Effects of aeration on the suspended matter from a tropical and eutrophic estuary

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ARTICLE INFO

Article history:
Received 16 February 2019
Revised 29 May 2019
Accepted 30 May 2019
Available online 11 June 2019

Keywords:
Mixed-pollution
Remediation
Acidification
Esterases enzymes
Biopolymer

ABSTRACT

A comprehensive understanding of the complex biogeochemical interactions between organic matter and persistent contaminants in the suspended matter is vital for eco-efficient estuary recovery. However, little is known regarding aeration effects in suspended particulate aggregates. Therefore, this study aimed to investigate the effects of aeration on the suspended matter from a Tropical and Eutrophic estuarine environment. Anoxic water with 60 g/L of suspended particulate matter (SPM) was collected from Guanabara Bay, Rio de Janeiro, Brazil, transferred to experimental boxes and aerated for 61 days. SPM aggregates monitoring included abiotic variables measurements and, determination of total organic matter (TOM), biopolymers composition, bacterial activity, trace metals, and polycyclic aromatic hydrocarbons (PAHs) concentrations. The aeration enhanced dissolved oxygen (DO) concentration and the redox potential (Eh). However, from days 0 to 61 the predominant bacterial activities were denitrification and fermentation. Electron transport system activity increased after day 10, and aerobic activity was detected after day 19. In summary, aeration increased aerobic bacterial activity, lipids (LIP) and trace metal concentrations, although diminished protein/carbohydrate ratio and PAH concentration. Trace metals concentration (Ni, Pb, Cu, Cr, Mn, and Fe) were the highest on day 19 when the pH was 5.9. Copper presented toxic values (Cu > 20.0 μg/g). The pH showed a strong negative correlation with Eh (r = −0.94; p < 0.001). Acidic environment (pH ≤ 5.9) in marine ecosystems with high loads of toxic trace metals is unsafe for biota. Therefore, managers must be aware of the environmental and biological risks of introducing the aeration technique into a eutrophic marine environment.

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Introduction

The direct wastewaters discharge from urban effluents, industries, and agriculture can carry organic and inorganic nutrients into the estuary in the form of suspended or dissolved particles (Cotovicz et al., 2018). The suspended particles (SP) are fundamental to the transfer of chemical constituents between the water, food chain, and bed sediment in different aquatic environments (Che et al., 2003; Gong et al., 2014; Turner and Millward, 2002). As they hold an intermediate position between these compartments, estuarine suspended particles can present high loads of terrestrial organic carbon (Cotovicz et al., 2018; Middelburg and Herman, 2007). In eutrophic estuaries, suspended particles can also contain pollutants including lead and copper (Sabadini-Santos et al., 2014) and polycyclic aromatic hydrocarbon (Christensen et al., 2010; Gong et al., 2014; Yang et al., 2012). If ingested by marine biota these pollutants can be accumulated, magnifying through the food web (Bryan and Langston, 1992) and damaging marine biota and human health (Mulligan et al., 2001).

In areas with high turbidity and limited light penetration, there is a predominance of heterotrophic bacterial metabolism rather than primary production (Cotovicz et al., 2018; Gallizia et al., 2004; Middelburg and Herman, 2007). The heterotrophic bacteria are vital to the organic matter cycling through biopolymers degradation promoted by extracellular enzymes such as the esterases (Fontana et al., 2010; Gallizia et al., 2004; Sabadini-Santos et al., 2014; Tselepidis et al., 2000). Therefore, the predominance of heterotrophic metabolism can increase CO$_2$ produced in the estuaries, contributing to atmosphere CO$_2$ emission (Cotovicz et al., 2018). Dissolved carbon dioxide increase in a eutrophic marine system can lead to ocean acidification with loss of biodiversity (Doney et al., 2009; Melzner et al., 2013).

Suspended particulate matter (SPM) composition are subjected to cycles of oxic-anoxic deposition and resuspension (Middelburg and Herman, 2007; Turner and Millward, 2002). These recurrent changes may influence the composition and degradability of SPM organic matter (Middelburg and Herman, 2007) and may also affect the bioavailability of pollutants (Christensen, 1998; Christensen et al., 2010; Gong et al., 2014; Mulligan et al., 2001; Zoumis et al., 2001). Many bacteria are subjected to biogeochemical conditions since microorganisms can attach to SPM. Thus, processes including nitrification and denitrification are stimulated by the SPM dynamics (Middelburg and Herman, 2007).

Although researches still underestimate the importance of SPM for environmental health, this compartment can be a harmful pollutant for aquatic systems (Bilotta et al., 2012). Thus, it should be investigated to control micropollutant bioavailability and biomagnification. Therefore, the development of efficient biotreatments for the marine environment still requires a comprehensive understanding of the complex biogeochemical interactions between organic matter and contaminants in the SPM (Cotovicz et al., 2018; Fonti et al., 2015).

The bioavailability of trace metals depends not only on its concentrations but on particle components to which it is sorbed (Zoumis et al., 2001). Because of their solubilities and persistence, trace metals can adsorb onto many surfaces including soils and suspended particles, reaching rivers, lakes or groundwater, and ocean waters. The US Environmental Protection Agency’s (EPA) includes cadmium, copper, lead, mercury, nickel, and zinc as the most dangerous metals (Cameron, 1992). Their accumulated effects can damage marine biota and human health (Mulligan et al., 2001). Another class of persistent pollutant that can be found at the SPM are the polycyclic aromatic hydrocarbon (PAH) (Christensen et al., 2010; Gong et al., 2014; Spasojevic et al., 2015; Yang et al., 2012). In estuaries, PAH may form oil-suspended particles aggregates (OSAs) interfering with its transport and weathering (Gong et al., 2014).

In this direction, we investigated the effects of aeration on the suspended matter from a Tropical and eutrophic estuary. To reach this goal, suspended particulate matter (SPM) were aerated under a controlled environment for 61 days. Abiotic variables were monitored during aeration. Monitoring also included SPM-organic matter (TOM), biopolymers, bacterial activity, trace metals, and PAH concentration. Our study is the first one to approach these variables altogether in a controlled experiment with suspended matter from a Tropical and eutrophic estuary.

1. Materials and methods

1.1. Samples collection and preparation

Estuarine water with suspended particulate matter (SPM) sampling occurred at 3.9 m depth from the northern portion of the Guanabara Bay, Rio de Janeiro, Brazil at coordinates 22°45′34″ S; 43°11′23″ W (Fig. 1). Guanabara Bay is flagged by massive eutrophication, including improper sewage disposal (Fistarol et al., 2015; Ribeiro and Kjerfve, 2002; Soares-Gomes et al., 2016), trace metals (Perin et al., 1997; Rangel et al., 2011; Soares-Gomes et al., 2016), and petroleum derivates (Fontana et al., 2010; Kjerfve et al., 1997; Michel, 2000; Ribeiro and Kjerfve, 2002). Despite the development of pollution control plans, studies show that the western portion of the bay and the harbor areas are the most eutrophic for the last few decades (Aguiar et al., 2011; Perin et al., 1997; Rangel et al., 2011; Ribeiro and Kjerfve, 2002; Soares-Gomes et al., 2016).

A water pump with 1.5 horsepower (hp) connected to 22 mm diameter PVC pipes pumped 1500 L water with suspended particle matter into 50 L closed containers. The filled gallons were transported to the laboratory, avoiding undesirable oxidation. Field water-SPM abiotic variables measurement included salinity (model 10419, American Optical, USA), temperature, pH, redox potential (El; CG 837, Schott Gerate, Germany), and dissolved oxygen (DO; CG 867, Schott Gerate, Germany). The day 0 sample represented the baseline before aeration.

1.2. Experimental design

In a dark room with controlled temperature (19°C), three replicas of glass boxes (B1, B2, and B3) sizing 0.5 m length, 0.5 m width and 1.60 m height were filled with 375 L of the
collected material. The external side walls of the boxes were covered with a black mantle to restrict apical light penetration. The boxes were illuminated from the top, every 12 hr with artificial 15 W fluorescent lamps (254 nm). A total of 2.36 m³/min of atmospheric air were supplied for 61 days by independent 1.5 hp air pumps connected to 50 mm PVC perforated pipes (Fig. 2).

Previous studies reported the necessity of microbial acclimation period before organic matter mineralization could be detected (Fontana et al., 2006; Wiggins et al., 1987). Thus, samples from each box were collected on days 3, 10, 19, 31, 45, 54 and 61 for SPM analysis.

1.3. Suspended particulate matter (SPM) aggregates characterization

1.3.1. Bacterial activity
The electron transport system activity (ETSA) was measured using 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride (INT) as an artificial electron acceptor following previously published methodology (Houri-Davignon et al., 1989; Trevors, 1984). The INT (2 g) was incubated in closed sterile tubes with 1 mL of SPM for 2 hr, at 20°C in darkness. The reduced INT (INT-formazan) was extracted three times with 5 mL of methanol for 10 min at 10°C. A reagent blank was prepared with 2% of INT solution in sterile distilled water and methanol. INT-formazan was quantified in triplicate by spectrophotometer at 480 nm. Values were expressed in μg O₂/hr/mL of SPM.

The esterase enzyme activity (EST) was determined by the addition of 1 mL of SPM into a 19 mL solution of phosphate

![Fig. 1 – Location of the study area and sampling site (red dot) of suspended matter in Guanabara Bay, Rio de Janeiro, Brazil for aeration assay.](image1)

![Fig. 2 – Schematic diagram and dimensions of the experimental aeration setup.](image2)
buffer (0.1 mol/L; pH 7.6) according to previous studies (Stubberfield and Shaw, 1990). A 0.1 mL volume of fluorescein diacetate (FDA) solution prepared in acetone (0.0048 mol/L) was added into the tubes, that were closed and shaken for 2 hr at 20°C. A control with buffer was prepared to reveal undesirable FDA hydrolysis. After the incubation period, the tubes were transferred into an ice bath to stop the reaction. To remove cell debris samples were filtered through a cellulose nitrate membrane filter with 0.22 μm pore size, 13 mm diameter, and 150 μm thickness (Millipore, Sigma-Aldrich, Germany). The absorbance of the filtrates was read at 490 nm in triplicate. The results were expressed in μg of fluorescein/hr/mL of SPM.

The bacterial metabolism (aerobic - AER, fermentative - FER, denitrification - DNT or sulfate-reduction - SR) was monitored through the aeration assay. Enrichment growth media formula for each bacterial group followed literature (Alef, 1995). For aerobic, fermentative and sulfate reduction the growth media was supplied with 10 g/L of peptone, 10 g of meat extract and 5 g of NaCl, pH 7.0–7.2 in distilled water. For the fermentation and sulfate-reduction reading, a redox indicator solution with 0.03% of methylene blue and resazurin (0.0003%) was used respectively. For denitrifying bacteria enrichment, the growth media contained 10.0 g/L sodium nitrate membrane filter with 0.9 mL of a NaOH 0.5 mol/L solution containing potassium sodium tartrate (2 g/L) and Na2CO3 (100 g/L). After a 10 min water bathing (50°C), the tubes were cooled at 25°C and treated with a second solution (0.1 mL) prepared with potassium sodium tartrate (2%), CuSO4•5H2O (1%), and 10 mL of NaOH 1 mol/L. The samples rested for 10 min at 25°C, and a third solution (3 mL) was added vigorously to force mixing. This solution (pH 10) contained 1 mL of Folin-Ciocalteu reagent (Sigma–Aldrich, USA) diluted into 15 mL of water. The sample tubes returned to the 50°C water bath for 10 min, then cooled at 25°C. Absorbances were read at 650 nm.

The lipid (LIP) determination of SPM followed methodology previously described (Marsh and Weinstein, 1966). Sulfuric acid (2 mL) was added to tubes containing 1 mL of SPM samples in triplicate. The tubes were placed on a heating plate (200°C) for 15 min, transferred for 15 sec to water (25°C) and then, to an ice bath for 5 min. Distilled water (3 mL) was added to each tube. After 10 sec vigorously mixing, the tubes were returned to the ice bath. Once cooled, tubes were placed at room temperature for 10 min before absorbance reading at 375 nm.

CHO, PTN, and LIP were expressed, respectively, like glucose, albumin, and tripalmitin equivalents. A reagent control without SPM samples was conducted in all analysis in triplicates. The conversion of carbohydrates, protein and lipids concentrations to carbon equivalent in mg C/g of SPM followed the conversion factors: 0.40, 0.49 and 0.75 respectively (Dell’Anno et al., 2002). The biopolymeric carbon (BPC) was the sum of carbohydrate, protein, and lipid carbon (Fichez, 1991). It represents the bioavailable carbon present in the SPM (Sabadini-Santos et al., 2014).

1.3.5. Trace metals

SPM-trace metals quantification was carried out by Atomic Absorption Spectroscopy (AAS) (model 1475, Varian, USA) equipped with copper (Cu), chromium (Cr), Manganese (Mn), lead (Pb), nickel (Ni), iron (Fe) and zinc (Zn) lamps. The sample preparation consisted of acid digestion (Rego et al., 1993) of the SPM matrix. Triplicates of SPM samples were heat-dried at 42°C for 48 hr and then macerated. The macerates (0.2 g) were digested with 4 mL of hydrofluoric acid and 5 mL of nitric acid at 120°C for 12 hr. The digests were evaporated in a sand bath (~230°C), then hydrochloric acid was added and evaporated again. After the second evaporation, the digested samples were transferred into tubes and resuspended to a final volume of 12 mL in hydrochloric acid solution (0.1 mol/L). Two replicas of reagent blank and reference sample (IAEA-356) for polluted marine sediment run together with samples digestion. The recuperation of IAEA-356 reference sample validated the method within 93.04% and 100%. Total trace metals values unit was μg/g.
1.3.6. Polycyclic aromatic hydrocarbon (PAH)
The PAH was quantified by High-Performance Liquid Chromatography (LC-10AS, Shimadzu, Japan) equipped with a fluorescence detector (RF-10 AXL, Shimadzu, Japan) and two peristaltic bombs (LC-10 AT and LC-10AS, Shimadzu, Japan) on days 0, 3, 10, 19, 45, 54 and 61. PAH analytical procedure and the chromatographic method were conducted according to a previous study (Meire et al., 2008). The analyzed PAH were Naphthalene (NAH), Fluorene (FLU), Acenaphthylene (ACY), Phenanthrene (PHE), Anthracene (ANT), Fluoranthene (FLA), Pyrene (PYR), Benzo[a]anthracene (BaANT), Benzo[b]fluoranthene (BbFLA), Benzo[k]fluoranthene (BkFLA), Benzo[a]pyrene (BaPYR), Dibenzo[a,h]anthracene (DBahANT), Benzo[g,h,i]perylene (BghiPER), Indeno[1,2,3-cd]pyrene (IPYR). The chromatogram integration and pollutant concentration calculation by the software Borwin 1.2. PAH values were as μg/g. Total PAH (PAHt) represents the sum of all 14 PAH detected.

Reagent blanks followed sample preparation and analytical steps. The limits of PAH detection calculated were three times the standard deviation of blank (Meire et al., 2008). The detection limits were 1.31 ng/g for naphthalene and 0.71 ng/g for fluoranthene and were between 0.01 and 0.47 ng/g for the other PAH compounds. The PAH standard reference material used was from NIST-1647c. The mean PAH recovery and other PAH compounds. The PAH standard reference material for fluoranthene and were between 0.01 and 0.47 ng/g for the detection limits were 1.31 ng/g for naphthalene and 0.71 ng/g times the standard deviation of blank (Meire et al., 2008). The limits of PAH detection calculus were three

1.3.7. Quality control
All analytical measurements were performed in triplicate under strict quality-control guidelines. Bacterial activity quantification was performed with sterile material under anseptic conditions. All glassware for PAH quantification were immersed in 5% Extran neutral (Merk, Alemanha), heat dried at 100°C for 24 hr, rinsed with acetone ACS (Tedia, Brazil) and 95% n-hexane. For TOM and heavy metal quantification besides Extran immersion cleaning, glassware was dried at 100°C for 24 hr, rinsed with acetone ACS (Tedia, Brazil), and 95% n-hexane. For TOM and heavy metal quantification was performed with sterile material under strict quality-control guidelines. Bacterial activity (ETSA and EST) and metabolism (DNT, FER, SR, AER) under 61 days of aeration. Results are the average concentration and the redox potential (Eh) with time. Natural water evaporation increased salinity, and the temperature reached equilibrium with room temperature at 20°C. Multivariate analysis of data revealed a strong negative correlation between pH and Eh (r = −0.94; p < 0.001). As the dissolved oxygen (DO) and the redox potential (Eh) increased, aerobic bacterial metabolism emerged, and water became very acidic (Table 1). This discussion will be addressed in Section 2.3.

2. Results and discussion

2.1. Suspended particulate matter aggregates
The estuarine water collected presented 60 g/L of suspended particulate matter (SPM). Table 1 shows abiotic variables and SPM aggregates quantification of total organic matter (TOM), bacterial activity (ETSA and EST) and metabolism (DNT, FER, SR, AER) under 61 days of aeration.

The aeration improved the dissolved oxygen (DO) concentration and the redox potential (Eh) with time. Natural water evaporation increased salinity, and the temperature reached equilibrium with room temperature at 20°C. Multivariate analysis of data revealed a strong negative correlation between pH and Eh (r = −0.94; p < 0.001). As the dissolved oxygen (DO) and the redox potential (Eh) increased, aerobic bacterial metabolism emerged, and water became very acidic (Table 1). This discussion will be addressed in Section 2.3.

2.2. Total organic matter
The TOM percentage was relatively constant under 61 days of aeration. TOM values ranged slightly between 18.5% and 21.4% (Table 1). However, with the aeration, the biopolymeric constitution of TOM changed. Table 2 presents the average concentration of carbohydrate (CHO), protein (PTN), lipids (LIP), PTN/CHO ratio and biopolymeric carbon (BPC) quantified in the SPM aggregate during the monitoring. The data show a predominance of CHO in the SPM, followed by PTN and low amounts of LIP. Although the CHO, LIP, and BPC quantity increased, PTN values oscillated with aeration (Table 2).

Table 1 - Quantification of abiotic variables (SAL, T, Eh, DO and pH), suspended particulate matter aggregates (TOM), bacterial activity (ETSA and EST) and metabolism (DNT, FER, SR, AER) under 61 days of aeration. Results are the average values of B1, B2, and B3.

<table>
<thead>
<tr>
<th>DAY</th>
<th>SAL</th>
<th>T</th>
<th>Eh</th>
<th>DO</th>
<th>pH</th>
<th>TOM</th>
<th>ETSA</th>
<th>EST</th>
<th>DNT</th>
<th>FER</th>
<th>SR</th>
<th>AER</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>29.0</td>
<td>24.0</td>
<td>20.0</td>
<td>0.0</td>
<td>7.8</td>
<td>21.2</td>
<td>0.0</td>
<td>0.0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>3</td>
<td>31.0</td>
<td>18.0</td>
<td>58.3</td>
<td>3.8</td>
<td>6.5</td>
<td>18.5</td>
<td>0.8</td>
<td>0.6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>10</td>
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<td>20.0</td>
<td>181.0</td>
<td>2.0</td>
<td>5.9</td>
<td>19.8</td>
<td>2.5</td>
<td>0.9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>19</td>
<td>32.0</td>
<td>19.0</td>
<td>177.7</td>
<td>2.5</td>
<td>5.7</td>
<td>20.4</td>
<td>5.0</td>
<td>1.1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>45</td>
<td>32.0</td>
<td>19.0</td>
<td>253.2</td>
<td>7.9</td>
<td>5.2</td>
<td>21.0</td>
<td>11.3</td>
<td>1.6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>54</td>
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<td>19.0</td>
<td>278.8</td>
<td>8.8</td>
<td>4.3</td>
<td>20.4</td>
<td>13.5</td>
<td>2.0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>61</td>
<td>32.0</td>
<td>19.0</td>
<td>266.7</td>
<td>7.0</td>
<td>4.1</td>
<td>21.4</td>
<td>15.3</td>
<td>0.4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

SAL: salinity; T: temperature in °C; Eh: redox potential in mV; DO: dissolved oxygen in mg/L; TOM: total organic matter in %; ETSA: Electron transport system activity in μg O₂/hr/mL of SPM; EST: Esterase enzyme activity in μg fluorescein/hr/mL of SPM; DNT: denitrification; FER: fermentation; SR: Sulfate reduction and AER: aerobic metabolism.
Concerning the high organic matter content (18.5% <TOM > 21.4%; Table 1), SPM presented low amounts of protein and lipids. During the experiment, carbohydrates represented 70% of BPC, followed by proteins (23.1% of BPC) and lipids (6.2% of BPC). In a previous study at the Mediterranean Sea, carbohydrates represented 54% to 74% of BPC in sediments (Dell’Anno et al., 2002). Indeed, researchers reported higher protein to carbohydrate ratio in highly productive areas decreasing toward oligotrophic sites in all benthic systems studied (Danovaro et al., 1993; Dell’Anno et al., 2002; Fabiano et al., 1995; Tselepides et al., 2000).

In our data, the PTN/CHO ratio lowered from 0.7 to 0.1 during aeration (Table 2). A low protein to carbohydrate ratio (<0.1) was previously reported in the Mediterranean Sea, indicating the presence of large amounts of non-living or aged organic matter (Pusceddu et al., 1999). It is well established that lower PTN/CHO ratio (<0.1) is characteristic of oligotrophic or detrital areas (Danovaro et al., 1993), although higher ratio (>10) are predominant in coastal Antarctic sediments (Pusceddu et al., 1999). Therefore, the low PTN/CHO ratio (<1) detected on SPM indicates the prevalence of refractory organic matter with low nutritional quality. Thus, suggesting protein as the limiting factor for the pelagic and benthonic life in Guanabara Bay.

### 2.3. Bacterial activity

The ETSA and EST activity started in low intensity (<1 μg O₂/hr/mL and μg fluorescein/hr/mL of STM) but increased with aeration, reaching their maximum on days 31 and 61 (Table 1). Corresponding to the literature, ETSA represents the dehydrogenase activity of all living micro-meiobenthic organisms (Cammen et al., 1990; Houri-Davignon et al., 1989; Relexans et al., 1992; Trevors, 1984). Moreover, as ETSA exists both in aerobic and in anaerobic organisms, its activity is a universal index of metabolism measurement (Fontana et al., 2010; Houri-Davignon et al., 1989; Relexans et al., 1992; Sabadini-Santos et al., 2014). The effects of substrate and oxygen concentrations, temperature and pH on microbial dehydrogenase activity may significantly affect the outcome of electron transport system activity (ETSA) measurements (Trevors, 1984). The optimum pH range for ETSA is between 7.4 and 8.6 (Ross, 1971; Trevors, 1984). Therefore, low Eh and DO from 0 to 10 may explain the low EST and ETSA enzymatic activity. After day 19, we detected higher bacterial metabolism (AER, EST and ETSA).

Concerning EST activity, our results showed an increase from day 3 to 54 and a decrease on day 61 (Table 1). The EST decrease can be related to the low quality of biopolymers detected by the PTN/CHO ratio on day 61 (Table 2). Esterase enzymes (EST) act on biopolymer and transform them into low-molecular-weight organic carbon (Alef and Nannipieri, 1995; Houri-Davignon et al., 1989). The most degradable (i.e., biopolymers) are mineralized faster than the less degradable complex organic matter. The complex organic matter includes complex carbohydrates and, fulvic and humic acids with low nutritional quality (Danovaro et al., 1993; Fabiano et al., 1995; Fichez, 1991; Pusceddu et al., 1999; Relexans et al., 1992). Furthermore, the EST technique only stains active microbial cells but not spores or cells in the stationary growth phase (Mapelli et al., 2017). Thus, the stationary cells were not computed as biomass on our data.

Both DNT and FER were detected during all the aeration experiment, while SR stopped on day 54 (Table 1). Bacterial denitrification (DNT), fermentation (FER) and sulfate-reducing (SR) are processes undertaken in the absence of oxygen with acid production (Hugh and Leifson, 1953). Fermentative bacteria are facultative (capable of growing in the presence or absence of oxygen). However, anaerobic fermentation of carbohydrates produces more acid than the aerobic oxidation (Hugh and Leifson, 1953). During the fermentation yeasts and bacteria can also produce CO₂ (Nelson et al., 2000). On eutrophic marine systems, the increase of dissolved CO₂ can contribute to atmosphere CO₂ emission (Cotovicz et al., 2018) and lead to ocean acidification, causing loss of biodiversity and diminishing dissolved oxygen (Doney et al., 2009; Melzner et al., 2013). Consequently, this acidification can change trace metal biosorption and bioleaching equilibria (Lors et al., 2004) as will be discussed in Section 2.4.

Most of the fermentative strains reduce nitrate to nitrite. The denitrifying bacteria use the nitrate as a terminal electron acceptor under anaerobic conditions, with the production of N₂ as the end product (Alef, 1995). In the presence of sulfur, autotrophic denitrification occurs with H⁺ production (Chen et al., 2018). The SR bacteria can impact the water pH through consumption of sulfate and production of hydrogen sulfide during anaerobic organic matter decomposition. In marine systems with poor carbonate buffering, this chemical process leads to water acidification (Baumgartner et al., 2006). A pH decrease during aeration was also reported previously in sediment studies (Lors et al., 2004). Contrastingly, other study detected ~160 mV of redox potential and, pH 8.0 after 60 days of enzymatic activity stimulation by oxygen supply (Gallizia et al., 2004). In respect to the microbial community, these authors concluded that oxygen supply enhanced bacterial metabolism (Gallizia et al., 2004). Multivariate analysis of the monitoring data revealed a strong positive correlation between ETSA and CHO (r = 0.92, p < 0.05), ETSA and Eh (r = 0.91, p < 0.001), ETSA and DO (r = 0.85, p < 0.01); and a strong negative correlation between ETSA and pH (r = −0.92, p < 0.001). Thus, water acidification may be explained by bacterial activity (ETSA) increase represented by fermentation, denitrification, and sulfate-reducing of the organic matter. With the aeration, dissolved oxygen, Eh, carbohydrates, and electron transport system activity (ETSA) increased, although sulfate-reducing bacteria (anaerobes) was inhibited after day 54. However, carbohydrate (primarily glucose) can be decomposed by fermentation.

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**Table 2 - Quantification of carbohydrate (CHO), protein (PTN), and lipids (LIP) in μg m/L, PTN/CHO ratio and biopolymeric carbon (BPC) in mg C/g of suspended particulate matter aggregate during the 61 days of aeration. Results are the average values of triplicates (B1, B2 and, B3).**

<table>
<thead>
<tr>
<th>DAY</th>
<th>CHO</th>
<th>PTN</th>
<th>LIP</th>
<th>PTN/CHO</th>
<th>BPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>98.9</td>
<td>67.8</td>
<td>7.2</td>
<td>0.7</td>
<td>78.2</td>
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<tr>
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<td>9.4</td>
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<tr>
<td>19</td>
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<td>65.3</td>
<td>15.5</td>
<td>0.2</td>
<td>162.0</td>
</tr>
<tr>
<td>31</td>
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<td>14.7</td>
<td>0.1</td>
<td>156.9</td>
</tr>
<tr>
<td>61</td>
<td>448.9</td>
<td>51.1</td>
<td>15.0</td>
<td>0.1</td>
<td>215.9</td>
</tr>
</tbody>
</table>
and/or aerobic oxidation, contributing to the severe acidification of water. Indeed, a bivariate fit of CHO by pH also revealed a strong negative correlation between carbohydrates and pH ($r = -0.996$; $p < 0.001$). Thus, as the quantity of the carbohydrate increased in the SPM, bacterial activity and water acidification also increased. Trace metal biosorption and bioleaching equilibria related to acidification will be presented in Section 2.4.

2.4. Trace metals

Fig. 3 shows the average of each trace metal (TM) quantified in the SPM aggregate. The order of TM abundance in SPM was Fe > Mn > Zn > Cr > Cu > Pb > Ni. The iron (Fe) concentration was 1000 times higher than the other trace metals analyzed. Day 19 showed the highest SPM-trace metals values for Ni, Pb, Cu, Cr, Mn, and iron (Appendix A Table S1).

In the right proportions metals like iron, manganese, zinc, copper, and nickel are essential elements for living organisms (Mulligan et al., 2001; Wood, 1987). However in high levels can cause deleterious effects to life (Agency for Toxic Substances and Disease Registry, 2012). Toxic copper levels vary from 20 to 100 $\mu$g/g (Mulligan et al., 2001). Therefore, the Cu concentration (>20 $\mu$g/g) detected in SPM is toxic for life (Appendix A Table S1).

After iron, manganese data presented the highest SPM-concentration. Mn is present as oxides, carbonates or silicates in aquatic systems (Agency for Toxic Substances and Disease Registry, 2012). Nickel forms stable and insoluble complexes with sulfide ions and with thiolates (Gallizia et al., 2004; Lors et al., 2004; Wood, 1987). However, several bacteria (including the sulfate-reducing bacteria) and algae can reduce sulfate to sulfide, causing the direct precipitation of nickel sulfides (Bryan and Langston, 1992; Lors et al., 2004; Wood, 1987). As discussed previously in Section 2.3, sulphate-reducing bacteria can also impact pH, promoting calcium carbonate precipitation and dissolution during organic matter decomposition (Baumgartner et al., 2006). Therefore, changes at pH and Eh can influence the nature, solubility, and reactivities of

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**Fig. 3** – Trace metals including iron (Fe), manganese (Mn), zinc (Zn), chromium (Cr), copper (Cu), lead (Pb) and nickel (Ni) quantified in the SPM aggregate by time. Bars represent mean values of B1, B2, and B3.

**Fig. 4** – Total Polycyclic aromatic hydrocarbon (TPAH) concentration in $\mu$g/g by the number of rings during 61 days of aeration.
chemical species (Wood, 1987), including the net charge of elements such as Mn and Ni.

The increase of dissolved oxygen and redox potential (Eh) stimulated aerobic bacterial activity (AER) on day 19, although SR, FER, and DNT were present concomitantly. Consequently, water acidification, LIP, Ni, Pb, Cu, Cr, Mn, and Fe boosted on this day (Tables 1, 2 and Fig. 3). Contrastingly to the other trace metals, in the absence of free oxygen, ZnS can precipitate forming ZnOH⁺, ZnCO₃, and ZnCl⁺ (Mulligan et al., 2001; Wood, 1987). Under acidic conditions, zinc is usually divalent and quite mobile. Zinc hydrolyzes at pH 7.0–7.5, forming Zn(OH)₂ at a pH higher than 8 (Bryan and Langston, 1992; Lors et al., 2004). This kinetics may explain Zn availability until day 3 (Fig. 3). Indeed, bivariate fit analyzes showed a strong negative correlation of Zn by Eh (r = −0.858; p < 0.01). While Eh increased with aeration (Table 1), Zn-SPM concentration decreased (Fig. 3 and Appendix A Table S1).

Considering the toxic copper levels detected in SPM, future researches should focus attention on SPM geochemistry rather than on sediments. As they hold an intermediate position between water and sediments, estuarine suspended particles can present high loads of micropollutants, including trace metals (Cotovicz et al., 2018; Middelburg and Herman, 2007; Turner and Millward, 2002). The bacterial fermentation and oxidation of carbohydrates coupled with sulfate-reducing metabolism may contribute to water acidification and trace metals mobilization. The most critical sources of available metals to the water column are fine-grained, oxidized particles (Bryan and Langston, 1992) such as SPM. If ingested by marine biota these pollutants associated with the SPM can be accumulated, magnifying through the food web (Bryan and Langston, 1992) and damaging marine biota and human health (Mulligan et al., 2001). Therefore, speciation of the trace elements can be induced when anoxic suspended particulate matter becomes progressively aerated (Lors et al., 2004) and water acidified. In summary, trace metal bioavailability, micropollutants magnification through food web, human health damage, and biodiversity loss can be cited as the most relevant potential environmental risk of marine waters acidification.

2.5. Polycyclic aromatic hydrocarbon

Total PAH concentration decreased with aeration (Fig. 4). The highest PAH₁ values were on day 10 due to compounds such as NAH, FLU, ANT, PYR, BaANT, BbFLA, BkFLA, DBahANT, and

![Fig. 5 - Polycyclic aromatic hydrocarbon (PAH) quantification of Naphthalene (NAH), Fluorene (FLU), Acenaphthylene (ACY), Phenanthrene (PHE), Anthracene (ANT), Fluoranthene (FLA), Pyrene (PYR), Benzo[a]anthracene (BaANT), Benzo[b]fluoranthene (BbFLA) during 61 days of aeration. Bars represent mean values of B1, B2, and B3.](image-url)
The polycyclic aromatic hydrocarbons (PAH) shows high bioaccumulation potential and low removal feasibility by traditional treatment processes (Christensen et al., 2010; Hurst et al., 1996; Meire et al., 2008). Though recalcitrant, PAH decline either through abiotic or biotic processes. The abiotic means include leaching, photodegradation, and volatilization (Hurst et al., 1996; Lamichhane et al., 2016; Lee, 2003; Quantin et al., 2005; Spasojević et al., 2015). The interactions between environmental factors often interfere with biodegradation efficiency. Those factors include temperature, pH, dissolved oxygen, redox potential, and the presence of other substrates (Abdel-Shafy and Mansour, 2016; Christensen et al., 2010; Gong et al., 2014; Huesemann and Truex, 1996; Hurst et al., 1996). However, the dissolved oxygen concentration is often the limiting factor for PAH biodegradation when nutrients are enough (Hurst et al., 1996).

It is well established that the PAH biodegradation relies on the interaction between electron donors and electron acceptors (Mulligan et al., 2001). Metabolically active microorganisms mediate this interaction. The first step for PAH molecule cleavage involves oxygen in a process called dihydroxylation (Hurst et al., 1996; Zhu et al., 2001). After molecular oxygen incorporation into the PAH molecule, the molecule is biologically active and can be the substrate for ring fission (Hurst et al., 1996). Indeed, bivariate fit analysis of FLA and PYR by DO showed, respectively, strong positive ($r = 0.91; p < 0.05$) and negative ($r = -0.93; p < 0.01$) correlation. Moreover, total PAH concentration decreased after day 10 (Fig. 4), when DO was 2.5 mg/L, and bacterial aerobic metabolism was active (Table 1).

At the beginning of the experiment, the PAH with four aromatic rings was predominant, followed by 3- and 5-rings compounds (Fig. 4). At the end of 61 days, however, 3- and 4-rings PAH were predominant at low concentration ($\leq 8.4$ μg/g). The 5- and 6-rings PAH compounds were close to 0 μg/g on day 61 (Fig. 7). In theory, biodegradation of low molecular weight PAH is faster when the correct microorganisms are present (Hurst et al., 1996). However, the 3-rings PAH biodegradation is slow. Even though, the biodegradation of compounds with more than four rings occurs as co-substrates in the co-oxidation process (Abdel-Shafy and Mansour, 2016; Hurst et al., 1996; Meckenstock et al., 2004).

The interaction among trace metals and PAH can affect their bioremediation (Baltrons et al., 2018; Liu et al., 2017; Shen et al., 2006, 2005; Thavamani et al., 2012). As a general observation, the higher the metal concentration, the lower the 3–4 rings PAH biodegradation (Baltrons et al., 2018). However, our results showed a decrease in 3–4 rings PAH, followed by microbial

**Fig. 6** – Polycyclic aromatic hydrocarbon (PAH) quantification of Benzo[k]fluoranthene (BkFLA), Benzo[aj]pyrene (BaPYR), Dibenzo[a,l]anthracene (DBahANT), and Benzo[g,h,i]perylene (BghiPER) during 61 days of aeration. Bars represent mean values of B1, B2, and B3.

**Fig. 7** – Concentrations in μg/g of polycyclic aromatic hydrocarbon (PAH) with five and six rings detected after 45 days of aeration.
activity increase in the presence of metals (Tables 1, 2 and Fig. 3). Under reduced circumstances, the iron and manganese are available to microbial metabolism as alternate electron acceptors (Baltrons et al., 2018; Hurst et al., 1996). Indeed, our results support this explanation. Considering the Fe and Mn increase from day 0 to 3 (Fig. 3) coupled to pyrene and fluoranthene decrease (Fig. 5), lower oxygen concentrations benefit alternate electron acceptors for microbial use corroborating with a previous study (Hurst et al., 1996).

3. Conclusions

The suspended matter can be a source of essential allochthonous nutrients and pollutants for the estuarine environment. Our results suggest that the trace metals in SPM did not affect PAH degradation and microbial metabolism. There were no noticeable synergic or antagonistic effects between contaminants in this study.

Furthermore, the long-term aeration of anoxic sediment induced changes in the redox potential (Eh), pH and increase of the dominant trace elements (Ni, Pb, Cu, Cr, Mn, and Fe). The physical–chemistry changes in DO and Eh promoted the growth of aerobic bacteria, although fermentation and denitrification occurred concomitantly. Those metabolic activities coupled with sulfate-reducing bacteria can play a role in water acidification, metal mobilization, and PAH degradation.

However, considering the nutritional importance of SPM and the toxic copper levels detected in it, future researches should focus attention on SPM geochemistry rather than on sediments. Undoubtedly, this knowledge is vital to reduce the impact of ocean acidification on marine biota. Moreover, estuarine remediation strategies should focus on eco-efficient technologies instead of aeration.

Acknowledgments

The authors declare no competing financial interest. This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil (CNPq Nos. 160053/2008-0 and 380487/2007-1). Special thanks to Pereira, D. C. for technical assistance, Silveira, R. for manuscript review and suggestions, and Silva, S. F. for map design. The authors appreciated the constructive comments of the two anonymous reviewers.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2019.05.030.

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