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Effect of membrane bioreactor configurations on sludge structure and microbial activity

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The aim of this paper was to determine the effect of two different membrane bioreactor (MBR) configurations (external/immersed) on sludge structure and microbial activity. Sludge structure was deduced from rheological measurements. The high shear stress induced by the recirculation pump in the external MBR was shown to result in decreasing viscosity due to activated sludge (AS) deflocculation. Besides, soluble microbial products (SMP) release was higher in the external MBR (5 $mg_{COD}\ g_{MLVSS}^{-1}$) than in the immersed configuration (2 $mg_{COD}\ g_{MLVSS}^{-1}$). Microbial activity was followed from respirometry tests by focusing on the distinction between heterotrophs and autotrophs. An easier autotrophic microbe development was then observed in the immersed MBR compared to the external one. However, the external MBR was shown to allow better heterotrophic microbe development.

Keywords: Membrane bioreactor, Heterotrophic, Autotrophic,, Soluble microbial products, Viscosity

1. Introduction

Compared to a conventional activated sludge (CAS) system, membrane bioreactor (MBR) technology presents many advantages but some drawbacks (Judd, 2008; Wisniewski, 2007). The complete separation of biomass and pollutants adsorbed on suspended solids allows high and constant quality of the effluent in MBRs, while CAS systems provide an effluent quality dependent on the settling ability of the biomass. One of the main drawbacks with the use of membrane technology is the fouling; this is particularly a problem with MBRs because of the activated sludge (AS) complexity. The exo-polysaccharides (EPS) of the microbial flocs and the soluble microbial products (SMP) released by biological metabolism have been indicated by some authors as primary foulants (Nataraj et al., 2008; Rosenberger and Kraume, 2002). Other authors specified that EPS and SMP are not reliable indicators of membrane fouling (Guglielmi et al., 2007; Pollice et al., 2005). These conflicting conclusions make the understanding and the control of the fouling in MBRs difficult (Le-Clech et al., 2006). However, authors agree there is a correlation between membrane fouling and AS structure/activity.

The research on MBR technology has been primarily focused on membrane fouling whereas microbial activity has been less studied. Some of the advantages of the MBR include higher sludge retention times (SRTs) with lower sludge production (Kim et al., 2001) and a more diversified biomass. Indeed, MBR technology has been demonstrated to increase microbial activity (Lee et al., 2003) with a more effective treatment of pollutants (Xing et al., 2000) than CAS systems. The higher SRTs are favourable to micro-organisms with slow growth, such as autotrophs, which are particularly interesting for the removal of an endocrine disrupter of great concern, the synthetic hormone 17α-ethinylestradiol (Clouzot et al., 2008). Only one study has been found on autotrophic and heterotrophic activities in MBR systems (Liang et al., 2010). Microbial activity was measured in immersed MBRs but no comparison with an external configuration was made.

The two MBR configurations (external/immersed) are characterized by different operating conditions (membrane material, filtration mode, shear stress, etc.). The aim of this paper was to determine the effect of these two different processes on sludge structure and microbial activity. Consequently, the biological parameters (SRT and food-to-mass (F/M) ratio) were fixed at the same values for both MBRs. With regard to the easily assimilated influent used (glucose as carbon source), the different hydraulic retention times (HRTs) applied to the external and to the immersed MBRs were considered to have no impact on microbial activity.

The biological activity was followed from respirometry tests by focusing on the distinction between heterotrophs and autotrophs. In the literature, physico-chemical parameters of AS were correlated to their rheological properties (Seyssiecq et al., 2003). Indeed, rheological parameters are linked to shear thinning of bacterial

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flocs through their ability to store or release water (Seyssiecq et al., 2008). During flocculation, water is stored whereas deflocculation induces a release of water responsible for decreasing viscosity. Therefore, in this paper, sludge structure was deduced from rheological measurements.

2. Methods

2.1. The external MBR

The external MBR (Polymem, France) was composed of a ceramic membrane (microfiltration, Novasep-Orelis, France) and an 11-L bioreactor equipped with a temperature regulator (approximately 24 °C) (Fig. 1A). Crossflow filtration was operated with a centrifugal pump that recycled sludge back to the membrane. The influent was provided to the bioreactor with a feed pump connected to a level regulator. The membranes used for the whole experiments had the same initial water permeability of approximately 270 L h^{-1} m^{-2} bar^{-1}.

During the biomass acclimation to the external MBR, the MLVSS was stabilized at 8 g L^{-1} and the crossflow velocity was held constant at 4 m s^{-1}, based on the results of a previous study (Fig. 2A). The impact of the crossflow velocity (2–5 m s^{-1}) on membrane fouling was previously evaluated in the external MBR with MLVSS maintained at 8 g L^{-1}. A maximum fouling was reached at a TMP of 1 bar for the minimal crossflow velocities comprised between 2 and 3 m s^{-1}. The membrane fouling decreased when the crossflow velocity was increased to 4 or 5 m s^{-1}. An increase to 3–4 m s^{-1} resulted in a 33% increase of the permeate flux (TMP 2.5 bar) whereas from 4 to 5 m s^{-1}, the increase was only 13%. Therefore, a rate of 4 m s^{-1} appeared to be an optimal choice for this MBR.

The critical pressure was determined between 0.7 and 0.9 bar from the method developed by Espinasse et al. (2002), for a crossflow velocity of 4 m s^{-1} and a MLVSS concentration of 8 g L^{-1}. During the biomass acclimation to the external MBR, the TMP was around 0.4 bar and the permeate flow was 0.6 L h^{-1}, which fixed a 18-h HRT. The process was then kept stable during 3 months

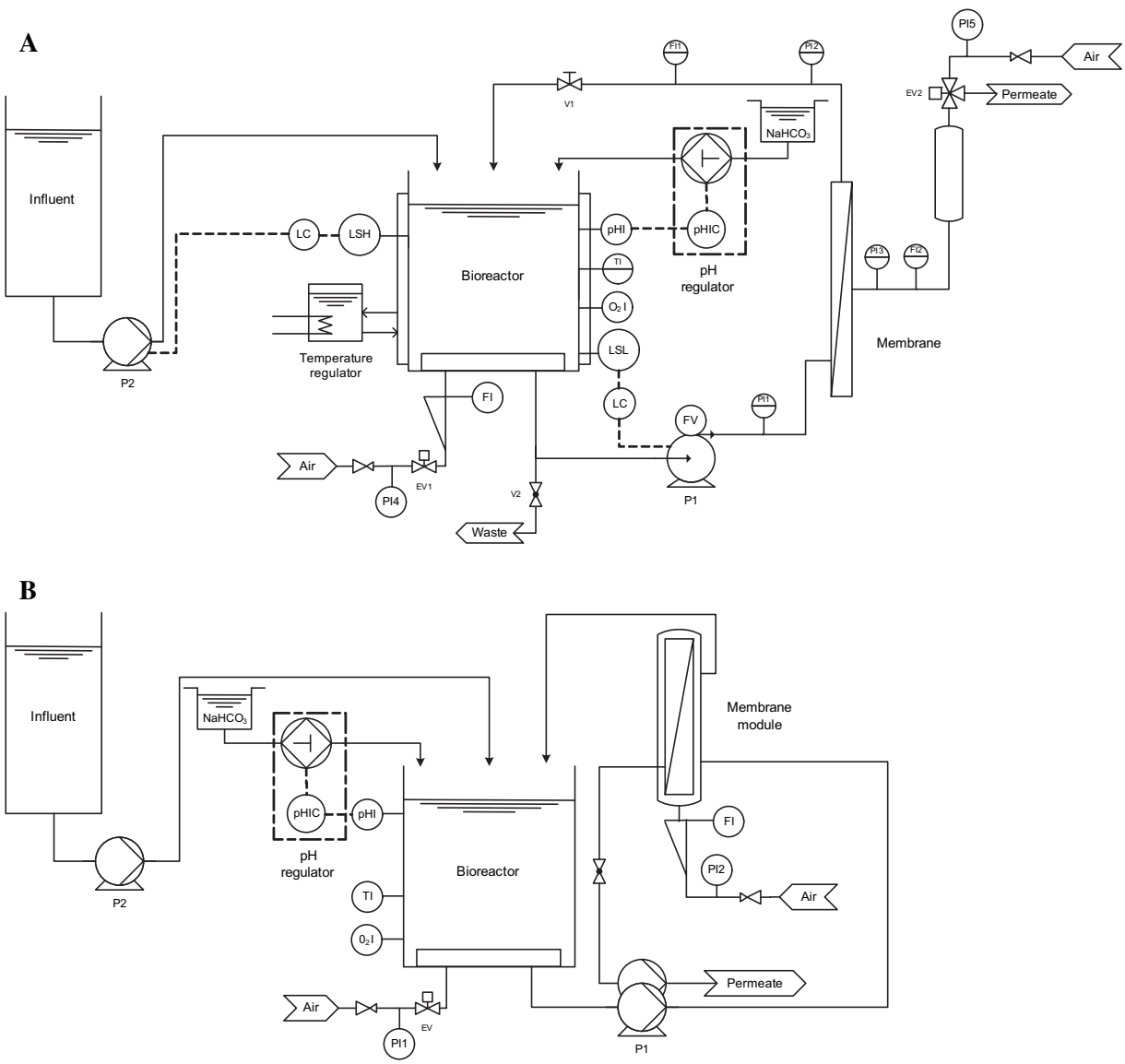

Fig. 1. The lab-scale MBRs: (A) external and (B) outside immersed configurations. P: Pump, V: Valve, EV: ElectoValve, LC: Level Controller, LSH: Level Security High, LSL: Level Security Low, FV: Frequency Variator, PI: Pressure Indicator, FI: Flow Indicator, pHI: pH Inidcator, pHIC: pH Indicator Controller.

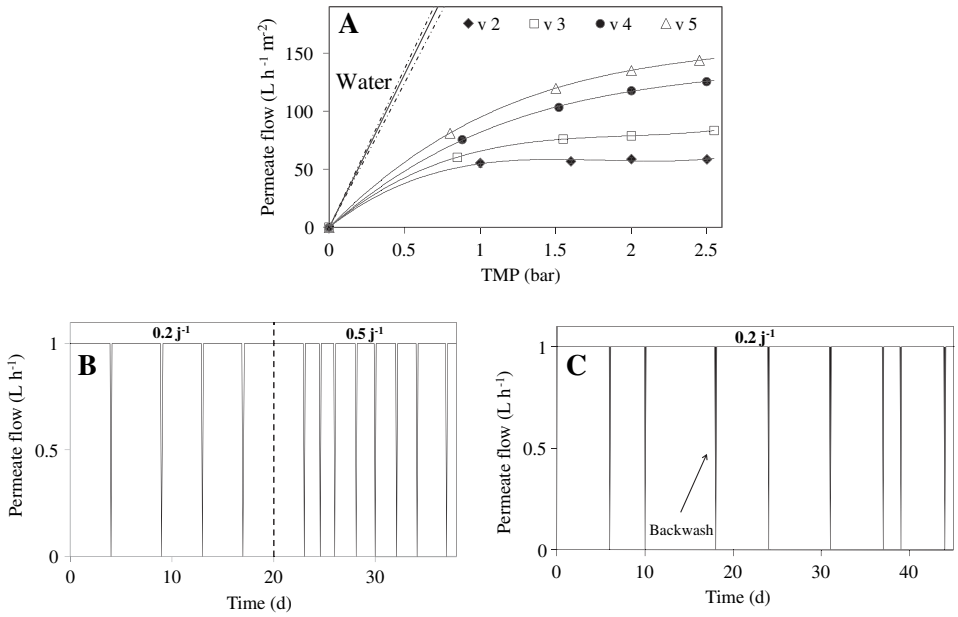

Fig. 2. Operating conditions of the lab-scale MBRs. The external MBR: (A) the permeate flux versus the recirculation rate (2/3/4/5 m s^{-1}). The immersed MBRs: Backwashes frequency for (B) the inside immersed MBR and (C) the outside immersed MBR.

because of the chosen operating conditions that limited the membrane fouling.

2.2. The immersed MBRs

The immersed MBRs were composed of organic hollow fibres (polysulfone, microfiltration, Polymem, France) and a 10-L bioreactor (temperature at approximately 24 °C). Peristaltic pumps were used for the influent and for the dead-end filtration. The hollow fibres were directly immersed in the bioreactor for the inside immersed MBR whereas the fibres were immersed in an external carter (300 mL) for the outside immersed MBR (Fig. 1B). An additional peristaltic pump was used in the outside configuration for the sludge recycle back to the membrane module. Besides, membrane fouling was limited by air sparging (6 L min^{-1}) in the external carter.

Backwashes of 5-L distilled water were regularly applied to maintain a constant permeate flow of 1 L h^{-1}. The inside immersed MBR can be operated during 20 days with a backwashes frequency of 0.2 J^{-1} (Fig. 2B). However, membrane fouling problems appeared after 20 days induced an increase of the backwashes frequency to 0.5 J^{-1}. With the outside immersed configuration, the backwashes frequency can be maintained constant at 0.2 J^{-1} over a period of 45 days without membrane cleaning (Fig. 2C). Thus, the outside configuration allowed an easier control of membrane fouling than the inside configuration.

The biomass acclimation to the immersed MBR was then performed in the outside configuration with MLVSS stabilized at approximately 9 g L^{-1} after 63 days. The permeate flow was around 0.4 L h^{-1}, which resulted in a 25-h HRT. Only two backwashes were used during the first 10 days but a second membrane module (identical to the first one) had to be added after 50 days of acclimation to maintain the stability of the process.

2.3. Biomass acclimation to the MBR processes

The lab-scale MBRs were started with AS collected from an urban WWTP operated with nitrification and denitrification tanks (165,000 population equivalents, Aix-en-provence, France). The AS was concentrated in the MBRs and the SRT was fixed at 50 days. A balanced synthetic sewage influent (C/N/P ratio = 100/10/2) was prepared with mass ratios of 2.1 $C_6H_{12}O_6$, 1.0 $(NH_4)_2SO_4$, 0.2 KH_2PO_4, 0.4 $NaHCO_3$, 0.1 $MgSO_4$ and 0.02 $CaCl_2$ (nutrients supplied by Chem-Lab, Belgium). The food-to-mass ratio (F/M) was maintained around 0.2 kg_{COD} kg_{MLVSS}^{-1} J^{-1} by adjusting the nutrient concentrations to the MLVSS contents. Oxygenation cycles of 2 h with air and 1 h without air were programmed to allow nitrification and denitrification reactions (dissolved oxygen concentration between 0 and 4 mg L^{-1}). The pH was maintained constant at 7 by an automatic regulator with a $NaHCO_3$ solution (60 g L^{-1}).

2.4. Analytical methods

The AS was regularly sampled in the bioreactor (30 mL), and samples were centrifuged for 30 min at 16,000*g* to separate suspended solids from the dissolved matrix. Ammonium and nitrate were analyzed in the supernatant by spectrophotometry (Spectro Aquamate, Thermo spectronic, UK) with reagent kits obtained from Merk (Germany). The chemical oxygen demand (COD) were quantified in the bioreactor supernatant and in the permeate to evaluate the fraction retained by the membrane. Reagent kits purchased from Aqua Lytic (Germany) were used for the COD.

2.5. Respirometry tests

The biological activity was followed from respirometry tests used to measure autotrophic and heterotrophic activity by differentiating endogenous and exogenous respiration. An aerated reactor was filled with 1 L of AS and every two minutes, 50 mL was sampled and injected into another reactor without oxygenation to measure the oxygen uptake rate (OUR) with a continuous dissolved oxygen probe (HQ 40d, Hach LDO, Düsseldorf, Germany). Then, specific nutrients or inhibitor were successively added to the aerated reactor according to four steps. Endogenous respirations of autotrophic and heterotrophic micro-organisms were first measured over a period of 1 h. Secondly, ammonium was added (0.02 g_{N-NH4} g_{MLVSS}^{-1}) to measure the maximum activity of autotrophic micro-organisms (ammonium removal was confirmed to

be correlated with nitrate production). Thirdly, autotrophic micro-organisms were inhibited with allylthiourea (inhibitor of the nitrifying ammonium monooxygenase (AMO) enzyme) to isolate heterotrophic endogenous respiration. A concentration of 0.1 $g_{allylthiourea}\ g_{MLVSS}^{-1}$ was shown to inhibit the respirometry activity, and nitrates were not produced after ammonium addition (0.02 $g_{N-NH4}\ g_{MLVSS}^{-1}$), which validated the autotrophic inhibition. Lastly, maximum activity of heterotrophic micro-organisms was quantified after glucose addition (equivalent to 0.3 $g_{COD}\ g_{MLVSS}^{-1}$).

2.6. Sludge characterization

A rotational and computer controlled stress rheometer (AR550, TA Instrument, France) coupled with a helical ribbon impeller (impeller Ø 14 mm, stator Ø 15 mm, immersed height 31 mm) was used to measure the AS viscosity. The method of the Couette analogy was applied to determine the constants of shear rate (6.85 rad^{-1}) and shear stress (27,103 Pa $N^{-1}\ m^{-1}$) (the method was detailed in Mori et al., 2006). With MLVSS concentrations below 26 g L^{-1}, the Ostwald model was chosen to calculate rheological parameters from two continuous stress ramps (from 0 to 20 Pa with 240 points in 40 min) separated by a 1 min isobar at 20 Pa.

3. Results and discussion

3.1. Biomass development

The biomass development was divided into four steps in both MBR configurations (Fig. 3A and B). The food to mass (F/M) ratio was initially fixed at approximately 0.2 $kg_{COD}\ kg_{MLVSS}^{-1}\ d^{-1}$ to resemble the WWTP conditions. The MLVSS concentration decreased during a first step (I) followed by a lag phase that was characteristic of an adaptation period of micro-organisms to the MBR process and to the synthetic influent. The smaller decrease in MLVSS observed in the outside immersed configuration highlighted milder operating conditions than those of the external MBR. Then, growth of the biomass defined the second step (II), where the nutrient concentration had to be increased. These two first steps were imposed by the microbial system whereas the next two steps were controlled to stabilize the bioprocess. The nutrient concentration was decreased during the third step (III) until the MLVSS concentrations were maintained at a constant level (step IV). At the end of the acclimation, the higher MLVSS concentrations obtained in the outside immersed configuration explain the different values of the F/M ratio.

The daily production of sludge (P_x), calculated by a simple mass balance (Eq. (1), Appendix A), is another indicator of the biomass development used in the literature for a similar AS acclimation to an external MBR with a 50-day SRT (Fernando Delgado Zambrano, 2009). The authors stipulated that a linear accumulated production ($P_{x\ accumulated}$) (Eq. (2), Appendix A) indicates the process stability. The two MBR configurations were subjected to a same lag phase of 20 days (Fig. 3C). However, the higher accumulated production in the outside immersed MBR confirmed an easier biomass development because of milder conditions, even if differences were not strongly significant. The process stability was confirmed for both MBRs at the end of the acclimation periods by the linear $P_{x\ accumulated}$.

3.2. Sludge structure

Sludge structure was deduced from rheological measurements and compared between the AS from the two MBR configurations and CAS concentrated at various MLVSS contents (Fig. 4). The viscosity of the AS acclimated in the external MBR (14.7 $g_{MLVSS}\ L^{-1}$) was lower than that of the CAS (12.2 $g_{MLVSS}\ L^{-1}$), whereas the MLVSS concentration was higher. Therefore, operating conditions of the external MBR resulted in a decrease of the AS viscosity. The viscosity of the AS developed in the outside immersed process (19.0 $g_{MLVSS}\ L^{-1}$) was between the viscosities of CAS with MLVSS concentrations between 16.7 $g_{MLVSS}\ L^{-1}$ and 21.0 $g_{MLVSS}\ L^{-1}$. Thus, the acclimation to the outside immersed MBR did not change significantly the sludge viscosity.

The flow index (n) did not reveal significant differences between the studied AS, but the consistency index (K) completed the viscosity interpretation (Table 1). Rheological parameters are linked to shear thinning of bacterial flocs through their ability to

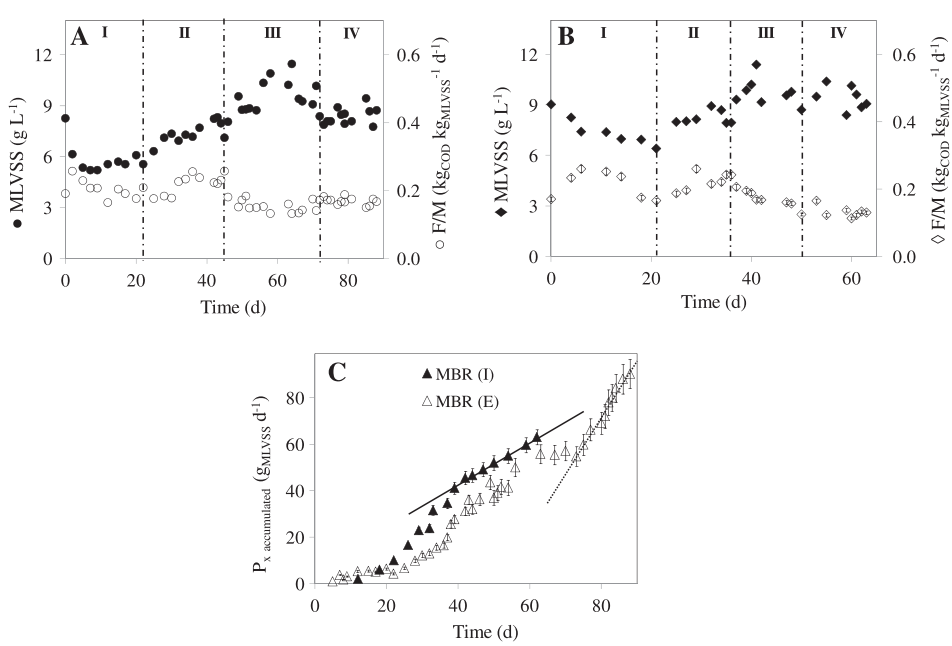

Fig. 3. Biomass development during the acclimations (A) MLVSS (●) and F/M ratio (○) in the external (E) MBR (B) MLVSS (♦) and F/M ratio (◇) in the outside immersed (I) MBR (C) accumulated sludge production in both MBR configurations.

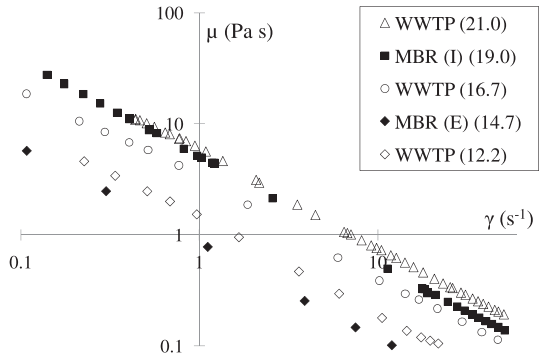

Fig. 4. Viscosity of the acclimated AS (E: external MBR and I: outside immersed MBR) and CAS. (The number inside brackets is the MLVSS concentration in g L^{-1}.)

Table 1
Rheological parameters for the CAS and the AS acclimated to the outside immersed (I) or to the external (E) MBR.

	WWTP	MBR (I)	WWTP	MBR (E)	WWTP
MLVSS (g L^{-1})	21.0	19.0	16.7	14.7	12.2
K (Pa s^n)	5.7 ± 0.5	4.8 ± 0.4	3.0 ± 0.2	0.9 ± 0.1	1.4 ± 0.1
n (−)	0.13 ± 0.01	0.09 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.16 ± 0.01

store or release water (Seyssiecq et al., 2008). During flocculation, water is stored whereas deflocculation induces a release of water responsible for decreasing viscosity. The consistency index was reduced for the AS acclimated to the external MBR, which underlines sludge deflocculation. Therefore, the shear stress induced by the recirculation pump used in the external MBR resulted in deflocculation of the AS. On the other hand, operating conditions of the outside immersed MBR did not induce deflocculation.

3.3. SMP release

Microbial activity was responsible for SMP release primarily composed of protein and PS which were analyzed within the COD (Fig. 5). The fraction retained by the membrane was determined by analyzing SMP in the bioreactor and the permeate. During the AS acclimation to the external MBR, high SMP concentrations were released in the bioreactor at the beginning (Fig. 5, Adaptation), followed by a decrease until a plateau was reached (Fig. 6, Stabilization). Stabilization was obtained after the same adaptation period revealed by the accumulated sludge production (Fig. 3C) (20 days of acclimation). For the outside immersed MBR, SMP concentrations were kept low during the entire acclimation. At the end of both acclimations, SMP concentrations were higher in the external MBR (at approximately 5 $mg_{COD}\ g_{MLVSS}^{-1}$) than in the outside immersed process (at approximately 2 $mg_{COD}\ g_{MLVSS}^{-1}$). Thus, operating conditions of the outside immersed MBR induced less SMP release than the external MBR. However, for both MBRs, low and constant SMP concentrations in the permeate confirmed the main advantage of the MBR technology, which is the high and constant quality of the effluent.

3.4. Biological activity

Purification rates were compared between the two MBR processes for organic carbon, ammonium and nitrate. The COD removal was maintained around 100% for both acclimations on the basis of similar F/M ratios (Fig. 6A). The nitrogen treatment was also effective with ammonium concentrations below 3 $mg_{N-NH4}\ g_{MLVSS}^{-1}$ and nitrate below 4 $mg_{N-NO3}\ g_{MLVSS}^{-1}$. Therefore, the AS from both MBRs was adapted to the purification of carbon and nitrogen without any differences between the two configurations.

Exogenous respiration of heterotrophic and autotrophic micro-organisms was followed during the acclimations to characterize the microbial activity (Fig. 6B and C). The two dotted horizontal lines represent the activities measured with the CAS (average of activities measured from CAS sampled throughout one year). In the external MBR, heterotrophic activity increased during the first several days until a plateau was reached (Fig. 6B). However, the high respiration of micro-organisms resulted in decreasing dissolved oxygen concentrations in the bioreactor, which explained the activity decrease observed after 40 days of acclimation. At that point, the superficial gas velocity was increased and the heterotrophic respiration stabilized at the end of the acclimation at values above those measured within the CAS. Contrary to the heterotrophic biomass, autotrophic bacteria were subjected to an adaptation period with an activity decrease. Eventually, their respiration was below or close to the value characterizing the CAS. The autotrophic biomass is defined by few species with slow growth and high sensitivity to the medium conditions, which can explain the slower adaptation to the MBR technology than heterotrophic micro-organisms. Moreover, the high organic F/M ratio characteristic of WWTP conditions (0.2 $kg_{COD}\ kg_{MLVSS}^{-1}\ d^{-1}$) was not well-adapted to the autotrophic micro-organisms (inorganic carbon required).

In the outside immersed configuration, the increase of heterotrophic activity was slower than in the external MBR, with lower maximum values (Fig. 6C). After 20 days of acclimation, an activity decrease was also measured. Compared to the external process, the final respiration values were below those of the CAS. Therefore, development of the heterotrophic biomass was better in the external MBR. However, the milder conditions of the outside immersed MBR resulted in a lower initial decrease of the autotrophic activity.

The biological activities obtained at the end of the two acclimations were compared with the CAS respiration (Table 2). The exogenous respiration of heterotrophic micro-organisms acclimated in

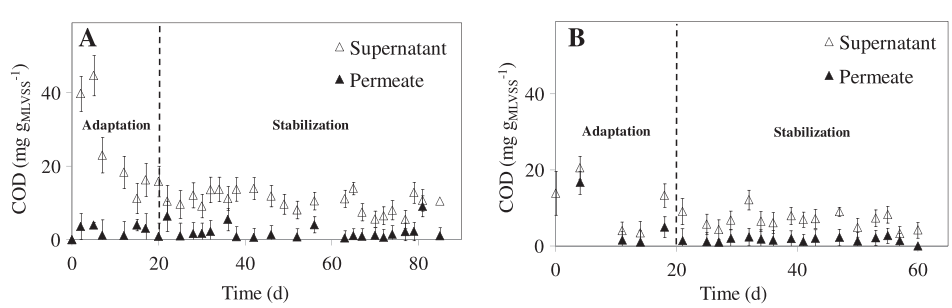

Fig. 5. SMP accumulated during the acclimations (A) to the external MBR and (B) to the outside immersed MBR. Concentrations of COD were measured in the bioreactor supernatant and in the permeate.

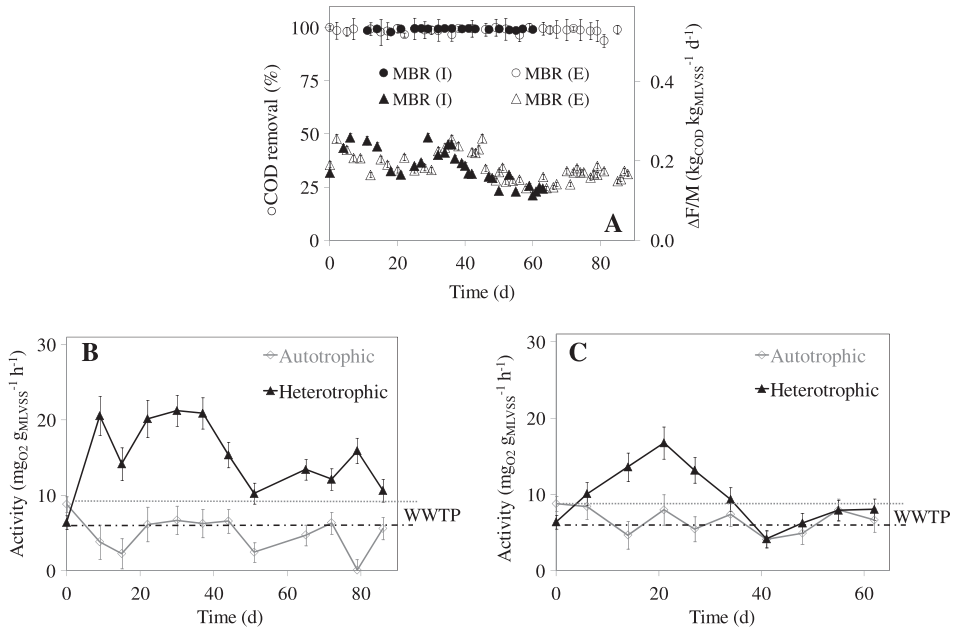

Fig. 6. Biological activity during the acclimations (A), COD removal (○) and F/M ratio (Δ) during both acclimations.B and C Exogenous respiration of heterotrophic and autotrophic micro-organisms for the AS acclimated to (B) the external and (C) the outside immersed configurations. (The two dotted lines represent the activities measured in AS taken from WWTP: grey for autotrophic and black for heterotrophic.)

Table 2
Respirometry activities of the CAS and the AS acclimated to the outside immersed (I) or to the external (E) MBR.

	$mgO_2\ g_{MLVSS}^{-1}\ h^{-1}$	WWTP	MBR (I)	MBR (E)
Hetero	Exogenous	6.4 ± 0.9	8.0 ± 1.4	10.6 ± 1.5
	Endogenous	2.7 ± 0.8	1.7 ± 0.2	1.4 ± 0.2
Auto	Exogenous	8.8 ± 1.0	6.6 ± 1.6	5.6 ± 1.5
	Endogenous	1.6 ± 0.4	2.6 ± 0.5	1.6 ± 0.5

the outside immersed MBR was similar to the CAS activity. On the other hand, the external MBR was adapted to the development of the heterotrophic biomass, as compared to the CAS system. Therefore, the acclimations performed for this study highlighted the beneficial impact of the external MBR process on heterotrophic micro-organisms. The endogenous respiration decreased when the heterotrophic activity increased because the micro-organisms were in a growth state. An increase in the endogenous respiration would indicate a protective state of the biomass.

At the beginning of the acclimation, the outside immersed MBR was better for autotrophic bacteria than the external process but in the end, no significant differences were noticed between the two acclimated AS and the CAS. The hypothesis proposed is that a longer acclimation with a high SRT may have revealed a better development of autotrophic biomass in the MBRs than in the CAS system.

4. Conclusion

The high shear stress induced by the recirculation pump in the external configuration resulted in decreasing viscosity due to AS deflocculation and increasing SMP release. With regard to the microbial activity, an easier autotrophic development was observed in the outside immersed MBR. However, the external MBR was shown to allow a better heterotrophic development. Therefore, the choice of the MBR configuration has to be made by considering the influent characteristics and the micro-organisms responsible for pollutant degradation.

Acknowledgements

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Appendix A

$$P_x = Q_p \cdot \mathrm{MIVSS_R} + V_R \cdot \Delta\mathrm{MLVSS_R}/\Delta t \quad (1)$$

$$P_{x\ \mathrm{accumulated}} = \sum_{i=1}^{n} P_{xi} \quad (2)$$

where, P_x: Daily production of sludge ($g\,d^{-1}$),Q_p: Flow of sludge wasted ($L\,d^{-1}$), $MLVSS_R$: MLVSS concentration in the bioreactor ($g\,L^{-1}$), V_R: Reactor volume (L), $\Delta MLVSS_R$: Variation of the MLVSS concentrations in the bioreactor ($g\,L^{-1}$), Δt: Time variation (d).

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