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ORIGINAL PAPER

Consequences of anaerobic biotreatments of contaminated sediments on metal mobility

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Abstract This study deals with anaerobic biotreatments of sediments contaminated with toxic metals carried out in slurry reactor. The sediment samples used for the investigation came from two Italian ports, and they were mainly contaminated with inorganic compounds (zinc, nickel and chromium). The treatments were aimed at assessing the responses of the autochthonous microbial community in relation to the geochemistry of the sediments (mainly organic carbon bioavailability) and the addition of organic and inorganic substrates. It was observed that the bioavailable carbon in the sediments can greatly influence microbial growth but without a significant effect on metal mobilization. By contrast, the supply of inorganic nutrients to the sediments did not have a major effect on microbial growth although important changes in metal mobility were observed. Our results provide new insights on the effects of anaerobic biotreatments on changes in metal partitioning in contaminated sediments, highlighting that, under certain conditions, an increase of metals in the more mobile fractions can occur.

Keywords Anaerobic treatments · Bioreactor · Bioremediation · Marine sediments · Metals

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Introduction

Sediment contamination is a widespread environmental problem and represents a major concern for the detrimental effects on ecosystem health. Despite many field and laboratory experiments aimed at the development of an effective strategy for the reclamation of sedimentary matrices contaminated with organic and/or inorganic compounds, a univocal solution has not been identified yet. Different treatments, more or less environmentally friendly, can be applied with the aim of reducing the contamination associated with a sedimentary matrix. Chemical, thermal, physical and biological strategies are the most common technologies used for this purpose. In the first attempts of bioremediation on polluted sediments, microbial metabolism has been exploited for organic contaminant degradation, and subsequently also for metal reclamation, since microbes can influence the speciation and solubility of metals to a large extent (Lovley and Coates 1997; Pal and Paul 2008).

Many bioremediation experiments have been carried out in aerobic conditions, mostly with linear and cyclic hydrocarbons as the major pollutants. Bioremediation under anaerobic conditions has been generally applied to recalcitrant compounds, such as pesticides, polyaromatic hydrocarbons, chlorinated solvents and polychlorinated biphenyls (Vidali 2001). Also biotreatments based on sequential steps with anaerobic and aerobic conditions have been used to increase the bioremediation performance of different compounds. As an example, soils contaminated with dichlorodiphenyl trichloroethane (DDT) and the polychlorinated biphenyl Aroclor 1260, and sediments contaminated with Tetrabromobisphenol A (TBBPA), have been treated in anaerobic–aerobic conditions with the use of microorganisms to enhance the

degradation of these contaminants (Corona-Cruz et al. 1999; Master et al. 2002; Ronen and Abeliovich 2000). Besides contamination by organic compounds, sediments can also be characterized by high concentrations of metals. Differently from organic contaminants, metals cannot be degraded, but they can display different oxidation states and can be associated with certain fractions of the sediment which influence their availability and toxicity. Variations in the redox conditions determined by the experimental conditions and by microbial metabolism can change the speciation and partitioning of metals which can be present at high concentrations together with organic pollutants. This is an important aspect that should be taken into consideration to assess the potential risk of metal mobilization during the application of bioremediation strategies (Dell'Anno et al. 2009). Indeed, the biostimulation of microbial metabolism, from one side can increase the biodegradation efficiencies both of organic contaminants and organic matter within the sediment, but from the other can have major effects on metal mobility (Van Hullebusch et al. 2005; Warren and Haack 2001). However, the consequences of bioremediation treatments on metal mobility are still largely unknown.

In this study, changes in metal partitioning during bioremediation experiments of contaminated marine sediments were investigated under anaerobic conditions in a bioreactor. More specifically, the effect of organic and inorganic addition on microbial growth and metal partitioning were investigated on sediments collected from the ports of Ancona and Livorno (Italy) in 2008. A semi-empirical approach for modeling the microbial responses in relation to the consumption of organic compounds and the potential consequences on metal mobility was used.

Materials and methods

Sampling and sample processing

Sediment samples were collected in July 2008 by means of a Van Veen grab from the port of Ancona, Adriatic Sea (43°37′7.93″N, 13°30′6.30″E) and from the port of Livorno, Tyrrhenian Sea (43°33′28.94″N, 10°17′38.07″E). The sediments were reduced and black. After sediment collection, values of pH and Eh were determined. Sediment sub-samples were collected for the analysis of grain size, water, total organic matter (TOM), carbonates, biopolymeric carbon, total petroleum hydrocarbons (TPHs) and metal concentrations, metal partitioning as well as for the determination of prokaryotic abundance.

Grain size, water content, total organic matter, carbonates and biopolymeric carbon

Grain size was determined by sieving technique. Sediment water content was calculated as the difference between wet and dry weight, normalized to wet weight and expressed as a percentage. For TOM analyses, sediment samples were treated with an excess of 10 % HCl (Buchanan 1971). TOM was determined as the difference between dry weight (60 °C, 24 h) of the sediment and weight of the residue after combustion for 2 h at 450 °C, referred to sediment dry weight (Parker 1983). The percentage of carbonates was determined as the difference between sediment dry weight and weight after removal of carbonates by acid dissolution and referred to sediment dry weight. Biopolymeric carbon pool was estimated as the sum of C equivalents of proteins, carbohydrates and lipids in the sediments (Fabiano et al. 1995; Pusceddu et al. 2003).

pH and redox potential

During the experiments, the values of pH and redox potential were monitored by using an inoLab Multi 720 precision measuring instrument by WTW; the ORP probe was a platinum electrode combined with a Ag/AgCl reference electrode in a 3 M KCl solution.

Chemical analyses

TPHs were analyzed according to the method UNI EN 14039: 2005. Total Zn, Cr and Ni content in the sediment was determined after acid digestion by inductively coupled plasma-atomic emission spectrometry. Analyses of Zn, Cr and Ni partitioning were carried out by means of a selective extraction procedure (Quevauviller 1998).

Total prokaryotic counts

Prokaryotic abundances were determined as described by Rocchetti et al. (2012), diluting sub-samples from 100 to 500 times. Cells were stained with Acridine orange and then observed under epifluorescence microscopy.

Experimental design

A mechanically stirred (Stuart SS110) 5-L bioreactor was used for the experiments carried out in this study. The bioreactor was filled with sediment (20 % dry weight) and 0.2 μm-filtered seawater. Anaerobic conditions were established and maintained for 14 days by purging the system with N_2. Then, the sediment in the bioreactor was allowed to switch to aerobic conditions for 2 days by

Table 1 Experimental plan of the treatments in slurry bioreactor (sediment 20 % w/v, volume 5 L, room temperature, anaerobiosis for 14 days with N_2 insufflation then shift to aerobiosis with air for 2 days)

Treatment	Sample	Amendment
LI-MNA	Livorno	No
AN-MNA	Ancona	No
AN-LAC	Ancona	Lactose
AN-NUT	Ancona	Inorganic nutrients

purging the system with air. During all the length of the treatment, the slurry in the bioreactor was mildly mixed, and every day 50 mL of deionized water were added to balance the amount of water lost by evaporation.

According to Table 1, four experiments were set up. For the sediments collected from the ports of Livorno, a treatment of monitored natural attenuation (LI-MNA) was done. For the sediments of Ancona, displaying the lowest content of organic carbon, not only monitored natural attenuation was simulated (AN-MNA) but also other experiments were performed, adding to the system either lactose (AN-LAC) or inorganic nutrients (AN-NUT) as amendments (Table 1). For this latter experiment, at the beginning of the test, $(NH_4)_2SO_4$ and K_2HPO_4 were added in order to obtain a C:N:P ratio equal to 100:10:1, where carbon content was considered as half of TOM (Schumacher 2002). Furthermore, 50 mL of deionized water supplemented with $\times 10$ concentrated nutrients were daily added to sustain the growth of microbial assemblages.

Results and discussion

Sediment characterization

The sediments collected from the ports of Livorno and Ancona were characterized by the dominance of the silt–clay fraction (80 % for Livorno and 90 % for Ancona; Table 2), high organic C content (especially for sediments sampled from the port of Livorno, displaying values of TOM equal to 56 ± 4 mg/g sediment, about twofold the amount detected in the sediments from Ancona) and relatively high metal concentrations. In the sediments from Livorno, the concentration of Zn was 62 ± 6 μg/g, Cr was 114 ± 9 μg/g and Ni 103 ± 8 μg/g (Table 2). In the sediments sampled from the port of Ancona 143 ± 8 μg/g of Zn, 150 ± 10 μg/g of Cr and 72 ± 8 μg/g of Ni were found (Table 2). As concerns the metal partitioning, Ni was mainly found in the residual fraction (84 ± 6 %) of the sediments of Ancona, and to a lesser extent in the others (10.0 ± 0.7 % in the oxidizable fraction, 3.1 ± 0.2 in the reducible fraction and 2.7 ± 0.2 in the exchangeable/carbonatic fraction). In the sample collected from the port of Livorno, Ni partitioning was quite similar: main differences were found in the lower percentage of this element associated with the residual fraction (77 ± 6 %) in favor of the exchangeable/carbonatic fraction (7.3 ± 0.4 %). More than 90 % of Cr was associated with the residual fraction both in the sediments of Ancona and Livorno, and 3.4–6.5 % of it was associated with the oxidizable fraction. Also Zn was preferentially found in the residual fraction (66 ± 5 % for Ancona and 60 ± 4 % for Livorno), and quite fairly distributed in the other three fractions of the sediment. As a whole, the physical–chemical characterization (Table 2) confirms data reported elsewhere for sediments sampled in different sites of the same ports (Beolchini et al. 2013; Fonti et al. 2013) and evidences that the main differences in the geochemical characteristics are due to carbonates and to TOM content.

Microbial activity

The redox conditions were similar for the two sediment samples, with −240 and −200 mV for Livorno and Ancona, respectively (Table 2). During the treatments, redox potential significantly decreased and it was in the range −480 to −360 mV for all the experiments. The initial pH was 8.4 and 7.6 for Livorno and Ancona, respectively, and it was stable during time. After the shift under aerobic conditions, as expected, positive values of redox potential were measured (about 200 mV). Figure 1 shows the temporal profiles of the prokaryotic abundances and the biopolymeric carbon during the 14 days of incubation under anaerobic conditions. A significant cell growth was observed only in the treatment of natural attenuation on the sediment sampled from the port of Livorno (LI-MNA; Fig. 1a) and in the one with lactose on the Ancona sample (AN-LAC; FIG. 1c). In any case, after an increase in the first week, with maximum values of 47 ± 3 and $24 \pm 3 \times 10^8$ cell/g for LI-MNA (Fig. 1a) and AN-LAC (Fig. 1c), respectively, a remarkable decrease of the microbial abundance was evident, with final values comparable to the initial ones. For the other two treatments on the Ancona sample, natural attenuation (AN-MNA; Fig. 1b) and amendment with nutrients (AN-NUT; Fig. 1d), the microbial abundance could be considered basically constant during time, with values around 2–5 10^8 cell/g.

Despite these differences in the prokaryotic abundances, in all the experiments, a decreasing trend for biopolymeric carbon was observed, supporting the hypothesis of active

microbial assemblages during all the treatments, even without a significant increase of their abundances. Initial values for the two sediment samples were different, as previously observed for the total organic carbon (Table 2), being 7.7 ± 0.7 and 3.6 ± 0.9 mg/g for the ports of Livorno and Ancona, respectively. The decreasing profile was continuous for the 2 weeks under anaerobic conditions in all the experiments, with about a 50 % reduction for the natural attenuation treatment of Livorno (LI-MNA) and around 20, 40 and 60 % in the natural attenuation (AN-MNA), lactose (AN-LAC) and nutrient (AN-NUT) amendment treatments on Ancona sediment samples, respectively.

Table 2 Physical–chemical characteristics of sediments sampled from the ports of Livorno and Ancona

Parameter	Livorno	Ancona
Sand (%)	20 ± 2	10 ± 2
Pelite (%)	80 ± 3	90 ± 3
Water content (%)	36 ± 5	37 ± 3
Total organic matter (mg/g)	56 ± 4	27 ± 1
Carbonates (%)	21 ± 2	12 ± 1
pH	8.40 ± 0.01	7.60 ± 0.01
Redox potential (mV)	−240 ± 1	−200 ± 1
Zn (μg/g)	62 ± 6	143 ± 8
Cr (μg/g)	114 ± 9	150 ± 10
Ni (μg/g)	103 ± 8	72 ± 8
Total hydrocarbons (μg/g)	10 ± 5	29 ± 5

Metal fate

For all the investigated treatments, no significant metal mobilization in solution was recorded, neither for anaerobic nor for aerobic conditions. On the other hand, some changes have been observed in metal partitioning in the residual fraction of the sediment, which is representative of metals within the crystal lattice of minerals (Hlavay et al. 2004). Figures 2, 3 and 4 show the partitioning of metals in the sediment, during the

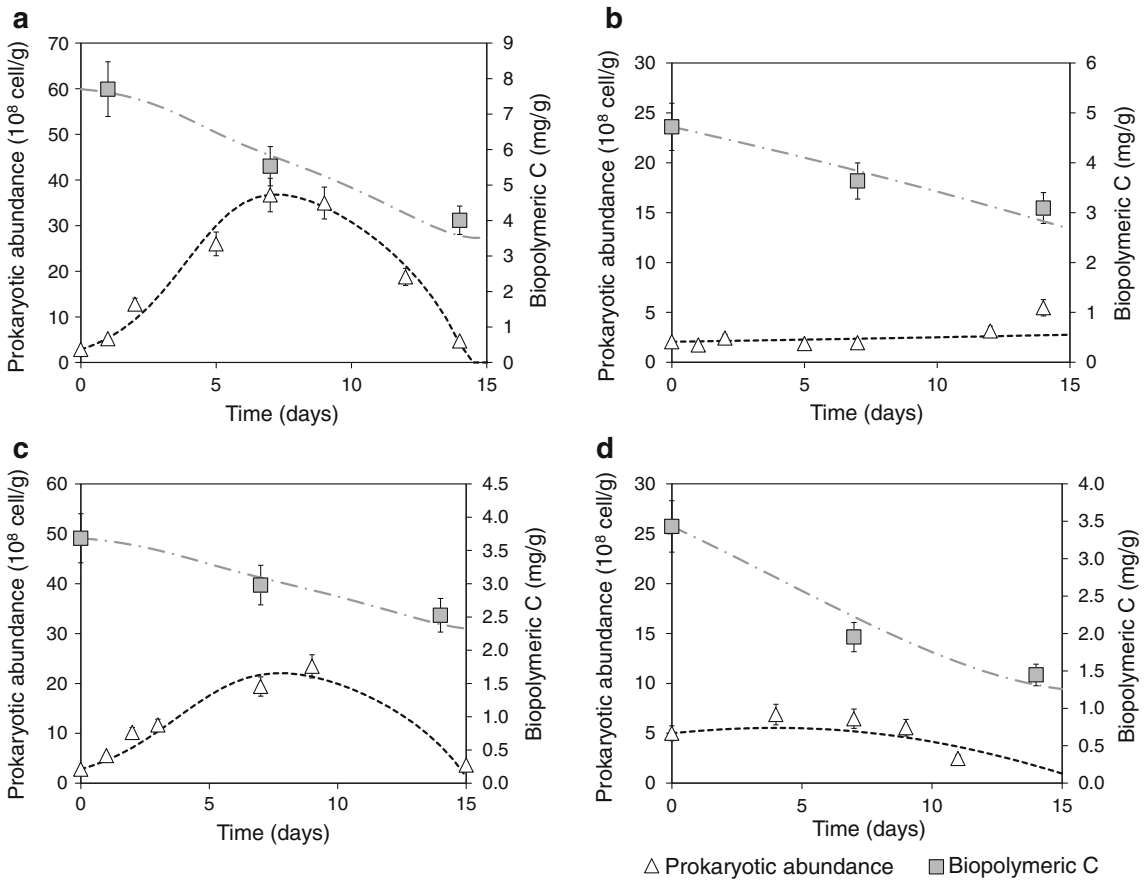

Fig. 1 Temporal changes of prokaryotic abundances and biopolymeric carbon content in the experiments carried out under anaerobic conditions in slurry bioreactor (volume 5 L, room temperature). The treatments are as follows: **a** LI-MNA, **b** AN-MNA, **c** AN-LAC, **d** AN-NUT. *Lines* have been calculated by Eq. 1 (see text for details)

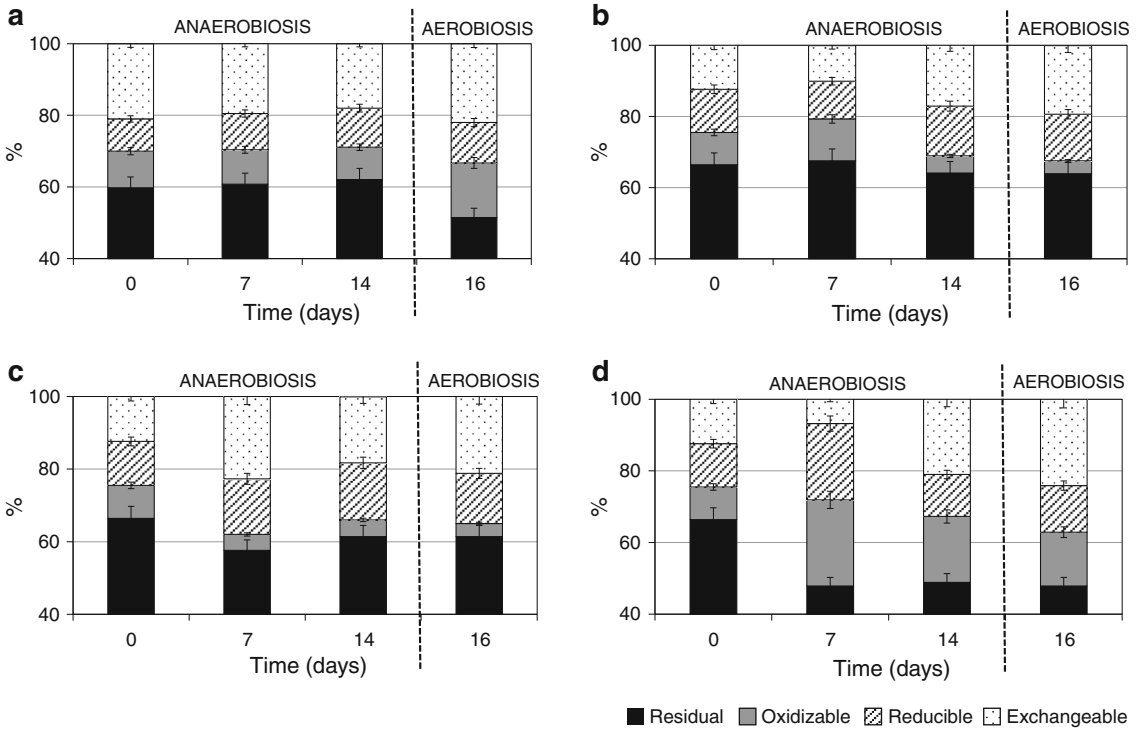

Fig. 2 Zn partitioning in the sediment during the anaerobic treatment in slurry bioreactor and after the aerobic shift (volume 5 L, room temperature). Total Zn was 62 and 143 μg/g in Livorno (LI) and Ancona (AN) sediment samples, respectively. The treatments are as follows: **a** LI-MNA, **b** AN-MNA, **c** AN-LAC, **d** AN-NUT

anaerobic treatment and after the 2 days aerobic shift, for Zn, Cr and Ni, respectively. As concerns the 2 weeks under anaerobic conditions, no significant changes are evident in metal partitioning during the natural attenuation of both sediment samples (LI-MNA, AN-MNA; Fig. 2a, b, 3a, b, 4a, b). On the other hand, a decrease of the residual fraction of all metals can be observed in the two treatments with an amendment (AN-LAC, AN-NUT; Figs. 2c, d, 3c, d, 4c, d) already after the first week of incubation under anaerobic conditions. Such decrease appears to be more remarkable when inorganic nutrients were added (AN-NUT; Figs. 2d, 3d, 4d), with a consequent significant increase of the oxidizable fraction.

After 2 weeks of incubation under anaerobic conditions, sediments were brought to aerobic conditions for 2 days, and possible changes in metal partitioning were assessed. Data in Figs. 2 to 4 show that this shift anaerobic–aerobic has a significant effect only for the decrease of Zn residual fraction in the natural attenuation treatment of the Livorno sediment (LI-MNA; Fig. 2a), while no changes are evident for other metals in all the treatments investigated on the Ancona sediment.

The stimulation of microbial metabolism, which is a common strategy to increase the biodegradation performance toward organic pollutants of contaminated sediments, can have important consequences also on the mobility of metals (Dell'Anno et al. 2009; Rocchetti et al. 2012). However, the effects of biotreatments on sediments also characterized by high metal contents are still largely unknown (Dell'Anno et al. 2009), but this information is needed for a better understanding of the potential risk associated with changes in metal mobility. Moreover, the development of efficient and eco-compatible strategies of bioremediation requires a thorough understanding of the complex relationships that develop over time between prokaryotic activity and contaminants. Based on the data set acquired in the present study, a semi-empirical approach was applied for the development of a kinetic model, using the biopolymeric carbon and the microbial abundance as dependent variables. There is a definite need for robust kinetic models which can contribute to explain the factors influencing biodegradation rates of contaminants in the environment and may ultimately be used to predict timescales and effects of remediation applications (Beolchini et al. 2001; Li et al. 1995; Vegliò

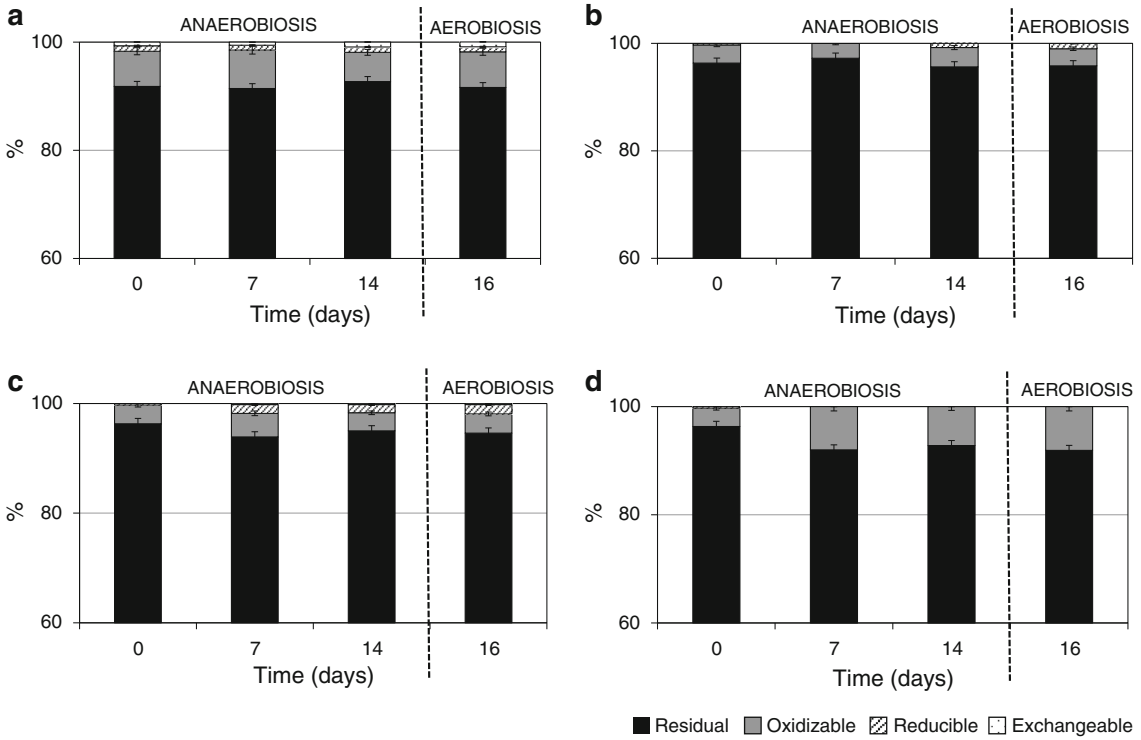

Fig. 3 Cr partitioning in the sediment during the anaerobic treatment in slurry bioreactor and after the aerobic shift (volume 5 L, room temperature). Total Cr was 114 and 150 μg/g in Livorno (LI) and Ancona (AN) sediment samples, respectively. The treatments are as follows: **a** LI-MNA, **b** AN-MNA, **c** AN-LAC, **d** AN-NUT

et al. 2010). A deterministic approach would request the analyses of temporal changes not only of the available carbon and the microbial abundances but also of nutrient concentrations (at least the limiting ones) required to sustain the biodegradation processes over time. The achieved temporal profiles (Fig. 1) suggest that biopolymeric carbon has a relevant role in sustaining microbial activity, even if it cannot be identified as the limiting substrate. Therefore, the results obtained here cannot be generalized and parameter estimation would be requested for each specific site under investigation. A similar approach has already been applied elsewhere (Beolchini et al. 2010) for marine sediment contaminated with hydrocarbons in aerobic conditions. The present study can be considered an improvement of the previous work, due to the fact that the major available carbon sources (i.e., biopolymeric carbon) are taken into account rather than hydrocarbons (no significant hydrocarbon contamination has been detected in these sediment samples). Furthermore, a comparison between microbial performance under aerobic (Beolchini et al. 2010) and anaerobic (this study) conditions would add relevant information for the understanding and design of sediment bioremediation processes. The following semi-empirical equations have been fitted to experimental data describing temporal changes in prokaryotic abundance (X) and total biopolymeric carbon (C):

$$\begin{cases} \dfrac{dX}{dt} = kX(1-\beta X) - K_0\left|\displaystyle\int_0^t X(t)dt\right| \\ \dfrac{dC}{dt} = \dfrac{1}{Y}kX(1-\beta X) \\ \end{cases} \qquad X(0) = X_0;\ C(0) = C_0 \tag{1}$$

with

- C biopolymeric carbon (mg biopolymeric carbon/g sediment)
- k rate constant (d^{-1})
- K_0 adjustable parameter related to a decrease in microbial abundance (d^{-2})
- t time (d)
- X prokaryotic abundance (10^8 cells/g sediment)
- Y adjustable parameter related to yield coefficient (10^8 cells/mg biopolymeric carbon)
- ß adjustable parameter related to inhibiting factor (g sediment/10^8 cells)

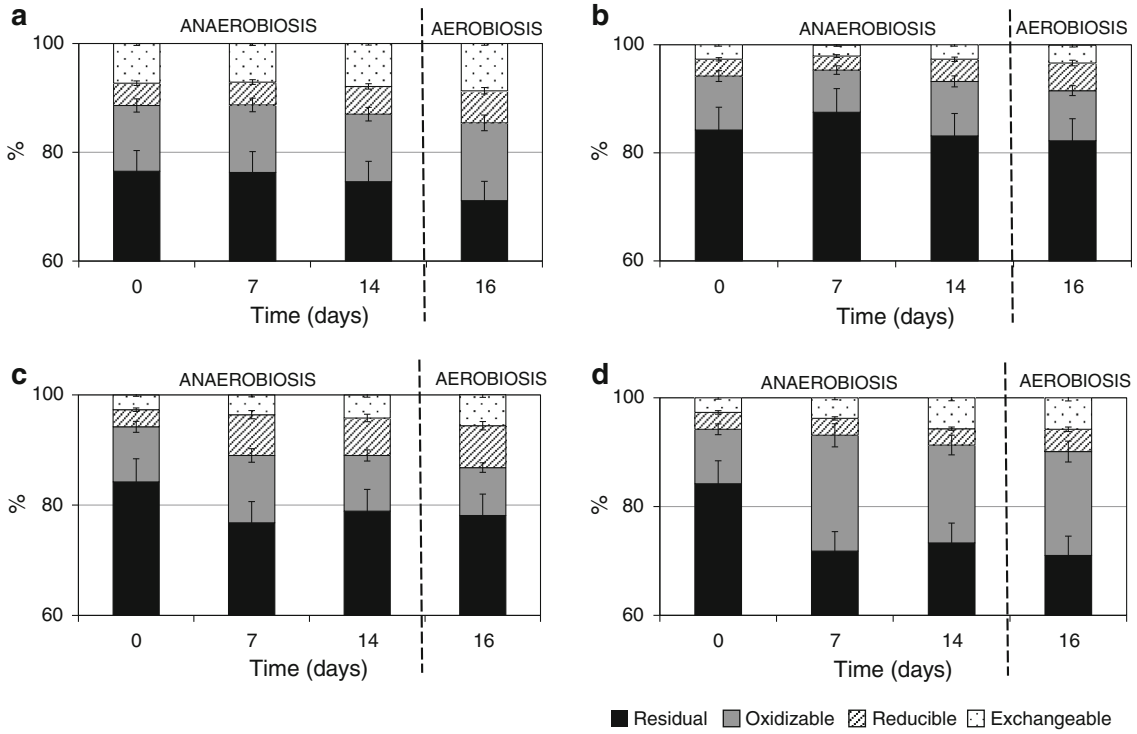

Fig. 4 Ni partitioning in the sediment during the anaerobic treatment in slurry bioreactor and after the aerobic shift (volume 5 L, room temperature). Total Ni was 103 and 72 μg/g in Livorno (LI) and Ancona (AN) sediment samples, respectively. The treatments are as follows: **a** LI-MNA, **b** AN-MNA, **c** AN-LAC, **d** AN-NUT

In Eq. (1), a modified version of the empirical logistic equation was used to mathematically describe the microbial growth rate (dX/dt), taking into account also the potential decrease of microbial abundance after the stationary phase (due to either a bottom-up control—nutrient limitation or top-down control due to predation). As concerns the biopolymeric carbon, its degradation rate (dC/dt) was supposed to be associated only to the logistic term of microbial growth through a yield factor, Y, hypothesizing that the microbial decrease term was not relevant for carbon biodegradation.

Equation (1) was fitted to experimental data (Fig. 1), and the four adjustable parameters k, K_0, β and Y were estimated through a nonlinear regression technique, minimizing the objective function, during resolution by Runge–Kutta algorithms (Beolchini et al. 2010). The estimated values for the different parameters and the regression coefficients are reported in Table 3. The relatively high values of the regression coefficients confirm the suitability of Eq. 1 to mathematically describe the temporal changes of microbial abundance and biopolymeric carbon, especially for three out of four treatments (LI-MNA, AN-LAC, AN-NUT; Fig 1a–d) investigated in the present study.

The estimated values for the kinetic constant (k) were relatively high in the experimental systems with a relatively high content of organic carbon, either characteristic of the sediment itself (port of Livorno) or added as lactose (port of Ancona), with 0.81 and 0.64 day^{-1} estimates for LI-MNA and AN-LAC, respectively (Table 3). Such estimates allowed us to calculate prokaryotic doubling times between 0.8 and 1.1 day, which are typically observed in highly eutrophic marine sediments (Luna et al. 2002). These high values for the rate constant k are counterbalanced by relatively high values of the microbial decrease constant, K_0, that were estimated at 0.05 and 0.04 day^{-2} in treatments LI-MNA and AN-LAC, respectively (Table 3). This is the mathematical translation of the initial significant increase of the microbial abundance, followed by an equally significant decrease after the first 7–10 days of incubation (Fig. 4). A comparison with the aerobic conditions (Beolchini et al. 2010) shows similar performance, with even faster growth rate in anaerobic conditions. This behavior, that

Table 3 Parameters (k, K_0, β and Y) estimated by nonlinear regression analyses in the different experimental systems

Experimental systems	k (1/day)	K_0 (1/day^2)	β (g/10^8 cells)	Y (10^8 cells/μg biopolymeric C)	Y (g C biomass/g biopolymeric C)	R^2
LI-MNA	0.81	0.05	0.02	26.5	0.053	0.99
AN-MNA	0.02	0	0.01	0.3	0.001	0.70
AN-LAC	0.64	0.04	0.03	46.4	0.093	0.96
AN-NUT	0.06	0.01	0.03	1.5	0.003	0.81

R^2 coefficients are also reported

goes against what was expected, could be related to different scales and efficiencies in mixing (5 L mechanically stirred bioreactor, in anaerobiosis, versus 250 mL shaken flasks in aerobiosis; Beolchini et al. 2010), with an improvement of the mass transfer processes in the experimental system used for anaerobic treatments. As recently reviewed, when a process is scaled-up, there is the possibility of changes in the performance (Garcia-Ochoa and Gomez 2009). In the case described in the present work, changing the scale the operational conditions varied, and in the bioreactor mixing and mass transfer increased. The estimated values for the yield factor Y in these high initial organic carbon treatments were 26.5 and 46.4 10^8 cells/mg biopolymeric carbon (Table 3), for LI-MNA and AN-LAC, respectively, that corresponds to 0.053 and 0.093 g C biomass/g biopolymeric carbon indicating that most of the organic substrate is used to sustain respiration processes (corresponding to a bacterial growth efficiency in the range 5–10 %; del Giorgio and Cole 1998).

As concerns the other two treatments, where no significant microbial growth was observed (Fig. 1), the estimated values for the parameters rate constant were low, as expected, and they were estimated at 0.02 and 0.06 day^{-1}, for AN-MNA and AN-NUT, respectively (Table 3). These two treatments were characterized by an initial relatively low organic carbon availability, since no carbon amendments were performed and the sediment sampled in the port of Ancona showed quite a low content of organic carbon (Table 2) if compared to Livorno port sample. The estimated values for the yield parameters in these cases (0.001 and 0.003 g C biomass/g biopolymeric carbon; (Table 3) suggest that carbon is used to a very minor extent to produce prokaryotic biomass.

As a whole, it was observed that the anaerobic biostimulation of microbial communities activates different biogeochemical processes, according to the initial availability of organic carbon and the amendment of inorganic nutrients. The first factor (bio-available carbon) appears to influence mainly the microbial growth: This was suggested both by the temporal profiles of the microbial abundances and by the estimated values for the kinetic model parameters. In spite of this effect, the initial availability of organic carbon apparently did not have a significant influence on metal mobilization. Indeed, it is known that there is affinity between metals and organic matter, even if some metals can display a different behavior (Sudhanandh et al. 2011, and citations therein). At least a decrease of the metals associated with the organic fraction (oxidizable fraction) would have been expected, as a consequence of the observed decrease of the biopolymeric carbon. Such decrease was not observed probably due to a simultaneous increase of metal sulfides (also included in the oxidizable fraction), due to the stimulation of sulfate reduction pathways (Muyzer and Stams 2008). To this regard, previous studies have shown that metal pyritization takes place in decreasing order Ni > Zn > Cr (Morse and Luther 1999).The second factor (the continuous amendment of inorganic nutrients), even if it was not relevant for sustaining the microbial growth, stimulated in any case the microbial activity, with a consequent relevant effect on metal partitioning. Indeed, an increase of metals in the oxidizable fraction was observed, with a parallel decrease in the residual fraction. The presence of nutrients is able to increase the microbial activity (van Loosdrecht et al. 1990), determining effects on the near environment. It cannot be excluded that also the changes observed in the residual fraction were a consequence of the biological activity of the microbial community. Indeed, although the residual fraction should refer only to metals in the crystalline lattice of minerals, variations in this fraction associated with the microbial activity were already observed in the scientific literature (Fonti et al. 2013; Prica et al. 2010; Sabra et al. 2012). Furthermore, highly insoluble metal salts can be classified as residual metals by selective sequential extraction procedure (Bacon and Davidson 2008; Park et al. 2011) and these could be involved in the processes occurring in the bioreactor.

Conclusion

Our study adds a relevant know-how dealing with anaerobic bioremediation of sediments contaminated with metals, alerting that, under certain conditions, an increase of metals in the more mobile fractions could take place, even without metal solubilization.

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