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Removal of 17-β estradiol from wastewater: comparison between a laboratory scale conventional activated sludge and a membrane bioreactor

R. López-Fernández[a,*], F.V.F. Tavares[a], M. Gómez[a], R. Irusta[b], P. Le-Clech[c]

[a]*Environmental Division, CARTIF Centro Tecnológico, Parque Tecnológico de Boecillo, 205, 47151 Valladolid, Spain*
Tel. +34 983 546504; Fax: +34 983 546521; email: raqlop@cartif.es
[b]*Department of Chemical Engineering and Environmental Technology, Higher Technical College of Industrial Engineering, University of Valladolid, Paseo del Cauce 59, 47011 Valladolid, Spain*
[c]*UNESCO Centre for Membrane Science and Technology, School of Chemical Engineering and Industrial Chemistry, University of New South Wales, Sydney 2052, Australia*

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ABSTRACT

This work reports the performance of a laboratory scale membrane bioreactor (MBR) and a conventional activated sludge (CAS) treating a real industrial–municipal mixed wastewater, spiked with 17-β estradiol (E2) in micrograms amounts. In order to compare the steady- state conditions of both systems, different sludge retention times (SRT) of 10 and 20 days were applied, while the other operational parameters were maintained unchanged. Results regarding the removal of chemical oxygen demand (COD), nitrogen and E2 have been assessed with statistics analysis using the Mann–Whitney test. MBR outperforms the CAS treatment in terms of COD and nitrogen removal, and the increase in the SRT generally improves the performance of each system. However, E2 was almost completely eliminated in both systems and SRTs applied, leading to removal rates above 99%. Thus, this study shows that the biological elimination of E2 is not directly related to the nitrification rate, and other process or operational parameters should be of importance.

Keywords: 17-β estradiol; Membrane bioreactor; Conventional activated sludge; Sludge retention time; Statistics

1. Introduction

There is a growing interest in removing emerging pollutants such as endocrine disrupting compounds (EDCs) from wastewater. Even in nanogram levels, EDCs may cause genetic anomalies due to their ability to mimic or antagonize the effect of endogenous hormones, and disrupt their synthesis and metabolism in aquatic wildlife [1]. Comparing to other EDCs, natural and synthetic hormones, such as estrone (E1), 17-β estradiol (E2) and 17-β ethynylestradiol (EE2), have an endocrine potential several times higher and could produce adverse effects in environmental realistic concentrations [2].

Several works dealing with the elimination of those micropollutants by conventional and advanced technologies have been carried out. Since the first observations of nitrifying biomass ability to biodegrade estrogens, other studies have been conducted in order to establish the operational conditions that

*Corresponding author.

support estrogens biodegradation in conventional wastewater treatment plants. A correlation between nitrification potential and estrogens elimination was observed when monitoring ammonia concentration during summer and winter stations [3]. It was found that, under the low temperatures that reduce the nitrifying biomass metabolism, estrogen concentrations were higher than in summer. This evidence was suggested to justify the remarkable season variation of EE2 concentration in effluents during season changes.

The ability of nitrifying activated sludge (NAS) to metabolize estrogens was confirmed later when batch test using ammonium-oxidizing inhibitor was applied on pure cultures of *Nitrosomonas europeae* and mixed nitrifying biomass [4]. This first observation suggested that bacteria strain of *N. europeae* was able to biodegrade estrogens in a solution containing ammonia and estrogens as organic carbon. However, when an ammonium-oxidizing inhibitor was applied in a mixed culture of NAS and *N. europeae*, it was only observed a reduction of the estrogen concentration, which strongly suggest that not only ammonium-oxidizing bacteria is capable to degrade estrogens, but their biodegradation could be played by co-metabolism of a diverse biomass present in activated sludge plants with a nitrification process.

Although estrogens can be eliminated under aerobic conditions, competition for carbon source seems to play an important role that may influence the estrogen biodegradation. It has been suggested that higher availability of carbon reduces the estrogen degradation, as it was observed when comparing the E2 removals in cascade of stirred reactors [5]. When the removal of E1, E2, and EE2 was studied using sludge cultivated on aerobic and alternated anoxic/aerobic conditions, it was observed that denitrifying biomass was able to promote incomplete estrogen removal, producing 17-α-estradiol from the degradation of E1, which still maintains estrogen activity [6]. However, the removal of the three estrogens was improved with the aerobic sludge, and the higher the nitrification rate, the higher the estrogen removal rate.

As several studies have supported the hypothesis of specialized microorganisms able to degrade persistent pollutants like EDCs, it is important to elucidate the parameters to control the biomass community. It has been published that above a critical sludge retention time (SRT) of 10 days at 10˚C, low concentrations of E1 and E2 could be reached in wastewater treatment plants effluents [7]. However, in that study, EE2 and others pharmaceuticals compounds such as diclofenac shown contradictory results. Therefore, beside the SRT, other parameters seem to be of importance.

In this way, the influence of hydraulic retention time (HRT), SRT, and temperature was investigated on E2-equivalents reduction in a full-scale biological nutrient removal plant [8]. In spite of the complex inter-relationship of all operational conditions, a clear and linear relationship between E2-equivalent removal and temperature was found rather than other control parameters, and the lowest estrogen concentrations were observed in warm conditions.

On the other hand, many studies have dealt with the comparison between membrane bioreactor (MBR) and conventional activated sludge (CAS) technologies. MBR process was perceived superior for removing micropollutants due to operational factors such as higher mixed liquor suspended solids concentration (better adsorption) or complete retention of slow-growing specialist microorganisms (better biodegradation) [9,10]. However, most of the recent investigations comparing both technologies agree that for a given operation conditions of organic load, SRT and temperature, the micropollutants removal are very similar [7,11–14].

The results related to the fate of estrogens in wastewater treatment plants reported in literature indicate that biological elimination of these compounds still remains an open challenge. In this way, this work aims to investigate the relation between the nitrification rate (controlled by the SRT) and the estrogen removal. In order to clarify this connection, the removal of the natural estrogen E2 present in microgram level in a real municipal–industrial wastewater will be studied using two different SRT of 10 and 20 days. Furthermore, this study try to provide a rigorous comparison between two laboratory-scale MBR and CAS systems working at the same operation conditions of HRT, organic load, and temperature. Finally, as a distinctive feature, the results will be assessed using statistics analysis. As environmental results do not usually fits to a normal statistical distribution, the Mann–Whitney test will be used, as a non-parametric test applied for paired and independent group of samples [15].

2. Materials and methods

2.1. Feed wastewater

Raw wastewater was obtained from a full-scale WWTP treating mixed municipal–industrial wastewater and was collected just before the activated sludge reactor. It was filtered by a 0.5 mm screen to remove large particles and characterized according to the standard methods (Table 1) [16]. Then, it was stored at 4˚C prior to use.

Table 1
Feed wastewater characterization

Parameter	Average value (±SD)
pH	7.05 (0.42)
Total alkalinity ($mg\,L^{-1}$)	212.50 (88.39)
tCOD ($mg\,L^{-1}$)	1339.18 (443.88)
sCOD ($mg\,L^{-1}$)	1098.74 (345.69)
BOD_5 ($mg\,L^{-1}$)	535.17 (94.57)
SST ($mg\,L^{-1}$)	0.06 (0.02)
TKN ($mg\,L^{-1}$)	33.58 (14.06)
NH_4–N ($mg\,L^{-1}$)	20.42 (7.26)
NO_3–N ($mg\,L^{-1}$)	2.77 (1.31)
P ($mg\,L^{-1}$)	2.99 (0.93)
E2 ($\mu g\,L^{-1}$)	0.173 (0.098)

2.2. Laboratory-scale design and operation

The laboratory-scale MBR comprised an organic ultrafiltration (UF) hollow fiber membrane, supplied by Zenon (ZW1), with a filtration area of $0.047\,m^2$, that was submerged in a 3 L tank (T1) (Fig. 1a). The system was connected to a reverse speed peristaltic pump (P1), in which permeate extraction and backwash were conducted in a 4.5 and 0.5 min cycle for both operation processes respectively. A manometer (PI) located in the permeate line was used to control the membrane fouling. The wastewater was fed into the reactor by a feed pump (P2), and the sludge was withdrawn by an additional pump (P3). Aeration was provided by a constant airflow through the membrane module to promote membrane scouring and by an air diffuser located on the bottom of the reactor for the organic matter oxidation. A water level sensor (LI) was used to keep a constant volume in the reactor.

The CAS system consisted in a 6 L laboratory scale tank (T) and a secondary clarifier (SC), (Fig. 1b). Peristaltic pumps were connected to the system in order to control influent flow (P1), sludge recirculation to aeration tank (P2) and sludge purge (P3). As described previously for MBR system, aeration was supplied by air diffusers located at the bottom of aeration tank, and oxygen concentration was kept approximated in $2.0\,mg\,L^{-1}$.

Laboratory-scale plants were inoculated with biomass ($12.5\,gVSS\,L^{-1}$) obtained from the sludge recirculation line of the WWTP. The HRT was set on 12 h for both reactors and the volumetric loading rate (VLR) on $1.4\,gCOD\,L^{-1}\,d^{-1}$ by diluting the real sewage with tap water to fix a constant influent chemical oxygen demand (COD) concentration of $700\,mgCOD\,L^{-1}$. Nitrogen and phosphorus were added when necessary as NH_4Cl, KH_2PO_4, and Na_2HPO_4 to maintain a typical C:N:P ratio of a domestic wastewater influent (100:5:1). E2 was added in order to set a constant concentration of $10\,\mu g\,L^{-1}$ in the feed wastewater. Under these conditions, SRTs of 10 and 20 days were imposed to the system through changing the purge flow.

2.3. Analytical methods

Control parameters such as temperature, pH, or dissolved oxygen, tCOD, sCOD, NH_4^+–N, NO_3^-–N, SST and SSV were taken three times per week and measured according to Standard Methods [16].

E2 analysis was determined by liquid chromatography coupled with a mass spectrometer (LC/MS–$MS^{\wedge n}$). Quantification was preceded by a clean-up solid-phase extraction (SPE) protocol in which Bond Elut Plexa cartridges (Varian Inc.) pre-packed with 200 mg of solid-phase material was applied. 100 mL of samples were previously acidified with HCl (3 M) and heated for 1 h at 60°C. A NaOH basic solution was added to set pH to 3.5. The cartridges were conditioned using 5 mL of methanol and 5 mL of ultrapure water and then the filtration was carried out by a manifold connected to a vacuum pump at a flow rate between 5.0 and $10\,mL\,min^{-1}$. Then, elution was performed by adding 3 mL of methanol, and the solution

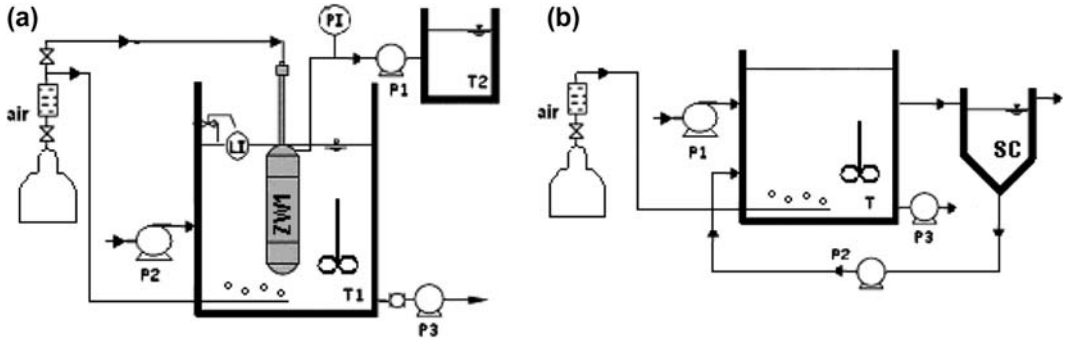

Fig. 1. (a) MBR and (b) CAS laboratory-scale treatment plants.

formed was collected in a conical bottom culture tube. Before LC-MS/MS injection, samples were evaporated to dryness by a gentle nitrogen stream, and the extracts were reconstituted in 1 mL of MeOH. Liquid chromatography (LC) was carried out by a ProStar 400 (Varian) equipped with a degasser, an autosampler and a binary pump. An octadecyl reverse phase column (RP-C18) with 150 mm of length and 2.0 mm of inner diameter was applied for the analyte separation. Organic mobile phases were constituted by methanol and water HPLC grade (90:10), whereas aqueous phases were constituted by water, acetic acid (0.2%), and ammonium acetate (5 μM). The mobile phase flow was set to 250 μm min^{-1}. LC was coupled with a Varian 500 MS mass spectrometer equipped with an atmospheric pressure chemical ionization source (APCI) working at positive mode. The implementation of the method was performed by an automatic optimization function of MS software through infusion of standard solutions. The precursor ion (m/z) formed by E2 ionization was 255 $[M + H–H_2O]^+$ which in turn fragmented in the 159 (m/z) ion. Subsequent fragmentation produces the ions 131, 141 and 144, which shown the greatest signal and relative abundance over the others ions produced and were the ion reference for E2 quantification.

3. Results and discussion

3.1. MBR and CAS performance

The initial biomass concentrations (X) selected for the SRT of 10 days were 10.0 and 5.0 g VSS L^{-1} for the MBR and CAS reactors, respectively. In both cases, the X decreased until reaching stable values in the steady state. Then the operation was extended for three more months. The operation of the second stage of 20 days was conducted directly without new inoculation and following the same procedure.

In order to establish comparable conditions, operational influent parameters were maintained constant over the whole period of monitoring. Mean temperature value was 19 ± 1 ℃ and pH kept between 7.0 and 8.0 for both reactors.

The steady-state values of the organic loading rate (OLR) and yield (Y) obtained in each reactor and SRT are gathered in the Table 2. The adoption of a constant VLR of 1.4 g COD L^{-1} d^{-1}, resulted in the decrease of the OLR for increasing the steady biomass concentrations when the higher SRT was used. As a consequence, a lower Y was also obtained with the SRT of 20 days. Yield values are similar to other published results obtained for MBR operating under similar conditions [17]. Comparing the MBR and CAS systems, the latter showed higher Y in the two SRT operated, probably due to some uncontrolled biomass wash out.

E2 concentrations, as well as tCOD, NH_4^+–N and NO_3^-–N, were monitored during the operation in order to verify the SRT influence (Figs. 2–5). Although it was not expected a remarkable improvement on reactors performance, the SRT increases provided a strengthening of the distributions and a general increment on ammonia removal rates. On the other hand, the MBR reactor seemed to be more robust and independent on experimental incidents.

According to Fig. 2, the mean E2 effluent concentrations for both reactors were in a range below 120 ng L^{-1} during all monitored period. Operating at the higher SRT favors the E2 removal in both systems and causes a strengthening of the distributions. Although a moderate concentration was spiked to the wastewater, both systems were capable to remove E2 almost completely from liquid phase (>99%).

COD profile suggests a better MBR performance in comparison with the CAS treatment (Fig. 3), leading to removal rates about 98% and 94%, respectively. However, the SRT does not seem to influence the COD removal. According to the median values, CAS effluent concentrations were in a range of 30 to 40 mg tCOD L^{-1}, while the MBR ones were quite close to 10 mg L^{-1} in both SRTs operated. These results could be attributed to the reduction of food/microorganism ratio in MBR systems, which increases competition and carbon subtracts elimination.

Regarding the ammonia effluent concentrations (Fig. 4), it was observed the same previous behavior obtained for the E2 and COD, where the increase of the SRT favored the overall performance of the

Table 2
Influence of SRT in the biomass growth in CAS and MBR systems

Parameters	CAS		MBR	
	SRT = 10 d	SRT = 20 d	SRT = 10 d	SRT = 20 d
OLR (gCOD $gVSS^{-1}$ d^{-1})	0.84	0.67	0.68	0.56
Y (gVSS $gCOD^{-1}$)	0.15	0.09	0.18	0.11

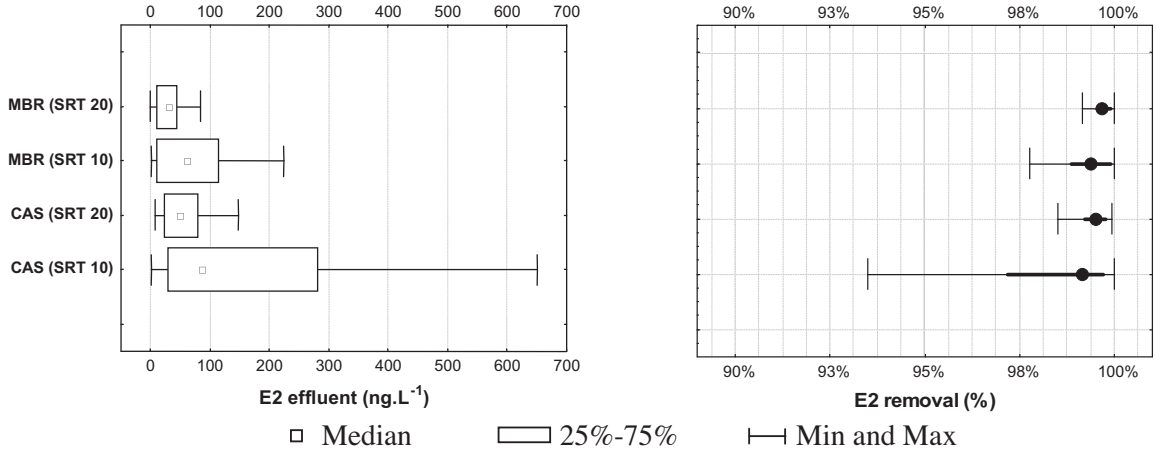

Fig. 2. E2 effluents concentrations and removal rates for CAS and MBR systems.

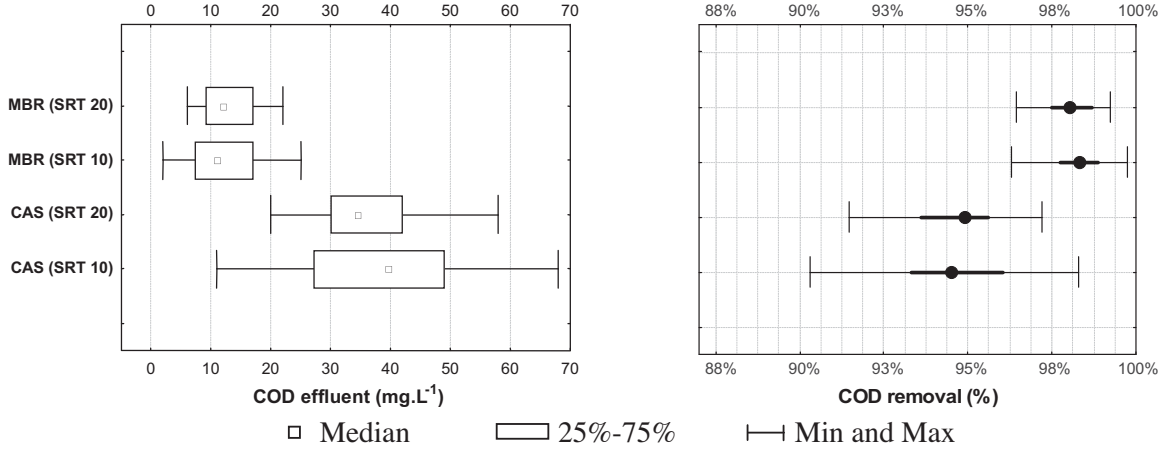

Fig. 3. COD effluents concentrations and removal rates for CAS and MBR systems.

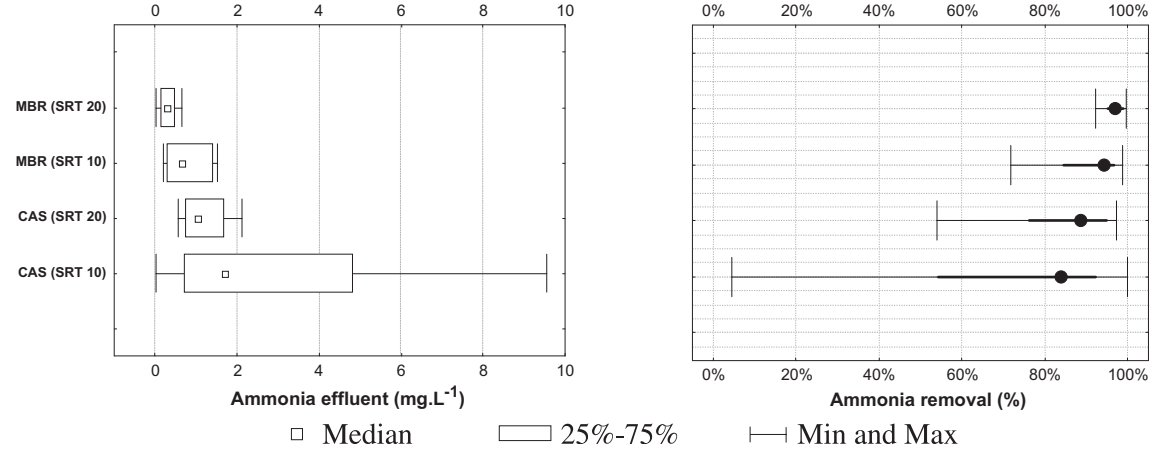

Fig. 4. NH_4^+-N concentration and removal rates for CAS and MBR systems.

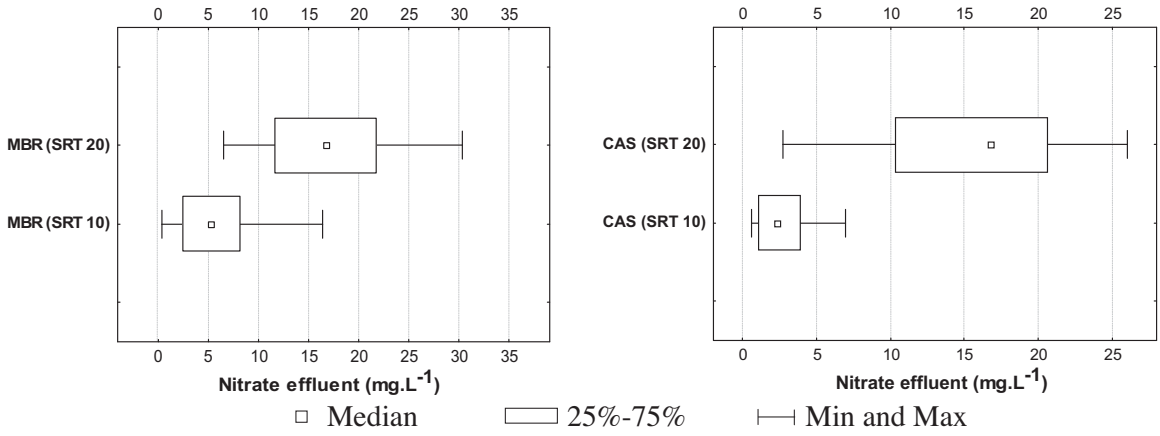

Fig. 5. NO_3^--N concentration for CAS and MBR systems.

reactors. Nitrification rate distributions were again narrower using the SRT of 20 days, especially for the CAS reactor. Even if nitrification could be completely reached below a SRT of 20 days, this higher sludge age seemed to favor a more stable and constant nitrification for both reactors, and mean removal rates increased from 92.7 to 96.4% and 94.6 to 98.2%, for the CAS and MBR systems, respectively. Finally, Fig. 5 collects the corresponding nitrate effluent concentrations and shows the improvement in the nitrification when 20 days of SRT was used.

3.2. Statistical analysis

In order to test the initial hypothesis of the influence of sludge retention time on the elimination of E2, Mann–Whitney test was applied as a statistical tool to compare the operational data. According to Table 3, parameters concentration discussed previously were in good agreement with some preliminary conclusions, though others could be refuted.

E2 behavior in the liquid phase does not seem to be dependent of the sludge age and treatment type under the conditions applied. No statistical differences were obtained from the test so above the sludge retention time of 10 days, both reactors are able to remove E2 from the liquid phase in percentages higher than 99%.

It was observed that COD performance removal was different between the two reactors once statistical results suggested differences between reactors concentrations in both conditions, although the increase in the sludge age did not seem to improve the performance itself.

Ammonia results also pointed to the same COD conclusion, and the mean concentrations were suggested to be different in both reactors. However, according to the test, the increase of the sludge age only favored the MBR performance but not the CAS, as no significant difference was obtained in this case for the two SRTs. It has to be taken into account that when higher SRT were operated, competition for nitrogen sources in MBR systems could be stronger

Table 3
Mann–Whitney test results for CAS and MBR under operational conditions

System and operational conditions tested	Mann–Whitney U test (p level α: 5%)							
	17-β-estradiol (E2)		COD		NH_4^+-N		NO_3^--N	
CAS (10 and 20 d SRT)	0.12	No SD	0.41	No SD	0.32	No SD	0.07	No SD
MBR (10 and 20 d SRT)	0.31	No SD	0.49	No SD	<0.05	SD	0.59	No SD
CAS and MBR (10 d SRT)	0.15	No SD	<0.05	SD	<0.05	SD	<0.05	SD
CAS and MBR (20 d SRT)	0.27	No SD	<0.05	SD	<0.05	SD	0.75	No SD

*No significant difference (No SD) and significant difference (SD).

than in the CAS, as the sludge was completely retained on MBR, while the activated sludge settler eventually suffered of biomass wash out.

On the other hand, nitrate effluent concentrations shown in the Fig. 5, suggested that the nitrification performs better in the MBR for the two SRT analyzed; however, attending to the analysis of Table 3, statistical difference between reactors was only observed for the SRT of 10 days. Two hypotheses can be suggested to explain this performance. One, nitrification process was incomplete due to lack of dissolved oxygen for nitrite oxidation, or an anoxic ambient were eventually reached inside the MBR's flocks, which could lead to denitrification.

4. Conclusions

The results have demonstrated that MBR technology outperforms the CAS treatment in terms of COD and nitrogen removal when treating a municipal –industrial mixed wastewater operating with two different SRTs and under the same operational conditions (HRT, OLR, and temperature). COD removals achieved in the MBR system were higher than in the CAS (98 and 94%, respectively) but the increase in the sludge age did not seem to affect the performance itself in each reactor. Regarding the nitrification process, MBR shows higher ammonia removal rates. However, according to the Mann–Whitney test, the increase of the sludge age only favored the MBR performance and no significant difference was observed in the CAS under the two SRTs applied.

Regarding the E2 biological removal, it has been observed that above a sludge retention time of 10 days, this estrogen can be easily eliminated in both systems. Thus, the results obtained in this paper shows that E2 removal rates are not directly related to the nitrification or the COD elimination and other processes have to influence the biological elimination of micropollutants.

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