BIOACTIVATORS AS A POTENTIAL STRATEGY FOR DREDGED MARINE SEDIMENT RECOVERY

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ABSTRACT

Sediment dredging from harbors and water bodies in order to maintain the navigation is a necessity worldwide; however, the storage and treatment of sediments is a problem for harbor managers. Sediment decontamination could represent a sustainable approach for turning them into a new source of environmentally reusable material.

To manage the sediments dredged from the Livorno harbour, several possible recycling techniques are being explored, including sediment washing and bioremediation. A combination of these two techniques can also be taken into consideration.

This study examines the feasibility of an enzyme enhanced bioremediation technology used as it is, or in combination with the sediment washing. Specifically, we applied an enhanced bioremediation approach to both raw sediments and two derivate granulometric fractions separated by a pilot sediment washing facility: a silt-clay fraction (< 63 μm) and a sand fraction (>63 μm<200 μm). The preliminary sediment washing was effective in concentrating the organic and inorganic contamination into a smaller volume of fine sediment particles (silt-clay).

The bioremediation experiment, carried out in triplicate at mesoscale level, consisted in setting up containers of about 0.2 m^3 each, filled with the three matrices (raw sediment, clay-silt and sand fractions) treated and untreated (control) with bioactivators (a mixture of microorganisms, enzymes and synergists). The physical, chemical and biological properties of sediments were determined at the initial sampling time (t_0) and after three months (t_{90}) from the beginning of the experimentation. The bioactivator application, providing specialized microorganisms and stimulating the growth of indigenous microorganisms, determined the increase in microbial respiration and in hydrolytic enzyme activities in all the treated matrices, in particular in the silt-clay fraction. However, this fraction both treated and untreated, has not been able to degrade significant amount of organic pollutants. This is probably due to the burial of contaminats in micropores making them inaccessible to microorganisms and extracellular enzymes. On the contrary, a significant reduction in total petroleum hydrocarbon was observed in sand and raw sediment matrices after three months from remediation strategy application (about 50%), indicating the efficiency of the bioremediation technology.

Keywords: sediment washing, bioremediation, enzyme activity, hydrocarbon degradation

1. Introduction

A large amount of sediments is dredged every year from ports and waterways in order to maintain adequate depths for ship navigation, but the fate of these sediments is an issue worldwide recognized. Usually, dredged sediments are disposed of in specific facilities and may cause environmental problems due to their contamination. Metals and organic compounds, in fact, accumulate in sediments due to the limited hydrodynamic energy on the inside portions of

harbors. The principle factor responsible for increased adsorption of contaminants is the fine fraction of sediments and the organic matter (Burton, 1991). Sand, which has a low specific surface area and a low surface charge density, is not very reactive and it has often a lower contamination than fine material. Sediment washing is a relatively simple and useful ex situ remediation technology, which is based on the separation and volume reduction processes. In view of this, sediment washing may be used to separate and concentrate the contamination into a smaller volume of fine sediment particles. This technology is usually used in combination with other technologies. The combination of sediment washing with natural techniques could represent, when suitable, the most convenient economic solution. Bioremediation technique is based on the capacity of microorganisms to degrade the organic compounds and to reduce their toxicity or concentration. In the bioremediation approach, the natural process of organic compounds degradation is accelerated by creating optimal environmental conditions (such as temperature, pH and nutrients) for autochthonous microorganisms activity (biostimulation), or by introducing microorganisms with specific capacity of degradation (bioaugmentation).

In this work, the mechanical grain size separation of dredged sediments was carried out in order to reduce the volume of contaminated sediments to be treated and to make the material more homogeneous. After the sediment separation pre-treatment, the effectiveness of the bioremediation technology on the decontamination of the two sediment derivate granulometric fractions (sand and silt-clay), as well as of the raw sediment, was evaluated.

2. Experimental layout

Sediment washing was conducted in a pilot separation installation. Fresh water-sediment slurry was sieved by a vibrating screen (4 mm size mesh) and conveyed in a hydrocyclone which allowed the recovery of the particles having a diameter lower than 200 μm. This fraction (sediment fraction <200 μm) containing the finer fraction (fine sand, silt and clay) was pumped in another hydrocyclone where a further mechanical separation was produced by centrifugal force. This hydrocyclone consists in a conical shell with a tangential inlet for feed (water-sediment mixture), an outlet at the top (overflow), and another outlet at the bottom (underflow). The overflow is enriched in water and fine fraction (< 63 μm), whereas the underflow concentrates the remaining sandy fraction (>63 μm<200 μm). The <63 μm fraction was allowed to settle for 24 h; after which, the supernatant was removed. The two resulting solid fractions; sand (>63 μm<200 μm) and silt-clay (<63 μm), as well as the water effluent and raw sediment were analyzed (Table 1).

Table 1: Characterization of the three matrices and water effluent after sediment washing.

	Unit	Sediment	Sand >63 μm <200 μm	Silt-clay <63 μm	Water effluent
Sand	%	63	95	45	
Silt	%	19	3	32	
Clay	%	18	2	23	
pH		8,3	8,7	8,1	
C.E.	dS m^{-1}	16,8	1,1	2,2	
TOC	%	1,93	1,01	2,42	
TN	%	0,384	0,214	0,397	
TP	mgP kg^{-1}	650	610	590	
Ammonia	mgNH_4^+ kg^{-1}	14,1	7,9	41,0	
Nitrate	mgNO_3^- kg^{-1}	20,0	2,4	5,0	
Cu	mg Kg^{-1}	123	55	83	0,094
Zn	mg Kg^{-1}	230	94	170	0,334
Cd	mg Kg^{-1}	n.d.	n.d.	n.d.	n.d.
Cr	mg Kg^{-1}	64,0	21,1	52,6	n.d.
Ni	mg Kg^{-1}	49,1	16,3	44,1	0,265
Pb	mg Kg^{-1}	59,7	22,3	47,5	0,006
TPH	mg Kg^{-1}	5447	3973	5648	

E.C., electrical conductivity; TOC, total organic carbon; TN, total nitrogen; TP, total phosphorus; TPH, total petroleum hydrocarbon; n.d. not detected.

For the bioremediation, 20 kg of polluted sediments (raw sediments, sand fraction and silt-clay fraction) were placed in plastic containers (mesocosms). All containers were maintained under controlled temperature and humidity for three months. For each of the three matrices, the bioremediation treatments carried out in triplicate were the following: (1) a mixture of microorganisms-enzymes-nutrients (bioactivator treatment); (2) sediment without treatment (control sediment). In the bioactivator treatment, 100 g of a commercial product containing a mixture of microorganisms, enzymes and nutrients was added. Chemical, biochemical and biological parameters were determined immediately after bioactivator application (t_0) and after three months (t_{90}) from the beginning of the experimentation.

3. Methods

Granulometry was measured using the pipette method (Indorante *et al*., 1990). Electrical conductivity (E.C.) and pH were measured in a 1/10 (w/v) aqueous solution incubated for 1 hour under shaking at 25 °C using selective electrodes (E.C.: Conmet 2, Hanna Instruments Italia; pH: Titroprocessor 672, Methron Switzerland).Total organic carbon (TOC) was measured with a LECO, U.S.A. RC-412 Multiphase Carbon/Hydrogen/Moisture Determinator. Total Nitrogen (TN) content was determined by a LECO, U.S.A. FP-528 Protein/Nitrogen Determinator. Total phosphorus (TP) was measured using the method reported by Murphy and Riley (1962). The ammonium and nitrate concentrations were measured by an ammonium and nitrate selective electrode, respectively (SevenMulti, Mettler Toledo). Total heavy metal concentration analysis was performed, after acid digestion with nitric-perchloric acids (HNO3:HClO4, 5:2) in microwave, by atomic absorption spectrometry using a ContrAA300 (Analytical Jena) spectrometer with air/acetylene flame. Total hydrocarbon (TPH) content was estimated by the gravimetric method 1664 by weighing the dry residue after solvent evaporation under nitrogen flow (APHA – American Public Health Association 1992). Evaluation of either fungal and bacterial populations was obtained through direct count on agarized media, R2A and MALT for bacteria and fungi, respectively (Andreolli *et al*., 2015). The respiratory activity of the sediment was determined using a modified version of the Isermayer method (1952). Sediment β-glucosidase activity was measured according to the methods of Marx *et al*. (2001) and Vepsäläinen *et al*. (2001), based on the use of fluorogenic methylumbelliferyl (MUF)-substrate (4-MUF-β-D-glucoside). Fluorescence (excitation 360 nm; emission 450 nm) was measured with an automated fluorimetric plate-reader (Infinite® F200PRO Tecan) after different incubation times at 30 °C.

4. Results

The sediment separation was crucial to concentrate the organic and inorganic contamination into a smaller volume of fine sediment particles (silt-clay). Treated and untreated sand fraction showed, in fact, significant lower values of heavy metals (HM) and organic contaminants (TPH) with respect to the silt-clay and raw sediment samples (Table 2). Generally, TOC, TN and TP did not significantly changed over time, and higher values of these parameters were shown in the treated matrices, especially in the silt-clay fraction. The mineral forms of nitrogen, which represent a nutrient available for microorganisms inside the sediments, increased in all the treated matrices due to bioactivator addition. Furthermore, the better oxygenation in the sand fraction is the reason for the lower ammonia and the higher nitrate content with respect to the other matrices (silt-clay and raw sediment).

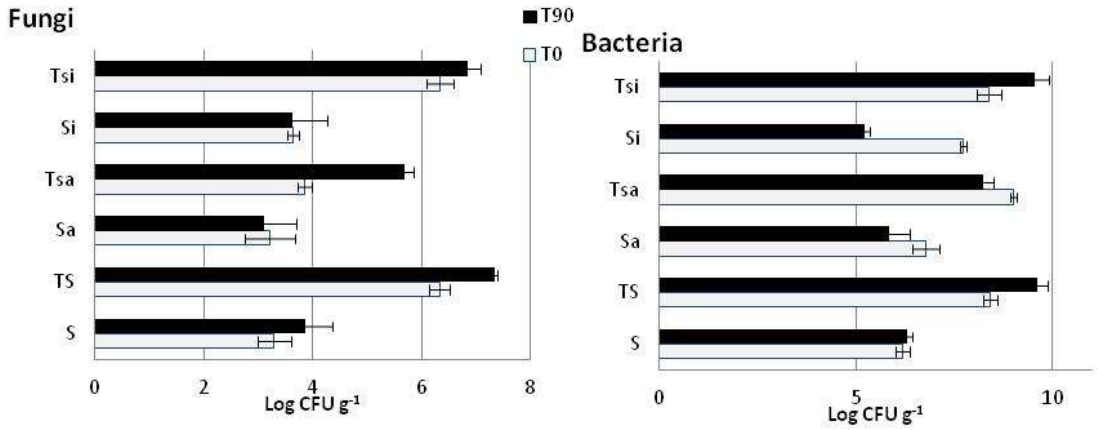
TOC, total organic carbon (%); TN, total nitrogen (%); TP, total phosphorus (g kg^{-1}); Nitr., NO_3^- ($mgNO_3^-$ kg^{-1}); Amm., NH_4^+ ($mgNH_4^+$ kg^{-1}); HM, Total heavy metals ($meqHM$ kg^{-1}); TPH, total petroleum hydrocarbon ($mgTPH$ kg^{-1}); Mic. resp., microbial respiration ($mgCO_2$ kg^{-1}); β-glu, β-glucosidase activity (mmol kg^{-1} h^{-1}).

Data obtained by means of total count method evidenced that addition with the biostimulating product enhanced the initial population of both fungi and bacteria when compared with untreated trials (Figure 1). Moreover, a general increase in biomass of both bacteria and fungi were observed in all the treated samples at the end (T_{90}) respect to the beginning (T0) of the experimentation.

Table 2: Chemical and biochemical parameters at the beginning (t_0) and at the end (t_{90}) of the bioremediation treatments.

	Sediment		Treated Sediment		Sand		Treated Sand		Silt-clay		Treated Silt-clay	
	t_0	t_{90}	t_0	t_{90}	t_0	t_{90}	t_0	t_{90}	t_0	t_{90}	t_0	t_{90}
TOC	1,94	1,91	2,60	3,00	1,00	1,06	1,8	1,41	2,19	2,07	4,03	4,3
TN	0,18	0,18	0,26	0,30	0,11	0,11	0,18	0,20	0,20	0,23	0,38	0,43
TP	0,80	0,85	1,29	1,51	0,70	0,68	0,97	1,32	0,90	0,75	1,93	2,09
Nitr.	9,35	6,35	37,7	26,6	2,15	3,09	38,6	40,4	5,34	1,20	13,9	29,1
Amm.	12,9	2,95	247	468	6,51	1,98	124	29,7	55,6	10,5	300	248
HM	17,4	16,7	19,3	19,3	7,40	7,90	7,70	7,80	16,5	15,7	17,2	16,6
TPH	5114	4365	5066	2732	3898	2999	3982	1783	5597	5523	5522	5298
Mic. resp.	103	43,1	4518	1594	49,2	27,3	7065	2840	242	111	14406	6170
β-glu.	14,6	223	116	741	1,64	12,4	34,5	120,4	19,1	91	75,5	1193

Figure 1: Microbial total count for Fungi and Bacteria obtained from Sediment (S), Sand (Sa), and Silt-clay (Si) samples, either treated (T) or not, at t_0 and t_{90}.



Carbon dioxide evolution, which represents microbial catabolic metabolism, showed higher concentrations in all treated with respect to untreated matrices, especially in silt-clay fraction, due to the addition of microorganisms and available substrates (Table 1). The stimulation of sediment microbial number and activity in treated matrices was also confirmed by the trend of hydrolytic enzymes, such as β-glucosidase activity, which provides an indication of the potential for soil organic matter decomposition in sediment. This enzyme showed the highest value in silt-clay treated fraction at t_{90} sampling time. Silt-clay fraction is probably more efficient in binding microorganisms and extracellular enzymes and in preserving them by proteolysis and chemical degradation (Lähdesmäki, 1992). However, this fraction, both treated and untreated, has not been able to degrade significant amount of organic pollutants (reduction percentage of TPH lower than 5%) (Table 1). Probably, in silt-clay fraction the availability and, thus, the potential of hydrocarbon biodegradation is reduced since the pollutants could be entraped in the micropores, thus becoming inaccessible to microbes and their extracellular enzymes (Cheng *et al*. 2012). The higher adsorption of the organic pollutants on clay fraction can be also the reason for their lower toxic effect on microbial activity (McBride 1994). In raw sediment and sand fraction, the treatment with bioactivators allowed a 46% and 55% removal of hydrocarbon, respectively, whilst natural attenuation in the untreated matrices allowed only a 15 and 23% removal of the starting hydrocarbons, respectively.

5. Conclusions

These results seem very promising considering the complexity of the material to be decontaminated and the apparent difficulty of creating acceptable habitat for the operation of a biological active system. The preliminary sediment washing represented an effective technology for concentrating the organic and inorganic contamination into a smaller volume of fine sediment particles (silt-clay). In addition, the combination of this technology with the addition of bioactivators enhanced the microbial degradation of organic contaminants. The hydrocarbon removal reached at t_{90} sampling time 46% and 55% in raw sediment and sand fraction treated with bioactivators, respectively, while natural attenuation (control sediment and sand) allowed only a 15 and 23% removal, respectively. The silt-clay fraction was not able to degrade significant amount of this contaminants. In conclusion, the results indicated that biactivator treatment significantly reduced the time required for the remediation of raw sediment and sand fraction, most likely because of the enhancement of microbial degradation of organic contaminants through the introduction of microorganisms and the improvement of nutrient balance.

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