Effects of inorganic nitrogen (NH_4Cl) and biodegradable organic carbon (CH_3COONa) additions on a pilot-scale seawater biofilter

F. Xavier Simon[1*], Elisabet Rudé[1], Elisa Berdalet[2], Joan Llorens[1], Sylvie Baig[3]

[1]Department of Chemical Engineering, University of Barcelona, Martí i Franquès 1, 08028 Barcelona, Spain

[2]Institut Ciències del Mar (CSIC), Passeig Marítim de la Barceloneta, 37-49, 08003 Barcelona, Catalunya, Spain

[3]Degrémont SA, 183 avenue du 18 juin 1940, 92508 Rueil-Malmaison cedex, France

Abstract

Biofilters degrade ~~only~~ a small fraction of the natural organic matter (NOM) contained in seawater which is the leading cause of biofouling in downstream processes. This work studies the effects of chemical additions on NOM biodegradation by biofilters. In this work, biofiltration of seawater with an empty bed contact time (EBCT) of 6 min and a hydraulic loading rate of 10 m h^{-1} reduces the biological oxygen demand (BOD_7) by 8%, the dissolved organic carbon (DOC) by 6% and the UV absorbance at 254 nm (A_{254}) by 7%. Different amounts of ammonium chloride are added to the seawater (up to twice the total dissolved nitrogen in untreated seawater) to study its possible effect on the removal of NOM by a pilot-scale biofilter. Seawater is amended with different amounts of easily biodegradable dissolved organic carbon (BDOC) supplied as sodium acetate (up to twice the DOC) for the same purpose.

* To whom correspondence should be addressed.

E-mail: xsimon@ub.edu

Tel: +34 934020155; Fax: +34 934021291

The results of this work reveal that the ammonium chloride additions do not significantly affect NOM removal and the sodium acetate is completely consumed by the biofiltration process. For both types of chemical additions, the BOD_7, DOC and A_{254} in the outlet stream of the biofilter are similar to the values for the untreated control. These results indicate that this biofilter easily removes the BDOC from the seawater when the EBCT is not above 6 min. Furthermore, nitrogen does not limit the NOM biodegradation in seawater under these experimental conditions.

1. Introduction

Freshwater biofiltration is widely used in water treatment plants (Cohen, 2001; Huck, et al., 1994; Servais, et al., 1991; Urfer, et al., 1997). Biofiltration is an effective means to reduce the level of biodegradable natural organic matter (NOM) of freshwater streams (Henze, 2008). Moreover, biofiltration limits the regrowth of microorganisms in pipelines, or biofouling, in downstream treatments (Hu, et al., 2005). Biofilters are often located downstream from ozone treatment modules where they consume ozonation by-products (Carlson and Amy, 1997; Servais, et al., 1994; Yavich, et al., 2004). However, relatively few publications address seawater biofiltration (Chinu, et al., 2009; Visvanathan, et al., 2003). Biofiltration of seawater can remove the easy biodegradable NOM that can cause biofouling in downstream facilities (desalination, cooling tower, heat exchangers, etc.) that are susceptible to biofouling. Therefore, it is important to understand NOM biodegradation by seawater biofilters. The biofiltration of seawater involves the growth of marine bacteria on porous media (Cohen, 2001) fed with seawater containing carbon, nitrogen, phosphorous and other elements necessary for biosynthesis. Catabolic reactions consume some of the biodegradable carbon and respire it as CO_2 under aerobic conditions, and anabolic biosynthetic cellular reactions use the remainder of the biodegradable carbon in cellular biosynthesis (del Giorgio and Cole, 1998; Goldman, et al., 1987). In the coastal and open Mediterranean Sea, the organic carbon content of the seawater is lower than in other water sources (Doval, et al., 1999),

and most of the NOM is biologically refractory (Amon and Benner, 1994; Amon and Benner, 1996). In fact, Søndergaard and Middelboe (1995) reported that an average of 19% of seawater dissolved organic carbon (DOC) is oxidised by autochthonous bacteria, and Simon, et al. (2011) found that only 8% of DOC in coastal Mediterranean seawater is biologically labile. However, biodegradation by marine bacteria may be limited by the availability of essential elements such as nitrogen (Cherrier, et al., 1996; Karleskint, et al., 2009) and/or phosphorous (Berdalet, et al., 1996; Pomeroy, et al., 1995; Zohary and Robarts, 1998; Zweifel, et al., 1993).

Marine bacteria in different habitats use various forms of nitrogen. For instance, Jørgensen, et al. (1993) reported the preferential utilisation of dissolved amino acids followed by ammonia, while nitrate was the least significant source of nitrogen. In contrast, Goldman, et al. (1987) observed that amino acids frequently do not provide all the nitrogen required by marine bacteria. Nearly all types of amino acids were consumed by prokaryotes, while ammonia was utilised by both prokaryotes and eukaryotes. Approximately 78% of the total ammonium uptake was by prokaryotes, and a significant portion of this was due specifically to heterotrophic bacteria (Wheeler and Kirchman, 1986). Moreover, Zweifel, et al. (1993) observed the preferential consumption of inorganic nitrogen and phosphorous by heterotrophic bacteria growth on short time scales. In any case, the inorganic nitrogen content in untreated seawater is very low (~2.4 10^{-2} mg $L^{-1}N$). Therefore, we investigated whether increasing the amount of inorganic nitrogen in seawater also increased NOM consumption. The purpose of our study was to investigate whether dissolved nitrogen and BDOC additions improved NOM removal of NOM from seawater through biofiltration. We performed a series of experiments where dissolved nitrogen (ammonium chloride) and/or organic carbon (sodium acetate) were supplied to a seawater biofiltration system. The performance of the biofiltration process at different C:N ratios was analysed in terms of the biochemical oxygen demand (BOD_7), DOC and A_{254}. Furthermore, inorganic and organic nitrogen were measured in the seawater before and after biofiltration.

2. Materials and methods

2.1. Seawater and biofilters

All experiments were performed in a pilot-scale biofilter at the "Zona d'Aquaris Experimentals" of the Institut de Ciències del Mar (CSIC) in Barcelona. Untreated seawater at a depth of 10 m was pumped from 300 m offshore into the system and biofiltered with an EBCT of 6 min and a constant superficial velocity of 10 m h^{-1}, which corresponded to organic loading rates of 47 and 67 mg L^{-1} of DOC and BOD, respectively. The seawater was not pretreated before it was biofiltered. The properties of untreated seawater are summarised in Table 1.

Table 1

The pilot-scale biofilter consisted of a column of PVC (height: 1.60 m; diameter: 0.80 m) with a fixed packed bed of expanded clay, Biolite®L2.7 (Degrémont). The external and internal surface areas per unit volume of the packed bed medium were 1300 and 2 10^5 m^2 m^{-3}, respectively. The biofilter was operated in the downflow direction, and it was backwashed with both air and water three times a week (Amirtharajah, 1993). The EBCT (and the hydraulic loading rate) were regulated with a flow meter and a flow control valve. Figure 1 shows a diagram and a photograph of the plant and the packed bed.

Figure 1

The packed bed medium used for biofiltration was Biolite® L2.7 (Degrémont) expanded clay, which has a large interfacial area. However, its specific surface area is very low (0.6 m^2 g^{-1}) in

97 comparison with typical adsorbent materials such as activated carbon (1 000 m^2 g^{-1}). Biomass
98 colonisation and acclimation were achieved because the biofilter operated for 10 months. There was
99 negligible NOM adsorption because of the combination of low specific surface area of Biolite® and
100 the duration of operation (Hidalgo and García-Encina, 2002; Persson, et al., 2006).
101 It was not necessary to introduce additional oxygen during any of these experiments. Dissolved
102 oxygen in untreated seawater was sufficient to support the heterotrophic biological activity, even
103 when ammonium chloride and sodium acetate were added. All analyses were performed on the inlet
104 and outlet streams of the biofilter. Water samples were collected 24 h after (250 bed volumes) the
105 step-wise addition of ammonium chloride or sodium acetate in order to evaluate the steady-state
106 response of the biofilter.
107

108 **2.2. Analysis**

109 Due to the small amount of organic matter measured in our experiments, all glassware was cleaned
110 thoroughly prior to use by soaking in aqueous HCl solution (10% v/v) for 24 hours and then rinsing
111 with MilliQ water. The glassware was covered with aluminium foil and heated at 550ºC for 4 hours.
112 All plasticware was cleaned thoroughly prior to sterilisation in an autoclave (QM 4000 SA-202X,
113 Quirumed Spain) for 30 min at 121ºC.
114 Biochemical oxygen demand at seven days (BOD_7) was determined as proposed by Simon, et al.
115 (2011) using concentrated autochthonous inoculum in an adaptation of the *Closed Bottle Method*
116 (EPA, 1998) and Standard Methods 5210 (Standard Methods, 1999). Seawater samples were
117 incubated for 7 days at 20 ± 1ºC (Medilow Selecta, Spain), and dissolved oxygen was measured
118 before and after incubation with an IntelliCAL™ LDO probe connected to an HQ40d multimeter
119 (Hach, USA). BOD_7 results were expressed in mg L^{-1} O_2.
120 The DOC was determined with a high-temperature catalytic oxidation method (HTCO), according
121 to Álvarez-Salgado and Miller (1998). First, the samples were filtered through pre-combusted

(450ºC, 24 hours) GF/F filters (Whatman, Spain) and collected in 10 mL glass ampoules. To preserve the organic materials, the samples were acidified to pH = 2 – 3 with 50 µL of 2 M H_3PO_4 Finally, the glass ampoules were heat-sealed and stored in a cool (4°C) dark place until they were evaluated with a TOC-V_{CSH} + TNM-1 analyser (Shimadzu) at the ICM (CSIC). This instrument allows the simultaneous estimation of both DOC and total dissolved nitrogen (TDN). The instrument sensitivity was checked regularly by analysing low carbon (1 µM DOC and 0 µM N, below the detection limit) and deep (700 m Florida Strait, 41-44 µM DOC, 31-33 µM TDN) water samples (Certified Reference Materials –CRM- for Dissolved Organic Carbon Analysis, Batch 10 – Lot 05-2010) supplied by Dr. Hansell and Dr. W. Chen (University of Miami, US). The detection limits of the methods were 50 µg L^{-1} for DOC and 20 µg L^{-1} for TDN, with a coefficient of variance of 1.5% and 3.0%, respectively.

Dissolved inorganic nitrogen (DIN, the sum of NH_4^+, NO_2^- and NO_3^-) was measured by following the standard procedure described by Grasshoff, et al. (1999) using an auto-analyser AA3 (Bran+Luebbe, Germany) at the ICM (CSIC). Therefore, dissolved organic nitrogen (DON) was calculated from TDN – DIN.

The UV absorbance at 254 nm (A_{254}) is commonly used to quantify aromatic and other unsaturated compounds in aquatic environments (Weishaar, et al., 2003). Matilainen, et al. (2011) proposed A_{254} as an alternative means to measure the DOC, although not all DOC is aromatic. In this work, a Perkin-Elmer UV-Vis lambda 20 spectrophotometer, with 10 cm path length quartz cell, was used for the A_{254} (m^{-1}) determinations.

Adenosine Triphosphate (ATP) is a biomolecule present in all viable microorganisms (Hammes, et al., 2010; Holm-Hansen and Booth, 1966), and it has been proposed as an indicator of biomass content. Total ATP concentration (ATP_{TOTAL}) is present in the ecosystem in two different forms: cellular ATP (ATP_{CELL}), which is directly associated with living cells, and free ATP (ATP_{FREE}), which is not confined to intracellular environments and is present in seawater (Karl, 1980).

147 ATP_{TOTAL} and ATP_{FREE} were measured using the *BacTiter-Glo™ Microbial Cell Viability Assay*
148 (Promega Biotech Iberica, Spain) and a GloMax® 20/20 luminometer (Promega Biotech Iberica,
149 Spain). For the ATP_{TOTAL} analysis, the ATP reagent (100 μL) and seawater samples (100 μL) were
150 transferred to *Eppendorf* tubes. The mixtures were continuously agitated with an orbital shaker at
151 room temperature for 3 min. Finally, the luminescence signals were measured in relative light units
152 and recorded 3.5 min after the ATP reagent additions. A prefiltration step with 0.22 μm filters was
153 used to estimate ATP_{FREE}. The ATP_{CELL} was determined as $ATP_{TOTAL} - ATP_{FREE}$, and the %
154 ATP_{CELL} was calculated from (ATP_{CELL} / ATP_{TOTAL}) · 100. ~~The ATP_{CELL} was determined as~~
155 ~~$ATP_{TOTAL} - ATP_{FREE}$, and the % ATP_{CELL} was calculated from (ATP_{CELL} / ATP_{TOTAL}) × 100.~~
156 **(Aquesta frase l'has escrit dues vegades)**
157

158 **2.3. Experimental design**

159 A dosing pump (ProMinent® BT4a, Germany) and PTFE tubing were used to enrich the seawater
160 with ammonium chloride or sodium acetate to meet the target C:N mass ratios. Three different
161 types of experiments were carried out:

162 *i)* experiments with no additions (*Control*),
163 *ii)* experiments with of ammonium chloride addition (*N series*)
164 *iii)* experiments with sodium acetate addition (*C series*)
165

166 Table 2 shows the concentrations of carbon (DOC) and nitrogen (TDN and $N\text{-}NH_4$) and the
167 corresponding C:N ratios. The atomic ratio of carbon (C), nitrogen (N) and phosphorus (P) found in
168 plankton and throughout the deep oceans is given by the Redfield ratio (C:N:P = 106:16:1). The
169 C:N ratio for nutrient-sufficient phytoplankton (7.1) is close to the Redfield ratio (6.6). However,
170 bacteria generally have a much lower C:N ratio of approximately 3 – 4.

In this work, different C:N ratios are tested for effects on microorganism growth. The tested ratios range from 9.1, for the untreated seawater, to the lowest value of 3.8 (see Table 2).

Table 2

The concentration of phosphorous in seawater was also measured, and it remained at 3.4 – 4.0 μg L^{-1} P during these experiments.

3. Results and discussion

For each series of experiments, BOD_7, DOC and A_{254} were measured at the inlet and outlet streams of the biofilter and differences between values for the inlet and outlet streams were used to evaluate the biofilter performance. These experiments are labelled according to their C:N ratios, and the results of the experiments with no chemical additions (C:N = 10:1.1) are presented for comparison. Error bars are shown for the values in the graphs. Despite the natural fluctuations of BOD_7, DOC and UV_{254} values in seawater, it is noted that these parameters are significantly reduced when water passes through the biofilter. The phosphorous level in the biofilter outlet stream was equal to the level in the untreated seawater and did not vary with the chemical additions.

3.1. Additions of ammonium-nitrogen

The BOD_7 results for the control samples revealed the presence of very low levels of biodegradable organic matter in the seawater. Hence, the BOD_7 was always less than 1.4 mg O_2 L^{-1} and, after 6 min of biofiltration, the reduction in BOD_7 was ~8%. The accuracy of individual determinations of BOD_7 was ± 0.1 mg L^{-1}. However, BOD_7 determinations for each experiment were performed in triplicate. In these circumstances, the average BOD_7 value has an accuracy of ± 0.07 mg L^{-1}.

Therefore, a reduction of 8% (~ 0.11 mg L^{-1}) in BOD_7 is significant. However, no significant differences were observed in the BOD_7 reduction for biofiltration with or without the addition of NH_4Cl. The same 8% reduction in BOD_7 was observed regardless of the C:N mass ratio (see Figure 2a). Therefore, we conclude that after 6 min of biofiltration, almost 10% of the BOD_7 is removed, regardless of the ammonium-nitrogen content of the seawater. The DOC results for the control samples revealed the presence of very low levels of organic matter. DOC values of 1 – 0.8 mg L^{-1} C were found in both the inlet and outlet streams of the biofilter. After 6 min of biofiltration, analyses of DOC in the output current of the biofilter showed almost the same reduction in DOC (~5%), regardless of the ammonium-nitrogen content in the seawater (see Figure 2b). However, the experiment with a C:N ratio equal to 10:2.0 did not follow the general trend, and there was a greater reduction in DOC. This could be caused by a slightly higher concentration of easily biodegradable organic matter in the untreated seawater. This explanation is based on the assumption that the slightly higher DOC content at the inlet of the biofilter consists of easily biodegradable organic matter, which is eliminated entirely in the biofilter. This assumption is supported by the results obtained from the experiments performed with the additions of biodegradable organic carbon, as discussed below. The results obtained by measuring the A_{254} in the inlet and outlet streams of the biofilter are consistent with the previous results obtained with the BOD_7 and DOC (see Figure 2c). In this case, the reductions of A_{254} were approximately 7 %.

Figure 2

Important consumption of DIN was not observed, regardless of the DIN content of the seawater (Figure 3a). In contrast, we observed a significant consumption of DON in the filter, which indicates that the organic nitrogen is the nitrogen source required for microorganisms in this biofilter. Although inorganic nitrogen was unchanged by the biofiltration process, internal changes

occurred in the total inorganic nitrogen pool. A significant proportion of ammonium-nitrogen was converted to nitrate during biofiltration (Figure 3b). In contrast, the small proportion of nitrite was the same in the inlet and outlet of the biofilter. Therefore, a nitrification process occurs during the biofiltration process. Chemoautotrophs are nitrifying microorganisms that use carbon dioxide as their carbon source for growth (Grady, et al., 1999; Hellinga, et al., 1999). Therefore, the addition of ammonium-nitrogen to the untreated seawater stimulated the growth of autotrophic microorganisms and did not favour heterotrophic microorganisms. Therefore, the consumption of organic carbon was unaltered, as seen above.

Figure 3

3.2. Additions of readily biodegradable organic carbon

Measurements of BOD_7 were performed on the seawater before and after the addition of sodium acetate and at the outlet stream after the biofiltration process, Figure 4a. All of the readily biodegradable organic carbon added to the raw seawater (0.85 mg L^{-1} and 1.79 mg L^{-1} of BOD_7 as sodium acetate) was consumed by the biofilter. ~~The removal of BOD_7 during the biofiltration process corresponds to the BOD_7 from the added sodium acetate because sodium acetate is already known to be a readily biodegradable substance.~~ The removal of BOD_7 during the biofiltration process corresponds to the BOD_7 added as sodium acetate in full accordance with the ready biodegradability of this substance. On the other hand, when there is no addition of sodium acetate, the removal of BOD_7 is approximately 10%. These results suggest that only readily biodegradable NOM is consumed during the short period of biofiltration (6 min). Furthermore, the BOD_7 of the fresh raw seawater varies from one day to another due to various uncontrollable natural causes. Therefore, the total content of readily biodegradable organic matter in the fresh untreated seawater can also vary from day to day.

Measurements of DOC and A_{254} were performed in the untreated seawater before and after the addition of sodium acetate and in the outlet stream after the biofiltration process. The DOC and A_{254} results are shown in Figure 4b and Figure 4c, respectively. The DOC analysis confirms that the readily biodegradable organic carbon, which was added to the untreated seawater, was biodegraded in the biofilter because the expanded clay has low adsorptive properties. The A_{254} analysis also indicates that some of the aromatic readily biodegradable organic carbon present in the untreated seawater is consumed. This result suggests that these aromatic compounds are peptides and aromatic amino acids (that contain tyrosine, phenylalanine, tryptophan, etc.) rather than humic and fulvic acids.

Figure 4

With the additions of sodium acetate, it was observed that the concentrations of the DON in the input and output streams of the biofilter are similar to the control experiment, which means that there was not much additional cellular growth. Presumably, the additional BDOC is catabolised, although anabolism and catabolism occur simultaneously in cells, as stated by Del Giorgio and Cole (1998).

Figure 5 shows the concentration of ATP_{CELL} and % ATP_{CELL} at the outlet of the biofilter for all of these experiments. The additions of both ammonium chloride and sodium acetate have positive impacts on cellular activity. Note that % ATP_{CELL} can highlight the impact of the additions on the biological activity by ignoring the effect of daily seawater ATP fluctuations. The additions of ammonium chloride resulted in higher cellular activities than the additions of sodium acetate in the studied range. As mentioned above, this higher cellular activity corresponds to autotrophic nitrification.

Figure 5

4. Conclusions

The addition of ammonium chloride to raw seawater activates the autotrophic nitrification. Increasing the concentration of inorganic nitrogen does not ~~necessarily~~ imply a higher heterotrophic activity and, therefore, greater biodegradation of organic matter present in the untreated seawater. Hence, the biofilter heterotrophic activity is limited by the biodegradability of the DOC. Furthermore, an organic source of nitrogen was preferred to satisfy the heterotrophic metabolism in the biofilter.

The sodium acetate added to raw seawater was degraded during biofiltration. Additions of sodium acetate equivalent to the amount of all DOC present in untreated seawater are completely biodegraded with an EBCT of 6 min. The organic nitrogen present in untreated seawater is not limiting the biodegradation process, even in the experiments using seawater amended with sodium acetate.

The results of this work show that the easily biodegradable NOM in seawater is consumed in the biofiltration process. The biofiltration time may be relatively short (~6 min), regardless of the amount of readily biodegradable organic matter in the seawater. Biofiltered seawater that contains lower concentrations of biodegradable substances will markedly reduce biofouling consequences.

Acknowledgements

The authors are grateful to the Spanish Ministry of Industry (CDTI) for their financial support within the framework of the Project CENIT: CEN20071039. DOC and TDN analyses were

294 performed by M.I. Abad in coordination with Dr. E. Berdalet at the Technical Services Unit of the
295 Institut de Ciències del Mar (CSIC). Certified Reference Materials for DOC analysis were obtained
296 from Dr. Hansell and Dr. W. Chen (University of Miami, US).
297

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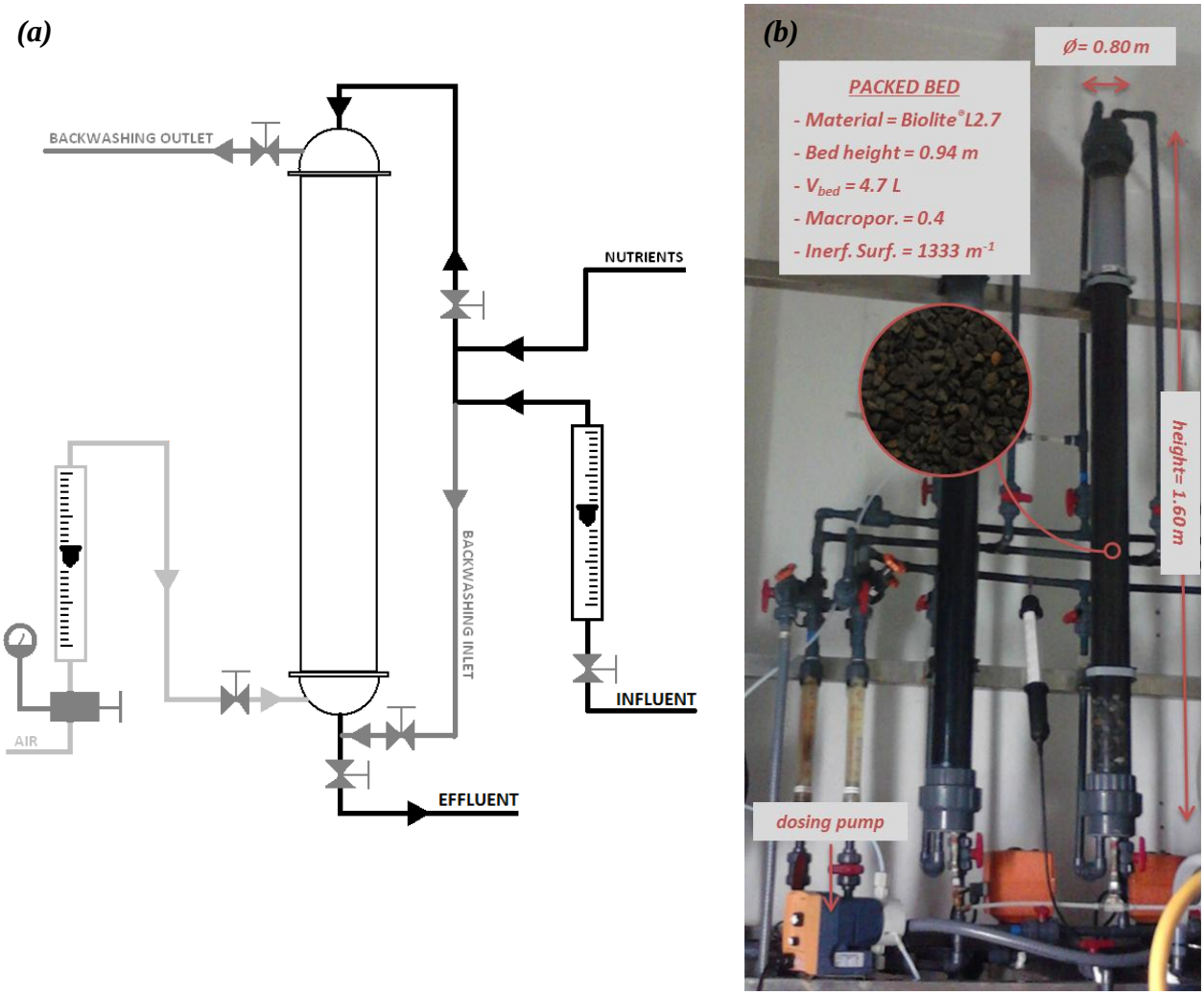

Figure 3. *(a)* Scheme of the biofiltration plant; *(b)* Photograph of the pilot biofilter

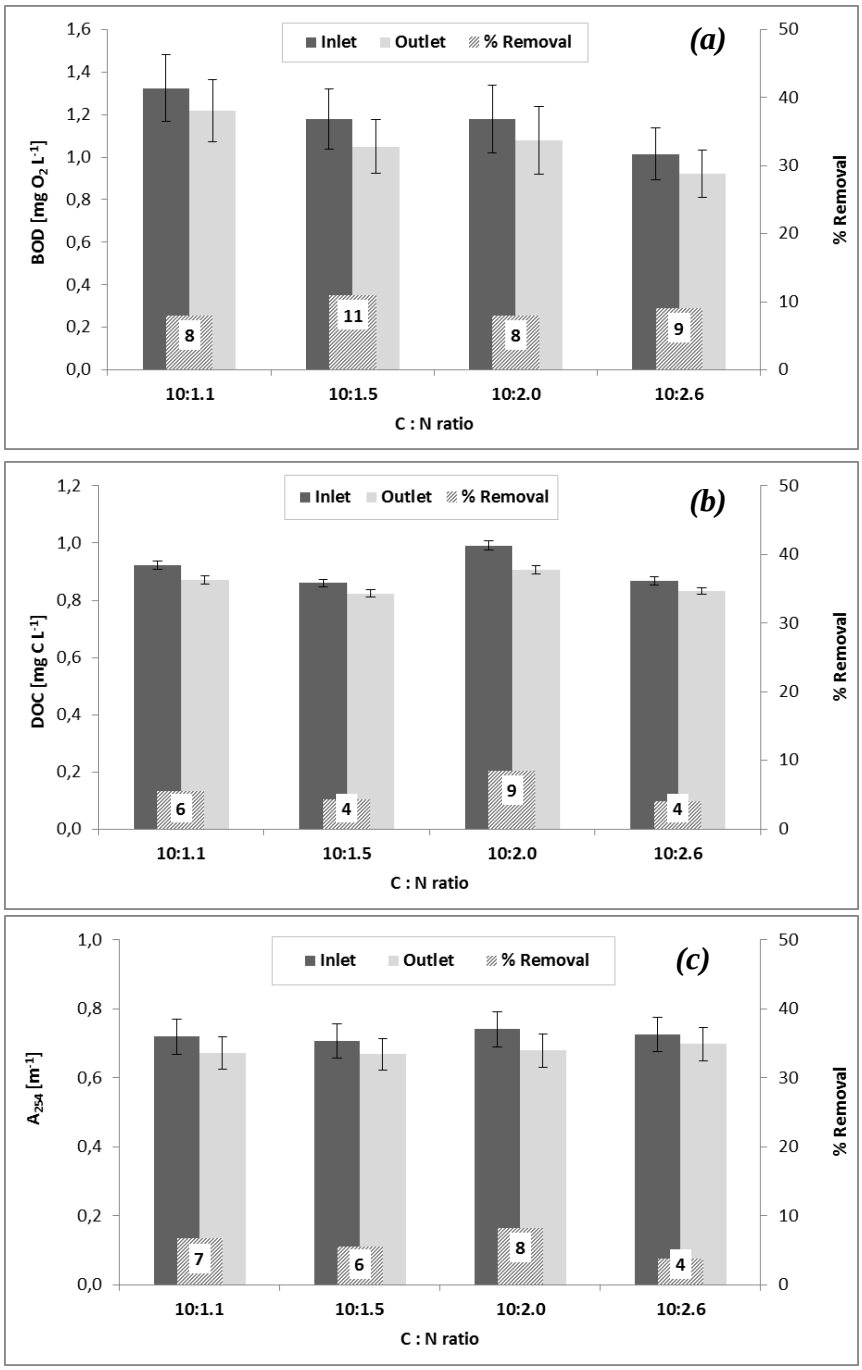

Figure 4. Biofiltration with different additions of NH_4Cl (see Table 2): ***(a)*** BOD_7 at the inlet/outlet; ***(b)*** DOC at the inlet/outlet; ***(c)*** A_{254} at the inlet/outlet. The numbers presented in each graph correspond to the % of removal of BOD_7, DOC and A_{254}, respectively. Error bars mean the standard deviation.

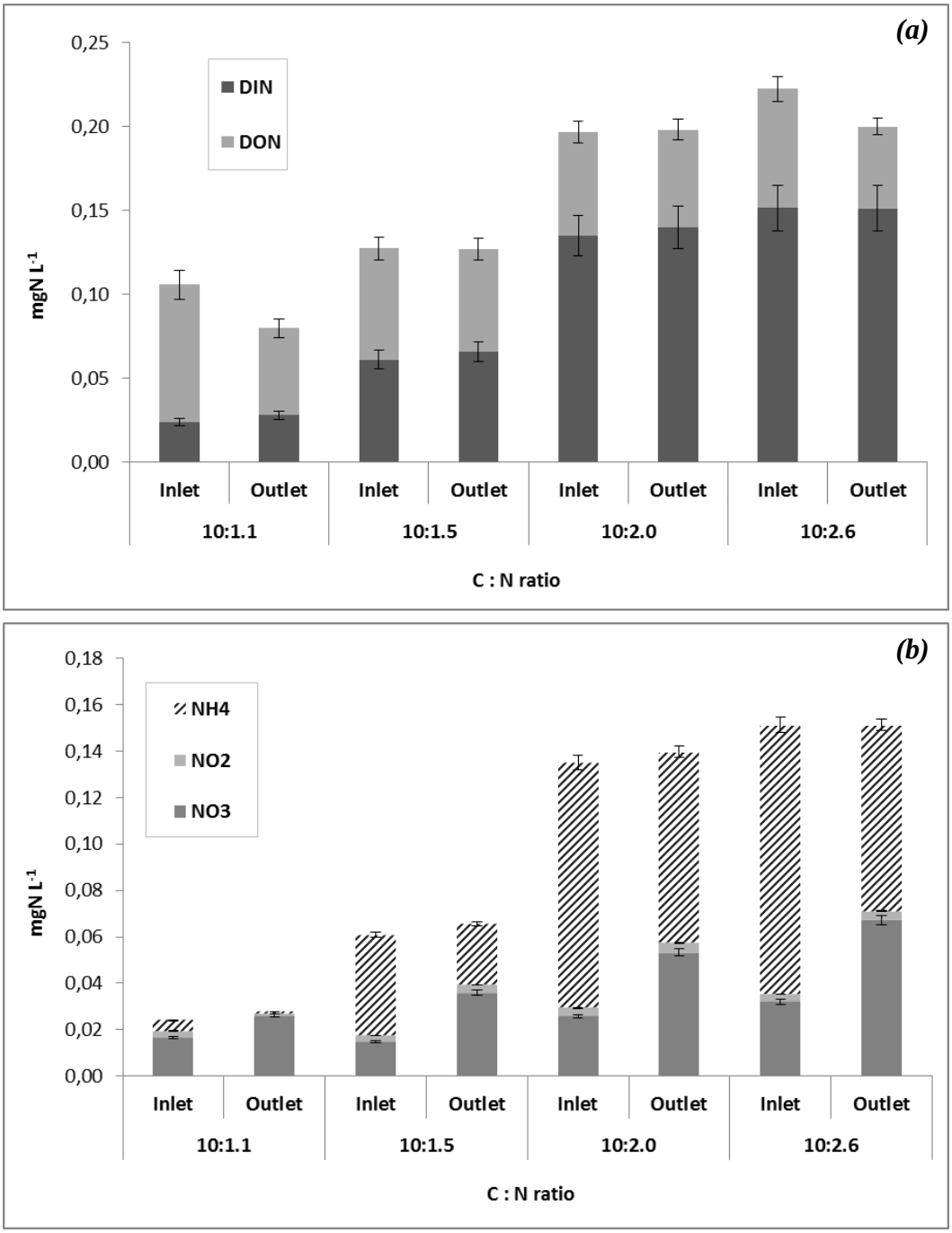

Figure 3. ***(a)*** Dissolved inorganic and organic nitrogen (DIN and DON, respectively) in the inlet and outlet streams of the biofilter with different additions of NH_4Cl; ***(b)*** Concentrations of NH_4^+, NO_2^- and NO_3^- in the inlet and outlet streams of the biofilter with different additions of NH_4Cl. Error bars mean standard deviation. Further information is given in Table 2.

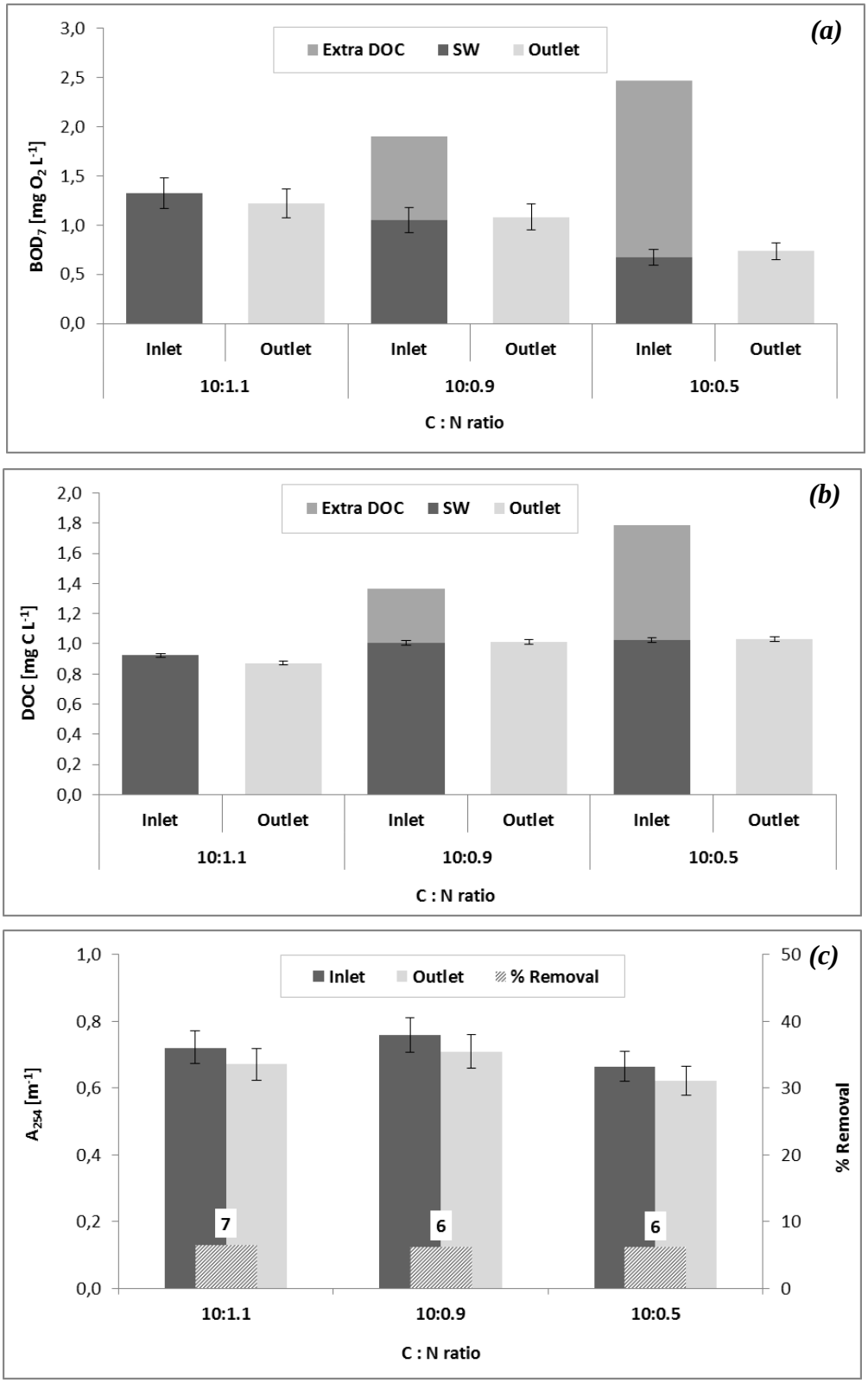

Figure 4. Biofiltration with different additions of sodium acetate (see Table 2) in the raw seawater (SW): ***(a)*** BOD_7 at the inlet/outlet; ***(b)*** DOC at the inlet/outlet; ***(c)*** A_{254} at the inlet/outlet. The numbers presented in each graph correspond to the % of removal of BOD_7, DOC and A_{254}, respectively. Error bars mean standard deviation and the numbers presented in *(c)* mean A_{254} removal.

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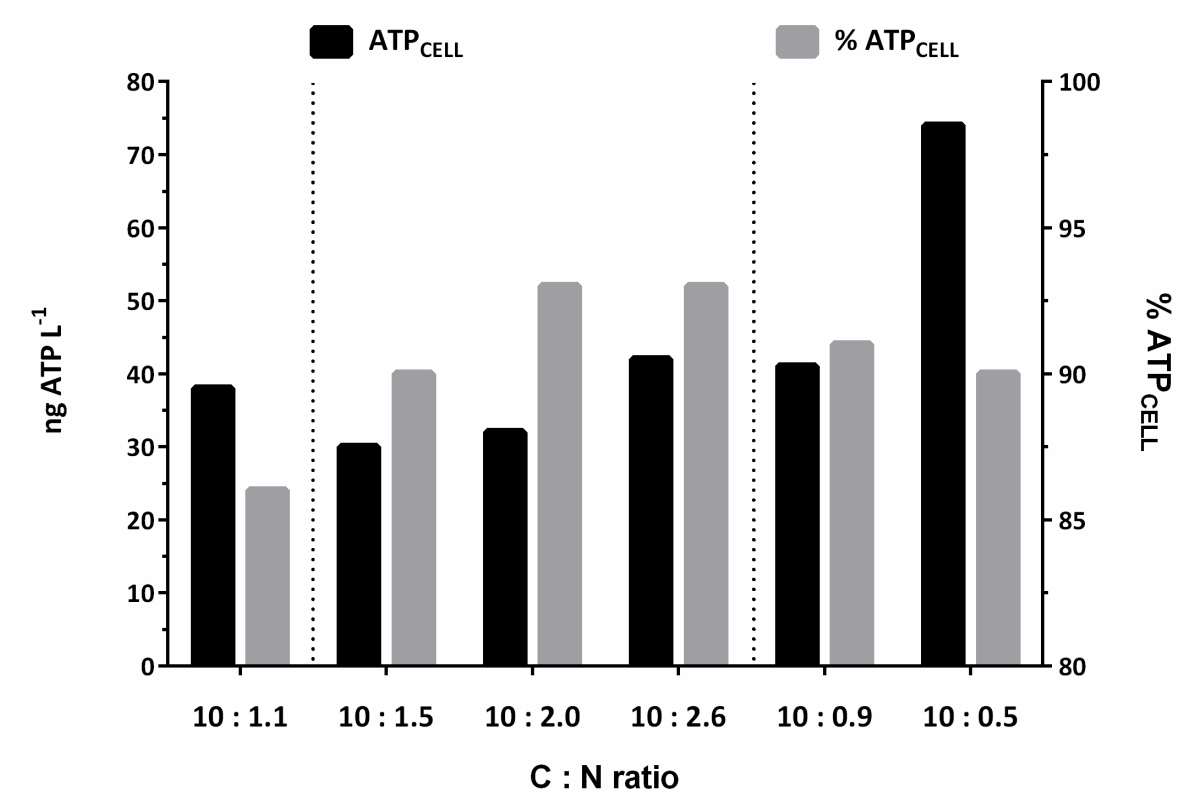

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509 **Figure 5.** ATP_{CELL} and $\%ATP_{CELL}$ at the outlet of the biofilter. C:N ratios are specified in