Bioresource Technology 189 (2015) 250–257

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Effect of C/N shock variation on the performances of a moving bed membrane bioreactor

Daniele Di Trapani [a,*], Gaetano Di Bella [b], Giorgio Mannina [a], Michele Torregrossa [a], Gaspare Viviani [a]

[a] Dipartimento di Ingegneria Civile, Ambientale, Aerospaziale, dei Materiali – Università degli Studi di Palermo, Viale delle Scienze, 90128 Palermo, Italy
[b] Facoltà di Ingegneria e Architettura – Università di Enna "Kore", Cittadella Universitaria, 94100 Enna, Italy

HIGHLIGHTS

- A MB-MBR pilot plant subject to C/N shock variation was investigated.
- Respirometry showed that nitrification was significantly affected by C/N and ammonia.
- Nitrite oxidizing bacteria (NOB) highly suffered the C/N shock variations.
- Extracellular polymeric substances (EPSs) strongly influenced membrane fouling.

ARTICLE INFO

Article history:
Received 8 February 2015
Received in revised form 26 March 2015
Accepted 29 March 2015
Available online 2 April 2015

Keywords:
C/N ratio
OLR
Moving bed membrane bioreactor
Nitrification

ABSTRACT

The effect of a sharp variation of C/N ratio in a moving bed membrane bioreactor (MB-MBR) pilot plant treating high strength wastewater has been investigated. The experimental campaign was divided into two periods, each characterized by a different C/N ratio (namely, 2.5 and 15, Period 1 and Period 2, respectively). The MB-MBR system was analyzed in terms of organic carbon removal, nitrification efficiency, biokinetic activity and fouling behavior. The results showed that the nitrification process was severely affected by lower C/N value and by high concentration of ammonia. It was noticed an extensive stress effect on the autotrophic bacteria. Furthermore, it was observed an increase of the resistance related to particle deposition into membrane pores, likely due to a worsening of the cake layer features, with a reduction of the "pre-filter" effect, also related to the increase of the total Extracellular Polymeric Substances production with the C/N ratio.

1. Introduction

Several industrial processes may generate wastewater characterized by high organic and ammonium concentration (such as dairy, agro-industry, fish processing, etc.); these effluents should be properly treated before discharging into the receiving water bodies, since their high organic matter and nitrogen content may provide serious injuries to the environment. The C/N ratio of the influent wastewater is one of the most critical parameters for wastewater nitrogen removal process, because it might directly affect functional microorganism species, including autotrophic populations, such as ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), as well as heterotrophic denitrifying bacterial species (Fu et al., 2009). In detail, C/N ratio fluctuations, mainly due to inlet ammonia variations, can severely hamper nitrification and/or nitrogen removal. Choi et al. (2008) investigated the effects of C/N ratios on an intermittently aerated MBR. Choi and co-workers found that C/N ratio over 7 is required for nitrogen removal, while a C/N ratio of 4.5 provided a much lower nitrogen removal capacity. Indeed, high ammonia loading rates coupled to low C/N values can significantly reduce the activity of specific nitrifying populations. In this context, the use of new technologies, characterized by high robustness, could be useful to sustain high removal efficiencies, for the treatment of high strength industrial wastewater or even in the case of co-treatment of domestic and industrial wastewater, characterized by sudden changes of C/N ratio. Among these technologies, recently the moving bed membrane bioreactor (MB-MBR) gave rise the interest of the scientific and technical community and several studies have been carried out in the last years (among others, Leyva-Díaz et al., 2013; Di Trapani et al., 2014; Yang et al., 2014). This technology relies on the combination of a moving bed biofilm reactor

* Corresponding author. Tel.: +39 091 23896552; fax: +39 091 23860810.
E-mail address: daniele.ditrapani@unipa.it (D. Di Trapani).

http://dx.doi.org/10.1016/j.biortech.2015.03.143
0960-8524/

(MBBR) coupled to a membrane bioreactor (MBR), with the aim to take advantage of the peculiarities of both technologies (biofilm and activated sludge processes). Basically, the MBBR process relies on the use of small plastic elements for biofilm growth, kept in constant motion throughout the overall volume of the reactor (Ødegaard, 2006; Di Trapani et al., 2008; Di Trapani et al., 2010). On the other hand, MBR systems have been widely used for the treatment of domestic as well as industrial wastewater, significantly improving the effluent quality compared to conventional activated sludge (CAS) processes (Stephenson et al., 2000). However, despite efforts have been spent in order to understand the potentiality of the combined system, MB-MBRs are relatively new and there is still a lack of knowledge relating to kinetics and system performance, especially when treating specific high strength industrial wastewater (e.g. wastewater deriving from oily shipboard activities, aquaculture, sediment washing, manure digestate, etc.). In particular, to authors' knowledge, no studies have been reported concerning the influence that shock C/N variations can exert on MB-MBR systems. Therefore, the aim of the study is to gain insight about the behavior of this new technology when subject to C/N shock variation. In detail, the objective is to assess the potential stress effect exerted by C/N variation in terms of carbon removal, nitrification ability, biomass activity and membrane fouling tendency.

2. Methods

2.1. MB-MBR system description

The MB-MBR pilot plant was built at the Laboratory of Environmental and Sanitary Engineering of Palermo University (IT) and it was similar to that one reported in Di Trapani et al. (2014). The pilot plant layout is available as Fig. S.1 in the Supplementary Information (SI). The pilot plant was divided into two compartments by a perforated wall: one was the bioreactor (volume 17 L) filled with the biofilm carriers while the other one (volume 7 L) contained the membrane module. This configuration was adopted in order to prevent any damage of the membrane module due to the collisions with the suspended carriers. Concerning the bioreactor, it was filled with AnoxKaldnes™ K1 carriers, with a 50% filling fraction, corresponding to a net surface area in the reactor of 250 $m^2\ m^{-3}$. The membrane compartment was equipped with an ultrafiltration (UF) hollow fiber membrane module (Zee-Weed™ 01, with surface area equal to 0.1 m^2 and nominal porosity of 0.04 μm). The membrane flux was kept almost equal to 15 $L\ m^{-2}\ h^{-1}$. The membrane was subject to ordinary backwashing (every 5 min for a period of 1 min) by pumping a fraction of permeate back through the membrane module. The membrane module was periodically subjected to extraordinary cleaning procedures (either physical or chemical) as soon as the transmembrane pressure (TMP) reached 0.5–0.6 bar, as suggested by the membrane manufacturer.

The pilot plant was operated for almost 60 days and it was fed with high strength synthetic wastewater. The overall experimental campaign was divided into two periods, each characterized by a different C/N ratio: Period 1 (duration: 28 days) characterized by a C/N ratio equal to 2.5 and Period 2 (duration: 32 days) characterized by a C/N ratio almost equal to 15.

Before running the experiments, a cultivation period (8 weeks) was carried out in order to acclimate the inoculated biomass to synthetic wastewater as well as to enhance the growth of the biofilm on the suspended carriers. In this period, the analyses were carried out only sporadically, with the main aim to evaluate biofilm growth on the suspended carriers.

2.2. Analytical methods

During the overall duration of the experimental campaign, the inlet synthetic wastewater, the mixed liquor and the membrane permeate were sampled and analyzed for the following parameters: total and volatile suspended solid (TSS and VSS), chemical oxygen demand (COD), ammonium nitrogen (NH_4-N), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N). It is worth noting that, excepting TSS and VSS, the analyses of the mixed liquor section were carried out on the supernatant filtered at 0.45 μm. Therefore, it was possible to assess the "biological" removal efficiency (evaluated at the upstream of the membrane) from the "total" removal efficiency (at the downstream of the membrane), referring in particular to COD and nitrogen compounds, according to Di Trapani et al. (2014). The analyses were carried out according to the Standard Methods (APHA, 2005). The concentration of free ammonia (FA) was estimated by means of the following equation (Eq. (1)), derived from Hansen et al. (1998):

$$\frac{NH_3\text{-}N}{NH_4^+\text{-}N} = \left(1 + \frac{10^{-pH}}{10^{-\left(0.09018 + \frac{2729.92}{T(k)}\right)}}\right)^{-1} \quad (1)$$

To evaluate the biofilm growth on the suspended carriers, a 20 carriers sample was periodically taken from the bioreactor and dried in an oven and then weighted (W1). After biofilm was removed, the carriers were dried and then weighted again (W2); the amount of the attached biomass was then calculated as W1 − W2. For further details on the aforementioned procedure, the reader is referred to literature (Mannina et al., 2011b).

The MB-MBR pilot plant was started-up with an initial suspended biomass concentration close to 5 g TSS L^{-1} and then was subjected to regular sludge withdrawals, in order to maintain the suspended biomass concentration in the range of 5–6 g TSS L^{-1}. It has to be stressed that the suspended carriers, subjected to the initial cultivation period before starting the experiments, presented a biofilm volumetric concentration almost equal to 4 g TS L^{-1}. In Table 1, the average characteristics of wastewater as well as the main operational features of the system are summarized.

2.3. Microscopic observation

Microscopic observations were carried out for filamentous bacteria identification as well as to observe the potential effects caused by C/N ratio variation. A microscope phase contrast (100× and 1000× magnifications) was used for the observations. The

Table 1
Average influent characteristics in the experimental periods.

Influent composition			
System	Preliminary cultivation	Period 1	Period 2
C/N	10	2.5	15
COD [g L^{-1}]	450	1974	2207
NH_4-N [g L^{-1}]	40	800	150
Conductivity [mS cm^{-1}]	1.9	10.2	5.5
HRT [h]	24	24	24
SRT [d^{-1}]	Indefinite	25	25
pH	7.15	6.91	7.57
Biomass features at the end of the cultivation phase			
Mixed liquor suspended solids [g L^{-1}]	5		
Biofilm [g ST L^{-1}]	4		
Extracellular polymeric substances [mg g^{-1}SS]	200		

filamentous microorganisms were morphologically identified using the Eikelboom classification system. Filamentous microorganism abundance and dominance were estimated using the criteria suggested by Jenkins et al. (2003).

2.4. Respirometric and AUR test analyses

Respirometric experiments were carried out on a "flowing gas/static-liquid" batch respirometer, either on suspended biomass and biofilm samples, taken from the bioreactor of the MB-MBR system. Referring to suspended biomass, the samples were transferred to the respirometer and eventually diluted with permeate, if necessary, in order to obtain a mixed liquor volatile suspended solid (MLVSS) concentration in the range of 2.0–3.0 g VSS L^{-1}. On the other hand, the batch tests on biofilm were carried out with suspended carriers and permeate, by imposing in the respirometer the same filling ratio of the MB-MBR pilot plant. Before running the respirometric test, each sample was aerated until endogenous conditions were reached. For further details on the adopted procedure, the reader is referred to literature (Di Trapani et al., 2011). In the batch tests aimed to evaluate the heterotrophic biokinetic parameters, the nitrifying biomass was inhibited by adding 10 mg L^{-1} of Allylthiourea (ATU), while the exogenous oxygen uptake rate (OUR) was enhanced by the addition of a readily biodegradable organic substrate (sodium acetate in this case). The substrate biodegradation rate was assumed proportional to the exogenous OUR, according to the following expression:

$$\Delta COD = \frac{\Delta O_2}{1 - f_{cv} \cdot Y_H} \tag{2}$$

where f_{cv} is the conversion coefficient from COD to VSS, assumed equal to 1.42 mg COD mg^{-1} VSS, while Y_H is the yield coefficient [mg VSS mg^{-1} COD]. The yield coefficient Y_H has been derived from the integral of the exogenous OUR chart, according to the methodology suggested by Vanrolleghem et al. (1999). Furthermore, the maximum heterotrophic growth rate $\mu_{H,max}$ (d^{-1}) and the half saturation coefficient K_S (mg COD L^{-1}) were evaluated by solving the Monod-type kinetic expression with the finite difference procedure, by fitting the following equation:

$$\frac{\Delta COD}{\Delta t} = \frac{\mu_{H,max}}{Y_H} \cdot \frac{COD}{(K_S + COD)} \cdot X_H \tag{3}$$

where COD is the carbonaceous substrate concentration (mg L^{-1}) at time t, X_H is the biomass active fraction (mg VSS L^{-1}), while $\mu_{H,max}$ and K_S have been previously defined. The estimation of the endogenous decay coefficient b_H and the heterotrophic active fraction were carried out according to the "single batch test" (among others Ramdani et al., 2010; Di Trapani et al., 2014). Briefly, the biomass samples were subjected to aerobic digestion for several days (at least 5, in the present study) without external substrate addition and the endogenous respiration rate was monitored; b_H was then derived from the slope of the respiration/time linear regression curve.

The estimation of the kinetic parameters for the autotrophic population was carried out with the same procedure. Nevertheless, in this case no inhibiting substance like ATU was added and ammonium chloride (NH_4Cl) was spiked to evaluate the biokinetic parameters. During the batch tests, the pH values were constantly monitored to avoid inhibition of the process; the conversion factor between oxygen and ammonium (NOD: nitrogen oxygen demand) is equal to:

$$\Delta NH_4\text{-}N = \frac{\Delta O_2}{4.57} \tag{4}$$

Furthermore, in order to highlight the nitrification behavior of both suspended biomass and biofilm, ammonium uptake rate (AUR) tests were performed by adopting a modified protocol derived by Kristensen et al. (1992). For further details on AUR test the reader is addressed to literature (Di Trapani et al., 2013).

2.5. EPS and fouling resistance analysis

The Extracellular Polymeric Substances (EPSs) were measured during the whole experimental campaign; the soluble EPSs or soluble microbial products (SMP) were obtained by centrifugation at 5000 rpm for 5 min, while the bound EPS (EPS_{Bound}) content was extracted by means of the thermal extraction method (among others Zhang et al., 1999; Cosenza et al., 2013). The extracted EPS_{Bound} and the SMP were analysed for proteins by using the Folin method with bovine serum albumin as the standard (Lowry et al., 1951), whereas the carbohydrates according to DuBois et al. (1956), which yields results as glucose equivalent. Moreover, the sum of proteins and carbohydrates content was considered as the total EPSs (EPS_T), according to the following equation:

$$EPS_T = \underbrace{EPS_P + EPS_C}_{EPS_{Bound}} + \underbrace{SMP_P + SMP_C}_{SMP} \tag{5}$$

where the subscripts "P" and "C" indicate the content of proteins and carbohydrates, respectively in the EPS_{Bound} and SMP, that typically constitute the main fractions (Drews et al., 2007). For further details on EPSs measurement, the reader is addressed to literature (Di Bella et al., 2011).

Concerning the analysis of membrane fouling, the total resistance to filtration (R_T) was described by the general form of the Darcy's law:

$$R_T = \frac{TMP}{J\,\mu} \tag{6}$$

where R_T is the total fouling resistance (10^{12} m^{-1}) calculated by the general form of Darcy's Law, TMP is the transmembrane pressure (Pa), μ the permeate viscosity (Pa s), and J the permeation flux (m s^{-1}). Furthermore, in order to investigate the specific deposition mechanisms, the resistance-in-series (RIS) model, based on cake layer removal with "extraordinary physical cleaning", was employed (among others Meng et al., 2005; Meng and Yang, 2007; Zhiwei et al., 2009). In particular, according to this approach the membrane fouling mechanisms can be evaluated by means of ordinary cleaning actions (e.g. backwashing) and extraordinary physical cleaning actions (e.g. hydraulic washing and mechanical scrubbing). The superficial cake deposition (irreversible or reversible) is determined by the calculation of permeate flux and TMP measurement before and after cake layer removal from the membrane surface (see for instance Mannina and Di Bella, 2012). More specifically, during normal operation of the system the total resistance to filtration is defined by:

$$R_T = R_m \underbrace{+R_{PB} + R_{c,irr} + R_{c,rev}}_{R_F} \tag{7}$$

where R_m is the intrinsic resistance of membrane and was estimated by measuring the water flux of ultrapure water; R_{PB} is the fouling resistance related to particle deposition into membrane pores, that can be partially removed by chemical cleaning only; $R_{c,irr}$ is the fouling resistance related to superficial cake deposition that can be removed by an extraordinary physical cleaning (hydraulic/water washing); $R_{c,rev}$ is the fouling resistance related to superficial cake deposition that can be simply removed by ordinary backwashing. These contributions can be evaluated by applying the aforementioned RIS model, according to the analysis discussed in Di Trapani et al. (2014).

3. Results and discussion

3.1. MB-MBR pilot performance

In Fig. 1 the inlet, mixed liquor supernatant and permeate COD concentrations (Fig. 1a) as well as nitrogen forms (Fig. 1b) are reported. The experimental results suggested that the C/N ratio and the high strength of wastewater (especially in terms of ammonia loading rate) had a significant influence on system performances. Particularly, due to the high loading rates, relevant differences were observed in the biological and hydraulic performances.

As noticeable from Fig. 1(a), the COD removal efficiencies during both the Period 1 and 2 remained high, despite the low C/N ratio and the high strength wastewater, with average COD removal efficiency equal to 95–96%. In particular, the biological efficiency was 90% (as average) or higher in both periods. Consequently, the COD and BOD concentrations in the effluent were well within the Italian standards for the discharge of treated wastewater, during the whole experiments. More specifically, the high COD removal was likely due to a double effect: one was the contribution of both attached and suspended biomass; the second one was the membrane filtration which worked as a physical barrier toward all pollutants characterized by an average dimension bigger than the membrane pores. Therefore, from the beginning of each period, even though the microorganisms were not perfectly acclimated to the new influent features (due to the C/N variation), the membrane operated by retaining/trapping, inside the bioreactor, most of non-biodegraded particulate COD and a portion of the soluble COD (in the range of 0.04–0.45 μm, respectively membrane pores and porosity of filters used in the laboratory for soluble COD determination).

On the contrary, the C/N ratio played a relevant role in the biological nitrogen utilization process, exerting a significant stress effect on the bacterial populations, as shown in Fig. 1(b). As discussed above, the influent NH_4-N and N_{tot} concentrations were significantly different compared to conventional municipal wastewater. Nevertheless, some interesting aspects could be drawn from the analysis of the results obtained in both experimental periods, especially for the nitrification process.

More specifically, despite it was supposed to have significant autotrophic bacteria concentration, due to the complete retention operated by the membrane, the nitrification performance in Period 1 was quite low. In particular, both elevated pH (average value in Period 1 equal to 8.33) and high ammonium nitrogen concentration (close to 800 mg NH_4-N L^{-1}) contributed to significant levels of FA in the mixed liquor (32.7 mg NH_3-N L^{-1}, as average). This condition could have caused a stress effect on the growth of nitrifying populations, referring in particular to NOB species, which are highly sensitive to FA (Cydzik-Kwiatkowska et al., 2013). Indeed, according to Cydzik-Kwiatkowska and co-workers, NOB species are inhibited at as low FA concentration as 0.1–1.0 mg NH_3-N L^{-1}, whereas AOB are less sensitive since they are inhibited in the range of 10–150 mg NH_3-N L^{-1}. From Fig. 1(b) it is possible to notice, besides the high ammonium nitrogen concentrations in the permeate, the accumulation of nitrite nitrogen, while no nitrate nitrogen was contained in the permeate. Moreover, also the salinity level (indeed, ammonium nitrogen was added as NH_4Cl) could have influenced the activity of nitrifying bacteria, depressing in particular the NOB species. Previous studies revealed that FA, free nitrous acid (FNA) and salinity level can exert a huge stress on NOB population (Qiao et al., 2010; Yogalakshmi and Joseph, 2010; Cydzik-Kwiatkowska et al., 2013). Therefore, the high ammonia loading rate could likely promote a shortened nitrification with nitrite accumulation as final product. Indeed, nitrite concentrations represented the 75% of the sum of oxidized nitrogen forms in Period 1. On the contrary, in Period 2 the residual nitrogen in the permeate is almost all in the oxidized form (nitrites plus nitrates) and the majority of the inlet ammonium is removed for satisfying the metabolic needs of heterotrophs (5% of the organic content removed, ~95–100 mg NH_4-N L^{-1}). Actually, the overall nitrification efficiency was in the range of 40–50%, value that is lower respect to previous literature studies (Di Bella et al., 2010). In the lights of the above comments, such a result was likely related to the inhibition of NOB species, referring to both suspended biomass and biofilm. This aspect is of importance since biofilm should be more specialized in the nitrification process, considering the high retention time of the biofilm inside the bioreactor (Di Trapani et al., 2014).

However, as better outlined in the following Section 3.4 (respiratory activity and biokinetic parameters), it was noticed a significant difference in the behavior of suspended biomass and biofilm, in terms of AOB activity. Indeed, despite the reduced levels of FA in the bulk liquid (12.42 mg NH_3-N L^{-1}, as average), the activity of AOB in the suspended biomass was still depressed, whereas the AOB activity within the biofilm was restored in Period 2, with high oxidation rates. Furthermore, it was noticed a slow increase of NOB activity inside the biofilm producing as a result a reduction of the percentage of nitrite concentrations compared to Period 1, with average value equal to 65% of the oxidized nitrogen forms.

In is worth mentioning that no denitrification process occurred and the total nitrogen removal was mainly due to biological assimilation by bacteria for cellular synthesis processes. In Table 2, the performance of AOB and NOB microorganisms (average values) are shown.

In conclusion, comparing the results of the overall experimental campaign, it is worth noting that nitrification was significantly influenced in both periods, contrarily to organic matter removal.

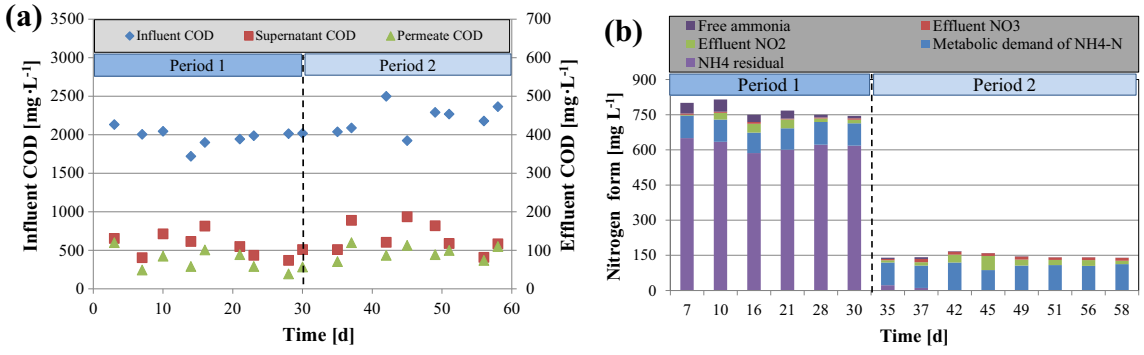

Fig. 1. COD concentration trends (a) and nitrogen forms (b), respectively.

Table 2
Average performance of AOB and NOB.

Microorganism	Phenomenon	End of cultivation (%)	Period 1 (%)	Period 2 (%)
AOB	η NH_4-N → NO_2-N	95%	25–30%	95%
NOB	η NO_2-N → NO_3-N	98%	15–20% (of nitrite previously formed)	40–50% (of nitrite previously formed)
AOB + NOB	η NH_4-N → NO_3-N	95%	<10%	40–45%

In Period 1, with C/N = 2.5, the high concentration of ammonium in the influent depressed the nitrification kinetic and only a small part of NH_4-N could be oxidized with the established HRT. On the other hand, when the C/N ratio was increased to 15, all the influent ammonium was oxidized to nitrite but only a limited portion was further oxidized to nitrate due to the inhibition of NOB. It can be suggested that longer durations are required to restore the whole nitrification ability of the system.

3.2. MLSS distribution and biofilm growth

As aforementioned, the experimentation carried out with the MB-MBR pilot plant started up with a sludge concentration equal to 5 g TSS L^{-1} and then it was maintained close to 6 g TSS L^{-1} throughout the whole experimental campaign. Concerning the biofilm, after the cultivation phase its volumetric concentration was equal to 4 g TS L^{-1} at the beginning of the experiments. However, at the beginning of Period 1, likely due to the high ammonium and saline levels, it was noticed a significant detachment from the carriers, with biofilm concentrations that decreased to 2.25 g TS L^{-1}. Such a result could be likely related to a stress effect caused by the environmental conditions on the biofilm. In Period 2, it was observed a gradual increase of biofilm concentrations, with values up to 3.77 g TS L^{-1}. This result could be likely due to the more suitable environmental conditions in Period 2, that enhanced biofilm acclimation and growth. The data of MLSS and biofilm concentration are shown in Fig. 2.

3.3. Microscopic observations

As aforementioned, qualitative microscopic observations were carried out on mixed liquor samples; they revealed in Period 1 the presence of "Type 021N" as dominant filamentous microorganism; its abundance could be justified by the features of the inlet wastewater, characterized by high loading rate of readily biodegradable COD (sodium acetate). In Period 2, on the contrary, it was noticed a relative abundance of filamentous species, only

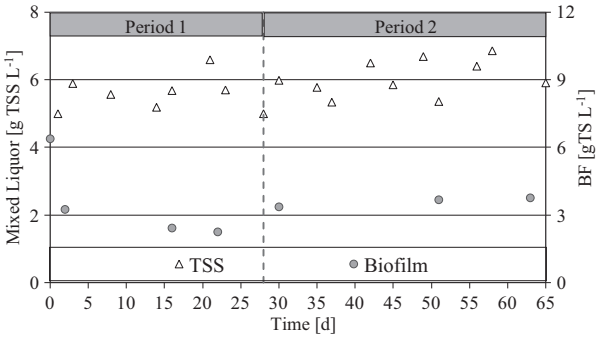

Fig. 2. Biofilm and suspended biomass growth.

apparently similar to Nocardioform organisms, belonging to *bacillus* gram stain positive, that sometimes can grow as filamentous organisms.

3.4. Biomass respiratory activity and biokinetic parameters

In Table 3 the biokinetic parameters (as average values) referred to both suspended biomass and biofilm are summarized, whereas an example of the typical respirogram charts obtained, respectively in Period 1 and Period 2, is available as Fig. S.2 in the SI.

The observed results reported in Table 3 highlighted that the C/N ratio did not exert a significant influence on the heterotrophic respiratory activity, with the respirogram charts featuring the typical exogenous and endogenous respiration phases after substrate spiking. Indeed, the biokinetic parameters were almost in the range of what reported in the technical literature (Hauduc et al., 2011), with high specific respiration rates and biomass growth, referring in particular to the suspended biomass, likely due to the high organic strength of the inlet wastewater. According to previous results (Di Trapani et al., 2014), it was noticed a sort of "specialization" of the suspended biomass that seemed more

Table 3
Summary of biokinetic parameters throughout the experimental campaign.

	Period 1 (C/N = 2.5)		Period 2 (C/N = 15)	
	Susp. biomass	Biofilm	Susp. biomass	Biofilm
Heterotrophic				
Y_H [mg COD mg^{-1} COD]	0.50 (±0.06)	0.51 (±0.01)	0.47 (±0.09)	0.49 (±0.05)
Y_{STO} [mg COD mg^{-1} COD]	0.75 (±0.03)	0.69 (±0.05)	0.75 (±0.02)	0.74 (±0.1)
$\mu_{H,max}$ [d^{-1}]	15.81 (±3.29)	3.45 (±1.78)	13.44 (±0.04)	5.64 (±2.32)
K_S [mg COD L^{-1}]	7.08 (±2.17)	8.39 (±5.88)	36.11 (±36.92)	7.50 (±3.54)
b_H [d^{-1}]	0.71 (±0.25)	0.41 (±0.19)	0.63 (±0.21)	0.64 (±0.15)
$SOUR_{max}$ [mg O_2 g^{-1} TSS h^{-1}]	78.54 (±22.92)	18.23 (±12.43)	86.43 (±16.93)	19.63 (±4.99)
Autotrophic				
Y_A [mg COD mg^{-1} N]	–	–	–	0.21 (±0.03)
$\mu_{A,max}$ [d^{-1}]	–	–	–	0.32 (±0.11)
K_{NH} [mg NH_4-N L^{-1}]	–	–	–	2.49 (±1.83)
$SOUR_{max}$ [mgO_2 g^{-1} TSS h^{-1}]	–	–	–	8.72 (±4.41)
Nitrif. rate [mg NH_4-N L^{-1} h^{-1}]	–	–	–	6.54 (±3.65)

Y_H = heterotrophic yield coefficient, Y_{STO} = storage yield coefficient, $\mu_{H,max}$ = maximum heterotrophic growth rate, K_S = heterotrophic half-saturation coefficient, b_H = heterotrophic decay coefficient, $SOUR_{max}$ = specific respiration rate (for both heterotrophic and autotrophic populations), Y_A = autotrophic yield coefficient, $\mu_{A,max}$ = maximum autotrophic growth rate, K_{NH} = autotrophic half-saturation coefficient.

competitive in organic matter removal. Furthermore, in the heterotrophic batch tests it was observed a "storage" phenomenon, related to the dynamic conditions occurring in the bioreactor. This situation likely enhanced the growth of bacterial groups able to rapidly convert the organic substrate into storage products. Such a behavior is clearly depicted in Fig. S2(a,c,e,g), reporting typical respirogram charts of heterotrophic species. The storage yield coefficient Y_{STO} was evaluated according to the procedure proposed by Karahan-Gül et al. (2002). Concerning autotrophic population, according to Section 3.1, the respirometric batch tests highlighted that in Period 1 no significant nitrification occurred, referring to both suspended biomass and biofilm. As observed above, the high ammonium concentration coupled to the salinity of the inlet wastewater likely inhibited the activity of nitrifying populations. On the contrary, in Period 2 (C/N ratio equal to 15), when either ammonium loading rate and salt concentration were decreased, a different behavior was observed. Indeed, while the suspended biomass was again characterized by a negligible nitrifying activity, inside the biofilm a gradual growth of nitrifiers was observed, as highlighted in the respirogram chart reported in Fig. S2(h), suggesting a higher robustness of biofilm compared to the activated sludge.

These results were confirmed by the AUR batch tests performed in Period 2. In Fig. 3, as an example, the results of a typical AUR test carried out either on activated sludge (Fig. 3a) and biofilm (Fig. 3b) are shown. Concerning the suspended biomass, no significant nitrifying activity was observed, with ammonium, nitrite and nitrate concentrations almost constant during the whole batch test duration (ammonia oxidation rate: 0.33 mg NH_4-N g^{-1} VSS h^{-1}). On the contrary, the biofilm showed a different behavior, highlighting a decrease of ammonium concentration with a consequent accumulation of nitrite nitrogen (ammonia oxidation rate: 5.79 mg NH_4-N g^{-1} VSS h^{-1}), suggesting that the AOB activity in the biofilm was restored in Period 2. Contrarily, the nitrate concentrations were almost constant during the batch test, suggesting that NOB species were inhibited even in Period 2 when ammonium and salinity were decreased, confirming the higher sensitivity of NOB to environmental conditions (Sudarno et al., 2011). Therefore, it is possible to conclude that a longer time would be required to recover NOB activity, in order to sustain a complete nitrification process.

3.5. Membrane fouling and identification of causes

Fig. 4 reports the fouling resistance trend, in terms of total and specific contributions.

As shown in Fig. 4(a), during the experiments nine extraordinary cleaning operations were conducted. These actions were necessary in order to prevent the TMP exceeding the critical values defined by the membrane manufacturer (0.5–0.6 bar). In general, as reported in Fig. 4(b), the irreversible resistance due to superficial cake deposition represented the majority of total hydraulic resistance to filtration. When the C/N ratio was increased (Period 2), the contribution of the internal irreversible fouling (pore blocking) changed according to the values reported in the Fig. 4(c) (in terms of percentage of total fouling). More specifically, in Period 1 the percentage of membrane fouling due to pore blocking mechanism (R_{PB}) was in the range of 3–5% of the total resistance (R_T). Conversely, at the end of Period 2 this contribution was 3 times higher compared to the average value obtained in Period 1, reaching a value close to 18% of the R_T.

This circumstance was likely due to the different concentration of EPS_{Bound} and SMP in the two experimental periods. The general trend of EPS_T as well as the specific contribution in terms of bound and soluble products are reported in Fig. 5(a and b).

In particular, from Fig. 5(a and b) it is possible to notice that the concentration of SMP significantly increased in Period 2, whereas the average concentration of EPS_{Bound} remained almost constant. In particular, at the end of Period 2 (day 45 to day 60), carbohydrates and proteins in the soluble fraction reached a value almost equal to 15% of EPS_T, while in Period 1 it was lower than 3%, as average. This variation of EPS_T composition, mainly as SMP fraction, could likely promote the increase of the mixed liquor hydrophobicity. Indeed, the sharp variation of the C/N ratio could produce a release of polymeric substances into the mixed liquor, as a consequence of a stress effect on the biomass. Consequently, a worsening of the "biological filter" effect of the cake layer on the membrane surface could compromise the filtration efficiency and the effectiveness of the pre-filter action (Mannina et al., 2011a).

The strong relation between fouling resistance and EPSs concentration is confirmed by the graphs reported in Fig. 6, where the main correlations between specific EPS fractions and resistance contributions are shown.

In particular, according to the technical literature (Judd, 2011), when the concentration of EPS_T in the mixed liquor increased, the membrane resistance increased, highlighting a worsening of the hydraulic performance of the system (see Fig. 6a). However, the relationship between EPSs production and membrane fouling tendency is a complex topic that deserves to be further investigated (Lin et al., 2014).

In particular, it is worth noting that a detailed analysis of the correlation between EPS fractions (both bound and soluble) and the specific fouling mechanisms (e.g. $R_{c,rev}$ and/or R_{PB}), revealed a particular behavior that is quite interesting. Indeed, the $R_{c,rev}$ is significantly lower when the EPS_T concentration (mainly bound, as shown in Fig. S3 in the SI) increased. This result was only apparently surprising: indeed, it is in good agreement with the aforementioned considerations. In fact, the increase of the C/N ratio caused the increase of EPS_T concentration (as average) in the mixed liquor, mainly due to a significant increase of the protein content, which is the most hydrophobic fraction (during experiments the average values of sludge hydrophobicity were equal to 93.13%).

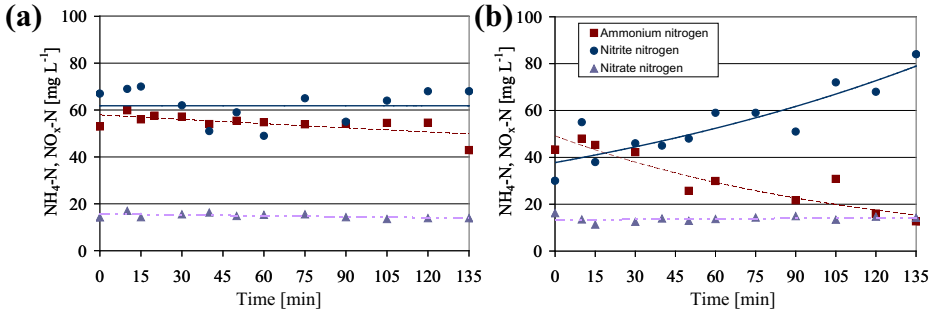

Fig. 3. Result of a typical AUR test carried out either on (a) suspended biomass and (b) biofilm, respectively.

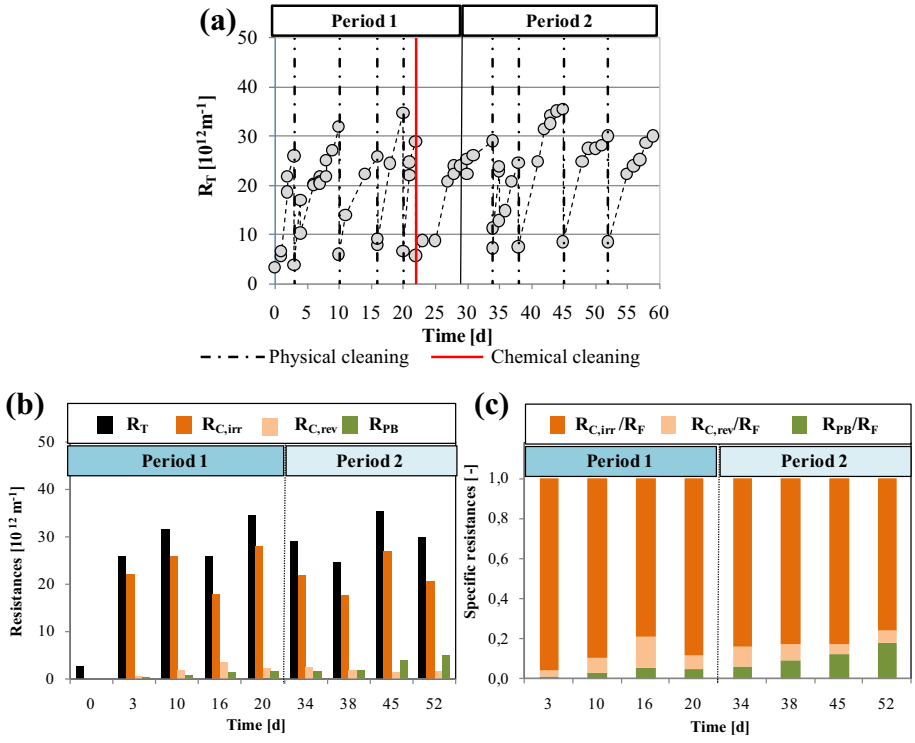

Fig. 4. Total (a) and specific (b, c) fouling resistances, respectively.

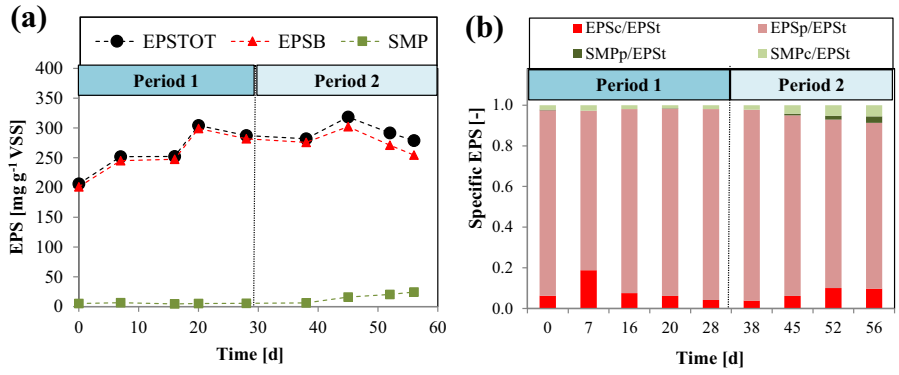

Fig. 5. Total (a) and specific (b) EPSs, respectively.

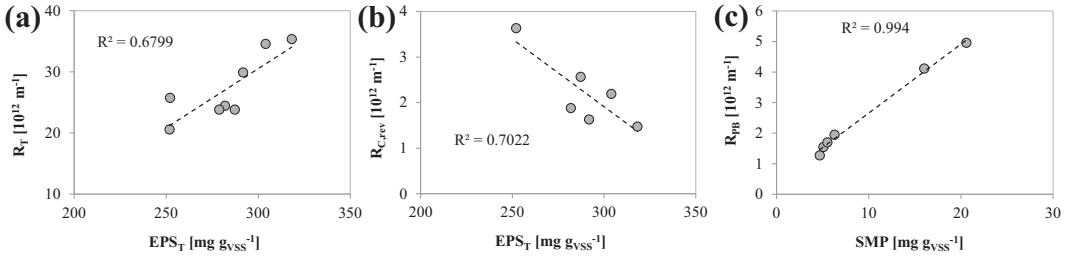

Fig. 6. Correlation between total resistance and EPS_T (a), reversible resistance of cake and EPS_T (b), and resistance of pore blocking and SMP (c).

The activated sludge, and consequently the cake layer, is more "bloated", similarly to what happens in the bulking phenomenon that occurs in CAS plants (Jenkins et al., 2003): therefore, the activated sludge deposited on the membrane surface (cake layer) is characterized by a low resistance (Mannina et al., 2011a). This circumstance negatively affects (as previously mentioned) the pre-filter effect of the cake layer (biological membrane). Consequently, the foulants can reach more easily the internal pores of the membrane, thus compromising its service life. The observed data highlight that the increase of SMP in Period 2 provides a net increase of the R_{PB} (see Fig. 6c). As final remark, with the increase of the C/N ratio the membrane fouling did not worsen, in terms of

total resistance to filtration, but it was observed a change of the specific deposition mechanisms. In particular, the contribution of the irreversible deposition increased more quickly than the reversible one. This result seems to be related primarily to an increase of the protein fraction of EPS_T, referring in particular to the soluble fraction.

4. Conclusions

The effect of a C/N variation in a MB-MBR pilot plant treating high strength wastewater was investigated. The plant showed good removal efficiencies referring to organic matter. Contrarily, nitrification was severely affected by the low C/N value and high ammonia concentrations. Referring to membrane fouling it was noticed an increase of R_{PB}, likely due to a worsening of cake layer features, reducing the "pre-filter" effect, also related to the increase of EPS_T with the C/N ratio. These findings can be useful information for a proper operation of MB-MBR systems characterized by sharp variation of C/N and high ammonium loading rates.

Acknowledgements

This research was funded by the National Operational Programme for Research and Competitiveness 2007–2013. Project STI-TAM (Sviluppo di Tecnologie Innovative per il trattamento di rifiuti liquidi della navigazione finalizzate alla Tutela dell'Ambiente Marino) – PON 02_00153_2849085 – CUP B61C12000840005; Italian Ministry of Education, University and Research and Ministry of Economic Development. Part of this research has been also financially supported by the Italian Ministry of Education, University and Research with the Research project of national interest PRIN 2010–2011 (D.M. 1152/ric 27/12/2011, prot. 2010 WLNFYZ) entitled 'Emerging contaminants in air, soil, and water: from source to the marine environment'.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2015.03.143.

References

APHA, 2005. Standard Methods for the Examination of Water and Wastewater. APHA, AWWA and WPCF, Washington, DC, USA.
Choi, C., Lee, J., Kwangho Lee, K., Kim, M., 2008. The effects on operation conditions of sludge retention time and carbon/nitrogen ratio in an intermittently aerated membrane bioreactor (IAMBR). Bioresour. Technol. 99, 5397–5401.
Cosenza, A., Di Bella, G., Mannina, G., Torregrossa, 2013. The role of EPS in fouling and foaming phenomena for a membrane bioreactor. Bioresour. Technol. 147, 184–192.
Cydzik-Kwiatkowska, A., Zielińska, M., Bernat, K., Wojnowska-Baryła, I., Truchan, T., 2013. Treatment of high-ammonium anaerobic digester supernatant by aerobic granular sludge and ultrafiltration processes. Chemosphere 90, 2208–2215.
Di Bella, G., Durante, F., Torregrossa, M., Viviani, G., 2010. Start-up with or without inoculum? Analysis of an SMBR pilot plant. Desalination 260, 79–90.
Di Bella, G., Torregrossa, M., Viviani, G., 2011. The role of EPS concentration in MBR foaming: analysis of a submerged pilot plant. Bioresour. Technol. 102, 1628–1635.
Di Trapani, D., Mannina, G., Torregrossa, M., Viviani, G., 2008. Hybrid moving bed biofilm reactors: A pilot plant experiment. Water Sci. Technol. 57, 1539–1545.
Di Trapani, D., Mannina, G., Torregrossa, M., Viviani, G., 2010. Comparison between hybrid moving bed biofilm reactor and activated sludge system: A pilot plant experiment. Water Sci. Technol. 61, 891–902.
Di Trapani, D., Capodici, M., Cosenza, A., Di Bella, G., Mannina, G., Torregrossa, M., Viviani, G., 2011. Evaluation of biomass activity and wastewater characterization in a UCT-MBR pilot plant by means of respirometric techniques. Desalination 269, 190–197.
Di Trapani, D., Christensson, M., Torregrossa, M., Viviani, G., Ødegaard, H., 2013. Performance of a hybrid activated sludge/biofilm process for wastewater treatment in a cold climate region: influence of operating conditions. Biochem. Eng. J. 77, 214–219.
Di Trapani, D., Di Bella, G., Mannina, G., Torregrossa, M., Viviani, G., 2014. Performance of a MBR pilot plant treating high strength wastewater subject to salinity increase: analysis of biomass activity and fouling behaviour. Bioresour. Technol. 162, 60–69.
Drews, A., Mante, J., Iversen, V., Vocks, M., Lesjean, B., Kraume, M., 2007. Impact of ambient conditions on SMP elimination and rejection in MBRs. Water Res. 41, 3850–3858.
DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.
Fu, Z., Yang, F., Zhou, F., Xue, Y., 2009. Control of COD/N ratio for nutrient removal in a modified membrane bioreactor (MBR) treating high strength wastewater. Bioresour. Technol. 100, 136–141.
Hansen, K.H., Angelidaki, I., Ahring, B.K., 1998. Anaerobic digestion of swine manure: inhibition by ammonia. Water Res. 32, 5–12.
Hauduc, H., Rieger, L., Ohtsuki, T., Shaw, A., Takács, I., Winkler, S., Héduit, A., Vanrolleghem, P.-A., Gillot, S., 2011. Activated sludge modelling: development and potential use of a practical applications database. Water Sci. Technol. 63, 2164–2182.
Jenkins, D., Richard, M.G., Daigger, G.T., 2003. Manual on the Causes and Control of Activated Sludge Bulking, Foaming and Other Solids Separation Problems. IWA Publishing, London.
Judd, S., 2011. The MBR Book: Principles and Applications of Membrane Bioreactors in Water and Treatment, second ed. Elsevier, Oxford.
Karahan-Gül, Ö., Artan, N., Orhon, D., Henze, M., van Loosdrecht, M.C.M., 2002. Respirometric assessment of storage yield for different substrates. Water Sci. Technol. 46, 345–352.
Kristensen, G.H., Jørgensen, P.E., Henze, M., 1992. Characterization of functional microorganism groups and substrate in activated sludge and wastewater by AUR, NUR and OUR. Water Sci. Technol. 25, 43–57.
Leyva-Díaz, J.C., Calderón, K., Rodríguez, F.A., González-López, J., Hontoria, E., Poyatos, J.M., 2013. Comparative kinetic study between moving bed biofilm reactor-membrane bioreactor and membrane bioreactor systems and their influence on organic matter and nutrients removal. Biochem. Eng. J. 77, 28–40.
Lin, H., Zhang, M., Wang, F., Meng, F., Liao, B.-Q., Hong, H., Chen, J., Gao, W., 2014. A critical review of extracellular polymeric substances (EPSs) in membrane bioreactors: characteristics, roles in membrane fouling and control strategies. J. Membr. Sci. 460, 110–125.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.
Mannina, G., Di Bella, G., 2012. Comparing two start-up strategies for MBRs: experimental study and mathematical modelling. Biochem. Eng. J. 68, 91–103.
Mannina, G., Di Bella, G., Viviani, G., 2011a. An integrated model for biological and physical processes in membrane bioreactors (MBR). J. Membr. Sci. 376, 56–69.
Mannina, G., Di Trapani, D., Viviani, G., Ødegaard, H., 2011b. Modelling and dynamic simulation of hybrid moving bed biofilm reactors: model concepts and application to a pilot plant. Biochem. Eng. J. 56 (1–2), 23–36.
Meng, F., Yang, F., 2007. Fouling mechanisms of deflocculated sludge, normal sludge, and bulking sludge in membrane bioreactor. J. Membr. Sci. 305, 48–56.
Meng, F., Zhang, H.M., Li, Y.S., Zhang, X.W., Yang, F.L., Xiao, J.N., 2005. Cake layer morphology in microfiltration of activated sludge wastewater based on fractal analysis. Sep. Purif. Technol. 44, 250–257.
Ødegaard, H., 2006. Innovations in wastewater treatment: the moving bed biofilm process. Water Sci. Technol. 53, 17–33.
Qiao, S., Matsumoto, N., Shinohara, T., Nishiyama, T., Fujii, T., Bhatti, Z., Furukawa, K., 2010. High-rate partial nitrification performance of high ammonium containing wastewater under low temperatures. Bioresour. Technol. 101, 111–117.
Ramdani, A., Dold, P., Déléris, S., Lamarre, D., Gadbois, A., Comeau, Y., 2010. Biodegradation of the endogenous residue of activated sludge. Water Res. 44, 2179–2188.
Stephenson, T., Judd, S., Jefferson, B., Brindle, K., 2000. Membrane Bioreactors for Wastewater Treatment. IWA Publishing, London, UK.
Sudarno, U., Winter, J., Gallert, C., 2011. Effect of varying salinity, temperature, ammonia and nitrous acid concentrations on nitrification of saline wastewater in fixed-bed reactors. Bioresour. Technol. 102, 5665–5673.
Vanrolleghem, P.A., Spanjers, H., Petersen, B., Ginestet, P., Takacs, I., 1999. Estimating (combination of) Activated Sludge Model No. 1 parameters and component by respirometry. Water Sci. Technol. 39, 195–214.
Yang, W., Syed, W., Zhou, H., 2014. Comparative study on membrane fouling between membrane-coupled moving bed biofilm reactor and conventional membrane bioreactor for municipal wastewater treatment. Water Sci. Technol. 69, 1021–1027.
Yogalakshmi, K.N., Joseph, K., 2010. Effect of transient sodium chloride shock loads on the performance of submerged membrane bioreactor. Bioresour. Technol. 101, 7054–7061.
Zhang, X.Q., Bishop, P.L., Kinkle, B.K., 1999. Comparison of extraction methods for quantifying extracellular polymers in biofilms. Water Sci. Technol. 39, 211–218.
Zhiwei, W., Zhichao, W., Shujuan, T., 2009. Extracellular polymeric substances (EPS) properties and their effects on membrane fouling in a submerged membrane bioreactor. Water Res. 43, 2504–2512.