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Analysis of Biomass Characteristics in MBR and MB-MBR Systems Fed with Synthetic Wastewater: Influence of a Gradual Salinity Increase

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The paper presents the results of a field gathering campaign carried out on two different pilot scale membrane bioreactor (MBR) systems, treating synthetic wastewater subject to a gradual increase of salinity. One was a conventional MBR system, while the other was a moving bed biofilm membrane bioreactor (MB-MBR), which combines suspended biomass and biofilm. Indeed, the presence of suspended carriers inside the bioreactor seems to give benefits due to the collisions between the circulating media and the membrane. The aim of the study was the comparison of two configurations in terms of biomass activity characterization and performance (pollutants removal and hydraulic behaviour). The results highlighted a significant biomass activity for both heterotrophic and autotrophic populations. Such behaviour was emphasized in MB-MBR system, likely due to the presence of biofilm attached on suspended sponge carriers, which is characterized by high retention times, thus improving through the "seeding" effect the nitrification ability of the whole system.

1. Introduction

In recent years, MBRs have been also used for the treatment of specific saline water, such as wastewater produced from shipboard activities, aquaculture wastewater or wastewater deriving by sediment washing treatment (Sun et al., 2010; Di Bella et al., 2013). In presence of high salinity, a modification of biomass characteristics may occur. This circumstance might exert a significant impact in terms of biokinetic as well as membrane fouling behaviour (Yang et al, 2012). Several studies have been carried out on MBRs treating saline wastewater, in order to evaluate the effect of salinity on performance and fouling behaviour (Pendashteh et al., 2012; Jang et al, 2013; Johir et al., 2013). Recently, a combination of MBR and biofilm systems has been proposed, usually referred to as moving bed biofilm membrane bioreactor (MB-MBR) (Leyva-Diaz et al., 2013; Khan et al., 2012). Indeed, biofilm processes have been proved to be reliable for organic matter and nutrients removal without suffering the typical problems of suspended biomass processes (Ødegaard, 2006). However, a knowledge gap still exists from the point of view of kinetics and system performance, since MB-MBRs are relatively new and few experiences have been reported in the technical literature so far, especially regarding the effect of salinity (Artiga et al., 2008). In this context, the present study was aimed at comparing the behaviour of two different MBR configurations: a conventional MBR process and a MB-MBR system. In detail, the MB-MBR pilot scale was characterized by the presence of soft polyurethane sponges, freely moving inside the bioreactor, as carrier material for biofilm attachment and growth. The major aim was to assess the effect of a gradual salinity increase on the performance of both systems, in terms of carbon and ammonium removal as well as to evaluate the biokinetic parameters by means of respirometry. A secondary aim was to analyze the extracellular polymeric substances (EPSs) production and the membrane filtration resistance in both systems, in order to evaluate if the suspended carriers could provide a benefit in terms of fouling behaviour. In the paper, the first results of an experimental campaign are presented and discussed.

2. Materials and Methods

2.1 MBR and MB-MBR pilot plants description

The pilot plants were built at the Laboratory of Sanitary and Environmental Engineering of Enna Kore University (Italy). One was a conventional MBR system and the other one was a MB-MBR system, characterized by the presence of soft polyurethane sponges as carrier material for biofilm growth. The bioreactor of the MB-MBR line was fed with Linpor[®] carriers, with a 25% filling fraction. Both plants were characterized by identical volumes (17 L) and solid-liquid separation phase, the latter realized with an Ultra Filtration (UF) hollow fiber membrane module (*ZeeWeedTM 01*, with specific area equal to 0.1 m² and nominal porosity of 0.04 μ m). The membrane was periodically backwashed (every 5 min for a period of 1 min) by pumping a fraction of permeate back through the membrane module, while the permeate flux was constantly kept at 15 L m⁻² h⁻¹. In Figure 1, the layout of the pilot plants is reported.



Figure 1. Picture and lay-out scheme of the MBR and MB-MBR pilot plant.

The pilot plants have been operated for 65 days and were fed with synthetic wastewater with chemical oxygen demand (COD) and ammonium (NH₄-N) concentrations respectively equal to 700 mg L⁻¹ of 35 mg L⁻¹ (as average). The overall campaign was divided in five phases, each characterized by a different NaCl dosage to the synthetic wastewater (phase I: no salt addition, phase II: 2 g NaCl L⁻¹; phase III: 5 g NaCl L⁻¹; phase IV: 10 g NaCl L⁻¹; phase V: 15 g NaCl L⁻¹). In more detail, from day 0 to day 30, the salinity of the feed wastewater was gradually increased up to 15 g NaCl L⁻¹, which represented the designed steady state value; afterwards, the latter was maintained constant from day 30 to day 65.

2.2 Analytical Methods

During the whole period of operations, the influent wastewater, mixed liquor and effluent permeate have been sampled 3 times per week and analyzed for total and volatile suspended solids (TSS and VSS), COD, NH₄-N, nitrite nitrogen (NO₂-N), nitrate nitrogen (NO₃-N) and phosphate (PO₄-P). All analyses have been carried out according to the Standard Methods (APHA, 2005). Periodically, samples of suspended carriers were taken and analyzed for TSS and VSS in order to evaluate the biofilm growth on carriers; for the details on the adopted procedure, the reader is referred to literature (Di Trapani et al., 2013).

2.3 Description of the respirometric batch test

Respirometric batch experiments were conducted using a "flowing gas/static-liquid" type as batch respirometer (Spanjers et al., 1996). During the batch tests, the biomass samples were maintained at a constant temperature of 20 ± 1 °C with a thermostatic cryostat. For further details on the adopted procedure, the reader is referred to literature (Di Trapani et al., 2011). In the batch tests aimed at evaluating the heterotrophic biokinetic parameters, the nitrifying biomass has been inhibited by adding 10 mg L⁻¹ of Allylthiourea (ATU), while the exogenous oxygen uptake rate (OUR) was enhanced by addition of sodium acetate (CH₃COONa) as readily biodegradable organic substrate. Concerning the nitrifying biomass, the estimation of the kinetic parameters has been carried out with the same procedure, bearing in mind that no inhibiting substance like ATU has been added and that ammonium chloride (NH₄Cl) has been spiked to evaluate the biokinetic parameters. The autotrophic specific yield coefficient has been evaluated according to the protocol suggested by Chandran and Smets (2001). The respirometric survey started during phase V, which was characterized by influent salinity of 15 g NaCl L⁻¹.

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2.4 EPS extraction and analysis

The EPS was extracted by thermal extraction method (Zhang et al., 1999). More specifically, through this protocol the Soluble Microbial Products (SMPs), that represents the soluble portion of EPS, as well as the EPS_{bound} were measured. In particular, the sum of proteins and carbohydrates content was considered as the total EPS (EPS_T), according to the following equation:

$$EPS_{T} = \underbrace{EPS_{P} + EPS_{C}}_{EPS_{bound}} + \underbrace{SMP_{P} + SMP_{C}}_{Soluble}$$
(1)

where the subscript symbol "P" or "C" indicates the relative content of proteins or carbohydrates respectively in the EPS_{bound} and SMPs. Carbohydrates in the EPS_T were determined according to the phenol–sulphuric acid method with glucose as the standard (DuBois et al., 1956). Proteins were determined by the Folin method with bovine serum albumin as the standard (Lowry et al., 1951).

3. Results and discussion

3.1 Pilot plants performances

Table 1 summarizes the removal efficiencies of the different phases (as average values). During the first days of operation, both plants showed very good performance, with organic matter removals in the range of 80-90%. Furthermore, the contribution of biological removal (referring to the supernatant of mixed liquor filtered at 0.45 µm) was about 86-88% of the total COD removal. After the salinity was gradually increased, a decrease of COD removal was noticed (down to 70%), but only when the salinity reached 10 gNaCl L⁻¹ (phase IV). Such behaviour was more emphasized in the MB-MBR plant likely due to the partial detachment of biofilm. Nevertheless, an additional increase of salinity to 15 g NaCl L⁻¹ influenced negatively the performance of the MBR line only. Due to the hybrid biomass adaptation in the MB-MBR reactor, the performance improved again during phase V, especially referring to nitrification (probably due to biofilm acclimation to a saline environment); this behaviour was not observed in the MBR line.

Indeed, the presence of biofilm in the MB-MBR line enabled very good nitrification (the autotrophic populations inside the biofilm could better respond to the environmental stress exerted by salinity); furthermore, a significant denitrification process was observed in the MB-MBR line, likely due to the establishment of anoxic conditions in the internal portion of biofilm, deriving from limitation of oxygen penetration.

		MBR			MB-MBR				
	Phases	η _{сор-вор} (%)	η_{сор-тот} (%)	η _N (%)	η _{DEN} (%)	η _{сод-вод} (%)	η _{сор-тот} (%)	η _ℕ (%)	η _{DEN} (%)
	I (0 g _{NaCl} L ⁻¹)	82.5	95.3	0	0	72.8	81.8	0	0
Gradual salinity	II (2 g _{NaCl} L ⁻¹)	78.6	88.5	21.3	0	79.2	84.9	27.7	0
increase	III (5 g _{NaCl} L ⁻¹)	89.3	97.3	99.0	0	85.0	86.7	98.1	0
	IV (10 g _{NaCl} L ⁻¹)	66.7	76.6	37.3	0	58.5	67.4	29.1	37.0
Steady-state	V (15 g _{NaCl} L ⁻¹)	57.2	75.8	17.9	6.7	77.4	81.2	63.2	40.0

Table 1. Pilot plant performance

Fig. 2 (a and b) shows the trend of TSS and VSS TSS⁻¹ ratio in the whole experimental period; the VSS TSS⁻¹ ratio is a useful parameter to evaluate if any accumulation of inert matter occurrs in the activated sludge. MBR and MB-MBR were started-up with the same MLSS concentration (about 3.5 g TSS L⁻¹), and it was aimed at maintaining similar MLSS concentrations in both lines during the whole experimentation. However, due to the higher "vitality" of biomass in the MB-MBR line compared to the MBR one (as better discussed in the next paragraph), more frequent sludge withdrawals were necessary in the MB-MBR line. For this reason, the sludge retention time (SRT) of MB-MBR was lower than that of MBR (about 25 and 45 days respectively). It is worth mentioning that the sludge of MBR was therefore subject to a gradual phenomenon of mineralization, which was more evident compared to MB-MBR. Concerning the biofilm, at the end of experimental period (Phase V), the volumetric concentration was about 10 gTS L⁻¹ clearly contributing to improve the total biomass amount in the system as well as the overall performance (with particular reference to nitrogen removal, due to simultaneous nitrification/denitrification). More specifically, the biofilm concentration in the suspended carriers reached values equal to 4.5 gTS L⁻¹ at the end of Phase II, 8.5 gTS L⁻¹ in the phase III and 9.3 gTS L⁻¹ at the end of phase IV.



Figure 2. MLSS (-=-) concentration and VSS/TSS (----) ratio in the MBR (a) and MB-MBR (b) system.

3.2 Biokinetic behaviour and respiratory activity of suspended biomass

Respirometric batch tests were performed in phase V (salinity 15 gNaCl L⁻¹) under steady state conditions, since they were aimed at evaluating the biomass activity in both plants after the acclimation of biomass to salinity. The kinetic and stoichiometric parameters (average values) of both heterotrophic and autotrophic populations are reported in Table 2. Referring to the heterotrophic characterization, the biomass respiratory activity of MB-MBR system showed an increasing trend during the experimental period, highlighting a satisfactory acclimation to saline conditions. Such a result is confirmed by the trend of both maximum and endogenous respiration rates. In the conventional MBR system, the specific respiration rates of heterotrophic biomass showed a slight decrease during the whole observation period, highlighting a lower biomass activity.

Table 2. Average values of kinetic and	d stoichiometric parameters	s in the overall observ	ation period for
both pilot lines (in brackets the standa	ard deviation)		

	MBR Suspended biomass	MB-MBR Suspended biomass
Heterotrophic		
Y _H [mg COD mg ⁻¹ COD]	0.74 (±0.06)	0.69 (±0.06)
Y _{STO} [mg COD mg ⁻¹ COD]	0.76 (±0.02)	0.75 (±0.06)
μ _{H,max} [d ⁻¹]	2.19 (±0.80)	6.71 (±2.04)
K _s [mg COD L ⁻¹]	5.05 (±2.88)	9.92 (±8.30)
SOUR _{max} [mg O ₂ g ⁻¹ TSS h ⁻¹]	7.11 (±2.38)	21.08 (±7.20)
Autotrophic		
Y _A [mg COD mg ⁻¹ N]	0.38 (±0.06)	0.32 (±0.12)
$\mu_{A,max}[d^{-1}]$	0.25 (±0.08)	0.31 (±0.08)
Nitrif. rate [mg NH ₄ -N L ⁻¹ h ⁻¹]	1.11 (±0.36)	1.92 (±0.79)
K _{NH} [mg NH ₄ -N L ⁻¹]	0.38 (±0.25)	1.82 (±1.92)
SOUR _{max} [mg O ₂ g ⁻¹ TSS h ⁻¹]	3.54 (±0.43)	5.16 (±1.87)

The maximum growth rate $\mu_{H,max}$ reflected the SOUR (Specific Oxygen Uptake Rate) trend, with values more than twice as high in MB-MBR compared to the MBR one. However, both plants showed average values well in the range of what reported in the technical literature for conventional activated sludge plant (Henze et al., 1987). Referring to autotrophic populations activity, the obtained results suggested a good development of nitrification activity in both plants, despite the not negligible salinity of the feeding wastewater in phase V (15 gNaCl L⁻¹). This aspect highlights that the nitrifying biomass, which usually suffers sharp increases of salinity (Di Bella et al., 2013), could well acclimate to a gradual increase of the inlet saline concentration. In detail, it was noticed a higher autotrophic activity, in terms of respiration rates, in the MB-MBR line, despite the latter was characterized by slight lower mixed liquor SRT compared to the MBR line. This result could be likely due to the "seeding" effect of nitrifiers from the detached biofilm to the mixed liquor, which contributed to increase the nitrification activity of the whole system, according to what reported in the technical literature (Di Trapani et al., 2013). Also in this case, the maximum autotrophic growth rates showed an increasing trend during the observation period, with higher values for the MBR line, thus confirming what observed with the SOUR values.

3.3 Extracellular polymeric substance (EPS) production and composition

Figure 3 illustrates the concentration of EPS components (bound and SMP), in the mixed liquor of both plants. Until 30th experimental day, characterized by the gradual salinity variation, a slight increase of EPS concentration was noticed, which was more pronounced in the MBR line. Previous studies reported that

increasing saline concentrations greatly influence the EPS production, especially at the beginning of operations with the saline water (Jang et al., 2013; Reid et al., 2006).

From day 30 till the end of the operations, a quite stable concentration was reached, which suggests the occurrence of biomass acclimation. Furthermore, at the beginning of plant operations, the total EPS was represented by the bound portion, while no SMPs were recognized in the mixed liquor. On the contrary, the SMPs production increased along with salinity, mainly as carbohydrate fraction. This behaviour is in good agreement with previous experiences, which highlighted that microorganisms respond to a salt shock by a release of organic cellular constituents, such as SMPs, by secretion and cells autolysis (Sun et al., 2010; Yogalakshmi and Joseph, 2010; Johir et al., 2013).



Figure 3. EPS distribution respectively in MBR (a) and in MB-MBR (b) lines

3.4 Membrane filtration characteristics in MBR and MB-MBR

To illustrate the characteristics of membrane filtration in each MBRs, the transmembrane pressure (TMP) was monitored at constant flux. In particular, the total fouling resistance (R_T) can be described by the general form of Darcy's law:

$$R_{\tau} = \frac{TMP}{J\mu}$$
(2)

where TMP is the transmembrane pressure (Pa), μ the permeate viscosity (Pa·s), and J the permeation flux (m s⁻¹). The flux was kept at 15 L m⁻² h⁻¹. Figure 4 shows the profiles of membranes fouling over time.



Figure 4. Total resistance fouling and fouling Rate in the two plants.

The trend of resistance and the daily fouling rate (FR) show that the fouling tendency in the MBR system was much more pronounced than that of the MB-MBR one. Therefore, considering that the operating conditions were comparable (especially the concentration of EPS and TSS in the mixed liquor, with the same influent wastewater), the difference in fouling tendency can be imputable to the "cleaning" effect due to the collisions between the polyurethane foam carriers and the membrane surface. Such result is in good agreement with Yang et al. (2012). Furthermore, the lower fouling tendency in MB-MBR line is likely due to a lower hydrophobicity of the MLSS, characterized by higher biomass activity and lower SRT values.

4. Conclusions

The effects of a gradual increase of salinity on a MBR and a MB-MBR pilot plants treating synthetic wastewater were investigated. The pilot plants were analyzed in terms of organic matter and ammonium removal, biokinetic activity, EPS production and membrane fouling tendency. The results showed that the gradual increase of salinity enabled a good acclimation of the biomass. Both plants provided good removal efficiencies also under the highest saline level (15 g NaCl L⁻¹); the MB-MBR line showed the best results,

referring in particular to nitrification (due to the high retention times of the biofilm), also providing a good denitrification efficiency, likely due to the establishment of anoxic conditions in the internal portion of biofilm. The respiratory activity of the suspended biomass was significantly higher in the MB-MBR line, referring to both heterotrophic and autotrophic population, The latter was probably influenced by the seeding effect of nitrifiers from the detached biofilm. The increasing salinity caused a release of soluble EPS, as a response of microorganisms to a salt shock. Furthermore, the fouling tendency was significantly more pronounced in the MBR line, while in the MB-MBR pilot, the collisions between the suspended carriers and the membrane surface contributed to reduce the fouling tendency. Finally, on the basis of the observed results, the MB-MBR system provides high potentiality that should be useful for the treatment of high strength industrial or saline wastewater.

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