Hospital Wastewater Treatment by Membrane Bioreactor: Performance and Impact on the Biomasses

Mousaab Alrhmoun, Claire Carrion, Magali Casellas, and Christophe Dagot

***Abstract*--**A pilot-scale membrane bioreactor (MBR) was installed and operated for 75 days at laboratory. It was fed an influent directly from the hospital drainage collection system. To study pharmaceutics compounds toxicity with micropollutant concentrations ranging from low ng /L to mg/L, toward activated sludge. Lab scale experiments were conducted and microscopic techniques as confocal laser scanning microscopy (CLSM). Samples were observed using confocal scanning laser microscopy to characterize the extracellular polymeric substances of sludge (EPS content).

The study was focused on efficacy the membrane bioreactor in treatment the hospital effluent and the extra cellular polymeric substances (EPS) as indicator of bacteria sensitivity to toxic agents. Results suggested that after 20 days of exposure the pharmaceuticals compounds induced a significant increase of concentration soluble EPS in flocs, the pharmaceuticals compounds not inhibited COD removal and the nitrification during the experiments.

These findings are in agreement with the microscopic studies, which showed a significant increase of concentration EPS. The presence of the pharmaceuticals compounds and its main metabolites stimulates mechanisms of protection and production of EPS with a slightly higher production of proteins. The removal of PPCPs by biological treatment processes including membrane bioreactor (MBR) was studied during the experience. The performance of MBR was demonstrated to be stable after 20 days of the experience. Although presence the fouling, the membrane look as technique very important for elimination the organics micrpolluents from the hospital wastewater.

***Keywords*---**Confocal laser scanning microscopy, membrane bioreactor (MBR), the extracellular polymeric substances (EPS), the organic micropolluants.

I. Introduction

THE treatment of hospital wastewater is one of the main prerequisites for reducing harmful impact on the environment. Hospitals are important sources of these compounds: a great variety of micro-contaminants result from diagnostic, laboratory and research activities on one side and medicine excretion by patients on the other [1], [2]

M. ALRHMOUN is with laboratory of GRESE EA 4330, university of Limoges (e-mail: moussab1984@hotmail.com).

C. Carrion is with Laboratory of CNRS, university of Limoges. (e-mail: claire.carrion@unilim.fr).

M. Casellas was with laboratory of GRESE EA 4330, university of Limoges (e-mail: Casellas@ensil.unilim.fr).

C. DAGOT is with the laboratory of GRESE EA 4330, university of Limoges (e-mail: Dagot@ensil.unilim.fr).

While in some countries (e.g., Japan, China, Greece) 1/3 wastewater from big hospitals is pre-treated or biologically treated on-site, in many other countries, including Switzerland, it is connected directly to a municipal sewer and treated at municipal wastewater treatment plants (WWTP). Treatment of the wastewater at the source has advantages of avoiding dilution due to mixing with the urban sewage and avoiding losses into the environment due to sewer leakage and combined sewer over flows. In the case of hospital wastewater, concerns are to avoid spread of (multiresistant or pathogenic) bacteria, viruses, and parasite eggs as well as to avoid input of pharmaceuticals, diagnostic agents, and disinfectants.

The membrane bioreactor (MBR) system can be regarded as an alternative way to achieve the design of compact wastewater treatment plants. The bioreactor which combines membrane system and biological treatment processes into a single unit, is designed to remove particulate, colloidal and dissolved substances from the solutions [3]. It is generally accepted that fouling reduces the performance of membrane. When the fouling occurs, a thick gel layer and cake layer are formed on and into the membrane, causing the permeate flux to decline and increasing the operating costs due to needs for cleaning or replacing the membrane. Fouling is usually attributed to a number of parameters, such as sludge particle deposition, adhesion of macromolecules such as extra cellular polymeric substances (EPS) and pore clogging by small molecules [4], [5]. Soluble EPS (soluble macromolecule and colloid) can enter the membrane pores and then build up on the pore wall, leading to a reduction of total section area of membrane pore, causing pore plugging into membrane and eventually increasing the membrane resistance [6]. The nature of fouling are strongly influenced by biomass characteristics, operating conditions and membrane characteristics. The membrane performance can be monitored through a number of factors such as membrane fouling, EPS production, treated effluent quality, biomass characteristic and microbial activity [7], [8]. These factors are dependent on hydraulic retention time (HRT), sludge retention time (SRT), organic loading rate (OLR), process configuration, membrane material and biomass concentration [3], [9].

According to previous studies, proteins and polysaccharides are the main EPS components of bacterial aggregates. They can be produced by the microorganisms or provided by the wastewater and have different functions in the aggregates. Some of them, having hydrolytic activity, can participate in organic matter removal and provide nutrients for bacterial growth. Since it was shown previously that confocal laser

scanning microscopy (CLSM) is a suitable method for visualization of floc structure [10], we combined CLSM with image analysis to provide a direct determination of floc volume and architecture. We applied this approach to characterize activated sludge flocs with average and poor settling properties.

The objective of this work was to provide data on the general performance and micropollutant elimination efficiency of an on-site biological wastewater treatment at hospitals. To meet this objective: (i) a pilot-scale MBR was installed to receive and treat wastewater originating in a Limoges hospital and was in continuous operation for 75 d; (ii) an efficient and representative sampling campaign was designed and employed to representatively collect influent and effluent samples from the MBR; and (iii) an HPLC- MS/MS analytical method was used to quantify the concentrations of approximately 52 target analyses including pharmaceuticals, human metabolites, and industrial chemicals. In addition, this work is to evaluate the influence the pharmaceuticals and their metabolites on the nutrient removal performances and on biomass in a membrane bioreactor system (MBRs).

II. Materials And Methods

A. Study area

The hospital effluent (HE) samples used in this study were collected from the sewerage system which comprises only sewers from clinical activities of the hospital. Average characteristics of wastewater and activated sludge used as inoculums during the experiments are detailed in the table 1.

TABLE I
PHYSICOCHEMICAL CHARACTERISTICS OF THE HOSPITAL EFFLUENT (HE), AND ACTIVATED SLUDGE (AS)

	COD (MG/L)		N (MG/L)			
	Total	**Soluble**	**Total**	**Soluble**	SM (G/L)	VM (G/L)
HE	318.415	185.5	122.451	80	0.1884	0.054
AS	1174	144	-	-	5.9854	0.121

B. Reactors and operating conditions

The reactor consisted of a membrane bioreactor with a working volume of 400 L and a membrane module in an external circulation loop. The membrane module was a Polypropylene and type of fibres cruses (MF) membrane with 1m2 of surface area and pore size of 0.2µm (ALTING, MICRODYN, France). A Ruston turbine (80-120 rpm) was installed to keep the bioreactor completely mixed. An identical lab-scale cross-flow MBR was run and inoculated with activated sludge from a municipal wastewater treatment plant (dry weight, 2.5 g/L). The influent was a hospital effluent (average flux 100 L/ day). The hydraulic retention time (HRT) was 22 h, temperature was 18-25 ◦C and pH was 7–8. The sludge retention time (SRT) was around 20 days. Treatment was operated in aerobic/anoxic conditions to allow nitrification and denitrification of the influent. Dissolved oxygen levels were maintained between 1 and 4.5mgO2/ L. The aeration cycle was automatic based on tow limits. Pressures were measured at the inlet (P1), outlet (P2), and permeate side of the membrane (P3) in order to determine the Trans- membrane pressure (TMP). At constant permeate flux, TMP indicates the extent of membrane fouling and it was calculated as follows:

$$Tmp = [(P1 + P2) /2 - P3] \quad (1)$$

TABLE II
KEY OPERATIONAL PARAMETERS OF MBR SYSTEMS INVESTIGATED.

Condition of bioreactor operation	
Operating parameters	**Operating range**
Concentration of oxygen	1- 5 mg O2/L
PH	6,9 -8
T C°	14,5- 20
Agitation	80-120 tr /min
Reaction Volume (L)	400 L
flow of outlet (L.d-1)	1300-1700 L / j
SRT (d)	15-20 days
HST	22 h
Aeration	Auto - 1-5 mg.O2/L
Time of presence O2 (h)	6
Flux outlet (14-20°C) (L.m-2.h-1)	40-50 L/h
Mode of filtration	position horizontal- vitesse tangentielle Interne-Externe T
Tangential speed along the membrane (m / s)	0,286m/s
Type of initial treatment	Decantation
Cycle of operation	
Time of decantation	20 min
Time of transport	20 min
Temps of filtration	20 min
Temps of alimentation	**40 min**
Volume of tank	150 L
Volume of tank the washing	150 L
Flow of pump Booster	900 L/h
flow of pomp of circulation	800-950 L/h
flow of Intel	4,25 L/ h
TMP	0,1 - 0,25 bar

C. Analytical methods

Wastewaters and sludge physic-chemical characteristic measurements were done every two day. Measurements of total and volatile suspended solids (TSS and VSS) were done according to the normalized method (AFNOR, NF T 90-105). Chemical Oxygen Demand (COD) was measured by the closed reflux colorimetric method (ISO 15705:2002), and total nitrogen (TN) was assessed using the alkaline persulfate digestion with colorimetric reactive (Hatch company). The COD and TN were carried out on both total and soluble fraction (after samples filtrated at 1.2µm). Ionic species in solution were determined on samples filtrated at 0.22µm using

ion chromatography (DIONEX 120) according to the standard method (AFNOR, NF EN ISO 10304-1). The used detector was conducted metric, and the analytical error was ±5%.

D. Confocal laser scanning microscopy and EPS staining

SYTO® 9 BacLight™ bacterial stains was used according to the manufacturer's instructions (Molecular Probes, Eugene, Oregon, USA). The kit provides a three colours fluorescence assay of bacterial relying on membrane integrity: viable bacteria are stained by SYTO® 9 and fluoresce green, while damaged bacteria are stained by propidium iodide and fluoresce red. Protocol established by [11], [12] was performed: 1 mL of undiluted biomass suspension was mixed with 3µL of a mixture of equal parts of SYTO® 9 and propidium iodide. This short staining protocol allowed direct observation of the original floc structure and the time-lapse microscopy. Microscopic observations started 15 min after staining. Excitation maxima for SYTO® 9 and propidium iodide bound to DNA are 480 and 540 nm, respectively [13]. For the image series, a Zeiss LCM 710 NLO confocal microscope equipped with 488 and 532 nm laser diode was used with an HCX 5×0.5. The band width of the detected fluorescence wave lengths has been optimized to uniquely channel the maximum emission in sequential mode to avoid potential cross-talking (502–530 nm for SYTO® 9 and 600–630 nm for propidium iodide). Fluorescence emissions were recorded within 1 Airy disk confocal pinhole opening and 1024 × 1024 images at a 1.36mm (x,y) pixel size were obtained. Instead of selecting a constant step size in the vertical direction, the step size was determined by choosing start and end points in the z-direction of the flocs, and by then selecting a number of optical sections.

PS and PN staining was carried out according to the modified procedure of [14]. Bio samples were centrifuged to remove supernatant, washed twice with 1× phosphate-buffered saline (PBS) buffer (pH 7.2) and kept fully hydrated in 2 mL centrifuge tubes covered with aluminium foil. For PS staining, 100 µL of concanavalin A conjugated with tetra - methylrhodamine (Con A, 250 mg L−1, Molecular Probes, and Carlsbad, CA, USA) was first dripped onto the sample and incubated for 30 min to stain α-mannopyranosyl and α glucopyranosyl sugar residues. For PN staining, 100 µL of sodium bicarbonate buffer (0.1 M) was introduced to the sample to maintain the amine groups in non-protonated form. Subsequently, 100 µL of fluorescein isothiocyanate solution (FITC, 1 g L−1, Fluka) was supplemented and incubated for 1 h to bind to proteins. Samples were washed tow times with 1× PBS buffer after each staining stage to remove loosely bound and excess dyes. Finally, sectioned granule or biofloc samples were mounted onto microscopic glass slides for observation of the distribution of PS and PN by a confocal laser scanning microscopy equipped with an Ar–He–Ne laser unit and three barrier filters. The image acquisition settings, such as laser intensity, numerical aperture, gain and offset settings were adjusted according to [15] and the levels were kept constant through observation. Samples were visualized with a ×10 objective and analyzed with the start LSM image browser confocal software.

E. Digital image analysis

Image analysis was performed with the freely available software Image J version 1.39i including the LSM-Reader plug in to open LSM5 formatted image stacks created by the microscope software. The tool Image J Analyzer 1.1, which is based on the performance of Image J and handles LSM5 formatted image stacks, was programmed for quantitative analysis. By setting a threshold, pixels with intensity below the threshold were assigned to the background. All other pixels were set to the foreground. Due to the individual image adjustment during the image stack acquisition, the threshold was chosen manually for each image stack. It has to be stressed that the pitfalls of threshold setting by the operator is well known [16], [17].

III. Results And Discussion

A. The Process Performances

The total and soluble COD removal efficiency was always respectively greater than 87.9 % and 86.9. During start-up TSS and VSS concentrations in the MBR increased almost continuously (depending on our wastewater characteristics the increased was slowly and not very remarkable). Effluent solids concentrations were always very low (<0.0012 g/L) confirming the excellent solids removal of micro-filtration systems. The removal of TSS was 99.5 % obtained only by the filtration by membrane and that indicate to the perfect solids retention capacity of the membranes. By the way, more than 97% of the VSS influent was removed. Particular attention has to be paid to the nitrogen removal efficiencies. The total and soluble Nitrogen removal efficiency was always respectively greater than 91% and 90%. It is not worth to confirm that the denitrification potential of a wastewater is linked not only to the COD availability in the influent but also to its ready biodegradability

Table III
Evolution The Efficiency Removal Of Organics Pollutants By Mbr

Efficiency of removal %	TSS	VSS	TCOD	SCOD	TN	SN
MBR	99,5	97,4	87,9	86,9	91	90,4

The (Fig. 2) shows that after 12 days of exposure the hospital effluent induced a significant increase in the concentration of EPS in the "soluble" and "Total" phase then a decrease and stabilization after 45 days of operation. This result is supported by the observations made by confocal microcopy. This could be attributed to the response of plants to stress sludge toxic type for the presence of different compounds in the effluent hospital. Also, Reference [18] reported that a high COD/N ratio promotes the production of more EPS. In another wards, increasing concentration of COD mean decreasing concentration of N by comparison and low ratio the nitrification, and as result, increasing EPS production.

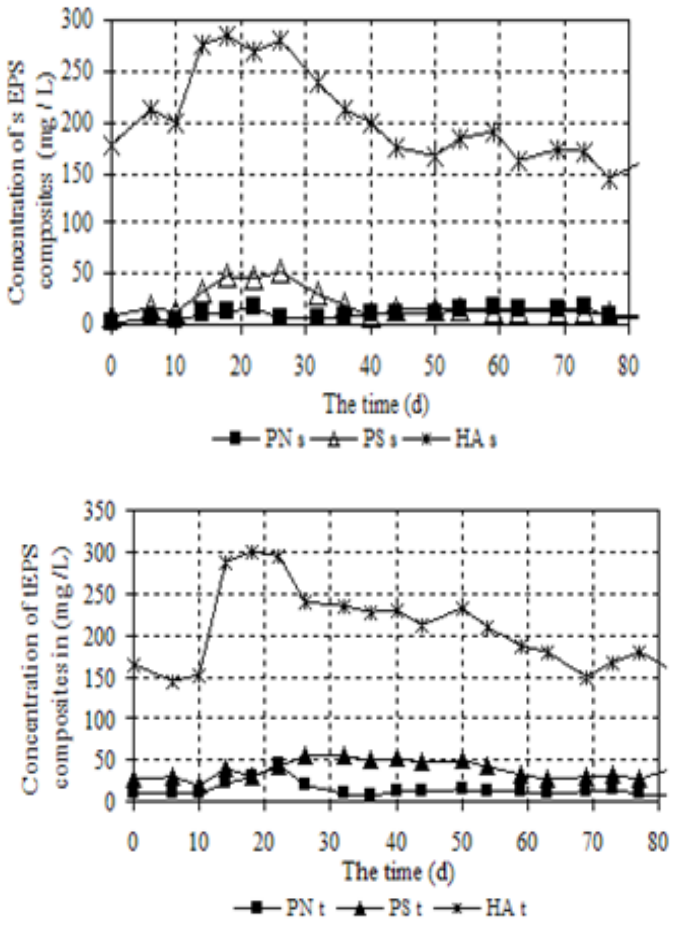

Fig.2. Evolution of the concentrations of EPS compounds (proteins, polysaccharides and humic acids) in the reactor in two phases (total and soluble).

B. EPS analysis with confocal laser scanning

EPS constituent localization within mixed microbial community Visualizations of flocs collected in the EMBR reactor after 2 and 45 days of exposure time to the HE are presented in Fig. (3) (C, D), (G, H), (K, L). In general, through visual inspection, an increase in EPS constituents (PN, PS and AH) was observed, especially in the first days by comparison with the first image between 2 and 45 days of exposure. This finding according with our chemicals results in increasing concentration of EPS with the time from 15 until 45 days.

The increase of green and red signals fluorescence (A, E) it can be deduced that the polysaccharides and the proteins compounds increased and that according with the figures (B and F). The increase in EPS constitutes in the reactor could be due to a better way to protect the bacteria from the toxic effect of hospital effluents. This is corresponds with the increase of filamentous bacteria.

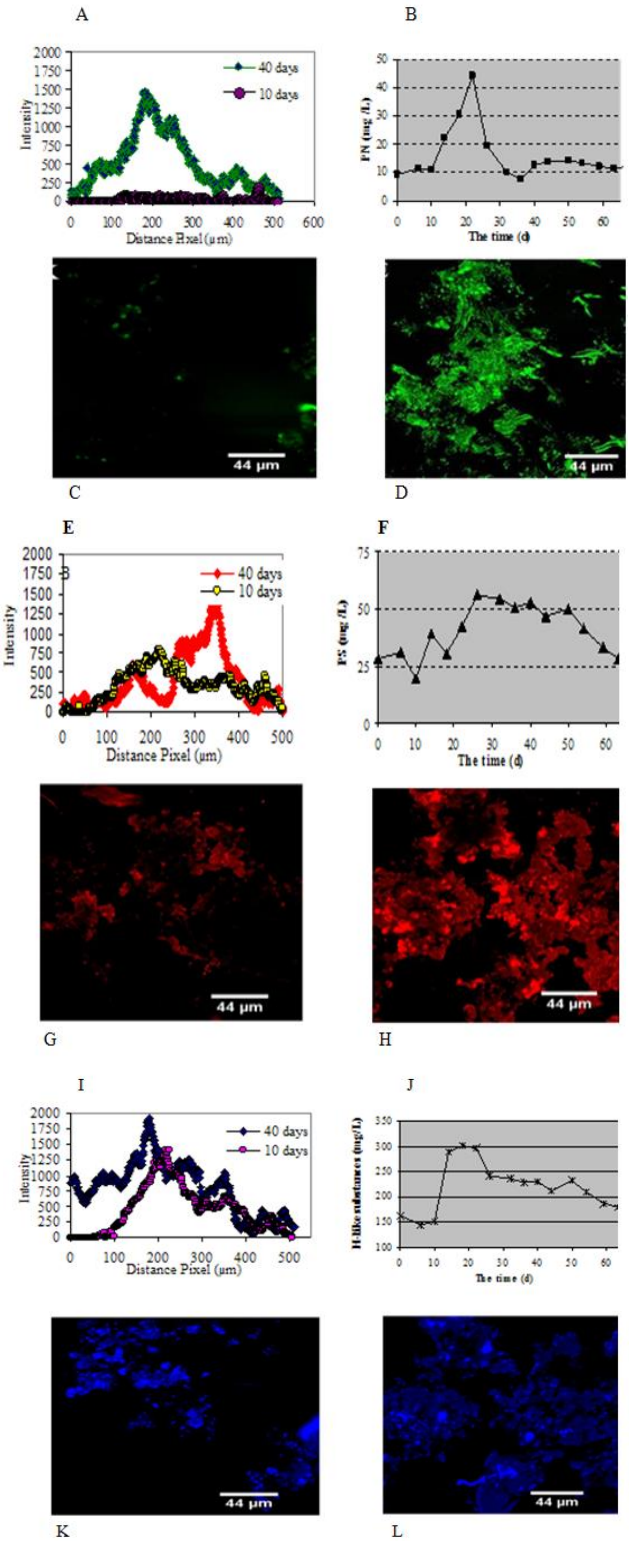

Fig. 3 CLSM images of the bound EPS distribution within MBR flocs. Images were obtained at 10 X magnification. (1) FITC staining universal protein was in (Green), (2) ConcA staining α-mannopyranosyl and α glucopyranosyl (red) and (3) Humic substances –like in bleu. Images are representative of 5-10 flocs examined. Images (C, D), (G, H) and (K, L) for 2 and 45 days respectively. The figures (A, E and I) represent intensity of Proteins, polysaccharides and Humic substances-Like in versus of surface the sample. The figures (B, F, and G) represent the EPS consentient versus the time in sludge from the experimental tests.

C. Sludge morphology

Any changes in morphology of floc were observed during the first days of experience. However, after 20 day a significant decrease in the proportion of fragments of floc and increase of filaments was observed (Fig. 4). These changes were consistent with the stabilization of the carbon (COD) of the effluent treated by the same reactor.

The hospital effluents are characterized by a high concentration of surfactant and pharmaceuticals [2] causing a decrease in the average size of flocs of activated sludge (Fig.5) [19], [20], [21]. The death of some bacteria and their hydrolysis can then weaken the structure of flocs. Therefore, the decrease in the concentration of floc fragments could be explained in several ways: by their leaching survives reactor, poor detection by image analysis, or re-aggregation in activated sludge. In addition, the wastewater quality directly impacts the morphology of flocs, as observed during the process of "bulking" [22]. The increase in the proportion of filament measured in sludge fed with hospital effluent clearly resembles this type of process. Poor settling properties (i.e. high SVI) and filamentous bacteria development were observed in the MBR associated to a release of polysaccharides and proteins in the supernatant. The table (4) showed the general distribution of floc particles in the reactor.

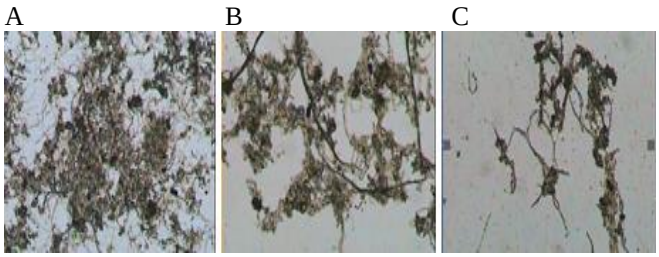

Fig. 4 Example of the morphology of activated sludge floc after (A) 2 days (B), 20 days (C) and 70 days of experience.

TABLE IV
ESTIMATED TELL FLOC PARTICLES TO DEPEND ON THE SURFACE

Time (d)	< de 50µm	50 -150 µm	150-255 µm	Fragments / total	Filaments / total
2	923960	3873226	7678917	0,074	0,615
24	1079878	3221492	9376326	0,078	0,685
70	760351	1985763	10438526	0,057	0,791

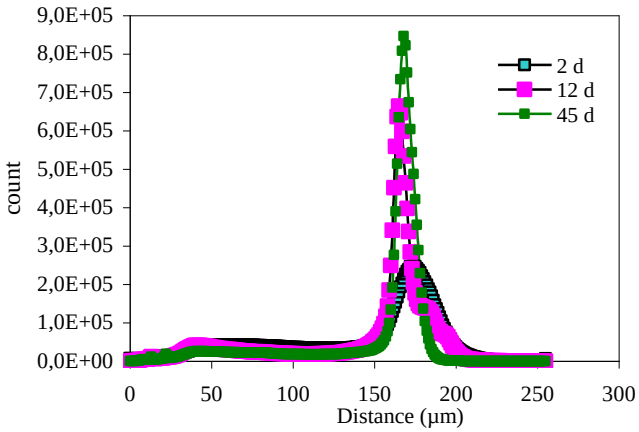

Fig. 5 Distribution the flocs (par taille) in the activated sludge after 2, 20 and 40 days of experience.

D. Live/dead assessment within mixed microbial flocs

Propidium iodide (PPI) stains dead cells and extracellular DNA but not intact cells in the floc. Syto9 was used to stain total available DNA (present in live cells, dead cells and extracellular DNA in the flocs). In general for all floc samples, the Syto9 and PPI signals were distributed throughout the flocs sections. However, the intensity of the two signals varied from one region of a floc to another, likely due to the differential number and localization of live versus dead cells within the flocs. Stacks of images were imported into the image software to mathematically compute relative intensities of the Syto9 and PPI signals, and to calculate the relative percentile of live cells per floc. Visual inspection in Figure (6) reveals that there is a increasing in bleu signal intensity during the time of experience and it is very significant. These modifications were attributed to a protection mechanism of the bacteria against toxic effluent [23]. In addition to decrease of live bacteria with increasing the time of contact although the toxics compounds in the hospital effluents.

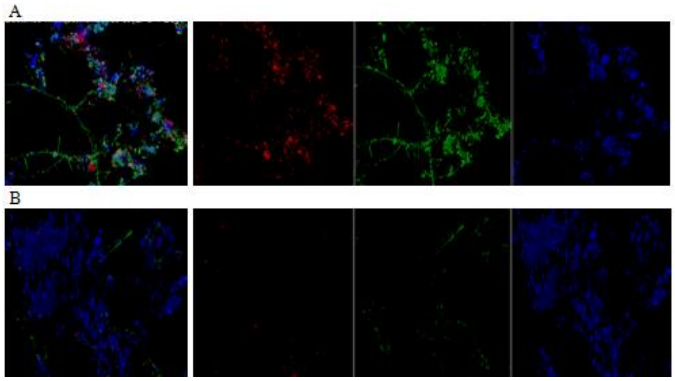

Fig. 6 CLSM image of live cell distribution within MBR flocs (A and B) for (2 and 45 days respectively). (Flocs were stained with syto9 for total available DNA (green) and PPI staining DNA for dead cells (red) and EPS DNA in (bleu) (.Images obtained at 10x magnification. These representative images are based upon the examination of 5-10 flocs per sample.

E. Occurrence and removal of PPCPs in hospital wastewaters.

The total removal efficiency (sludge sorption+ biodegradation + membrane retention) of each pharmaceutical compound was determined for MBR according to Eq. (1):

$$R\ (\%) = (C2 - C1) / C2 \times 100 \qquad (1)$$

Where:

C1: the experimental concentration determined for each pharmaceutical compound in each reactor influent by LC / (MS-MS) analysis.

C2: the experimental concentration of each pharmaceutical compound in each reactor effluent by the LC / (MS-MS) analysis. R: the removal (%)

The concentrations of the various pharmaceutical compounds and their transformation products during the spiking period were determined by LC-MS applying electrospray ionization (ESI) under high resolution MS conditions. The table (5) shows the concentration of the PPCPs and the removal for MBR. It can be clearly observed the highest removal efficiency (around 90) or complete removal of Paracétamol, Naproxen, Ibuprofen Sulfamétoxaz - ole, Trimethoprim, Caffeine, Acide fenofibrate, Metoprolol, Iopromide and Atenolol in the MBR

TABLEV
THE CONCENTRATION OF PHARMACEUTICS COMPOUNDS (PPCPS) IN INFLUENT AND EFFLUENT FOR MBR.

Pharmaceutical compound	Influent (µg/L)	Removal (%)	References
Codéine	0,18	0	
Ketoprofen	6,4	62	(30%), [24]
Paracétamol	177	100	
Diclofenac	0,1	0	(5–45 %), [25]
Naproxen	3,7	90	(55-85 %), [25]
Ibuprofen	1,4	95	(90-100 %), [25]
Roxithromycin	0,21	0	(<60 %), [26]
Sulfamétoxazole	1,6	92	
Metronidazole	4,8	33	15–18 % [24]
Trimethoprim	1,4	98	90%, [26]
Hydrochlorothiazide	2,8	0	
Furosemide	5,1	61	35–40 %, [24]
Caféine	41	100	
Gemfibrozil	0	0	
Pravastatin	0,42	55	45 %, [24]
Metoprolol	0,1	100	
Atenolol	0,77	100	70 %, [24]
Acide Fénofibrique	2,1	100	
Carboxy-ibuprofen	16	82	80 %, [25]
Iopromide	1,1	100	60-80 %, [24]
Iohexol	167	0	

The elimination of pharmaceutical compounds can occur through various mechanisms in MBR and CAS. Sorption onto sludge is one of the mechanisms that take into account the absorption and adsorption factors. According to [27], adsorption refers to the hydrophobic interactions of the aliphatic and aromatic groups of a compound with fats present in the sludge or with the lipophilic cell membrane of the microorganisms (depending on their Kow value), while adsorption refers to the electrostatic interactions of positively charged groups of dissolved chemicals with the negatively charged surfaces of the microorganisms (characterized by the dissociation constant pKa). Another mechanism responsible for the removal of pharmaceutical compounds in MBR is the physical retention by the membranes.

IV. CONCLUSION

This work evaluated performances of extern MBR, treating the effluents hospitalises operating over a little range of SRT (15 days) and studied the influence of pharmaceuticals compounds present in the effluents hospitalises on the physicochemical and properties of the activated sludge. The analyses performed on the supernatant and activated sludge bioreactors allow us to draw the following conclusions:

1. The MBR was able to achieve very good organic removal efficiencies. Deterioration of activated sludge effluent quality was especially observed after 20 days of experiment (filamentous bacteria, increase in protein and polysaccharide release).
2. Despite the low concentration studied, the toxicity of the pharmaceutical compounds on activated sludge altered the characteristics of the biological matrix. The presence of the pharmaceutical compounds stimulated the mechanisms of survival (higher production of EPS).
3. Simultaneously, confocal laser scanning observations and image analyses showed significant modifications of sludge morphology. (Higher production of soluble EPS).
4. The results obtained of dosage the compounds pharmaceuticals showed that the MBR presented high removal efficiencies for more of 10 different compounds.

Finally, this study shed new light on the efficacies of membrane bioreactors MBR in the treatment of effluents hospitalises containing pharmaceuticals compounds which, despite their low concentrations, modify the biological suspension behaviour. And shed new light on the subject which will be useful for further development and optimisation of MBR and these findings will need to be correlated to fouling tendency in further work.

REFERENCES

[1] Kümmerer K. Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources a review. 2001 Chemosphere, 45, (6–7), 957-969 http://dx.doi.org/10.1016/S0045-6535(01)00144-8

[2] Carballa, M., Omil, F., Lema, J.M., Llompart, M., Garcia-Jares, C., Rodriguez, I., Gomez, M., Chromatography–mass spectrometry: methods and preliminary results including toxicity studies with Vibrio fischeri. J. Chromatogr. A 2004- 938, 187–197.

[3] Chang I. S. and Lee C. H. (1998) Membrane filtration characteristics in membrane coupled activated sludge system the effect physiological

states of activated sludge on membrane fouling. Desalination 120, 221–233.
http://dx.doi.org/10.1016/S0011-9164(98)00220-3

[4] Nagaoka H., Ueda S. and Miya A. (1996) Influence of bacterial extracellular polymers on the membrane separation activated sludge process. Water Sci. Technol. 34(9), 165–172.
http://dx.doi.org/10.1016/S0273-1223(96)00800-1

[5] Bouhabila E H, Epuration d'eaux usees par bioreacteur aere a membranes immergees Interaction suspension floculee/membrane, These de doctorat INSA Toulouse (1999)

[6] Xing C.H, Qian Y., Wen X.H., Wu W.Z., Sun D.. Physical and biological characteristics of a tangential-flow MBR for municipal wastewater treatment. Journal of Membrane Science 191 (2001), pages 31-42. (Xing et al. 2001).

[7] Kim Jae-Seok, Lee Chung-Hak, Chang In-Soung. Effect of pump shear on the performance of a crossflow membrane bioreactor. Water Research Vol. 35 No. 9, pp 2137-2144 (2001). (Kim Jae Seok et al., 2001).

[8] Lee W., Kang S., Shin H. Sludge Characteristics and their contribution to microfiltration in submerged membrane bioreactors. Journal of membrane Science, 216, (2003), pages 217-227. (Lee et al., 2003).

[9] Judd S. A review of fouling of membrane bioreactors in sewage treatment. Water Science and Technology, 49 (2004), 229-235. (Judd, 2004).

[10] Liss SN, Droppo IG, Flannigan DT, Leppard GG. Floc architecture in wastewater and natural riverine systems. Environ Sci Technol 1996;30:680–6.
http://dx.doi.org/10.1021/es950426r

[11] Lopez C, Pons MN, Morgenroth E. Evaluation of microscopic techniques (epifluorescence microscopy, CLSM, TPE-LSM) as a basis for the quantitative image analysis of activated sludge. Water Research 2005; 39:456–68.
http://dx.doi.org/10.1016/j.watres.2004.10.009

[12] Baker, A., Inverarity, R., 2004. Protein-like fluorescence intensity as a possible tool for determining river water quality. Hydrol. Process. 18, 2927–2945. Biodegradation. 20, 441-466.

[13] Reynolds, D.M., 2002. The differentiation of biodegradable and non-biodegradable dissolved organic matter in waste waters using fluorescence spectroscopy. J. Chem. Technol. Biotechnol.77, 965–972
http://dx.doi.org/10.1002/jctb.664.

[14] M.Y. Chen, D.J. Lee, J.H. Tay, Distribution of extracellular polymeric substances in aerobic granules, Applied Microbiology Biotechnology 73 (2007) 1463–1469
http://dx.doi.org/10.1007/s00253-006-0617-x

[15] Toh, S.K., Tay, J.H., Moy, B.Y.P., Ivanov, V., Tay, S.T.L., 2003. Size effect on the physical characteristics of the aerobic granule in a SBR. Appl. Microbiol. Biotechnol. 60, 687–695.
http://dx.doi.org/10.1007/s00253-002-1145-y

[16] Staudt, C., Horn, H., Hempel, D.C., Neu, T.R., 2004. Volumetric measurements of bacterial cells, extracellular polymeric substance glycoconjugates in biofilms. Biotechnol. Bioeng. 88 (5), 585–592
http://dx.doi.org/10.1002/bit.20241.

[17] Yang, X., Beyenal, H., Harkin, G., Lewandowski, Z., 2001. Evaluation of biofilm image thresholding methods. Water Res. 35, 1149–1158.
http://dx.doi.org/10.1016/S0043-1354(00)00361-4

[18] A.P. Miqueleto, C.C. Dolosic, E. Pozzi, E. Foresti, M. Zaiat, Influence of carbon sources and C/N ratio on EPS production in anaerobic sequencing batch biofilm reactors for wast water treatment, Bioresources technology, February 2010, Pages 1324–1330.

[19] Louvet, J.N., Giammarino, C., Potier, O., Pons, M.N. 2010. Adverse effects of erythromycin on the structure and chemistry of activated sludge. Environmental Pollution 158 (3), pp. 688-693
http://dx.doi.org/10.1016/j.envpol.2009.10.021

[20] Liwarska-Bizukojc E., Marcin Bizukojc. 2007 .The influence of the selected nonionic surfactants on the activated sludge morphology and kinetics of the organic matter removal in the flow system Enzyme and Microbial Technology, Volume 41, (1–2), 26 34
http://dx.doi.org/10.1016/j.enzmictec.2006.11.016

[21] Liwarska-Bizukojc E., Marcin Bizukojc M. 2005. Effect of selected anionic surfactants on activated sludge flocs. Enzyme and Microbial Technology, 39,(4), 660-668
http://dx.doi.org/10.1016/j.enzmictec.2005.11.020

[22] Martins, A.M.P., Pagilla, K., Heijnen, J.J., Van Loosdrecht, M.C.M. 2004. Filamentous bulking sludge - A critical review. Water Research 38 (4), pp. 793-817
http://dx.doi.org/10.1016/j.watres.2003.11.005

[23] Avella, A.C., Delgado, L.F., Gorner, T., Albasi, C., Galmiche, M., De Donato, Ph., 2010. Effect of cytostatic drug presence on extracellular polymeric substances formation in municipal wastewater treated by membrane bioreactor. Bioresour. Technol. 101 (2), 518–526
http://dx.doi.org/10.1016/j.biortech.2009.08.057

[24] Jelic A, Gros M, Ginebrenda A, Cespedes-Sánchez R, Ventura F, Petrovic M, et al. Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. Water Res 2011; 45:1165–76.
http://dx.doi.org/10.1016/j.watres.2010.11.010

[25] Joss A, Keller E, Alder A, Göbel A, Mcardell C, Ternes T, et al. Removal of pharmaceuticals and fragrances in biological wastewater treatment. Water Res 2005; 39: 3139–52.
http://dx.doi.org/10.1016/j.watres.2005.05.031

[26] Göbel A, Mcardell CS, Joss A, Siegrist H, Giger W. Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies. Sci Total Environ n2007; 372:361–71.

[27] M. Carballa, F. Omil, J.M. Lema, Removal of cosmetic ingredients and pharmaceuticals in sewage primary treatment, Water Res. 39 (2005) 4790–4796
http://dx.doi.org/10.1016/j.watres.2005.09.018