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Maud Villain, Benoît Marrot. Influence of sludge retention time at constant food to microorganisms ratio on membrane bioreactor performances under stable and unstable state conditions. *Bioresource Technology*, 2013, 128, pp.134-144. 10.1016/j.biortech.2012.10.108 . hal-00995702

HAL Id: hal-00995702

<https://hal.science/hal-00995702v1>

Submitted on 19 Dec 2024

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Influence of sludge retention time at constant food to microorganisms ratio on membrane bioreactor performances under stable and unstable state conditions

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Food to microorganisms ratio (F/M) and sludge retention time (SRT) are known to affect in different ways biomass growth, bioactivities and foulants characteristics. Thus the aim of this study was to dissociate the effects of SRT from those of F/M ratio on lab-scale membrane bioreactors performances during stable and unstable state. Two acclimations were stabilized at a SRT of either 20 or 50 d with a constant F/M ratio of $0.2 \text{ kg}_{\text{COD}} \text{ kg}_{\text{MLVSS}}^{-1} \text{ d}^{-1}$. During stable state, a higher N-NH_4^+ removal rate (78%) was obtained at SRT of 50 d as an easier autotroph development was observed. Soluble microbial products (SMPs) release was double at 50 d with a majority of polysaccharides (49% of total SMP). The unstable conditions consisted in F/M ratio changes and operation without air and nutrient. Autotrophs were highly affected by the tested disturbances and SMP retention on membrane surface exhibited consistent changes during the performed stresses.

1. Introduction

The growth rate of membrane bioreactor (MBR) is higher (almost 10.9% per annum) compared to other advanced wastewater treatment technologies and more than any other membrane technologies (Drews, 2010). The MBR system has drawn a lot of attention because of the numerous advantages over the conventional wastewater treatment plant (WWTP), such as a smaller footprint, process flexibility and complete retention of the biomass. As sludge retention time (SRT) affects in the same time biomass characteristics (e.g. sludge structure and biomass activity) (Han et al., 2005), membrane biofouling (Grelier et al., 2006) and outlet effluent quality, SRT is considered as one of the target operating factors to control (Meng et al., 2009). Thus, in the past ten years the rate of publications on the SRT in MBR and its influence on bioactivities of microorganisms (Han et al., 2005; Huang et al., 2001), sludge

structure (Laera et al., 2007), dissolved organic matter as well as ammonium removal efficiencies (Hocaoglu et al., 2011) and membrane fouling (Van den Broeck et al., 2012) has increased continuously.

In aerobic bioprocesses there are heterotrophic and autotrophic bacteria which prevail. Because the oxidation of inorganic material does not yield as much energy as the oxidation of organic carbon sources, autotrophs have a much slower growth rate than heterotrophs. Thus, it is preferable to work at a high sludge rate to achieve a high ammonium removal rate.

As far as membrane biofouling is concerned, the extracellular polymeric substances (EPS) in either bound or soluble form are currently considered as the predominant cause of fouling in MBRs (Le-Clech et al., 2006; Meng et al., 2009). Bound EPS consist of proteins, polysaccharides and humic substances, which are the main components, and lipids, nucleic and uronic acids in fewer proportions (Wingender et al., 1999). They are located at the cell surface or outside. Soluble microbial products (SMPs) can be defined as the pool of organic compounds that are released into the solution from

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substrate metabolism (usually with biomass growth) and biomass decay (Barker and Stuckey, 1999). Laspidou and Rittmann (2002) proposed a unified theory to define the complex interrelations between bound EPS and SMP. They subdivided SMP into two categories: substrate-associated products (UAP), which are produced directly during substrate metabolism and biomass-associated products (BAP) which are formed from biomass, presumably as part of decay. Cells use electrons from the electron-donor substrate to build active biomass and they produce bound EPS and UAP in the process. Part of the bound EPS can be hydrolyzed to BAP. Some SMP can be used by active biomass as recycled electron donors; and some can be adsorbed by the biomass flocs and then become bound EPS. No consensus has been found about the influence of SRT on membrane biofouling. Many studies found that bound EPS content decreased and consequently membrane fouling whereas SRT increased from 10 to 60 d (Ahmed et al., 2007; Massé et al., 2006). However, a too long SRT (above 60 d) was also found and resulted in excessive membrane fouling. Lee et al. (2003) noticed that the overall fouling resistance increased when SRT increased from 20 to 40 and 60 d. These reported results indicate that in order to control bound EPS concentration and thus membrane fouling with high removal efficiencies, the optimum SRT of MBRs should be controlled between 20 and 50 d (Meng et al., 2009). Several studies however reported that bound EPS had little correlation with membrane fouling. Instead, SMP have a great impact on the membrane fouling as due to the membrane rejection, SMP are more easily accumulated in MBRs (Drews et al., 2008; Jang et al., 2007). In most of the studies SMP concentration decreased together with increasing SRT (Lee et al., 2003; Liang et al., 2007). Therefore, SRT does not need to be too long.

However in a large part of these researches, because biomass requires at least 2 or 3 months to be stabilized in MBR process, only one acclimation is realized (Huang et al., 2001; Massé et al., 2006; Hocaoglu et al., 2011). SRT is progressively increased with a reduction of sludge extraction. Thus a biomass concentration augmentation is observed. Accordingly as feedwater characteristics are let constant, food to microorganisms (F/M) ratio progressively decreases with increasing SRT and microbial bioactivities reduce still maintaining high removal rates of COD and ammonium.

Thus, as F/M ratio is known to directly affect biomass growth, biomass activity (Johir et al., 2011) and EPS production (Laspidou and Rittmann, 2002), it is clearly useful to perform a study at different SRTs with a constant F/M ratio to dissociate both effects of SRT and F/M ratio on foulants characteristics, bioactivities and removal rates.

Furthermore, in wastewater treatment processes it is not rare to operate in unstable conditions due to diurnal, seasonal and irregular variations in substrate strength and flow rate. The unstable operational condition often causes serious deterioration of sludge flocs, microbial activities and treatment performance (Sponza, 2003). The EPS abundance of the sludge can be sensitive to the environmental variations, such as changes in organic loading rate, aerobic or anaerobic conditions (Sheng et al., 2010). In case of stress conditions, EPS release was reported to directly affect the quality of outlet effluent (Jang et al., 2007). However, the links between autotrophic and heterotrophic activities with EPS production and removal rates under unstable conditions at different SRTs have not been well characterized. The effects of EPS released on membrane rejection at different SRTs during unstable MBR operation also need to be investigated.

The present work was first carried out to dissociate the effects of SRT on biomass activity (e.g. autotrophs and heterotrophs), removal rates, SMP release and membrane biofouling from the effects of F/M ratio on the same parameters. Therefore, two entire acclimations in MBR were realized and SRT was stabilized directly

at either 20 or 50 d with a constant F/M ratio. The biological activity was followed from respirometry tests focusing on the distinction between autotrophs and heterotrophs. The objectives of the second part of this study were to determine the effects of three different operational stresses (F/M ratio increase, F/M ratio decrease, 24 h without air and nutrient) on the evolution of biomass activities, treatment performances, SMP release and their membrane rejection for both biomass acclimated at SRT of 20 and 50 d.

2. Methods

2.1. The MBR setup

The external MBR (Polymem, France) was composed of a 18 L bioreactor equipped with a cooling coil to maintain biomass at approximately 25 °C (Fig. 1). The membrane system used was a tubular ceramic membrane (ultrafiltration, Novasep-Orelis, France) made with ZrO₂-TiO₂ was characterized by a 150 kDa cut off and a 0.02 m² filtration area. Crossflow filtration was operated with a centrifugal pump which 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 110 L h⁻¹ m⁻² bar⁻¹.

During both acclimations in the external MBR, the mixed liquor volatile suspended solid (MLVSS) was stabilized around 8 g L⁻¹ and the crossflow velocity was held constant at 4 m s⁻¹, based on the results of a previous study performed in the same type of bioreactor to limit membrane biofouling (Clouzot et al., 2011). The constant flow rate operating mode was used. The permeate flux was 0.75 L h⁻¹ which fixed a hydraulic retention time (HRT) of 24 h. The increase in transmembrane pressure (TMP) was monitored with manometer sets at the inlet and outlet of the membrane module.

2.2. Biomass acclimations to the MBR process

Both acclimations were realized with AS sampled from the same municipal wastewater treatment plant (Aix en Provence, France, 175,000 inhabitant equivalent, 35,000 m³ d⁻¹, organic load 0.12 kg_{BOD5} kg_{MLVSS}⁻¹ d⁻¹). The initial Mixed Liquor Suspended Solids (MLSS) concentration ranged from 3 to 4 g L⁻¹ and the amount of MLVSS varied from 75% to 80%. Samples were taken from the recirculating loop between the aerated tanks and the secondary clarifiers and then transported to the laboratory with no aeration (30 min). The AS was concentrated around 8 g_{MLSS} L⁻¹ in the MBR. A balanced synthetic sewage influent (C/N/P ratio = 100/10/2) was prepared with mass ratios of 2.1 C₆H₁₂O₆, 1.0 (NH₄)₂SO₄, 0.2 KH₂PO₄, 0.4 NaHCO₃, 0.1 MgSO₄ and 0.02 CaCl₂. The food-to-mass ratio (F/M) was maintained around 0.2 kg_{COD} kg_{MLVSS}⁻¹ d⁻¹ with adjustment of the nutrient concentrations in the feed to the MLVSS contents. Oxygenation cycles of 2 h with air and 1 h without air were programmed to allow nitrification and denitrification reactions. The pH was maintained constant at 7 by an automatic regulator with NaHCO₃ solution (60 g L⁻¹).

The SRT was fixed at 20 days in the first acclimation (Run I) and at 50 days in the second one (Run II) due to a control of the amount of waste sludge. The amount of waste sludge is inversely proportional to the desired sludge age (Eq. (1)):

$$V_{\text{waste}} = V_{\text{R}}/\text{SRT} \quad (1)$$

With V_{waste} : the volume of waste sludge per waste event (L), V_{R} : the total volume of MBR (L), SRT: the desired sludge retention time (d).

AS was wasted every day from the drain of the bioreactor. In order to reduce the effect of discontinuous sludge wasting on sludge

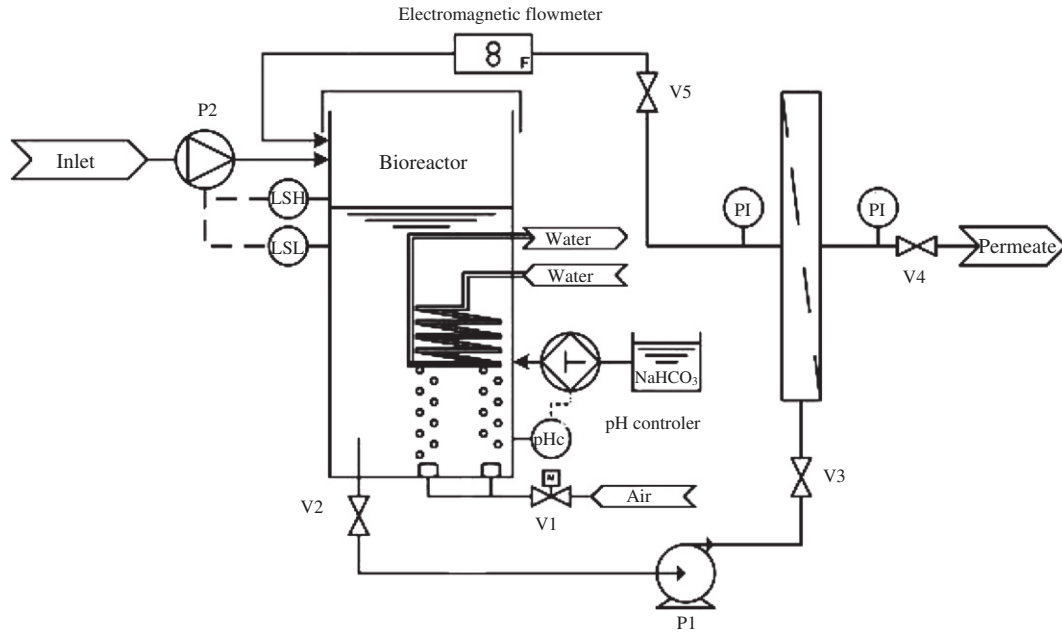


Fig. 1. Experimental set up of membrane bioreactor. P: pump, V: valve, LSH: level security high, LSL: level security low, PI: pressure indicator, pHc: pH controller.

analysis, all sludge samples were collected just before sludge wasting.

The daily production of sludge (P_x) was calculated with the following mass balance (Eq. (2))

$$P_x = Q_p \text{MLVSS}_R + V_R \Delta \text{MLVSS}_R / \Delta t \quad (2)$$

With P_x : daily production of sludge ($\text{g}_{\text{MLVSS}} \text{d}^{-1}$), Q_p : flow of sludge wasted (L d^{-1}), MLVSS_R : MLVSS concentration in the bioreactor ($\text{g}_{\text{MLVSS}} \text{L}^{-1}$), V_R : the total volume of MBR (L), ΔMLVSS_R : variation of the MLVSS concentration in the bioreactor ($\text{g}_{\text{MLVSS}} \text{L}^{-1}$), Δt : time variation (d).

Process was considered as stable as soon as the accumulated daily production of sludge (P_x accumulated) was linear (Delgado, 2009) (Eq. (3))

$$P_x \text{ accumulated} = \sum_{i=1}^n P_{xi} \quad (3)$$

2.3. Stress conditions

Three different stresses typically observed either on effluent discharge in WWTP (low F/M ratio and high F/M ratio) or either on power supply (oxygen cut off with nutrient depletion) were performed on both MBRs stabilized at 20 and 50 days respectively. They were provoked in the same order for both MBRs with a period of 10 days between each stress which was evaluated as the necessary period to get a stable process (e.g. sludge production, removal rates of COD and ammonium) back. The first stress was a reduction of the F/M ratio from $0.2 \text{ kg}_{\text{COD}} \text{ kg}_{\text{MLVSS}}^{-1} \text{ d}^{-1}$ to $0.1 \text{ kg}_{\text{COD}} \text{ kg}_{\text{MLVSS}}^{-1} \text{ d}^{-1}$ during one day. The second one was an increase in the F/M ratio from $0.2 \text{ kg}_{\text{COD}} \text{ kg}_{\text{MLVSS}}^{-1} \text{ d}^{-1}$ to $0.8 \text{ kg}_{\text{COD}} \text{ kg}_{\text{MLVSS}}^{-1} \text{ d}^{-1}$ during one day. The last one was a 24 h oxygen cut off with no nutrient supply. For both F/M ratio changes, mass ratios of 2.1 $\text{C}_6\text{H}_{12}\text{O}_6$, 1.0 $(\text{NH}_4)_2\text{SO}_4$, 0.2 KH_2PO_4 , 0.4 NaHCO_3 , 0.1 MgSO_4 and 0.02 CaCl_2 were maintained. To evaluate the effect of each stress on both MBR performances, removal rates of COD and ammonium, respirations and activities of heterotrophic and autotrophic microorganisms were quantified. SMP contents (proteins, polysaccharides and humic substances) were

measured in bioreactor supernatant and in the permeate to assess their contribution on membrane fouling. For all these measurements, sampling was done for both MBRs at the same time after the stress beginning e.g. for F/M ratio changes, sampling was performed 24 h after the stress beginning and for stress III 2 h after air return and substrate at normal F/M ratio injection.

2.4. Analytical methods

The AS was regularly sampled in the bioreactor (30 mL), and samples were centrifuged for 15 min at 16,000g to separate the dissolved matrix from the suspended solids. COD and ammonium were analyzed in the inlet and the permeate to determine their removal rates. Nitrates were quantified in the bioreactor supernatant. As for ammonium, nitrates were evaluated by spectrophotometry (Spectro Aquamate, Thermo spectronic, UK) with reagent kits obtained from Merck (Germany). Reagent kits purchased from Aqua Lytic (Germany) were used for the COD. Like the major components of SMP contents, polysaccharides (Dubois et al., 1956), proteins and humic substances (Frolund et al., 1995) were quantified with colorimetric methods in the bioreactor supernatant and in the permeate to evaluate the fraction retained by the membrane; glucose (Sigma-Aldrich, 99.5%) was used for polysaccharides, bovine serum albumin (Sigma-Aldrich, 98%) was used as a standard for proteins and humic acids (Sigma-Aldrich) for humic substances contents.

2.5. Respirometry tests

The biological activity was followed from respirometry tests used to measure autotrophic and heterotrophic activities thanks to a differentiation of endogenous and exogenous activities. The endogenous activity is reached when no external substrate (e.g. an organic carbon source for heterotrophic bacteria and an ammonium source for autotrophic biomass) is available; bacteria are forced to biodegrade their intracellular carbon (bound EPS) as substrate to provide necessary energy for cell maintenance and their respiration is minimal. The exogenous activity is obtained after substrate addition; bacteria use the substrate available to supply their energy demand, and the resulting respiration is at its highest

value. A 1 L reactor was filled with AS from MBR and maintained aerated during 4 h to guarantee endogenous respiration of the biomass. After this period 50 mL of AS was sampled every 2 min and injected into another reactor without oxygenation to measure the specific oxygen uptake rate (SOUR) with continuous dissolved oxygen probe (HQ 40d, Hach LDO, Germany). Then specific nutrients or inhibitor were added to the aerated reactor according to four different steps. Endogenous respirations of autotrophic and heterotrophic microorganisms were first measured over a period of 1 h. Secondly, ammonium was added ($0.02 \cdot g_{N-NH_4^+} \cdot g_{MLVSS}^{-1}$) to measure the maximum activity of autotrophic microorganisms (ammonium removal was confirmed to be correlated with nitrate production). Thirdly, autotrophic microorganisms were inhibited with allylthiourea (inhibitor of the nitrifying ammonium monooxygenase enzyme) to isolate heterotrophic endogenous respiration. A concentration of $0.1 g_{allylthiourea} g_{MLVSS}^{-1}$ was shown to be sufficient to inhibit the respirometry activity of autotrophic microorganisms, and nitrates were not produced after ammonium addition ($0.02 \cdot g_{N-NH_4^+} \cdot g_{MLVSS}^{-1}$), which validated the autotrophic inhibition. Lastly, maximum activity of heterotrophic microorganisms was measured after glucose addition ($0.3 g_{COD} g_{MLVSS}^{-1}$).

3. Results and discussion

3.1. Biomass development and characteristics after process stabilization

The biomass development was divided into four steps for both acclimations (Fig. 2A). The F/M ratio was initially fixed at $0.2 kg_{COD} kg_{MLVSS}^{-1} d^{-1}$ to approximate 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 microorganisms to the synthetic wastewater and the MBR process. Then, the biomass growth defined the second step (II), where the nutrient concentration had to be increased. The applied experimental conditions were strictly the same in both acclimations. Nevertheless, the experiments exhibit a slightly different transition behavior in phase II with a faster growth during run I. These two first steps were imposed by the microbial system and it is very difficult to determine what causes the transient period to be different from one another since the physical and biochemical interactions in an AS population are extremely complex (Van den Broeck et al., 2009). Steps III and IV were controlled to stabilize the bioprocess. During the third step, nutrient concentration was

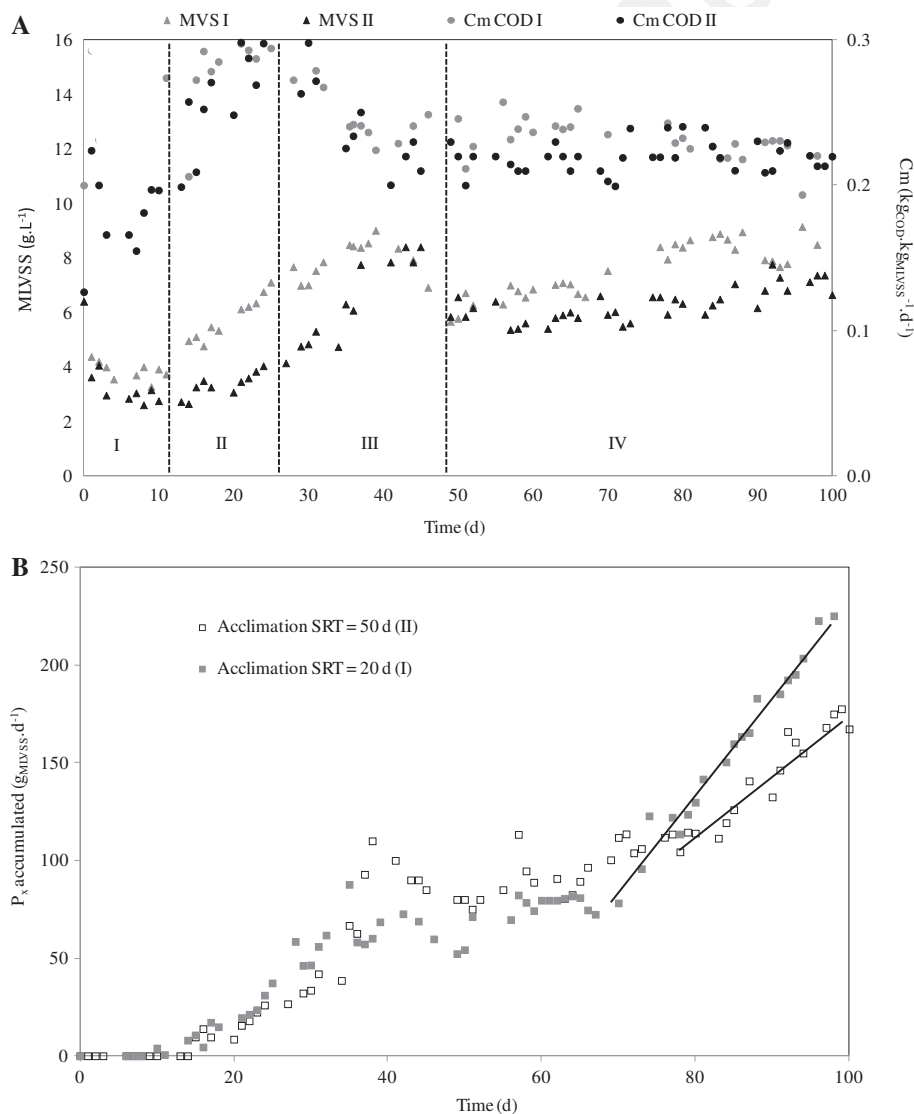


Fig. 2. Biomass development during the acclimations. (A) MLVSS and F/M ratio in the external MBR with stabilization at SRT of 20 d (I) and 50 d (II). (B) accumulated sludge production in both MBR experiments.

voluntarily reduced until the MLVSS concentrations were maintained at a constant value (step IV). Process stability was confirmed for both MBRs at the end of the transient period whereas the accumulated daily production of sludge (P_x accumulated) was linear (Fig. 2B). Sludge production rate of 0.42 and 0.12 $\text{kg}_{\text{MLVSS}} \text{m}^{-3} \text{d}^{-1}$ were obtained for SRT of 20 and 50 d respectively.

At the end of both acclimations, a slightly higher MLVSS concentration ($8.2 \pm 0.7 \text{ g}_{\text{MLVSS}} \text{L}^{-1}$) was reached for run I stabilized at a SRT of 20 d compared to run II at 50 d (MLVSS concentration = $7.0 \pm 0.7 \text{ g}_{\text{MLVSS}} \text{L}^{-1}$). The ratio MLVSS/MLSS was constant at 0.8 ± 0.02 during both experiments indicating that no mineralization occurred over time. This difference in MLVSS concentration was explained by the F/M ratio slightly higher during run I ($0.23 \pm 0.02 \text{ kg}_{\text{COD}} \text{ kg}_{\text{MLVSS}}^{-1} \text{d}^{-1}$) compared to run II ($0.22 \pm 0.01 \text{ kg}_{\text{COD}} \text{ kg}_{\text{MLVSS}}^{-1} \text{d}^{-1}$) and also by the easier growth of biomass observed during transient period of run I (step II, Fig. 2A).

Exogenous and endogenous activities of heterotrophic and autotrophic microorganisms were assessed during biomass development and after stabilization to characterize the microbial activity (Fig. 3). The initial points showed the activities measured with the CAS sampled to perform both acclimations in the MBR. In both runs, the heterotrophic exogenous activities never stopped increasing during the acclimations and reached a plateau after biomass stabilization with mean value of $43 \pm 3 \text{ mg}_{\text{O}_2} \text{ g}_{\text{MLVSS}}^{-1} \text{h}^{-1}$ for a SRT of 20 d and $49 \pm 5 \text{ mg}_{\text{O}_2} \text{ g}_{\text{MLVSS}}^{-1} \text{h}^{-1}$ at 50 d (Fig. 3A). The obtained values of heterotrophic exogenous activities were relatively high and only one study was found with value in the same range at SRT of 20 d (Liang et al., 2010) but no studies were found with such high data at a SRT of 50 d. Indeed, in most of the studies the main reason is that the F/M ratio decreases as soon as the SRT increases. Thus, heterotrophic activity shows a reduction not because of the increasing SRT but due to the decrease in F/M ratio. These evolutions of heterotrophic exogenous activity for both SRT with COD removal always maintained higher than 95% indicated that this type of microbial community well-adapted itself to the MBR process. The higher heterotrophic activity at 50 d showed that this microbial population biodegraded the COD pollution faster than at 20 d. This conclusion was confirmed (Fig. 3C) because for substrate addition higher than $0.02 \text{ g}_{\text{substrate}} \text{ g}_{\text{MLVSS}}^{-1}$, stabilized biomass at SRT of 50 d always biodegraded substrate faster (SOUR was higher) than biomass acclimated at 20 d.

The endogenous activity of heterotrophic microorganisms was constant around $2 \text{ mg}_{\text{O}_2} \text{ g}_{\text{MLVSS}}^{-1} \text{h}^{-1}$ for both runs during the first 40 days and after stabilization (Fig. 3B). The same increase was observed from day 40 to 60 which corresponded to the end of step III and beginning of step IV when F/M ratio was reduced to stabilize the bioprocess. This F/M ratio reduction has created a stress on heterotrophic biomass and thus an increase in their endogenous activity. Contrary to heterotrophic bacteria, autotrophic biomass was subjected to an adaptation period with an exogenous activity decrease during the first 5 days revealing their high sensitivity to the hydrodynamic conditions of the MBR. After this decrease, in run I autotrophic exogenous activity was maintained at a really low value around $0.2 \pm 0.1 \text{ mg}_{\text{O}_2} \text{ g}_{\text{MLVSS}}^{-1} \text{h}^{-1}$ whereas a slight increase was observed during run II and a stabilization at a mean value of $0.9 \pm 0.2 \text{ mg}_{\text{O}_2} \text{ g}_{\text{MLVSS}}^{-1} \text{h}^{-1}$. With N-NH_4^+ removal rate of $78\% \pm 4$ at a SRT of 50 d compared to $71\% \pm 3$ at 20 d, the exogenous autotrophic activities well indicated that a higher SRT was adapted to the autotrophic biomass which is defined by few species with slow growth. As soon as the waste sludge events have started to stabilize the process at either 20 or 50 d, an augmentation of autotrophic exogenous activity was observed and correlated with the increase in heterotrophic exogenous activity (part I, Fig. 4). As heterotrophic bacteria increased their exogenous activity, they biodegraded faster the substrate (Fig. 3C) releasing more CO_2 available as inorganic carbon substrate for autotrophic biomass

development. Thus, there was a synergy between both types of microorganisms (e.g. autotrophic and heterotrophic). After the process stabilization (part II, Fig. 4) the autotrophic exogenous activity was constant and independent of the augmentation of heterotrophic exogenous activity.

Autotrophic endogenous activities of both processes showed a differentiation after 40 days of operation (Fig. 3B). As in run I, autotrophic endogenous activity remained constant at $0.7 \pm 0.1 \text{ mg}_{\text{O}_2} \text{ g}_{\text{MLVSS}}^{-1} \text{h}^{-1}$, a stabilization around $2.1 \pm 0.5 \text{ mg}_{\text{O}_2} \text{ g}_{\text{MLVSS}}^{-1} \text{h}^{-1}$ was obtained in run II. As a better development of autotrophic microbial communities occurred at a SRT of 50 d, the convincing explanation seems to be that the percentage of autotrophic biomass per gram of MLVSS was higher. Thus the observed endogenous activity tripled at SRT of 50 compared to 20 d.

3.2. Influence of stress situations on biomass characteristics

Both biomasses acclimated at SRT of 20 and 50 d were subjected to the same three different stresses and the influence of each stress on MLVSS concentration, COD, N-NH_4^+ removal rates and exogenous and endogenous activities of heterotrophic and autotrophic biomasses were assessed and were reported in Table 1.

Stress I was a decrease of the F/M ratio from $0.2 \text{ kg}_{\text{COD}} \text{ kg}_{\text{MLVSS}}^{-1} \text{d}^{-1}$ to $0.1 \text{ kg}_{\text{COD}} \text{ kg}_{\text{MLVSS}}^{-1} \text{d}^{-1}$ during one day. Due to this unexpected shortage of food compared to the concentration of microorganisms present in the system, in both experiments MLVSS concentrations decreased. Autotrophic and heterotrophic bacteria increased their endogenous activities and simultaneously decreased their exogenous activities. This suggests that a portion of energy resources are diverted from growth and used in stress management and protection of the microorganisms.

While COD removal rate was maintained at it higher level in both experiments, a decrease of N-NH_4^+ removal rate of at least 25% was observed. Heterotrophic bacteria successfully adapted themselves to this starvation condition and used all the substrate available in the medium to maintain a high COD removal rate in both experiments. On the contrary autotrophic microorganisms seem to be stressed by this sudden lack of substrate increasing much more their endogenous activities than the heterotrophic bacteria. This F/M ratio decrease seems to have generated more oxygen needs for stress management for the autotrophic population of AS at both SRT.

The stress II was a rapid augmentation of F/M ratio from $0.2 \text{ kg}_{\text{COD}} \text{ kg}_{\text{MLVSS}}^{-1} \text{d}^{-1}$ to $0.8 \text{ kg}_{\text{COD}} \text{ kg}_{\text{MLVSS}}^{-1} \text{d}^{-1}$ during 24 h. This increase in four times of the initial F/M ratio has brought sufficient food to allow microorganisms to increase their anabolism. After one day, MLVSS measured at a SRT of 20 d have shown a growth of 20% and 37% at 50 d.

Heterotrophic bacteria were able to increase their exogenous activities to confront this excess of substrate and always achieved COD removal rate up to 90%. In both runs, heterotrophic endogenous activities were double compared with the one observed under stable conditions. This seems to implicate that with this excess of substrate bacteria have also increased their catabolism and synthesized more storage products used as substrate in endogenous conditions.

The behavior of autotrophic biomass was completely different compared to heterotrophic bacteria. As a result of F/M ratio increase in both experiments N-NH_4^+ removal rate decreased of 48%. Other studies also reported a lower removal of inorganic nutrients with higher F/M ratios (Johir et al., 2011). They explained this variation by a lower $\text{N-NO}_3^-/\text{N-NH}_4^+$ ratio at high F/M ratio. A higher value of $\text{N-NO}_3^-/\text{N-NH}_4^+$ ratio indicates higher nitrification (e.g. conversion of N-NH_4^+ into N-NO_3^- is high). This could be due to the effect of competition between heterotrophs and autotrophs because more organic carbon was present in the bioreactor under

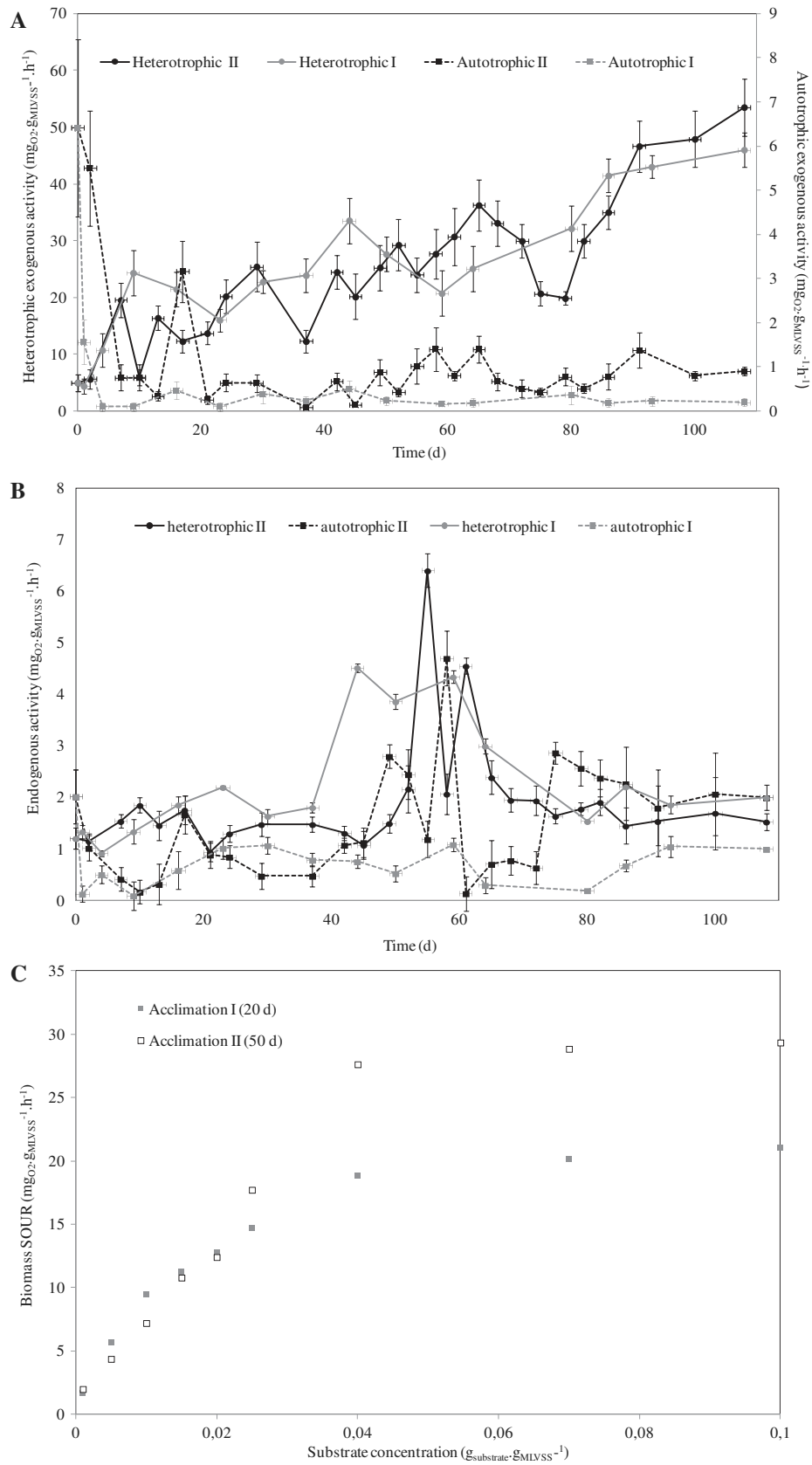


Fig. 3. Effect of SRT on biological exogenous (A) and endogenous (B) activities of heterotrophic and autotrophic bacteria during the acclimations and after stabilization (I = SRT of 20 and II = SRT of 50 d). Effect of SRT on biomass SOUR for different substrate concentrations (C).

high F/M ratio. Another explanation came from biological activity of autotrophs. Their exogenous activities significantly decreased

and the endogenous activities increased which could signify that autotrophic microorganisms were in a protective state managing

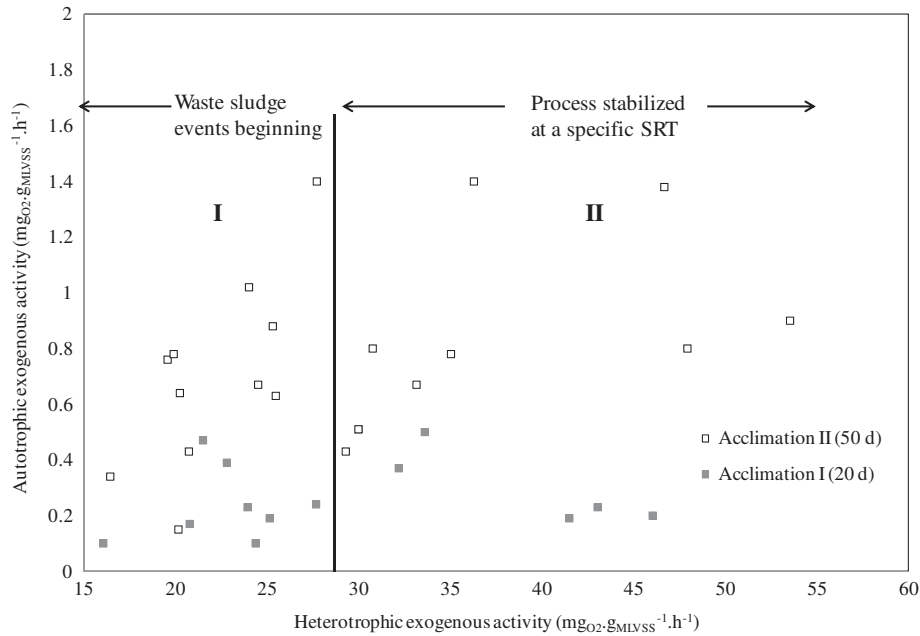


Fig. 4. Influence of heterotrophic exogenous activity on autotrophic exogenous activity before and after stabilization at a specific SRT.

Table 1
Evolutions of MLVSS concentrations, biological activities and removal rates with SRT and stress applied.

SRT (d)	Normal conditions (process stable)		Stress I (F/M ratio decrease)		Stress II (F/M ratio increase)		Stress III (24 h without air and nutrient)		
	20	50	20	50	20	50	20	50	
<i>Removal rate (%)</i>									
COD	99 ± 1	96 ± 3	Stable	-3% ± 1	-7% ± 4	+3% ± 1	-1% ± 1	+1% ± 1	
N-NH ₄ ⁺	71 ± 3	78 ± 4	-25% ± 3	-27% ± 4	-48% ± 1	-48% ± 2	-8% ± 3	-10% ± 2	
<i>Respirometry activities (mgO₂.gMLVSS⁻¹.h⁻¹)</i>									
Heterotrophic	Exogenous	43.5 ± 3.2	49.3 ± 3.6	-34% ± 2	-43% ± 5	+45% ± 5	+16% ± 3	-34% ± 3	-35% ± 2
	Endogenous	2.0 ± 0.1	1.7 ± 0.4	+50% ± 3	+24% ± 2	+100% ± 9	+112% ± 9	+75% ± 8	+153% ± 9
Autotrophic	Exogenous	0.2 ± 0.1	0.9 ± 0.2	-33% ± 2	-50% ± 9	-64% ± 7	-86% ± 7	-33% ± 4	-14% ± 1
	Endogenous	0.7 ± 0.1	2.1 ± 0.5	+86% ± 4	+123% ± 6	+200% ± 10	+50% ± 2	-57% ± 4	-19% ± 2
MLVSS (g L ⁻¹)	8.2 ± 0.7	7.0 ± 0.7	-6% ± 2	-11% ± 2	+20% ± 2	+37% ± 2	-13% ± 2	-15% ± 2	

the created stress. Inhibition of autotrophic populations by high concentration of N-NH₄⁺ was reported in the literature (Gagnaire, 2010). Like just after the F/M ratio increase, concentrations up to 400 mg_{N-NH₄⁺} L⁻¹ were measured in the bioreactor, this explanation is also plausible.

Thanks to this results analysis, it could be assumed that most of the biomass created during this stress was of heterotrophic type.

The latter stress created was a 24 h oxygen cut off with no nutrient supply during that time. After this period, biomass was subjected to 2 h of normal aeration and F/M ratio conditions before COD and N-NH₄⁺ removal rates, respirometry tests and MLVSS concentrations were measured.

A drop of MLVSS concentration comparable for the both SRT was observed. In this situation storage products use for cellular maintenance was favored.

As heterotrophic bacteria are facultative aerobes, this situation was not stressful for them. They have just increased their endogenous activities to consume storage products and reduced their exogenous activities because there was not enough substrate to maintain its high level. The same COD removal rates were observed despite the exogenous activity decrease.

For autotrophic biomass, they are obligate aerobes which means that oxygen is required for all of their metabolism needs (e.g. substrate consumption and maintenance actions). Because of this absence of air during 24 h, their global oxygen consumption (endogenous + exogenous activities) slowed down. The N-NH₄⁺ removal rate was not really disturbed compared to the two other stresses performed. The small diminution observed could be attributed to the reduction of the exogenous consumption rate of oxygen by autotrophic bacteria decreasing their rate of used ammonium simultaneously.

Unlike stresses I and II, endogenous activities of autotrophic bacteria decreased due to stress III. The feast/famine process developed in aerobic/anoxic bioreactor may offer a possible explanation for this obtained difference (Lee and Oleszkiewicz, 2003). Autotrophic bacteria in the alternating aerobic/anoxic system generally cope with the fact that energy cannot be derived from the biodegradation of exogenous substrate as oxygen is missing. Thus they may have an improved tolerance to this type of starvation conditions. In other words, autotrophic bacteria got used to this situation which had not created any additional structural damages, thus endogenous activity did not increased.

3.3. SMP release and membrane biofouling

Double total concentrations of SMP were found in the bioreactor at a SRT of 50 d compared to a SRT of 20 d during stable conditions (Fig. 5). The specificity of this study was to perform two different acclimations maintaining SRT at either 20 or 50 d with a constant F/M ratio around $0.2 \text{ kg}_{\text{COD}} \text{ kg}_{\text{MLVSS}}^{-1} \text{ d}^{-1}$. This result of the evolution of total SMP content with SRT is in contradiction with most of the literature found on this subject. Usually studies carried out on the influence of SRT with SMP production in a MBR system worked only with one stabilized biomass and changed the sludge age progressively with a diminution of purged biomass volume (Huang et al., 2001). Long SRT conditions implying less extracted sludge are conducive to an increase in sludge concentration. As substrate quantities remained unchanged all along this type of experiment, fewer nutrients are available for the biomass which is able to consume part of the SMP content to counter the substrate scarcity. Thus, as in this study SRT and F/M ratio are completely dissociated, the advantage is to be able to observe direct influence of SRT on SMP release. Then, an increase in SMP quantities with SRT is obtained.

Augmentations of the three main component quantities were noticed at 50 d with a relevant increase in polysaccharide concentration from $2.2 \pm 0.4 \text{ mg g}_{\text{MLVSS}}^{-1}$ to $7.8 \pm 1.4 \text{ mg g}_{\text{MLVSS}}^{-1}$.

The major component of SMP present in the MBR at a SRT of 20 d was proteins which represented 59% of the total SMP content. Polysaccharides were the main component at 50 d with 45% of the total SMP concentration.

The retention of these SMP by the membrane was slightly higher at SRT of 50 d (82%) compared to 76% at 20 d. The molecules with molecular weights lower than the membrane cut off of 150 kDa and able to go through the membrane were mainly composed of proteins at 20 d and humic substances at 50 d.

As regards to the stress periods, the higher loss of SMP retention by the membrane was obtained after the 24 h without air and nutrient (stress III). For both SRTs, almost the part of protein content analyzed in the bioreactor supernatant was also found in the permeate and more than 60% of polysaccharides and humic substances were detected in the bioreactor outlet at 50 d whereas these components were absent at 20 d. During this stress at both SRTs, similar drop of MLVSS concentration was observed. Due to the high shear rate in this type of bioreactor cellular content of

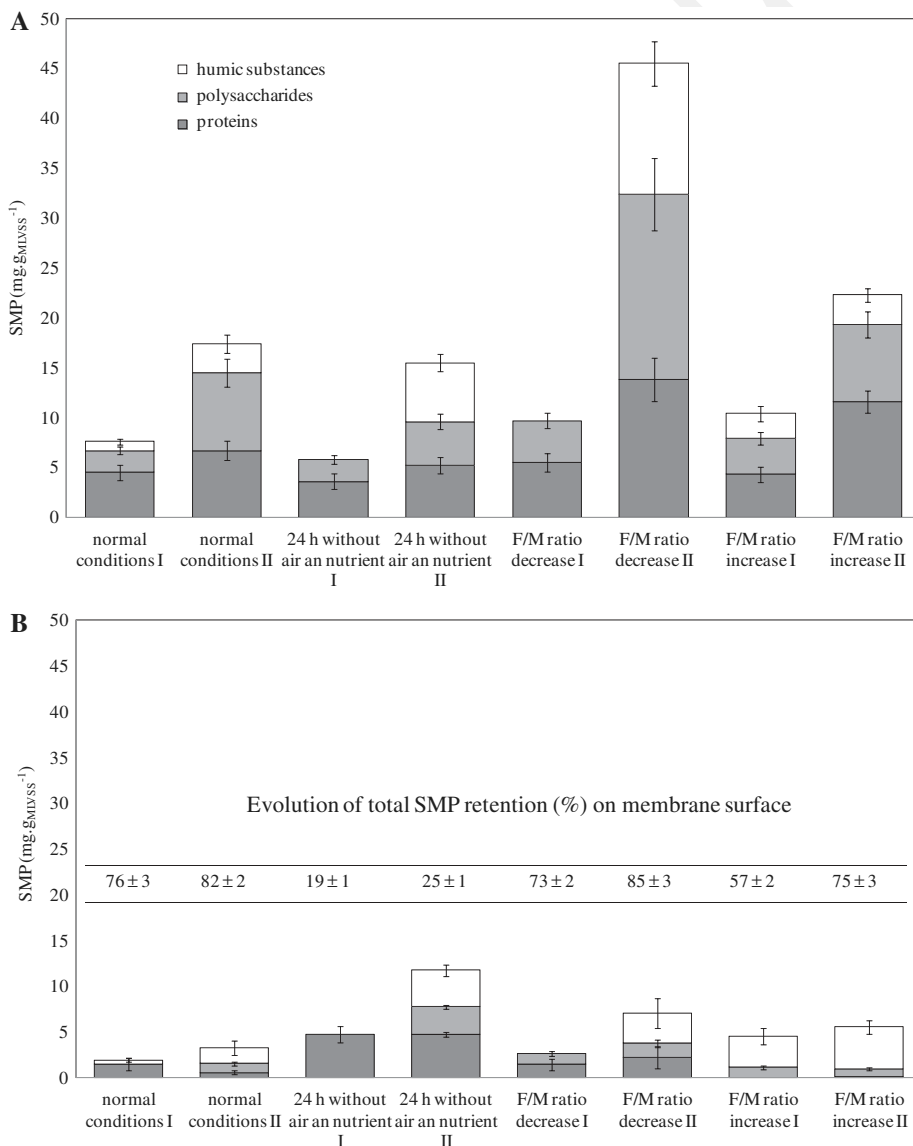


Fig. 5. Effect of SRT on the SMP quantities (e.g. proteins, polysaccharides, humic substances) extracted from the MBR sludge (A) and in the permeate (B) during stable conditions, after 24 h without air and nutrient, after a F/M ratio decrease and a F/M ratio increase (I = SRT of 20 and II = SRT of 50 d).

dead cell and membrane fragments were released in the liquid medium. With SMP already in the liquid as well as this new content mainly composed of proteins and polysaccharides, bacteria seem to have used this substrate as carbon and energy sources in case of external substrate depletion to survive (Zhang and Bishop, 2003). Nielsen et al. (1997) have also reported that under the first three days of anaerobic conditions of a 12 days period, polysaccharide and protein contents decreased. It is reported that activated sludge flocs tend to disintegrate because of the suppression of EPS production as well as the hydrolysis of EPS. In this study, an increase in endogenous activity of heterotrophic bacteria was noticed after this stress (Table 1). Thus, EPS production was probably increased and hydrolyzed by the bacteria during that stress (e.g. stress proteins production (Bott and Love, 2001) to make them smaller enough to pass through the membrane.

The F/M ratio decrease (stress I) caused an intense increase in SMP release at SRT equal to 50 d (total content multiplied by 2.5) with an augmentation of the three SMP components and in a weaker but relevant proportion at 20 d a release of proteins (+22%) and polysaccharides (+90%). As autotrophic and heterotrophic bacteria increased their endogenous activity during this stress (Table 1), they may have produced more EPS. This SMP augmentation in the MBR supernatant might also be caused by the cell lysis observed after that stress as a MLVSS concentration decrease was noted.

Compared to stress III where 24 h of nutrient and air depletion were performed, in the case of stress I bacteria disposed of an F/M ratio equal to $0.1 \text{ kg}_{\text{COD}} \text{ kg}_{\text{MLVSS}}^{-1} \text{ d}^{-1}$. Thus, microorganisms may have not biodegraded all the EPS produced and less hydrolysis products were formed well explaining the total SMP retention on the membrane surface similar to the one observed during stable conditions in both experiments.

The F/M ratio increase from $0.2 \text{ kg}_{\text{COD}} \text{ kg}_{\text{MLVSS}}^{-1} \text{ d}^{-1}$ to $0.8 \text{ kg}_{\text{COD}} \text{ kg}_{\text{MLVSS}}^{-1} \text{ d}^{-1}$ has caused an augmentation of protein content in the bioreactor supernatant during run II (73%) while protein concentration remained constant in run I and polysaccharides increased of (64%) as well as humic substances which tripled.

One of the main metabolic pathways of glucose degradation is the glycolysis. This glucose biodegradation requires several enzymes to produce the energy needed to maintain bacterial catabolism. The augmentation of both heterotrophic exogenous and endogenous activities during this stress could be the visible characteristic of an increase in enzyme synthesis to biodegrade this excess of substrate. Furthermore, a MLVSS increase was observed in both experiments. The energy produced from this excess of food was mainly intended to the synthesis of new cells.

Similar results were obtained in other studies (Jang et al., 2007) about the augmentation of both polysaccharide and humic substances contents at SRT of 20 d. Authors assumed that SMP from the influent and bulk solution could be more adsorbed by the EPS matrix with higher F/M ratio. Wingender et al. (1999) explained that it is one of the functions of EPS matrix to accumulate dissolved organic matters from the surrounding water.

The same particularity in SMP content of the permeate was observed for both runs after this stress: no proteins were released. Compared with proteins present in the bulk solution of all the other tested conditions, in this specific case of F/M ratio increase, proteins may have molecular weights higher than the membrane cut off. This seems to indicate that due to the excess of substrate, bacteria do not have hydrolyzed proteins.

As the main components of SMP are part of the dissolved organic matter measured in the bulk solution and in the MBR outlet, changes in COD removal rates (e.g. effluent quality) during all the conditions tested might be allocated to the variations of SMP rejection.

The effect of SRT on membrane fouling is presented in Fig. 6. As for the membrane biomass development, four different steps were distinguished and highly influenced by the MLVSS concentration in the MBRs. In step I after a rapid decline of the ratio membrane permeability during operation (L_p) on initial membrane permeability (L_{p0}) due to the introduction of biomass concentrated at $8 \text{ g}_{\text{MLVSS}} \text{ L}^{-1}$ in the reactor, this ratio increased as the biomass was in the lag phase (MLVSS decrease) (Fig. 6A). Afterwards this ratio decreased in step II because of the exponential growth of the biomass. Step III corresponded to an alternative augmentation and diminution of the permeability ratio as the biomass growth rate was tried to get controlled in order to stabilize the process. After stabilization at both SRTs the same low permeability decrease was observed (step IV). Membrane fouling was mainly limited due to the application of a high crossflow velocity of 4 m s^{-1} . In these stabilized conditions, the difference of measured SMP concentrations between SRT of 20 and 50 d do not seem to accelerate membrane biofouling.

It was also the case during the different stresses tested for both runs. The observed changes of MLVSS concentrations and SMP releases do not create intensive biofouling.

With the use of the three different kinds of fouling defined by Meng et al. (2009), the fouling obtained after the stable conditions (e.g. before stresses applications) at both SRT was characterized. These authors differentiate removable, irremovable and irreversible fouling. The former can be removed by hydraulic cleaning. Irremovable fouling cannot be removed by simple hydraulic cleaning and requires intensive chemical cleaning. Irreversible fouling cannot be removed by any approach.

To distinguish the three types of fouling, a succession of cleaning was applied. First a simple cleaning with distilled water was performed to remove removable fouling. After chemical cleanings (with NaOH 40 g L^{-1} followed by HNO_3 concentrated at 68%) were done. Between each cleaning step, the membrane permeability was assessed. With the use of Eq. (4), hydraulic resistances due to each fouling kinds previously defined can be calculated and contribution of each fouling type can be obtained. Membrane hydraulic resistance was previously measured and is equal to $3.3 \times 10^{12} \text{ m}^{-1}$.

$$L_p = \frac{1}{\mu \times (R_m + R_f)} \quad (4)$$

With L_p : membrane permeability ($\text{m}^3 \text{ m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$), R_f : hydraulic resistance to the fouling (m^{-1}), R_m : membrane hydraulic resistance (m^{-1}), μ : dynamic viscosity of the permeate at 20°C (Pa s).

After process operation of around 100 days in both experiments, the main part of fouling can be attributed to irremovable fouling with 90% at 50 d and 85% at 20 d. Irreversible fouling was a little higher at 20 d and it represents 3% of the total fouling whereas it was only 1% at 50 d. Finally removable fouling was responsible for 12% of total fouling at 20 d and 9% at 50 d. Thus, depending on the SRT, fouling exhibited differences. With the SRT, activated sludge characteristics as flocs size, bound EPS and SMP quantities and characteristics showed variations which influenced fouling mechanisms. Irreversible fouling can be attributed to adsorption of small molecules on or into the membrane pores. The proportion of small particles should be higher at 20 d. As high crossflow velocity of 4 m s^{-1} was applied all along the experiments, at both SRTs only a small part of the fouling was removable. Concerning the irremovable fraction of fouling, Metzger et al. (2007) proved that it was mainly caused by SMP and highly composed of proteins. As in the MBR, SMP concentration was higher at 50 d ($18 \text{ mg}_{\text{g}_{\text{MLVSS}}^{-1}}$) than at 20 d ($7 \text{ mg}_{\text{g}_{\text{MLVSS}}^{-1}}$), the higher irremovable fouling obtained was certainly caused by the more abundant concentration of SMP at 50 d.

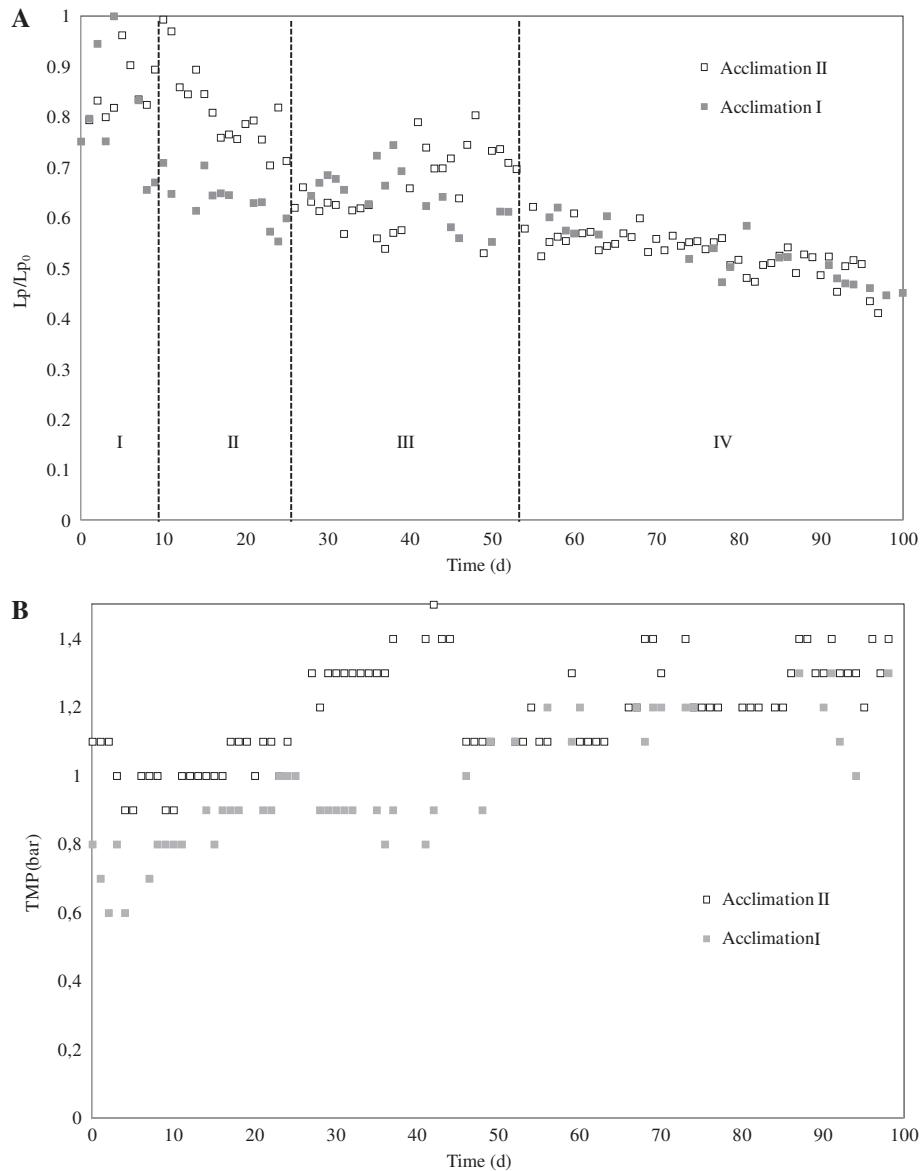


Fig. 6. Evolution of (A) membrane permeability (I = SRT of 20 and II = SRT of 50 d) and (B) TMP with time.

Mannina and Di Bella (2012) studied the development of membrane fouling depending on MBR start-up method. They proved that membrane permeability and fouling development were influenced by the start-up protocol. Even if during the stable period of operation of both MBRs, TMP and corresponding membrane permeability were the same, TMP and membrane permeability showed differences between the two acclimations before stabilization. The biggest difference was observed between the phase II and III (from day 20 to 45). While TMP was stable around 0.9 bar during acclimation I, an increase of TMP was obtained from 1 to 1.4 bar in acclimation II. This result can be related to the evolution of membrane permeability during step II. The decrease of permeability was faster during the second acclimation whereas MLVSS was lower during this period. Then, characteristics of biomass during this time implying SMP characteristics could have influenced fouling development.

In conclusion MBR start-up has to be controlled because it can influence membrane fouling development and membrane permeability.

4. Conclusion

With dissociation of SRT from F/M ratio which was maintained constant, the interest of working at 50 d was demonstrated as heterotrophs adapted well themselves with faster substrate biodegradation that at 20 d. Autotrophs showed easier development with $N-NH_4^+$ removal efficiency up to 78%. SMP release doubled at 50 d with a majority of polysaccharides but no additional membrane fouling was noticed. For each stress tested autotrophs were affected with the same decrease of $N-NH_4^+$ removal rate whatever the SRT. Composition of SMP and their retention on membrane surface changed with the applied stress.

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