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Treatment of Contaminated Sediments by Bio-Slurry Reactors: Study on the Effect of Erythromycin Antibiotic

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The biological treatment with bio-slurry reactors has been effectively considered as a remediation technology for the removal of organic pollutants from soils or sediments, characterized mainly by sandy and clayey fractions. In this context, the treatment of marine sediments contaminated by hydrocarbons, through a remediation technique, represent a topic of particular interest for the scientific community. In this work, bio-slurry technology has been studied for the treatment of marine contaminated sediments with a Total Petroleum Hydrocarbons (TPH) content of 888.57 mg kg^{-1}. The experimental campaign was divided into three phases, for a period of about 75 days. TPH removal efficiency was evaluated in two reactors, operating in parallel and in batch mode. In the first phase, reactors had the same characteristics and operating conditions while, in the second phase, the erythromycin antibiotic was added into one of the two reactors. Surprisingly, the addition of erythromycin improved the TPH removal efficiencies and reduced treatment times. Finally, the third phase was characterized by the substitution of the liquid phase, present in the reactors, with saline water, in order to deepen the study on the removal and transfer mechanisms typical of the bio-slurry systems.

1. Introduction

Bioremediation is one of the most widely used and cost-effective treatment technologies for petroleum-contaminated sediments or soils with from low to moderate levels of organic contamination (Huesemann et al., 2002). This technique uses naturally occurring fungi or plants and bacteria to detoxify and degrade substances hazardous to human health and the environment. The slurry bioreactor treatment is an *ex situ* (off-site) technology that consists in the biological treatment of contaminated soil or sediments in a reactor, which is provided with the conditions to enhance natural attenuation of a great variety of organic pollutants in slurry phase. In general, slurry reactors are chosen as sediment or soil remediation treatment when safe and fast remediation is needed or when suitable conditions for more conventional biological technologies are not given. The main advantages of this technology are: increased rates of pollutant biodegradation compared to *in situ* bioremediation or to *ad situ* solid phase biotreatment; increased mass transfer rates and increased contact microorganisms/contaminants/nutrients; control and optimization of environmental parameters (such as pH, temperature, etc.); (effective use of bioaugumentation and biostimulation increase contaminant availability and desorption through the addition of solvents and surfactants (Gan et al., 2009; Nano et al., 2003). Bioreactors are usually operated in semi-continuos (Sequencing Batch Reactor or SBR) or batch mode because they facilitate the handling of sediments and slurries (Mohammad Reza 2014; Prasanna et al. 2008). The operation in continuous mode is also possible but remains uncommon (Robles-González et al., 2008). Distinctive features of bio-slurry reactors are that sediment is treated in aqueous suspension (typically 10 to 30% w/v) and that pneumatic or mechanical mixing is provided, in order to maintain homogeneous conditions. Mechanical mixing is usually provided by a stirrer connected to a motor. (Machín-Ramírez et al. 2008; Mustafa et al., 2015). Additionally, diffusers are often installed in the reactor with the purpose of ensuring aerobic conditions (Robles-González et al., 2008).

This communication reports experimental data pertaining to bio-slurry phase reactor performance with respect to TPH degradation in the sediments of Augusta Bay (Syracuse, Italy). In particular, the main objective of this

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work was to compare the effectiveness of this technology in the removal of organic compounds in bio-slurry reactors, evaluating the performance of the treatment also in presence of erythromycin, is one of the most commonly used antibiotics for the treatment of human and animal infection diseases and, as a consequence, one of the most detected antibiotic in the environment. In particular, erythromycin is introduced into the environment primarily by excretion in faces and urine of humans and animals, thus, reaching wastewater treatment plants (WWTPs). There, erythromycin accumulated due to its capability to resist to the removal processes. Therefore, WWTP effluents are contributors to the spread erythromycin and other antibiotics in the environment (Schafhauser et al., 2018). Nowadays it is recognised that the presence of environmental antibiotics can have serious consequences for the spreading of resistance genes and antibiotic-resistant bacteria, including human pathogens (Schafhauser et al., 2018).

2. Materials and methods

2.1 Sediment characterization and analysis methodology

The coastal sediment samples used for the experiments were collected from the northern part of Augusta Bay (Site of National Interest of Priolo), in a highly impacted zone in front of petrochemical plants. The chemical-physical properties of the sediments are well as their contaminant content were assessed as follows. The granulometric analysis was assessed by ASTM D 421-85, the density was assessed using ASTM D854-92 method whereas the moisture content was assessed by the “AnD” type “ML-50” analyser (ASTM D 2216-80). The organic matter concentration was determined by Walkley-Black method (Gutiérrez Castorena et al., 2017). Metal concentration was assessed with US-EPA 6010C and 3051A methods, using ICP-AES (OPTIMA 4300DV, Perkin Elmer®). Hydrocarbon concentration, expressed as mg kg^{-1}, was assessed by GC–FID (Agilent 6890) as several Total Petroleum Hydrocarbons (TPH) (US-EPA 8015C). EPA 3545 A method “Pressurized Fluid Exaction (PFE) and the “Speed Extraxtor E-916” were used for the TPH extraction procedure from sediments while TPH extraction procedure from liquid phase was determined by US-EPA 3510C. Total Organic Carbon (TOC), expressed in mg L^{-1} was assessed by (TOC-V_{CSH} SHIMADZU).

Finally, the monitoring of the operating parameters (pH, conductivity, temperature and dissolved oxygen) was carried out through a multi-parameter probe type “PCD650”.

2.2 Bio-slurry reactors and operation

Two bio-slurry phase reactors having a total working volume of 4 L were used in bioremediation experiments. Reactors were fabricated using plexiglass with H/D ratio (height / diameter) equal to 10. Air diffusers connected to sparger network using silicon tubing were provided at the bottom of reactor (2 mm above the reactor bottom) to facilitate uniform distribution of air through the system and air flow rate was set at 3 L min^{-1}. This value was choses on the basis of mixing tests conducted with varyng air flow.

In the study, 400 g of sediment with nutrients was added to 4 l of water and active sludge (sediment/solution ratio 1:10), previously acclimatized to salt condition.

Nutrients (CH_3COONa [35 g L^{-1}], NH_4Cl [7 g L^{-1}], K_2HPO_4 [2 g L^{-1}], $CaCl_2$ [1.5 g L^{-1}], $MgSO_4$ [1.2 g L^{-1}]) were added at the beginning of the study to achieve a carbon:nitrogen:phosphorus (C:N:P) molar ratio of 100:10:1 based on the hydrocarbon loading as a measure of C (Maddela et al., 2016; Vázquez et al., 2009) and in order to ensure that there is no nutrient limitation (Robles-González et al., 2008; Woo, Jeon, & Park, 2004). Sediment samples were taken every two weeks and were analysed for TPH concentration and organic matter in triplicate. Liquid phase sample were taken at 45th, 60th e 75th day and TPH concentration were analysed.

The experimental campaign, divided into three phases for a period of about 75 days. The first phase of the work, lasting about 30 days, was conducted by assessing the performance of the fixed treatment for both the same operating conditions, such us volume, flow rate of aeration and solid-liquid ratio; the second phase, also lasting 30 days, was characterized by the addition of the *erythromycin* antibiotic into one of the columns in order to evaluate any possible effects on on the removal kinetic in a dosage dependent manner; finally, the third phase is consistent with the replacement of the liquid component present in the reactors with saline water in order to deepen the study on bacterial kinetic.

2.3 Microcosms tests

At the beginning of the experiment, preliminary batch tests were conducted in microcosm aimed at identifying the optimal erythromycin antibiotic concentration and at evaluating its effect on the biodegradation activity of pollutants by a specifically halotolerant biomass inoculated. The latter was previously acclimatized through a cultivation with a semi-continuous supply of civil wastewater with increasing salinity (from 0 to 20 mg L^{-1} in NaCl). The "batch tests" have been divided into two phases. The first phase was conducted by adding different concentrations of antibiotic (0.05 μg mL^{-1}, 0.1 μg mL^{-1}, 0.5 μg mL^{-1}, 1 μg mL^{-1}, 5 μg mL^{-1}) in five different flasks, each containing activated sludge and water in liquid/liquid ratio of 1:10. The solution was kept stirring by a orbital shaker at a speed of 150 RPM. In order to evaluate the biodegradative activity of bacteria under the effect of antibiotic, it was decided to monitor, in this first phase, the variation of the concentrations of Total Organic Carbon (TOC) after 30 ', 1 h, 24 h and 96 h. The results obtained were compared with the values of the control test in which no antibiotic dosage was performed. The second phase continued and was planned based on the results of the first. More specifically, with reference to the "optimal dosage" obtained from the first battery of tests (Phase 1), the study was deepened by evaluating the effect of the presence of antibiotic on the biodegradation of TPH by the same bacteria. For this purpose, the test was carried out in a volume of 1L consisting of water and activated sludge, in which 100 g of sediment were added (in a solid/liquid ratio equal to 1:10) and the antibiotic was dosed to optimal concentration value (0.5 µg mL^{-1}, see results section below). Also in this case, the sample was stirred and the variations of TOC concentrations (at the contact time of 1 h, 24 h and 96 h) and the residual concentrations of TPH present in the sediment and in the supernatant at the end of the test were analyzed.

3. Results and discussions

3.1 Characterization of dredged sediment sample

The analysis of dredged sediments revealed a slightly acidic in nature (pH 6.60) with a higher content of sand (42.36 %), followed by silt (35.38 %) and clay (22.27 %). The sediment has a high concentration of moisture content (48.6%), organic matter (4.7%), (2.57 g cm-3) and conductivity (5 mS cm^{-1}).
In terms of contaminant content, sediments were affected by a severe hydrocarbon contamination with a detected TPH level of 888.57 mg kg$^{−1}$, whereas low metal concentrations were found. For each investigated metal, its concentration was below the respective Italian regulatory limits, whereas TPH level (TPH fraction C>12) was largely higher than the limit of 750 mg kg$^{−1}$. This clearly emphasizes the seriousness of the problem and the need for the development of suitable and effective specific clean-up tools.

3.2 Microcosm results

The following tables, with attached graphics, show the values corresponding to the analyses carried out in the first step (without sediment), corresponding to the Total Carbon (TC) (Figure 1a) and Total Organic Carbon (TOC) (Figure 1b). The values of inorganic carbon, were considered not influential and in any case obtainable as a difference from the value of TC for each test.

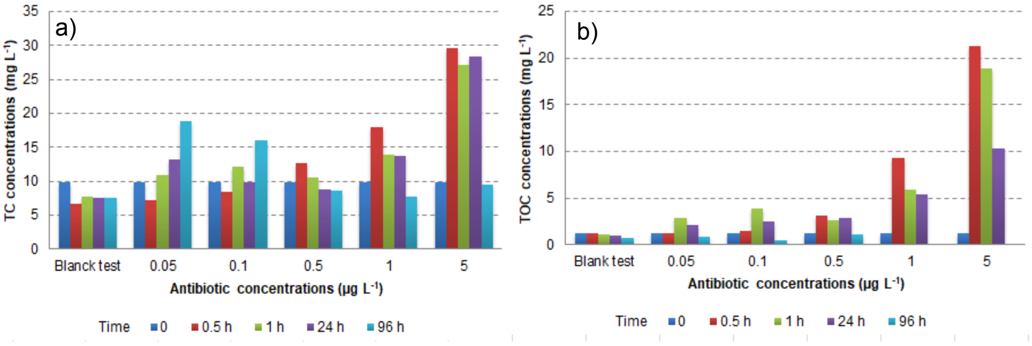

Figure 1: TC concentrations in the first phase of microcosm tests (a); TOC concentrations in the first phase of the microcosm tests (b).

The first phase of the tests in microcosm show significant results for the antibiotic concentration at 0.1 µg mL^{-1}, 0.5 µg mL^{-1} and 1 µg mL^{-1}, since the TC concentrations remain much more stable and constant than the TC values obtained for the concentrations at 0.05 µg mL^{-1} and 5 µg mL^{-1}. On the base of these promising preliminary results, the variations of the TOC was evaluated at the same concentrations and exposure times in order to identify an optimal value of antibiotic concentration. The results of TOC evaluation show that the antibiotic has the ability to stimulate the biological process and microbial growth. In fact, at 0.1 µg mL^{-1}, 0.5 µg mL^{-1} and 1 µg mL^{-1} TOCs increased compared to the initial value of 1.18 mg L^{-1} at 24 h; while the antibiotic concentration at 0.05 µg mL^{-1} did not show any bacterial growth. Analyzing the TOC concentration at 96 h, a reduction in the values to 0 mg L^{-1} was noted for the antibiotic concentrations at 1 and 5, as they were probably too high and harmful for bacteria growth. The graphs show that the best result is the intermediate antibiotic concentration (0.5 µg mL-1) because, as the contact time varies, the TOC concentrations tend to stabilize, going from one to four days, similar to the TC trend. Based on the results of the individual batch tests, as described above, microcosm testing continued by analyzing a further batch test, mixing water and sediments in a larger volume (1 L) and adding the antibiotic based on the best dosage previously detected. In this context, the analysis of TC and TOC data showed a certain consistency of results. In particular, the TOC increased rapidly from 1.5 µg mL^{-1} to 2.44 µg mL^{-1} after the first 24 hours, remaining stable at around 2.2-2.3 µg mL^{-1} in the following three days. This last result could be an indirect confirmation of an active microbial growth, also promoted by an important contextual removal of TPH from the microcosm sediments in just 4 days, in the order of 12-15 % (going from 888 mg_{TPH} kg^{-1} to 777 mg_{TPH} kg^{-1}). The results obtained from this second microcosm test confirm and validate the data obtained in the first phase. In particular, it appears that by injecting 0.5 µg mL^{-1} of erythromycin the microbial cells of the bio-slurry manage to survive and, based on the temporal evolution of the TOC and TPH values, seem to adapt to the presence of TPH in the sediments. On the basis of this further validation, it was decided to dose the antibiotic directly in the column and study the effect of erythromycin during the continuous treatment of sediments with bio-slurry.

3.3 Bio-slurry phase reactors performance

The performance of bio-slurry phase reactors with respect to TPH degradation was evaluated estimating the substrate (TPH) degradation efficiency (ξa) as explained in Eq(1):

$$\xi a\ (\%) = \left(\frac{Co-Ct}{Co}\right) \times 100 \qquad (1)$$

Where, C0 represents the initial TPH concentration (mg kg^{-1}), Ct denotes TPH concentration (mg kg^{-1}) at a given time ‘t’ during the reactor operation. The degradation patterns of TPH in sediment and liquid phase, for the reactors R1 e R2, are shown in Figure 2a.

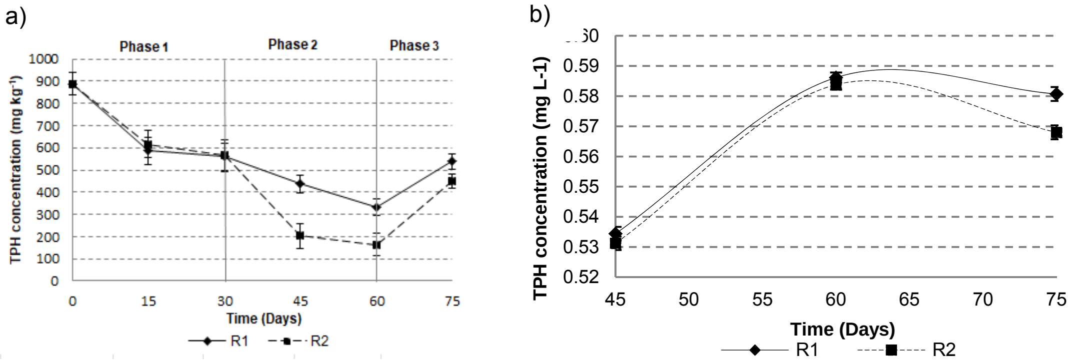

Figure 2: TPH residual concentrations in sediment (a) and liquid phase (b).

In the first phase of the experimentation, both reactors present the same operating conditions and in this case TPH residual concentrations are resulted be 558.93 mg kg^{-1} for R1 and 564.38 mg kg^{-1} for R2 with a removal efficiency of 60% in R1 and 62 % in R2. Subsequently, in the second phase, in R2 reactor the erythromycin antibiotic was added to the concentration of 0.5 µg L^{-1}, chosen on the basis of microcosms tests. In this phase, the removal efficiency are increased in both reactors specially in R2 reactor. These results are probably due to the addition of antibiotic, which causes the increase of removal efficiency in R2 compared to R1 without antibiotic. Finally, at the end of the experimentation, the liquid phase of both reactors with tap water (in which NaCl was added, in order to obtain the same conductivity present in the reactors) was replaced. In this phase, TPH residual concentrations in both reactors increased and that is probably due at the change of the operating condition. This change resulted in the release of TPH sequestered by non-degraders (present in mixed cultures) or by other substances present in the bioflocs or biofilms structures (such as extracellular polymeric substances or EPS) (Adav & Lee, 2008; Aksu, 2005). This phenomenon is known as “biosorption” and is one of the solid liquid mass transfer mechanisms, typical of the slurry phase systems. Despite the occurrence of TPH biosorption, residual concentration values remained lower than the initial values as it was, probably, effectively removed within the bio-slurry system. Therefore, the R2 reactor proved to be more performing, compared to R1, thanks to the addition of the antibiotic.

TPH concentrations are also been evaluated in liquid phase in order to obtain a mass balance (Figure 2b). In this experimentation, TPH concentrations in liquid phase has been measured on 45 th, 60 th and 75 th days of work. The results showed a link with the results obtained in sediment phase. In fact, TPH concentration in liquid phase increase (second phase) when TPH concentration in sediment decrease, reaching concentration values of 0.59 mg L^{-1} for R1 and 0.58 mg L^{-1} for R2. Subsequently, during the third phase, TPH concentration values in the liquid phase decreased.

3.3.1 Bioprocess monitoring

One distinctive and advantageous feature of SBs is the manipulation and control of environmental conditions that leads to biodegradation optimization and better process performance. In this regard, several operational variables can be monitored and controlled: pH, temperature and dissolved oxygen in aerobic SBs. pH is one of the important parameters which influences the activity and type of microflora and substrate mobility. A rise in the pH values was observed during the first phase of slurry phase systems (from 7 to 8.4) and subsequently it has remained constant. Temperature remained in a range between 25°C and 30°C, optimal value for treatment (Pino-Herrera et al., 2017) while dissolved oxygen (DO) concentration has remained constant (7.3 g L^{-1}) in both reactors during all the experimentation. During the three phases of the experiment, the variations of the sediment organic matter (SOM) in both reactors were analyzed (Figure 3a). During the first phase, the results obtained show very similar values in both reactors, with an increase from 4.7% to about 6.75%. In the second phase, SOM remained almost constant (about 6.6% in both reactors) while it decreased in the third phase (6.28% for the reactor R2 and 6.16 for the reactor R1). Additionally, mineral-SOM aggregates can be formed, in which TPH can diffuse and sorbed, reducing their bioavailability.

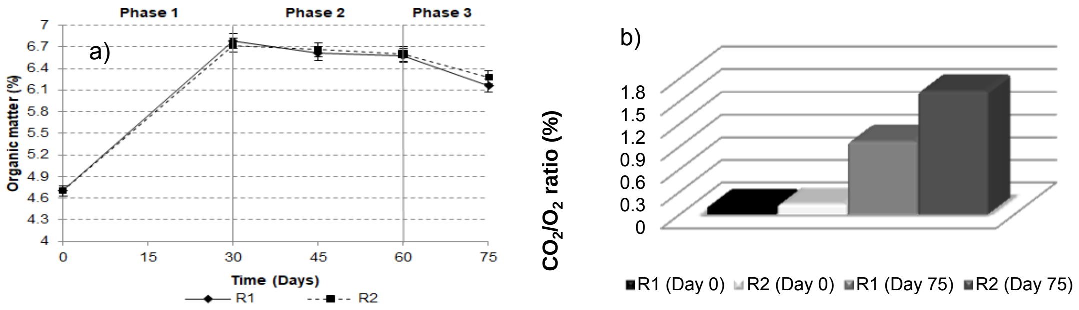

Figure 3: Variation in organic matter concentration during the experimentation of the bio-slurry phase reactors (a); CO_2 production at the end of the experimentation (b).

Finally, at the end of the experimentation, CO_2 production was evaluated (Figure 3b). The aerobic biodegradation process also known as aerobic respiration is the breakdown of contaminants by microorganisms in the presence of oxygen. Aerobic bacteria use oxygen as an electron acceptor to breakdown both the organic and inorganic matters into smaller compounds, often producing carbon dioxide and water as the final product (Gan et al., 2009). Figure 3 shows the results about CO_2 production both reactors at the end of experimentation. Results show a greater production of CO_2 in the reactor R2 compared to the reactor R1 and this could be due to an increased microbial metabolism following the addition of the antibiotic.

4. Conclusions

Petroleum hydrocarbons pollutants are priority pollutants as they are resistant to degradation due to their low reactivity. These contaminants (TPH) pose serious threat to the human and environmental health. Biological treatments are recognized as an efficient, versatile and economic alternative to physic-chemical and thermal treatments. This paper has attempted to utilize the bio-slurry treatment to degrade TPH in marine contaminated sediment. The study was conducted using two reactors with the same operating conditions but erythromycin antibiotic addition to one of the two. Surprisingly, the results obtained show that highest TPH removal was observed in the reactor with antibiotic, both in the sediment and in the liquid phase. The reason of this enhancement is not clear: one possibility can rely on the fact that serendipitously the antibiotic can select the most performing microorganisms capable of degrading TPH or that antibiotic can exert a stimulative effect towards the microbial physiology by promoting horizontal gene transfer and increasing genetic variability (Andersson and Hughes, 2014) or both. Anyhow, this very interesting aspect is and constitutes a novelty in the remediation of sediments contaminated by hydrocarbons. These evidences can be further investigated by microbiological analyses based on “omic-technologies (Narayanasamy et al., 2015) aimed at characterizing the microbial community structure and functionalities in the reactors.

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