in the membrane filter. To form colonies of sufficient size to be viewed by eye, coliforms are fed with a culture media (food) as specific as possible. Under these conditions, in one day (24 hours) coliforms have increased their number so that the colony of each individual can be seen. With test conditions the colonies will be light pink. Total and faecal coliforms were enumerated according to Standard Methods 9222B and 9230D (APHA, 1998).

The culture medium used for each of the microorganisms, as well as the incubation temperature, can be seen in Table 2-1.

Table 2-1 Culture media and incubation temperature

Microorganism	Culture media	Temperature
Total Coliforms	Chromocult®	35 °C
Fecal Coliforms	Bactokit®	44 °C

2.5.2 Identification of bacteria populations by FISH

The abundance of the difference populations of microorganisms present in the sludge was researched using Fluorescent In Situ Hybridization (FISH). In this technique specific regions in 23S or 16S rRNA are detected with fluorescently labelled probes. If the corresponding domain, phylum, genus or species is present, the probe hybridizes to the targeted sequence and can later be detected microscopically. According to (Amann, 1995) a typical FISH protocol includes four steps (Fig. 2-6): a) biomass fixation; b) immobilization of the sample; c) hybridization of the targeted sequence to the probe; d) washing steps to remove unbound probe and the detection of labelled cells by microscopy or flow cytometry. This protocol must be applied to flocculent, granular and biofilm biomass; therefore, the attached biomass must be detached before starting the procedure. To achieve the biofilm detachment, a piece of support media (with biomass) was sonicated for 1 min at 65% of amplitude using a probe sonicator (UP200s, Dr. Hielscher) or similar.

During hybridization the cells were exposed to high temperatures, detergents and osmotic gradients. Thus fixation of the cells essential in order to maintain the morphological integrity of the cells. Fixation of cells with glutaraldehyde resulted in considerable autofluorescence of the specimen. Autofluorescence was minimized by fixation in freshly prepared (not older than 24 h) 4% paraformaldehyde solution in Phosphate Buffer Solution (PBS).

After fixation, the cells were immobilized on a microscopic slide and used for hybridization with 16S rDNA probes. In order to avoid non-specific binding of the rDNA probes, the hybridization was done at stringent conditions (46 °C, 0 – 65% formamide) and specimens were washed with wash buffer (48 °C). The targeted organisms could be detected by the characteristic fluorescence.

The fluorochromes used to detect the hybridized rRNA were fluos (5(6)-carboxyfluorescein-Nhydroxysuccinimide ester) and Cy3 (indocarbocyanine). To visualize all cells in a sample the stain 4,6-diamidino-2-phenylindole (DAPI) was used. Its application can provide insight into the existence of archaeobacteria and eukaryotes, like protozoa. For analysis of the slides an epifluorescence microscope (Axioskop 2 plus, Zeiss) in combination with a digital camera (Coolsnap, Roper Scientific Photometrics) was used. The probes applied in this Thesis are listed and detailed in the corresponding chapters.

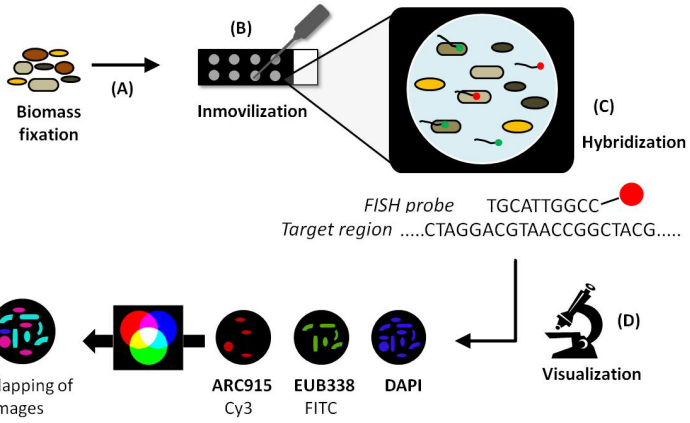

Figure 2-6 Basic steps of FISH technique (source: Amann and Fuchs, 2008) .

For further discussion it has to be kept in mind that samples can never be 100% representative. Thus the fact that no bacteria of a certain kind were present in the sample can sometimes be attributed to unrepresentative sampling or error during procedure (e.g., hybridization process).

2.6 MEMBRANE PERFORMANCE

2.6.1 Flux and Permeability

During this work, the membrane modules used were different with range from 0.9 m^2 to 2.0 m^2 per module. Fluxes were calculated as following:

$$J = Q/S \quad \text{Eq. 2-25}$$

Where:

J: Specific flux (L m^{-2} h^{-1});

Q: Flux (L h^{-1});

S: membrane area (m^2).

Therefore, the permeability can be calculated as:

$$P = J/TMP \quad \text{Eq. 2-26}$$

Where:

P: permeability (L m^{-2} h^{-1} bar^{-1});

TMP: Transmembrane Pressure (bar).

2.6.2 Filterability

Different filterability test were conducted in this work, which are described in section 2.4.2.

2.6.3 Critical flux

The critical flux hypothesis is that during 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 (Drews, 2010). 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 should be higher than 10 Pa min^{-1} (Le-Clech et al., 2003).

2.6.4 Biopolymer clusters (BPC)

Recently, a pool of biopolymer clusters (BPC) has been identified in the liquid phase of the MBR sludge and in the cake sludge on the membrane surfaces. According to the confocal laser scanning microscopy (CLSM) examination, BPC are free and independent organic solutes that are different from biomass flocs and EPS and much larger than SMP (Wang and Li, 2008; Sun et al., 2008). In this Thesis only the colloidal fraction of BPC (cBPC) was considered.

The colloidal fraction of BPC in the liquid phase of the sludge mixture suspension was estimated by calculating the difference in organic matter concentration between the sludge mixture after filtration through a 0.45 µm filter and the permeate (Sánchez et al., 2013). Organic matter can be measured in terms of Dissolved Organic Carbon (DOC) (as in Sánchez et al., (2013)) (chapter 6), or Dissolved Chemical Oxygen Demand (COD_s) (as in Lin et al., (2009) (chapter 5).

2.6.5 Maintenance of the membrane modules

Membrane washing performed in the experimental set ups were mechanical (physical) washing with tap water, and, when necessary, chemical cleaning. The different chemical cleaning strategies are briefly described in this section; other details are presented in the corresponding chapters:

Maintenance cleaning

The maintenance chemical cleaning is performed in situ ("cleaning in place" or CIP) and is an intermediate chemically enhanced backwashing that uses a low chemical dosage at ambient temperature. The procedure was the following:

1) Mechanical (physical) cleaning with tap water, and

2) Backwashing with chlorinated water (250 – 500 ppm hypochlorite, up to 1000 ppm of sodium hypochlorite solution).

In practice, when the reactor is sufficiently small (laboratory or bench-scale), the maintenance cleaning can be also performed out of place.

Intensive chemical cleaning

Intensive chemical cleaning is carried out using high chemical concentration and can be performed either in situ (CIP) or ex situ ("cleaning out of place" or COP). Intensive chemical cleaning was performed outside the reactor only when permeability value was below 50 L $m^{-2}{\cdot}h^{-1}{\cdot}bar^{-1}$, approximately. This cleaning

implies soaking the module of membrane with higher concentration of chemicals in clean water. This procedure was:

1) Physical cleaning by rinsing with tap water;

2.a) Submerging the membrane module in chlorinated water (500 ppm hypochlorite) for 8 h (in chapters 3, 4, 5);

2.b) Submerging the membrane in chlorinated water (2000 ppm hypochlorite) for 2 h and backwashing with chlorinated water (2000 ppm hypochlorite) for 1 h (in chapter 6).

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Chapter 3

Evaluation of a hybrid vertical membrane bioreactor (HMBR) at bench-scale for wastewater treatment[1]

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

L. Rodríguez-Hernández, A.L. Esteban-García, A. Lobo, J. Temprano, C. Álvaro, A. Mariel and I. Tejero (2012). *Evaluation of a hybrid membrane bioreactor (HVMBR) for wastewater treatmen*t. Water Science and Technology 65 (6), 1109 - 1115.

L. Rodríguez-Hernández, L. De Florio, I. Tejero (2011). *Scale-up of membrane biological reactor configuration including biofilm activity: bench and pilot scale trials of a hybrid MBR*. 6th IWA Specialist Conference on Membrane Technology for Water and Wastewater Treatment (Book of abstracts), 3rd best YWP Poster, Aachen (Germany), 509-510.

OUTLINE

SUMMARY

A new hybrid membrane bioreactor (HMBR) has been developed to obtain a compact module, with a small footprint and low requirement for aeration. The aim of this research was to pre-evaluate its performance at bench-scale. The system consists of a single vertical reactor with a filtration membrane unit (MF) and, above this, a sponge fixed bed as support medium. The aeration system was located under the membrane unit, allowing for membrane cleaning, oxygenation, biofilm thickness control and bulk liquid mixing. Operated under continuous aeration, a bench-scale reactor (70 L) was fed with pre-treated, raw (unsettled) municipal wastewater.

Biological oxygen demand (BOD_5) and suspended solids removal efficiencies (96% and 99% respectively) were comparable to those obtained with other membrane bioreactors (MBRs). Total nitrogen removal efficiencies of 80% were achieved through simultaneous nitrification and denitrification (SND) process within the biofilm. These results are better than those obtained in other HMBRs and similar to the values reached using more complex MBRs with extra anoxic tanks, intermittent aeration or internal deflectors. Additionally, the recirculation rate seems to play an important role in the operating system, mainly in nitrogen removal.

3.1 INTRODUCTION

Low energy consumption, low maintenance compact equipment and, usually, small footprint are desirable characteristics for waste water treatment plants. Many of these plants are expected to produce a high quality effluent, enabling water reuse, among other objectives. Win this aim, membrane bioreactors (MBRs) have emerged from the combination of two basic processes: biological degradation and membrane separation. The use of membranes has several advantages such as higher solids retention time (SRT), a suspended biomass concentration in the reactor between 6 and 15 g SS L^{-1} (Rosenberger et al., 2006; Judd, 2011), and a reduction in the food/microorganisms (F/M) ratio compared to conventional activated sludge (CAS) treatment. A low F/M ratio reduces the production of sludge, thereby minimizing the number of purges required and a high SRT allows the development of specialized degrading microorganisms. Furthermore, membrane fouling is a major concern in MBRs and it has been a major obstacle to the widespread use of this technology. Since the liquid suspension (i.e., activated sludge and/or excess of biofilm) is rather complex it is

still unclear which fraction or compounds are mostly responsible for membrane fouling in MBR (Judd, 2011; Drews, 2010). Different strategies have been proposed to control fouling (Meng et al., 2009), such as hydraulic control (lower hydraulic retention time [HRT], higher aeration, backwashing and low flux operation), chemical control (adding activated carbon or a membrane fouling reducer, flocculation/coagulation and chemically enhanced backwashing) and biological control (increasing SRT and reducing the mixed liquor suspended solids concentrations [MLSS], F/M ratio or filamentous bacteria).

Another challenge for MBRs is total nitrogen removal. To obtain high rates of denitrification, conventional MBRs require some modifications, i.e., internal recirculation of the mixed liquor to a pre-anoxic tank to obtain good denitrification rates (Gander et al., 2000). In a less common scenario, a single tank can be used for both anoxic and aerobic biological degradation considering the use of intermittent aeration (Yeom et al., 1999)or the addition of baffles to create an anoxic zone inside the aerobic reactor (Kimura et al., 2008).

The addition of attached biomass to these systems is intended to overcome the aforementioned two major weaknesses of conventional MBRs. The biofilm can have an anoxic zone, which facilitates the denitrification process, thereby improving total nitrogen (TN) removal (Guo et al., 2010b; Liu et al., 2010). In addition, the high retention time achieved by using two kinds of biomass, suspended and attached, favours the growth of microorganisms specialized in the type of wastewater to be treated. In general, most studies have reported that membrane performance (i.e., less fouling) is also greatly improved in this type of configuration. The improvement is attributed to a reduction in suspended biomass in (Shuo et al., 2008). Nonetheless, not only the concentration of the suspended material is of significance but also the composition and characteristics of the material (Meng et al., 2009; Liu et al., 2010; Liu et al., 2012) , whereby some other researchers have reported the opposite behavior (Lee et al., 2001). Therefore more studies are needed to know the effect of bio-solids concentration in these systems.

Two main configurations of MBR with attached biomass have been studied so far: biofilm membrane bioreactors (BF-MBRs), also called pure biofilm based MBR (pBF-MBR) by (Ivanovic and Leiknes, 2012) and hybrid biofilm-suspended biomass membrane bioreactors (HMBRs) or assisted biofilm MBR (aBF-MBR) by (Ivanovic and Leiknes, 2012). Although some authors use BF-MBR and HMBR interchangeably, e.g., (Yang et al., 2009) and (Phattaranawik and Leiknes, 2010), we prefer differentiate between them. In BF-MBRs, most of the biomass is attached and the activity of suspended matter is neglected due to very low concentrations and low biologically active MLSS in the bioreactor (Lee et al.,

2001). In contrast, the amounts of suspended and attached biomass are in the same range in HMBRs, and the biodegradation is carried out by both kinds of biomass. In fact, the same reactor is capable of operating as a BF-MBR or HMBR depending on the operating strategy (with/without excess sludge removal). Moreover, in both configurations, biofilm support can be arranged in a fixed bed (Shuo et al., 2008; Lee et al., 2001; Tejero and Cuevas, 2005) or, more commonly, in some type of mobile biofilm carrier (e.g., Leiknes and Odegaard, (2007)). The membrane can be placed in a separate reactor (Artiga et al., 2005) or submerged in the same reactor, with a perforated separator (Yang et al., 2010) or without it (Shuo et al., 2008; Tejero and Cuevas, 2005).

For this study, a new configuration of hybrid membrane bioreactor was designed to simultaneously remove organic carbon and nitrogen in wastewater. With the purpose of minimizing the air flow required and reducing the footprint, two strategies were combined. On the one hand, the reactor is vertical, with the biofilm support medium located above the filtration membrane unit and the aeration system. Given this, the air supplied for membrane cleaning also serves to oxygenate the biofilm, to mix the bulk liquid and to control biofilm thickness. On the other hand, the biofilm support medium employed, a fixed bed of small, randomly oriented, flexible sponge cubes, was designed to encourage bubbles to follow a tortuous path. This type of path arrangement tends to increase the turbulence and retention time of the bubbles, thereby increasing oxygen transfer (Gómez, 2010) even if coarse bubbles are used.

This research has an antecedent in the studies of Cuevas and Tejero, (2003), who worked with anaerobic pre-fermenter and HMBR systems at bench-scale. The results obtained were related to operating conditions and were essential for the construction and design of the HMBR system used in this work.

Note that other authors, e.g., Guo et al., (2008), Ngo et al., (2008) and Yang et al., (2006), have also proposed the use of sponges for microbial attachment in HMBR, but in those studies they were used as mobile carriers intended to collide with the membranes and mitigate the formation of biofouling.

3.2 OBJECTIVES

In this study, the main objective was to assess the performance at bench-scale of a new configuration consisting of a hybrid vertical membrane bioreactor (HMBR)

for the treatment of municipal wastewater, as an alternative system to other conventional configurations.

Furthermore, other specific objectives were:

- Evaluate whether the aeration flow delivered to clean the membranes is sufficient to provide adequate mixing in the reactor and supply enough oxygen for the removal of organic matter and ammonia, and at the same time the system is able to remove total nitrogen via SDN.

- Assess if it is possible to obtain an effluent with quality for reuse.

- Study the effect of adding a recirculation flow from the membranes zone to the head of the reactor.

- Evaluate the performance of the membrane permeability during the operational time and check whether the vertical configuration allows filtering by gravity only.

3.3 MATERIALS AND METHODS

3.3.1 Description of the bench-scale plant

In this chapter the study of a hybrid membrane bioreactor was conducted at bench-scale. The proposed system was constructed at the site of the Santander (Spain) municipal waste water treatment plant (428,000 equivalent inhabitants, combined sewer system and average flow of 7668 m^3 h^{-1}). A submerged pump was used to extract the wastewater used to feed the reactor after pre-treatment by the waste water treatment plant (coarse screen, 3 mm fine screen, grit and grease removal).

A diagram and a general picture of the system studied is shown in Figure 3-1.

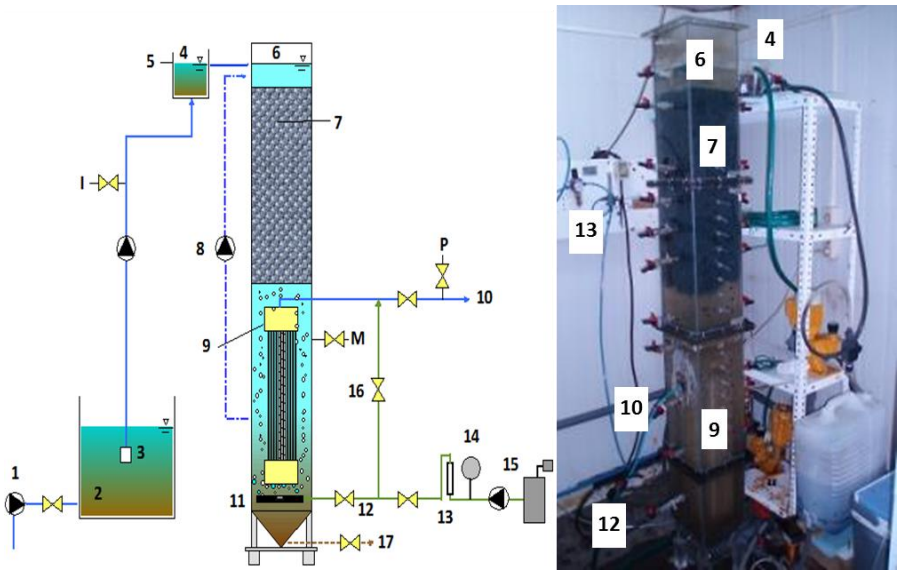

Figure 3-1 Schematic diagram (left) and general picture (right) of the bench-scale HMBR system. (1) Influent pump; (2) Regulation tank; (3) Fine screen; (4) Distribution tank; (5) Overflow weir; (6) Biofilm reactor; (7) Sponge fixed bed; (8) Recirculation; (9) Membrane module; (10) Effluent; (11) Air diffuser; (12) Aeration pipe; (13) Rotameter; (14) Pressure valve; (15) Air compressor; (16) Membrane backwash pipe; (17) Excess sludge. Sample points: (I) Influent; (M) Membrane zone; (P) Permeate.

Basically, it consists of a vertical membrane bioreactor, with a bed to support the biofilm above the membrane (Tejero and Cuevas, 2005). The feed pump (1) directs the pre-treated water to a 50 L equalization tank (2) where fine screening (1.5 mm circular opening) (3) is performed. From there, the water is pumped into a small tank with a weir (4) that feeds the reactor and keeps the water level constant. In this way, the treatment flow rate is fixed by the permeate flow rate.

The reactor is 2 m high, has an internal cross-section of 0.2 x 0.2 m and holds a net volume of 70 L (6). The aeration system is located at the bottom of the reactor (which allows the reactor to be very compact) and is used mainly for membrane cleaning. Moreover, the cleaning bubbles also serve to aerate and mix the contents of the reactor. It is a coarse bubble system that is composed of perforated tubes (11). The air, which comes from a compressor (15), passes through a pressure drop valve (14). A rotameter (13), whose measurements are confirmed precisely with a gasometer, is used to ensure a constant flow rate. The air supply (6 L min^{-1}) is determined by the need for sufficient and continuous stirring in the membrane zone, which controls membrane fouling. This flow rate

results in a high bulk liquid oxygen concentration of about 6 mg L^{-1} throughout the system and a specific aeration demand per membrane area (SAD_m) of 0.2 Nm^3 m^{-2} h^{-1} (manufacturer´s recommendations between 0.2 and 0.8) and 60 m^3 air m^{-3} permeate produced (SAD_p). The continuous flow rate applied to the membrane, corresponded to a superficial velocity in the compartment of 9 m h^{-1}. In addition, the aeration system has a shunt (16) located behind the pressure drop valve and the rotameter and connected to the permeate output pipe. Thus, during the membrane backwashing, air is provided through this shunt at a pressure of 1 kg cm^{-2} for 10 min every 24 h (superficial velocity in the compartment > 22.5 m h^{-1}).

A Spanish commercial membrane module (Porous Fibers; Leioa, Spain) was used in this research with an active length of hollow fiber of 550 mm, forming a membrane surface of 2 m^2. The size of the pores fell within the range of microfiltration (0.4 µm). Other membrane information is shown in Table 3-1 and Figure 3-2.

Table 3-1 Technical characteristics of Porous Fibers membranes

Characteristics	**Micronet R®**
Material	PVDF
Free volume of pores in the fibers	65%
External diameter of the fiber (mm)	2.40
Internal diameter of the fiber (mm)	1.10
Explosion Prove (internal pressure) (Kg cm^{-2})	60

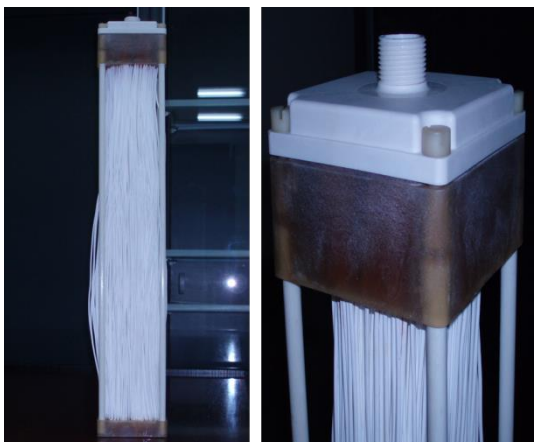

Figure 3-2 A Picture and detail of Membrane Module

Vertical configuration allows the operation to be performed by gravity (in the study covered by this chapter, there was 1 m water column above the membrane module which favoured the permeation) or by suction (chapters 4, 5), depending on hydraulic requirements.

3.3.2 Biofilm support medium

The support media consisted of 2 x 1 x 1 cm polyester sponge cubes (density of the material: 0.986 g cm^{-3}; density of the packed bed: 0.024 g cm^{-3}), which was cut from 100 x 100 x 5 cm sheets (Aqua Medic aquarium filters). The total volume of material placed in the reactor was 11,850 cm^3, which represented an apparent volume of 32 L (46% of the total reactor volume) and filled a depth of 0.8 m. This biofilm bed was separated from the membrane module, underneath it, by a horizontal mesh (10 mm openings) that keeps the cubes out of the membrane fibers. It was held submerged by another similar horizontal holding mesh that was placed 5 cm below the water level to prevent air bubbles from causing the cubes to float. In this way, cubes are allowed to have little or no movement inside the packed bed. The support was not acclimatized before being introduced in the reactor and the system was started-up without addition of inoculum. The aim was to develop the biocenosis inside the HMBR from the microorganisms present in the wastewater.

Figure 3-3 Aqua Medic support media without biofilm

3.3.3 Operational conditions

In this chapter, the experiment was divided into two periods according to the operational mode, namely, one period without (Period I) and one with (Period II) recirculation of 18 L h^{-1}, which is equivalent to 300% of the average permeate flow rate. The first experiment lasted 120 d, and the second 60 d. The average operational conditions during each period are reported in detail in Table 3-2. The study was carried out during the winter season, when wastewater temperature ranges from 11 to 17 °C.

Table 3-2 Average operational conditions of the HMBR reactor

Parameter	Units	Period I	Period II
Solids retention time, SRT	d	<120	<60
Hydraulic retention time, HRT	h	12	12
Membrane surface area	m^2	2	2
Transmembrane pressure	kPa	10	10
Effluent flow	L h^{-1}	6	6
Specific permeability	L m^{-2} h^{-1} kPa^{-1}	0.3	0.3
Air flow	Nm^3 h^{-1} m^{-2} membrane	0.18	0.18
Aeration velocity	Nm^3 h^{-1} m^{-2}	9	9
Recirculation flow	L h^{-1}	-	18
Organic Loading Rate, OLR	kg COD m^{-3} d^{-1}	1.114	2.686
Organic Loading Rate, OLR	kg BOD m^{-3} d^{-1}	0.450	0.83
Nitrogen Loading Rate, NLR	kg TKN m^{-3} d^{-1}	0.079	0.079
Nitrogen Loading Rate, NLR	kg NH_4^+-N m^{-3} d^{-1}	0.037	0.045

3.3.4 Analytical methods

Twenty-four hour composite samples were taken twice a week. The samples were kept cool until laboratory analysis was performed. The sample points are shown in Figure 3-1 and include the influent (I), the membrane area within the reactor (M) and the permeate (P).

The analytical determinations of chemical oxygen demand (COD), soluble chemical oxygen demand (COD_s), biochemical oxygen demand (BOD_5), mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) and Total Kjeldahl Nitrogen (TKN) were performed according to the Standard Methods (Eaton et al., 2005). Nitrate and nitrite were determined using an ion-chromatography system (761 COMPACT-IC METROHM); the amount of ammonia was analyzed by an ammonia selective electrode (ORION, model 95-12).

Microbiological analyses to be performed are total coliforms and fecal coliforms according to Standard Methods 9222B and 9230D (APHA, 1998). The technique used is membrane filtration.

The dissolved oxygen (DO) concentration was measured using a portable meter (HQ40d meter with a LDO101 probe, HACH, Co); pH was determined using a glass electrode pH meter (WTW, model SENTIX 21); and turbidity was measured with a turbidimeter (HACH, model 2100P ISO).

Further information regarding analytical methods is provided in chapter 2.

3.3.5 Mass balances in the HMBR reactor

A simplified to mass balance was performed for the HMBR to assess the capacity of the system to remove the nitrogenous compounds.

In this system, where there was no excess sludge (purge), and based on nitrogen removal theory, nitrogen removal can be achieved by two main processes: assimilation by microorganisms and nitrification-denitrification (see Figure 3-4).

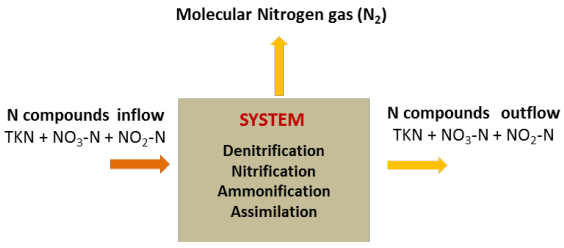

Figure 3-4 Nitrogen mass balance for this study

The amount of nitrogen mass balance in the HMBR system in this study is presented as follows:

$$(TKN + NO_2^- + NO_3^-)_{in} = (TKN + NO_2^- + NO_3^-)_{ef} + N_{ass} + N_{den} \qquad \text{Eq. 3-1}$$

Where subindex *in* is influent, *ef* is effluent, *ass* is assimilated and *den* is denitrified.

For nitrogen assimilation, it is accepted that during the aerobic process the cells grow and there is a linear relationship between the amount of biomass produced and the amount of substrate consumed. The biomass yield

considered was 0.4 mg VSS mg COD_s^{-1} and the nitrogen content measured as N is 0.12 mg N mg VSS^{-1} (Tchobanoglous et al., 2003).

$$Nitrogen\ lost\ by\ assimilation\ (mg\ L^{-1}) = biomass\ yield \times N\ content\ in\ biomass \times \left(CODs_{in} - CODs_{ef}\right) \quad \text{Eq. 3-2}$$

In the nitrification process, ammonium nitrogen is converted to nitrite and nitrate by nitrifying bacteria under aerobic conditions. Nitrification can be calculated as follows:

$$Nitrification = N_{den} + (NO_{2\ ef}^{-} - NO_{2\ in}^{-}) + (NO_{3\ ef}^{-} - NO_{3\ in}^{-}) \quad \text{Eq. 3-3}$$

Denitrified nitrogen is obtained solving equation 3-1.

Phenomena of endogenous respiration and autotrophic assimilation were considered negligible in the mass balance.

3.4 RESULTS AND DISCUSSION

3.4.1 Organic matter removal

During experimentation the organic load applied (OLR) to the system was highly variable, showing significant peak loads. The average values were approximately 1.11 kg COD m^{-3} d^{-1}, in Period I, and 2.69 kg COD m^{-3} d^{-1}, in Period II, which corresponded to COD concentrations of 581 ± 244 and 1,470 ± 800 mg L^{-1}, respectively. These intense fluctuations were due to operating the system with municipal wastewater, which is subject to daily variations of existing sanitation system itself. Nevertheless, the HRTs were constant in both periods.

Despite the great variability of applied load, high average efficiencies of chemical oxygen demand (COD) and five-day biochemical oxygen demand (BOD_5) removal were achieved, showing that these peaks were mostly buffered in the reactor effluent.

Table 3-3 shows a summary of the results obtained during the experiment.

Parameter	Period	Influent			Permeate				Efficiency[c] (%)
		Maximum	Minimum	Average[a]	Maximum	Minimum	Average[a]	Percentile[b]	
COD	I	1873	118	581 ± 244	65	15	37 ± 6	53	90
(mg L^{-1})	II	3251	403	1470 ± 800	93	37	67 ± 13	93	91
BOD_5	I	420	95	228 ± 49	12	3	7 ± 1	11	96
(mg L^{-1})	II	620	300	427 ± 136	4	0	2 ± 2	3	99
TSS	I	472	56	183 ± 62	4	1	2 ± 0.4	3	98
(mg L^{-1})	II	3540	258	1428 ± 820	6	2	4 ± 1	6	99
VSS	I	310	25	92 ± 35	3	1	2 ± 0.4	3	97
(mg L^{-1})	II	2212	184	874 ± 479	7	0	3 ± 2	5	99
TKN	I	68.7	21.8	37.7 ± 6.4	4.6	0.2	1.7 ± 0.6	4.3	95
(mg L^{-1})	II	54.2	26.6	35.6 ± 6.8	5.6	1.4	2.8 ± 1.1	4.3	92
NH_4^+- N	I	30.0	7.2	17.2 ± 2.9	4.3	0.2	1.5 ± 0.6	3.6	91
(mg L^{-1})	II	31.5	13.8	20.6 ± 4.5	1.0	0.1	0.5 ± 0.2	0.9	98
NO_3^- - N	I	5.0	0.3	1.6 ± 0.6	17.0	4.0	9.8 ± 1.7	14.3	N/A
(mg L^{-1})	II	1.6	0	0.4 ± 0.6	6.2	1.9	3.4 ± 1.0	4.8	N/A
NO_2^- - N	I	0.9	0	0.29 ± 0.1	1.3	0.1	0.5 ± 0.2	1	N/A
(mg L^{-1})	II	1.9	0	0.5 ± 0.7	1.9	0	1.0 ± 0.7	1.9	N/A
TN	I	70.2	23.6	39.6 ± 6.1	20.3	5.3	12.1 ± 1.8	16.3	69
(mg L^{-1})	II	54.2	26.6	36.5 ± 6.7	9.3	4.7	7.3 ± 1.1	8.8	80
Turbidity	I	780	27	299 ± 305	2.5	0.2	1.0 ± 0.6	1.4	99
(NTU)	II	856	238	393 ± 133	1.2	0.2	0.5 ± 0.2	0.7	99

[a] Average values with 95% confidence intervals.

[b] 90^{th} percentile of the data (value for comparison to legal threshold for wastewater reuse in Spain, Real Decreto (2007)).

[c] Calculated as the average of sample efficiencies

Averages, percentiles and efficiencies were calculated considering the steady state of the system in each period (i.e., after three weeks of experimentation). For nitrogen, last date were obtained on days 112 and 50 for Period I and Period II respectively.

During Period I, the average BOD_5 concentration (228 mg L^{-1}) was higher than 100 – 200 mg L^{-1}, which is the recommended threshold to avoid overloading in a SFBBR using the same fixed bed (Santamaría, 1998). Nevertheless, no signs of overloading (increase in head losses, development of whitish biofilm) were observed. This could be due to the mixing caused by the continuous aeration of the reactor (necessary to reduce membrane fouling and to provide oxygen to the biomass), which would have diluted the influent concentrations, thereby avoiding possible overloading, something that does not occur in trickling filters. The results obtained prove that the proposed vertical design, with or without a recirculation flow, can directly treat pre-treated raw wastewater. This is in contrast to other biofilm processes, which require primary sedimentation.

In spite of the fact that the organic loading rate in Period II was much higher than in Period I (Figure 3-3), recirculation allowed a slight significant improvement in removal efficiencies with respect to BOD_5, increasing average efficiencies from 96% to 99% (See Table 3-3). A similar positive effect of recirculation in fixed-bed bioreactors was observed by Santamaría, (1998). The improvement is considered to be mainly attributable to two factors: better control of biofilm thickness (as a result of dilution, i.e., lower concentrations of organic compounds, and higher shear forces) that, in turn, enables greater contact between the biofilm and the wastewater, and greater biofilm uniformity across the fixed support medium. Thus, in fixed bed biofilm reactor, the recirculation rate is an important parameter to consider.

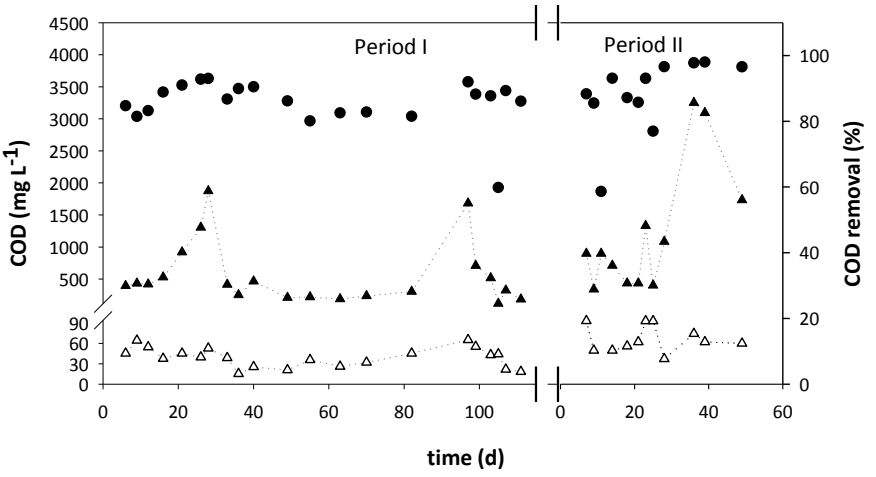

Figure 3-5 Evolution of influent concentration (▲), effluent concentration (Δ) and removal (●) of COD in HMBR during both periods.

The average efficiencies of COD removal were similar without and with recirculation (90 – 91 %), no differences were observed.

It can be concluded that, for the organic loads, aeration rate and recirculation applied, continuous aeration was more determining than recirculation. Nevertheless, recirculation should be considered in the reactor design with higher loads or lower aeration in fixed bed supports (Santamaría, 1998).

The carbon removal rates obtained here are slightly lower than the values of 97.7% (Liang et al., 2010) and more than 97% (Guo et al., 2008) found in other studies of HMBRs and synthetic wastewater with higher mixed liquor suspended solids (MLSS) (10 g L^{-1}). On the other hand, our results are in line with those obtained in an HMBR operating with raw domestic wastewater, 94.2% (Liu et al., 2010), and other studies performed using conventional MBRs, in which efficiencies of COD removal between 85% and 92% (Kimura et al., 2008; Leiknes and Odegaard, 2007; Ueda et al., 1996) have been obtained.

3.4.2 Nitrogen removal

The total nitrogen and ammonia concentrations in the influent were typical of municipal medium-strength wastewater (Tchobanoglous et al., 2003) with an average TN concentration of 39 mg L^{-1} in both periods and average ammonium concentrations of 17 and 21 mg L^{-1}, respectively (Table 3-3).

Regarding ammonia removal (Figure 3-6), great efficiencies were obtained in the HMBR system after about 15 days of the start of the pilot plant and these efficiencies were maintained during all operation. Despite the strong fluctuations and low temperature registered (11 – 17 °C), nitrification was not substantially altered or inhibited. Probably, once the biofilm was formed onto support media, it was able to protect biomass against load and temperature fluctuations (Water Environment Federation, 2010), which would explain the good results obtained.

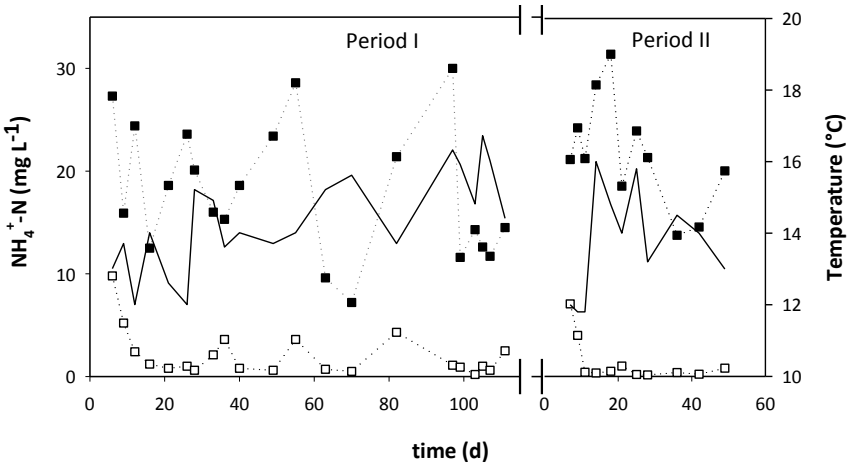

Figure 3-6 NH_4^+-N concentration influent (■), effluent (□) and variations of temperature (—) in HMBR during both periods.

The influent and effluent TN and nitrate concentrations during the two periods of the study are plotted in Figure 3-7. The high TN removal efficiencies (Table 3-3) achieved even without recirculation (69%) could be due to the fact that aeration provided mixing in the system that allowed for denitrification under the applied organic load. This means that nitrates formed in the lower part of the biofilm bed were taken to the top, contributing to nitrate removal by anoxic denitrification in the deepest layer of the biofilm, in spite of the high dissolved oxygen concentrations (6 mg L^{-1}).

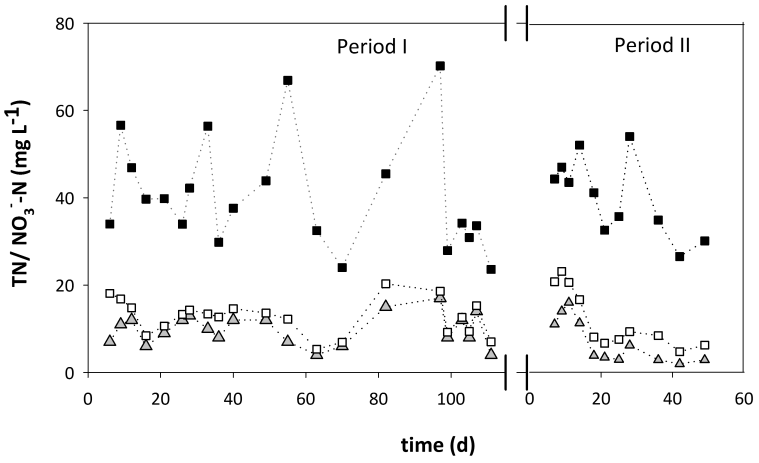

Figure 3-7 TN concentration influent (■), effluent (□) and NO_3^- -N effluent concentration (▲) in HMBR during both periods.

Considerable better average nitrogen removal efficiencies were achieved in Period II, with ammonium and total nitrogen removals of 98% and 80% vs. 91% and 69% in Period I (Table 3-3).

In addition to the effect of recirculation on biofilm thickness and uniformity mentioned above, it can be hypothesized that the nitrifying fraction of the sponge fixed bed volume increased and the nitrification improved because, in this down-flow configuration, more carbonaceous matter is removed at the top of the bed. Moreover, it seems likely that the performance of the system could be enhanced with intermittent aeration or by reducing the air flow rate, but any modification in air supply should be carefully conducted so that membrane performance is not compromised.

In both periods, the system stabilized in approximately the third week of operation. After stabilization in Period II, average values of 7.3 mg TN L^{-1} and 3.4 mg NO_3^-- N L^{-1} were obtained. Considering that the pattern of stability in Period II was very similar to what had been observed in Period I, it was not considered necessary to continue Period II beyond 60 days.

The effluent quality obtained by this system meets the physical-chemical parameters established by the European Directive concerning Urban Wastewater Treatment 91/271/EEC, the Spanish regulations for Water Reuse (Royal Decree 1620/2007) and the values established by the U.S. Environmental Protection Agency for water reuse (US EPA, 2004) (except for indirect potable reuse).

The total nitrogen removal efficiencies obtained in this study were much higher than the value of 51% obtained with other HMBRs that were fed with pre-treated raw wastewater (Liu et al., 2010) and they were higher than or comparable to the efficiencies reached in HMBRs treating synthetic wastewater: 41% (Liang et al., 2010) and 88.4% (for 3 mg DO L^{-1}) or 65.3% (for 6 mg DO L^{-1}) (Yang et al., 2009). Furthermore, the results are similar to those obtained in modified MBRs. Specifically, with an extra anoxic tank in the system (Côté et al., 1998; Ueda and Hata, 1999; Rosenberger et al., 2002) efficiencies between 79% and 82% were attained with similar retention times, and intermittent aeration in a single reactor and similar retention times 83% efficiency was achieved (Yeom et al., 1999; Ueda et al., 1996), while an MBR with internal deflectors (Kimura et al., 2008) had an efficiency of 77%.

3.4.3 Total suspended solids (TSS) concentrations

The maximum biomass concentration in our reactor throughout the study was 5 g MLSS L^{-1}, with averages of 2.4 g MLSS L^{-1} in Period I and 3.4 g MLSS L^{-1} in Period II. The HMBR was operated with low MLSS concentrations compared with usual values reported for conventional MBR (Judd, 2011). Nevertheless, hybrid systems, which combine attached and suspended biomass in the same system, normally work with similar MLSS concentrations to this study. For instance, (Yang et al., 2009) operated a moving bed membrane bioreactor (MBMBR) with 4 g L^{-1} of MLSS and 1 g L^{-1} of biofilm. In our case, since the mixed liquor concentration was not too high during operation, it was not necessary to remove sludge from the reactor during either of the periods of the study. Note that the higher influent organic rate measured in Period II explains the differences in mixed liquor concentration between the periods.

Unlike most hybrid MBRs, which work with moving bed support, in this configuration with a fixed bed support the biomass in the biofilm was not determined due to the difficulties in the extraction of the support media. The lack of a standardized protocol for *in situ* quantification of attached biomass was a limitation.

With respect to solids in the effluent, the microfiltration membranes allowed high efficiencies in suspended solids removal of approximately 99% for total suspended solids removal and 98% for volatile suspended solids removal (Table 3-2). Similar values have been obtained by other systems, including Kubota, Zenon and Orelis, namely 99%, 99% and 96.5% respectively (Stephenson, 2000).

3.4.4 Microbiological quality

It is generally accepted that MBRs provide excellent treated water quality producing a rather disinfected effluent. Under normal operating conditions, MBR technology achieves more than 6.6 log-removals of total coliforms, making it suitable for the post-treatment of effluents discharged in sensitive water bodies (van Nieuwenhuijzen et al., 2008). However, pathogens could pass through the membrane and contaminate the permeate if membrane integrity is compromised (e.g., broken fiber, fiber degradation, etc.).

The removal of microorganisms is only effective if the membranes are intact. Membrane integrity can be evaluated with direct methods, in which the magnitude measured is a direct function of membrane breaches (e.g., changes in

pressure) or via alteration in water quality parameters such as turbidity of particle counts (WEF, 2006).

Although pressure decay is the most frequently used direct method for evaluating membrane integrity, it can provide false-negative results (Guo et al., 2010a). Therefore, two parameters were used to determine membrane integrity in this study: turbidity and bacterial contamination.

In the samples collected during this experimentation, the average influent concentrations were 2.2 10^7 CFU/100 mL total coliforms and 3.9 10^6 CFU/100 mL fecal coliforms, whilst in effluent the average concentrations were 22.45 CFU/100 mL and 2.64 CFU/100 mL respectively. In addition, turbidity was always lower than 1 NTU.

These results indicate that the integrity of the membranes was not compromised and hence there were not leaks during the study.

3.4.5 Membrane performance

The reactor was first filled with tap water to measure the water flow rate through the clean membranes before beginning the experiment. The initial permeability with clean water was 1,035 L m^{-2} h^{-1} bar^{-1}. Next, the reactor was emptied and restarted; this time, it was fed with effluent from the Santander waste water treatment plant. The initial permeability was 216 L m^{-2} h^{-1} bar^{-1}. Once again, the reactor was emptied and filled with pre-treated raw wastewater, the permeability falling to 63 L m^{-2} h^{-1} bar^{-1}. The permeability was measured daily for the first week, during which a progressive decrease was observed. For the rest of the study, the permeability remained close to 30 L m^{-2} h^{-1} bar^{-1}. The permeability remained nearly constant, probably because of the relatively low suspended biomass concentrations in the reactor. The uniformity of the permeate flow rate made possible to maintain a 12 h HRT throughout the study even though in this chapter the system was operated by gravity.

Each period had its own start up, as the membrane module was chemically cleaned (section 2.6.5 chapter 2 Materials and methods), and the biofilm support material was replaced at the beginning of each period. The same first flow rate that was used in Period I, 12.6 L h^{-1} (permeability of 63 L m^{-2} h^{-1} bar^{-1}), was achieved after cleaning, and an evolution in the permeate flow rate similar to Period I was observed. Thus, no significant changes occurred in the membrane permeability conditions during the rest of the experiments.

In this phase of testing of the system, the main objective was not to investigate the behaviour of the membranes in depth, but rather to assess whether the configuration of the reactor could compete with other conventional and hybrid MBRs in terms of contaminant removal. However, the fact that throughout the two periods of experimentation permeability conditions remained constant could indicate good performance with respect to membrane fouling, and encouraged further research in this line.

Taking into account the results obtained in this chapter with pre-treated wastewater and bench-scale, the new vertical hybrid membrane bioreactor was scaled up and subsequent research studies were carried out (see chapters 4 and 5).

3.5 CONCLUSIONS

In this chapter a new hybrid membrane bioreactor (HMBR) with a compact vertical configuration, designed to simultaneously remove organic carbon and nitrogen, has been presented as an alternative to other MBRs.

This system is able to successfully process pre-treated raw municipal wastewater obtaining reuse-quality effluent, using a fixed-bed biofilm reactor without observing any signs of overloading.

The aeration system intended for membrane cleaning, also allowed for oxygenation, biofilm thickness control and bulk liquid mixing in a compact configuration.

The HMBR showed good performance in total nitrogen removal through simultaneous nitrification and denitrification (SND), despite the continuous aeration applied. Remarkably, average concentrations of 7.3 mg TN L^{-1} in the effluent were achieved.

Recirculation rate (300%) improved slightly organic carbon removal efficiencies (99% vs. 96% in BOD_5) and notably nitrogen removal (98% vs. 91% in NH_4^+-N; 80% vs. 69% in TN).

The new configuration can compete with other MBRs in terms of contaminant removal.

To obtain the optimum fixed bed hybrid membrane bioreactor, future investigations should focus on more specific aspects to identify the differences

between hybrid MBR and conventional MBR, and to determine the impact of the hybrid configuration on membrane fouling.

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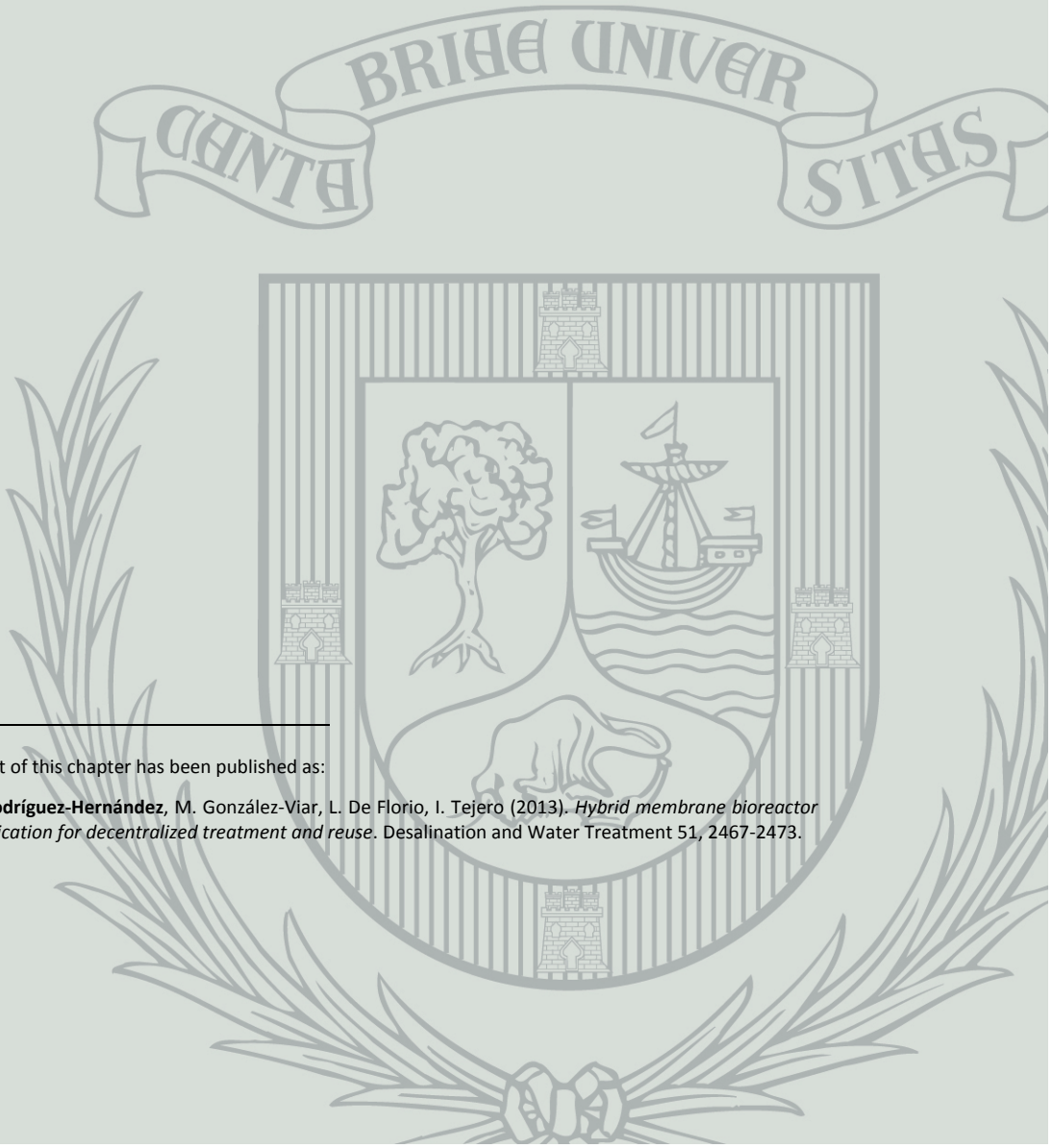
Chapter 4

Hybrid membrane bioreactor application for decentralized treatment and reuse[1]



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OUTLINE

SUMMARY

Membrane bioreactor (MBR) technology is worldwide recognized, and it is also being applied for reuse purposes. The addition of biofilm support media has already been suggested as pure biofilm as well as hybrid membrane bioreactor (HMBR) in order to get more efficient, compact and stable system. An original HMBR vertical configuration is here proposed for its feasibility as decentralized treatment, implementing submerged fixed bed biofilm support media (self-produced plastic nets filling the top part of the reactor) and submerged microfiltration membranes (collocated below the support media). The demonstrative treatment plant, tested at increasing loading rates (0.36 – 1.76 kg COD m^{-3} d^{-1}), was able to treat municipal wastewater without need of primary settling thus awarding high compactness as required to decentralized treatments.

The system maintained good overall performances at increasing loading rates with special regard to organic matter and ammonium removal. Denitrification and total nitrogen removal were slightly affected by the loading rate´s increase, until reaching stabilization to the new loading conditions. HMBR reliability in terms of stable effluent quality and the average characteristics of the effluent (among the others: [COD] < 55 mg L^{-1}, [SS] < 4 mg L^{-1}, [TN] < 10 mg L^{-1}, turbidity < 2 NTU) allow for discharge in sensitive areas as well as for reuse.

4.1 INTRODUCTION

The communities served with conventional sanitation facilities relay on a centralized, well-controlled, integrated management of water resources. Nevertheless, there are several drawbacks related to centralized systems. First of all, the elevate cost of the infrastructures (building up and maintenance of distribution and collection systems, which may be one order of magnitude greater than the treatment facility cost itself). Secondly, such big-scale systems are frequently subject to leakage causing the loss of fresh water as well as of harmful untreated wastewater. Furthermore the reuse of water (centralized reclamation) and resources thereby contained is hampered by the different nature of wastewaters, including in certain cases industrial wastewater. According to modelling performed by Fane et al., (2002) small scale reuse also reduces the risk of waterborne infection transmission. Furthermore, implementing reuse at local level is expected to save fresh water while avoiding the build-up of wastewater pipelines and pumping energy consumption. Anyway, new water sources must meet the water quality standards for actually safeguarding public health (US EPA, 2004).

In the proposal of decentralized wastewater treatment, compact technologies are desirable. With this aim, in spite of high aeration requirement, membrane biological reactors (MBR) appear to be suitable for on-site treatment and reuse (Jefferson et al., 2000; Meuler et al., 2008) when compared to other higher energy demanding processes assuring similar effluent quality, capable of achieving public acceptability (membrane barrier). However, the fouling of the membranes is one of the major drawbacks of MBR, limiting the efficacy of the process and escalating the costs.

An alternative to conventional MBR is the introduction of attached biomass in the system making it mainly biofilm type or hybrid (HMBR). This has been suggested by Artiga et al., (2005), Tejero and Cuevas, (2005) and Leiknes and Odegaard, (2007), among others (Ivanovic and Leiknes, 2012; Meng et al., 2012), most of whom utilize moving bed biofilm reactor (MBBR). A combination of MBBR, high rate separation (disk filter) and membrane ultrafiltration has also been proposed as compact tertiary treatment (Odegaard et al., 2012).

In HMBRs, biofilm attached to a support media (moving or fixed bed) and activated sludge biomass types coexist in the same reactor. Freely moving carriers allow for the utilization of the whole volume of the bioreactor while, on the other hand, fixed bed system are characterized by improved sludge characteristics (such as sludge volume index, SVI) (Water Environment Federation, 2010).

Namely, the novel configuration HMBR proposed in this chapter, previously tested at bench-scale (chapter 3), is made of an aerated mixed tank with submerged microfiltration membrane, in which a fixed support media for the biofilm attachment also takes place. The addition of biofilm type of biomass allows for achieving high biomass concentration and consequently high efficiency while keeping low suspended biomass concentrations in the reactor thus possibly reducing the effect of membrane fouling (Ivanovic and Leiknes, 2012; Meng et al., 2009; Liu et al., 2010). It also allows for the presence of nitrifying organisms without the need of extended aeration (volume requirements) since the solid retention time (SRT) is uncoupled with hydraulic retention time (HRT). The nitrogen removal may not be a priority in the treatment of wastewater aimed at reuse, especially when it is for irrigation purposes since the soil could positively profit the nutrients thereby contained; as a matter of fact, legislation typically require total nitrogen removal only for groundwater recharge application. Nevertheless, in case the reuse is not immediately after the treatment, a storage unit may be required which asks for controlled nutrient content in order to avoid undesired algae explosion. Furthermore, the possibility of controlling nitrogen removal and regulate the level of nitrification/denitrification may be desired

according to the agronomic necessities of the irrigation field (season variability, balance of phosphorous and nitrogen content). With this in mind, HMBR is proposed as a manner of providing a non-conventional water source by sewer mining or serving as decentralized facility.

4.2 OBJECTIVES

The aim of the study was to build up, to start up and to assess the feasibility of the hybrid membrane bioreactor at pilot scale working with pre-treated wastewater from a real WWTP.

Several experimental campaigns have been carried out with real wastewater to verify the reuse feasibility for small water systems.

Preliminary results of the first campaign are reported and discussed in this chapter.

4.3 MATERIALS AND METHODS

4.3.1 Experimental plant configuration

The demonstrative pilot plant was located in the municipal waste water treatment plant of Santander (Cantabria), Spain, thus being fed with raw unsettled wastewater after the pre-treatment unit (coarse screen, 3-mm fine screen, grit and grease removal). In addition, the pilot plant had a pre-treatment unit (1.5 mm fine screen and grit removal) before the MBR. A diagram of the pilot system is shown in Figure 4-1.

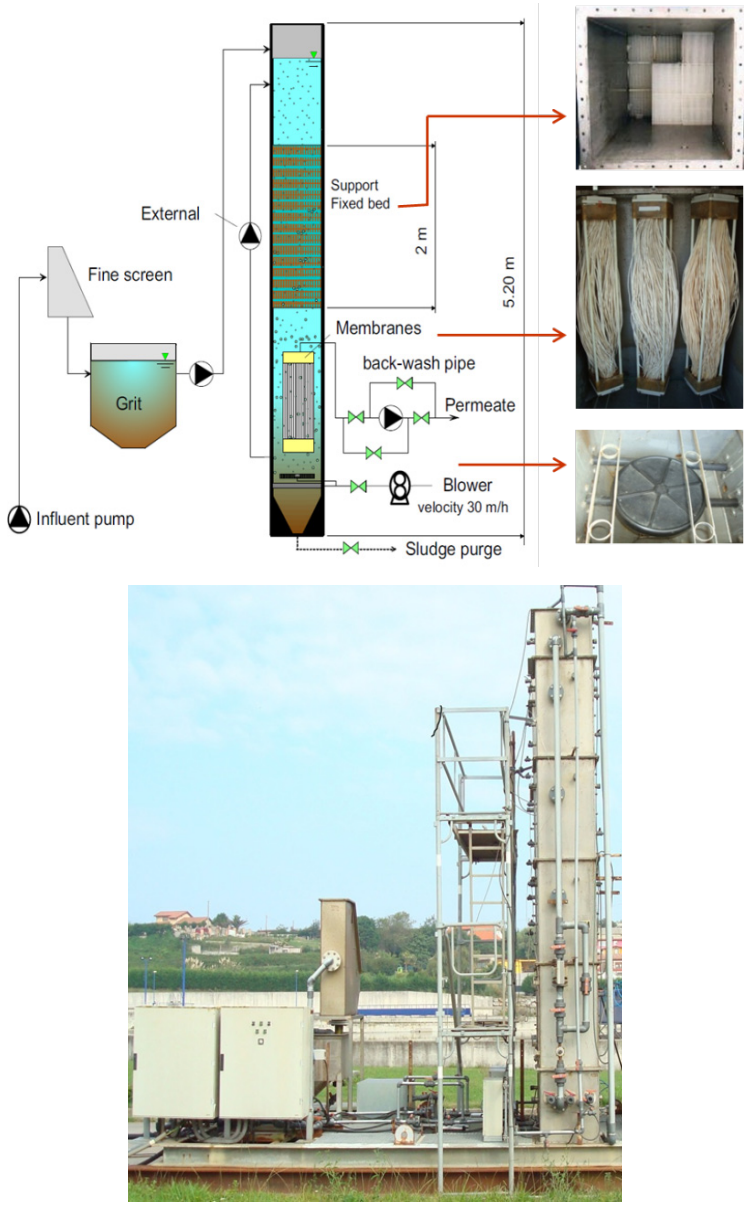


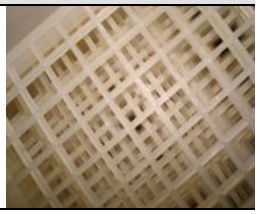
Figure 4-1 (up) Diagram and main components inside the reactor and (down) picture of the demonstrative plant configuration.

The vertical pilot plant (volume total of 1.8 m^3) is made of a stainless steel aeration tank in which an upper biofilm support fixed media zone takes place. Feeding and aeration systems, membrane modules, backwashing (with permeate)

system and recirculation pump complete the plant configuration. The tank has an internal square section of 0.60 m x 0.6 m and height of 5.20 m.

The submerged fixed biofilm support media was self-produced on a specific design (BLAS) (Tejero and Santamaría, 2000). It is made of flat rigid square meshes overlapping one another, with opening of voids in the mesh of 0.010 m and the separation between meshes of 0.013 m, resulting in a specific surface of 119 m^2 m^{-3}, which can reach up to 180 m^2 m^{-3} when biofilms grows on it (biofilm specific surface). 1,368 meshes were collocated inside the reactor to make up the 0.72 m^3 biofilm support bed. More characteristics of fixed support are summarized in Table 4-1.

Table 4-1 Characteristics and detail of fixed support media BLAS

	BLAS (patent [1])	
Material	polyethylene	
Geometry	square	
Configuration size (m)	0.2 x 0.2 x 0.013	
ensity (kg m^{-3})	950	
Pore size (m)	0.005	
Specific surface area (m^2 m^{-3})	119	

[1](Santamaría, 1998)

Six microfiltration polyvinylidene fluoride (PVDF) hollow fiber membrane modules (Porous Fiber, Leioa, Spain, pore size < 0.4 µm) are situated at the bottom of the bioreactor, offering an overall filtration surface of 12 m^2; their permeability was previously measured with clean water (at 20 °C) in laboratory (210 L m^{-2} h^{-1} bar^{-1}).

The aeration system is made of a course bubbles blower (aeration flowrate: 8 – 9 m^3 h^{-1}) which allows for the aeration of the two biomasses as well as the mixing of the bulk liquid, also improved by recirculation (300% flowrate); since the membrane module is located below the biofilm zone, the same aeration system is also used to perform air scouring on the membranes, thus unifying the triple action of aerating the biomass, mixing the system and reducing/controlling the biofouling on the membranes (see Figure 4-1).

Throughout the experimentation, the flowrate was set at about 120 L h^{-1}. During the experimental campaign, once obtained stable state, an increase in the loading was induced by reducing the volume occupied by bulk liquid in the reactor (passing approximately from 1.44 m^3 to 1.08 m^3 net volume), which produced a

change in the operational mode, as summarized in Table 4-2. This was aimed at observing the effect of an organic load applied increase over the demonstrative plant's performances.

Table 4-2 Operational conditions during the experimental campaign

Operational parameter	**Period I** (1 - 47 d)	**Period II** (48 - 74 d)
HRT (h)	12	9
SRT (d)[a]	Up to 47	Up to 74
MLSS (mg L^{-1})	< 1,000	< 3,000
Recycle rate (% of influent)	300	300
Temperature (°C)	8.4 - 14.7	9.2 - 14.6
Membrane Flux (L m^{-2} h^{-1})	10	10
Organic loading rate (kg BOD_5 m^{-3} d^{-1})	0.14 - 0.49	0.33 - 0.61
COD/ N/ P ratio[b]	165:40:3	149:39:4

[a]No sludge wastage
[b] COD_s, total nitrogen and phosphates

4.3.2 Analytical methods

Twenty-four hour composite samples were taken twice or three times per week. The analytical determinations of chemical oxygen demand (COD), soluble chemical oxygen demand (COD_s), biochemical oxygen demand (BOD), mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) and Total Kjeldahl Nitrogen (TKN) were performed according to the Standard Methods (APHA, 2005). NO_2^--N and NO_3^--N and PO_4^{3-} were determined using an ion-chromatography system (761 COMPACT-IC METROHM); the amount of ammonia was analyzed by an ammonia selective electrode (ORION, model 95-12); total Nitrogen (TN) was calculated by adding the nitrogen forms TKN, NO_2^--N and NO_3^--N; the dissolved oxygen (DO) concentration and temperature was measured inside the bioreactor above and below the biofilm support fixed bed, using a portable DO meter (HQ40d meter with a LDO101 probe, HACH, CO); pH was determined using a glass electrode pH meter (WTW, model SENTIX 21) and turbidity was measured with a turbidimeter (model 2100P ISO HACH, CO). With respect to membrane operation, transmembrane pressure (by means of a vacuum meter) was monitored continuously. More detailed are described in chapter 2.

4.3.3 Mass balances in the HMBR

A simplified to mass balance was performed for the HMBR. It is described in section 3.3.5.

4.4 RESULTS AND DISCUSSION

As a general evaluation, the results of the experimental campaign show that the system was able to treat pre-treated raw wastewater without primary sedimentation; clogging phenomena was not observed nor any increase of the hydraulic head loss through the system. This is contrast to other biofilm processes, which require primary sedimentation and it is a feature of the specifically designed fixed biofilm support media. Avoiding primary sedimentation is important to get a compact decentralized treatment system.

Throughout the operational period, it was possible to maintain an almost constant flux of about 10 L m^{-2} h^{-1}. In order to control membrane fouling, the strategy of washing was maintenance cleaning in situ (CIP). Permeability decrease observed during operation was similar to those obtained in other experimental runs with pilot-plant MBR, treating municipal wastewater (indicated values: 70 –50 L m^{-2} h^{-1} bar^{-1}) (Artiga et al., 2006) and somewhat lower than those obtained at bench scale, treating industrial wastewater (160 – 75 L m^{-2} h^{-1} bar^{-1}) (Sánchez et al., 2010), using the same membrane.

During the whole experimentation, the system was never purged since the suspended biomass growth was limited by the presence of the biofilm, typically characterized by low waste sludge production, as confirmed in other experiences with hybrid systems (Water Environment Federation, 2010).

In the followings, the average treatment performances of the demonstrative plant, not considering the first 30 days (start-up period), are reported (Table 4-3).

Table 4-3 Influent and effluent main parameters and removal efficiencies (RE) of the proposed HMBR.

Parameter	Influent (mg L^{-1})	Effluent (mg L-1)	Removal efficiency (%)
COD	372 ± 54	54 ± 4	84
COD_s	123 ± 20	29 ± 3	74
BOD_5	177 ± 25	4 ± 1	98
TSS	194 ± 27	4 ± 1	98
VSS	147 ± 20	3 ± 1	98
TKN	39.1 ± 1.6	3.1 ± 0.3	92
NH_4^+-N	24.1 ± 0.9	0.8 ± 0.3	97
NO_3^--N	0.2 ± 0.1	6.2 ± 1.6	-
NO_2^--N	0.4 ± 0.2	0.7 ± 0.3	-
TN	39.7 ± 1.5	9.9 ± 1.5	75
PO_4^-	3.5 ± 0.9	1.9 ± 0.3	42
Turbidity	213 ± 35	1.5 ± 0.4	99

4.4.1 Organic matter removal

The organic load applied to the system was in the range of 0.36 – 1.71 kg COD m^{-3} d^{-1}, varying due to the real influent wastewater fluctuations (combined sewer in wet weather coastal region) and to the change in operational conditions in the second period. The influent COD concentration showed peaks that were absorbed in the reactor effluent, even during the start-up period (Figure 4-2). Considering influent and effluent concentrations from day 30 on, the average percentage removals of organic matter for COD, COD_s and BOD_5 were satisfactory (84, 74 and 98%, respectively), showing a good response to the load increase.

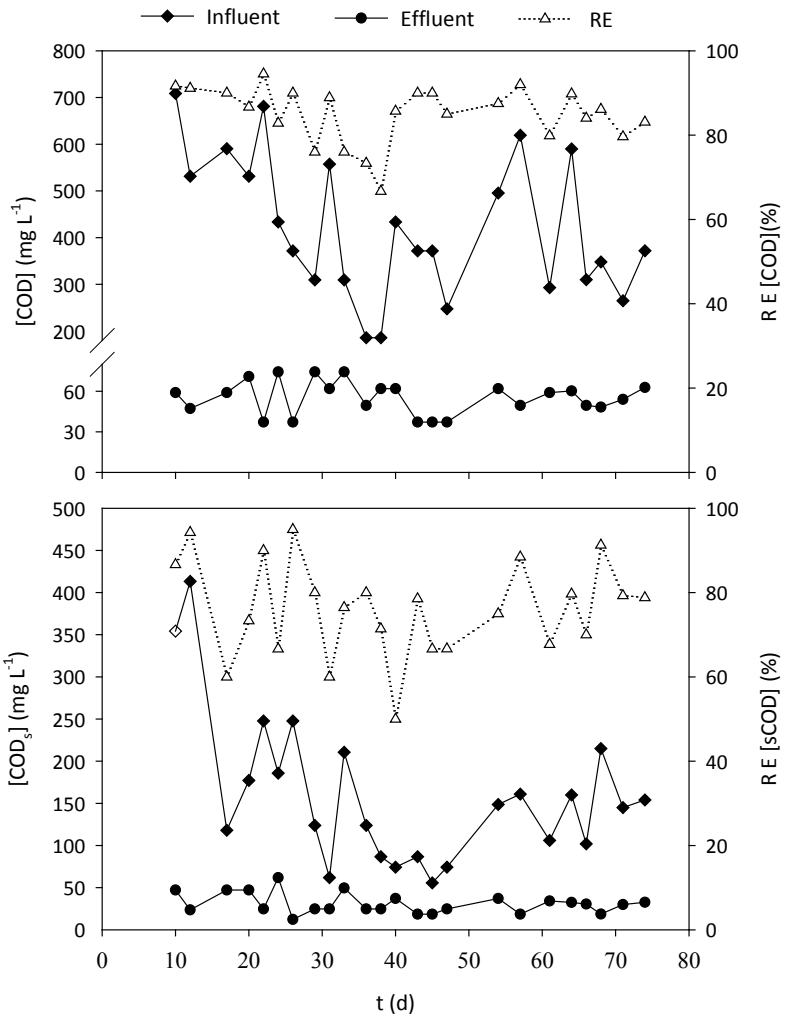


Figure 4-2 Influent and effluent concentrations and removal efficiencies (RE) of COD and COD_s during the experimental campaign.

In Figure 4-3, the organic load applied, in terms of COD, is correlated with the COD elimination capacity. It can be observed that the system did not reach its maximum treatment capacity (saturation) during the reported campaign so that it is possible to expect that the plant configuration be viable for higher organic load applied, as well.

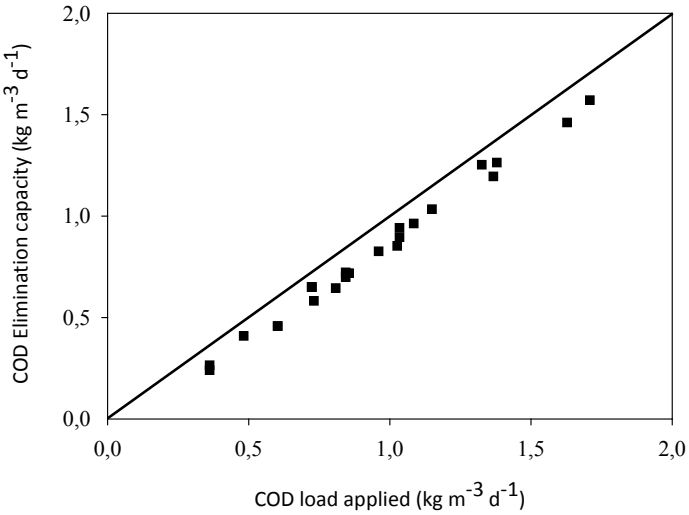


Figure 4-3 Organic load applied vs. elimination capacity in terms of COD (the axe´s bisector line representing the theoretical 100% removal).

4.4.2 Nutrients removal

In Figure 4-4, the trend of ammonium and total nitrogen removal is reported. Efficient nitrification was performed throughout the experimental trials (94%). It showed an improvement along with the campaign duration, as for the organic substance removal, in spite of the load applied increase. Starting from the day 30 – 35, the NH_4^+-N in the effluent was very low, with an average NH_4^+-N removal rate over 97%, indicating that the HMBR could enhance nitrification compared with conventional MBR, as observed also by (Jamal Khan et al., 2011) comparing a suspended–growth with an attached-growth MBR. This is due to the presence of attached biomass coexisting with suspended biomass; nitrifying microorganisms on the support medium are protected by the biofilm structure against shocks (in terms of load or contaminants), assuring stable performances (Water Environment Federation, 2010).

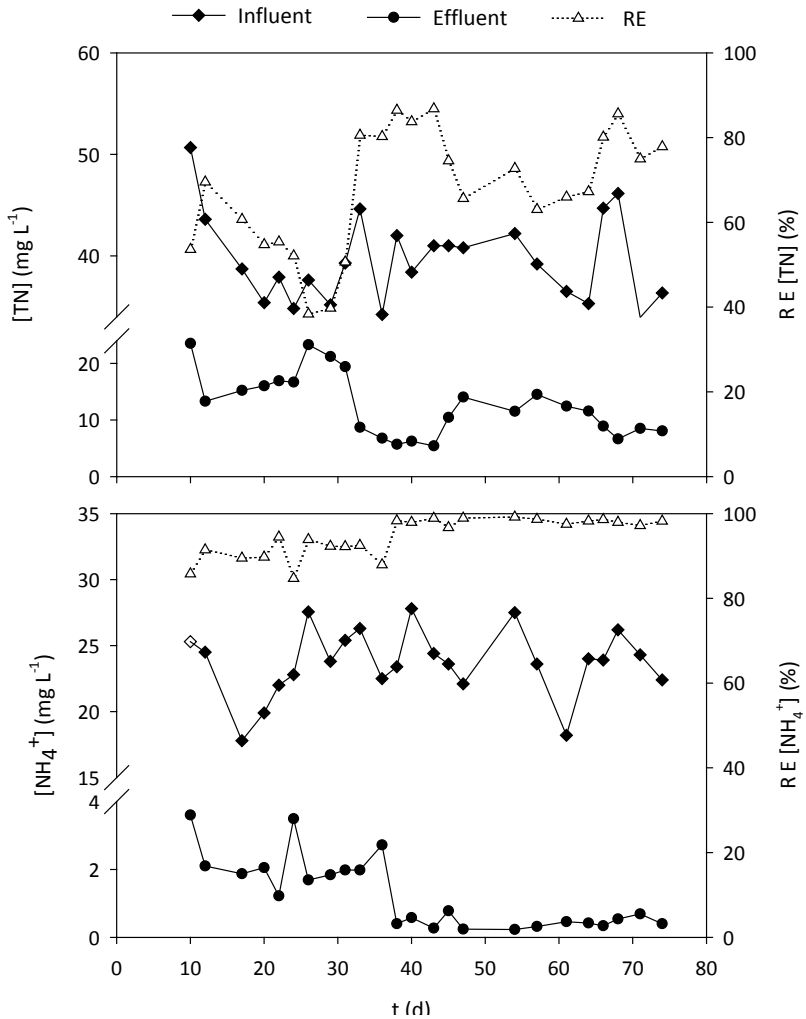


Figure 4-4 Influent and effluent concentrations and removal efficiencies (RE) of TN and NH_4^+-N during the experimental campaign.

In spite of not including any anoxic tank in the system configuration, increasing denitrification was observed along with the experimental campaign. This may be explained by the growth of biofilm thickness in the HMBR which provokes that dissolved oxygen transference into the inner part of the biofilm is increasingly hindered. Consequently, the outer biofilm layer is kept aerobic while the inner biofilm is subject to anoxic or anaerobic conditions. Nitrification takes place in the aerobic layer and in suspended biomass and denitrification may occur in the

anoxic layers of the biofilm. This phenomenon, known as simultaneous nitrification-denitrification (SND) (Jamal Khan et al., 2011; Pochana et al., 1999), became evident during the last 44 days of the experimental campaign, when TN removal efficiency increased significantly and average TN in the effluent was < 10 mg L^{-1}.

This result shows the efficacy of the system in removing nitrogen, in spite of continuous aeration resulting in DO concentration around saturation inside the bioreactor (both below and above the biofilm support fixed bed). Such elevate TN removal is not shown by conventional MBR given the small size of the flocs (Zhang et al., 1997; Henriques et al., 2005) which typically grow in the MBRs' activated sludge. TN removal related to the last 44 days experimentation averaged 75%, in spite of the slight worse removal efficiencies (RE) at the beginning of the second period (days 47 – 64), characterized by sudden load applied increase. Such slight decrease in TN removal may be explained by the higher competition among heterotrophs for the substrate utilization, which resulted in less organic matter available for denitrification, until reaching stabilization to the new loading conditions. As for denitrification, a mass balance gave values for period I and II of 48% and 59%, respectively.

It was also observed 42% removal of phosphates in the period from day 30 on, which, in principle, was not attributed to enhanced biological phosphorus removal (EBPR) but mainly to assimilation and biomass retention thanks to membrane filtration. However, other authors reported EBPR to occur in the biofilm, observing slightly higher percentage removal in an attached–growth MBR (Jamal Khan et al., 2011), indicating that phosphorus accumulating organisms (PAOs) may have developed within anoxic/anaerobic zones of the support media. Such conditions were not looked for in the design of the present HMBR in which the aeration system, located at the bottom of the vertical configuration, also performs a shear force on the upper biofilm fixed bed, enhancing the oxygen transference and limiting the biofilm thickness. As already mentioned, the reuse application of treated wastewater may not ask for nutrient removal, except for the storage conditions (which may induce algae bloom) and a few specific reuses. Limited phosphate concentrations in the effluent, nevertheless, may be desirable also to avoid scaling problems in the pipelines, while nutrients could be favourably recovered by controlled sludge application to the soil.

Table 4-4 HMBR effluent quality and limit values indicated in the Spanish reuse legislation.

		TSS ($mg\ L^{-1}$)	Turb (NTU)	OTHER POLLUTANTS	TP ($mg\ L^{-1}$)	TN ($mg\ L^{-1}$)	NO3 ($mg\ NO_3\ L^{-1}$)
Urban uses	Residential	<10	<2	According to discharge permit	--	--	--
	Urban services	<20	<10	(Annex II, RD 1620/2007)	--	--	--
Agricultural	Raw consumables	<20	<10		--	--	--
uses	Non raw consumables	<35	--		--	--	--
	Irrigation for industrial crops	<35	--		--	--	--
Recreational	Watering golf courses	<20	<10		--	--	--
uses	Not open to public ponds	<35	--		<2	--	--
Environm.	Aquifer recharge	<35	--	(Annex III, RD 1620/2007)	--	<10	<25
Uses	Aquifer recharge (direct)	<10	<2		--	<10	<25
	Irrigation woodland	<35	--		--	--	--
HMBR effluent average quality		4	<2	[COD] = 54 $mg\ L^{-1}$	--	<10	24[a]

[a] Without considering the destabilization caused by the load applied increase (days 47-64)

4.4.3 Quality of the effluent

The effluent quality obtained is compatible with the standards for reuse of treated water in terms of bacterial contamination, nitrogen, organic matter, suspended solids and turbidity (as reported in Table 4-3), satisfying the requirements established in the European legislation as well as the US EPA recommended values (US EPA, 2004). In Table 4-4, the physical-chemical parameters required for the possible end-uses, as established by the Spanish legislation (R.D. 1620/2007), are reported alongside the average value of such parameters in the treated effluent.

4.5 CONCLUSIONS

The assessment of the proposed technology's suitability to serve as a decentralized treatment facility has been carried out at demonstrative scale, treating wastewater from a real WWTP.

The application of the present HMBR configuration proved to be technically feasible and offered promising overall advantages:

- high quality of the effluent;
- no need for primary sedimentation with the tested real wastewater;
- capability to work with increasing load applied to the system, obtaining good removal efficiencies.

In steady state, average percentage removals of organic matter for COD and BOD_5 were 84% and 98%, respectively; nitrification was 97% and TN removal was 75% (in one single reactor); total suspended solids, volatile suspended solids and turbidity removal averaged 98, 98 and 99%, respectively, meeting the standards required by the legislation for reuse.

Neither clogging was observed in the submerged fixed bed, nor loss of flux through the membrane during the operational period, in spite of not carrying out intensive chemical cleaning.

The system obtained a stable quality effluent (e.g., COD concentration of 54 ± 4 mg L^{-1}) while treating a highly variable influent (372 ± 54 mg L^{-1}).

As observable from the TN removal percentage, the biofilm allows for the presence of anoxic zones at the most internal layers of the biofilm thus enabling nitrification and denitrification to occur simultaneously; TN removal slightly decreased when increasing the loading rate without hindering the good quality of

the effluent whose average TN concentration remained below 10 mg L^{-1} (not considering the start-up, first 30 days, as for the other parameters).

Neither clogging was observed in the submerged fixed bed nor loss of flux through the membrane during the operational period, indicating operation reliability.

The proposed technology application as decentralized treatment and reuse facility makes available an alternative valuable water source for the mentioned uses, especially in scenarios of water scarcity.

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Chapter 5

Comparison between a fixed bed hybrid membrane bioreactor and a conventional membrane bioreactor for municipal wastewater treatment: a pilot-scale study[1]

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OUTLINE

SUMMARY

Two pilot plants, a hybrid membrane bioreactor (HMBR) was developed, by adding biofilm support media into a conventional membrane bioreactor (CMBR), and operated in parallel with domestic wastewater under the same conditions. Results showed effluent quality was significantly better with the HMBR. The removal efficiencies of COD, BOD_5, $NH4^+$-N and TN with the HMBR were 84, 98, 97 and 75%, respectively, as compared to 80, 96, 93 and 38% with the CMBR. No substantial differences were found with respect to phosphorus removal. Regarding membrane performance, the fouling rate in the HMBR was on average only 57% of than in the CMBR. The lower concentration of colloidal biopolymer clusters (cBPC) in the HMBR sludge, probably due to their retention by the biofilm, could be partially responsible for this difference. Filterability and settleability of the sludge were also better in the HMBR. Consequently, it is concluded that the addition of fixed support media for biofilm growth can improve the performance of CMBRs.

5.1 INTRODUCTION

A membrane bioreactor (MBR) combines an activated sludge process with a solid-liquid separation by membrane filtration (ultra- or micro-) filtration the usual sedimentation step. As a result, MBRs have many advantages over conventional activated sludge treatments, including small footprint and reactor requirements, good disinfection capability, higher volumetric loading and less sludge production. The high sludge retention time (SRT) in MBRs, leads to the formation of a specialized bacterial community with particular degradation features (Drews et al., 2005) in relation to the organic substrate in the effluent. As a result, higher organic matter removal rates and better effluent quality are achieved compared to conventional activated sludge processes. MBR technology is therefore considered reliable and effective for removing many contaminants from wastewater in one step.

In aerobic MBRs almost complete nitrification can be achieved, while for denitrification needs the addition of an anoxic tank prior to the aeration tank with conventional recirculation (e.g., Patel et al., 2005), the modification of the reactor configuration (intermittent aeration, e.g., Hasar et al., 2002; baffled membrane bioreactors, e.g., Kimura et al., 2008) or simultaneous nitrification/denitrification (e.g., Liu et al., 2010; Rodríguez-Hernández et al., 2012).

Membrane fouling is still the main concern in the application of MBRs (Drews, 2010), reducing filtration performance, shortening the life of membranes and leading to higher operating costs. For a given MBR, fouling is directly related to sludge characteristics and hydrodynamic conditions, and indirectly related to operating conditions and feedwater (Meng et al., 2009), which in turn affect sludge characteristics (e.g., suspended solids, colloidal and soluble organic content or physical properties). Therefore, as the biological system is complex and there is a lack of standardization of methods and terminology (Drews, 2010), there is no consensus on which constituents fouling can primarily be attributed to. Many researchers have focused on sticky substances of the biomass that could govern the rate of fouling, such as extracellular polymeric substances (EPS) or soluble microbial products (SMP) (Drews, 2010). Recently, a new pool of organic substances in the sludge classified as biopolymer clusters (BPC), which are easier to measure than EPS or SMP, were found to be one of the major foulants in the membrane bioreactor system (Wang and Li, 2008). In addition, Sánchez et al. (2013) observed a strong correlation between the colloidal fraction of biopolymer clusters and membrane fouling.

An alternative to the conventional membrane bioreactor (CMBR) (with suspended biomass) involves its combination with a biofilm reactor (with attached biomass). When the biodegradation is carried out by both suspended and attached biomasses, this configuration has been called an assisted or hybrid biofilm membrane bioreactor (HMBR) (Ivanovic and Leiknes, 2012) (see chapter 1). In these systems, a hybrid growth configuration is achieved when both types of biomass grow simultaneously in the same reactor (Guo et al., 2010; Rodríguez-Hernández et al., 2012) or there is external recirculation from the aerated membrane filtration (Leyva-Díaz et al., 2013), obtaining mixed liquor suspended solids (MLSS) concentrations in the range of activated sludge processes. It is important to note that many other authors use the term hybrid MBR in a broader sense, namely for an MBR combined with any other technology, such as a granular activated carbon-sponge fluidized bed bioreactor (e.g., Nguyen et al., 2013) or a nanofiltration unit (Chon et al., 2013). In hybrid MBRs, the addition of biofilm support to the reactor has been mainly proposed with the goal of overcoming the aforementioned limitations, that is, to improve nutrient removal and reduce membrane fouling. In this regard, most researchers have obtained significantly better total nitrogen (TN) removal in HMBRs via simultaneous nitrification and denitrification (Liu et al., 2010; Yang et al., 2009b; Jamal Khan et al., 2011) by providing good anoxic conditions inside the biofilm. Enhanced phosphorus removal has also been reported (Ngo et al., 2006).

With respect to membrane fouling, lower fouling rates have been commonly obtained in HMBRs (Liu et al., 2010; Wang et al., 2012), although poorer membrane performance has also been reported (Yang et al., 2009a). Moreover, it is still unclear which factors are mostly responsible for the differences in fouling (Ivanovic and Leiknes, 2012). Hence, there is the need for more research in this field.

Most hybrid systems use moving carriers, but some configurations, that combine fixed beds with membrane reactors, have also been investigated (Lee et al., 2001; Tejero and Cuevas, 2005; Shuo et al., 2008). The hybrid MBR used in this work, as described in chapter 4, has proved to be capable of treating pre-treated raw wastewater, without observing any clogging phenomena or increase of the hydraulic head loss. This is in contrast to other fixed bed biofilm processes which require primary sedimentation, and it is a feature mainly attributed to the specifically designed support media.

Recently, there has been a growing interest in the comparison between the performance of hybrid and conventional MBRs, but to date few studies have been reported, and to the best of our knowledge, only the works of Wang and colleagues (Wang et al., 2012; Liu et al., 2012) and Leyva-Díaz et al., (2013), who worked with suspended carriers, have been conducted at a pilot-scale with real wastewater.

The study of this chapter provides further insight into the differences in behavior of hybrid and conventional MBRs, operated simultaneously at a pilot-scale fed with real municipal wastewater and with the hybrid MBR being a novel fixed bed-type.

5.2 OBJECTIVES

Specifically, the aim of this study was to compare the overall performance of a hybrid biofilm membrane bioreactor (HMBR) and a conventional membrane bioreactor (CMBR) for the treatment of municipal wastewater under the same operating conditions.

For this purpose, two pilot scale MBRs, identical in design except for the addition of a fixed bed in the HMBR, were constructed, characterized and operated in parallel with real wastewater.

The performance of the two reactors was assessed based on organic matter and nutrient removal at various stages of the process. Further studies were also carried out to compare membrane fouling and sludge characteristics.

5.3 MATERIALS AND METHODS

5.3.1 Pilot-scale MBR setup

Two demonstrative pilot-scale membrane bioreactors with identical volumes (1.8 m^3, cross-sectional are of 0.36 m^2 by height of 5.2 m), were constructed and operated in parallel. The setup of the MBR system is illustrated in Figure 5-1 (schematic diagram and picture). It consists of two column-shaped reactors made of stainless steel. After pre-treatment, wastewater was pumped into the reactors using volumetric pumps. The flow was controlled with an electrode-type level switch. The main components of MBRs system were enumerated to below: 1- municipal wastewater; 2- screen; 3- grit removal; 4- feed pump; 5- recirculation pump; 6- fixed bed support (BLAS); 7- membrane module; 8- permeate; 9- back-wash pipe; 10- air diffuser; 11- blower; 12- vacuum gauge; 13- sludge purge.

As shown in Figure 5-1, the aeration system was located at the bottom of the reactors and was used for membrane cleaning purposes. The cleaning bubbles also serve to aerate and mix the contents inside the reactors, and this allowed the system to be very compact. The air flow rate (8 – 9 $m^3 h^{-1}$) was determined by the need for sufficient continuous stirring in the membrane zone (specific air demand per membrane area, SAD_m of 0.75 $m^3 m^{-2} h^{-1}$), which corresponded to a superficial velocity in the reactors of 22 – 25 m h^{-1}. This controlled membrane fouling, resulting in an air/permeate flow ratio of 57 – 75 m^3 air m^{-3} permeate. The oxygen concentration in the bulk liquid was around saturation. The air came from a blower and was measured by several rotameters to maintain a constant flow rate.

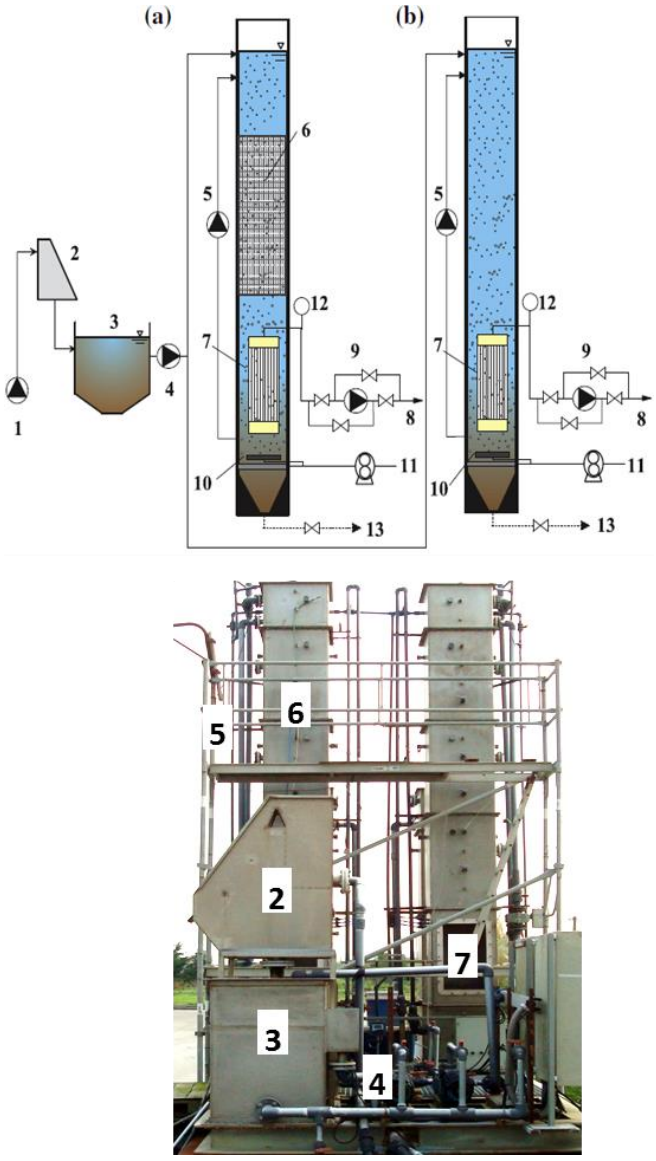

Figure 5-1 (up) Schematic diagram and (down) picture of the demonstrative pilot plants: (a) HMBR and (b) CMBR.

Each reactor had a recirculation pump of 700 L h^{-1} and both MBRs worked with recirculation of around 300% of the inflow. Hollow-fiber membranes used for both MBRs were made of polyvinylidene fluoride (PVDF) with a nominal pore size of 0.4 μm (Porous Fibers; Leioa, Spain) and a total membrane surface area of 2 m^2 per module. Six submerged membrane modules were installed in each reactor

(seven in period III); therefore, the total filtering surface per reactor was 12 (14 m^2 in period III). Effluent was extracted from the system by imposing a negative pressure on the membrane (i.e., a transmembrane pressure, TMP) using a volumetric pump. A digital compound pressure sensor ZSE80F (SMC Corporation) was used to monitor changes in TMP evolution in the bioreactors. The permeate was accumulated in a 200-L permeate tank for backwashing the membranes.

The only difference between the two configurations was that the HMBR included a support media for growth of attached biomass. The fixed biofilm support media used (called BLAS) was produced in house to a specific design (Tejero and Santamaría, 2000). This vertical configuration of HMBR has been tested previously in bench scale with other support (chapter 3) and later in pilot scale with BLAS support (chapter 4). For this work, several improvements were incorporated in the pilot plants. BLAS support is made of flat rigid polyethylene square meshes with a density of 950 kg m^{-3} and the dimensions of the mesh sheets are approximately 0.2 × 0.2 × 0.013 m. These mesh sheets overlap one another, resulting in a pore size of 0.005 m and a specific surface area of 119 m^2 m^{-3}.

The high biofilm surface area in the HMBR was obtained by adding this biofilm support media at a high filling fraction, corresponding to 1/2 of the effective reactor volume. The fixed support was not acclimatized before operation.

5.3.2 Wastewater and operating strategy

The study corresponding a this chapter, was performed in a waste water treatment plant, located in the province of Cantabria (Spain), with a population equivalent of about 428,000, combined sewer system and average flow of 7,668 m^3 h^{-1}. The pilot plants were operated with pre-treated wastewater (coarse screen, 3-mm fine screen, grit and grease removal) taken after one of the grit chambers of the facility. In addition, the pilot plants had a pre-treatment unit (1.5 mm in stages I and II, and 0.5 mm in stage III fine screen and grit removal) before the MBRs (in Figure 5-1).

The composition of the inlet wastewater was the following average values of 372 ± 135 mg L^{-1} total COD, 123 ± 50 mg L^{-1} soluble COD, 177 ± 63 mg L^{-1} BOD_5, 39 ± 4 mg L^{-1} TKN, 24 ± 2 mg L^{-1} ammonium, 0.4 ± 0.6 mg L^{-1} nitrite, 0.2 ± 0.3 mg L^{-1} nitrate, 40 ± 4 mg L^{-1} total nitrogen, 3.5 ± 1 mg L^{-1} orthophosphate, 200 ± 69 mg L^{-1} MLSS and pH of 7.4 ± 0.1.

The configurations proposed were physically characterized, in terms of their hydrodynamic behavior and the oxygen transfer coefficient (K_La). This characterization is explained in section 5.3.3.

Performance of the two MBRs in terms of organic matter and nutrient removal was evaluated during two stages in continuous operation in parallel (that is, both reactors operated with the same working parameters) for around three months. Stage I lasted 46 days. From day 47 to 80 (stage II), an increase in the loading applied was induced by reducing the volume occupied by bulk liquid in the reactor.

The last step (stage III) of this research lasted around four months and focused on comparing the membrane performance and the characteristics of settleability, filterability and dewaterability of the sludge obtained from the two reactors.

In period III, the membranes were operated in cycles of 10 min with a permeation period of 14 min and a backwashing period of 1 min.

Maintenance cleaning or cleaning in place (CIP) was performed every 7 - 9 days at the same time in both reactors, using between 250 and 350 mg L^{-1} of a sodium hypochlorite solution. In addition, physical cleaning and a cleaning out of place (COP) with higher concentration of chemicals, up to 800 mg L^{-1} of sodium hypochlorite solution in tap water, were performed in series on operating day 97 of stage III.

The water temperature throughout all the experiments ranged from 9 to 18 °C. Details of operating conditions are given in Table 5-1.

Table 5-1 Operating conditions of the two pilot-scale MBRs

Stage I	Stage II	Stage III
HRT: 12 h SRT: up to 47 d OLR: 0.36–1.38 kg COD m^{-3} d^{-1} (average 0.83 kg COD m^{-3} d^{-1}) Suspended biomass: HMBR: < 1000 mg SS L^{-1} CMBR: < 1000 mg SS L^{-1} Filling fraction HMBR: 50%	HRT: 9 h SRT: up to 80 d OLR: 0.73–1.71 kg COD m^{-3} d^{-1} (average 1.15 kg COD m^{-3} d^{-1}) Suspended biomass: HMBR: < 3000 mg SS L^{-1} CMBR: < 4000 mg SS L^{-1} Filling fraction HMBR: 63%	HRT: 9 - 10 h SRT: up to 130 d OLR: 0.54–3.87 kg COD m^{-3} d^{-1} (average 2.2 kg COD m^{-3} d^{-1}) Suspended biomass: HMBR: < 4300 mg SS L^{-1} CMBR: < 5900 mg SS L^{-1} Filling fraction HMBR: 55%
Flow rate: 120 L h^{-1}		Flow rate: 140 L h^{-1}
Membrane flux: 10 LMH – Backwash flux: 30 LHM		

5.3.3 Analytical methods and statistical analysis

The hydrodynamic evaluation of the liquid phase was performed using the tracer step input technique (Levenspiel, 1999) with rhodamine-WT as a tracer, this being measured with a Turner Designs Model 10-AU005 fluorometer. The $K_L a$ for the oxygen was measured using the non-steady-state modified method in accordance with the European Standard (UNE EN 12255-15, 2003) and was calculated according to the following formula:

$$k_L a = \frac{\frac{dC}{dt}}{C^* - C} \qquad \text{Eq. 5-1}$$

Where

C: is the oxygen concentration in the bulk liquid o the reactor (mg L^{-1});

*C**: is the oxygen saturation concentration (mg L^{-1}).

The characterization was carried out without biomass, prior to the feeding of wastewater.

During continuous operation, 24-hour composite samples were taken twice a week. Single samples for sludge characterisation were taken in the membrane zone. All samples were kept cool until laboratory analysis was performed. The sample points include: the wastewater inlet, area around the membranes and permeate, in both systems, and additionally above the support area in the HMBR.

COD_t, COD_s, BOD_5, mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) and total Kjeldahl nitrogen (TKN) were measured according to the Standard Methods (APHA, 1998). Ammonia nitrogen (NH_4^+-N) was analyzed by an ammonia selective electrode (ORION, model 95-12), and nitrite nitrogen (NO_2^--N) and nitrate nitrogen (NO_3^--N) concentrations were determined using an ion-chromatography system (761 COMPACT-IC METROHM). The details of the analytical methods are described in chapter 2.

Dissolved oxygen concentration and temperature were measured with a portable DO meter (HACH, LDO101), pH with a glass electrode pH meter (WTW, SENTIX 21) and turbidity with a turbidimeter (HACH, model 2100P ISO). With respect to membrane operation, transmembrane pressure (TMP) was monitored continuously using a vacuum meter.

The colloidal fraction of biopolymer clusters (cBPC) in the liquid phase of the sludge mixture suspension was estimated by calculating the difference in organic

matter concentration between the sludge mixture after filtration through 0.45-µm nitrocellulose membrane filters (HA, Millipore) and the permeate (Sánchez et al., 2013), measuring the organic matter in terms of COD (Lin et al., 2009).

Membrane fouling rates (KPa d^{-1}) were calculated as the increase in transmembrane pressure (TMP) during the interval between maintenance cleanings (dTMP/dt) (i.e., residual fouling, as defined in (Drews, 2010).

Sludge volume index (SVI) and specific resistance to filtration (SRF) were assessed according to modified Standard Methods ((APHA et al., 1998) for SVI; and (UNE EN 14701-2, 2006) for SRF) in order to evaluate settling, dewatering and filtering characteristics of the sludge. The SRF test was performed by recording the volume of filtrate vs. time using a negative pressure of 0.51 bar and Whatman No. 1 as filter media. The SRF was determined by plotting the ratio filtration time/filtrate volume (t/V) vs. the filtrate volume (V). Using the slope of the plot, the specific resistance to filtration was calculated with the following formula (UNE EN 14701-2, 2006):

$$\alpha = \left(\frac{2A^2P}{\mu w}\right) b \qquad \text{Eq. 5-2}$$

Where:

α: is the specific resistance to filtration (m kg^{-1});

P: the pressure of filtration (Pa);

A: the area of the filter paper (m^2);

μ: the viscosity of filtrate (Pa·s);

w: the weight of dry solids per volume of filtrate (kg m^{-3});

b: the slope of the plot.

(More detail sees section 2.4.2 chapter 2 Materials and methods).

Average treatment performances are reported, not considering the first 30 days (total nitrogen removal stabilization period). Statistical analysis was performed using SPSS (Version 20.0) with data for the whole operating period. The significance of differences between values obtained in the two configurations was assessed using the Student´s *t*-test. Values of $P \leq 0.05$ were considered significant.

5.3.4 Mass balances in the MBRs

A simplified to mass balance was performed for the HMBR. It is described in section 3.3.5.

5.4 RESULTS AND DISCUSSION

5.4.1 Physical characterization

A tracer study was conducted with the aim of examining the hydraulic and mixing characteristics of the configurations proposed. The two reactors exhibited similar hydrodynamic behaviour. The tracer returned more than 97% of the injected amount for HMBR and 96% for CMBR. The Morril index (MI) values, of 16.35 in HMBR and 19.31 in CMBR, suggest that both configurations (in the absence of biomass) had optimum mixing (Levenspiel, 1999). Nevertheless, hydraulic efficiency values of approximately 1.5 in both MBRs indicated the presence of dead zones (Levenspiel, 1999), which were attributed to the sedimentation area of the hopper. With respect to oxygen transfer, the K_La value (at 20 °C) was 1.85 times higher in the HMBR (33.9 h^{-1} vs. 18.3 h^{-1} in the CMBR). Such an improvement has been attributed to extended bubble retention time within fixed bed reactors (Stenstrom et al., 2008).

5.4.2 Organic matter removal

Through the experimental period, effluent concentrations of COD were consistently lower in the HMBR than the CMBR and basically unaffected by (real and artificially induced) influent wastewater fluctuations (0.36 - 1.71 kg COD m^{-3} d^{-1}) (Fig. 5-2). In the CMBR, the average effluent of COD, COD_s, and BOD_5 was 65 ± 16, 38 ± 17 and 6 ± 3 mg L^{-1}, which corresponded to removal rates of 80, 63 and 96%, respectively. Whereas, in the HMBR, the results obtained were COD 54 ± 11, COD_s 29 ± 9 and BOD_5 4 ± 3 mg L^{-1} (84, 74 and 98%, respectively).

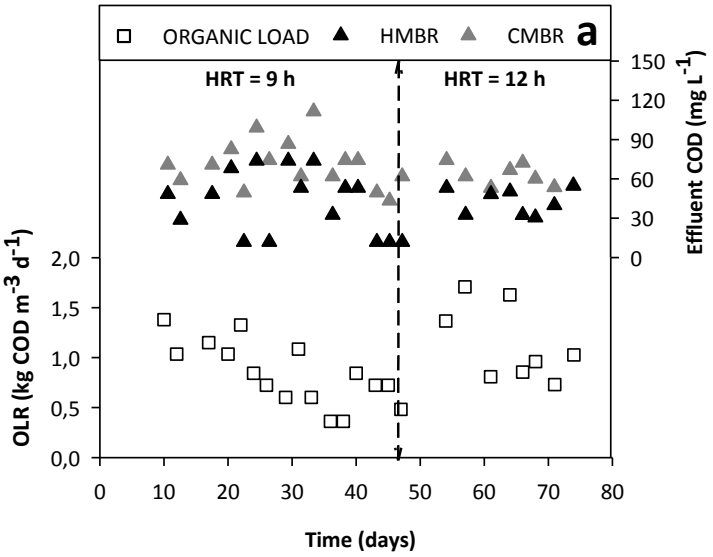

Figure 5-2 Evo

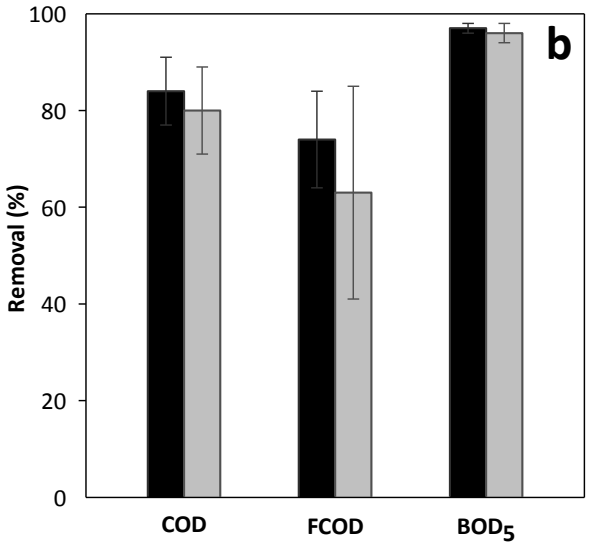

Figure 5-3 Average organic matter removal for systems: HMBR (■) and CMBR (■).

As showed in Figure 5-3, all parameters analysed in terms of organic matter were better in the HMBR than the CMBR ($P < 0.05$; $P = 0.07$ for COD_s removal; $P = 0.12$ for COD removal), a pattern also observed by other authors (Liu et al., 2010; Yang et al., 2009b; Wang et al., 2012). The main reason for better organic removal in an

MBR with attached growth compared to a conventional MBR is likely to the higher concentration or activity of the biomass attached.

5.4.3 Nutrient removal

Fig. 5-4 shows the ammonium removal over the experimental period for both MBRs. The average effluent ammonium concentrations of the HMBR and CMBR were 0.8 ± 0.3 and 1.7 ± 0.3 mg L^{-1}, respectively. Both systems showed excellent average removal efficiencies, being ammonium removal in the HMBR slightly better (P=0.03) than in the CMBR (97% vs. 93%) (Fig. 5-4).

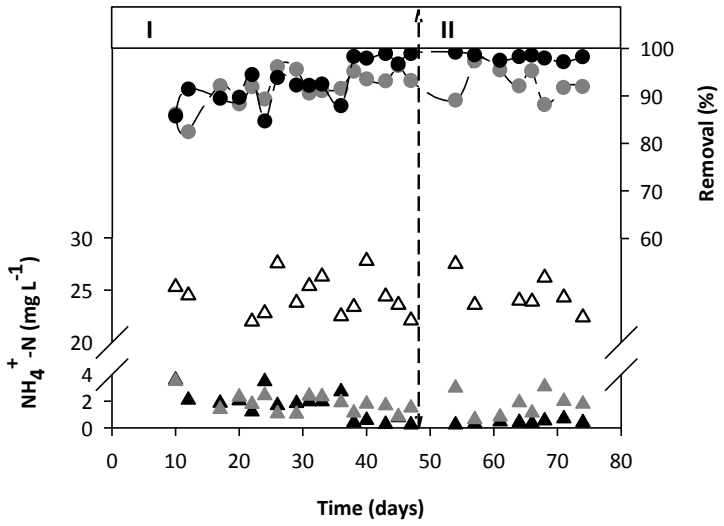

Figure 5-4 Overall concentrations and removal efficiencies of NH_4^+-N for the HMBR and CMBR systems: Influent (△); Effluent HMBR (▲); Effluent CMBR (▲); Removal HMBR (●); Removal CMBR (●).

Other authors observed similar improvements when combining attached and suspended biomass (Jamal Khan et al., 2011; Wang et al., 2012; Guo et al., 2009). These studies demonstrated disclosed that hybrid systems are better at eliminating ammonium and have greater resistance to shock loading than single activated sludge in an MBR. The attached biomass is more protected from both load and temperature variations than the suspended biomass. This fact promotes a greater growth of nitrifying microorganisms, mainly on the support media rather than suspension growth, resulting in more efficient ammonium elimination.

During the first 30 days, the effluent concentration of TN was high in both MBRs, (Fig. 5-5), but it substantially decreased over the rest of the study period. Average effluent nitrite concentrations in both systems were similar (≈ 0.7 ± 0.7 mg NO_2^--N L^{-1}). In contrast, both nitrate and TN concentrations in the HMBR effluent (average 6.2 ± 3.9 mg NO_3^--N L^{-1}; 9.9 ± 3.8 mg TN L^{-1}) were significantly lower (P << 0.05) than those obtained in the CMBR (average 19.1 ± 3.7 mg NO_3^--N L^{-1}; 24.5 ± 4.3 mg TN L^{-1}). The better TN removal in the HMBR (average 75 vs. 38%, in the CMBR), (Fig. 5-6) is attributable to simultaneous nitrification and denitrification (SND) in this reactor. As for denitrification, values obtained from a mass balance, were 63% in the HMBR and 28% in the CMBR.

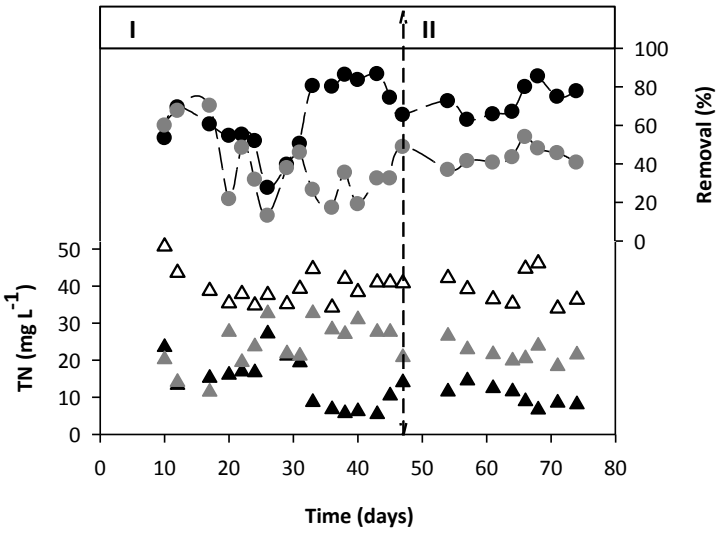

Figure 5-5 Removal efficiencies of TN for the HMBR and CMBR systems: Influent (Δ); Effluent HMBR (▲); Effluent CMBR (▲); Removal HMBR (●); Removal CMBR (●).

As both MBRs were operated continuously under aerobic condition (DO concentration around saturation), the biomass enriched gradually. Due to thickening of attached biofilm in the HMBR, the oxygen diffusion into the biofilm would have been hindered. Consequently, the outer biofilm layer would have been aerobic while the inner biofilm was subjected to anoxic/anaerobic conditions; in line with this, TN removal efficiency increased considerably in comparison to that in the CMBR. Further, the low TN removal obtained in the CMBR may be due to the aeration intensity (high ambient DO) in the reactor and/or small size flocs. Several authors have also reported better TN removal in HMBR systems using mobile bed supports than in CMBRs (Yang et al., 2009b;

Jamal Khan et al., 2011; Wang et al., 2012). However, with this kind of mobile support there is a risk of no significant biofilm formation and thus limited nitrogen removal via SND (Liang et al., 2010). Unlike the studies cited, in this setup the support media used was a fixed bed, which was exposed to less shear stress. Moreover, a biofilm thickness of approximately 1 mm was observed, which indicated that there was substantial thickness for active biomass working on and inside the biofilm. Although high TN removal and almost complete ammonium removal were achieved in the HMBR, complete denitrification was not obtained, which implies that there is scope for improvement and optimization of the process.

The average PO_4^{3-} removal in effluent of the HMBR was 2.0 ± 0.3 mg L^{-1} whereas in the CMBR it was 2.2 ± 0.3 mg L^{-1} (Fig. 5-4). PO_4^{3-} removal efficiencies were 42 and 37%, respectively (not significantly different, $P > 0.05$). Phosphorous can be removed by assimilation for biomass growth and by phosphorus accumulating organisms (PAOs). Liu et al. (2010), among others, reported better phosphorus removal in a HMBR compared to a CMBR. They attributed the enhanced biological phosphorus removal (EBPR) to PAOs, which may have developed within anoxic/anaerobic zones of the support media. In contrast, in the systems presented in this work, phosphorus removal by PAOs appears to be negligible because of the low influent PO_4^{3-} concentrations (Jamal Khan et al., 2011) and the long sludge retention times (SRT ≈ 47 – 80 d). Under these circumstances, phosphorus removal would be mainly due to assimilation by the microorganisms to meet their nutrient requirements, which consistent with the results obtained in the two MBRs. The slightly better performance observed in the HMBR could be due to a higher biomass concentration in this system.

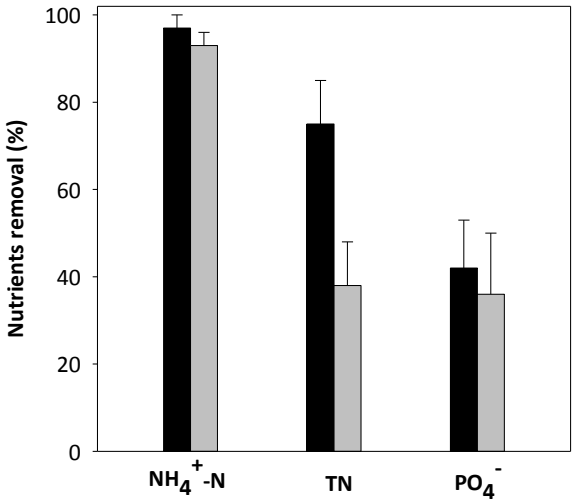

Figure 5-6 Average nutrients removal for systems: HMBR (■) and CMBR (■).

5.4.4 Sludge characterization

The pilot plants were started-up without an inoculum in order to develop an autochthonous biocenosis. During experimentation, the suspended solids (MLSS) concentration in both MBRs increased continuously (depending on wastewater characteristics). The sludge in the two MBRs appeared different: microscopic examination revealed a greater abundance of ciliate protozoans and metazoans in the HMBR than in the CMBR. The most common species were paramecia among the protozoans, and rotifers and nematodes among the metazoans. There was less variety and quantity of both groups in the CMBR. On the basis of these findings, it was concluded that the microbial community in the HMBR was richer than that in the CMBR.

As observed in this work, various studies have established a relationship between the presence of biofilm and sludge properties (Ivanovic and Leiknes, 2012). Indeed, the sludge filterability tests carried out, after the same period of operation, revealed that the HMBR is able to reduce the SRF compared to that in the CMBR, all the values obtained being lower (average $1.28 \cdot 10^{12}$ and $5.70 \cdot 10^{12}$ m kg^{-1} respectively). In addition, the sludge of the HMBR had significantly better settleability than that of the CMBR, with SVI average values of 52 and 174 mL g^{-1} respectively.

The results obtained in the HMBR were similar to others measured by the authors in integrated fixed film activated sludge processes (IFAS) with the same support (Presmanes et al., 2013) and both were markedly lower than previously reported

SVI values for hybrid growth MBRs with similar sludge concentrations (approximately 128 mL g^{-1}; Wang et al., 2012). Operating with real wastewater, SVI values lower than 100 mL g^{-1} have also been reported in a hybrid system in which a granular activated carbon-sponge fluidized bed bioreactor is followed by an MBR (Nguyen et al., 2013). Both measurements (SRF and SVF) indicate that the HMBR had potentially better filtering characteristics and floc structure, which appear to be more favorable for membrane filtration and sludge post-treatment.

5.4.5 Membrane performance

One of the objectives of the study of this chapter was to ascertain whether including attached biomass in a conventional MBR leads to less membrane fouling. Except for the addition of the support medium in the HMBR, all equipment and operating parameters were exactly the same in the two MBRs. Hence, other factors namely differences in the biomass characteristics of the HMBR and CMBR must underlie the differences in membrane performance. Clean water permeability (20 °C) was the same in all modules (average 210 L m^{-2} h^{-1} bar^{-1}). In operation, the permeability decreased gradually to 40 L m^{-2} h^{-1} bar^{-1} in the HMBR and to 30 L m^{-2} h^{-1} bar^{-1} in the CMBR. Average permeabilities were about 80 and 60 m^{-2} h^{-1} bar^{-1} in the HMBR and CMBR respectively. Other authors working with the same fibers obtained permeabilities in the same range: 70 – 50 L m^{-2} h^{-1} bar^{-1} with municipal wastewater and 160 – 75 L m^{-2} h^{-1} bar^{-1} with industrial wastewater (Sánchez et al., 2010).

The evolution of TMP at a constant flux (10 L m^{-2} h^{-1}) in the two MBRs is depicted in Fig. 5-7a. From the beginning of the experimental period, with clean membrane, TMP increases at a faster rate in the CMBR than the HMBR. The same trend occurs after the cleaning out of place (COP) on day 97. After the first maintenance cleaning, the fouling rates remained similar during a start-up period of approximately 4 – 5 weeks and, afterwards, the fouling rate in the HMBR was on average only 57% of the fouling rate in the CMBR (Fig. 5-7b). This result suggests that the presence of support media did notably improve the membrane performance of the HMBR. Similarly, other authors have observed longer filtration periods in hybrid (in this case, moving bed) MBRs compared to conventional MBRs (Wang et al., 2012; Liu et al., 2012; Jamal Khan et al., 2012).

The MLSS concentration and the accumulation of colloidal biopolymer clusters (cBPC) were analyzed in both MBRs in order to establish whether there were a relationship between these parameters and the fouling rate.

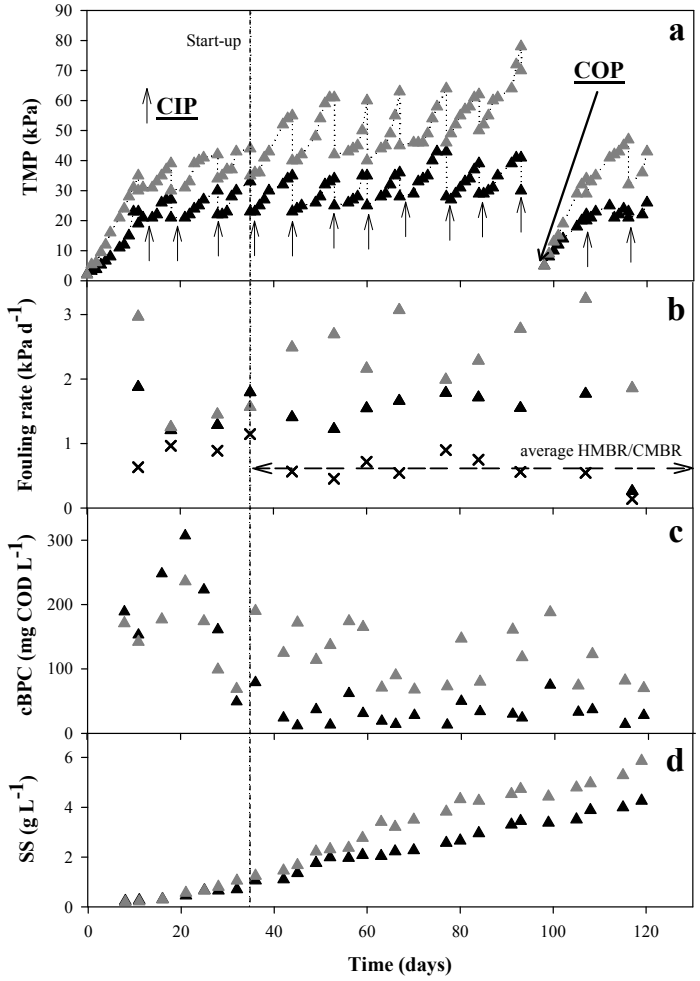

Fig. 5-7 Variations of the TMP (a), fouling rate (b), cBPC (c) and SS (d) in both MBRs during stage III: (▲) HMBR, (▲) CMBR and (**x**) HMBR/CMBR fouling rates ratio.

While the MLSS (alone) is a poor indicator of fouling propensity (Jefferson et al., 2004), it should not be excluded from consideration as a fouling parameter, since biological flocs play a key role in fouling (Le-Clech et al., 2006). In this study, however, though the MLSS concentration increased steadily in both MBRs (Fig. 5-5d), their fouling rates (Fig. 5-7c) did not increase. In addition, the same concentration of solids in the reactors led to different fouling rates. That is, for the range of operation tested (up to 6 g L^{-1}), no correlation was found between MLSS concentration and fouling rate.

As indicated above, in the present study MBRs were operated at high SRT (47 – 80 d) which has been identified (Drews, 2010) as one of the operating conditions responsible for a lack of correlation between fouling and the concentration of commonly studied foulants (such as soluble microbial products) in many studies. This, as well as the simplicity of its measurement, is why it was decided to study the recently proposed fraction classified as biopolymer clusters as an indicator of fouling.

Observing the changes in this fraction over time (Fig. 5-7c), there seems to be a certain relationship between fouling rate and cBPC concentration. With the exception of a start-up period, which appeared to be influenced by the process instability in both reactors, and especially the growing of the biofilm in the HMBR, cBPC concentrations were higher and more variable in the CMBR (121 ± 43 mg COD L^{-1}) than in the HMBR (33 ± 20 mg COD L^{-1}), which also corresponds to a faster and more variable fouling rate.

Wang and Li (2008) noted a substantial retention of biopolymer clusters within the sludge cake and indicated that under turbulent conditions in submerged MBRs, these may be detached from the membrane with the sludge cake and returned to the sludge suspension. This fact could explain the high fluctuations in cBPC in the CMBR. Conversely, when a full biofilm developed in HMBR (good denitrification rate from days 30 – 35), smaller fluctuations were observed in the cBPC. This biofilm might have retained some amount of cBPC. Moreover, it has been suggested that a reduction in colloidal biopolymer concentration could be related with the development of filtering organisms, such as protozoans, in the biofilm (Sánchez et al., 2013), which appeared abundantly in the microscopic observations of the HMBR sludge (section 5.4.4). According to these results, the cBPC differences could be partially responsible for the differences in membrane fouling between the two systems, and specifically the presence of biofilm support in the HMBR may lead to a positive impact by decreasing cBPC concentration. Given all this, further investigations into factors affecting fouling trends in conventional and hybrid MBRs are necessary. Moreover, additional research on fouling mechanisms needs to be conducted at a pilot scale with real wastewater, as in synthetic wastewater may not reflect the actual features of real inputs (Nguyen et al., 2013).

Considering the improvements over conventional reactors achieved using hybrid MBRs, observed in the present study in agreement with other cited references, it seems clear that future studies are required to assess the environmental and economic benefits of hybrid MBRs. Enhancement in effluent quality and cost

reductions associated both with the mitigation of membrane fouling and the improvement in sludge characteristics should be explored. In the hybrid configuration of the present study, the additional cost of the support would be very likely outweighed by the savings. Additional benefits could arise from better effluent quality, especially when stringent nutrient limitations are imposed.

5.5 CONCLUSIONS

A fixed bed HMBR and a CMBR were operated side by side at a pilot-scale with real wastewater.

Both MBRs showed good removal efficiency of organic matter and ammonia, but the HMBR performed better (by 4% in both parameters).

The HMBR also exhibited far better average TN removal compared to the CMBR, increased 37% the efficiency, which was attributed to simultaneous nitrification and denitrification (SND).

The HMBR exhibited a notably (43% decrease) lower membrane fouling rate.

The same trend was observed in physical properties of the sludge, that is, better dewatering and settleability.

All the improvements with the HMBR were attributable to the addition of fixed support media, which allowed biofilm to coexist with suspended biomass.

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Chapter 6

HMBR system for combined nitrification/denitrification coupled to aerobic/anoxic methane oxidation as a post-treatment of UASB reactors[1, 2]

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

A. Sánchez, **L. Rodríguez-Hernández**, D. Buntner, A.L. Esteban-García, I. Tejero and J.M. Garrido (2013). *Denitrification coupled with methane oxidation in a Membrane Bioreactor after a methanogenic pre-treatment*. Under revision in Journal of Hazardous Materials.

A. Sánchez, **L. Rodríguez-Hernández**, D. Buntner and J.M. Garrido (2013). *Denitrification of wastewaters in an MBR system using dissolved methane from methanogenic pre-treatment.* 13th World Congress on Anaerobic Digestion. June 25-28, Santiago de Compostela, Spain, 2013.

[2] Part of the experimentation carried out by the author was used for the discussion of the PhD Thesis:

A. Sánchez (2013). Combining submerged membrane technology with anaerobic and aerobic wastewater treatment, Universidad de Santiago de Compostela.

D. Buntner (2013). Combined UASB-MBR system for the treatment of low-strength wastewater at ambient temperature, Universidad de Santiago de Compostela.

OUTLINE

SUMMARY

The presence of dissolved methane, especially at low temperature, represents an important environmental concern in terms of greenhouse gases (GHG) emissions of wastewaters treated by using methanogenic bioreactors. Methane has a global warming potential of 25. For low strength wastewaters as municipal wastewater, dissolved methane might account up to 50% of the methane produced. The dissolved methane is easily desorbed from the effluents, especially if these are either released in the environment or post-treated by using aerobic bioreactors. The use of this dissolved methane as a carbon source for biological denitrification has already been proposed theoretically as an alternative to reduce both greenhouse gas emissions and nitrogen content of the treated wastewater. However, its feasibility had not been studied yet. In the study covered by this chapter, the effluent of an upflow anaerobic sludge blanket (UASB) reactor was post-treated in a two-compartment membrane bioreactor (MBR). The first compartment was an anoxic moving-bed reactor intended to use dissolved methane as carbon source for denitrification, while the second compartment was an aerobic membrane filtration reactor. Up to 60% and 95% nitrogen removal and methane consumption were observed, respectively.

The recirculation rate between the aerobic and the anoxic compartments and the concentration of dissolved methane were shown as the main important parameters governing the process. The lower recirculation ratios studied (between 0.5 and 1) showed the higher nitrogen removal and the lower methane emissions. The stripping of the dissolved methane present in the anaerobic UASB effluent led to a worsening of nitrogen removal in the MBR system. In addition, batch experiments and fluorescence in situ hybridization (FISH) analysis indicated the presence of microorganisms capable of denitrifying using the dissolved methane as a carbon source, both aerobically and anaerobically. Denitrification seems to be carried out by a consortium of aerobic and anaerobic methane oxidizing bacteria, anammox and heterotrophic bacteria.

Denitrification process seemed to influence membrane performance. The highest cBPC concentrations and the lowest permeabilities were observed when denitrification activity diminished.

The biofilm presence favoured the development of a wide variety of populations of microorganisms, which could be advantageous for the growth of those implicated in the denitrification process. In addition, the use of membranes allows for a complete retention of the slow growing bacteria involved in methane and

nitrogen removal. Thus, the HMBR proposed seems to be a suitable technology for the post-treatment of UASB reactors.

6.1 INTRODUCTION

Anaerobic treatment processes have been widely applied to various types of wastewaters thanks to such advantages 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 of 25. A fraction of the methane generated is present in the effluent. Dissolved methane can be estimated by considering that effluents are, at least, in equilibrium with the biogas formed by using Henry´s law. Thus, methane concentrations in the UASB effluent between 13.4 and 20.8 mg L^{-1} may be expected operating at 17 – 25 °C, with 60 – 80 % methane composition in the biogas at an operating pressure of 1 atm. For low strength wastewaters, such as municipal wastewater, treated in anaerobic reactors, dissolved methane could account around 50% of the produced methane (Noyola et al., 2006). Moreover, Souza et al., (2011) indicated methane losses accounting for 36 – 41 % of the methane produced in two pilot scale anaerobic reactors.

Methane may be emitted to the atmosphere by stripping, if the effluents are either aerobically post-treated or discharged in the environment without further post-treatment, thus increasing the environmental impact of anaerobic wastewater treatment as a result of GHG emissions. Cakir and Stenstrom, (2005) analyzed GHG emissions associated with anaerobic municipal wastewater treatment. These authors proved that the presence of dissolved methane in the effluent strongly increases GHG emissions, when 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 (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 mg CH_4 L^{-1} d^{-1} removal.

The methane present in the effluents of methanogenic bioreactors may also be used 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 a microbiological point of view, biological methane oxidation coupled to denitrification proceeds via two different pathways: aerobic and anaerobic (Modin et al., 2007):

Aerobic Methane Oxidation coupled to denitrification

Aerobic methane oxidation coupled to denitrification 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:

$$5CH_4 + 5O_2 + 4NO_3^- + 4H^+ \rightarrow 2N_2 + 12H_2O + 5CO_2 \qquad \text{Eq. 6-1}$$

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).

Anaerobic Methane Oxidation coupled to denitrification

Anaerobic/anoxic methane oxidation has been demonstrated in consortia of anaerobic methanotrophic (ANME) archaea and sulphate-reducing bacteria (SRB). In this process methane is oxidized to carbon dioxide and sulphate is reduced to hydrogen sulphide (Boetius et al., 2000; Knittel and Boetius, 2009). More recently, anaerobic/anoxic methane oxidation coupled to denitrification has been also identified through three different pathways: 1) Bacteria belonging to NC10 phylum named "*Candidatus* Methylomirabilis oxyfera" (Raghoebarsing et al., 2006; Ettwig et al., 2008; Luesken et al., 2011) with an archaeal partner. The newly discovered bacteria are denitrifying methanotrophs (Wu, 2012), and about 80% of the population consisted of these bacteria while a smaller fraction (less than 10%) was made of archaeal species. 2) Bacteria "M. oxyfera" are able to oxidize methane in anoxic conditions on its own, without an archaeal partner, coupling it to nitrite reduction (Ettwig et al., 2008). This mechanism involves the dismutation of nitric oxide (NO) into O_2 and N_2 (Ettwig et al., 2010 and Wu, 2012). 3) A syntrophic association in which nitrite produced by anaerobic methanogenic

(ANME) archaea while oxidizing methane is reduced to nitrogen gas by anammox bacteria (Haroon et al., 2013).

Stoichiometric representation of anoxic methane oxidation coupled to denitrification using either nitrite or nitrate is as follows:

$$5CH_4 + 8NO_3^- + 8H^+ \rightarrow 5CO_2 + 4N_2 + 14H_2O \quad \text{Eq. 6-2}$$

$$3CH_4 + 8NO_2^- + 8H^+ \rightarrow 3CO_2 + 4N_2 + 10H_2O \quad \text{Eq. 6-3}$$

In the case of the consortium of anaerobic methanogenic (ANME) archaea with anammox bacteria, the reduction of nitrate to nitrite is according to equation 6-4 (Haroon et al., 2013):

$$CH_4 + 4NO_3^- \rightarrow CO_2 + 4NO_2^- + 2H_2O \quad \text{Eq. 6-4}$$

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. However, 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. 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 yet. 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, a two compartment MBR would represent an interesting technological choice to limit methane emissions and promote denitrification coupled to methane oxidation. The liquid phase would be recirculated from the aerobic membrane compartment to an anoxic compartment, in such manner that ammonia is oxidized to nitrate/nitrite in the aerobic compartment and nitrates/nitrites are reduced in presence of methane in the anoxic. Thereby, the

negligible presence of nitrate in the effluents of methanogenic reactors (van Haandel and Lettinga, 1994) could be overcome.

Moreover, the use of MBR systems could be a good strategy to enhance denitrification coupled to methane oxidation as result of the high sludge concentration of these systems, typically between 8 – 12 g MLVSS L^{-1} for submerged MBR systems (Judd, 2011). Denitrification coupled to methane oxidation is characterized by its low specific denitrification activity. Different authors, using batch assays, found activities in between 15 and 90 mg N g $MLVSS^{-1}$ d^{-1} at temperatures around 20 – 25 °C (Lee et al., 2001; Khin and Annachhatre, 2004). These values are much lower than 250 mg N g $MLVSS^{-1}$ 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 under anoxic conditions and increasing the effective biomass concentration in this compartment. Kampman et al., (2012) estimated that around 50% of the biomass produced was washed out from a sequencing batch reactor in which the growth of the newly discovered anaerobic denitrifying methanotrophic biomass was promoted. Thus, the installation of a membrane in the aerobic compartment would allow complete microorganisms retention in the system.

6.2 OBJECTIVES

The main objective of this chapter was to study of an HMBR system as a post-treatment of UASB reactors. The system is aimed at reducing total nitrogen and methane emissions of UASB effluents by promoting the use of the dissolved methane as an electron donor in denitrification.

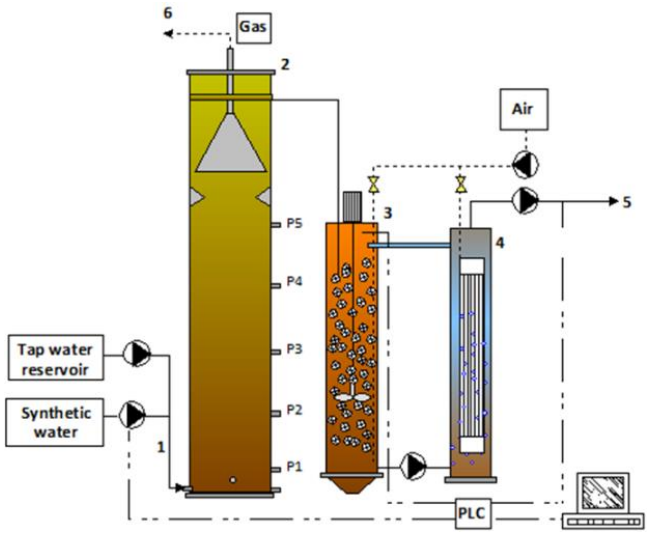
Two complementary objectives were to determine the effect of recirculation rate and dissolved methane presence on the process and to identify the possible mechanisms of methane oxidation coupled with denitrification in the system.

The behaviour of the membrane module and its relation with operation conditions and fouling indicators was also checked.

6.3 MATERIALS AND METHODS

6.3.1 Combined UASB-HMBR system

For the purpose of this chapter a combined UASB-HMBR system was investigated. The experimental set-up was located in the laboratory of the University of Santiago de Compostela. The system was composed of three compartments connected in series: a methanogenic UASB, an anoxic compartment with attached and suspended biomass and an aerobic membrane compartment. This experimental set-up is presented in Figure 6-1.



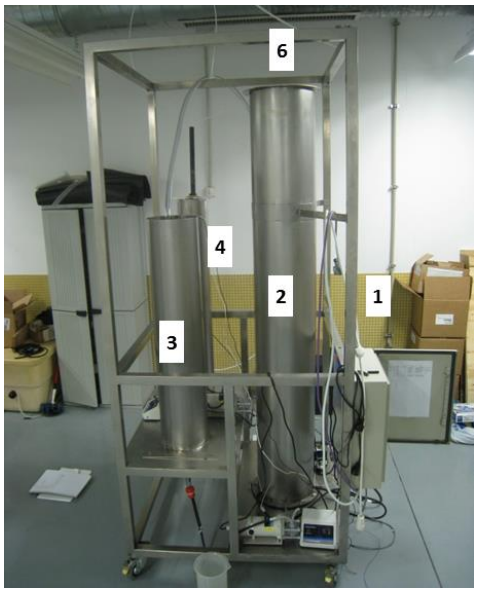


Figure 6-1 (up) Schematic diagram and (down) picture of the combined UASB-HMBR system: (1) Feeding, (2) UASB stage, (3) Anoxic compartment with Kaldnes K3 filter media, (4) Membrane filtration compartment, (5) Permeate (backwashing), (6) Biogas outlet.

The total and working volumes of each compartment are shown in Table 6-1.

Table 6-1 Total and working volumes of the system

Volume (L)	UASB	Anoxic compartment	Membrane filtration compartment
Total volume[1]	141	42	22
Empty working volume[2]	120	36	20

[1] Internal geometric volume of the reactor

[2] Total volume minus headspace

The methanogenic UASB reactor was seeded with 50 L of anaerobic biomass with a concentration of around 27 g VSS L^{-1}, originating from the anaerobic reactor of a brewery industry located in Galicia (Spain). The MBR system was inoculated with 5 L of aerobic biomass from a MBR pilot plant treating urban wastewater, which was preserved at 4 °C until the start-up of the reactor.

The effluent of the UASB reactor was led to a MBR reactor composed of two compartments. A first hybrid compartment, which consists of a 36 L anoxic bioreactor filled with biofilm carriers type K3 (made of polyethylene), supplied by AnoxKaldnes (Figure 6-2a). Filling fraction was 50% of the working volume. An internal recirculation from the aerobic membrane compartment was used to

return suspended solids and nitrates to the first hybrid compartment. A membrane ultrafiltration module Zenon® ZW10 (Figure 6-2b) with a surface area of 0.9 m^2 was employed in the aerobic membrane compartment to avoid the loss of biomass. This module consisted of PVDF hollow-fibre membranes, with a porous 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 aerobic membrane compartment 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 m^3 m^{-2} h^{-1}, which corresponded to a superficial velocity in the membrane compartment of 36 m h^{-1}. Transmembrane Pressure (TMP) data was measured with an analogue pressure sensor (Efector500 PN-2009) and collected in the PC by means of an analogue programmable logic controller (PLC) module Siemens EM 235.

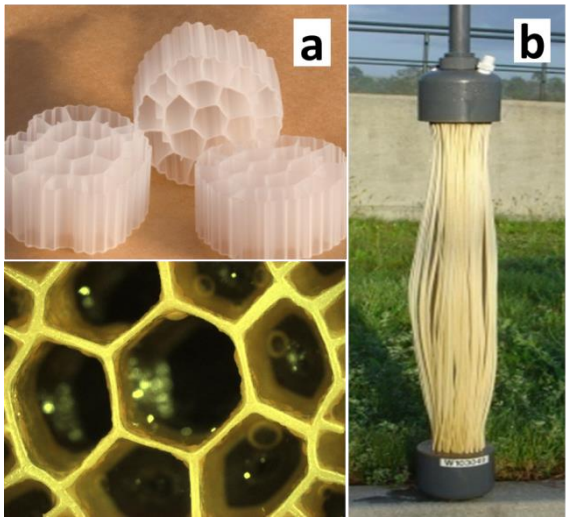


Figure 6-2 a) K3 support media without (up) and with biofilm (down); b) ZW10 Zenon membrane module.

During the experimental period, the system was operated at ambient temperature (17 – 23 °C). It was fed with synthetic wastewater stored in a refrigerator and composed of diluted skimmed milk, $NaHCO_3$ (200 mg L^{-1}), $FeCl_3 \cdot 6H_2O$ (1.5 mg L^{-1}), H_3BO_3 (0.15 mg L^{-1}), $CuSO_4 \cdot 5H_2O$ (0.03 mg L^{-1}), KI (0.03 mg L^{-1}), $ZnSO_4 \cdot 7H_2O$ (0.12 mg L^{-1}), $CoCl_2 \cdot 6H_2O$ (0.15 mg L^{-1}) and $MnCl_2 \cdot 4H_2O$ (0.12 mg L^{-1}). COD concentration in the feeding varied between 800 and 1300 mg L^{-1}.

The impact of internal recirculation ratio (R) in the MBR and the presence of methane in the UASB effluent were studied during six different periods, which are

described in Table 6-2. The hybrid compartment of the MBR system was continuously aerated during a first experimental period (period I) with the aim of establishing a base scenario for the emissions of total nitrogen and methane. Afterwards it was maintained under anoxic conditions in order to promote denitrification and investigate the impact of recirculation rate on nitrogen removal and methane emissions (periods II, III, V and VI). Additionally, the methane present in the effluent from the UASB reactor was stripped off with a rapid coarse aeration before entering the anoxic compartment of the MBR during period IV. The main objective within this period was to determine the denitrification rate caused by the remaining biodegradable COD fraction of this stream.

Table 6-2 Operational periods of the combined UASB-HMBR system

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 hybrid, first compartment of the MBR system

[2] Internal recirculation ratio from the membranes compartment to the hybrid compartment of the MBR

[3] Methane was stripped off from UASB effluent before entering the anoxic compartment

[4] From days 85 to 91 the recirculation rate was fixed in R=1

6.3.2 Analytical methods

Mixed Liquor Total Suspended Solids (MLSS) and Mixed Liquor Volatile Suspended Solids (MLVSS), total and soluble chemical oxygen demand (COD and COD_s), nitrite, nitrate and ammonia were determined according to the Standard Methods APHA, (1998). Total nitrogen (TN) was measured with a DN 1900 analyser (Rosemount, Dohrman) and it referred to the sum of nitrogen ions (ammonia, nitrate and nitrite) and soluble organic nitrogen. Volatile fatty acids (VFA) (i-butyric, n-butyric, i-valeric and n-valeric) were analyzed by gas chromatography (HP, 5890A) provided with a flame ionization detector (HP, 7673A). Biomass concentration in the biofilm attached to the plastic support was also determined. Five 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 MLTVSS were determined in the resulting mixed liquor and this concentration was referred to the surface of the plastic support.

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). The remaining methane dissolved in the liquid phase was estimated by Henry´s law. Methane is characterized by a Henry constant of 1.5 10^{-3} mol L^{-1} atm^{-1} at 25 °C (Sander, 1999). A sample (300 mL) was hand-shaked in a 500 mL Erlenmeyer. After three minutes of shaking, gas phase was analysed in the gas chromatograph.

The cBPC in the liquid phase of the sludge mixture suspension was estimated as in chapter 2 (see 2.6.4), but in this research organic matter is measured in terms of TOC (Sánchez et al., 2013).

Sludge filtration index (SFI) was measured according to the procedure developed by Thiemig (2012).

More information regarding analytical methods is provided in chapter 2.

A dissolved oxygen probe (AQUALITYC, model OXI-921) connected to a meter (M Design Instruments TM-3659) was used to control DO concentration in each compartment. The pH measurements were performed with an electrode of Crison Instruments S.A., 52-03, equipped with an automatic compensatory temperature device and a measure instrument (pH mV^{-1}).

6.3.3 Microbial population identification by FISH

The abundance of different populations of microorganisms present in the hybrid and aerobic membrane compartments was investigated by Fluorescent In Situ Hybridization (FISH), according to Amann, (1995). Two types of biomass were analysed in the anoxic compartment of MBR: suspended biomass and biofilm. The probes used are collected in Table 6-3.

Table 6-3 Specific probes used for the microorganism identification by FISH

Probe	Cyto.[1]	Probe sequence (5' → 3')	%F[2]	Target organisms
Amx368	cy3	CCTTTCGGGCATTGCGAA	15	All Anammox bacteria
ARCH915	cy3	GTGCTCCCCCGCCAATTCCT	20-35	Archaea
DARCH872	fluos	GGCTCCACCCGTTGTAGT	30	Various Euryarchaeota including ANME groups
DBACT1027	cy3	TCTCCACGCTCCCTTGCG	30	Bacteria belonging to NC10 phylum
DBACT193	cy3	CGCTCGCCCCCTTTGGTC	30	Bacteria belonging to NC10 phylum
EUB338mix	fluos	GC(T/A)GCC(T/A)CCCGTAG G(A/T)GT	...	Bacteria domain, Planctomycetales and Verrucomicrobiales
MA450	cy3	ATCCAGGTACCGTCATTATC	20	Type II methanotrophs (Methylosinus/Methylocystis spp.)
MG705	fluos	CTGGTGTTCCTTCAGATC	20	Type I methanotrophs
MG84	fluos	CCACTCGTCAGCGCCCGA	20	Type I methanotrophs

[1] Cytochrome

[2] % (v/v) Formamide

6.3.4 Batch experiments

6.3.4.1 Denitrification activity assays

Two different batch denitrification assays using methane and/or acetate as electron donor were performed using 500 mL flasks.

In the first assay denitrifying activity of both biomasses in suspension and biofilm was tested. Four flasks were filled with 400 mL of suspended biomass (2 g MLVSS L^{-1}) and 20 plastic carriers Kaldnes K3 (40% of working volume). In the second assay only biofilm activity was measured and therefore four bottles were filled with 50 plastic carriers Kaldnes K3 and 400 mL of phosphate buffer (0.143 g L^{-1} of KH_2PO_4 and 0.740 g L^{-1} of K_2HPO_4).

Both biofilm and suspended biomass samples were taken from the anoxic compartment of the MBR and were maintained under endogenous conditions for at least 12 h. In addition all samples were 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 gas depending on the conditions (Table 6-4), to guarantee anaerobic atmosphere.

Acetate substrate (5 mL of $NaC_2H_3O_2 \cdot 3H_2O$ 0.9M) was spiked as a carbon source in the corresponding flasks (Table 6-4) and nitrate (1 mL of KNO_3 0.86M) was spiked to each bottle at the beginning of the experiment.

The flasks were incubated at 25 °C 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). These batch experiments as well as the control assays were performed in duplicate and during period VI, thus making the biomass conditions specific from that period (Table 6-2).

Table 6-4 Denitrification batch tests

Flask	Headspace	Carbon Source
Blank	N_2	None
Methane	CH_4	CH_4
Acetate	N_2	Acetate
Methane + acetate	CH_4	Acetate + CH_4

6.3.4.2 Anammox activity assays

Batch anammox assays to measure the maximum Specific Anammox Activity (SAA) were performed according to the methodology described by Dapena-Mora et al., (2007). Completely closed flasks with a total volume of 38 mL and 25 mL of liquid sample volume were used.

For these assays the biomass was washed three times with phosphate buffer as in section 6.3.4.1. The pH was fixed at 7.8 and the temperature at T = 35 °C. Gas and liquid phases were purged with an inert gas (He) to remove O_2. The flasks were placed in a thermostatic shaker, at 150 rpm. After some minutes for thermal stabilization, substrates were added into the flasks. Initial concentrations of substrates were 70 mg NH_4^+-N L^{-1} and 70 mg NO_2^--N L^{-1}. The production of N_2 was measured (pressure transducer Centrepoint Electronics) in the gas phase as the increment of pressure in the headspace of the vials. Maximum Specific Anammox Activity (SAA) was estimated from the maximum slope of the curve described by the cumulative N_2 production along the time and related to the biomass concentration in the flasks. Since the values of the affinity constant of the

Anammox bacteria for the substrates ammonium and nitrite are each less than 0.1 mg N L^{-1} (Strous et al., 1999), it can be considered that the activity measured is the maximum activity for the range of nitrite and ammonium concentrations used.

6.3.5 Determination of methane and oxygen transfer in the anoxic MBR compartment

The methane emissions to the environment in the anoxic compartment 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 every 30 minutes and their 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 by performing a mass balance to the headspace of the anoxic compartment. There is no generation or output of methane in the headspace, so the accumulation of methane is just the result of its desorption from the bulk liquid.

$$m_{CH4} = V \cdot \left(\frac{dC_{CH4}}{dt}\right) \qquad \text{Eq. 6-4}$$

Where:

m_{CH4} is the mass flow of methane that is desorbed in the anoxic compartment (mg d^{-1});

V is the headspace volume of the anoxic compartment (L) (V=5L);

C_{CH4} is the concentration of methane in the headspace (mg L^{-1});

t is the time (d).

The desorbed methane mass flow (m_{CH4}) is calculated by plotting the methane concentration in the headspace versus time as the slope of the linear representation.

The mass transfer coefficient for methane can be obtained from Equation 6-5:

$$m_{CH4} = K_L a_{CH4} \cdot (C - C^*) \qquad \text{Eq. 6-5}$$

Where:

C is the dissolved methane concentration in the bulk liquid of the anoxic compartment, (mg L^{-1});

*C** is the methane concentration in equilibrium with air (considered as zero), (mg L^{-1});

$K_L a_{CH4}$ is the mass transfer coefficient for methane, (d^{-1}).

According to the penetration film theory (van't Riet and Tramper, 1991) the ratio of K_L of two different substances is equal to the square root of the ratio of their diffusion coefficients. Therefore, $K_L a$ for the oxygen ($K_L a_{O2}$) can also be calculated in our system. This value was used to estimate the amount of oxygen transferred from the surface air to the anoxic compartment according to Equation 6-6:

$$K_L a_{O2} = K_L a_{CH4} \cdot (D_{O2}/D_{CH4})^{0.5} \quad \text{Eq. 6-6}$$

Where:

D_{O2} and D_{CH4} are the diffusive coefficients for oxygen and methane ($1.97 \cdot 10^{-5}$ and $1.5 \cdot 10^{-5}$ cm^2 s^{-1}), respectively.

6.3.6 Mass balances in the anoxic compartment

Considering the anoxic compartment 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. Assuming a steady state, mass balances were performed to individual components according to Equation 6-7:

$$Q_{IN,i} \cdot C_{0i} + Q_{r,i} \cdot C_{ri} - Q_{OUT,i} \cdot C_i = V \cdot r_i + K_L a_i \cdot (C_i - C_i^*) \cdot V \quad \text{Eq. 6-7}$$

Where:

subindex *i* corresponds to each component (nitrogen anions, oxygen and methane) of the mass balance;

$Q_{IN,i}$ is the input flow to the anoxic compartment (L d^{-1});

C_{0i} is the input concentration (mg L^{-1});

$Q_{r,i}$ is the recirculation flow (L d^{-1});

C_{ri} is the recirculation concentration (mg L^{-1});

$Q_{OUT,i}$ is the output flow from the anoxic compartment (L d^{-1});

C_i is the output concentration (mg L^{-1});

V is the volume of the anoxic compartment (L);

r_i is the volumetric reaction rate (mg L^{-1} d^{-1});

C_i^* is the concentration of either methane or oxygen in equilibrium with air (mg L^{-1});

$K_L a_i$ is the mass transfer coefficient for either methane or oxygen (d^{-1}).

The last term of Equation 6-7 was only taken into account for methane and oxygen mass balances.

Data from stable periods operating with the same recirculation ratio were grouped. Average values of both input and output concentrations of dissolved methane, dissolved oxygen and nitrogen anions were calculated from experimental data for each recirculation scenario. The dissolved methane was measured in the UASB effluent and in the recirculation flow (from aerobic membrane to anoxic compartment). Due to the strong aeration in the membrane compartment, all the dissolved methane was released to the environment and for this reason the concentration was cero in the recirculation. The presence of nitrite and nitrate in the UASB effluent was negligible, which coincided with that reported by (van Haandel and Lettinga, 1994) for UASB effluents. The recirculation flow brought nitrogen anions and dissolved oxygen to the anoxic compartment. The input oxygen concentration was also negligible. Therefore the mass balance is fairly simplified: the only inflows in the anoxic compartment are UASB effluent for methane and recirculation for nitrogen anions and dissolved oxygen. The desorption of methane and the adsorption of oxygen through the liquid surface were calculated according to section 6.3.5.

6.4 RESULTS AND DISCUSSION

6.4.1 General results

The MLVSS concentrations in the three reactors, UASB, anoxic compartment and aerobic membrane compartment, 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 28 g MLVSS m^{-2}, which was equivalent to an MLVSS concentration of approximately 4.9 g L^{-1}. Regarding the MBR, sludge retention time (SRT) was maintained between 15 and 30 d during the whole experimentation. Anaerobic biomass was not purged from

the UASB reactor during the study. Volumetric loading rate applied to the MBR was around 0.29 g COD_s L^{-1} d^{-1} referred to non-methane soluble COD. The dissolved oxygen concentration measured in the anoxic compartment ranged between 0.1 and 0.3 mg L^{-1} from periods II to VI. This concentration varied between 2 and 6 mg L^{-1} during period I, when the anoxic compartment was aerated. With regard to the UASB reactor, continuous production between 40 and 60 L d^{-1} biogas with a methane content above 70% was observed during the whole operation, which approximately corresponded to 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).

6.4.2 Influence of dissolved methane on denitrification

The remaining non-methane biodegradable COD and dissolved methane in the effluent from the UASB can be used as carbon source for denitrification. Soluble COD measured in this effluent during the experiments was very low, 57 ± 34 mg L^{-1}. Moreover, the concentrations of VFAs in the UASB effluent were monitored during the six experimental periods, being always below the detection limit (20 mg L^{-1}). The methane dissolved in the UASB effluent (and influent to the MBR) was normally between 19 and 25 mg L^{-1}, except in period IV, when methane was stripped off and its concentration decreased to concentrations between 3 and 8 mg L^{-1}.

Total nitrogen was occasionally measured in the UASB effluent, showing that virtually all the TN was present in the form of soluble ammonia (35.7 ± 7.9 mg L^{-1}). Thus, the TN in UASB effluent and permeate was estimated as the sum of ammonia, nitrate and nitrite. In Figures 6-3 and 6-4 the evolution of TN and the generation of NO_X-N during the six different periods can be followed. During period I the anoxic compartment of the MBR was continuously aerated, becoming in fact an aerobic reactor, in order to establish a base scenario for nitrogen and methane emissions, as previously explained in materials and methods section. TN in the permeate was slightly higher than the TN concentration from UASB effluent (Figure 6-3), which could be due to processes of hydrolysis and ammonification. Regardless of this fact and assuming that both measures were similar, it is concluded that no nitrogen removal took place during this period as a consequence of the aeration. Nevertheless, the ammonia was fully nitrified as it is shown in Figure 6-4.

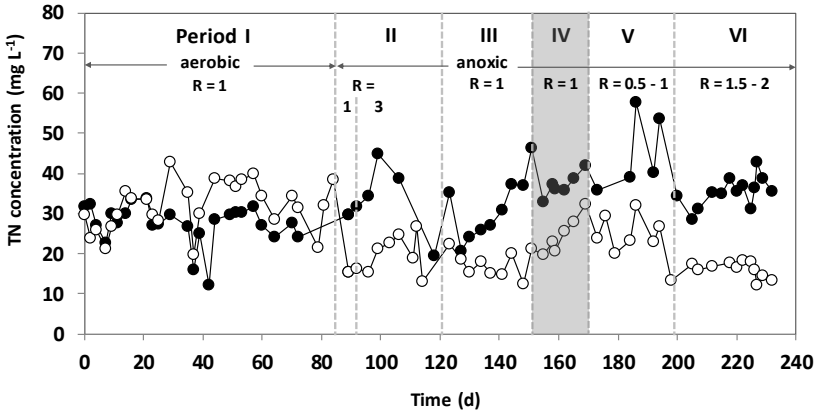


Figure 6-3 Total Nitrogen in the UASB-HMBR system during the six experimental periods: TN UASB effluent (●), TN permeate (○). The shaded area indicates the period when dissolved methane was stripped off.

On the other hand, during the first operating days in period II (when anoxic conditions were implemented), complete denitrification of nitrate was observed in the anoxic compartment. Later on, the concentration of this compound increased. Significant nitrogen removal was also observed during periods III, IV, V and VI, operating under anoxic conditions. This caused a remarkable diminution of TN in the permeate (Figure 6-3). Up to 60% nitrogen removal was observed before strip-off of methane. Once the stripping was stopped (from period V till the end of operation) nitrogen removal increased again up to the previous values. The nitrite and nitrate generated in the membrane or nitrification compartment were recycled to the anoxic compartment and partially reduced to nitrogen gas. Since the UASB effluent contained remaining biodegradable COD and dissolved methane, the denitrification could proceed using both carbon sources.

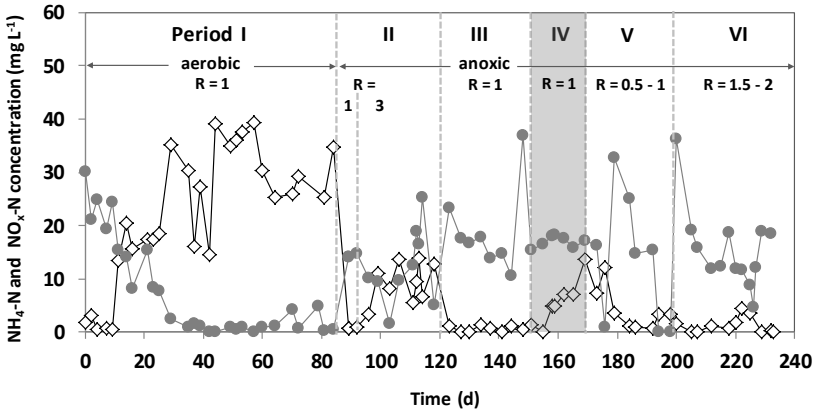


Figure 6-4 Evolution of the NH_4-N (●) and NO_x-N (◇) in the anoxic compartment during the six experimental periods. The shaded area indicates the period when dissolved methane was stripped off.

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 TN concentration in the permeate (Figure 6-3). Soluble COD in the UASB effluent during that period ranged between 21 and 27 mg L^{-1}. Figure 6-4 shows that NO_X-N concentration was almost zero in period III in the anoxic compartment. 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 anoxic compartment, thus indicating that the limiting factor within this period was the carbon source (Figure 6-4 and 6-5).

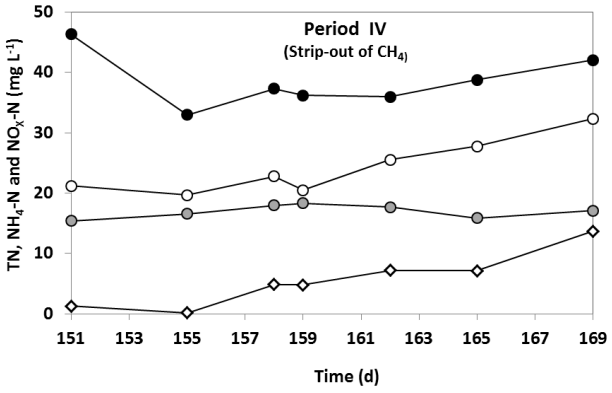


Figure 6-5 Evolution of the TN in the UASB effluent (●), permeate (○) and NH_4^+-N (●) and NOx-N concentration (◇) in the anoxic compartment during period IV.

From the date obtained at the end of period IV (Figure 6-5) a constant nitrogen removal rate of 73 mg N L^{-1} d^{-1} was obtained, whereas, considering the end of period III and the beginning of period IV, this nitrogen removal rate was around 164 mg N L^{-1} d^{-1}. The difference between both nitrogen removal rates could probably be due to dissolved methane. Up to 60% nitrogen removal was observed as indicated in previous paragraphs. Nevertheless, the nitrogen removal percentage at the end of period IV was, at most, 27%. Thus, the TN removal percentage coming from the oxidation of methane could account, at least, up to 33%. It should be taken into account that some dissolved methane was still remaining in the UASB effluent ($3 - 8$ mg L^{-1}), 60% of which was oxidized in the anoxic compartment during this period, according to methane mass balance When the stripping of methane was stopped in period V, nitrogen removal increased again up to the previous values observed during period III (60%). The difference between both nitrogen removal rates could probably be due to dissolved methane.

Although the aeration of the UASB effluent during the strip-off of methane (period IV) could have provoked the entrance of oxygen to the anoxic compartment, this input was considered negligible since the concentration of dissolved oxygen in this stream did not increase more than 0.5 mg L^{-1} with respect to a period without stripping of methane. It should be taken into account that some oxygen was always measured as a consequence of the contact of the sample with the environment. During all operation, except period IV, the tube of the UASB effluent stream was completely submerged in the anoxic compartment, preventing its oxygenation.

The results presented show that denitrification using methane as a carbon source is possible and feasible. Soluble COD present in the UASB effluent was used for conventional heterotrophic denitrification. Nevertheless, this low COD concentration may have 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 6-4, ammonia was also removed with no build-up of nitrate at least during periods II and VI, in which a reduction of ammonia concentration was observed in the anoxic compartment. The removal of ammonia in anoxic conditions might be explained by means of the anammox process.

When dissolved methane desorption was implemented (period IV), nitrogen removal did not decrease instantly but progressively, thus maintaining a certain denitrification capacity (Figure 6-5). This fact was probably related to a mechanism involving either endogenous respiration or biomass accumulation

products. Therefore, the impact of methane depletion increased with time, causing the increase of nitrate accumulation in the effluent.

The opposite was observed (Figure 6-4) in the recovery of denitrification capacity when methane desorption was stopped (period V). Thus 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 on methane emissions and denitrification

The role of recirculation ratio (R) in the behaviour of the system was evaluated by changing its value in periods II, III, V and VI (see Table 6-2) (period IV is intended to study the effect of dissolved methane, and thus it is not considered in this discussion). Methane is a gas that can be easily desorbed from the liquid phase by aeration. During the first period, where the conditions were not anoxic, 100% of the dissolved methane present in the UASB effluent was desorbed in the hybrid compartment, due to the aeration (Figure 6-6). When anoxic conditions were implemented in this compartment, a fraction of this dissolved methane was oxidized. Dissolved methane concentration in hybrid compartment ranged between 1 and 7 mg L^{-1}.

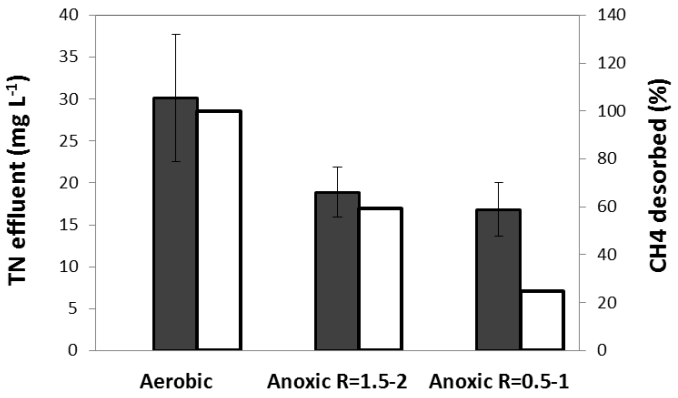


Figure 6-6 Average TN concentration in the permeate (■) and percentage of methane desorbed (□) during three different operational scenarios and recirculation rates (R).

Operational data from anoxic periods were grouped depending on the recirculation ratio. As can be observed in Figure 6-6, the lower the recirculation ratio (R) was, the lower the methane emissions became. The remaining methane that was not oxidized in the anoxic compartment was desorbed in the aerobic

membrane compartment, which was continuously aerated. Methane desorption in the anoxic compartment was negligible, representing at most only 2.4% of the total dissolved methane present in the UASB effluent. To obtain this value, the mass transfer coefficient for methane in the anoxic compartment was measured (0.97 d^{-1}).

Anoxic conditions allowed for total nitrogen removal, not significantly affected by R. Therefore, the best results in terms of both nitrogen removal and lower methane emissions were obtained by operating with lower R values (between 0.5 and 1).

Mass balances of methane, nitrogen (as nitrate and nitrite) and oxygen in the anoxic compartment were performed in order to clarify the methane oxidation and denitrification mechanisms at different recirculation ratios (Table 6-5). The mass transfer coefficient for oxygen in the anoxic compartment was measured (1.11 d^{-1}) in order to determine the oxygen transferred from the environment. This value was not negligible, representing 59%, 31% and 15% of the oxygen transferred with the recirculation flow from the aerobic membrane compartment at R of 0.5, 1.0 and 2.0 respectively.

Apparent specific denitrification rates at different recirculation ratios were similar, with a maximum value at R=1 (Table 6-5).

Table 6-5 Average denitrification, methane and oxygen apparent specific consumption rates and CH_4:O_2 molar ratio in the anoxic compartment.

R	mg N $g\ MLVSS^{-1}\ d^{-1}$	mg CH_4 $g\ MLVSS^{-1} \cdot d^{-1}$	mg O_2 $g\ MLVSS \cdot d^{-1}$	mol CH_4 $mol\ O_2^{-1}$
0.5	14.3	39.8	5.2	15.2
1.0	22.5	42.2	11.4	7.4
2.0	16.8	14.4	13.3	2.2

Neglecting period IV, the methane volumetric loading rate to the anoxic compartment was between 150 and 190 mg CH_4 L^{-1} d^{-1}. The calculated methane volumetric removal rate (Equation 6-7) was in between 50 and 160 mg CH_4 L^{-1} d^{-1}. The specific methane oxidation rates obtained (Table 6-5) were similar at R=0.5 and R=1 (39.8 and 42.2 mg CH_4 g $MLVSS^{-1}$ d^{-1}, respectively) but significantly lower at R=2 (14.4 mg CH_4 g $MLVSS^{-1}$ d^{-1}). As observed in Figure 6-6, the higher the

recirculation ratio, the lower the methane consumption, thus provoking higher methane emissions. Moreover, a remarkable raise in NO_x-N concentration in the anoxic compartment (Figure 6-4), similar to that observed when methane was desorbed from the UASB effluent during period IV, occurred in period II from day 91 onwards, when R increased from 1 to 3.

The observed effect of R on performance could be related with the fact that a rise in the recirculation ratio from the aerated membrane compartment also increased the amount of oxygen entering the anoxic compartment. More oxygen would improve the aerobic methane oxidation, but worse the anaerobic oxidation path. As methane oxidation decreased (Table 6-5), it would suggest that anaerobic oxidation is important.

The experimental molar ratio between the oxidized methane and the oxygen consumed was from 2.4 to 16.5, which is much higher than the theoretical molar relationship 1:1 according to the stoichiometry of the aerobic pathway (Equation 6-1), thus suggesting a combination of both, aerobic and anaerobic oxidation of methane. Other processes such as methanotrophy uncoupled to denitrification or heterotrophic oxygen consumption were probably present, affecting also the stoichiometry of the process. Nevertheless, the importance of anaerobic oxidation of methane should also be taken into account, especially at lower recirculation rates.

Given all this, the explanation of the important effect of recirculation ratio on this process remains unclear. As Waki et al., (2009) reported, 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. Therefore more research will need to be done on this subject.

6.4.4 Denitrification batch assays

The results presented before show that denitrification using methane as a complementary carbon source in the presence of the oxygen, was possible. Nevertheless, the denitrification mechanism might be complex, involving different pathways (Modin et al., 2007; Haroon et al., 2013). To determine the main denitrification mechanisms batch experiments were performed. In order to prove if anaerobic methane oxidation coupled to denitrification was feasible, the batch assays were performed in anaerobic conditions.

In Figure 6-7 the NO_3-N consumption is depicted depending on the substrate used (methane or acetate). Interestingly, the mixed (biofilm and suspended) biomass showed a relatively high endogenous denitrification rate (blank) of 20.0±14.3 mg N g $MLVSS^{-1}$ d^{-1}. Flasks fed with acetate showed higher denitrification rates, independently of the presence of methane, being 57.1±19.1 mg N g $MLVSS^{-1}$ d^{-1} (Figure 6-7a). Nevertheless, this activity was only three times higher than the activity under endogenous denitrification and significantly lower than those reported for activated sludge at 20 °C, using acetate as carbon source, being around 250 mg N g$MLVSS^{-1}$ d^{-1} (Henze et al., 2002). Additionally, some activity was also observed where methane was used as a sole carbon source. This activity was slightly higher than the one measured for the biomass without any substrate (the blank) and reached 28.2±11.2 mg N g $MLVSS^{-1}$ d^{-1}. This could indicate that anaerobic methane oxidation coupled to denitrification might have taken place.

In any case, the apparent specific denitrification rates observed during the continuous operation of the system (Table 6-5) were between 20 and 50% lower than observed in batch experiments with methane as a sole carbon source, which would be related with the optimum conditions in batch experiments. Furthermore, results regarding the obtained specific denitrification rates were of the same order of magnitude than those of 15 and 90 mg N g $MLVSS^{-1}$ d^{-1} reported by other authors (Lee et al., 2001; Khin and Annachhatre, 2004).

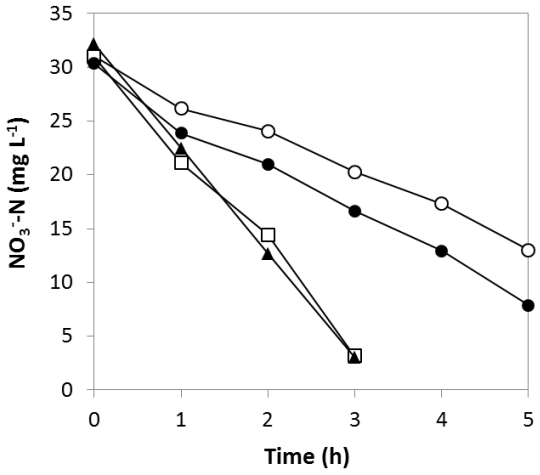


Figure 6-7 Batch denitrification assays with the presence of both suspended and biofilm biomass. Carbon sources employed were: blank test (○), acetate (□), methane (●) and methane and acetate (▲).

As MBR allows for complete biomass retention, the enrichment of denitrifying methanotrophic bacteria with time, and thus an increase in denitrification rates, is

expected. In fact, the capability for retaining slow growing bacteria would be an advantage of using MBR systems for methane oxidation coupled to denitrification (Kampman et al., 2012). In addition, the use of a support in the anoxic compartment increases the microbial diversity of the system (section 6.4.5). Longer experimentation periods would be required to confirm this hypothesis.

6.4.5 Microorganisms responsible for biological methane oxidation

FISH analyses were performed to determine the potential denitrification mechanisms and confirm the possibility of nitrite-driven methane oxidation. Abundant methanotrophs type I were found (Figure 6-8a) in both suspended and biofilm biomass. These bacteria can oxidize methane to methanol or acetate at low oxygen concentrations, which can be subsequently utilized by heterotrophs as carbon source for denitrification (Hanson and Hanson, 1996).

Taking into account the abundance of methanotrophic bacteria, it might be assumed that aerobic methane oxidation coupled to conventional heterotrophic denitrification was probably the dominant process in the presented system. This assumption would be in accordance with the literature (Rhee and Fuhs, 1978; Thalasso et al., 1997). Moreover, FISH analyses confirmed the presence of some archaeal species phylogenetically positioned between *Methanosaeta* and anaerobic methanotrophic archaea (ANME) (Figure 6-5b), which are normally found in anaerobic environments (Nauhaus et al., 2005). Therefore, the presence of these bacteria in MBR was probably caused by wash out of a fraction of anaerobic biomass from the UASB. ANME are known to be able to carry out reversed methanogenesis (Knittel and Boetius, 2009; Valentine and Reeburgh, 2000), where methane (and optionally CO_2) is converted into acetic acid (or acetate, if CO_2 is involved) and H_2. This acetic acid/acetate could serve as an electron donor for nitrate-reducing bacteria.

Reverse methanogenesis might occur in the anoxic compartment either during the low recirculation period (i.e. R=0.5) and/or deep inside the biofilm growing on the carriers, where anaerobic conditions would be maintained. If this is true, it could explain methane oxidation observed in the reactor even though the molar ratio between the methane oxidized and the oxygen consumed was always higher than the one given by stoichiometry observed in aerobic methane oxidation pathway (Eq. 6-1; Table 6-5), suggesting a combination of both, aerobic and anaerobic methane oxidation.

On the other hand, Raghoebarsing et al., (2006) demonstrated that the consortium of archaeal species with bacteria belonging to NC10 phylum could couple anaerobic methane oxidation to denitrification. In this process the reverse methanogenesis and electron shuttling to the denitrifying partner would be analogue to ANME and SRB syntrophic relations. Later, however, it was found that the process of nitrite-driven methane oxidation could be carried out without an archaeal partner (Ettwig et al., 2008; Wu, 2012).

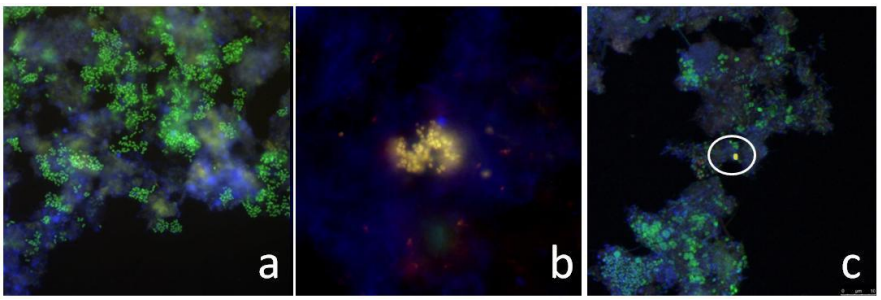


Figure 6-8 FISH analysis of microorganisms responsible for methane oxidation: (a) Type I methanotrophs in light green, as an effect of overlap of MG705 and MG84 Fluos (green) in combination with DAPI (blue), x4; (b) ANME, marked in yellow as an effect of overlap of DARCH872 fluos (green) in combination ARC915 cy3 (red) and DAPI (blue), x100; (c) Bacteria belonging to the NC10 phylum: marked in bright yellow as an effect of overlap of red (DBACT193 and DBACT1027), green (EUBmix) and blue (DAPI), x100. White circle marks a bacterium/group of bacteria that exhibited a positive signal with all the probes.

In the case of the present work, FISH analysis confirmed the presence of single bacteria belonging to NC10 phylum (Figure 6-8c), namely *Candidatus* Methylomirabilis oxyfera, and believed to be responsible for nitrite-driven methane oxidation. Their activity might be reflected by denitrification with methane as the sole carbon source in batch assays.

Moreover, FISH analyses indicated the abundance of large clusters of anammox bacteria in the biofilms, and the presence of a low amount of small clusters of these microorganisms in the suspended biomass (Figure 6-9). The presence of ANME and anammox bacteria might suggest the occurrence of the novel process reported by Haroon et al., (2013), where nitrite produced by archaeal is reduced to dinitrogen gas through a syntrophic relationship with anammox.

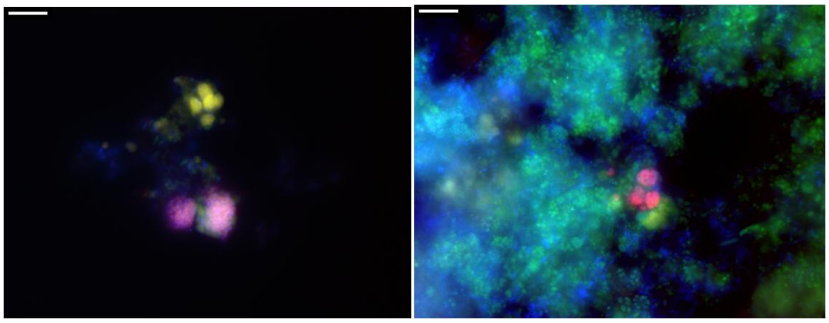


Figure 6-9 FISH analysis of anammox bacteria. The positive signal is marked in light pink, as an effect of overlap of two different probes: Amx820 (red) and EUBmix (green) and DAPI (blue): (a) biofilm biomass growing on Kaldnes support; (b) suspended biomass from the anoxic compartment. Scale bar corresponds to 10 µm.

The oxidation of methane is one of the most scientifically intriguing and controversial processes and there is no single mechanism of coupling it to denitrification. Apart from archaeal anaerobic activity, nitrogen removal in the presence of CH_4 and O_2 is, in most cases, a mixture between methanotrophic, denitrifying, ammonia-oxidizing and anammox activity, as it was previously stated by Waki et al., (2009) and Haroon et al., (2013), and confirmed in the present study.

In the case of the proposed UASB-HMBR system, anaerobic/anoxic denitrification with methane proved to be possible

6.4.6 Membrane performance

During the whole experimentation, the combined UASB-HMBR system treated an average flow rate of 270 L d^{-1}. The membrane flux was maintained around 15 L m^{-2} h^{-1} and was always below the critical flux (with average value of 20.8 ± 2.0 L m^{-2} h^{-1}). The membrane fluxes were lower than those typically reported with similar membrane modules in aerobic MBR (20 – 25 L m^{-2} h^{-1}) (Judd and Judd, 2006), but the permeability values obtained were similar to other researches (Judd, 2002). In normal operation (not considering the short-term effects of start-up and cleaning procedures), permeabilities ranged between 140 and 250 L m^{-2} h^{-1} bar^{-1} (see Figure 6-10).

As the flux applied was below the critical flux, it was expected that reversible fouling was predominant. Normally, with physical cleaning using tap water, the permeability was almost fully recovered. As shown in Figure 6-10, only two

maintenance chemical cleanings out of the reactor, at the beginning of periods III and V, were performed (1000 ppm of sodium hypochlorite solution).

The MLVSS concentration and colloidal BPC concentrations have been reported as possible fouling indicators (Drews, 2010). In this study, these parameters were measured in order to establish a possible relationship with operational conditions of the system and membrane fouling. The sludge filterability was also evaluated by obtaining the sludge filtration index (SFI).

The evolution of membrane permeability and colloidal BPC concentration in filtration compartment is depicted in Figure 6-10. Data of period I, when the environment in hybrid compartment of the MBR was aerobic, correspond to the research developed by Sánchez, (2013) and has been complemented in this study with anoxic periods II-VI. Regardless of a start-up period, which is generally influenced by the process instability (as reported in chapter 5), the lowest values of colloidal BPC concentration (cBPC) were obtained in period I. This fact coincided with MLVSS concentration around 2 g L^{-1} and the highest stable permeabilities of all experimentation.

At the beginning of period II, an increase in cBPC accumulation to around 25 mg COT L^{-1} was measured, when the environment in hybrid compartment of the MBR was changed. During anoxic periods (except period IV), the variations of the cBPC concentration did not appear to have a significant effect on membrane permeability. From period II till the end of operation, the MLVSS concentration was around 6 mg L^{-1} in filtration compartment. Sánchez et al. (2013) suggested that with a high MLVSS concentration, the membrane would be protected and the influence of the biopolymers released could be lower. The results obtained in chapter 5 might support this idea, since the MLVSS concentration in this HMBR reactor was much lower (average around 2 mg L^{-1}) and under these conditions, certain correlation was observed between cBPC concentration and fouling rate.

During period IV, the methane was desorbed from the UASB effluent and denitrification in the system was compromised. This disturbance coincided with a remarkable increase in cBPC concentration and significant drop in permeability, being necessary a chemical cleaning to recover the normal permeability values. Hence, it is possible that the MLVSS concentration cannot protect the membrane against the fouling provoked by sharp increases of cBPCs. During period V, the dissolved methane desorption was stopped. Although a sudden decrease was not observed, from this period the cBPC concentrations were progressively decreasing (period V-VI).

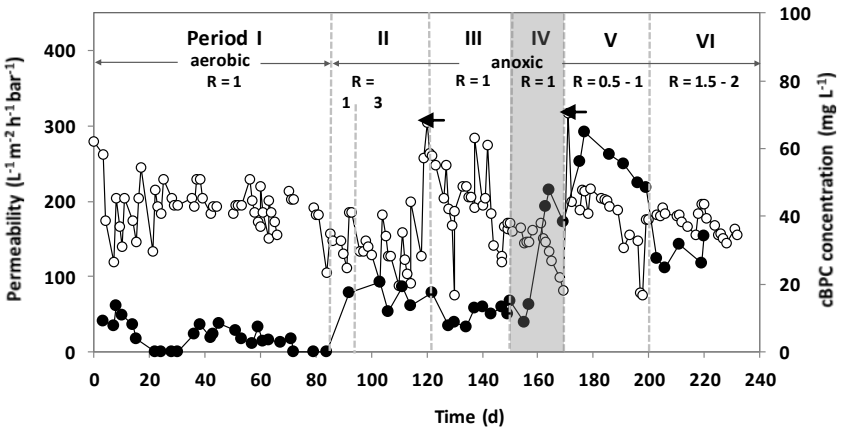


Figure 6-10 Membrane permeability (○), cBPC concentration (●) and maintenance chemical cleanings (←) during experimental periods. The shaded area indicates the period when dissolved methane was stripped off.

Published results indicate that the stress conditions (i.e. availability of oxygen sources, changes of temperature or nitrifying activity) play a decisive role in the production and release of polymeric substances by biomasses, and in fouling propensity (Drews, 2010; Drews et al., 2007). Therefore, the change in the operation conditions at the beginning of periods II and IV would explain the increase of cBPC concentration. Specifically, dissolved methane desorption might have had an indirect impact in the increasing of cBPC concentration through the loss of denitrification activity.

During operation the average values of SFI were around 116 seg $\%^{-1}$, whilst in period IV filterability worsened with values up to 442 seg $\%^{-1}$.

The influence of denitrification on membrane performance has not been extensively characterized and to date few studies have been reported. Nevertheless, the sharp increases in cBPC obtained in period IV were in accordance with results reported by Paetkau and Cicek, (2011). They studied nitrogen removal in a denitrification MBR and obtained that the highest TEP concentrations (25 mg L^{-1} Xantham gum) took place during an unstable denitrification period, which they attributed to cell die-off related with reduction in microbial activity.

In general, the cBPC did not presents clear correlation with membrane fouling except for period IV when a strong increase in fouling indicator coincided with an important worsening of the permeability. More research is therefore required to

elucidate the effects of denitrification in membrane fouling, and under which conditions the studied fouling indexes correlate with membrane performance.

6.5 TRENDS AND IMPLICATIONS FOR FUTURE RESEARCH

The findings of this research suggest that the configuration proposed is feasible for reducing GHG and nutrient emissions of the wastewaters anaerobically treated, especially for municipal wastewaters in sub-tropical countries, where wastewaters reach temperatures suitable for anaerobic treatment (van Lier, 2008). Souza et al., (2011) quantified the dissolved methane present in different UASB effluents through the treatment of domestic wastewater at ambient temperature in Brazil. These effluents were from 30 to 60% oversaturated with methane, reaching concentrations up to 22 mg L^{-1}. These values were very similar to those obtained in the present work (an average of 23 mg L^{-1}). Around 50 mg L^{-1} of total nitrogen can be expected in an anaerobically treated municipal wastewater effluent (Kampman et al., 2012; Van Haandel and Lettinga, 1994). According to the results of the present study, with dissolved methane of 24 mg L^{-1} in the anaerobic effluent and considering Equation 6-1, at least 16 mg L^{-1} of total nitrogen removal could be expected through aerobic methane oxidation. If anaerobic methane oxidation takes place this nitrogen removal could be increased up to 32 mg L^{-1} according to Equations 6-2 and 6-3 or even to 82 mg L^{-1} according to Equation 6-4 (depending on the anaerobic oxidation pathway and neglecting other denitrification processes).

6.6 CONCLUSIONS

Denitrification using methane as a carbon source was proved in an HMBR system as a post-treatment of UASB reactors. The findings that support this process are:

- The decrease of dissolved methane concentration in the hybrid compartment (from anoxic periods) cannot be attributed solely to the physical effect of desorption.
- The observed nitrogen removal cannot be carried out solely with the remaining biodegradable COD in the UASB effluent. Thus, it was

necessary to use the dissolved methane in this effluent as a carbon source.

- When the dissolved methane was very low in the anoxic compartment (during the strip-off of methane, period IV), the nitrate removal substantially decreased.
- FISH analysis confirmed the presence of microorganisms responsible for methane oxidation (Type I methanotrophs). Few bacteria belonging to NC10 phylum, capable of denitrifying with methane, were also detected.

Denitrification seems to be carried out by a consortium of aerobic and anaerobic methane oxidizing bacteria, anammox and heterotrophic bacteria. Specifically, anaerobic/anoxic methane oxidation coupled to denitrification activity is supported by the following results:

- Batch experiments confirmed the feasibility of anaerobic methane oxidation coupled to denitrification.
- FISH analysis revealed ANME microorganisms.
- The ratio between the oxidized methane and the oxygen consumed CH_4:O_2 (2:15) is greater than the theoretical ratio for aerobic oxidation (1:1).
- When recirculation rate (R) from the aerobic to the anoxic compartment increased from 0.5 to 2, denitrification capacity decreased. More oxygen availability may have inhibited anaerobic methane oxidation microorganisms.

Denitrification process seemed to influence membrane performance. The highest cBPC concentrations and the lowest permeabilities were observed when denitrification activity diminished.

Biofilm processes are known to favor the development of a wide variety of populations of microorganisms, which could be advantageous for the growth of those implicated in denitrification under anaerobic/anoxic conditions (conventional heterotrophic bacteria, “C. oxyfera”, anaerobic methanogenic archaea, anammox, etc). In addition, the use of membranes allows for a complete retention of the slow growing bacteria involved in methane and nitrogen removal. Thus, the HMBR proposed seems to be a suitable technology for the post-treatment of UASB reactors.

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Conclusiones y recomendaciones

Los resultados obtenidos, después de la operación de diferentes configuraciones de RBM híbridos, ponen de manifiesto que los reactores de biopelícula combinados con fangos activos convencionales y acoplados a un sistema RBM tienen aplicaciones potenciales en el tratamiento avanzado de las aguas residuales. Las principales conclusiones que lo sustentan se presentan a continuación, estructuradas de acuerdo con los principales objetivos de la presente Tesis Doctoral.

Resultados de la investigación

1. Desarrollo, construcción a escala de bancada y evaluación del funcionamiento de un nuevo RBM híbrido, muy compacto y con requisitos optimizados de aireación.

Se desarrolló un nuevo RBM híbrido de configuración vertical a escala de bancada, con un medio soporte fijo tipo esponja, no observándose signos de sobrecarga durante toda la experimentación. El caudal de aireación aplicado para la limpieza de la membrana fue asimismo suficiente para oxigenar ambas biomasas (biopelícula y en suspensión), mezclar el líquido del reactor y controlar el espesor de la biopelícula. A pesar de la gran variabilidad de la carga aplicada (valores medios de entre 1,1 y 2,7 kg DQO m^{-3} d^{-1}) se lograron altas eficiencias de eliminación de materia orgánica, con valores superiores al 90% en DQO y 96% en DBO_5.

La configuración mostró un buen rendimiento en la eliminación de nitrógeno total (NT) a través de la nitrificación y desnitrificación simultánea, a pesar de aplicarle una aireación continua.

Se encontró que la eliminación biológica de nitrógeno fue mejorada por la presencia de micro-zonas anóxicas que se desarrollaron en las regiones internas de la biopelícula del medio soporte.

La recirculación desde la zona de membranas a cabeza de reactor desempeñó un papel importante en el comportamiento del RBM híbrido. Con una recirculación del 300% se mejoró ligeramente la eficiencia de eliminación de carbono orgánico

(99% vs. 96% en DBO_5) y notablemente la eliminación de nitrógeno (98% vs. 91% en amonio; 80% vs. 69% en NT).

El estudio preliminar tratando agua residual municipal real indica que esta configuración puede ser considerada como un sistema alternativo a otros RBM convencionales.

2. Construcción y evaluación a escala piloto de un RBM híbrido, basado en los resultados obtenidos en escala de bancada. Estudio de su aplicabilidad para el tratamiento descentralizado.

Después de demostrar las ventajas de la configuración híbrida, se construyó y evaluó un nuevo RBM híbrido a escala piloto para estudiar su aplicabilidad como tratamiento descentralizado. A diferencia de la configuración a escala de bancada, el medio soporte fijo implementado en el reactor (llamado BLAS) fue de fabricación propia basado en un diseño específico desarrollado previamente por el Grupo de Ingeniería Ambiental de la Universidad de Cantabria. La planta piloto fue capaz de tratar un agua residual municipal sin necesidad de decantación primaria, lo cual le confiere la alta compacidad que requieren los tratamientos descentralizados.

Durante la experimentación, la carga orgánica aplicada osciló entre 0,36 y 1,76 kg DQO m^{-3} d^{-1}. Sin embargo, el sistema mantuvo buenos rendimientos generales. Las eliminaciones alcanzadas de materia orgánica en términos de DQO y DBO_5 fueron del 84% y 98%, respectivamente. Se obtuvieron altas eliminaciones de nitrógeno total (75%) a pesar de aplicar una aireación continua, debido al fenómeno de nitrificación y desnitrificación simultánea.

Las características medias del efluente obtenido fueron DQO < 55 mg L^{-1}, SS < 4 mg L^{-1}, NT < 10 mg L^{-1} y turbidez < 2 NTU, cumpliendo con los estándares para descarga en zonas sensibles y para reutilización.

3. Comparación en paralelo y a escala piloto del RBM híbrido propuesto con un RBM convencional, centrándose en la eliminación de carbono y nutrientes, el ensuciamiento de membranas y las propiedades del fango.

En cuanto a la caracterización física, un estudio de trazadores mostró para ambos reactores un comportamiento hidrodinámico similar, con mezcla completa. En cuanto al coeficiente de transferencia de oxígeno, K_La (a 20 °C), se observó una mejora en el RBM hibrido con respecto al RBM convencional (33,9 h^{-1} vs. 18,3 h^{-1}),

lo que se atribuyó al mayor tiempo de retención de las burbujas dentro del reactor de lecho fijo.

Las eficiencias de eliminación de DQO, DBO_5 y $N\text{-}NH_4^+$ en el RBM híbrido fueron del 84, 98 y 97%, mayores que en el RBM convencional. Esta mejora se asocia con la actividad de la biopelícula en el medio soporte del sistema de crecimiento híbrido.

Se encontró una diferencia significativa en la eliminación de NT entre el RBM híbrido y el convencional, con valores promedio de 75% y 38%, respectivamente. Este incremento se atribuyó a la existencia de nitrificación y desnitrificación simultánea (SND) en el RBM híbrido. Según los resultados obtenidos, la asimilación podría ser el mecanismo principal para la eliminación de fosfatos, con rendimientos de eliminación de 42% en el RBM híbrido, y de 37% en el RBM convencional, sin encontrarse diferencias estadísticas significativas.

Las características de los fangos con respecto al tipo de microorganismos, filtrabilidad y decantación, fueron diferentes en ambos reactores. El examen microscópico de los fangos reveló que la comunidad microbiana en el RBM híbrido era más abundante y más rica que en el RBM convencional. Principalmente se observaron ciliados, rotíferos y nematodos, los cuales podrían estar relacionados con la presencia de la biopelícula. Además, el fango del RBM híbrido obtuvo mejor filtrabilidad en comparación con el RBM convencional (promedio de $1{,}28 \cdot 10^{12}$ and $5{,}70 \cdot 10^{12}$ m kg^{-1}, respectivamente) y mejor decantabilidad (IVF con valores medios de 52 y 174 mL g^{-1}, respectivamente). Estos resultados sugieren que la configuración híbrida puede dar lugar a fangos con mejores propiedades, lo cual parece ser más favorable para la filtración de membranas y el tratamiento posterior de los fangos.

La tasa de ensuciamiento de las membranas en el RBM híbrido fue notablemente inferior (43% de disminución) que en el RBM convencional. Para el rango de operación de este trabajo (hasta 6 g L^{-1}), no se encontró correlación entre la concentración de SSLM y la tasa de ensuciamiento. Los cBPC tuvieron una mayor concentración y variabilidad en el RBM convencional, lo que se correspondió con una mayor y más variable tasa de ensuciamiento. Este comportamiento sugiere la existencia una cierta relación entre la tasa de ensuciamiento y la concentración de cBPC. En este sentido, la menor concentración de cBPC en el fango del RBM híbrido, probablemente debido a su retención por la biopelícula, pudo ser parcialmente responsable de la diferencia en el ensuciamiento.

Todas las mejoras observadas en el RBM híbrido son atribuidas al crecimiento híbrido que se consigue cuando la biopelícula y la biomasa suspendida crecen simultáneamente.

4. Evaluación a escala de bancada de un RBM híbrido como post-tratamiento de bioreactores metanogénicos.

Finalmente, en el presente trabajo se estudió un sistema RBM híbrido como post-tratamiento para biorreactores metanogénicos. El efluente de un reactor anaerobio de flujo ascendente (UASB) se trató en un RBM de dos compartimentos. El primer compartimento era un reactor de lecho móvil anóxico (con soportes K3) con el propósito de utilizar el metano disuelto como fuente de carbono para la desnitrificación. El segundo compartimento consistió en un reactor aerobio de filtración con membranas, que permite una retención completa de la biomasa.

Se observó una reducción de metano en el sistema de hasta el 95%, que no pudo ser atribuida únicamente a los efectos físicos de desorción en el reactor anóxico.

También se observó una eliminación de nitrógeno de hasta el 60%. Se demostró que esta eficiencia no pudo llevarse a cabo exclusivamente con la DQO biodegradable que quedaba en el efluente del UASB, siendo necesario el uso del metano disuelto como fuente de carbono.

Se llevó a cabo una desorción del metano disuelto del efluente del UASB, lo que condujo a un reducción del 60% al 27% en la eliminación de nitrógeno. Por tanto, el porcentaje de eliminación de nitrógeno procedente de la oxidación de metano pudo suponer hasta el 33% del total.

Además de la concentración de metano disuelto, la ratio de recirculación entre el compartimento aeróbico y el anóxico mostró ser otro parámetros importantes del proceso. Con los ratios de recirculación más bajos estudiados (entre 0,5 y 1) se obtuvieron la mayor eliminación de nitrógeno y las emisiones de metano más bajas.

Los análisis de FISH indicaron la presencia de microorganismos capaces de desnitrificar usando metano disuelto como fuente de carbono, tanto en condiciones aeróbicas como anaeróbicas. La desnitrificación parece llevarla a cabo un consorcio de bacterias oxidantes de metano aerobias y anaerobias, anammox y bacterias heterotróficas.

El estudio comportamiento de la membrana indicó que las mayores concentraciones de cBPC y las menores permeabilidades se obtuvieron cuando disminuyó la actividad desnitrificante.

El RBM híbrido propuesto parece ser una tecnología adecuada para el post-tratamiento de los reactores UASB. La presencia de biopelícula favoreció el desarrollo de una amplia variedad de poblaciones de microorganismos, lo que podría ser ventajoso para el crecimiento de aquellos implicados en el proceso de desnitrificación. Además, el uso de membranas permite una retención completa de bacterias de crecimiento lento que participan en la eliminación de nitrógeno y metano.

Recomendaciones para futuras investigaciones

Con base en los principales resultados obtenidos en este trabajo, surge un potencial de investigación importante en el campo de los sistemas HMBR. En concreto, se recomiendan las siguientes investigaciones:

- La actividad microbiana y la biocinética de la biopelícula y de la biomasa en suspensión debe ser estudiado con más detalle. La contribución de cada tipo de biomasa al comportamiento general del sistema necesita ser aclarado con el fin de conocer sus distribuciones y concentraciones óptimas (es decir, “el grado de hibridez”). La identificación y cuantificación de las especias microbianas (por ejemplo mediante técnicas como FISH/DGGE) podría ser una herramienta útil para este propósito.
- Se precisa comparar las eficiencias de eliminación y el ensuciamiento de membranas entre un RBM híbrido y uno convencional bajo condiciones de operación distintas a las estudiadas (por ejemplo con otras cargas orgánicas y tiempos de retención celular).
- Los resultados de este estudio sugieren que el RBM híbrido podría reducir el ensuciamiento de la membrana en comparación con el RBM convencional. Considerando que el ensuciamiento es hasta ahora el principal problema en la aplicación de los RBMs, la configuración híbrida podría ser una opción interesante para paliar esta limitación. Sin embargo, son necesarios más estudios sobre el ensuciamiento de la

membrana en estos sistemas para determinar los efectos y los mecanismos responsables de la adición de biomasa adherida.

En conclusión, los resultados obtenidos en este trabajo evidencian que los reactores RBM híbridos pueden llegar a ser una alternativa interesante para el tratamiento avanzado de las aguas residuales.

Conclusions and recommendations

Findings obtained, after of the operation of different configurations of hybrid MBR (HMBR), evidenced that biofilm reactors combined with conventional activated sludge and coupled to MBR system have potential applications in advanced wastewater treatment. The main findings that support it are presented below, structured according to the main objectives of the Doctoral Thesis.

Research Findings

1. Development, construction at bench-scale and performance evaluation of a new hybrid MBR, compact and with optimized requirements for aeration.

A new vertical HMBR configuration was developed and operated at bench-scale. A sponge fixed bed as support medium was employed and no signs of overloading were observed during the whole experimentation. The aeration flow delivered for membrane cleaning, was also sufficient for the aeration of two biomasses (biofilm and suspended) as well as the mixing of the bulk liquid in the reactor and the biofilm thickness control. In spite of the great variability of applied load (average 1.1 and 2.7 kg COD m^{-3} d^{-1}) high efficiencies of organic matter removal were achieved, with values above 90% in COD and 96% in BOD_5.

The configuration showed good performance in total nitrogen removal through simultaneous nitrification and denitrification (SND), regardless of the continuous aeration applied. It was found that biological nitrogen removal was enhanced by the presence of anoxic micro-zones that developed in the interior regions of biofilm of the support media.

The recirculation flow from the membranes zone to the head of the reactor seems to play an important role in the behavior of the hybrid MBR. With a recirculation rate of 300%, organic carbon removal efficiencies improved slightly (99% vs. 96% in BOD_5) and nitrogen removal values increased notably (98% vs. 91% in NH_4^+-N; 80% vs. 69% in TN).

The preliminary research treating real municipal wastewater indicates that this configuration can be regarded as an alternative system to other conventional MBRs.

2. Construction and evaluation at pilot-scale of the proposed hybrid MBR, based on the findings at bench-scale. Study of its feasibility for decentralized treatment.

After proving the advantages of the hybrid configuration, an original vertical HMBR reactor was built and assessed at pilot-scale to study its feasibility as decentralized treatment. Unlike in bench-scale configuration, the fixed biofilm support media (called BLAS) implemented in the reactor, was self-produced based on the specific design previously developed by the Group of Environmental Engineering of the University of Cantabria. The pilot-plant was able to treat municipal wastewater without need of primary settling thus awarding high compactness as required for decentralized treatment. During experimentation, applied organic loading rate increased between 0.36 and 1.76 kg COD m^{-3} d^{-1}. Nevertheless, the system maintained good overall performances. Specifically, removal efficiencies in the range of 84% for COD, 98% for BOD_5 and 97% for ammonium were achieved. High removal efficiencies for total nitrogen (75%) were obtained even though it was a single reactor continuously aerated, due to simultaneous nitrification and denitrification (SND) occurrence in the biofilm. The average characteristics of the effluent were COD < 55 mg L^{-1}, SS < 4 mg L^{-1}, TN < 10 mg L^{-1} and turbidity < 2 NTU, meeting the standards for discharge in sensitive areas as well as for reuse.

3. Comparison in parallel and at pilot-scale of the proposed hybrid MBR with a conventional MBR, focused on carbon and nutrient removal, membrane fouling and sludge properties.

Regarding physical characterization, a tracer study showed similar hydrodynamic behavior with optimum mixing for both reactors. An improvement in the oxygen transfer coefficient K_La (at 20 °C) in the HMBR with respect to the CMBR was observed (33.9 h^{-1} vs. 18.3 h^{-1}), being attributed to extended bubble retention time within fixed bed reactors.

The removal efficiencies of COD, BOD_5 and NH_4^+-N with the HMBR configuration were in the range of 84, 98 and 97%, respectively, which were greater than those

in the CMBR. The improvement is associated with the activity of the biofilm on the support media in the hybrid growth system.

Significant difference in TN removal efficiency was found between hybrid and conventional MBR systems with average 75% in the HMBR vs. 38% in the CMBR. This rise was attributed to simultaneous nitrification and denitrification (SND) in the hybrid growth system. Assimilation would be the main mechanisms for PO_4^{3-}, with removal efficiencies of 42% in the HMBR and 37% in the CMBR, not being statistically different.

The sludge characteristics of the two MBRs were found to be different in terms of type of microorganisms, sludge filterability and settleability. Microscopic examination revealed a greater abundance and diversity microbial in the HMBR than in the CMBR, mainly of ciliates, rotifers and nematodes, which could be related with the presence of the biofilm. The HMBR sludge had better filterability compared to the CMBR (average $1.28 \cdot 10^{12}$ and $5.70 \cdot 10^{12}$ m kg^{-1}, respectively). In addition, sludge from HMBR showed better settleability (with SVI average values of 52 compared to 174 mL g^{-1}). These results indicate that the hybrid configuration results in potentially better sludge properties, which seems to be more favorable for membrane filtration and sludge post-treatment.

The HMBR reactor with biofilm was found to have notably lower membrane fouling rate (43% decrease) as compared to CMBR. For the range of operation tested in this work (up to 6 g L^{-1}), no correlation was found between MLSS concentration and fouling rate. The higher concentration and variability of cBPC in the CMBR corresponded with higher and more variable fouling rates, which suggested a certain relationship between fouling rate and cBPC concentration. In this sense, the lower concentration of cBPC in the HMBR sludge, probably due to their retention by the biofilm, could be partially responsible for this difference in fouling.

All the improvements with the HMBR were attributable to the hybrid growth achieved when biofilm and suspended biomass grew simultaneously.

4. Evaluation at bench-scale of a hybrid MBR for the post-treatment of methanogenic bioreactors.

Finally, a hybrid MBR system is studied in this work as a post-treatment of methanogenic bioreactors, to reduce total nitrogen and methane emissions. The effluent of an upflow anaerobic sludge blanket (UASB) reactor was treated in a two-compartment membrane bioreactor (MBR). The first compartment was an

anoxic moving-bed reactor, with Kaldnes K3 carriers, intended to use dissolved methane as carbon source for denitrification, while the second compartment was an aerobic membrane filtration reactor that allows for a complete retention of the slow growing bacteria.

Up to 95% methane reduction was observed, which could not be attributed solely to the physical effect of desorption in the anoxic reactor.

In addition, up to 60% nitrogen removal was observed. It was proved that this efficiency could not be carried out solely with the remaining biodegradable COD in the UASB effluent, being necessary the use of the dissolved methane as a carbon source.

A stripping of the dissolved methane present in the anaerobic UASB effluent was carried out, which led to decrease in nitrogen removal from 60 to 27%. Thus, the percentage of nitrogen removal coming from the oxidation of methane could account up to 33%.

Besides the concentration of dissolved methane, the recirculation rate between the aerobic and the anoxic compartments was shown as another important parameter governing the process. The lower recirculation ratios studied (between 0.5 and 1) showed the higher nitrogen removal and the lower methane emissions.

FISH analysis confirmed the presence of microorganisms capable of denitrifying using the dissolved methane as a carbon source, both aerobically and anaerobically. Denitrification seems to be carried out by a consortium of aerobic and anaerobic methane oxidizing bacteria, anammox and heterotrophic bacteria

The influence of denitrification with methane on membrane performance was also studied. The highest cBPC concentrations and the lowest permeabilities were observed when denitrification activity diminished.

The HMBR proposed seems to be a suitable technology for the post-treatment of UASB reactors. The biofilm presence favoured the development of a wide variety of populations of microorganisms, which could be advantageous for the growth of those implicated in denitrification process. In addition, the use of membranes allows for a complete retention of the slow growing bacteria involved in methane and nitrogen removal.

Recommendations for further research

Based on the main results obtained from this work, there exists a significant research potential in the field of HMBR systems. Specifically, the following studies are recommended:

- The microbial activity and bio-kinetics of biofilm and suspended biomasses in the HMBR should be studied in more detail. The contribution of each type of biomass to the overall performance needs to be elucidated in order to find their optimum distribution and concentration (that is, the "degree of hybridity"). The identification and quantification of the microbial species (e.g., through techniques as FISH/DGGE) would be a useful tool for this purpose.
- It is necessary to compare the removal efficiency and fouling performance between hybrid and conventional MBR in other operation conditions (e.g., various organic loadings or SRTs).
- The results of this study suggest that the hybrid MBR could reduce membrane fouling as compared to the conventional MBR. Considering that fouling is still the main concern in the application of membrane bioreactors, hybrid configurations could be an interesting option to alleviate this limitation. However, more thorough studies on membrane fouling in HMBRs are needed to ascertain the mechanisms responsible for this observed effect of adding fixed biomass

To conclude, the results obtained in this work evidence that hybrid MBR may become an interesting option in advanced wastewater treatment.

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