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Fouling analysis and biomass distribution on a membrane bioreactor under low ratio COD/N

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Abstract. This paper deals with the influence of chemical oxygen demand to nitrogen ratio ((COD/N) ratio) on the performance of an membrane bioreactor. We aim at establishing relations between COD/N ratio, organisms' distribution and sludge properties (specific resistance to filtration (SRF) and membrane fouling). It is also essential to define new criteria to characterize the autotrophic microorganisms, as the measurements of apparent removal rates of ammonium seem irrelevant to characterize their specific activity. Two experiments (A and B) have been carried on a 30 L lab scale membrane bioreactor with low COD/N ratio (2.3 and 1.5). The obtained results clearly indicate the role of the COD/N ratio on the biomass distribution and performance of the membrane bioreactor. New specific criteria for characterising the autotrophic microorganisms activity, is also defined as the ratio of maximum ammonium rate to the specific oxygen uptake rate in the endogenous state for autotrophic bacteria which seem to be constant whatever the operating conditions are. They are about 24.5 to 23.8 $gN\text{-}NH_4^+/gO_2$, for run A and B, respectively. Moreover, the filterability of the biological suspension appear significantly lower, specific resistance to filtration and membrane fouling rate are less than 10^{14} m^{-2} and 0.07 10^{12} $m^{-1}.d^{-1}$ respectively, than in conventional MBR confirming the adv < antage of the membrane bioreactor functioning under low COD/N ratio.

Keywords: membrane bioreactor; autotrophic organisms; oxygen uptake rate; autotrophic-heterotrophic ratio; fouling

1. Introduction

Many waste treatment processes usually generate an effluent highly concentrated in ammonium (leachates, anaerobic digestion, etc.) whose uncontrolled disposal can significantly damage the environment. The dissolved ammonium is one of the worst pollutants for aquatic life (Krupa 2003). Its deleterious effects include toxicity to aquatic fauna, depletion of dissolved oxygen and reduction of chlorine-disinfection efficiency (Cooper *et al*. 1994). Furthermore, the colonization of surfaces by nitrifying microorganisms can induce corrosion and even destruction of the structures

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because of production of nitrous and nitric acids. For these reasons, ammonium removal is required.

Biological nitrogen removal (BNR) is frequently included in urban wastewater treatment facilities. The process involves two stages: conversion of ammonium into nitrate (nitrification) and subsequent transformation of nitrate into nitrogen (denitrification). Nitrification is an aerobic process, where autotrophic organisms use the oxidation of ammonium for their energy requirement. The nirification reaction includes two steps: ammonium is firstly converted into nitrite by Nitrosomonas species and the produced nitrite is converted into nitrate by nitrobacter species. The BNR could efficiently be achieved by using a membrane bioreactor (MBR) that combines a biological process and a separation by membrane filtration. Because high cell concentration can be achieved in a MBR, this reactor offers many advantages over conventional activated sludge: small foot-print and capacity to treat highly concentrated influent, while the effluent is of excellent quality notably in term of disinfection (Engelhardt *et al*. 2003).

These advantages do not hide the major problems of MBR, namely fouling membrane (Xu *et al*. 2015), (Zhang *et al*. 2014) and the high cost due to the chemical cleaning and operational cost linked to the air necessary for the process and membrane (Chua *et al*. 2006, Celine *et al*. 2011, Chang *et al*. 2001).

Different fouling mechanisms, such as macro-molecule adsorption, pore plugging and cake build-up (Gasmi *et al*. 2014). However, the particle matrix within the liquor of MBR is formed by a large range of living micro-organisms along with soluble and colloidal compound. The bulk biomass has different physiological characteristic such as total suspended solid (TSS), volatile suspended solid (VSS), extracellular polymeric substances (EPS), soluble microbial product (SMP), all these compounds change according to the MBR operating conditions; as a result,(i) fouling in MBR processes becomes very difficult to control and identify, (ii) the apparent ammonium removal rate expressed through the VSS (gN/gVSS) is irrelevant to characterize the activity of autotrophic bacteria in MBR caused by the big variation of VSS from one study to another (Campos *et al*. 1999, Wouter and Vandael 1999, Dytczak and Oleszkiewicz 2008).

It could be relevant, in the case of domestic wastewater, to associate an MBR to an upstream physico-chemical step to be able to abate the organic carbon. The wastewater feeding in an MBR is unconventional with a lower COD/N ratio which can alter the biomass composition and growth favouring nitrifying microorganisms.

The aim of this paper is to establish a relationship between COD/N ratio, microorganism distribution, specific sludge filtration, and membrane fouling. In addition it could be pertinent to understand the influence of operation condition, specially the low ratio COD/N, on the development and evolution of microorganisms (mainly autotrophic and heterotrophic bacteria), also to study this influence on fouling propensity and compare our results to those obtained by an conventional MBR.

2. Materials and methods

2.1 Experimental set up

The experimental set-up consists of an aerobic reactor with a 30 L working volume equipped with a continuous pH controller and a submerged hollow fibre membrane module (0.05 μm pore size and 0.2 m^2 of surface area) (Fig. 1). Due to the high mixing rate, the reactor is considered as

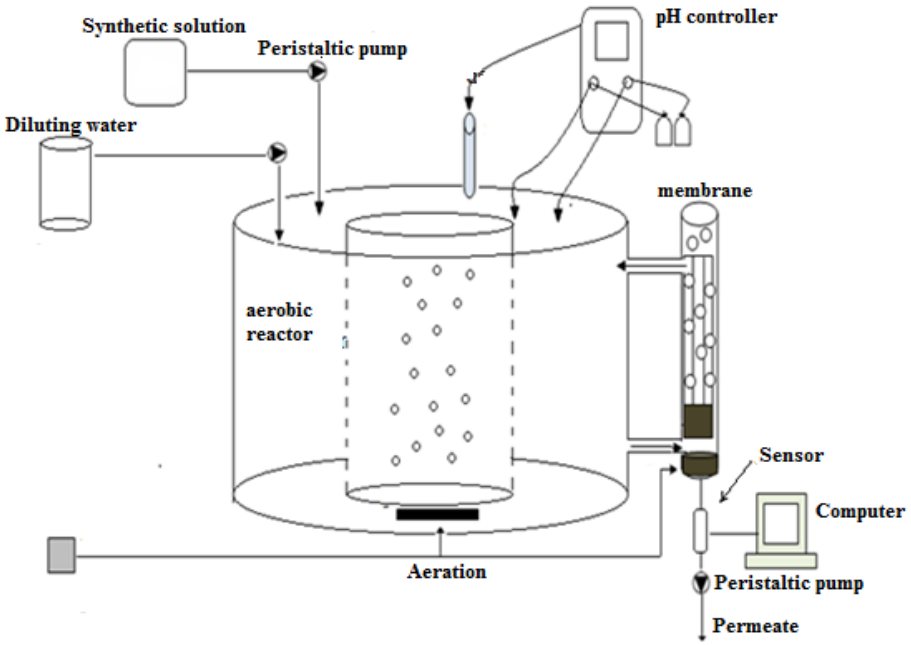


Fig. 1 Schematic representation of the experimental unit

Table 1 Operational conditions

Parameter	Run A	Run B
T (K)	293	293-297
Hydraulic retention time (HRT) (d)	0.625	0.625
pH	7	7
Conentration of dissolved oxygen (mg/L)	> 7	> 7
Time of experiments (d)	51	54
$N\text{-}NH_4^+$ influent $mgN.L^{-1}$	80	125
COD influent $mgCOD.L^{-1}$	188	188
Organic Loading Rate (OLR) ($kgCOD.m^{-3}.d^{-1}$)	0.3	0.3
Nitrogen Loading Rate (NLR) ($kgN.m^{-3}.d^{-1}$)	0.12	0.2
COD/N ratio	2.3	1.5
Sludge retention time (SRT) (d)	150	100
Alkalinity ($mgCaCO_3.L^{-1}$)	900	1200

perfectly mixed, including the membrane module. The concentrated synthetic feed solution, the diluting water and the permeate are injected or extracted by peristaltic pumps. The permeate pressure is recorded with sensors connected to a computer through a Labview Program, which enables us to monitor the Transmembrane Pressure (TMP). Aeration is continuously provided through membrane diffusers at the bottom of the reactor and just below the fibres in the membrane module enabling to operate without limitation of dissolved oxygen.

2.2 Biological conditions

Two successive series of experiments A and B are carried out under the operational conditions of Table 1. At the beginning of each run, the reactor is filled with sludge inoculums from a domestic wastewater plant operated with low organic loading rate (< 0.1 kg COD kg^{-1} VSS d^{-1}). The reactor is then fed with a synthetic solution containing 20.07 g/L of ammonium chloride (NH_4Cl) in the first run and 31.86 g/L for the second, 1.075g/L of diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) and 11.84 g/L of sodium acetate (CH_3COONa) as the sole organic compound. The other elements (Mg^{2+}, K^+, etc.) are supplied by tap water used as diluents. The inorganic carbon required for ANO is supplied with calcium carbonate ($CaCO_3$).

2.3 Analyses of nitrogen compounds and organic matter

All the concentration data correspond to samples taken inside the reactor, in the permeate or influent. Chemical analyses are carried out according to the Standard Methods APHA (1995).

Extracellular polymeric substances (EPS) soluble in solution called soluble microbial product (SMP), which are mostly composed of polysaccharides and proteins, has been measured according to Dubois *et al*. (1956) for polysaccharide and Lowry modified by Frolund *et al*. (1995) for proteins.

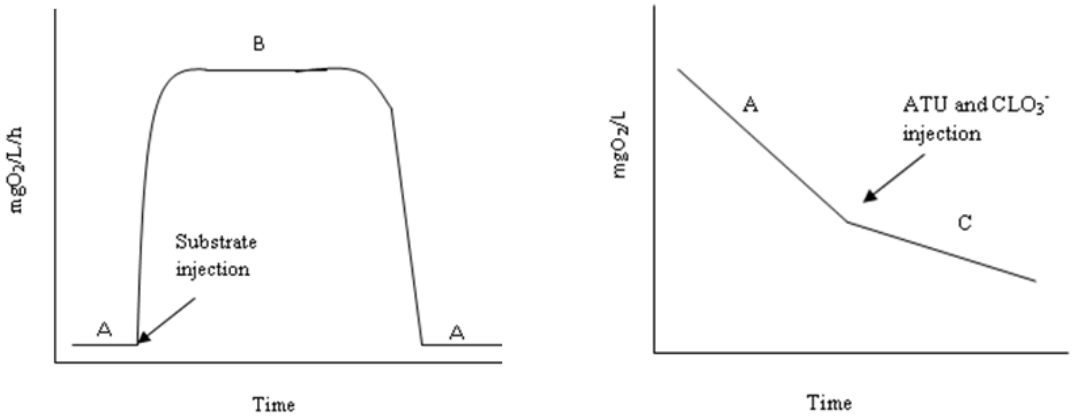
It is noticed that nitrate interferes with the polysaccharide measurement that is a brown colour observed instead of the expected green, and leads to a wrong value. In order to quantify this interference, three calibration curves are drawn in the range 0-150 mg/L of: (i) a solution containing only glucose; (ii) a solution of glucose with added KNO_3 (for three different concentrations: 35, 60 and 100 mg N L^{-1}); (iii) a glucose solution with added $NaNO_3$ (for 35, 60 and 100 mg $N^{-1}L^{-1}$). The deviation dispersion between the results is not significant when the nitrate concentration is less than 35 mg N L^{-1}. Above 60 mg N L^{-1}, the dispersion becomes highly significant. This is why the polysaccharide concentration is determined after sample dilution until nitrate concentration is lower than 35 mg N L^{-1}.

2.4 Biomass properties

Biological activity

Respirometry, frequently used to quantify biological activity (Munz *et al*. 2008), is carried out to measure the mass of oxygen consumed by the microorganisms in a 250 ml vessel. Before the respirometric measurements, samples are aerated during 24 hours without any addition enabling to reach the endogenous regime. Moreover, specific experiments are also carried out in endogenous condition after adding a solution of 10 mg/L allythiourea (ATU) (Aleem and Sewell 1981), (Surmacz-Gorska *et al*. 1996) and 10mM of sodium chlorate ($NaClO_3$) (Vanrolleghem *et al*. 1995), (Ljung 1987) to inhibit the Nitrosomonas and Nitrobacter bacteria respectively (Munz *et al*. 2008) which enables us to differentiate the oxygen uptake rate (OUR) for heterotrophic population (OHO) and autotrophic ones (ANO). In endogenous condition a specific concentration of NH_4Cl is injected to the bioreactor which leads to measure the exogenous OUR (OUR_{ex}) and this test is used also to calculate the nitrification maximum rate ($r_{nitrifmax}$) (Choubert *et al*. 2005).

Fig. 2(a) illustrates the different respirometric tests after macroinjection of NH_4Cl. Phase (A) represents the OUR in endogenous conditions (OUR_{end}) where both autotrophic and heterotrophic organisms consume oxygen. The addition of a nitrogen substrate (NH_4Cl) induces an increase of



(a) Addition of ammonium (b) Addition of ATU and ClO_3^-

Fig. 2 Respirometric test obtained after addition ammonium and autotrophic inhibitor (ATU, ClO_3^-)

oxygen consumption (level B). Then organisms come back to an endogenous regime (phase A); the monitoring of nitrogen forms with time can determine the $r_{\text{nitrifmax}}$.

Fig. 2(b) illustrates a test after ATU and $NaClO_3$ addition. The inhibition of ANO leads to an OUR decrease (phase C). The difference of slopes between the phases A and C enables us to differentiate between ANO and OHO respirometric activities which are translated by the % $\text{Activity}_{\text{ANO/OHO}}$ given by Eq. (1), and also we can calculate the OUR_{end} for ANO ($\text{OUR}_{\text{EndANO}}$) Eq. (2).

$$\%\text{Activity}_{\text{ANO/OHO}} = 1 - \frac{\text{OUR}_{\text{end+ATU+ClO}_3^-}}{\text{OUR}_{\text{end}}} \tag{1}$$

$$\text{OUR}_{\text{EndANO}} = \text{OUR}_{\text{end}} - \text{OUR}_{\text{end+ATU+ClO}_3^-} \tag{2}$$

2.5 Suspension filterability and membrane fouling

The filterability of the sludge is characterized using the specific cake resistance (SCR) α obtained from experiments at constant pressure or at constant flux using the cake filtration model.

For filtration at constant pressure, SCR is obtained as following Eq. (3) Bowen and Jenner (1995).

$$\frac{t}{V} = \frac{\mu\alpha C}{2\text{TMPS}^2}V + \frac{\mu R_m}{\text{TMPS}} \tag{3}$$

The experiments are carried out in a sartorius cell in dead end filtration. The pressure used is low (50 kPa) in order to have filtration conditions close to those of the MBR (Dominik *et al*. 2011). When plotting t/V versus V a straight line is obtained whose slope enables us to calculate the SCR.

Whereas its value in constant flux filtration is equal to Eq. (4).

$$\text{TMP} = \mu\alpha C J_w^2 + \mu R_m J_w \quad \text{or} \quad \frac{d\text{TMP}}{dt} = \mu\alpha C J_w^2 \tag{4}$$

TMP the transmembrane pressure, s the membranes area (m^{-2}), μ the viscosity Pa.s, J_w the permeate flux $m^3m^{-2}s^{-1}$, α specific cake resistance ($m.kg^{-1}$), V the filtrate collected volume (m^3), t the filtration time (s), R_m the intrinsic membrane resistance (m^{-1}). C is the quantity of accumulated matter on the membrane per volume of filtrated water ($kg.m^{-3}$). If this concentration is equal to bulk concentration in dead end filtration, the value of C cannot be obtained in most MBR systems. For that reason, only values of the product αC (Specific resistance to filtration (SRF)) can be acquired from MBR experimental data.

The fouling rate r_f ($r_f = dR/dt$) can be easily deduced from the TMP evolution Eq. (5).

$$\frac{dR}{dt} = \frac{1}{\mu J_w}\frac{d\text{TMP}}{dt} \tag{5}$$

3. Results and discussion

3.1 Overall performance

During the first days of run A and B, a significant decrease of TSS (Fig. 5) corresponds to the acclimation of the inoculums to the imposed conditions. The data is collected after reaching a steady state.

3.1.1 Organic carbon and nitrogen removal

Due to the membrane barrier, no solids are observed in the permeate which is significantly clarified: the turbidity is less than 1 NTU. The COD and ammonium concentrations in the supernatant, after a 2 hour settling, and the permeate are displayed in Figs. 3-4. The low values in the permeate demonstrate the interest of such a device: the ammonium is oxidised at more than 90% and the COD is abated at 88%. The apparent COD and $N\text{-}NH_4^+$ removal rate reaches average

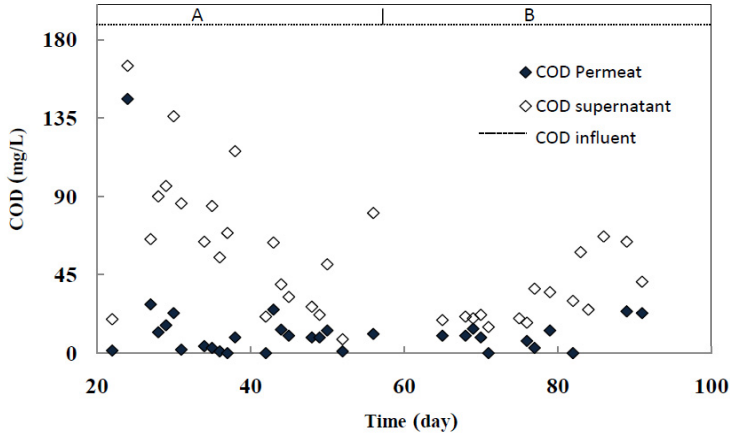


Fig. 3 Evolution of COD concentration during experiment

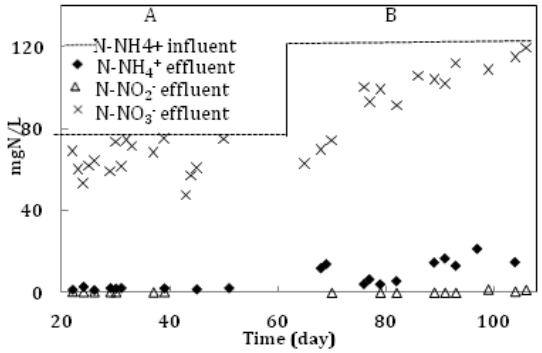


Fig. 4 Evolution of nitrogen concentrations

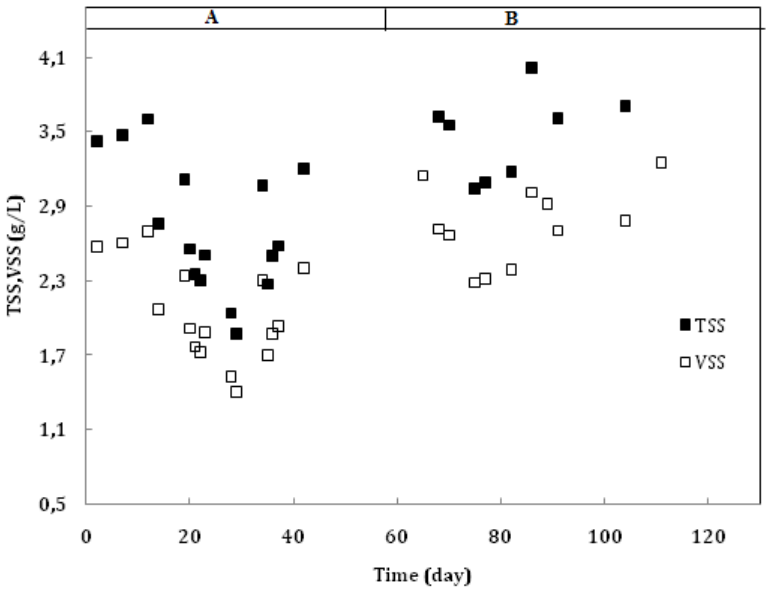


Fig. 5 TSS, VSS evolutions during run A and B

values of 0.21 to 0.24 kg COD.m^{-3}.d^{-1} and 0.10 to 0.18 kg N.m^{-3}.d^{-1} (for run A). The COD contained in the permeate is not residual acetate. Acetate is completely oxidised which shows that the permeate COD resulted from soluble microbial product (SMP) as noticed in other works (Lebegue *et al*. 2008). Though the permeate COD was low (< 30 mg/L), the unsettle-able COD is high probably because of: (i) the dismantling of floc particles under high level of turbulence; and (ii) the choice of the organic carbon source that favours insufficient flocculation. Under such appearing conditions; the membrane enables to get a high-quality permeate whatever the level of flocculation.

No nitrite or ammonium is found in the effluent of run A (Fig. 4); their concentration never exceeds 0.3 and 1 mg NL^{-1} respectively. At the beginning of run B where ammonium influent has increased from 80 to 125 mg NL^{-1}, ammonium nitrogen is noticeable in the effluent (10 mg NL^{-1}). The increase in ammonium concentration induces a progressive increase of nitrate concentration in

the effluent, which corresponds to the slow ANO growth. The concentration reaches a plateau after approximately 40 days. Ammonium concentration does not display a similar trend. The nitrogen balance for run A is equilibrated between 85 and 95%, whereas it is 72 to 92% balanced in run B. This mass balance does not include: (i) nitrification intermediate forms (hydroxylamine (NH_2OH) Kartik and Barth (2008), N_2O or N_2); (ii) nitrogen requirements for bacterial growth; and (iii) ANO ability to store ammonium.

3.2 Biomass evolution

The extraction of biomass is practised all along the experiments for analyses. The evolution of TSS and VSS concentration are given in (Fig. 5).

Except during the acclimation carried out at the beginning of each run, the TSS and VSS variations are not significant during the experiment. It could result from a low solid production, close to 0.051 kg $VSS.m^{-3}.d^{-1}$ with yields between 0.21 and 0.24 g $VSS_{product}.g^{-1}$ $COD_{removed}$ or 0.18 to 0.42 g $VSS_{product}.g^{-1}$ $N_{removed}$. Moreover, the specific removal ammonium (r_{nitrif}/VSS) are respectively comprised between 2.17 to 3.5 g $N.kgVSS^{1}.h^{-1}$. These values do not clearly show that the reactor becomes an MBR_{ANO}. Therefore the respirometry is used to better describe the biologic behaviour of each population.

3.3 Growth of autotrophic biomass

Respirometric analyses are carried out in order to identify the specific mixed liquor ANO and OHO activities. To carry on such investigations, different measurements of oxygen uptake rates (OUR) are achieved in controlled conditions: (i) OUR_{End} during endogenous conditions; (ii) $OUR_{End+ATU+ClO3^-}$ during endogenous conditions with ATU and $NaClO_3$ injection, these two respirometric are done several times during the investigation; and (iii) OUR_{ex} after macro injections of NH_4Cl for ANO population. The macroinjections are done after the establishment of the steady state. Fig. 6 presents the evolution of the OUR_{EndANO} and the ratio activity ANO/OHO during runs A and B. Results show that the percentage of ANO activity increases from 16 to 42%

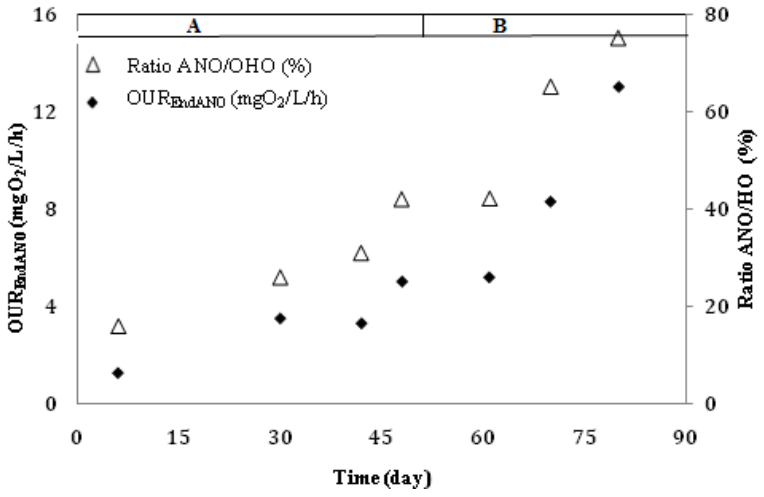


Fig. 6 Evolution of ANO/OHO ratio and OUREndANO during experiment

for run A and reaching 72% at the end of the run B. This observation confirms the MBR evolution from an heterotrophic MBR toward an MBR_{ANO}. These evolutions can be explained by: (i) the decreasing ratio COD/N-NH_4^+ in influent passing from an urban wastewater treatment plant (COD/N-NH_4^+ = 10) at day 0 to the synthetic influent (with COD/N-NH_4^+ = 2.4 during run A and 1.5 during run B); and (ii) by the fact that no sludge extraction, except for sample, is practised to favour autotrophic accumulation.

If the assumption that the OUR of ANO and OHO are linked to their associated loading rate Eq. (6).

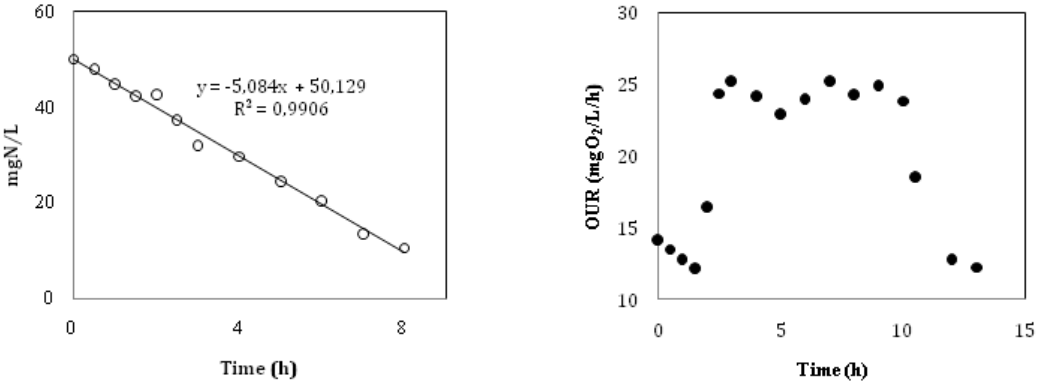
$$\frac{\mathrm{OUR}_{\mathrm{EndANO}}}{\mathrm{OUR}_{\mathrm{EndANO}}+\mathrm{OUR}_{\mathrm{EndOHO}}}=\frac{\mathrm{ANO}}{\mathrm{ANO}+\mathrm{OHO}}=\frac{\alpha\mathrm{NLR}}{\alpha\mathrm{NLR}+\beta\mathrm{OLR}} \tag{6}$$

This simple assumption allows the α and β coefficients determination (α = 1.39 gO_2 /gN-NH_4^+ and β = 0.54 gO_2/gCOD). The inverse of these coefficient translate the nitrification or organic carbon sludge removal potential: 0.72 gN-NH_4^+/gO_2 for autotrophic population and 1.83 gCOD/gO_2 for heterotrophic ones.

The endogenous respirometric needs are directly linked to the active part of the biomass. Then the measurement of $OUR_{end+ATU+ClO3-}$ and OUR_{end} allows the access to OUR_{EndANO} and the ANO/OHO ratio activity. Once this ratio is obtained, it can be interesting to compare the specific pollutant removal according to its own endogenous respirometric. This step will permit the definition of relevant criteria describing specific performances relatively to ANO defined as the ratio of maximum ammonium rate to the specific oxygen uptake rate for autotrophic micro organisms in the endogenous state ($r_{nitrifmax}$/ OUR_{EndANO}).

Fig. 7 gives an example of macro injection of 55 mgN/L practise in day 50, and the evolution of ammonium form during experience.

Table 2 gives an original data ($r_{nitrifmax}$/ OUR_{EndANO}) equal to 24.5 to 23.8 gN-NH_4^+/gO_2 for run A andB respectively. These criteria remain relatively constant for the two runs whatever the experimental condition are (acclimation, load, transient or permanent flow). These results highlight the interest of respirometric analysis to evaluate the active part of biomass in any bioreactor and then permit the calculation of $r_{nitrifmax}$ removal potential.



(a) Ammonium concentration (b) OUR monitoring

Fig. 7 Ammonium concentration and OUR monitoring during experience

Table 2 Results obtained for day 50 and 80

Parameter	Day 50 (run A)	Day 80 (run B)
OUR_{End} ($mgO_2.L^{-1}.d^{-1}$)	11.83	17.79
Injected concentration (mgN/L)	55	120
Ratio (ANO/OHO) (%)	42	72
OUR_{EndANO} ($mgO_2.L^{-1}.d^{-1}$)	4.97	12.81
$r_{nitrifmax}$($mgN.L^{-1}.d^{-1}$)	122	305.5
$r_{nitrifmax}/OUR_{EndANO}$ ($mgN.mgO_2^{-1}$)	24.5	23.8

3.4 Suspension filterability and membrane fouling

Mixed liquor filterability is characterized by measuring the SRF (αC) and the TMP evolutions.

3.4.1 Mixed liquor SRF (αC)

Fig. 8 shows the evolutions of the SRF and SMP concentration in supernatant during runs A and B. The SRF values are found in the range of 2.10^{13} to 9.10^{13} m^{-2}. These values are significantly lower than others obtained in conventional MBR for which the values range between 1.2 10^{14} to 10^{15} m^{-2} (Delrue *et al*. 2011, Stricot *et al*. 2010) confirming the interest to favour autotrophic development in order to improve the suspension filterability.

No trend is observed between polysaccharide evolution and punctual SRF whereas this kind of compounds is generally mentioned as fouling compounds. It is probably due to the procedure of SRF measuring, where both dead end filtration and short-term experiment are not able to

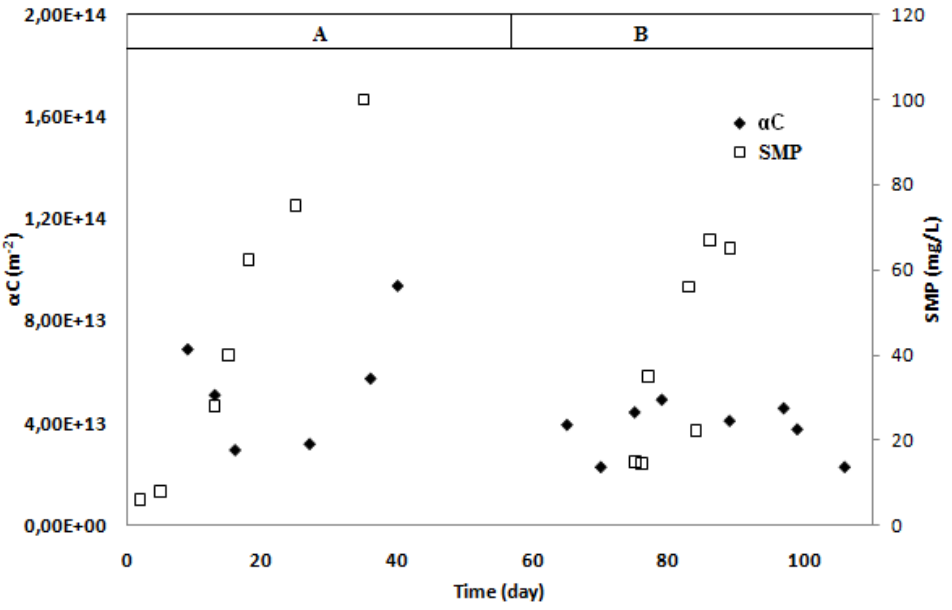


Fig. 8 Evolution of SRF (*αC*) and SMP concentration during experiments

characterize micro (biofilm) and nano-scale fouling (adsorption) in regards to the high macro-scale fouling generated. In fact, the frontal filtration mode is governed by the cake deposit onto the membrane depending mainly on the suspended solid and colloidal concentrations in the suspension. In opposite, the "continuous" shear stresses induced are closed to the membrane surface in MBRs favours the determining role of SMP and progressive biofilm development onto the membrane surface as generator of membrane fouling (Ng and Ng 2010).

3.5 TMP evolution

During continuous operation in the MBR_{ANO}, a membrane cleaning is only operated at the end of each run A and B. Fig. 9 shows the TMP and SMP concentration evolutions during runs A and B. We can notice a progressive evolution of TMP in direct relation with the SMP accumulation in the bioreactor.

As the SMP are mostly retained by the membrane barrier, their concentration increased in the reactor. It can be observed that their concentration increases as a linear function of time for both experiments but its evolution appears less important during run B where the COD/N ratio is lower. This observation also confirms that autotrophic communities have a benefit impact on the SMP production in MBR_{ANO}.

Eqs. (7)-(8) allows the calculation of the fouling rate expressed in ($m^{-1}d^{-1}$), where f_t is the temperature correction factor in order to report the fouling rate at 20°C.

$$\frac{dR}{dt} = \frac{1}{\mu J_w ft} \frac{d\mathrm{TMP}}{dt} \tag{7}$$

$$f_t = e^{-0.0239(\mathrm{T}-20)} \tag{8}$$

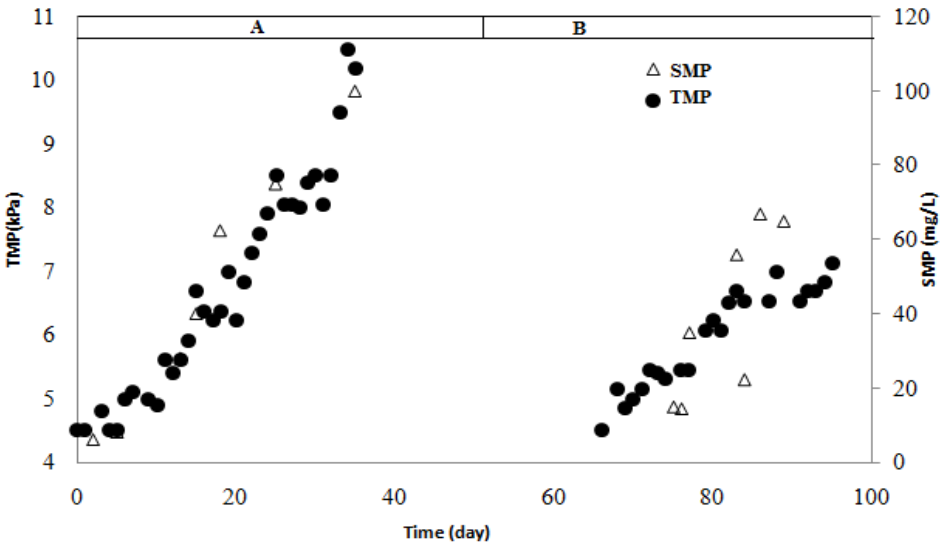


Fig. 9 TMP and SMP evolutions during experiment

Table 3 Fouling rate and cleaning membrane frequency in comparison with other investigations

Conditions	$\frac{dR}{dt}$ (10^{12} m^{-1} d^{-1})	Cleaning membrane frequency (d)	References
Hollow fiber membrane, T = 25°C, heterotrophic MBR, 0.18 kg COD kg TSS^{-1} d^{-1}	2.56	One time /15 d	(Castaing *et al*. 2011)
Hollow fiber membrane, T = 20°C, heterotrophic MBR 0.5 kg COD kgTSS^{-1} d^{-1}	931	-	(Delrue *et al*. 2011)
heterotrophic MBR, 1.7 kg DCO kg TSS^{-1} d^{-1}, Hollow fiber membrane	0.25	One time /15 d One time/30 d	(Lebegue *et al*. 2008)
Hollow fiber membrane, T = 20°C and 27°C, MBR_{ANO}, 0.1 kg COD TSS^{-1} d^{-1}	0.017-0.064	At the end of each run (one time / 50 d)	Our study

Table 3 shows the fouling rate for runs A and B and the cleaning membrane frequency in comparison with other investigations.

It is important to underline the lower fouling rate values obtained with this MBR_{ANO} in comparison with usual results obtained in MBR.

All these results confirm the interest to develop conditions favouring autotrophic population development that generate less fouling material.

4. Conclusions

Experiments are carried out to analyse the effect of low ratio COD/N, where the development of ANO is promoted, on membrane bioreactor performances. Results have showed a good nitrogen elimination without nitrite accumulation also results have confirmed the interest of the membrane barrier to insure a high level of permeate quality.

As the global measurement of VSS is not relevant, the interest of respirometric tools are underlined. These tools allowed the determination of ANO specific activity. The originality in this study is to define new criteria to characterize the ANO which is the ratio rnitrifmax/ OUREndANO, which seem to be constant over time whatever the operational condition is, and in average it is equal to 24.1 $mgN.mgO_2^{-1}$. These criteria permit the determination of nitrification potential removal only through the OUR_{EndANO} measurement. Moreover, the fouling propensity of biological suspension (SRF and membrane fouling rate) appear significantly lower than other conventional MBR.

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