Effect of salinity variation on the autotrophic kinetics of the start-up of a membrane bioreactor and hybrid moving bed biofilm reactor-membrane bioreactor at low hydraulic retention time

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ABSTRACT

A membrane bioreactor (MBR) and a hybrid moving bed biofilm reactor-membrane bioreactor (hybrid MBBR-MBR) for municipal wastewater treatment were studied to determine the effect of salinity on nitrogen removal and autotrophic kinetics. The biological systems were analyzed during the start-up phase with a hydraulic retention time (HRT) of 6 h, total biomass concentration of 2,500 mg L⁻¹ in the steady state, and electric conductivities of 1.05 mS cm⁻¹ for MBR and hybrid MBBR-MBR working under regular salinity and conductivity variations of 1.2–6.5 mS cm⁻¹ for MBR and hybrid MBBR-MBR operating at variable salinity. The variable salinity affected the autotrophic biomass, which caused a reduction of the nitrogen degradation rate, an increase of time to remove ammonium from municipal wastewater and longer duration of the start-up phase for the MBR and hybrid MBBR-MBR.

Key words | autotrophic kinetics, moving bed biofilm reactor, salinity

INTRODUCTION

The growing awareness of environmental protection has led to more stringent legislation regarding the treatment of saline wastewater before discharge. This aspect is especially important for activities such as those in the fish processing, petroleum, petrochemical and tannery industries, as well as the wastewater produced during shipboard activities that is characterized by high saline concentrations (Sun et al. 2010; Aslan & Simsek 2012; Abdollahzadeh Sharghi et al. 2014).

In recent years, membrane bioreactor (MBR) systems have been applied to treat saline wastewater (Di Bella et al. 2015). To improve the performance of MBRs regarding pollutant removal and membrane filtration, hybrid moving bed biofilm reactor-membrane bioreactor (hybrid MBBR-MBR) systems have been developed during the last years. These systems combine an MBR and a moving bed biofilm reactor (MBBR) and constitute an efficient treatment alternative based on the presence of a special biofilm inside the bioreactor (Leyva-Díaz et al. 2014). In addition to the technologies analyzed in this research, it is worth mentioning that there are combined systems, including different physical, chemical and biological processes, which are also used to treat saline wastewater (Ahmadun et al. 2009). In this regard, granular activated carbon (GAC) adsorption is probably the main alternative to biological treatment (Roccaro et al. 2015).

An analysis of available scientific literature shows a lack of knowledge in terms of autotrophic biomass respiratory activity in MBRs and hybrid MBBR-MBRs used to treat municipal wastewater under salinity variation. Salt is a stress factor that can negatively affect the microbial communities in wastewater treatment plants (WWTPs), e.g. the nitrification process is especially sensitive to salt inhibition (Moussa et al. 2006; Zhao et al. 2014). The destabilization of microbial biomass could be caused by the reduction of its metabolic activity, changes in the sludge flocs and biofilm structure and composition, and disruption of the oxygen transfer rate to the liquid phase (Van’t Riet & Tramper 1991; Bassin et al. 2011). Furthermore, it is worth mentioning that the effect of salinity on the nitrification process also
depends on the biomass present in the bioreactor (suspended or attached), and the introduction method for salt in the system, i.e., the salinity variation can appear as a pulse or gradually (Moussa et al. 2006). In light of this, kinetics of nitrogen removal in an MBR and hybrid MBBR-MBR was studied based on the Monod first order substrate removal model (Rittmann & McCarty 1980), taking into account the influence on kinetics due to the coexistence of two kinds of biomass, suspended and attached, in a hybrid MBBR-MBR (Di Trapani et al. 2010). In such a perspective, respirometry definitively represents a helpful method for kinetic modeling, providing kinetic parameters of autotrophs under controlled conditions of temperature and pH (Ferrai et al. 2010). The principles of respirometry for the characterization of these systems have been described in the literature (Leyva-Díaz et al. 2013). In light of this, kinetic modeling can be an important tool for design and operation of MBR and hybrid MBBR-MBR plants (Leyva-Díaz et al. 2014).

Consequently, the aim of this study was to assess the effect of salinity on the autotrophic kinetics in the start-up of an MBR and hybrid MBBR-MBR working under regular and variable salinity in order to compare their evolution up to the steady state. This paper provides initial results about the behavior of autotrophic biomass working under variable and regular salinity at low hydraulic retention time (HRT) (6 h) during the start-up of MBR and hybrid MBBR-MBR systems.

**MATERIALS AND METHODS**

**Description of the WWTP**

In this study, an MBR (Figure 1(a)) and a hybrid MBBR-MBR (Figure 1(b)), working in parallel, were fed with municipal wastewater containing regular (1.05 mS cm⁻¹) and variable salinity (1.2–6.5 mS cm⁻¹). Variable salinity cycles were achieved through the mixing of urban wastewater and tap water amended with NaCl with an electrical conductivity of 50 mS cm⁻¹ by means of an electronic control to get the desired electric conductivity each time. The salinity conditions forced on the bioreactors' influent followed a cycle consisting of 6 h of a mix with 6.5 mS cm⁻¹ and 6 h of urban wastewater. The conditions of the salinity-amended tap water provided that the mixture had a minimum of 90% urban wastewater. These systems included a bioreactor divided into four zones: one anoxic zone (C2) and three aerobic ones (C1, C3 and C4), as well as a membrane tank (C5). There was biomass recycling from the membrane tank.

![Figure 1](https://iwaponline.com/wst/article-pdf/77/3/714/212422/wst077030714.pdf)

**Figure 1** | Diagram of the experimental plants used in the study. (a) Membrane bioreactor (MBR). (b) Hybrid moving bed biofilm reactor-membrane bioreactor (hybrid MBBR-MBR).
to the bioreactor to get the working mixed liquor suspended solids (MLSS) concentration inside the bioreactor and the nitrogen removal. The total biomass concentration for the steady state was 2,500 mg L\(^{-1}\), resulting from the sum of suspended biomass and attached biomass as biofilm. The WWTPs operated under an HRT of 6 h, a flow rate of 4.70 L h\(^{-1}\) and a membrane flux of 23.5 L m\(^{-2}\) h\(^{-1}\). The working volumes of the bioreactor and the membrane tank were 24 L and 4.32 L, respectively, and the anoxic zone of the bioreactor had a volume of 6 L. Regarding the hybrid MBBR-MBR, the K1 carrier filling fraction had a value of 35% in the aerobic zone and the anoxic zone did not contain carriers. Furthermore, the dissolved oxygen (DO) in the aerobic zone varied between 2.0 and 2.5 mg O\(_2\) L\(^{-1}\) and DO in the anoxic zone fluctuated between 0.2 and 0.4 mg O\(_2\) L\(^{-1}\) for both bioreactors.

**Kinetic modeling**

Respirometric tests were carried out on suspended biomass for the MBR. In order to evaluate the effect of biomass adhered to carriers, respirometric assays were performed on suspended and attached biomass for the hybrid MBBR-MBR.

Kinetic parameters for autotrophic bacteria and the total nitrogen (TN) degradation rate (\(r_{su,A}\)) were evaluated through respirometric techniques according to Leyva-Díaz et al. (2015). One litre of mixed liquor was withdrawn from the MBR and the hybrid MBBR-MBR; apart from mixed liquor, carriers were obtained from the hybrid MBBR-MBR to simulate the 35% filling fraction. Afterwards, the biomass was aerated for 18–24 h to reach endogenous conditions and transferred to the BM Advance respirometer. Temperature was maintained at 20.0 ± 0.1 °C and the stirring rate was 2,000 rpm. An air pump generated an air flow rate of 0.906 ± 0.001 L min\(^{-1}\), which supplied the oxygen. The use of sulphuric acid (30% vol.) and/or sodium hydroxide (0.1 M) allowed pH to be maintained at 7.25 ± 0.50. A recyling from the bottom to the top of the respirometer was necessary to homogenize the mixed liquor. A stock solution of ammonium chloride (150 mg L\(^{-1}\)) was prepared and three dilutions (50, 80 and 100%) were used to evaluate the autotrophic kinetic parameters corresponding to the start-up of the systems working at regular and variable salinity. The values of ammonium concentration for the three dilutions were determined by ion chromatography (Leyva-Díaz et al. 2015). The evolution of the DO and the dynamic oxygen uptake rate (\(R_o\)) were registered for the three additions of substrate (Leyva-Díaz et al. 2015).

Furthermore, the concentrations of ammonium, nitrite and nitrate were also evaluated through ion chromatography for the influent and effluent of the experimental pilot plants to analyze the evolutions of NH\(_4\)-N, NO\(_2\)-N and NO\(_3\)-N. The concentrations of MLSS and mixed liquor volatile suspended solids (MLVSS) were calculated from Standard Methods (APHA 2012). The concentrations of autotrophic biomass (\(X_A\)) were determined by supposing the percentages of autotrophic bacteria that were obtained by Leyva-Díaz et al. (2015) for the same MBR and hybrid MBBR-MBR under similar operational conditions.

Thus, this respirometric test allowed the maximum specific growth rate (\(\mu_{m,A}\)) substrate half-saturation coefficient (\(K_{M,A}\)) and yield coefficient (\(Y_A\)) for autotrophic biomass to be assessed. In light of this, these kinetic parameters characterized the biomass growth during the working of the biological systems at regular and variable salinities. This kinetic analysis was carried out by following a normalized procedure without salinity variations with samples from the MBR and hybrid MBBR-MBR in order to compare the substrate consumption between the different start-up phases. Therefore, respirometric tests were conducted on biomass samples that had been subjected to regular and variable salinities during the start-up phases, which lasted 60 days for the systems working at regular salinity and 82 days at variable salinity. The assessment of these parameters was carried out in five steps.

1. Determination of the oxygen consumption (OC) through the integration of \(R_o\), as shown in Equation (1):

\[
OC = \int_{t_i}^{t_f} R_o \, dt \, (mgO_2 \, L^{-1})
\]  

2. Estimation of \(Y_A\) according to Equation (2) described by Helle (1999):

\[
Y_A = \frac{S - OC}{S_{NH} \cdot f_{cv}} \, (mgVSS \, mgTN^{-1})
\]

where \(S\) is the ammonium concentration expressed as oxygen (mgO\(_2\) L\(^{-1}\)), \(S_{NH}\) is the ammonium concentration expressed as TN (mgTN L\(^{-1}\)) and \(f_{cv}\) is a conversion factor (1.42 mgO\(_2\) mgVSS\(^{-1}\)). This factor is the same for both biological systems as it considers that BOD\(_{ss}\) of the cells (C\(_3\)H\(_7\)NO\(_2\)) is equivalent to 1.42 times the value of the cell concentration when they are completely oxidized (Metcalf 2003).
### RESULTS AND DISCUSSION

Table 1 shows the autotrophic kinetic parameters for the MBR and hybrid MBBR-MBR working at regular and variable salinities.

It should be noted that the values of $\mu_{m,A}$ are higher for the systems operating under regular salinity than those obtained for the technologies working under variable salinity, which favored the substrate degradation rate. Moreover, the values of $K_{M,A}$ were higher for the systems working under variable salinity, which could be due to the inability of autotrophic biomass to use the whole substrate (Rodríguez-Sánchez et al. 2017). However, the values of $Y_A$ were similar for both salinities, which meant that the biomass production per substrate consumed was similar under regular and variable salinity.

Di Bella et al. (2015) also worked with MBR and hybrid MBBR-MBR systems at HRT values of 12–15 h under salinity variation. These authors obtained similar values of $\mu_{m,A}$ to those obtained in this research during the biomass acclimation to salinity (0.25 day$^{-1}$ for the MBR and 0.31 day$^{-1}$ for the hybrid MBBR-MBR). Regarding the values of $K_{M,A}$, these values were much lower for the systems that were evaluated by Di Bella et al. (2015), i.e. 0.38 mgNH$_4$-N L$^{-1}$ and 1.82 mgNH$_4$-N L$^{-1}$ for the MBR and hybrid MBBR-MBR, respectively. Thus, the values of $r_{su,A}$ will probably be higher for the systems analyzed by Di Bella et al. (2015) due to the higher HRT, which implies a lower TN loading rate, more easily assimilable by the autotrophic biomass. Additionally, Di Bella et al. (2015) carried out the start-up of the systems under a gradual increase of salinity that could improve the adaptation of autotrophic biomass as the MBR and hybrid MBBR-MBR of this research were started up under variable salinity cycles.

Moreover, in general, the values of $\mu_{m,A}$ were higher than those obtained by Rodríguez-Sánchez et al. (2017) for a hybrid MBBR-MBR treating salinity-amended urban wastewater with $6.50 \pm 0.50$ mS cm$^{-1}$ of electrical conductivity under an HRT of 9.5 h during its start-up (0.0001, 0.0399 and 0.0015 h$^{-1}$). The MBR system analyzed by Rodríguez-Sánchez et al. (2017) had suspended biomass with a higher $\mu_{m,A}$ (0.0517 h$^{-1}$) than the MBR studied in this research (0.0145 h$^{-1}$). Regarding the values of $K_{M,A}$, they widely exceeded those obtained in this research (Table 1), with values of 488.3461 mgTN L$^{-1}$ for the MBR and values of 526.1619, 150.0080 and 54.8534 mgTN L$^{-1}$ for the hybrid MBBR-MBR. According to Equation (6), the $r_{su,A}$ was higher for the hybrid MBBR-MBR working at variable salinity due to its higher values of $\mu_{m,A}$ and lower values of $K_{M,A}$ (Table 1) regardless of the lower HRT (6 h).

<table>
<thead>
<tr>
<th>Salinity condition</th>
<th>WWTP</th>
<th>$Y_A$ (mgVSS mgTN$^{-1}$)</th>
<th>$\mu_{m,A}$ (h$^{-1}$)</th>
<th>$K_{M,A}$ (mgTN L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular (1.05 mS cm$^{-1}$)</td>
<td>MBR</td>
<td>$1.2684 \pm 0.1522$</td>
<td>$0.1607 \pm 0.0144$</td>
<td>$0.3887 \pm 0.0390$</td>
</tr>
<tr>
<td>Variable (1.2–6.5 mS cm$^{-1}$)</td>
<td>MBR</td>
<td>$1.5681 \pm 0.1725$</td>
<td>$0.3579 \pm 0.0376$</td>
<td>$3.9188 \pm 0.4311$</td>
</tr>
</tbody>
</table>

$Y_A$ (yield coefficient for autotrophic biomass), $\mu_{m,A}$ (maximum specific growth rate for autotrophic biomass), $K_{M,A}$ (half-saturation coefficient for total nitrogen).
Nevertheless, this conclusion could not be obtained for the MBR working under an amended salinity scenario at 6.50 ± 0.30 mS cm⁻¹ since both \( \mu_{m,A} \) and \( K_{M,A} \) values were lower for the variable salinity scenario (Table 1).

Two multivariate analyses were conducted to see the effect of the DO concentration and salinity over the kinetic parameters of the bioreactors studied (Figure 2(a) and 2(b), respectively). The kinetic parameters were transformed using \( \log(X + 1) \). The DO and salinity were not transformed due to the consistency of units in both analyses. The multivariate analysis linking the operational conditions of the MBR and hybrid MBBR-MBR systems working with municipal wastewater under regular and variable salinity with the Monod growth model parameters clearly showed the influence of salinity and DO concentration in the autotrophic kinetics of the systems (Figure 2). The yield coefficient for autotrophic biomass (\( Y_{A} \)) (purple triangle) and the half-saturation coefficient for ammonium nitrogen (\( K_{M,A} \)) (red triangle) had a positive correlation with conductivity and DO concentration in the aerobic zone. On the other hand, the maximum specific growth rate for autotrophic biomass (\( \mu_{m,A} \)) (yellow triangle) was negatively correlated with electric conductivity and DO concentration in the aerobic zone.

The TN degradation rate (\( r_{su,A} \)) values for the MBR and hybrid MBBR-MBR were analyzed in Figure 3 at regular and variable salinities.

It was clearly shown that higher electric conductivities led to lowest TN degradation rate (\( r_{su,A} \)) values concerning TN removal during the start-up of the MBR and hybrid MBBR-MBR under variable salinity (Figure 3). Thus, the autotrophic kinetics was slower for the systems working under variable salinity (Figure 3(b)). The hybrid MBBR-MBR working at regular salinity showed higher \( r_{su,A} \) than the MBR (Figure 3(a)), probably due to the development of more specialized biomass that could be favoured by the higher retention times characterizing the attached biomass on carriers as biofilm (Di Trapani et al. 2014; Leyva-Díaz et al. 2014). In this regard, it should be highlighted that autotrophic biomass required less time for ammonium oxidation during the start-up of the MBR and hybrid MBBR-MBR working under regular salinity due to their higher \( r_{su,A} \) (Figure 3). Thus, a longer time would be required to accomplish a steady state for the systems working under variable salinity (Figure 3(b)).

This was in accordance with the studies carried out by other authors. In this regard, Johir et al. (2013) demonstrated an inhibitory effect of salinity on ammonium removal efficiency for an MBR system. Jang et al. (2013) also found a reduction of the ammonium removal for an MBR under high salinity conditions due to the variation of the microbial community. In this regard, Di Bella et al. (2013) also found a significant decrease of nitrification rate for autotrophic biomass from an MBR due to the stress exerted by the increase of salinity. According to Yogalakshmi & Joseph (2010), prolonged acclimation periods are required by nitrifying bacteria in presence of salinity variation due to their high sensitivity.

Figure 4 shows the ammonium oxidation and TN removal rates for the MBR and hybrid MBBR-MBR working at normal and variable salinities.

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**Figure 2** | Multivariate redundancy analysis triplot linking the autotrophic kinetics of MBR and hybrid MBBR-MBR systems working under regular and variable salinity influent wastewater with the DO in the anoxic and aerobic chambers, DO Anoxic chamber and DO Aerobic chamber, respectively, (a), and the conductivity (b) of the bioreactors. Please refer to the online version of this paper to see this figure in color: http://dx.doi.org/10.2166/wst.2017.585.
The trends for ammonium oxidation and TN removal showed that the MBR and hybrid MBBR-MBR working at base salinity had higher conversion of ammonium to nitrate and higher efficiency of TN removal than these systems operating at variable salinity. This confirmed the results obtained regarding the evolution of \( r_{\text{m,A}} \) indicated in Figure 3. Moreover, it should be highlighted that the hybrid systems containing both suspended and attached biomasses had a slightly better performance than the MBRs (Figure 4). This also corroborated that the growth of attached biomass on carriers favored the biological process of nitrogen removal (Di Trapani et al. 2014; Leyva-Díaz et al. 2014).

**CONCLUSIONS**

The effect of salinity on the autotrophic biomass from an MBR and a hybrid MBBR-MBR was analyzed through an autotrophic kinetic study based on the Monod model during the start-up of both systems working at regular (1.05 mS cm\(^{-1}\)) and variable (1.2–6.5 mS cm\(^{-1}\)) salinities.

Variable salinity slowed down the nitrogen degradation rate as a consequence of a reduction in the value of maximum specific growth rate for autotrophic biomass and an increase in the value of the half-saturation coefficient for ammonium nitrogen. Thus, the autotrophic biomass could be inhibited by salinity, implying the inability of this kind of biomass to degrade ammonium substrate.

The MBR and hybrid MBBR-MBR systems that operated at regular salinity showed the highest values for TN degradation rate, which involved less time to oxidize ammonium during the start-up phase and to reach the steady state.

**ACKNOWLEDGEMENTS**

This research was supported by EMASAGRA and the Spanish Ministry of Economy and Competitiveness in the framework of the program ‘Convocatoria de ayudas a Proyectos de I + D Excelencia 2013’ (CTM2013-48154-P).

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First received 6 July 2017; accepted in revised form 6 November 2017. Available online 20 November 2017.