

Study on the removal of biodegradable NOM from seawater using biofiltration

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Abstract

Despite the low biodegradability of seawater NOM, problems associated with biofouling are common in facilities that handle seawater. In this work, a fixed-film aerobic biofilter is proposed as an effective unit for preventing biofouling in such facilities.

A packed-bed biofilter with an EBCT = 6 - 11 min was employed. The results demonstrated that the DOC is reduced by 6% and the BOD₇ is reduced up to 15%. The LC-OCD analysis revealed that biofiltration abates the LMW neutrals and biopolymer fractions by 33 and 17%, respectively. However, the fractionation with UF membrane showed that the biofiltration process is able to degrade the more biodegradable compounds that have molecular weights that are greater than 1 kDa and compounds with molecular weights of less than 1 kDa.

After biofiltration, the biological activity measured in terms of ATP removal was reduced by 60%. Finally, a test to evaluate the biofilm formation capacity of a water sample revealed reductions of ~94% when comparing biofiltered and non-biofiltered seawater. Therefore, a fixed-film aerobic biofiltration process could be a useful treatment for the removal of biodegradable organic matter from seawater and for improving the water quality in terms of less biofilm formation capacity.

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Nomenclature

A_{254} : Ultraviolet absorbance at 254 nm [m^{-1}]

ATP: Adenosine triphosphate [$ng\ ATP\ L^{-1}$]

ATP_{total}: Cellular and free adenosine triphosphate [$ng\ ATP\ L^{-1}$]

ATP_{cell}: Cellular adenosine triphosphate [$ng\ ATP\ L^{-1}$]

ATP_{free}: Extracellular adenosine triphosphate [$ng\ ATP\ L^{-1}$]

BFC: Biofilm formation capacity [$pg\ ATP\ cm^{-2}\ day^{-1}$]

BFR: Biofilm formation rate [$pg\ ATP\ L^{-1}\ day^{-1}$]

BOD₇: Biochemical oxygen demand at 7 days [$mg\ O_2\ L^{-1}$]

CMIMA: Mediterranean Center for Marine and Environmental Research

DOC: Dissolved organic carbon [$mg\ C\ L^{-1}$]

EBCT: Empty bed contact time [min]

F-EEM: Fluorescence excitation-emission matrix

HLR: Hydraulic loading rate [$m\ h^{-1}$]

HMS: High molecular size

HPC: Heterotrophic plate counts [CFU]

LC-OCD: Liquid chromatography coupled with organic carbon detection

LMS: Low molecular size

LMW: Low molecular weight

MF: Microfiltration

MFI: Modified fouling index [$s\ L^{-2}$]

MWCO: Molecular weight cut-off [kDa]

NH₄: Ammonia [mg N L⁻¹]

N_{inorg}: Dissolved inorganic nitrogen [mg N L⁻¹]

NO₂⁻: Nitrite [mg N L⁻¹]

NO₃⁻: Nitrate [mg N L⁻¹]

NOM: Natural organic matter

N_{org}: Dissolved organic nitrogen [mg N L⁻¹]

N_{total}: Total dissolved nitrogen [mg N L⁻¹]

RLU: Relative light units

RO: Reverse osmosis

RU: Raman units

S_{bead}: Bead surface [cm²]

SDI: Silt density index [% min⁻¹]

SUVA₂₅₄: Specific UV absorbance at 254 nm [L mg C⁻¹ m⁻¹]

SWRO: Seawater desalination by reverse osmosis

TDC: Total direct cell counts [cells mL⁻¹]

TDS: Total dissolved solids [g NaCl L⁻¹]

UF: Ultrafiltration

UV: Ultraviolet light

1. Introduction

Seawater desalination by reverse osmosis (SWRO) has currently emerged as an effective solution to obtain water for human consumption and for industrial and agricultural uses (Schneider and Mastrandrea, 2011). In addition to being used for desalinated water, seawater is also used, e.g., as a coolant in thermal plants (Parkash, 2003; Baker, 2004).

In seawater facilities and particularly in SWRO plants, the most common foulants obtained from SWRO membranes are a combination of both organic and microbial

deposition (Darton and Fazel, 2001; Xu et al., 2007). Consequently, microorganisms start growing at the expense of dissolved NOM and can develop a biofilm that can cause operational problems (Flemming, 1997; Matin et al., 2011; Griebe and Flemming, 1998; Flemming et al., 1998; Hu et al., 2005). Even with the low and mostly non-biodegradable seawater NOM (Volk et al., 1997; Penru et al., 2011), marine microorganisms are metabolically active (Egli, 2010). In SWRO plants the consequences of fouling include an increase of the membrane resistance coupled with a decrease in water production and water quality. All the above factors have an impact on the total cost of desalinated water (Flemming et al., 2007; Matin et al., 2011).

Pre-treatments in SWRO processes are designed to improve the water quality for stable and sustainable operation (Shon et al., 2008). A considerable number of reports can be found in the literature associated with conventional and membrane pre-treatments (Rosberg, 1997; Vial et al., 2003; Halpern et al., 2005; Bonnelye et al., 2008; Voutchkov, 2010, van Hoof et al., 1999; Brehant et al., 2002; Pearce et al., 2004; Kumar et al., 2006; Xu et al., 2007; Lorain et al., 2007; Bonnelye et al., 2008; Shon et al., 2008; Salinas Rodriguez et al., 2009). Nevertheless, dosages of biocides (often chlorinated substances) are the currently employed common strategy to prevent biofouling because biocides can kill nearly all microorganisms. However, debris are not removed from the aqueous phase and they can therefore serve as a substrate for the living microorganisms, which is why the use of biocides is only considered to be a short-term strategy (Flemming, 1997; Schneider et al., 2005). Moreover, microorganisms embedded in a biofilm are more resistant to biocides, which is most likely due to selection in favour of more biocide tolerant bacteria; this property confers a large inertia to the system (Griebe and Flemming, 1998; Chowdhury, 2012). Therefore, the frequent use of biocides results in microorganisms developing resistance to the biocides and may also aggravate the biofouling episodes (Flemming, 2002; Bereschenko et al., 2011).

[In this study, it is proposed the use of a fixed-film aerobic biofilter](#) (herein referred to as a biofilter) as an effective unit for preventing organic and biological fouling in SWRO plants and, in general, in facilities that handle seawater. As reported by Rittman (1990), biofilm technology is advantageous when the concentrations of biodegradable substances are low because the retention of cells by attachment allows a considerable accumulation of biomass. Furthermore, as emphasised by Cohen (2001), fixed-film (attached growth) has some advantages related to metabolic

activities compared with suspended growth. Therefore, aerobic degradation is proposed due to its simplicity and due to the dissolved oxygen content in seawater.

The purpose of this study is to evaluate the effectiveness of a biofilter for reducing the biodegradable substances in seawater. [Moreover, it is also quantified the reduction of biological activity downstream of the filter.](#)

2. Materials and Methods

2.1. Seawater and pilot biofilter

The raw seawater was captured from the NW Mediterranean Sea through an open intake that was located 300 m offshore at a depth of 10 m. No pre-treatment was performed to the raw seawater before it was introduced into the biofilter. Table 1 summarises the characteristics of the raw seawater.

Table 1. Characteristics of the raw seawater

The pilot biofilter was located in the Mediterranean Centre for Marine and Environmental Research (CMIMA, Barcelona). The biofilter was composed of a fixed packed bed of expanded clay (Biolite[®]L2.7). It was operated downflow, and it was backwashed with both air and water 3 times per week according to Ahmad and Amirtharajah, (1998).

A schematic illustration of the pilot biofilter is presented in Figure 1*a* while Figure 1*b* presents an image of the pilot biofilter. Figure 1*c* presents the detailed characteristics of the filter media (Biolite[®]L2.7) used in the packed-bed.

The different experimental conditions employed in this study are presented in Table 2. The characteristics of the column and the packed bed are summarised in Table 3.

Figure 1. (a) Schematic illustration of the pilot biofilter; (b) Image of the pilot plant;
(c) Expanded clay - Biolite[®]L2.7

Table 2. Experimental conditions

Table 3. Characteristics of the column and packed bed

Colonisation and acclimatisation of the biomass was achieved through a continuous flow of raw seawater for several months. The dissolved oxygen was continuously measured to monitor the full development of the biofilm. It was not necessary to introduce additional oxygen because the amount of dissolved oxygen in the raw seawater was sufficient for the heterotrophic biological activity.

All analyses were performed on the input and output currents of the biofilter. Due to the very small amounts of organic matter that was measured during the experiments of this work, all the glass materials were thoroughly cleaned before use. The glass materials were first soaked in an aqueous 10% (V/V) HCl solution for 24 hours and then rinsed with copious amounts of Milli-Q water. Subsequently, the glass materials were covered with aluminium foil and heated to 450 °C for 4 hours. All the plastic materials were also carefully and thoroughly cleaned before use. Then, these materials were sterilised by autoclaving (QM 4000 SA-202X, Quirumed Spain) for 30 min at 121°C.

2.2. Analysis

Among the general parameters used to quantify NOM (Matilainen et al, 2011) in this study, the conventional analytical techniques for measuring the DOC, BOD₇ and A₂₅₄ are used to quantify the NOM at the inlet and at the outlet streams of the biofilter and therefore, to assess the effectiveness of the biofilter in the removal of NOM.

The removal of biodegradable NOM was investigated using a seawater biofilter operating with an empty bed contact time (EBCT) ranging from 6 to 11 min (corresponding to hydraulic loading rates that range from 5 to 10 m h⁻¹). Furthermore, to elucidate the mechanisms for the operation of the biofilter for an EBCT = 8.1 min, more accurate techniques used, such as liquid chromatography coupled with organic carbon detection (LC-OCD), fluorescence excitation-emission matrices (F-EEM) and membrane fractionation. Finally, we analysed the evolution of nitrogen compounds in seawater as a result of the biofiltration process and the biofilm formation capacity (BFC) in treated and untreated seawater.

Dissolved organic carbon (DOC), total nitrogen (N_{total}), ammonia (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-)

The dissolved organic carbon (DOC) analysis consisted of first filtering the sample through a 0.7 μm nominal pore GF/F filter (Whatman, Spain), as proposed by Ogawa and Tanoue (2003), and then to prevent inorganic carbon, the samples were acidified with H_3PO_4 to a $\text{pH} = 2 - 3$ (50 μL ; 2 M). The samples were stored at 4 $^\circ\text{C}$ in 10 mL glass vials. The DOC was determined using the high-temperature catalytic oxidation method with a Shimadzu (TOC-V_{CSH}) analyser. The total dissolved nitrogen (N_{total}) was also analysed using a TN unit according to Álvarez-Salgado and Miller (1998). The inorganic forms of nitrogen (ammonia, NH_4^+ , nitrite, NO_2^- , and nitrate, NO_3^-) were measured following the standard procedure described in Grasshoff et al. (1999). A Bran-Luebbe (AA3) auto-analyser was used. The dissolved organic nitrogen (N_{org}) was calculated as the N_{total} minus the sum of the forms of inorganic nitrogen (N_{inorg}).

Biochemical oxygen demand at 7 days (BOD_7) and the biodegradability ratio (BOD_7/DOC)

The biochemical oxygen demand at 7 days (BOD_7) was determined using a previously described method (Simon et al., 2011). This method is an adaptation of the *Closed Bottle Method* (EPA, 1998) and the Standard Methods 5210 (Standard Methods, 1999), and it uses concentrated autochthonous inoculum. The seawater samples were incubated for 7 days at 20 ± 1 $^\circ\text{C}$ (Medilow Selecta, Spain), and the dissolved oxygen content was measured before and after incubation with an IntelliCAL™ LDO probe connected to a HQ40d multimeter (Hach, USA).

The biodegradability of the dissolved carbon was measured from the BOD_7/DOC ratio in units of $\text{mg O}_2 \text{ mg C}^{-1}$ (Liu and Lipták, 1997).

UV absorbance (A_{254}) and specific UV absorbance at 254 nm ($SUVA_{254}$)

The ultraviolet absorbance at 254 nm (A_{254}) was measured using a Perkin-Elmer UV-Vis Lambda 20 spectrophotometer with quartz cuvettes that had a 10-cm path length. The A_{254} was expressed in m^{-1} . The specific UV absorbance at 254 nm ($SUVA_{254}$), which is defined as the ratio between A_{254} and the concentration of DOC (Edzwald and Tobiason, 1999), was also calculated. The $SUVA_{254}$ was expressed in units of $\text{L m}^{-1} \text{ mg C}^{-1}$.

Measurements of biological activity

Adenosine Triphosphate (ATP)

ATP is a biomolecule that is present in all living organisms, and its concentration in seawater can be used as an indirect measure of the biological activity in seawater (Hammes et al., 2010). The total ATP (ATP_{total}) is present in the ecosystem in two different forms, which include cellular ATP (ATP_{cell}) that is directly associated with the living cells and free ATP (ATP_{free}) that is dissolved in the media and not contained in the cells, which is called extracellular ATP.

ATP_{total} and ATP_{free} were quantified using the BacTiter-Glo™ Microbial Cell Viability Assay (Promega Biotech Iberica, Spain) and a GloMax® 20/20 luminometer (Promega Biotech Iberica, Spain). The samples were first filtered through a 0.22 µm filter (to retain bacterial cells) before measuring the ATP_{free} . The ATP_{cell} was indirectly determined by subtracting the ATP_{free} from the ATP_{total} . The measurements were performed by mixing 100 µL of the seawater sample with 100 µL of an ATP reagent in an Eppendorf tube. The mixture was agitated with an orbital mixer at room temperature for 3 min, and after 30 s, the luminescence signal was recorded, which was measured as relative light units (RLU). The RLU values were converted to ng ATP L⁻¹ using ATP standard dilutions (A3377, Sigma-Aldrich). All the ATP analyses were performed in triplicate.

Biofilm formation capacity (BFC)

A test has been established to evaluate the biofilm formation capacity (BFC) in seawater. The assay is based on the biofilm formation rate (BFR) described elsewhere (van der Kooij et al., 1995a). The BFC test consists of a glass non-porous bead chain (bead surface area = 28.3 mm²) that is immersed in continuously flowing water in a vessel (10 × 12 × 15 cm) (see Figure 2), and the growth of biofilm on the glass beads is recorded over time in terms of the ATP concentration. Periodically, a pearl of the chain was carefully removed and analysed for its ATP content. The pearls were placed in an Eppendorf tube that contained 100 µL of phosphate buffer (pH = 7.0), and then 200 µL of an ATP reagent was added. After mixing for 3 minutes, 200 µL of the supernatant was collected and the luminescence signal (in RLU) after 30 seconds was recorded. Finally, the RLU values were converted to ATP content. The BFC is expressed as pg ATP cm⁻² day⁻¹.

Figure 2. Schematic of the BFC monitor

Liquid Chromatography coupled with Organic Carbon Detection (LC-OCD)

Liquid chromatography coupled with organic carbon detection (LC-OCD) allows the pool of NOM to be separated into major fractions of different sizes and chemical functionalities and to quantify these fractions on the basis of organic carbon (Huber et al., 2011). The fractions obtained with this analysis are biopolymers, humic substances, building blocks, low molecular weight (LMW) neutrals, and LMW acids (Table 4).

Table 4. Composition of the LC-OCD fractions

Fluorescence Excitation-Emission matrices (F-EEM)

Approximately 50% of NOM is fluorescent (Beck and Royal Society of Chemistry, 1993). In this study, excitation wavelengths were varied from 220 to 470 nm, and the emission wavelengths were varied from 280 to 580 nm. The fluorescence intensities were corrected by normalising with the Raman spectra of water according to Lawaetz and Stedmon (2009) and subtracting the signal of Milli-Q water.

The excitation and emission data are presented in the form of a fluorescence excitation-emission matrix (F-EEM) according to the fluorescent regions proposed by Chen et al. (2003) (see Table 5).

Table 5. Fluorescence regions (according to Chen et al., 2003)

Membrane fractionation

Samples of seawater before and after biofiltration were fractionated using a ceramic nanofiltration membrane with a molecular weight cut-off (MWCO) of 1 kDa (TAMI, France) in the tangential-flow mode.

The fraction that can pass through the membrane, which is also referred to as the permeate, is composed of NOM that has molecular weights less than 1 kDa (Amon and Benner, 1996; Ogawa and Tanoue, 2003; Kaiser and Benner, 2009). Therefore,

the permeate is the low molecular size fraction (LMS) (Equation 1). Then, to fulfil the mass balance, the fraction that has molecular weights greater than 1 kDa, which represents the high molecular size (HMS) fraction, was calculated using Equation 1. Note that DOC appears in Equation 1 and 2, but the same calculations are performed for BOD₇ and A₂₅₄.

$$\text{DOC}_{\text{LMS}} = \text{DOC}_{\text{permeate}} \quad [\text{Equation 1}]$$

$$\text{DOC}_{\text{HMS}} = \text{DOC}_{\text{seawater}} - \text{DOC}_{\text{permeate}} \quad [\text{Equation 2}]$$

The SUVA₂₅₄ (A₂₅₄/DOC) and biodegradability (BOD₇/DOC) ratios were also calculated using the previously described parameters.

3. Results and discussion

3.1. DOC, A₂₅₄, BOD, turbidity, biodegradability and SUVA₂₅₄ for different EBCT

Biofiltration was performed at three different EBCTs, including 5.7, 8.1 and 11.3 min. Table 1 presents the values of DOC, A₂₅₄, BOD₇, SUVA₂₅₄ and BOD₇/DOC obtained at the inlet and outlet streams of the biofilter for each EBCT. The removals for each parameter were also calculated and are presented in Table 6.

The first observation from Table 6 is the low content of organic matter in seawater (DOC = 0.93 – 1.13 mg C L⁻¹) compared to natural freshwater (1 – 30 mg C L⁻¹), (Jaffe et al., 2008; Baghoth et al., 2008). Additionally, the low values of BOD₇ (1.00 – 1.11 mg O₂) and BOD₇/DOC (0.98 – 1.17 mg O₂ mg C⁻¹) reveal that the seawater contains only a small fraction of biodegradable matter. Furthermore, the values of SUVA₂₅₄ < 1 reveal the lower hydrophobic character of NOM (Edzwald and Tobiasson, 1999). These results are consistent with those previously obtained by Penru et al. (2011). However, although most NOM observed in seawater is resistant to microbial oxidation (Ogawa et al., 2001), the biological filtration with EBCTs from values as low as 5.7 to 11.3 min allows the removal of DOC, A₂₅₄ and BOD₇ ranging from 4 to 15%. In the investigated EBCT range, the SUVA₂₅₄ at the inlet and at the outlet streams of the biofilter remain almost invariable due to no preferential removal

of UV absorbing substances. As expected, the biodegradability (BOD_7/DOC) at the output of the biofilter is always small in comparison to the input.

Low consumptions of DOC have also been reported by other authors (Sndergaard and Worm, 2001), and it has been observed that these consumptions are significantly affected by the source of NOM (Hozalski et al., 2001). The DOC in seawater is primarily recalcitrant and at most 19% ($SD = 12\%$) of this DOC can be used by heterotrophic microorganisms (Søndergaard and Middelboe, 1995).

Table 6. Inlet and outlet concentrations of the different analytical parameters and the removals attained in the biofiltration process

Note that the NOM removed by the biofiltration process corresponds to the easily biodegradable fraction, as evidenced by the fact that the removal of DOC is almost independent of the EBCT in the investigated range. Presumably, this fraction of NOM is the one that can cause biofouling in downstream processes (Carlson and Amy, 1997).

The hydraulic loading rates (HLR), which are directly associated with the EBCT, ranged from 5 to 10 m h^{-1} , and it does not appear to have any effect on the removal of NOM, as reported elsewhere (Carlson and Amy, 1998).

The results obtained in a seawater environment enable a classification of the overall NOM depending on the lability of the carbon (Carlson and Ducklow, 1996; Ogawa and Tanoue, 2003). Therefore, seawater NOM can be classified in three fractions that are named labile, semi-labile and recalcitrant. The use of a biofilter can remove the more labile fraction in few minutes (most likely less than 5 min). These labile compounds are those that support bacterial growth (Hozalski et al., 1995). The semi-labile fraction is susceptible to be removed depending on the applied EBCT. Finally, the refractory fraction cannot be removed by biofiltration and some type of pre-oxidation process could be required to remove this fraction (Lamsal et al., 2011).

3.2. Nitrogen

The measurements of the concentration of organic and inorganic nitrogen at the inlet and the outlet streams of the biofilter revealed the preferential use of organic nitrogen during the biological activity of the biofilter (

Figure 3). It was observed that 18% of organic nitrogen (N_{org}) was consumed in the biofilter, whereas the amount of inorganic nitrogen (N_{inorg}) remained almost constant. These results are consistent with those published by Cherrier et al. (1996).

Figure 3. Concentration of N_{inorg} and N_{org} at the inlet and outlet streams of the biofilter operating at EBCT = 8.1 min

Figure 4 provides a breakdown of the inorganic nitrogen composition. Nitrate (NO_3^-) is the predominant (70%) species in seawater. Furthermore, the amount of nitrate in the outlet of the biofilter is greater than in the inlet due to the biological oxidation of NH_4^+ and NO_2^- by the nitrification reactions (Hellinga et al., 1999). Therefore, autotrophic nitrifying bacteria are also present in the filter. However, due to the low concentration of ammonia in seawater, it may be concluded that autotrophic biomass represents only a small part of the total biomass of the biofilter.

Figure 4. Inorganic nitrogen (NH_4^+ , NO_2^- , and NO_3^-) distribution in the inlet and outlet streams of the biofilter operating at EBCT = 8.1 min

3.3. Evolution of ATP in the biofiltration process

Analyses of the ATP concentrations were performed at the inlet and the outlet of the biofilter. It was observed that the biofilter reduces the amount of ATP in seawater by 60%; the concentration of ATP at the outlet was $16 \text{ ng ATP}_{\text{cell}} \text{ L}^{-1}$ whereas that at the input was $41 \text{ ng ATP}_{\text{cell}} \text{ L}^{-1}$ (Figure 5). From the above mentioned ATP concentrations, it is possible to estimate the cell number at both the inlet and outlet of the biofilter. Assuming that a marine cell has an ATP content of $0.5 - 6.5 \cdot 10^{-6} \text{ ng ATP}$ (Hamilton and Holm-Hansen, 1967), the active cell number observed at the inlet is $6.3 \cdot 10^6 - 8.2 \cdot 10^7 \text{ cells L}^{-1}$, whereas in the outlet the active number of cells is $2.5 \cdot 10^6 - 3.2 \cdot 10^7 \text{ cells L}^{-1}$. However, the amount of ATP trapped in the biofilter during the period between two consecutive backwashings is considerably less than the amount of ATP extracted in the backwashing process itself. Therefore, autochthonous biomass is established and developed in the biofilter.

Figure 5. Concentrations of ATP_{cell} and ATP_{free} at the inlet and outlet streams of the biofilter operating at EBCT = 8.1 min.

3.4. Evolution of LC-OCD in the biofiltration process

The LC-OCD reveals that seawater has a low content of organic matter that is primarily composed of LMW neutrals, humic substances and building blocks, which represent approximately 90% of the total DOC (Baghoth et al., 2008; Penru et al., 2011; Simon et al., 2012). All fractions, except the LMW acids, were removed to some extent by the biofiltration process (Figure 6). The maximum removals were achieved for LMW neutrals (33%), which essentially consist of alcohols, aldehydes, ketones, sugars and amino acids (Huber et al., 2011). In principle, all these compounds can be easily degraded by heterotrophic biomass (Amon et al., 2001). Building blocks were better removed than humic substances (21% and 9%, respectively), which was most likely because of their lower complexity and greater hydrophilicity (Huck, 1999; Baghoth et al., 2008; Penru et al., 2011). For biopolymers with high molecular weights, the concentration of 64 $\mu\text{g C L}^{-1}$ in seawater was reduced to 53 $\mu\text{g C L}^{-1}$ by the biofilter.

Figure 6. Results of LC-OCD for the inlet and outlet streams of the biofilter operating at EBCT = 8.1 min

3.5. Membrane fractionation

The fractionation of the seawater using membranes revealed that the majority of NOM in seawater has a molecular weight that is less than 1 kDa (Figure 7). Therefore, it was observed that 67% BOD₇, 90% DOC and 77% of A₂₅₄ are in this majority fraction, which is consistent with the results published elsewhere (Kaiser and Benner, 2009; Ogawa and Tanoue, 2003; Amon and Benner, 1996; Dittmar and Kattner, 2003; Amon and Benner, 1996; Dittmar and Kattner, 2003). Furthermore, the results reveal that the biodegradability and the aromaticity of seawater contain a greater proportion of high molecular size fraction (HMS) material compared with the total of the DOC. Accordingly, although the LC-OCD analysis revealed that the *LMW neutrals* fraction was better reduced fraction (see Figure 6), this is only a slight

fraction of the DOC pool. Therefore, HMS are better degraded than LMS. All these results confirm the size-reactivity continuum model, which suggests that NOM found in HMS is more bioreactive than LMS (Tranvik, 1993; Amon and Benner, 1996; Ogawa and Tanoue, 2003; Kaiser and Benner, 2009).

Figure 7. LMS and HMS fractions distribution at the inlet and outlet streams of the biofilter, expressed in terms of BOD_7 , DOC, and A_{254} (EBCT = 8.1 min). Note that in the case of the outlet stream, the distribution between LMS and HMS is referred to inlet parameters.

3.6. Fluorescence excitation-emission matrices

Figure 8 presents the F-EEM of the seawater at the input and output of the biofilter when the EBCT was 8.1 min. **¡Error! No se encuentra el origen de la referencia.**7 shows the accumulated fluorescence intensities, expressed as Raman Units (RU), with their percentage on the five aforementioned groups and the fluorescence reduction due to the biofiltration process.

Figure 8. Density plots of fluorescence intensities for seawater at the input (*a*) and output (*b*) of the biofilter (EBCT = 8.1 min)

Table 7. Fluorescence intensity distribution at the inlet and outlet streams of the biofilter and removals operating at EBCT = 8.1 min

The fluorescence signal from seawater primarily arises from humic and fulvic acids, which represent approximately 90% of the total observed intensity. All five distinct regions of the fluorescence excitation-emission matrix undergo a reduction in intensity due to the biofiltration process, and the overall reduction is 12%. The highest reduction (21%) was observed in region [I], which is primarily composed of aromatic protein-like substances. Aromatic protein-like substances are susceptible to being biologically degraded and are hypothesised to be a principal foulant together with the polysaccharides (Salinas Rodriguez et al., 2009). Furthermore, the tyrosine-like and

tryptophan-like fluorescence peaks are contained in the protein-like regions, which were first described by Coble (1996) and are characteristic of microbial activity and associated with labile organic matter.

3.7. Biofilm formation capacity (BFC)

The potential capacity of seawater to cause biofouling was measured in seawater before and after the biofiltration process. To measure this capability, the BFC assay was used as described above. Figure 9 shows the concentration of ATP over 40 days of the test.

The average concentration of ATP in the biofilm exposed to unfiltered seawater was greater than $100 \text{ pg ATP cm}^{-2} \text{ day}^{-1}$. In contrast, the average concentration in the biofilm exposed to the biofiltered seawater was less than $7 \text{ pg ATP cm}^{-2} \text{ day}^{-1}$. Therefore, the biofilter is capable of reducing biological activity by approximately 95%.

Figure 9. ATP concentrations on the surface of the beads versus time obtained with the BFC assay performed in the seawater before (inlet) and after (outlet) the biofiltration process (EBCT = 8.1 min)

Vrouwenvelder et al.(2001; 2003) observed that ATP concentrations greater than $100 \text{ pg ATP cm}^{-2} \text{ day}^{-1}$, which were measured using the biofilm formation rate (BFR) test, involve problems related to biofouling. Although there are some differences between the BFR and BFC tests (e.g., in the BFR test, the supports are small circular bands, whereas in the BFC test, the supports are beads; furthermore, the hydraulic rates are greater in the BFR tests), the obtained values could be compared.

Using this comparison, in terms of biofouling capacity, it can be stated that the biofiltered seawater is better than treated freshwater (groundwater or river water) (van der Kooij et al., 1995a). However, biofiltration does not appear to reduce the biofouling capacity of seawater to less than $1 \text{ pg ATP cm}^{-2} \text{ day}^{-1}$, which can be observed in treated freshwaters that have very low contents of easily biodegradable substances (van der Kooij et al., 1995b).

4. Conclusions

A biofilter operated with an EBCT of 5.7 – 11.3 min is able to reduce the small amount of biodegradable NOM observed in seawater. These reductions correspond to removals of DOC, BOD₇ and A₂₅₄ ranging from 6 to 15%.

NOM fractions obtained by LC-OCD analysis in the inlet and outlet of the biofilter have shown reductions through the biofiltration process of 33% of LMW neutrals, 21 % of building blocks and 17% of biopolymers.

NOM fractions obtained by membrane separation in the inlet and outlet of the biofilter have shown that the biofiltration process reduces the molecular weight of the NOM. The percentage of compounds with molecular weights greater than 1 kDa is effectively reduced.

Fluorescent analysis (F-EEM) of seawater has shown the presence of fulvic acid-like substances, humic acid-like substances and aromatic protein-like compounds. Fulvic and humic acid-like substances are more abundant than the aromatic protein-like compounds, but in the biofiltration process, the aromatic protein-like compounds are reduced in a greater proportion than the fulvic and humic acid-like substances.

The organic nitrogen content of seawater is the nitrogen source to fulfil the metabolic processes of the microorganisms that developed in the biofilter. Furthermore, seawater contains a slight quantity of inorganic nitrogen as ammonia, which is almost fully oxidised to nitrate in the biofiltration process.

Biofiltration significantly reduces the ability of seawater to generate a biofilm.

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Table 2. Characteristics of raw seawater

Parameter	Unit	Value
pH	-	8.2 ± 0.1
TDS	g NaCl L ⁻¹	32.5 ± 0.6
Conductivity	mS cm ⁻¹	57.4 ± 0.4
BOD ₇	mg O ₂ L ⁻¹	1.11 ± 0.32
DOC	mg C L ⁻¹	0.96 ± 0.11
A ₂₅₄	m ⁻¹	0.67 ± 0.03
Temperature	°C	21 ± 4
Turbidity	NTU	1.06 ± 0.28

Table 3. Characteristics of the column and packed bed

Parameter	Units	Value
height (h)	mm	940
diameter (d)	mm	80
h / d	-	12
bed volume	L	4.7
porosity	-	0.4
interfacial area	m ⁻¹	1,333
bulk density	kg m ⁻³	0.73 – 0.90

Table 4. LC-OCD fraction composition

Fraction	Size Range (Da)	Composition
Biopolymers	> 20,000	Polysaccharides and protein
Humic substances	~ 1,000	Humic and fulvic acids
Building blocks	300 – 500	Weathering and oxidation products of humics
LMW neutrals	< 350	Alcohols, aldehydes, ketones and amino acids
LMW organic acids	< 350	Aliphatic low-molecular-weight organic acids

Table 5. Fluorescence regions (accordingly to Chen et al., 2003)

Region	Excitation	Emission	Description
I	220-250	280-332	Aromatic proteins I
II	220-250	332-380	Aromatic proteins II
III	220-250	380-580	Fulvic acid-like
IV	250-470	280-380	Microbial by-products
V	250-470	380-580	Humic acid-like

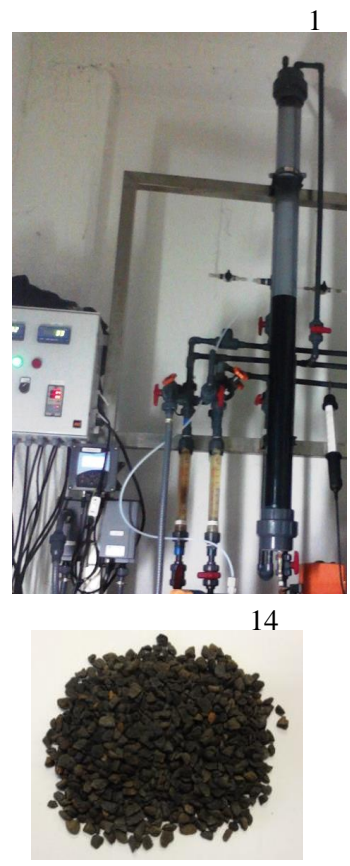
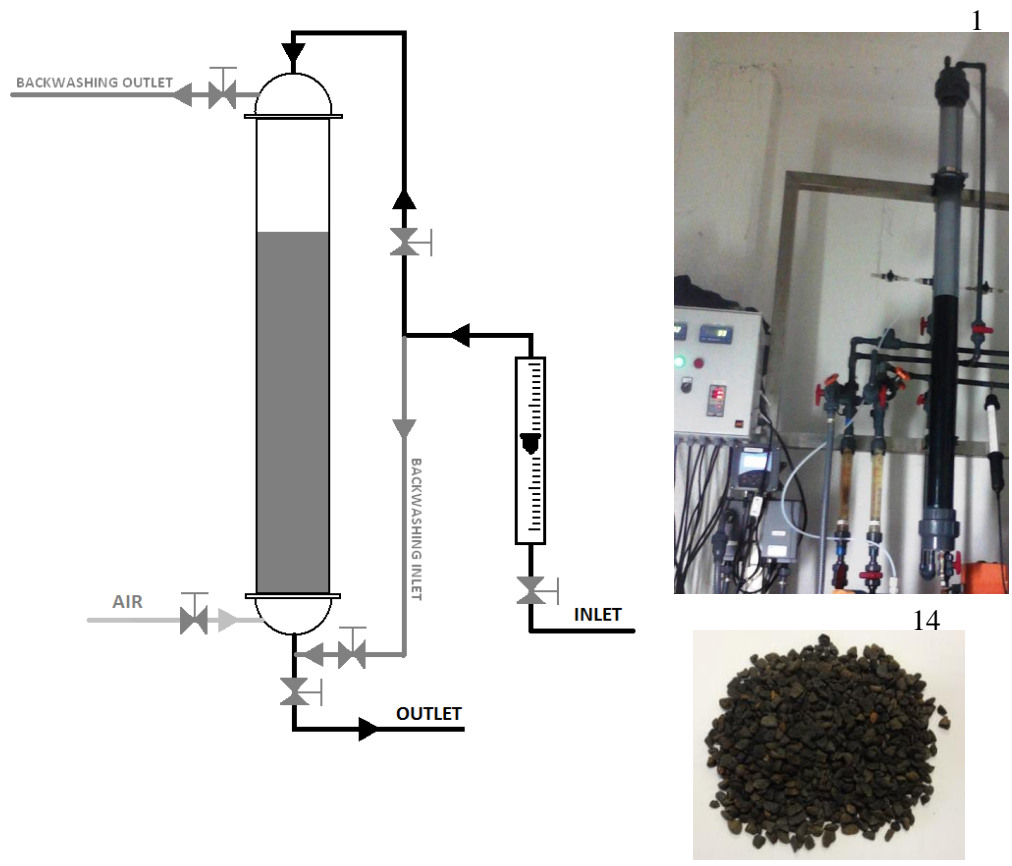
Table 6. Inlet and outlet concentrations of the different analytical parameters and the removals attained in the biofiltration process

<i>Parameter</i>	<i>Experiment 1</i> EBCT = 5.7 min			<i>Experiment 2</i> EBCT = 8.1 min			<i>Experiment 3</i> EBCT = 11.3 min		
	INLET	OUTLET	Removal [%]	INLET	OUTLET	Removal [%]	INLET	OUTLET	Removal [%]
DOC [mg C L ⁻¹]	0.94 (± 0.10)	0.88 (± 0.11)	6	1.13 (± 0.06)	1.07 (± 0.05)	5	0.93 (± 0.08)	0.87 (± 0.07)	6
A₂₅₄ [m ⁻¹]	0.68 (± 0.03)	0.65 (± 0.04)	4	0.65 (± 0.02)	0.61 (± 0.02)	6	0.68 (± 0.04)	0.64 (± 0.04)	6
BOD₇ [mg O ₂ L ⁻¹]	1.10 (± 0.21)	0.94 (± 0.21)	15	1.11 (± 0.26)	0.98 (± 0.32)	12	1.00 (± 0.23)	0.88 (± 0.20)	12
SUVA₂₅₄ [L mg C ⁻¹ m ⁻¹]	0.72	0.74	-2	0.58	0.57	1	0.73	0.74	-1
BOD₇/DOC [mg O ₂ mg C ⁻¹]	1.17	1.07	9	0.98	0.92	7	1.08	1.01	6

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Table 7. Fluorescence intensity distribution at the inlet and outlet streams of the biofilter and removals operating at EBCT = 8.1 min

Fractions	INLET Σ int [R.U.] (Distribution, %)	OUTLET Σ int [R.U.] (Distribution, %)	Removal [%]
I - Aromatic Protein I	132 (2)	105 (2)	21
II - Aromatic Protein II	404 (7)	361 (8)	11
III - Fulvic acid-like	3603 (66)	3215 (67)	11
IV - Microbial by-product	81 (2)	69 (1)	14
V - Humic acid-like	1268 (23)	1086 (22)	14
TOTAL	5489	4837	12

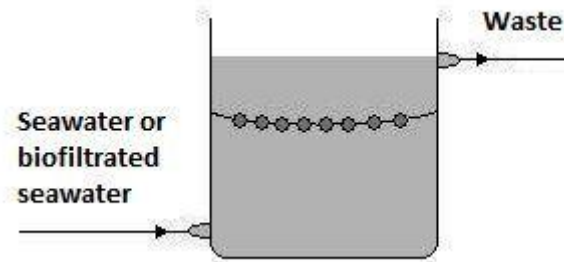


(c)

Figure 10. (a) Scheme of the biofiltration pilot; (b) Image of the pilot plant; (c) Expanded clay - Biolite®L2.7

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Figure 11. Scheme of the BFC monitor

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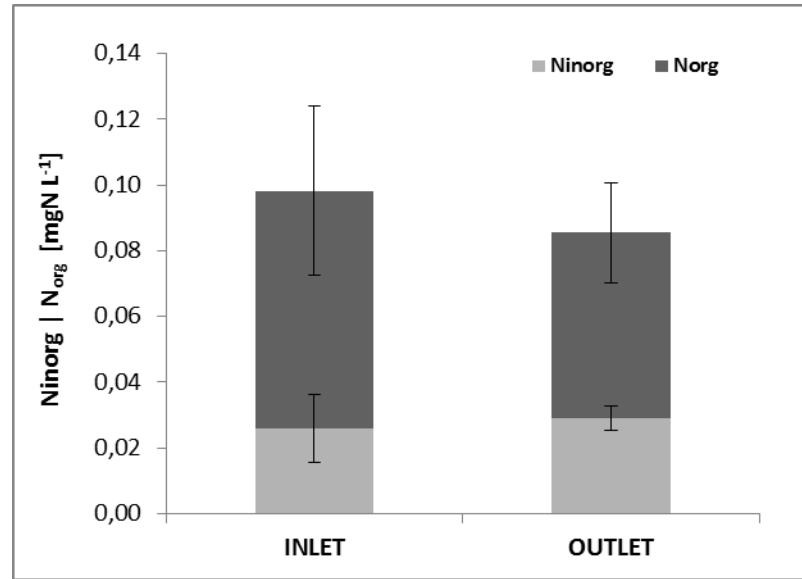


Figure 12. Concentration of N_{inorg} and N_{org} at the inlet and outlet streams of the biofilter operating at EBCT = 8.1 min

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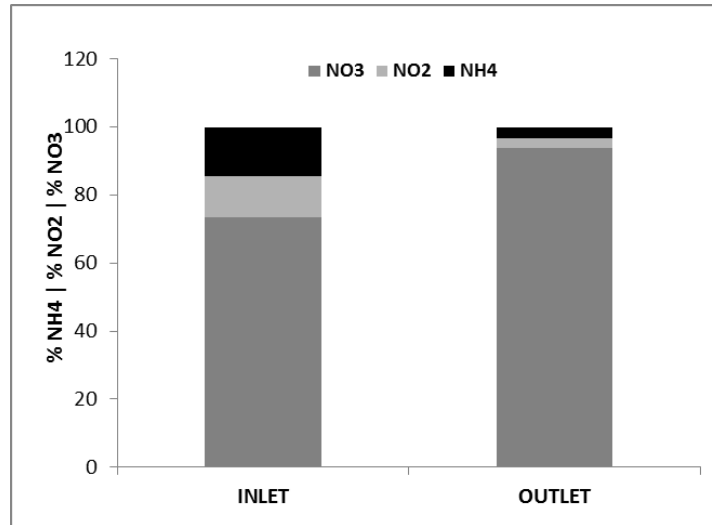


Figure 13. Inorganic nitrogen (NH_4^+ , NO_2^- , NO_3^-) distribution in the inlet and outlet streams of the biofilter operating at EBCT = 8.1 min

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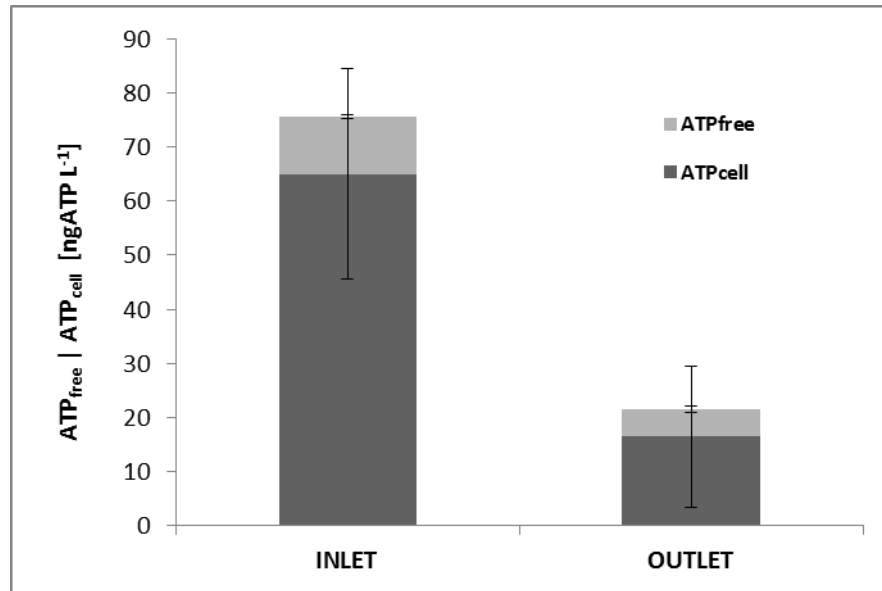


Figure 14. Concentrations of ATP_{cell} and ATP_{free} at the inlet and outlet streams of the biofilter operating at EBCT = 8.1 min.

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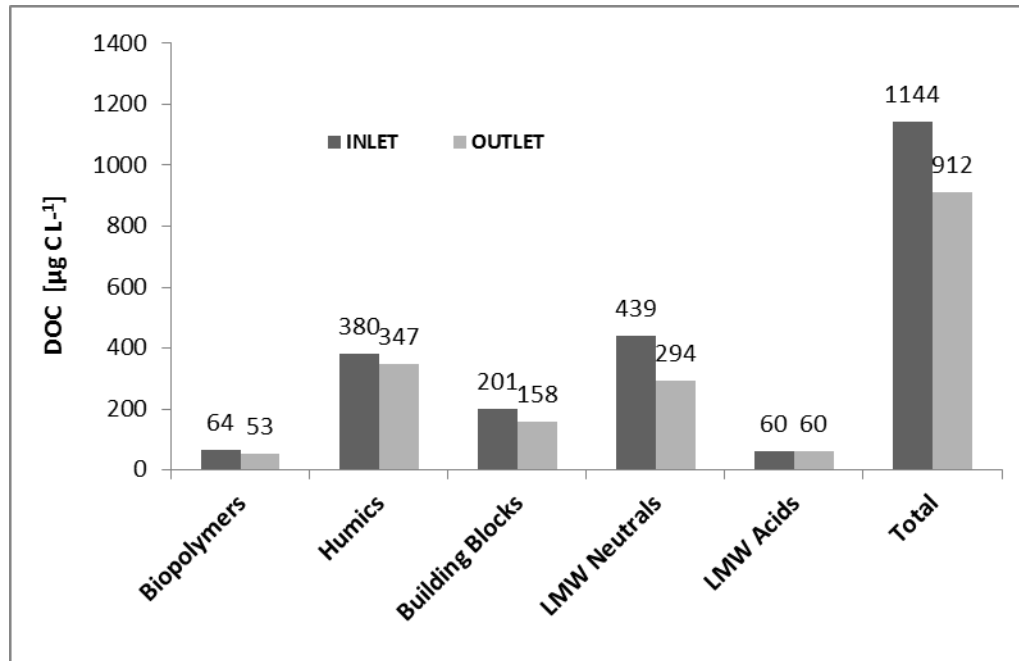
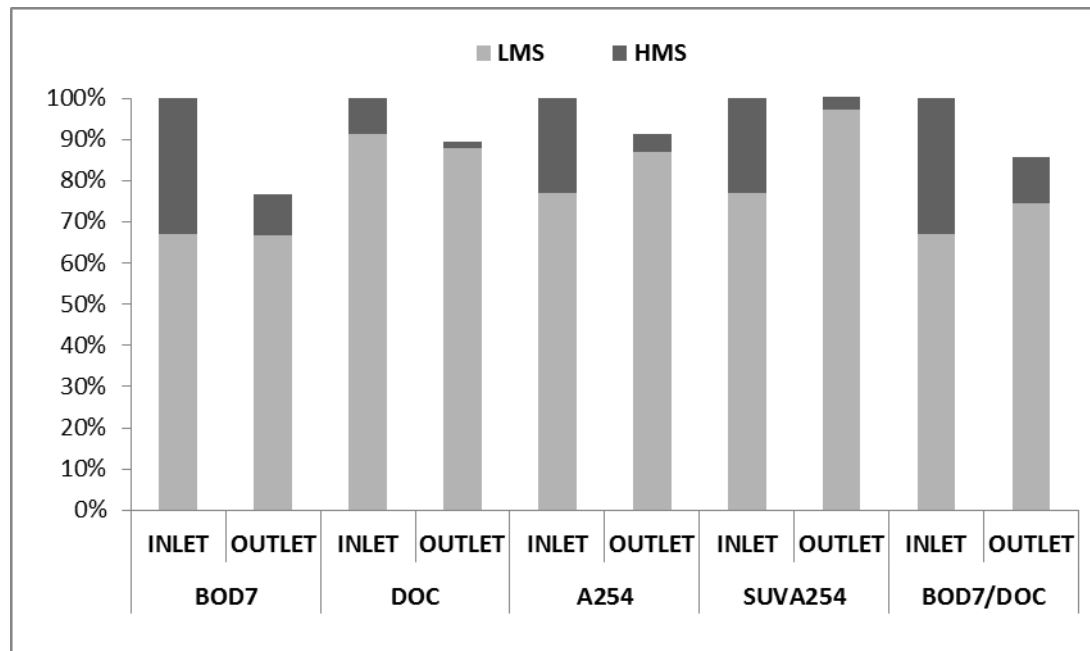


Figure 15. Results of LC-OCD for the inlet and outlet streams of the biofilter operating at EBCT = 8.1 min



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Figure 16. LMS and HMS fractions distribution at the inlet and outlet streams of the biofilter, expressed in terms of BOD₇, DOC, and A₂₅₄ (EBCT = 8.1 min). Note that in case of the outlet stream the distribution between LMS and HMS is referred to inlet parameters.

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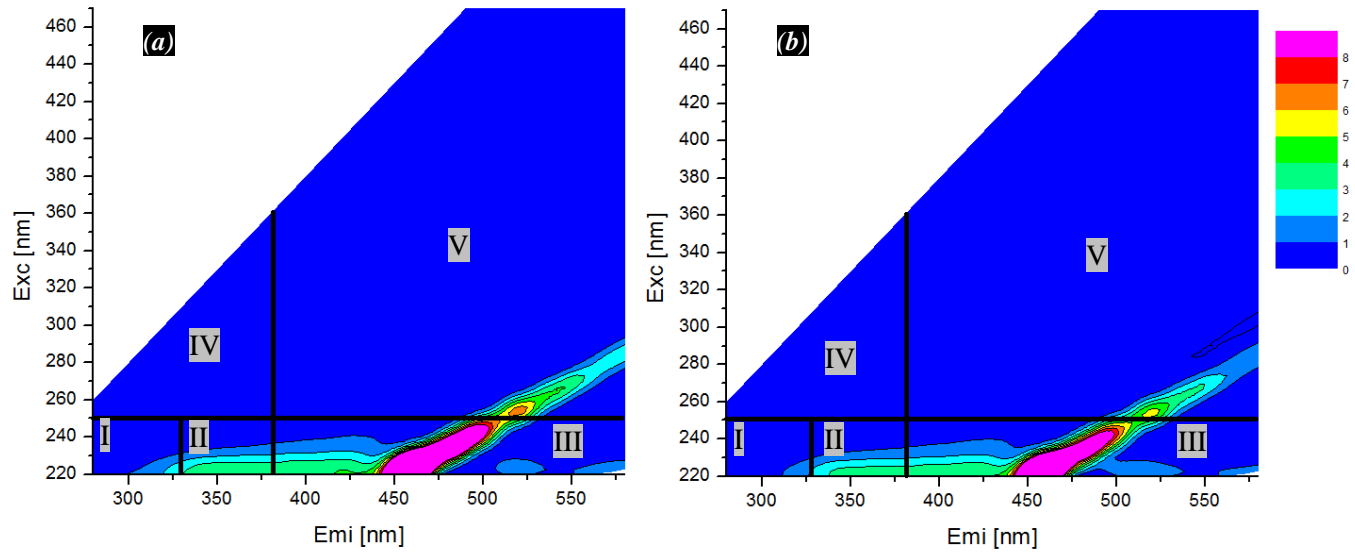


Figure 17. Density plots of fluorescence intensities for seawater at the input (a) and output (b) of the biofilter (EBCT = 8.1 min)

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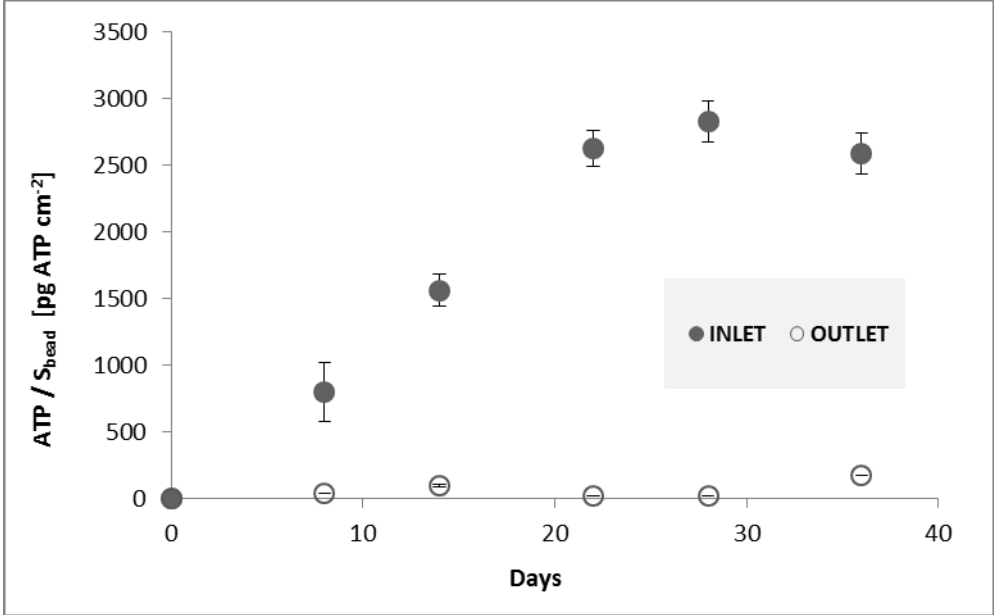


Figure 18. ATP concentrations on the surface of the beads versus time obtained with the assay BFC performed in the seawater before (inlet) and after (outlet) of biofiltration process (EBCT = 8.1 min).