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Sustainable Remediation Strategies for Contaminated Marine Sediments

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GENERAL INTRODUCTION

1. Overview

The marine environment is polluted by diverse contaminants among which are toxic metal, aliphatic and polycyclic aromatic hydrocarbons (PAHs) etc (Mai et al., 2018; Sun et al., 2018) for which marine sediments constitute a major reservoir. At various locations, the discharge of hazardous substances over many years has resulted in high levels of pollution (Dell'Anno et al., 2009)

PAHs are a group of organic compounds with a (two or more) fused benzene ring structure. They have low solubility in water but are highly lipophilic (Sun et al., 2018) There are over a hundred different compounds within this group and they mostly occur in complex mixtures.

PAHs can occur naturally in the environment as a result of incomplete combustion of organic materials such as coal, wood or oil (Abdel-Shafy and Mansour, 2016), however, frequent oil seeps and spills around the world, together with heavy industrial and other anthropogenic activities, have caused huge quantities of crude oil deposits in the marine environment. The first large marine oil spill in 1907 caused a release of over 7400 tons of paraffin oil off the coast of the UK. Since then, an estimate of over seven million tons of crude oil have been released in the marine environment following about 140 large oil spills (Li et al., 2016; Mapelli et al., 2017). The Deep Water Horizon (DWH) Oil spill alone, has caused a release of approximately 500,000tons of crude oil in the Gulf of Mexico (Brakstad et al., 2018; Scoma et al., 2016)

On another hand, intensive industrialisation and urbanisation resulting in industrial and sewage effluent discharges in the marine environments has also contributed to the environmental pollution in sediments especially with PAHs and toxic metals. A study by Qu et al. (2018), investigated on the spatial distribution of PAHs in the coastal sea sediments of the Bagnoli brownfield site within the Gulf of Naples, Southern Italy. Results showed concentrations of up to 768 µg/kg and 317 µg/kg of PAH in the Naples and Salerno Gulfs respectively.

Since PAHs do not easily biodegrade once introduced in the environment, and toxic metals cannot be biodegraded (Kronenberg et al., 2017) , a large proportion of these contaminants end up accumulating in marine sediments. Spilled crude oil travels into the marine food web and persists in the marine environment for many years, exerting detrimental effects on biological systems (Lee et al., 2018). Studies affirm that a year after then DWH oil spill, slicks of oil heavily enriched with PAHs were detected on the seashores (Mapelli et al., 2017)

PAHs and especially High Molecular Weight (HMW) PAHs containing five rings or more are even more persistent in the environment due to their more complex structure harder to biodegrade. Depending on their level of exposure, PAHs can have severe effect on aquatic life. Exposure to PAHs has been associated with diseases like cancer and reproductive disorders (Abdel-Shafy and Mansour, 2016). Benthic foraminifera is a highly abundant marine protozoan commonly used as environmental bioindicators. Studies have shown lower diversity as well as fewer and/ or deformed species in metal contaminated sediments (Caruso et al., 2011; Le Cadre and Debenay, 2006). Metals in the environment are absorbed by primary producers and are thereby introduced into the food chain with potential harmful effects on the entire biota (Javed, 2005; Sakan et al., 2009). Similarly, laboratory animals were affected with tumors after long exposure to certain PAHs. Also, high-levels of benzo[a]pyrene caused reproductive disorders in pregnant mice as well as their offspring who presented birth defects and reduced weights (Efeovbokhan and Anawe, 2011)

2. Current Remediation Strategies

Several remediation approaches have been proposed which can be classified under physico-chemical or biological remediation. They can be classified in-situ (when treatment is applied at the original contamination site) or ex-situ (involves the displacement of contaminated sediments to a different location prior to treatment). They are also classified depending on the nature of the treatment, as either physical, chemical or biological methods.

2.1. Physico-Chemical Remediation treatments

Generally adopted physico-chemical approaches in marine sediment remediation include dredging, active mixing or capping of contaminated areas (Taneez et al., 2018). Dredging involves the removal of contaminated sediments and disposal of it at another site. It is a relatively common practice to attempt a clean-up of contaminated sediments, however in the process, it creates a resuspension of buried contaminants and causes increased exposure for the affected ecosystem biota, and also affects the sediment-associated benthic ecosystem. Studies carried out on pre and post-dredging sediments at over 20 sites in the U.S. revealed that the outcome of this remediation practice were quite unpredictable. Some sites showed no change or decreases in contaminant concentrations, but others showed increased concentrations (Lofrano et al., 2016; National Research Council, 2007)

Capping consist of in-situ covering of contaminated sediments layer with suitable isolating material such as activated carbon (AC) or sand to limit leaching of contaminants to the upper water layer. Several studies have investigated on the potential application of different materials for capping. In addition to AC, materials such as organoclay, zeolites, coke, apatite and various others have been tested and among them AC, apatite and organoclay appear to be most promising. (Lofrano et al., 2016)

Another technic identified in the literature involves sediment stabilization/ solidification with addition of selected solid wastes. This method was proposed as a solution for the disposal of dredged sediments. (Wang et al., 2015)

Several Chemical oxidants are also employed to enhance chemical oxidation of PAHs into less toxic products. Commonly employed oxidants include hydrogen peroxide, ozone, sodium permanganate, sodium persulfate. These oxidants have been successfully applied in several instances for chemical degradation of PAHs. However, the use of chemical oxidants in contaminated sediments is often ex-situ because of the potential detrimental environmental effects.(Lofrano et al., 2016)

Generally, physico-chemical approaches are economically costly and do not exclude a risk of secondary contamination (Ghosh et al., 2011)

2.2. Biological Remediation Treatments

Biological remediation (Bioremediation) on another hand involves the use of biological agents for the decontamination (and possible complete degradation of hydrocarbon contaminants) of marine sediments and thus represents a more efficient solution with reduced environmental and economic cost. A number of approaches have been proposed which can be classified broadly under bioaugmentation, biostimulation or a combination of both. Some promising biostimulation approaches proposed include the addition of oxygen, and/or nutrients and microencapsulated nutrients (Chen et al., 2017; Haque and Kwon, 2017; Kalantary et al., 2014) the addition of biosurfactants (Ammami et al., 2015; Lee et al., 2018; Raddadi et al., 2017) which represent a more environmentally friendly alternative to the use of chemical surfactants. A more recent approach is that of bio-electrochemical systems which involves the introduction of inert electrodes in sediments to enhance bioremediation. Here the inert electrodes serve as final electron acceptors in the anoxic sediments, and thus enhance the oxidation of the organic contaminants. Two approaches often used are the oil spill snorkel (Cruz Viggi et al., 2017, 2015) and sediment microbial fuel cell systems (Abbas et al., 2017; Hamdan et al., 2017). Also, several studies propose a mixed approach consisting of a combination of biological and physico-chemical remediation.

2.3. Mixed Approach for Remediation of PAH Contaminated Sediments

Several studies have proposed a combination of different approaches for the remediation of PAH contaminated marine sediments. A mixed approach can include a combination of biological methods as well as biological and physicochemical methods, for example studies have tested the use of chemical oxidants as preliminary steps for subsequent bioremediation (Lofrano et al., 2016). This approach has been proposed to enhance the low bioavailability of weathered PAH in contaminated sites and has achieved some relative success with HMW hydrocarbons, however a good majority of these studies focused on soils (Chen et al., 2016; Nam and Kukor, 2000; Samsami et al., 2011).

PAH are always present in complex mixtures of various organic compounds, and a mixed approach could have the potential of yielding better results in terms of biodegradation. For example, bacteria and fungi are major microorganisms used in bioremediation and according to various studies, the diversity of enzymatic abilities of a microbial consortium is required for the degradation of complex hydrocarbons (Kachieng'a and Momba, 2018) Numerous physicochemical remediation approaches have been developed for marine environment, however, PAH contaminant eventually require the action of microorganisms for degradation (Mapelli et al., 2017).

3. Regulatory scenario

A rigorous environmental regulatory framework regarding sediment bioremediation specifically can greatly contribute to increase general awareness on bioremediation methods and thereby increase its application on the field.

In the US for example, the "Estuaries and Clean Waters Act of 2000" was enacted specifically to better understand and assess the efficiency of bioremediation strategies. According to this law, the USEPA (US Environmental Protection agency) was to undertake a two-year study on the efficiency of bioremediation products.(Zhu et al., 2004). Furthermore, the National Oil and Hazardous Substances Pollution Contingency Plan (NCP) which is the government's blueprint for responding to oil spills and release of hazardous substances requires the USEPA to prepare a list of remediation products and oil spill mitigating devices that may be used in emergency situations (US EPA., 2016). As at the time of this writing, 41 bioremediation products figure on this list out of 164 remediation products. This number steadily grew from 15 in 2004 to over 40 today. On another hand at European level, various legislations and international directives exist relating to sediment management focusing majorly on sediment quality categorisation according to contaminant concentrations.

This categorisation process enables to distinguish between low, medium and highly contaminated sediments and the possible disposal or treatment methods (Sekret Life, 2016). However, there is no information provided on potential bioremediation methods to promote their use for treatment of contaminated sediments rather, methods such as landfarming or disposal at sea for low contaminated sediments are mentioned (Röper and Netzband, 2011; Spadaro, 2011; SubSed, 2018). The European

Landfill directive (1999/31/EC) for example has been developed to regulate and minimise the environmental impacts of landfilling disposal by specifying requirements that ought to be met before considering disposal of a particular waste in landfills. Among these requirements are some form of pre-treatment which is not specified. Also, in the decree number 172 of 15th of July 2016 concerning dredging operations in sites of national interest (SNI), guidelines are provided on how to choose dredging technologies and how to reuse the dredged material and mitigate impacts associated with the dredged sediments still with no mention of potential bioremediation applications (Sekret Life, 2016). Meanwhile, there are several environmentally friendly approaches which constitute promising approaches that could be further implemented at large scale. Although assessing their efficiencies under field conditions can be a challenging task, there is need for more of these field investigations as this will greatly contribute to further advance the research aiming at unravelling the full potential of bioremediation.

4. Aim and objectives of the research activity during the Doctorate

The general objective of this study was to propose a sustainable multi-step remediation approach for contaminated sediments from the Bagnoli former industrial area, targeting specifically the removal of weathered PAHs and toxic metals.

To achieve this goal, a number of research questions were formulated at various stages of the research and addressed step by step, they were notably:

Which methods have been investigated in the literature for sediment bioremediation and which of these have been exploited to develop patented inventions?

How effective are the various bioremediation methods to enhance PAH biodegradation in contaminated sediments, notably biostimulation methods with addition of inorganic nutrients, biosurfactants, *A. niger* strains or other more sustainable nutrients such as activated sludge, or nutrient rich super compost?

Can Physico-chemical pretreatments such as ozonation or voltage application (Electrokinetic remediation) help enhance the efficiency of bioremediation potentially by breaking down the more complex HMW PAHs compounds?

How about the toxic metals abundantly present in the contaminated sediments? How is their mobility in the sediments influenced by these bioremediation treatments? Can the prior removal of these metals via Electrokinetic methods accelerate significantly the removal of PAHs during subsequent bioremediation applications?

Iron was identified as the most abundant metal in the sediments with concentrations of over 13000ppm. According to the literature iron was found to be an efficient binding agent for both inorganic and organic contaminants, hence another research question was with the considerable removal of Fe during the EK remediation, was it possible that some PAH removal was achieved simultaneously?

Different groups of experiments were carried out on highly contaminated sediments to investigate on these research questions. The first set of bioremediation experiments consisted of biostimulation with the addition of inorganic nutrients, bioaugmentation with the addition of fungi belonging to Aspergillus sp. and microbial fuel cell with the insertion of graphite electrodes in sediments to serve as electron acceptors and enhance the oxidation of the PAH contaminants. All of the above biotreatments achieved a significant decrease of PAH concentrations in a relatively short time interval with biostimulation being the most effective approach.

Secondly, another set of bioremediation experiment were carried out associated with ozonation of the contaminated sediments. The bioremediation methods consisted of addition of organic nutrients, the addition of rhamnolipid biosurfactants and the addition of a nutrient rich organic compost. Each of this bioremediation methods were tested on both ozonated and non-ozonated sediments to investigate on the specific effects of both the bioremediation and ozonation treatments.

Thirdly, Electrokinetic remediation was investigated on contaminated sediments for both metal and PAH removal, via a ten-day continuous voltage application process whereby metals in the sediments were mobilised from an anode to a cathode end.

Key Findings

Bioremediation has proved to be an efficient and sustainable approach for PAH degradation in sediments and throughout this PhD research, a number of approaches were investigated with objective to asses various methods and propose a method for a more rapid contaminant degradation of the Bagnoli sediments. In the first set of experiments, bioaugmentation with Aspergillus strains was investigated as well as with biostimulation with inorganic nutrients and natural attenuation. Highest effciciency was obtained with natural attenuation and biostimulation implying that good oxygenation alone might be a most crucial factor for optimization of biodegradation in the Bagnoli sediments at least at an early stage. The results obtained with Bioaugmentation was considerably lower.

In the second set of experiments, organic nutrients such as activated sludge and Supercompost were investigated as Biostimulating agents. Although Activated sludge can be considered as a bioaugmentation agent as well due to its high microbial content. The efficiency using these organic agents was considerably lower especially when comparing the inorganic nutrient (which yielded over 80% degradation) and the Supercompost organic nutrient (less than 10%) However, there were some differences in the experimental set-up that might be responsible for the lower efficiency of the organic nutrients such as the fact that the doses of supercompost added were insufficient (which is reflected by the low microbial growth). Also, the Supercompost was added in the form of a powder which might have been more difficult to assimilate for the microbial agents compared to the liquid inorganic nutrients.

These first two set of experiment confirmed the fact that natural attenuation with oxygenation seem to be a good bioremediation option for the Bagnoli sediments at least at an early stage. Also if nutrient are required, then Supercompost proposed in this study represents a more sustainable alternative to inorganic nutrients but should be applied in optimum doses and in a liquid form preferably. Activated sludge on another seemed to have no positive effect on the PAH degradation similarly to the bioaugmentation treatment with Aspergillus in the first experiment. This suggest that the natural microbial population is adapted and efficient enough for PAH degradation in the sediments.

Since the sediments were highly contaminated with metals, an electrokinetic experiment was carried out to observe metal removal from an anode to a cathode end and eventually a possible mobilization of the PAHs. With the high Fe concentrations and iron being known as a binding agents to both polar and non-polar compounds, the hypothesis here was that iron removal via an electrokinetic set up could induce the mobilisation of PAH bound to the metal fraction. Hence the experiment was carried without addition of any solubilizing agents and results seem to indicate some level of PAH mobilization and approximately 50% removal of Pb in the portion of sediments closest to the anode.

In conclusion, the key findings in this thesis are opening new perspectives for the sustainable remediation of sediments contaminated by polycyclic aromatic hydrocarbons and/or toxic metals. It is clear that not all the open questions have been answered, however, considering the current conditions of contaminated sites in the world, it is urgent that the research pushes innovation in the direction of sustainable remediation approaches.

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CHAPTER 1

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Bioremediation of sediments contaminated with polycyclic aromatic hydrocarbons: the technological innovation scenario

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Abstract

Sediment contamination with polycyclic aromatic hydrocarbons (PAHs) represents a major environmental concern worldwide. The different remediation strategies proposed are mainly based on physico-chemical and biological approaches. Physico-chemical methods (also referred to as conventional methods) although more efficient in several cases, are considered as less sustainable as a result of their higher environmental and economic costs. Biotechnological methods on another hand, which make use of microorganisms and/or their metabolic products, have received increased attention as a more environment friendly and less costly alternative, although usually more time consuming. The various biotechnological approaches developed and patented on bioremediation of sediments polluted with PAHs are presented in this review, as well as some soil bioremediation methods considered applicable to sediments. Patents on sediment microbial fuel cells (SMFC) and other electrokinetic approaches are also included as considerable advances have been made in this field. Over 150 patents dating from 1995 to 2019 were collected from Espacenet platform, a worldwide patent database and considered in the present review. Overall, this review highlighting strengths and weaknesses of the various biotechnological approaches developed and patented, so far, can be useful to address further studies to improve bioremediation performance and can represent a benchmark of information for bioremediation companies to identify and explore the most promising field applicable approaches.

1. Introduction

The marine environment is polluted by diverse contaminants among which polycyclic aromatic hydrocarbons (PAHs), a group of organic compounds with two or more fused benzene rings structure. PAHs enter marine systems through a wide variety of anthropogenic sources such as oil spills, urban runoff, shipping and industrial activities (Abdel-Shafy and Mansour, 2016; Beolchini et al., 2009). Because of their relatively low solubility in water and highly lipophilic properties (Mai et al., 2018; Sun et al., 2018), once introduced in the marine environment tend to accumulate in the sediment (Akcil et al., 2015; Kronenberg et al., 2017). PAHs accumulated in the sediments pose serious concerns for the environmental and human health, thus demanding effective and eco-sustainable remediation solutions (Beolchini et al., 2020). Several remediation approaches have been proposed including physico-chemical or biological methods. Remediation solutions can be applied in-situ (i.e., directly on contaminated matrices) or ex-situ (involving the displacement of contaminated sediments to a different location prior to treatment). Generally adopted physico-chemical approaches in marine sediment remediation include dredging, active mixing or capping of contaminated areas (Taneez et al., 2018). Dredging involves the removal of contaminated sediments and their disposal at another site. It is a relatively common practice adopted to attempt a clean-up of contaminated sediments, and also as a measure to maintain required navigation depth and facilitate sailing in coastal environments (Akcil et al., 2015). However, in the process, it creates a resuspension of buried contaminants and causes increased exposure for the affected biota, also affecting the sediment-associated benthic ecosystem. Studies carried out on pre- and post-dredging sediments at over 20 sites in the U.S. revealed that the outcome of this remediation practice was quite unpredictable. Some sites showed no change or decrease in contaminant concentrations, but others showed increased concentrations (Lofrano et al., 2016). Active mixing involves the mixing of contaminated sediments with natural substrates or other strongly binding inert materials. In the process, both the contaminant bioavailability and movement to the water layer are reduced (Ghosh et al., 2011). Capping consist of in-situ covering of contaminated sediments layer with suitable isolating material such as activated carbon or sand to limit leaching of contaminants to the upper water layer. Several studies have investigated the potential application of different materials for capping. In addition to activated carbon, materials such as organoclay, zeolites, coke, apatite and various others have been tested and among them, apatite and organoclay appear to be most promising (Lofrano et al. 2016; USEPA 2013). Another physicochemical approach identified in the literature involves sediment stabilization and solidification with the addition of specific compounds. This method was proposed as a solution for the disposal of dredged sediments (L. Wang et al., 2015). Different chemical oxidants were also proposed to enhance chemical oxidation of PAHs into less toxic products. Commonly employed oxidants include hydrogen peroxide, ozone, sodium permanganate and sodium persulfate. These oxidants have been successfully applied in several instances for chemical degradation of PAHs. However, the use of chemical oxidants in contaminated sediments is often ex-situ because of the potential detrimental environmental effects (Lofrano et al., 2016; Shih et al., 2016). Generally, physico-chemical approaches are economically costly and do not exclude a risk of secondary contamination (Ghosh et al. 2011). On other hand, bioremediation involves the use of biological agents for the decontamination (and possible complete degradation of hydrocarbon contaminants) of marine sediments, and thus represents a more efficient solution with the reduction of both environmental and economic costs (Jorfi et al., 2014; Kalantary et al., 2014). A number of approaches have been proposed which can be broadly classified under bioaugmentation, biostimulation or a combination of both. Some promising biostimulation approaches proposed include the addition of oxygen, and/or nutrients and microencapsulated nutrients (Chen et al., 2017; Haque and Kwon, 2017;

Kalantary et al., 2014), the addition of biosurfactants (Ammami et al., 2015; Lee et al., 2018; Raddadi et al., 2017), which represent a more environmentally friendly alternative to the use of chemical surfactants. A more recent approach is that of bioelectrochemical system involving inert electrodes buried in the contaminated sediments to enhance bioremediation. The biodegradation process relies on electroactive bacteria able to transfer electrons directly to inert electrodes. Hence, the buried electrode (also referred to as anode) serves as final electron acceptors in the anoxic sediments, and thus enhance the oxidation of the organic contaminants. The anode is connected to a cathode placed in the overlying oxic waters to form an electric circuit (Abbas et al., 2017; Hamdan et al., 2017; Kronenberg et al., 2017; Logan et al., 2006). Another configuration proposed is the oil spill snorkel whereby a single electrode is partly buried in the anoxic sediments and partly in contact with the oxic zone (which represents the overlying water containing dissolved O_2) so as to serve as an electrochemical bridge (Cruz Viggi et al., 2017, 2015). Also different studies propose a mixed approach consisting of a combination of biological and physico-chemical remediation methods. With such extensive research carried out in the field of bioremediation, it is relevant to comprehend how much of these research studies have been translated into field especially as this was found to be scarce in the literature, hence the present review was prepared with that objective.

2. Structure and Scope of the review

The various inventions identified on the subject of PAH contaminated sediment bioremediation were classified under five major broad topics based on the bioremediation approach adopted (as illustrated in Figure 1??):

- Patents focusing on biostimulation
- Patents focusing on bioaugmentation
- Bioremediation integrated with chemical/physical treatments
- Patents on equipment/apparatus for sediment bioremediation
- Patents on bioelectrochemical systems

During the literature review and patent search, the general objective was to identify the patented bioremediation methods applicable to sediments contaminated with PAHs.

Nevertheless, some more generic patents such as those on PAH contaminated soils, reporting to be equally applicable to other environments were also considered, in agreement with (Gan et al., 2009). However not exhaustively, but only when the invention described was considered applicable to sediments. Also, several patents used terms such as crude oil, diesel oil or petroleum hydrocarbon which refer to complex mixtures of hydrocarbon compounds including aliphatic, aromatic, paraffins, asphaltenes, cyclic or polycyclic aromatic in various concentrations. PAH contamination in the field almost always occur together with several other hydrocarbon compounds, in presence of which the bioremediation products especially microbial remediation agents should be able to thrive (An et al., 2017). In this regard, patents which mentioned PAHs among other petroleum compounds were considered or patents using the term heavy oil, petroleum derivatives, and in many cases only total petroleum degradation rates were reported. Furthermore, inventions on bioelectrochemical systems which essentially addressed organic compounds biodegradation were examined as this method is considered a promising approach for in situ bioremediation of PAH contaminated sediments (Kronenberg et al., 2017). In these patents, degradation rates reported are generally in terms of total organic carbon degradation and not PAH specifically. As previously mentioned, bioremediation methods have proved to be more environmentally sustainable and were therefore the main focus. Purely physico-chemical remediation methods were not considered within the scope of this review, as the focus was majorly on microbial remediation related methods. However patents proposing these methods in combination with bioremediation approaches were considered. The patents consulted for this review date from the year 1995 to 2019.

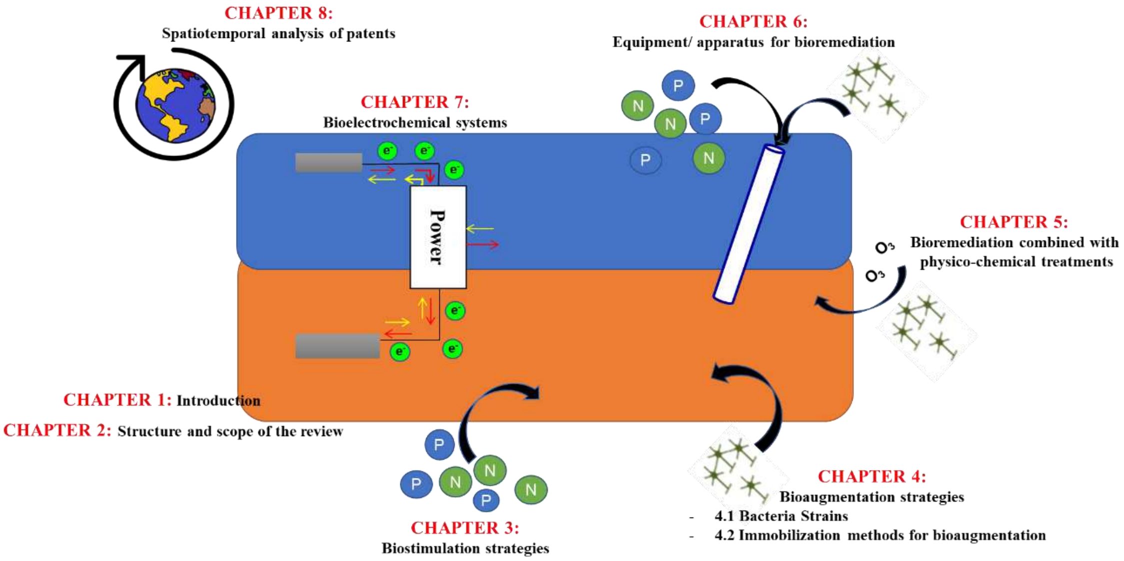

Figure 1: Roadmap of the patent review

3. Biostimulation strategies

Generally, the autochthonous microbial assemblages are considered to be the most efficient for contaminant degradation due to the lack of need to adapt to environmental conditions as in the case of using allochthonous microbes and to avoid their potential harmful effects in the long-term especially when applied in-situ (Mapelli et al., 2017). Several biostimulation agents have been developed for the remediation of PAH contaminated sites, as schematically represented in Figure 2. It should be noted that the nutrients or various other biostimulation agents essential for PAHs degrading microorganisms are also required for microorganisms degrading other petroleum contaminants. Hence, the patents on biostimulation in this section are generally described as applicable for enhanced biodegradation of petroleum products as a whole or sometimes even a wider range of contaminants and will scarcely mention PAHs specifically. Among the exceptions, patent JP2009195821(A) specifically address PAH contaminated sites proposing a method of monitoring PAH concentration through a PAH dioxygenase gene (Fukunaga et al., 2009). Also, in patent WO2008012578(A2), the nutrient mixture is described as efficient to enhance PAH contaminant degradation (Jones et al., 2008). Regarding the environmental matrix, the biostimulation agents in most of the patents identified were prescribed for contaminated soils or water, while sediments are scarcely mentioned. In patent US2008041776(A1) the biostimulation method is described as applicable for both soil and sediments (Wu, 2008). Even though mentioning contaminated soils only, these patents were considered in this review since biostimulation agents enhancing microbial degradation in soils should equally be efficient in sediments.

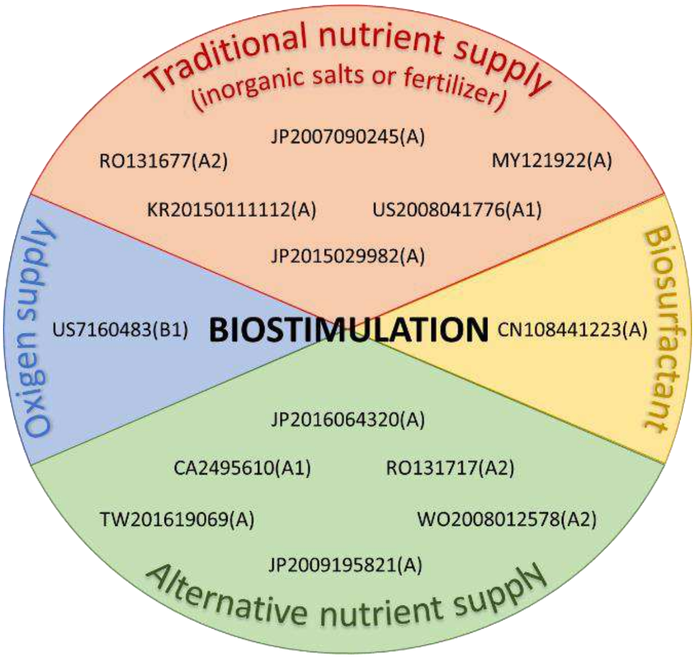

Figure 2: Summary of biostimulation methods

Several biostimulation nutrients have been formulated, mainly consisting of nitrogen and phosphorus based organic and inorganic compounds. Also, some cheap, sustainable and readily available bioproducts such as beech tree leaves, or treated sewage sludge have been proposed~~,~~ as alternative to the use of nitrate and phosphate compounds. Moreover, biostimulation can also be achieved through the addition of compounds that create an aerobic environment (e.g. oxygen, oxides, oxidising agents) and through the addition of biosurfactants to enhance the PAH bioavailability. Hence, all the biostimulation approaches aim at creating optimum conditions for PAH-degrading microbes (Mapelli et al., 2017; Perelo, 2010). Patent US7160483(B1) developed methods for a continuous release of oxygen in contaminated sites and thus enhanced biodegradation activity (Hince 2007). The products described are prepared as granules, tablets, capsules or pellets or other similar forms and can be made to disintegrate upon contact with water. BTEX (Benzene, Toluene, Ethylbenzene and Xylenes) degradation rate was approximately 67% in contaminated groundwater after 52 days. KR20150111112(A) formulated a biostimulating complex specifically for in-situ application in contaminated sediments, made of several nutrients such as nitrates, phosphates, potassium, acetic acid mixed with a biosurfactant and coated with a polymer material (cellulose or polysulfone) (Song and Woo, 2015). This product once introduced in the sediments slowly releases trace amounts of nutrients to sustain a prolonged activity of the degrading microorganisms in situ. The efficiency of this method is measured in terms of how slowly the release of nutrients was in sediments, and dissolution rates ranged between 23 and 43% after 5 days under turbulent conditions. A similar invention was proposed by patent MY121922(A) which consisted of a biostimulating nutrient product for soils capable of continuously releasing nutrients over up to six months at 25°C (Drake 2006). Furthermore, JP2015029982(A) and RO131677(A2) developed nitrogen and phosphorus rich nutrients. JP2015029982(A) proposed an inorganic compound at specific appropriate ratios for contaminated soils. Biodegradation rates were approximately 41% after 14 days with optimum biodegradation results achieved with C:N:P ratio of 100:5:2 (Kawagoe et al., 2015). RO131677(A2) provides a nutritive biostimulation product comprising essential nutrients such as nitrogen, phosphorus pentoxide, potassium oxide, and microelements including iron, copper, zinc, magnesium, manganese, boron, sulphur and organic substances, including humic substances (Burtan et al., 2017). Some patents proposed innovative and readily available nutrient rich substrates such as treated sewage effluent, with which was achieved 40% biodegradation after 82 days thanks to the promotion of the number and growth of naturally occurring microorganisms which accelerates the remediation. In patent CA2495610(A1), a mixture of a kind of distilled liquor called Awamori and a phosphate rich nutrient achieved 80% degradation in contaminated soil after 5 weeks against 20% in the control (JP2016064320 (A)) (Mackrell and Hutlet 2005; Tokashiki et al. 2016). A mixture of beech tree leaves extracts, horse chestnut and seed extracts (RO131717(A2)), a nutritious gel-like biostimulation agent (TW201619069(A)) (Radu and Voina 2017; Chen et al. 2016) were proposed as alternatives. A nutrient control process is described by JP2009195821(A), consisting of monitoring the PAHs concentration in the petroleum-contaminated soil by means of a PAH dioxynase gene and maintaining the concentration of the gene in the soil within a specific optimum range. This is achieved by the addition of nutrients for microbial growth in quantities just enough to maintain that optimum range (Fukunaga et al. 2009). This microbial growth promoter containing alanine and alginate, phosphate and ammonium was applied on a contaminated soil in a field scale experiment, and 77% biodegradation rate was achieved in 71 days, with total petroleum hydrocarbon (TPH) concentration reducing from 1628 mg/kg to 374 mg/kg. The specific objective of this invention was to achieve bioremediation without introducing microorganisms in the contaminated site. Other biostimulation inventions such as patent WO2008012578(A2) proposed mixing the contaminated soil with nutrients and biodiesel and leaving the mixture for up to twelve months. The biodiesel is a nontoxic, diesel equivalent fuel made from vegetable oils or animal fats, and according to the patent, when added to a contaminated soil, it solubilises the PAHs and makes them more bioavailable for microbial degradation. Complete remediation was obtained within two to three months in many cases, and within twelve months in other instances. The use of activated carbon for contaminant immobilisation is proposed in this patent, in cases where some residual fraction of pollutants might be remaining in the biotreated soil (Jones et al., 2008). Patent US2008041776(A1) described a downwards washing process with application of physical force and addition of fertilizers and biostabilizing material (Wu 2008) for bioremediation of PAH contaminated sediments. Using this bioremediation system, complete remediation was obtained in three and a half months in a contaminated field where initial TPH concentrations ranging between 2000-10000 ppm decreased to 0-100 ppm. In patent JP2007090245(A), Matsumoto and Nunji (2007) proposed the addition of 2 to 3% by weight of smoked coal for accelerating microbial growth in contaminated soils together with nitrogen and phosphorus based nutrient salts. According to this patent, petroleum degradation was obtained in six weeks with the application of the Khun charcoal against 14 weeks in the control (Matsumoto and Junji, 2007). Biosurfactants such as a rhamnolipid or a sophorolipid and sodium salt are proposed in patent CN108441223(A) in a biostimulation formulation (Li et al., 2018). The application of this biotreatment resulted in up to 90-98% total petroleum removal in laboratory scale experiments, within 3 hours with constant stirring. Also, this solution was reported to have no secondary pollution effect. Generally, the identified biostimulation methods majorly consisted of the use of nutrients. This is because macronutrients such as nitrogen and phosphorus are key elements for the success of microbial degradation, and several studies have demonstrated that in their absence or low concentrations, bioremediation is limited (Liu et al., 2017). In this review, some cheaper, more sustainable and readily available bioproducts such as beech tree leaves or treated sewage sludge have been proposed, as seen in Figure 2, as alternatives to the use of nitrate and phosphate inorganic salts. Similarly, in the literature, either inorganic nutrients or commercial fertilizers are commonly employed as biostimulation agents (Kalantary et al., 2014; Wick et al., 2011). Furthermore, Yi and Crowley (2007) investigating on more sustainable biostimulation agents, using various plant based nutrients, found that crushed root tissues of celery, radish, potato and carrot were efficient to enhance bioremediation of pyrene contaminated soils within 40 days (Yi and Crowley, 2007). On another hand in some cases, the contaminated matrix is not nutrient deficient and rather require other stimulation agents such as oxygen (or other electron acceptor) or biosurfactants. Oxygen is very often provided in the form of oxygen releasing compounds to enhance the growth of aerobic microbial strains which are generally more efficient than anaerobic strains (Salminen et al., 2004). Biosurfactants, on another hand, are efficient to solubilise the PAHs to enhance their bioavailability. Also, they have been reported to have good emulsification properties and remain stable under various in-situ turbulent conditions. They represent a more sustainable alternative to chemical surfactant, that are more difficult to degrade in the environment and hence represent a potential source of secondary pollution (Marchant and Banat, 2012).

Table 1: Patents on biostimulation methods

Patent Code	Patent Title	Efficiency	Reference
CN108441223(A)	Leaching agent used for restoration of petroleum hydrocarbon polluted soil and preparation and application thereof	Up to 90-98% petroleum removal after 4 hours	(Li et al., 2018)
RO131717(A2)	Biostimulator and process for bioremediation of soils polluted with mineral oils and other xenobiotic products	Not available	(Radu and Voina, 2017)
RO131677(A2)	Nutritive product to be used in bioremediation of soils polluted with petroleum products	Not available	(Burtan et al., 2017)
TW201619069(A)	Method for bioremediation of contaminated soils and contaminated groundwater	Not available	(C.-Y. Chen et al., 2016)
JP2016064320(A)	Bioremediation agent	30% heavy oil degradation after 35 days	(Tokashiki et al., 2016)
JP2015029982(A)	Contaminated soil remediation method	70% oil degradation, after 14 days	(Kawagoe et al., 2015)
KR20150111112(A)	Biostimulating agents for in-situ bioremediation of contaminanted sediment	Not available	(Song and Woo, 2015)
JP2009195821(A)	Bioremediation method	Over 75% petroleum degradation, after 71 days	(Fukunaga et al., 2009)
WO2008012578(A2)	Bioremediation method	Not available	(Jones et al., 2008)
US2008041776(A1)	Downwash process bioremediation system	Not available	(Wu, 2008)
US7160483(B1)	Solid-chemical composition for the non-exothermic chemical oxidation and aerobic bioremediation of environmental contaminant	67% BTEX biodegradation after 52 weeks	(Hince, 2007)
JP2007090245(A)	Method for purifying soil polluted by petroleum-based hydrocarbon	Degradation after 6 weeks	(Matsumoto and Junji, 2007)
MY121922(A)	Bioremediation of hydrocarbon contaminated soils and water.	Not available	(Drake, 2006)
CA2495610(A1)	Method, process, apparatus, and product for remediation of hydrocarbon contamination	34% hydrocarbon degradation after 59 days	(Mackrell and Hutlet, 2005)

4. Bioaugmentation strategies

Bioaugmentation strategies are based on the addition of efficient degrading microbial strains in order to boost PAH biodegradation (Hou et al., 2005; Tyagi et al., 2011). Different studies have demonstrated the potential of specific microbial strains for PAH degradation in environmental matrices including sediments (Mapelli et al., 2017; Mercer and Trevors, 2011; Perelo, 2010). The most used strains include *Pseudomonas* and *Bacillus sps*.; however, some inventions are also based on the use of fungi, yeasts and of less common bacterial strains (Figure 3). The details are reported below, for each group of biological agents, including a section at the end of the chapter dedicated to bioaugmentation assessed on liquid media: Indeed this is considered also relevant for the potential of PAH remediation in contaminated sediments.

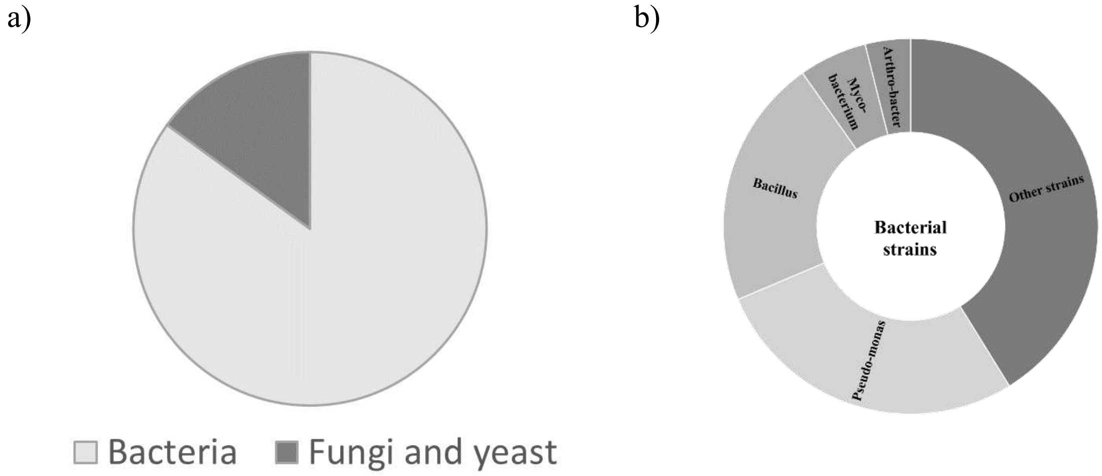

Figure 3: a) Various microorganisms used as bioremediation agents for PAH contaminated soils/sediments; b) Most common bacteria species used for patented microbial remediation preparations for PAH contaminated soils/sediments

4.1 Bacteria Strains

Generally, under the patents describing bioaugmentation approaches, bacteria strains were the most commonly used among microbes for the formulation of bioremediation products as illustrated in Figure 3a. This is most likely due to their highly ubiquitous nature and good adaptation capacity to hostile conditions such as in environments with extreme temperatures, pressure or salinity (Füssel et al., 2017; Ryan et al., 2009). Furthermore, bacteria strain cultivation and conservation methods over long periods have been extensively investigated and several studies and patents can be found on the subject. On the other hand, patents using yeast or fungi strains were relatively scarce, although high biodegradation efficiencies were observed at laboratory scale.

4.1.1 Pseudomonas Strains

Pseudomonas strains have been isolated from various contaminated environments and proposed for remediation of diverse PAH contaminated sites, including sediments (related inventions are reported in Table 2). In some cases, degradation rates in the description of the invention are expressed as TPH and not specifically for the polycyclic aromatics. However, they have been included in this review since the approach is considered potentially effective also for PAH. Patent US2018056346(A1) proposed a microbial bioremediation solution made of at least one strain of *Pseudomonas putida, Pseudomonas fluorescens, Pseudomonas sp. LD2, Pseudomonas aeruginosa*, or any combination of these (Reynolds and Mciver 2018). According to this invention, the microbial mixture constitutes a simple, cheap and efficient bioremediation solution with capacity to degrade a wide range of PAHs.

The TPH degradation rates reported vary between 70-95% in 90 days. This study also proposed a mixture of the microbial solution with either readily degradable surfactants or biosurfactants. Patents AU2016101966(A4) and US2018194654(A1) both used *Pseudomonas* with other strains, such as *Alcaligenes*, *Rhodococcus*, *Bacillus licheniformis, Bacillus subtilis, Bacillus amyloliquefaciens* and *Lactobacillus acidophilus* to formulate microbial remediation products. AU2016101966(A4) obtained 33% TPH degradation in an experiment compared to 5% in the control, after 60 days (Zhiqiang, 2016). The composition by US2018194654(A1),which also contains several enzymatic and nutritive components, was applied in the Kalamazoo River, where an estimate of over 4 million liters of heavy crude oil was spilled, and within minutes of its application it clarified the areas of surface water it contacted (Angel, 2018). CN108795834(A) proposed a genetically engineered *Pseudomonas aeruginosa* strain with no pyocyanin production during metabolism that can be used for large-scale production of rhamnolipid for bioremediation of PAH contaminated sites, with 85% fluorantrene degradation after 9 days (Yanling et al., 2018). Yanling et al. (2018) also proposed microbial- based remediation products using *Pseudomonas* strains were for soil bioremediation; however, several inventions are considered suitable for the sediment treatments as well. Inventions RO132554(A0) and KR20090030897(A) include a microbial formulation based on a mixture of *Pseudomonas* and *Bacillus* strains, that allows the achievement of total petroleum degradation rates of 83% and 44% after 12 weeks and 3.5 weeks, respectively (Micle et al., 2018; Park and Kim, 2009). A mixture of a fast growth strains (including *Pseudomonas*, *Acinetobacter* and *Bacillus*) and a slow growth strain (such as *Nocardia nova*, *Cellulomona sturbata*, and *Flavobacterium*) with the addition of an enzyme expression accelerator was proposed in the invention KR20080046301 (Park et al., 2008). This mixture was applied on contaminated soil with initial crude oil concentration of 8,050 ppm and 44% degradation efficiency was achieved after 31 days, at laboratory scale. A high degradation potential for aromatic hydrocarbons (97% degradation efficiency in 40 days) was demonstrated by a mixture of the three *Pseudomonas* strains: *P. putida* IOC5a11, *P. putida* IOCR11, *P. fulva* IOCSAM11 US2010274069(A1), (Kumar et al., 2010). Several other patents have used *Pseudomonas* strains in combination with diverse methods (An et al., 2017; Hutchings et al., 2007; Yu et al., 2010), and are discussed below.

Table 2: Patents on *Pseudomonas* as remediation agent in bioaugmentation

Patent Number	Title	Efficiency rate	Reference
US2018056346(A1)	Methods for bioremediation of hydrocarbon contaminated media	Up to 70-95% PAH degradation after 90 days	(Reynolds and Mciver, 2018)
US2018194654(A1)	Composition and methods of use	Not available	(Angel, 2018)
RO132554 (A0)	Process for ex-situ bioremediation of hydrocarbon-polluted soils, using *pseudomonas* and *bacillus* micro-organisms	83% total hydrocarbon degradation after 12 weeks	(Micle et al., 2018)
AU2016101966(A4)	A process for the bioremediation of hydrocarbons in contaminated soil or sediment	33% total petroleum degradation after 60 days	(Zhiqiang, 2016)
US2010274069	Method for bioremediation of highly aromatic hydrocarbon wastes	90% PAH degradation after 6 weeks	(Kumar et al., 2010)
KR20090030897(A)	Liquid composition of microorganisms for bioremediation of hydrocarbon-contaminated soil, method of preparing the same, and bioremediation using the same	44% petroleum degradation after 31days	(Park and Kim, 2009)
KR20080046301	Liquid composition of microorganisms for bioremediation of hydrocarbon-	65-70% oil degradation after about 6 weeks	(Park et al., 2008)

	contaminated soil, method of preparing the same, and bioremediation using the same		

4.1.2 Bacilllus Strains

A number of inventions involved the use of *Bacillus* strains (Table 3). In patent WO2019118982(A1), a 72% total petroleum degradation rate was achieved within 12 weeks, in situ, on a contaminated soil using a microbial consortium of various *Bacillus* strains (Penet et al. 2019). In patent RU2266958(C2), full remediation was achieved in contaminated soils after 20-30 days using *Bacillus* strains in consortium with *Athrobacter* and *Zoogloea* Sp. These strains were described as oil destructive (Pugachev et al., 2005). RU2538125(C1) proposed the use of poultry manure with application of probiotics and *Bacillus subtilis* strains for bioremediation of oil contaminated cryogenic soils (Neustroev et al., 2015). A temperature resistant *Geobacillus* bacteria was used by US2014042087(A1) and 95% degradation of benzo(a)pyrene was achieved with contaminated sand after 96 hours, with constant mixing. However, the application of this product requires a sediment preheating step since the bacteria can grow only above 60°C (O'driscoll et al., 2014). On the other hand, *Bacillus mycoides* NS1020 or bacterial strain *Sarcina sp* OA10 isolated from refinery wastewater are involved in the process described in patent PL403931 (Śmigielski et al., 2014).

Table 3: Patents on *Bacillus* remediation agent in bioaugmentation

Patent Number	Title	Efficiency	Reference
WO2019118982(A1)	Microbe combinations for bioremediation and methods of using the same	50-99% PAH degradation after 90-120 days	(Penet et al., 2019)
RO132554(A0)	Process for ex-situ bioremediation of hydrocarbon-polluted soils, using *Pseudomonas* and *Bacillus* micro-organisms	83% total petroleum degradation after 12 weeks	(Micle et al., 2018)
AU2016101966(A4)	A process for the bioremediation of hydrocarbons in contaminated soil or sediment	33% total petroleum degradation after 60 days	(Zhiqiang, 2016)
RU2538125(C1)	Oil-contaminated cryogenic soils bioremediation method	Up to 60% oil degradation after 3 months	(Neustroev et al., 2015)
US2014042087(A1)	Bioremediation of persistent organic pollutants using thermophilic bacteria	95% benzo[a]pyrene (BaP) degradation after 4 days	(O'driscoll et al., 2014)
PL403931(A1)	Method for bioremediation of the land with the contaminated diesel oil	Not available	(Śmigielski et al., 2014)
KR20090030897(A)	Liquid composition of microorganisms for bioremediation of hydrocarbon-contaminated soil, method of preparing the same, and bioremediation using the same	44% total petroleum degradation after 31days	(Park et al., 2008)

RU2266958(C2)	Destructor microorganism strains *Zoogloea Sp.* 14 h, *Arthrobacter Sp.* 13 h, *Arthrobacter Sp.* 15 h, *Bacillus Sp.* 3 h, *Bacillus Sp.* 12 h, useful in remediation of ponds and soils contaminated with petroleum and petroleum product(s), and strain association based on the same	60-70% petroleum degradation rate after 60 days	(Śmigielski et al., 2014)

4.1.3 Other Bacteria Strains

Several other bacterial strains were used for formulation of microbial-based remediation products (Table 4). *Arthrobacter* was active in microbial formulations developed by CN103215204, with ability to grow on phenanthrene, anthracene and naphthalene as sole carbon sources, and capable of over 98% phenanthrene degradation, after about 40 days (Ji et al., 2013). *Ochrobactrum* sp. was proposed by CN108949634(A). This strain was capable of using heavy crude oil with high content of asphalt and resins as sole carbon source, as well as removing petroleum pollutants including PAHs (Chen et al., 2018). TW200606253(A) proposed a microbial mix formula for the enhancement of bioremediation of crude oil contaminated environments (containing both aromatic and aliphatic compounds) (Lin et al. 2006). CN106315868(A) proposed a *Rhodococcus* strain product and achieved 55-100% degradation of PAH in contaminated soil (Ding et al., 2017). In patents US2010216219(A1) and CA2735944(A1), methods for isolation and identification of PAH degrading strains were presented, coupled with in situ autochthonous bioaugmentation in contaminated anoxic sites. Degrading strains are isolated from the contaminated site and grown in a chemostat bioreactor. Efficient anaerobic degrading strains can equally be grown in anoxic conditions and then reinjected in the contaminated site (Hendrickson et al. 2015; 2010). For bioremediation of oil contaminated deep sea environments, CN104403947(A) proposed a method for screening and isolating of barophilic petroleum degrading bacteria (Yang and Wang 2015).

Table 4: Patents on bacterial strains other than *Pseudomonas* and *Bacillus* as remediation agents in bioaugmentation

Patent Number	Title	Efficiency	Reference
CN108949634(A)	Petroleum degrading bacterium capable of degrading heavy crude oil as well as separation method and application of petroleum degrading bacterium	20% total petroleum degradation rate after 7 days	(Chen et al., 2018)
CN106315868(A)	Application of degrading bacterium capable of metabolizing various hydrocarbons to petroleum pollutant disposal	100% PAH degradation after 11 days	(Ding et al., 2017)
US2010216219(A1)	Method of in situ bioremediation of hydrocarbon-contaminated sites using an enriched anaerobic steady state microbial consortium	Not available	(Hendrickson et al., 2015)
CN104403947(A)	Method for treating deep-sea spilled oils by using barophilic bacteria	50-80% petroleum degradation after 30 days	(Yang and Wang, 2015)
CN103215204	*Arthrobacter* strain highly effectively degrading phenanthrene, and application thereof	98% phenanthrene degradation after 40 days	(Ji et al., 2013)

Patent Number	Title	Efficiency	Reference
CA2735944(A1)	Identification, characterization, and application of *Thauera* s*p.* Al9:8 useful in microbially enhanced oil recovery	Not available	(Hendrickson et al., 2010)
TW200606253(A)	Local microbial formulation applied to the hydrocarbon pollutant treatment	Not available	(Lin et al., 2006)

4.2 Yeast and Fungi Strains

As an alternative to bacteria, several inventions include yeast and fungi species (Table 5), sometimes together with their metabolic products, such as biosurfactants, as described in patent WO2018107162(A1) (Adams et al., 2018). The PAH degrading yeast *Candida tropicalis* G-94, isolated from contaminated soil in an oil production area, was used by CN108130291(A) (Ma et al. 2018), and live yeast culture and yeast metabolites with polysaccharides were proposed in a microbial formulation by US2017232490(A1) (Smith, et al., 2017). Other compositions involving a mixture of bacteria with fungi strains were also reported. Indeed, according to CN106244479(A), degradation of the complex and numerous PAH compounds required different strains with various metabolism abilities; hence the introduction of selected degrading bacteria and fungi strains constituted a promising approach, reaching a total petroleum degradation in soil above 90% using a bioremediation mix consisting of bacteria and fungi strains, and a biosurfactant for complete remediation of deep oil contaminated soils (Zhang 2016). CN105170644(A) comprised the addition of biodegrading strains and maintaining soil moisture at 10-30% (Li, 2015). The bioremediation product by MY145799(A) consists of various autochthonous degrading strains mixed with nutrients, surfactants, pH stabilisers (Zakaria and Yusoff, 2012). A mixture of degrading strains is also proposed by DE102005048904(A1), that developed a method for the bioremediation of PAH contaminated abandoned landfills and soils, consisting of a mixture of photosynthetic microorganisms, a luminescent bacteria, a hyphae forming organism, combined with a PAH marker (strigolactones, 5-deoxystrigol) (Uphoff, 2007). Similarly, CN104607460(A) described a multistep soil bioremediation process consisting of crushing, stirring with addition of organic fertilizer and nutrients and addition of selected efficient biodegrading bacteria and fungi strains and surfactants. Constant humidity level is also maintained in the treated soil (Gao and Cao, 2015). WO2018164648(A1) described a successful bioremediation process using a composition made of *Bjerkandera adusta* and *Trametes versicolor* fungi strains following nutrients addition in the contaminated soils and mixture with clean forest soil (Aydin et al., 2018). The optimum C:N:P ratio in this formulation was found to be 100:15:1 and the highest efficiency in total petroleum biodegradation was 36%.

Table 5: Patents on yeast and fungi as remediation agents in bioaugmentation

Patent Number	Title	Efficiency	Reference
WO2018107162(A1)	Microbial products and their use in bioremediation and to remove paraffin and other contaminating substances from oil and gas production and processing equipment	Not available	(Adams et al., 2018)
WO2018164648(A1)	Method for bioremediation of petroleum-contaminated soils	36% total petroleum biodegradation after 60 days	(Aydin et al., 2018)

US2017232490(A1)	Bioremediation enhancing agents and methods of use	over 95% TPH biodegradation after 50 days	(Smith et al., 2017)
CN106244479(A)	Composite inoculant for deep oil contaminated soil bioremediation process	over 90% petroleum hydrocarbons biodegradation after some weeks	(Zhang, 2016)
CN105855287(A)	Method for ex-situ remediation of organic contaminated soil by virtue of sodium percarbonate-biological pile technique	over 90% phenanthrene, naphthalene, anthracene and pyrene biodegradation rates after 36 days	(JIang et al., 2016)
CN104607460(A)	Bioremediation method of organic matter contaminated soil	Up to 90% degradation in benzene and aniline after 60 days	(Gao and Cao, 2015)
CN105170644(A)	Bioremediation method for petroleum-polluted soil	96% TPH biodegradation after 3 weeks	(Li, 2015)
MY145799(A)	In situ bioremediation of contaminated site	Not available	(Zakaria and Yusoff, 2012)
DE102005048904(A1)	Bioremediation of abandoned landfills and contaminated sites in soil, comprises inserting PAH marker for labeling site in medium and hyphae-forming organism combined with material for bioremediation into medium	Not available	(Uphoff, 2007)

4.3 Bioaugmentation on liquid media

A number of patents deal with bioaugmentation assessed on contaminated liquid media only, very often on minimal media with PAH compounds as the sole carbon source. The assumption made in these patents is that PAH degrading strains that are efficient in such contaminated minimal medium can also be efficient in other solid matrices like soils or sediments. Such patents, which specifically target PAH contaminated water environments are also included in this review, since the microbial strains used equally have a great potential for contaminated sediment remediation (Table 6).

These include a bioremediation product proposed by CN102533589(A) with *Pseudomonas aeruginosa,* able to enhance PAH degradation (Zhengshun et al., 2012). Fluorantrene and phenantrene degradation efficiency of 83% in an inorganic salt medium were obtained with 2% (w/v) initial petroleum concentrations by Zhengshun et al. (2012). Similarly, other microbial formulation containing *Pseudomonas* strains with high degradation rates were proposed in patent CN104388352(A) that reported a complete napthalene degradation after only 4 days of incubation (Yan et al., 2015). The invention in RU2272071(C2) is based on a mixture *Saccaromyces* and *Pseudomonas* that can provide a 50% naphthalene and anthracene degradation in 20 days. *Achromobacter* sp. strain W143 showed rapid degradation to phenanthrene, naphthalene, fluorene, fluoranthene and other organic pollutants. In patents CN104099266(A) and CN104046580, approximately 95% phenanthrene degradation was achieved in mineral salt medium with initial phenanthrene concentration of 500 mg/L and sole carbon source (Chen et al., 2014b, 2014a) Bioremediation formulations with *Mycobacterium gilvum* were proposed by KR20010073276(A), CN104946568 and CN101423806(A) for polycyclic aromatic hydrocarbon degradation with PAH degradation rates around 98%, after 9 days culture in a liquid medium. KR20010073276(A) obtained over 80% pyrene degradation in 28 days in minimal salt medium containing initial 500 mg/L of pyrene (Ahn and Lee, 2001; Cai et al., 2015; Zhencheng et al., 2009a). Less common bacteria strains were also used to formulate microbial remediation preparations with high degradation efficiencies, such as described in CN102120976(A) using *Sphingobium yanoikuyae* (Ji et al., 2011). A phenanthrene degradation rate above 90% was reported after 4 days incubation in inorganic salt media, at laboratory scale. Patents CN105695360(A) and CN103013841(A) proposed microbial remediation products using respectively *Acinetobacter* and *Microbacterium* strains for one and *Scopulariopsis brevicaulis* strains for the other and obtained 80% phenanthrene degradation in 18 days and 60-80% degradation of phenanthrene, anthracene, fluoranthene and benzopyrene after 6 weeks (Li et al., 2016; Mao et al., 2013). Similarly, US2011129902(A1) developed a formulation containing *Alteromonas* strains highly efficient for naphthalene and phenanthrene degradation (Jeon et al., 2011). A radiation-resistant *Deinococcus actinosclerus* was proposed by CN109504618(A) in a microbial product which enhanced by 50% phenanthrene degradation within 7 days in inorganic medium containing 100 mg/L of phenanthrene (Liang and Xiong, 2019). CN102732429(A) invention was based on the use of *Mucor* sp. strain with degradation efficiencies up to 90%, after 10 days, against approximately 26% in controls for phenanthrene, anthracene and benzo[a]pyrene (Peidong et al., 2012).

Table 6: Patents on bioaugmentation methods on liquid media

Patent Number	Title	Efficiency rate	Reference
CN109504618(A)	Radiation-resistant D*einococcus actinosclerus* capable of efficiently decomposing estrogen and PAHs	50% phenanthrene degradation, after 7 days	(Liang and Xiong, 2019)
CN108130291(A)	Petroleum aromatic hydrocarbon degradation yeast	70% PAHs degradation after 30 days	(Ma et al., 2018)
CN108795834(A)	Attenuated *Pseudomonas aeruginosa* genetically engineered bacteria	85% fluoranthene degradation after 9 days	(Yanling et al., 2018)
CN105695360(A)	Phenanthrene-degrading strain *Acinetobacter tandoii* LJ-5 and application thereof	80% phenanthrene degradation after 18 days	(Li et al., 2016)
CN104946568	Heavy metal resisting polycyclic aromatic hydrocarbon degrading bacteria	Up to 97% PAH degradation after 9 days	(Cai et al., 2015)
CN104388352(A)	*Pseudomonas stutzeri* capable of simultaneously degrading chlorpyrifos and naphthalene	100% naphthalene degradation after 4 days	(Yan et al., 2015)
CN104046580	*Sphingobacterium* strain for degrading polycyclic aromatic hydrocarbon organic pollutant	95% phenanthrene degradation, after 4 days	(Chen et al., 2014a)
CN104099266(A)	*Achromobacter* sp. strain for degradation of polycyclic aromatic hydrocarbon organic pollutants	95% phenanthrene degradation after 4 days	(Chen et al., 2014b)
CN103013841(A)	*Scopulariopsis brevicaulis* and application thereof	80% PAH biodegradation after 42 weeks	(Mao et al., 2013)
CN102732429(A)	Polycyclic aromatic hydrocarbon-degrading bacteria	Over 90% PAH degradation after 9 days	(Peidong et al., 2012)
CN102533589(A)	*Pseudomonas aeruginosa* and application thereof	83% PHA biodegradation. after 7 days	(Zhengshun et al., 2012)
US2011129902(A1)	Microbial strain *Alteromonas sp*. SN2 for degrading polycyclic aromatic hydrocarbon	Complete degradation of naphthalene and phenanthrene after 2 weeks	(Jeon et al., 2011)
CN102120976(A)	*Sphingobium yanoikuyae* and application thereof in degrading polycyclic aromatic hydrocarbon	92% phenanthrene degradation after 4 days	(Ji et al., 2011)

CN101423806(A)	Organic aromatic compound degrading bacteria	99% phenanthrene degradation after 7 days	(Zhencheng et al., 2009a)
RU2272071(C2)	*Saccaromyces sp.* and *Pseudomonas sp.*, uses thereof as decomposers in bioremediation	50% naphthalene and anthracene degradation after 20 days	(Andreeva et al., 2005)
KR20010073276(A)	High molecular weight polycyclic aromatic hydrocarbon degrading bacteria for bioremediation	80% PAHs degradation in 28 days	(Ahn and Lee, 2001)

4.4 General considerations on bioaugmentation strategies

The analysis reported above (summarised in Figure 4) has evidenced that the most used strains for bioaugmentation approaches are *Pseudomonas* and *Bacillus.* Both can live in PAH contaminated environments. Indeed, many *Pseudomonas* species have been isolated from diverse contaminated sources and some *Bacillus* species have been found to be able to withstand extreme temperatures, making them promising candidates for bioremediation of extreme environments. Furthermore, *Pseudomonas* can also be exploited for the ability to produce rhamnolipid biosurfactants, which favour the solubilisation of the hydrophobic and recalcitrant PAH compounds and thus increasing bioavailability for microbial degradation (Guansheng Liu et al., 2018; Randhawa et al., 2014). Other commonly strains used are *Arthrobacter* and *Mycobacterium,* which give a satisfactory performance on PAH degradation. In general, as expected, relatively high degradation rates are observed at laboratory scale with liquid contaminated media. However, under more real conditions using soil or sediment matrices, the biodegradation rates are relatively low, with the process taking quite a long time. Generally, these various patents on microbial remediation correlate well with the literature, where similar strains including *Pseudomonas*, *Mycobacterium*, *Bacillus* or *Arthrobacter* are commonly referred to as efficient degrading strains for recalcitrant compounds including PAHs (Tyagi et al. 2011; Wick et al. 2011; Lang et al. 2016). Also similarly to literature, *Pseudomonas* strains are considered the most promising strains (Fulekar, 2017).

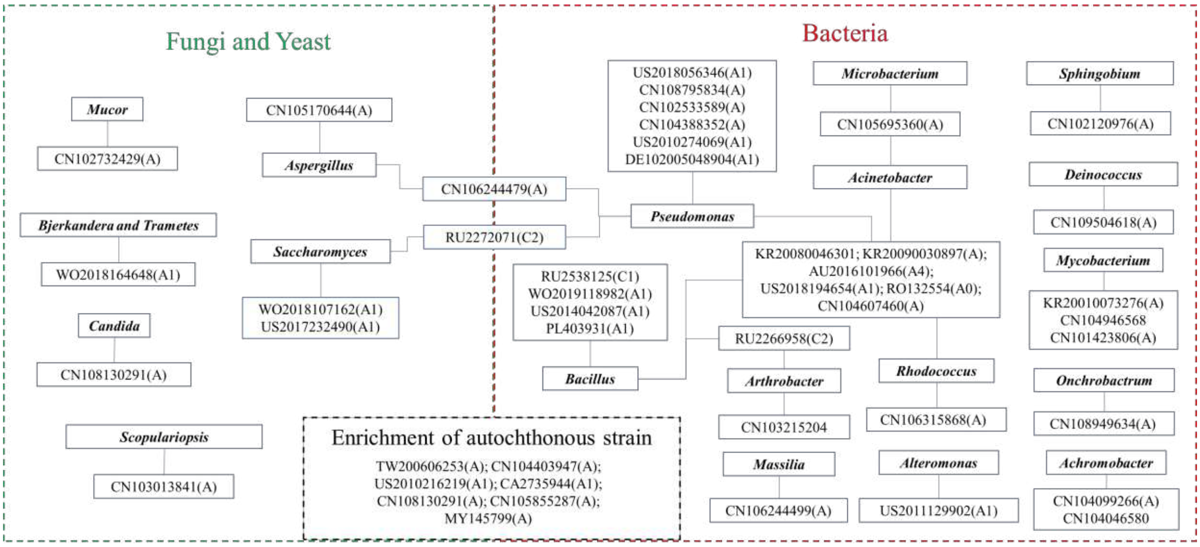

Figure 4. Summary of bioaugmentation methods

4.5 Immobilization methods for bioaugmentation

Bioaugmentation approaches have proven to be efficient, economic, sustainable and versatile alternatives to most physicochemical remediation treatments (Xu and Lu, 2010). However, their efficiency can be limited under the less predictable and often turbulent field conditions. To both improve the microorganisms survival and ensure the stability of the bioremediation agents in the field and thus optimize their contact time with contaminants, immobilization methods have been developed consisting of embedded microbial preparations and nutrients in an environmentally friendly solid material (Bayat et al., 2015). In this regard, several inventions (listed in Table 7) on various immobilization methods were identified, making use of cheaply available materials (see Figure 5). The immobilization substrate used promotes the microbe action, because it protects the cells from natural competition with soil microflora and the possible toxicity due to environmental conditions (Wang et al., 2019; Yang et al., 2018). In this regard, patents CN107151663(A) and CN101717725 described the use of low cost and environment friendly materials. In the first case, the kelp residues, combined with kaolin and an adhesive, served both as PAH adsorbent and bacteria immobilising agent. The immobilizing material protects the bacteria allowing the growth in polluted area for a long time, accelerating the PAH biodegradation. The second patent used a material composed of diatomite, kaolin, peat, and chitin, suitable for several strains, also for synergistic action of different microbes (Yu et al., 2010). Alternatively, CN107129940(A) included the immobilization of *Pseudomonas sp.* and *Alcaligenaceae sp*. consortia in a beef paste peptone medium (An et al., 2017). The use of corncob was reported in patents CN108212109, CN101423807(A) and CN104312944(A) (Dang et al., 2015; Yang and Yang, 2018; Zhencheng et al., 2009b). This material is a farmland waste, often cause of environmental pollution due to burning straws and haze. Its use, in agreement with the circular economy principles, could increase the economic income of farmers with an additional ecological advantage. Furthermore, the corn cob is rich in nutrients, with a high content of nitrogen, phosphorus and potassium (Yang and Yang 2018). The invention CN108212109 immobilized fungi, bacteria (*Bacillus*) and chain microbes (*actinomycetes*). More in detail, the genus *Microbacterium* releases enzymes for the fermentation reaction, and the macromolecule is initially decomposed into small molecules. *Bacillus* genus adsorbs molecules and performs multi-fermentation/respiration such as ion exchange. The temperature increases up to 60°C, and pathogenic bacteria and bacteria are eliminated. The chain microbes have a further action of decomposing, with a final degradation around 90%, after 3 weeks (Yang and Yang, 2018). Comparable degradation efficiencies were reportedd in the other two inventions, which immobolized zymotic fluid of *Mycobacterium sp.* strain (CN101423807 (A)) and a mix of *Burkholderia cepacia*, *Sphingomonas sp.* and *Pandoraea pnomenusa* (CN104312944) (Dang et al., 2015; Zhencheng et al., 2009b). The addition of charcoal was proposed in patent GB2431926(A), with the introduction of a bioremediation agent consisting of a charred biological material inoculated with suitable biodegrading strains including bacteria and fungi species. According to this invention, charcoal constitutes an inert and protective material which promote the microorganisms colonization with final density levels between 100 and 1000 times greater than a soil (Hutchings et al., 2007). Other immobilising matrices included polyvinyl alcohol (PVA), vermiculite and polyurethane in patent WO9508513(A1) (Lin et al., 1995), with degradation efficiency of 60% againts 40% in the non immobilised experiments. Comparable materials were described in patents CN103611508, CN103613208, and CN102408160 for the production of adsorption balls (the firstone) and plates (the second and the third inventions) (Dou et al., 2014b, 2014a, 2012). The substrates were composed of NH_4HCO_3, $Ca(NO_3)_2$, $(NH_4)_2HPO_4$, urea water solution, ethylenediaminetetraacetic acid, calcium sodium solution, poly beta-hydroxybutyrate chloroform and a polyethylene glycol chloroform. A physical process including a freezing step produced the structures, suitable for the culture adsorption with over 91% degradation efficiency (Dou et al., 2014a). The immobilized microsphere has the advantages of nutrition, protection, slow release, high activity and stability, environmental protection and safety. Alginate material was used in patents CN102199589(A), CN103849615(A), CN103320346(A) and US2018243716(A1) (Fan et al., 2013; Hongqi et al., 2011; Xu et al., 2014; Yang et al., 2018), with degradation rates above 80%. In the first three inventions the resulting substate had a ball structure which allowed a more easy transport than bacteria liquid from the production facility to the affected site. Furthermore, the spherical shape can sink to the sea bottom for the treatment of deep sites (Fan et al., 2013). On the other hand, the fourth patent described the use of calcium alginate and chitosan to encapsulate the bacterial broth in a microcapsule. The environmentally safe and biocompatible material proved to be able to maintain efficient degradation performance at different environmental conditions, with a high adaptability level. The invention reported a pyrene biodegradation rate over 81% within 40 days in an experiment on PAH contaminated soils using these microcapsules, against 42% using free non immobilised bacteria. Alginate material, combined with polyvinyl acetate as immobilized carriers, was also used in patent CN103849615(A) which included a bacterium symbiotic system between physiological functions of algae and bacteria. This system aimed at solving many limits of bacteria action. Indeed, the algae photosynthesis supply the oxygen necessary for bacteria respiration (Xu et al., 2014). Furthermore, in the absence of nitrogen, algae can fix it from the atmosphere, providing both nitrogen and organic matter to improve the bacteria growth. At the same time, the symbiotic system provides favorable conditions for algae growth. The CO_2 from bacterial respiration can be utilized by algae as well as the organic matter resulting from bacteria degradation action. An additional advantage is the possible preliminary enrich of pollutants in algae cells. The symbiosis advantage is due to the providing of both energy and nutrients to each other, with degradation rates between 37-79% using different combination of algae and strains. A biodegradable plastic consisting of polylactic acid in fibrous form was proposed in patent KR20170016223(A) obtaining a degradation efficiency of 73%. The immobilizing system allows a log-term storage (up to 1 year at 4°C) (Lee et al., 2017). On the other hand, Soda et al. (2016) proposed in patent JP2016077176 (A) an incapsulation by acrylate oligomer materials with a final product suitable to be placed on the contaminated sediment (Soda et al., 2016). In order to solve the criticality of low temperature which could affect the degradation efficiency, intention RU2565549(C2) proposed a product designed for cold regions. The biopreparation system included a solid substrate and a consortium of microorganisms immobilized on its surface, including *Bacillus vallismoris*, *Exguobacterium mexicanum*, *Serratia plymuthica*, *Rhodococcus sp.*, and the mineral nutrient for bacteria (Erofeevskaja and Gljaznetsova, 2015).

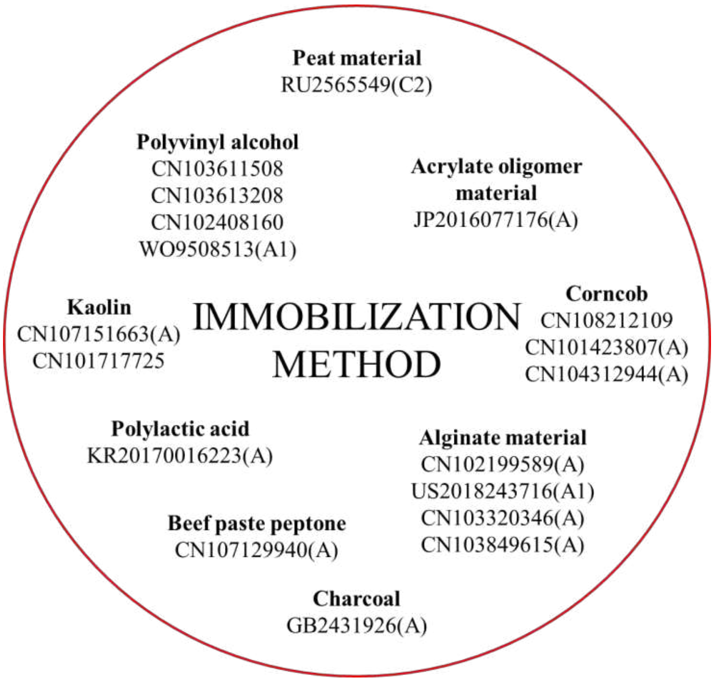

Figure 5: Summary of immobilization methods in bioaugmentation

The immobilization method represents a promising approach for bioremediation as confirmed by the patents described in this section. This method addresses one common challenge faced during field applications, which is the limited contact between the biodegrading agent, nutrients and the target contaminant. In the literature, several studies demonstrated higher bioremediation efficiency rates compared to free cells (Bayat et al., 2015; Jin et al., 2017; Liu et al., 2015). The main reason is the possibility to maintain the high biomass of bacterial populations, one of the key points of bioremediation techniques. Furthermore, this technique ensures the longest operating lifetime and enhance both the stability and survival of the cells (Bayat et al., 2015; Cassidy et al., 1996). Overall, the main characteristics of a good immobilization material should be the environmentally friendly composition, the resistance to toxic contaminants and its capability to provide favorable conditions for the bioremediation agents (Bayat et al., 2015).

Table 7: Patents on immobilisation methods in bioaugmentation

Patent Number	Patent Title	Efficiency	Reference
CN108212109	Oil pollution collecting adsorbent, using method and application of recycling product thereof	95% oil degradation after 1 week	(Yang and Yang, 2018)
US2018243716(A1)	Microcapsule material capable of reducing pollution containing polycyclic aromatic hydrocarbon, and preparation method and application thereof	Over 81% pyrene degradation after 40 days	(Yang et al., 2018)
KR20170016223(A)	The composition for contaminated soil remediation and in-situ bioremediation method using the same	73% total petroleum degradation after 42 days	(Lee et al., 2017)

Patent Number	Patent Title	Efficiency	Reference
CN107151663(A)	Petroleum pollution remediation immobilized microbial inoculum prepared from kelp residues	40% total petroleum degradation after 21 days	(Zheng et al., 2017)
CN107129940(A)	Multiple microorganisms for repairing petroleum-contaminated soil, fungicide, culturing and immobilization method and application	Over 90% total petroleum degradation after 4 weeks	(An et al., 2017)
JP2016077176(A)	Environmental clean-up capsule including environmental pollutant-degrading microorganisms	Not available	(Soda et al., 2016)
CN104312944(A)	Microbial preparation, and preparation method and application thereof	98% petroleum removal after 5 days	(Dang et al., 2015)
RU2565549(C2)	Biopreparation for bioremediation of oil-contaminated soils for climatic conditions of far north	87-88% total petroleum degradation after 60 days	(Erofeevskaja and Gljaznetsova, 2015)
CN103611508	Copolymer adsorption ball with enhanced biodegradation function against various organic pollutants and preparation process thereof	62% total petroleum degradation after 150 days	(Dou et al., 2014b)
CN103849615(A)	Immobilized algal-bacterial symbiotic system for processing petroleum pollutants and application thereof	Up to 79% total petroleum degradation after 7 days	(Xu et al., 2014)
CN103320346(A)	Method for screening and immobilizing efficient petroleum hydrocarbon degradation flora	45% TPH degradation after 30 days	(Fan et al., 2013)
CN102199589(A)	Immobilized microspheres for remediation of petroleum contaminated soil, preparation method thereof and application thereof	Up to 90% total petroleum degradation after 30 days	Hongqiet al. 2011
CN102408160	Biodegradable perforated plate for adsorption and biodegradation of petroleum hydrocarbon pollutants as well as its preparation method	More than 95% petroleum hydrocarbons degradation after 35 days	(Dou et al., 2012)
CN101717725	Microbial inoculum for bioremediating coastline polluted by oil spilling and preparation method thereof	Not available	(Yu et al., 2010)

Patent Number	Patent Title	Efficiency	Reference
CN101423807(A)	Method for preparing polycyclic aromatic hydrocarbon degrading bacteria	95% and 91% phenanthrene and anthracene respective degradation	(Zhencheng et al., 2009b)
GB2431926 (A)	Charred biological material carrying microbes	Not available	(Hutchings et al., 2007)
WO9508513 (A1)	Biocomposite comprising a microorganism and an additive in a formulation matrix for bioremediation and pollution control	60% phenanthrene degradation after 20 days	(Lin et al., 1995)

5. Bioremediation combined with physico-chemical treatments

Bioremediation have been generally accepted as more environmentally and economically sustainable. However, very often under field conditions, they present several limitations such as low predictability and long degradation periods. This is mainly due to the complex, hydrophobic and hardly biodegradable structure of PAH compounds, as well as their weathered state (in the numerous cases of long-term pollution) rendering them even less bioavailable (Gan et al., 2009; Gou et al., 2020; Perelo, 2010; Sutton et al., 2011). To help overcome this issue, various inventions have proposed the integration of physico-chemical methods to convert these complex organic compounds into more easily biodegradable products and/or enhance their low bioavailability. In this regard, patent CN105855287(A) proposed a method for ex-situ remediation by a percarbonate-biological pile technique (JIang et al., 2016) for PAH contaminated soils with high concentrations of phenanthrene, naphthalene, anthracene and pyrene (over 2,300 mg/kg of each contaminant). The treatment allows a pre-oxidation of the contaminated soil pile thanks to the action of a sodium percarbonate solution mutually complemented. The integration of the chemical agent with the biological treatment is translated into the rapid pollutant oxidation and the efficient degradation of the resistant PAH. Overall, the invention is suitable for large scale applications and shows high efficiency (over 90% after three cycles), short bioremediation time, low cost and the avoided production of secondary pollution. The use of a reducing agent was included also in patent CN108746201 (A), which described a multi step bioremediation process including an aerobic step, in the presence of zero-valent iron powder or a pyrite powder, followed by an anaerobic stage. The invetion included a mechanical stirring, able to ensure no dead corner, to avoid the production of hazardous by-products during the remediation (Zhu et al., 2018). Zhu et al. (2018) reported a 67-74% degradation of benzo(a)pyrene after 3 months. Iron was also proposed in patent CN105363771(A) as well as a preliminary hydrogen peroxide treatment to serve as an oxidative agent to enhance the subsequent bioremediation. During the treatment, able to combine the chemical effect with a biostimulation approach, a large quantity of soluble nutrition can be released by the contaminated soil, avoiding the cost due to the necessary nutrient substances. The degradation rates reached up to 67%, after 80 days, thanks to the addition of soluble iron which further enhanced the bioavailability of dissolved nutrients present in the contaminated soil (Xu et al., 2016). A different approach is described in patent US2018361444(A1), where the physical aspects played a key role. The invention provided a bioremediation formula combining microbes and surfactants with positive effect on both efficiency and time. Indeed, the surfactants action produced small spherical suspensions of contaminants promoting the bioremediation mechanisms. An additional co-absorbent material holds the by-products for the further degradation by microbes. Another peculiarity of the formula was a low impact abrasive ingredient to physically promote the contaminant removal (Franssen and Franssen, 2018). On another hand, a purely physical treatment (without any chemical agent) with microbial remediation was proposed in patent IE20160132(A1), consisting of an ex-situ bioremediation method where the contaminated soil was moved to a bioremediation facility having a series of heated bays for the soil treatment. The contaminants were degraded by a microbial cleaner containing a microbial blend consisting of microorganisms such as *Pseudomonas* and *Bacillus* (Mcdonnell, 2016).

Table 8: Patents involving bioremediation integrated with physico-chemical methods

Patent Number	Patent Title	Efficiency	Reference
US2018361444(A1)	Compositions and methods for cleaning contaminated solids and liquids	Not available	(Franssen and Franssen, 2018)
CN108746201 (A)	Bioremediation method of organic matter contaminated soil	67-74% degradation of benzo(a) pyrene after 3 months	(Zhu et al., 2018)
IE20160132(A1)	A remediation process	Not available	(Mcdonnell, 2016)
CN105363771 (A)	Method capable of facilitating bioremediation of oil-contaminated soil	Up to 67% TPH degradation after 80 days	(Xu et al., 2016)
CN105855287 (A)	Method for ex-situ remediation of organic contaminated soil by virtue of sodium percarbonate-biological pile technique	90% phenol degradation after 36 days	(JIang et al., 2016)

The integration of physico-chemical methods with bioremediation represents a most promising approach as illustrated in the section (Gan et al., 2009; Gou et al., 2020; Maletić et al., 2019). Similarly, literature proposed many techniques addressed to solve the PAHs tendency to bind and persist in the sediments, often including the use of strong chemical agents (such as H_2O_2, ozone, permanganate, etc.) or physical pre-treatments (i.e. heating, mixing, crushing). Various combinations of these approaches were widely studied (T. Chen et al., 2016; Gill et al., 2014; Mahony et al., 2006; Samsami et al., 2011; Zou et al., 2016). The physico-chemical remediation of PAH contaminated sediments which aims at completely mineralising the contaminants solely via chemical methods remains a common practice nowadays. Nevertheless, their use as pre-treatment integrated with bioremediation strategies, seems to be the most sustainable and a cost-saving alternative in many cases (T. Chen et al., 2016).

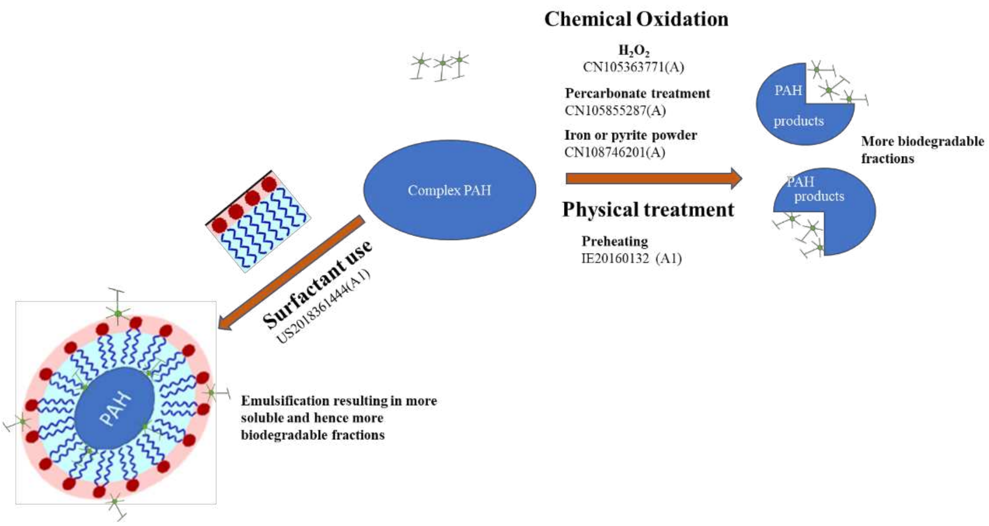

Figure 6: Summary of bioremediation methods combined with physico-chemical treatments

6. Equipment and apparatus for bioremediation

Several environmental aspects influence the effectiveness of the bioremediation, such as temperature, humidity and oxygenation, affecting the microorganism number and the biological enzyme activity. Therefore, bioremediation technologies for contaminated sediments also include several equipment and devices developed to further assist and enhance the efficiency of biostimulation and/or bioaugmentation methods, as summarized in Table 8. These process setups ensure an optimum exposure and contact time between the contaminant and the bioremediation agent. Some patents were developed for the treatment of different matrices. In other cases, the preliminary operations of dredging and dewatering are necessary to make soil technologies suitable for marine sediments too. A biological plug directly insertable in a contaminated site is described in patent US2011207204(A1) specifically for in situ application in contaminated soils and sediments. It can have various possible configurations to convey selected microbial compositions for bioremediation into the affected sites (Mack, 2011). This bioplug provide oxygen, air and/or other gases, nutrients, and a porous immobilization surface on which selected organisms can grow. Injection apparatus are also proposed in patents JP2014205112(A) and KR101433257(B1) by Shimada et al. (2014) and Kim et al. (2014), which described an uniform distribution of bioremediation products in contaminated matrix (Kim et al., 2014; Shimada et al., 2014). On the other hand, patents CN205165344(U), CN108500058(A) and CN204638709(U) provided soil bioremediation devices which enables for good stirring, enhancing contact between microorganisms and the contaminated matrix and greatly increasing the biodegradation process (Guo et al., 2018; Li et al., 2015; Zhen et al., 2016). The first bioreactor is equipped with both microwave heating and a vertical mixing system, and a water spray nozzle controls the humidity. The reactor in the invention CN108500058(A) shows a horizontal rotating structure (Guo et al., 2018). Also, Chen et al. (2018) in patent CN207386156(U) developped a litlle and easy to operate rotating soil bioremediation apparatus. The equipment comprises a collecting plate and a mixing device so as to increase the degradation rate of organic contaminants (Chen Kan et al., 2018). A completely different setup was described in patent CN207308565(U), which system includes sequential sections: a soil feeding box, a pyrolysis tank, a bioremediation tank, a homogenizer, a cleaning tank, and a drying box (Guang Liu et al., 2018). The device proposed in patent CN105036354(A) involves the immobilization of slow-release solid nutrients and granular bacteria on a simple support composed of plastic and two wire meshes (Xia et al., 2015) and Li et al.

(2015). Patents CN206779142 and TW201513948 were developed for soil, nevertheless, after assuming the preliminary dredging and dewatering steps, they could be considered suitable also for sediments (Shimada et al., 2015; Zhong et al., 2017). More in detail, authors described the possibility of the contaminated matrix circulation by a system of feeder apparatus and pumps. The whole setup includes both the air injection and liquid nutrient dosing devices. On another hand, inventions CN206591127(U) and US2016017373(A1) aimed at solving a problem of bioremediation approaches (mainly in open water pollution cases): the availability of large quantity of microorganisms (Ganti, 2016; Zhao, 2017). Zhao (2017) in the first invention developped an equipment for microbial inoculum preparation which consist of a cell body, a sterilisation apparatus (UV lamp), a temperature regulating device, an agitating unit and air blast device. According to the description, the equipment has a simple structure, is convenient for the preparation of microorganisms which are efficient for bioremediation. Similarly, in patent US2016017373(A1), Ganti (2016) proposed a method for large scale production and storage of bioremediation microorganisms in which the bioremediation product has a typical shelf life of 2 years or more.

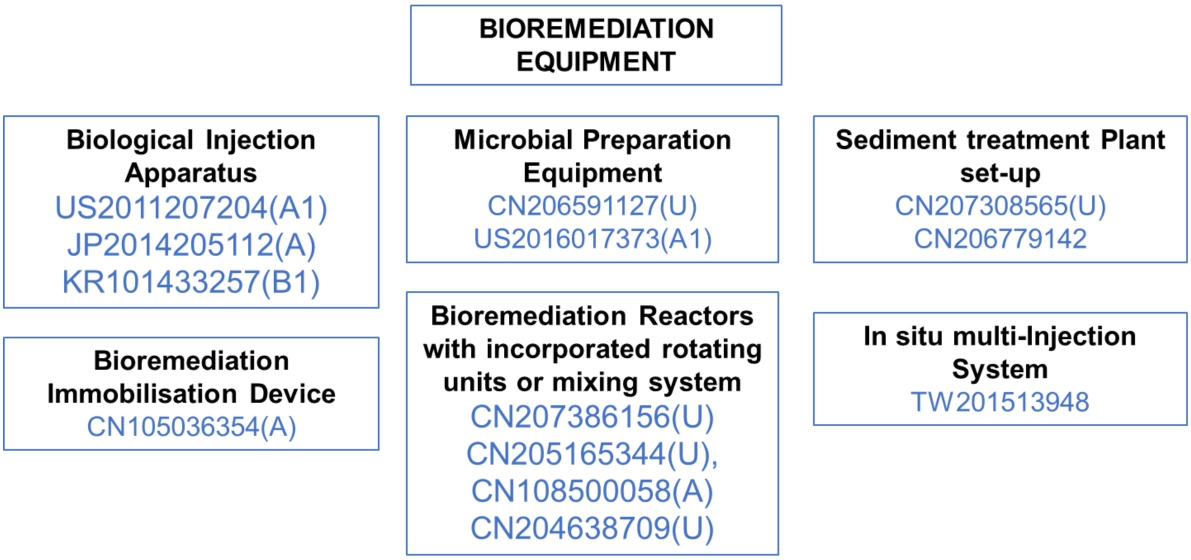

Figure 7: Patented bioremediation equipment and apparatus

Table 9 : Bioremediation Apparatus

Patent Number	Patent Title	Reference
CN207386156(U)	Little bioremediation polluted soil earth device	(Chen Kan et al., 2018)
CN108500058(A)	Sealed thermal insulation type rotary bioreactor	(Guo et al., 2018)
CN207308565(U)	Repair equipment of polluted soil earth	(Guang Liu et al., 2018)
CN206591127(U)	Microorganism solid microbial inoculum preparation facilities	(Zhao, 2017)
CN206779142	bioremediation device to be applied to circulation of organic contaminated site soil	(Zhong et al., 2017)
US2016017373(A1)	Bulk availability and production of bioremediation products	(Ganti, 2016)

CN205165344(U)	Little bioremediation pollutes device of soil	(Zhen et al., 2016)
TW201513948(A)	In situ purification method utilizing multi-point injection	(Shimada et al., 2015)
CN105036354(A)	Device for bioremediation of petroleum-polluted sand beach	(Shimada et al., 2015)
CN204638709(U)	Prosthetic devices of contaminated soil	(Li et al., 2015)
KR101433257(B1)	Spray injection apparatus and method of gas and liquid mixtures containing microbial growth substrate and microbes for in situ bioremediation of contaminated ground water	(Kim et al., 2014)
JP2014205112(A)	Original-position decontamination method by multi-point injection	(Shimada et al., 2014)
US2011207204(A1)	Method and apparatus for bioremediation of soils and sediments	(Mack, 2011)

7. Bioelectrochemical systems

Bioelectrochemical approaches have also been proposed for the bioremediation of contaminated sediments (Table 10). In this regard, the terms sediment or benthic microbial fuel cell (SMFC or BMFC) are generally used to describe bioelectrochemical devices that use electro-active bacteria present in the contaminated environment to oxidise the organic contaminant and in the process generate electricity (Logan et al., 2006). SMFC systems typically consist of anodes (electrodes buried in the sediments and serving as electron acceptor for organic contaminant oxidation by microbes) and cathodes (electrodes in contact with aerobic surface) (Bass et al., 2007; Santoro et al., 2017; Wang et al., 2014). This approach identifies the ocean as a favorable environment thanks to the strong conductivity of the seawater (50,000 μS/cm vs 500 μS/cm of freshwater) (Lines and Kestin, 2004). Therefore, it is suitable for in-situ remediation and it could avoid the criticalities connected to the dredging activities (J. Wang et al., 2015). Although SMCF has good application perspectives, existing systems usually have low conductivity and often require large current intensities. The power loss is relevant and the selection of both the design and the choice of anode materials and structures play an essential role on the decontamination effect (J. Wang et al., 2015). As described in the patents below, the anode modification can significantly improve its conductivity.

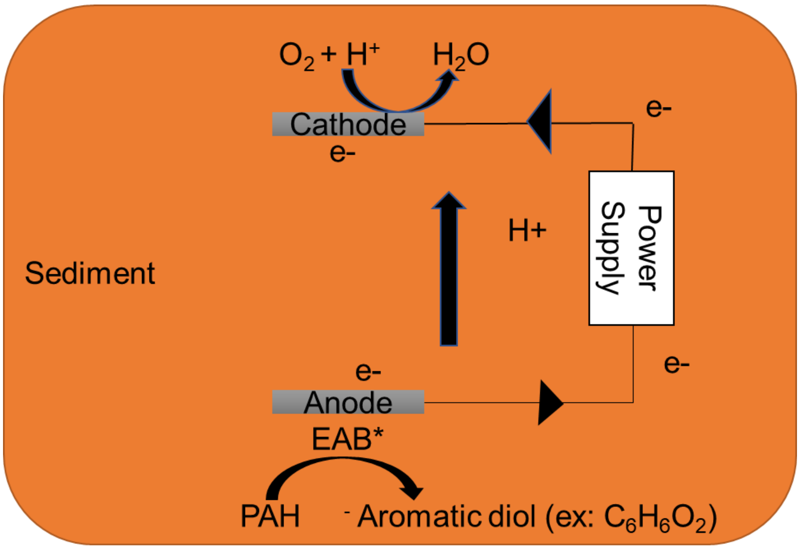

Figure 8: Schematic representation of bioelectrochemical systems

The system described by Huang and Chang (2018) in patent CN108996661(A) modified the traditional carbon felt anode material using iron oxide nanomaterial, by a brushing method, for improving the efficiency of SMFC for oxidation of organic matter. According to this low-cost invention, the anode modified SMFC was able to improve total organic matter removal by 15% compared to the non-modified anode, and by 35% compared to natural degradation (Huang and Chang, 2018). On the other hand, Wang J. (2015) proposed in patent CN104466201(A) an anode modification method which involves the polishing of the surface of the graphite using abrasive paper and coating with Mn, Fe and Ni materials (J. Wang et al., 2015). The efficiency of this apparatus was measured in terms of the grown conductivity of the modified anode, the anode microbial kinetic activity was improved, the extracellular electron transport rate was accelerated, and the microorganism action increased. A SMFC device described in patent CN106299431(A) included an ion exchange membrane and an aeration system to increase the dissolved oxygen at the cathode, with the consequent decrease of the internal resistance and improving the efficiency so as to be able to adapt to various complicated water conditions. The whole system, adaptable to the complicated water conditions, allows to obtain a total organic matter degradation of 31%, after one month (Li et al., 2017). The oxygenation represents a priority also in patent CN104787900(A), which proposed a SMFC device with an automatic control aeration system which can be powered by the energy produced by the device. One major limitation of these systems is their inefficiency to operate at greater depths and their usage is often limited to shallow water sediments such as in rivers and lakes (Li and Li, 2015). To overcome this criticality, US9660286(B1) mainly focused on the development of a SMFC which may be deployed at a water-sediment interface utilizing a cable, without the need for burying the anode under the seabed. This system does not require the divers work during the deployment on the ocean floor with a relevant decrease of the costs (Mcneilly, 2017). The inventions CN103224313(A) and US9484589(B1) included additional agitation system to further improve the degradation efficiency (Hsu et al., 2016; Zhao et al., 2013). Nevertheless, the incorporated devices were only suitable for a scarce depth. On the other hand, patent CN106898804(A) described a device with adjustable movable cathode plates able to follow variable water surface locations. This equipment can be used for application in sea sediments with adjustment of the cathode according to the depth of the water (Yang et al., 2017). Other inventions also proposed various anode and/or cathode materials, configurations to enhance the efficiency of the system such as the three electrode microbial fuel system proposed in US2012064416(A1) by Chang et al. (2017). More in detail, the method includes an anode placed in the sediment, on the bottom of a contaminated water zone, an intermediate electrode acting as an anode or a cathode and placed in water, and a floating electrode acting as a cathode and placed adjacent to a water surface (Chang and An, 2012). The second electrode acts as anode or cathode depending on the contamination level in sediments, to optimise the degradation and electricity production output. Multy electode setups were proposed in patents CN103401009(A), with multi-anode SMFC and US2018097237(A1) with a multi-cathode unit (Chadwick et al., 2018; Yang et al., 2013). Other inventions in this field have been proposed such as US2012276418(A1), where MFCs can be connected in series or in parallel to obtain better effects; WO2012082670(A2) which described a durable low cost equipment, US8430601(B1) with a simple installation equipment, without divers support (Chadwick et al., 2013; Chang and An, 2012; Nealson and He, 2010; Tender, 2012; Zhou et al., 2012).

Generally, patents on electrochemical methods specifically applied to organic contaminants are relatively few, since this kind of methods mainly address metal contamination (Birger et al., 1995). Hence, this is a relatively new technology and several challenges are still to be overcame to arrive at successful field-scale implementation. The possible improvements are confirmed by the scientific literature which has extensively investigated this method with relatively high bioremediation outputs obtained in laboratory scale experiments although mostly localised around the anode (Adelaja et al., 2017; Bach et al., 2005; Jiang et al., 2014; Kronenberg et al., 2017; Morris and Jin, 2012; Zhou et al., 2012). Furthermore, electrokinetic remediation which involves current intensities application represents an alternative to enhance the efficiency of bioremediation. This method addresses the major limitations of biostimulation, increasing the contaminant mobilisation and bioavailability. An electrokinetic approach allows the homogenous mixing of nutrients, biosurfactants or various electron acceptors (Ammami et al., 2015; Gill et al., 2014; Hongwen et al., 2012a) in the contaminated sediments. This method was proposed in patents CN102652955(A) and CN102463254(A) (Hongwen et al., 2012b, 2012a). The first invention had the purpose of overcoming the criticalities of low solubility, strong adsorption capacity and low degradation of some pollutants, during the bioremediation implementation. The described technique combined the use of a surfactant with an electric remediation with high efficiencies and a relevant speedup of degradation (Hongwen et al., 2012b). The setup of the equipment described in patent CN102463254(A) allows an effectiveness distribution of both nutrients and microorganisms into the remediation area, and it solves the problem of the possible pH change in the matrix, obtaining efficiencies by 55% (Hongwen et al., 2012a).

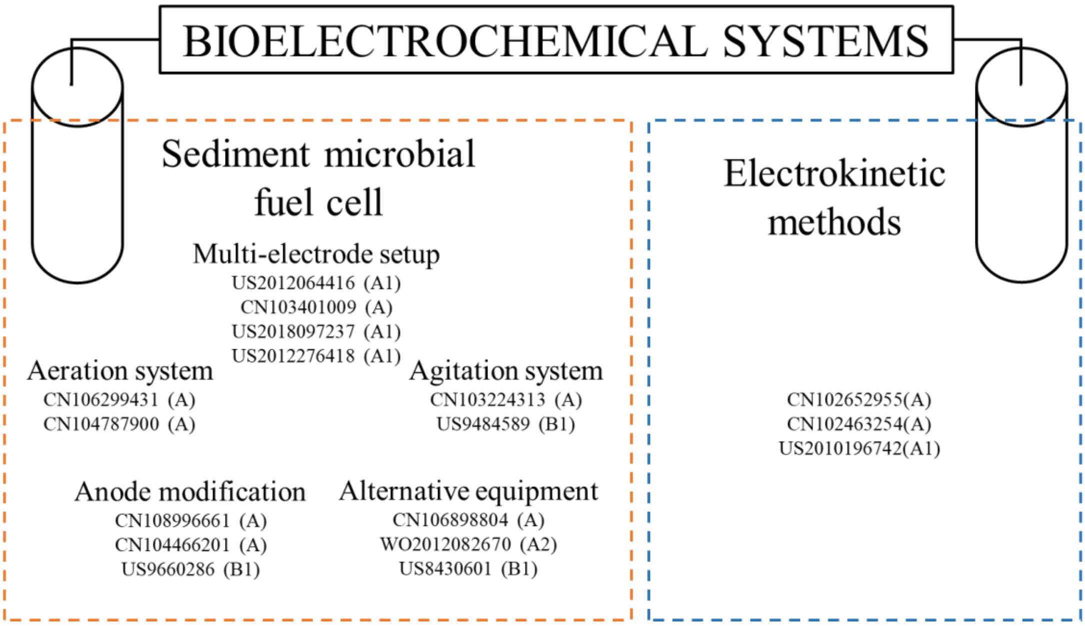

Figure 9: Patented bioelectrochemical systems Electrokinetic methods is really very vague like it is written. Please revise and at least add "e.g." before the patent numbers!

Table 10: Patents dealing with bioelectrochemical systems

Patent Number	Patent Title	Efficiency	Reference
US2018097237(A1)	System and method for improving power production in linear array benthic microbial fuel cells	Not available	(Chadwick et al., 2018)
CN108996661(A)	Method for improving degradation performance of sediment microbial fuel cell	41% and 76% removal rates of TOC and DOC resp. after 50 days	(Huang and Chang, 2018)
CN106898804(A)	Sediment microbial fuel cell with adjustable movable pole plates	Not available	(Yang et al., 2017)

CN106299431(A)	Electrochemical device for in-situ remediation of river channel bottom mud and method thereof	31% organic matter degradation after 1 month	(Li et al., 2017)
US9484589(B1)	Microbial fuel cell with sediment agitator	Not available	(J. Wang et al., 2015)
CN104466201(A)	Preparation method of modified anode of marine sediment microbial fuel cell	Not available	(J. Wang et al., 2015)
CN104787900(A)	Automatic control aeration system for strengthening both water and sediment purification based on electrochemistry	Not available	(Li and Li, 2015)
US9660286(B1)	Method for assembly of a microbial fuel cell	Not available	(Mcneilly, 2017)
US8430601(B1)	System for deploying fabric cloth in marine sediment	Not available	(Chadwick et al., 2013)
CN103224313(A)	Sediment in-situ treatment device and method utilizing microbial fuel cell	Not available	(Zhao et al., 2013)
CN103401009(A)	Sediment type microbial fuel cell	Not available	(Yang et al., 2013)
US2012064416(A1)	Three electrode type of microbial fuel cell and a method for operating the same	Not available	(Chang and An, 2012)
CN102652955(A)	Novel process and technology for strengthening electric remediation and bioremediation of polycyclic aromatic hydrocarbon contaminated soil by using surfactant	70% of phenanthrene and pyrene resp. degradation after 40 days	(Hongwen et al., 2012b)
CN102463254(A)	Novel electrokinetic intensified technology and process for biologically remedying polycyclic aromatic hydrocarbon polluted soil	80% pyrene degradation after 70 days	(Hongwen et al., 2012a)
WO2012082670(A2)	Advanced apparatus for generating electrical power from aquatic sediment/water interfaces	Not available	(Tender, 2012)
US2012276418(A1)	Methods and devices for in-situ treatment of sediment simultaneous with microbial electricity generation	36.2% and 94.4% removal of COD and acid volatile sulphides, respectively	(Zhou et al., 2012)
US2010196742(A1)	Electricity generation using phototrophic microbial fuel cells	Not available	(Nealson and He, 2010)

8. Spatiotemporal analysis of patents

The study of the technological innovation change is essential to integrate the scientific literature for a complete overview of a specific topic. As confirmed by the reviews present in the literature, the patent state-of-art (an excellent index of the worldwide technological activity) allows the identification of all the inventions developed for the scale-up (Amato et al., 2020; Amato and Beolchini, 2018; Archibugi and Planta, 1996; Rocchetti et al., 2018). A further focus on the patent topics and their origin (Figure 8) was useful to better understand the emerging technologies on PAH biodegradation methods and the most active countries.

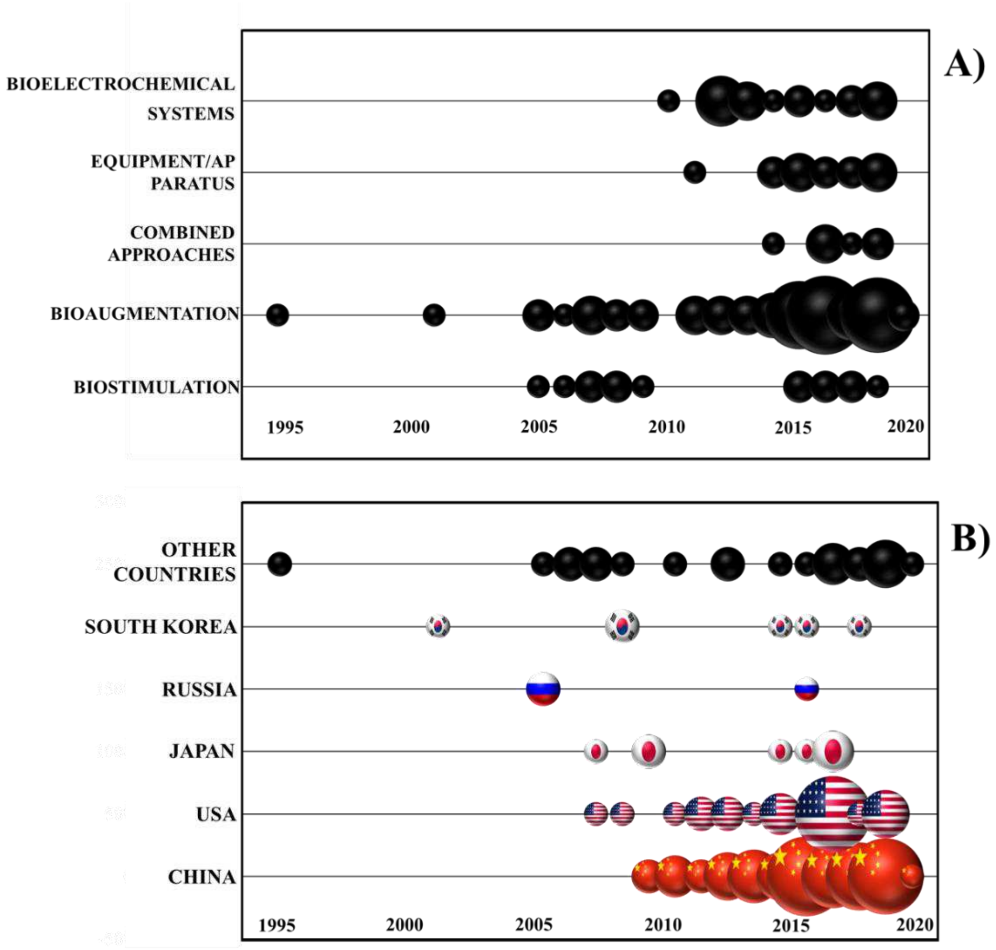

Figure 8: Trend in a) patented bioremediation approaches; a) per country overtime

As showed in Figure 8a), bioaugmentation methods are the most investigated with various microbial remediation products developed overtime as efficient PAH degrading strains are being isolated and tested. In this regard, research has evolved mainly from the use of individual strains to the application of microbial consortia consisting of diverse combinations of bacteria, fungi and/or yeast strains. Among these inventions, about 30% involved immobilization methods, able to increase the degradation efficiency using environmental friendly materials, often enhancing agriculture waste, in agreement with the circular economy principles. On the other hand, biostimulation have been less investigated with major innovations consisting of natural nutrient rich organic substrate many of which are cheaply available. Patents related to bioelectrochemical system and bioremediation devices are more recent, often developed for in-situ treatments, aiming at the increasing of degradation yield. Regarding, the spatial distribution of these inventions, USA and China are the major players in the development of bioremediation technologies, and together account for over 65% of all the patents investigated in this study. This data is due to the high industrialization levels of both countries and the consequent need of efficient and low impact technologies to apply in case of necessity. Overall, the numerous patented technologies described in the present review provide evidence of the considerable research advances made to address the limitations in the application of in situ or large-scale bioremediation of PAH contaminated sediments. With an estimate of 100-200 million cubic metres of dredged sediments in Europe every year (Akcil et al., 2015), the relevance of the present technological innovation change overview is evident. Nevertheless, in addition to robust field testing of promising bioremediation approaches, environmental regulations should largely contribute to promote the real application of the innovative bioremediation methods by a rigorous environmental regulatory framework on sediment bioremediation.

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CHAPTER 2

Assessing the efficiency and eco-sustainability of bioremediation strategies for the reclamation of highly contaminated marine sediments

This chapter is a version of a paper with the same title published in the Marine Environmental Research Journal in 2020 by Antonio Dell'Anno, Francesca Beolchini, Cinzia Corinaldesi, Alessia Amato, Alessandro Becci, Eugenio Rastelli, Melanie Hekeu, Francesco Regoli, Enrico Astarita, S.Greco, L. Musco, Roberto Danovaro

1.Introduction

Environmental contamination by polycyclic aromatic hydrocarbons (PAHs) and metals is reaching a global dimension and represents a serious risk for the sustainable provision of ecosystems' goods and services and for human wellbeing (Johnston, 2015). Chemical contamination can impair biological components at different trophic levels, from microbes to top predators (Gupta, 2019 and citations therein). Recent findings highlighted the presence of approximately 2.5 million potentially contaminated sites, only in Europe, two third of which polluted by hydrocarbons and metals (van Liedekerke et al., 2014). Marine sediments of coastal areas characterized by high anthropogenic inputs and low hydrodynamic regimes can accumulate large amounts of PAHs and metals and require efficient remediation solutions (Bandowe et al., 2014;Das and Das, 2015;Sun et al., 2018). Sediment removal by dredging is commonly used to maintain navigation depths and can be also used as a clean-up solution to reduce ecological hazards determined by chemical contamination (Akcil et al., 2015). The management of dredged contaminated sediments all over the world is mainly based on landfill disposal and/or confined aquatic disposal (Bortone et al., 2004). However, these strategies have several disadvantages, such as limited space capacity, high cost, and low environmental sustainability and compatibility (Rulkens, 2005; Akcil et al., 2015;Lofrano et al., 2017). There is an urgent need for strategies that can reduce contaminant concentrations of dredged materials below threshold levels to allow their safe re-use. Bioremediation is an environmental-friendly strategy gaining increasing attention for its potential to clean-up hydrocarbon contaminated marine sediments (Akcil et al., 2015;Beolchini et al., 2010; Dell'Anno et al., 2012; Head et al., 2006; Chen et al., 2017; Kronenberg et al., 2017). Several field and laboratory experiments demonstrated that the biodegradation of hydrocarbons in the sediments can be accelerated through the addition of inorganic nutrients and/or different electron acceptors/donors able to stimulate the autochthonous microbial assemblages (Atlas, 1995; Head and Swannell, 1999; Kasai et al., 2002; Head et al., 2006; Atlas and Bragg, 2009; Dell'Anno et al., 2012; Kalantary et al., 2014). In this regard, microbial fuel cell-based strategies employing solid-state electrodes as electron donors or acceptors have been recently proposed to stimulate biodegradation processes in contaminated sediments (Cruz Viggi et al., 2015, 2017; Kronenberg et al., 2017; Nastro et al., 2019). However, PAHs in aged contaminated sediments can be recalcitrant to biodegradation due to adsorption to sediment particles, which reduces availability for microbial degradation (Dandie et al., 2010). Therefore, optimization of bioremediation strategies can be needed to reclaim historically contaminated sediments.

Also bioaugmentation strategies, based on the addition of specific microbial taxa to enhance biodegradation processes, can be effective for the remediation of sediment contaminated with hydrocarbons (Jacques et al., 2008; Yu et al., 2005), but to date only a few bacterial or fungal taxa have been tested (Dell'Anno et al., 2018). Among them, fungi belonging to the *Aspergillus* genus have been previously reported to efficiently degrade hydrocarbons (Li et al., 2009; Wu et al., 2009; Ye et al., 2011; Haritash and Kaushik, 2016).

Although bioremediation can be effective for removing hydrocarbons, this strategy could induce important changes in the partitioning, mobility and bioavailability of heavy metals in the sediment, possibly increasing environmental risk (Dell'Anno et al., 2009; Rocchetti et al., 2012; Fonti et al., 2015). Therefore, an accurate risk analysis should be conducted to assess the contextual effects of the biotreatments on sediments characterized by mixed chemical contamination (due to the presence of both organic and inorganic contaminants).

The Bagnoli-Coroglio bay, in the Gulf of Naples (Tyrrhenian Sea, Mediterranean Sea), is a typical example of coastal area chronically contaminated by PAHs and metals, which have been released for decades by industrial activities and stopped at the beginning of nineties (Arienzo et al., 2017; Romano et al., 2004, 2008; Trifuoggi et al., 2017). Here, PAH concentrations can be three to four orders of magnitude higher than those reported from several marine benthic systems worldwide (Arienzo et al., 2017; de Vivo and Lima, 2018).

In this study we tested the efficiency of different bioremediation approaches for PAH degradation on marine contaminated sediments collected in the Bagnoli-Coroglio bay by comparing: i) biostimulation (by the addition of inorganic nutrients), ii) bioaugmentation (by the addition of fungi belonging to *Aspergillus* sp., previously isolated from the Bagnoli sediment) and iii) microbial fuel cell-based strategies. At the same time, we investigated the effects of biotreatments on heavy metal partitioning, coupled with a life cycle analysis of the potential environmental sustainability of *ex situ* biotreatments for sediment re-use vs. current options for the disposal of contaminated dredged sediments.

2.Materials and methods

2.1.Study area and sediment sampling

The study area of Bagnoli-Coroglio, located within the Gulf of Naples (Southern Tyrrhenian Sea), is listed among the Italian contaminated Sites of National Interest (SIN) for the high levels of chemical contamination due to industrial activities. Such industrial activities in the Bagnoli-Coroglio area, mainly represented by a steel plant using fossil coal, iron and limestone, began in 1905 and ended at the beginning of nineties when the plant was dismissed (De Vivo and Lima, 2008; Qu et al., 2018). Previous investigations carried out in marine sediments in front of the plant report very high concentrations of different metals and PAHs due to the industrial activities (Albanese et al., 2010; Arienzo et al., 2017; De Vivo and Lima, 2008; Romano et al., 2017, 2009; Trifuoggi et al., 2017). Sediment samples (from the sediment surface down to 50 cm depth) were collected in November 2017 in front of the industrial plant using a vibro-corer. After collection, sediment samples were homogenised, and sub-samples were collected for the analysis of polycyclic aromatic hydrocarbon and heavy metal concentrations as well as of metals associated with different geo-chemical phases.

2.2.Bioremediation experiments

Three different bioremediation strategies were compared: i) biostimulation, based on inorganic nutrient addition, ii) bioaugmentation with fungal strains, and iii) bio-electrochemical stimulation based on the use of solid-state electrodes.

The biostimulation experiments were performed in 500mL Erlenmeyer flasks containing 40g wet sediment samples and 200mL pre-filtered 0.2µm seawater. Replicate microcosms (n=3) were added with $(NH_4)_2SO_4$ and K_2HPO_4(final concentrations of 50 and 5mM of nitrogen and phosphorus, respectively). The final concentrations of N and P were defined on the basis of the organic carbon content in the sediment according to a molar C:N:P ratio of 100:10:1 (Beolchini et al., 2010). Additional microcosms (n=3) prepared in the same way, but without inorganic nutrient addition, were used as controls. All flasks were incubated at a constant temperature of 20°C in a shaking incubator (Stuart, orbital incubator SI50) at a speed of 120rpm for 4 weeks. During the incubation, the small loss of water due to evaporation (overall <4%) was compensated by supplying weekly 2mL of pre-filtered 0.2µm seawater in all microcosms. Thus, the effects of changes in salinity due to seawater addition were likely negligible. Subsamples were collected for prokaryotic counts (2.5 mL of sediment slurry) and PAH determinations (15mL of sediment slurry) at the beginning of the experiment (time 0) and after 1, 2 and 4 weeks. Additional sub-samples (5 mL of sediment slurry) were collected for the determination of fungal biomass at the beginning (time 0) and at the end of the experiments (4 weeks).

Bioaugmentation experiments were also performed in 500mL Erlenmeyer flasks containing 40g wet sediment samples and 200mL pre-filtered 0.2µm seawater. Replicate microcosms (n=3) were supplied with 10% glucose, 1% yeast extract, 0.25% $(NH_4)_2SO_4$ and 0.15% K_2HPO_4 and 5 mg of fungal spores belonging to *Aspergillus* sp. Previously isolated and characterized from Bagnoli-Coroglio sediments (see below for details). Additional microcosms (n=3) prepared in the same way, but without addition of fungi, were used as controls. All flasks were incubated at a constant temperature of 20 C in a shaking incubator (Stuart, orbital incubator SI50) at a speed of 120rpm for 4 weeks. Sub-samples were collected for the determination of fungal biomass, PAH and heavy metal concentrations and metals associated with different geo-chemical phases at the beginning (time 0) and at the end of the experiment (4 weeks).

For the bio-electrochemical stimulation, a dual chamber microbial fuel cell system was realised: the anode consisted of a graphite electrode (10cm length, 0.9cm diameter) inserted vertically into 250g of sediments mixed with 100mL pre-filtered 0.2µm seawater; the cathode consisted of a graphite electrode with the same characteristics of the anode, inserted into 500mL of a 2M NaCl solution. The experiment was started by supplying oxygen to the cathode, and sediment sub-samples were collected after 1, 4 and 6 weeks for the determination of PAH concentrations. and metal concentrations as well as metals associated with different geo-chemical phases. At the end of the experiment (6 weeks), heavy metal concentrations and metals associated with different geo-chemical phases were also determined.

2.3.Microbiological analyses

For the determination of the prokaryotic abundance, sediment slurries (2.5mL) were treated with pyrophosphate (5mM final concentration) and ultrasound (three times for 1min; Danovaro et al., 2002). For prokaryotic counts, sub-samples (1mL) were diluted 50–100 times, stained with Acridine Orange (0.01% final concentration) and filtered on black Nuclepore 0.2µm filters. Filters were

analysed under epifluorescence microscopy using a Zeiss Axioplan microscope equipped with a 50-W lamp. Ten to 50 fields were viewed at 1000×magnification and a minimum of 400cells were counted. Prokaryotic counts were normalized to sediment dry weight after desiccation (60 C, 24h).
To detect and quantify fungi in the sediment samples, fluorescence in-situ hybridisation (FISH) coupled with Calcofluor white staining (which targets chitin, cellulose and carboxylated polysaccharides) have been used following procedures previously described (Barone et al., 2018; Bochdansky et al., 2017). Samples were observed under epifluorescence microscopy, and the length and width of fungal-like structures were measured. The average width and cumulative length of each identified fungal structure were converted to a cylinder with halfspheres at ends, and the biovolume was converted into fungal biomass according to the following equation (Menden-Deuer and Lessard, 2000):
(1) Biomass (pg C)=$0.216\times\text{biovolume}^{0.811}$
where the biovolume is expressed in μm^3.

Fungi used for the bioaugmentation experiment were isolated from sediment sub-samples by using the dilution plating technique using marine agar added with 0.3mg mL^{-1} rifampicin to prevent bacterial contamination. The resulting fungal colonies were identified by amplification and sequencing of the 18 S rRNA using ns1 (5′-GTAGTCATATGCTTGTCTC) and ns2 (5′-GGCTGCTGGCACCAGACTTGC) primers and of the ITS gene using fungal-specific ITS 1 F (5′-TCCGTAGGTGAACCTGCGG) and ITS 4 (5′-TCCTCCGCTTATTGATATGC) primers (White et al., 1990; Nilsson et al., 2019). Based on the taxonomic identification of the fungal strains followed by experiments testing their ability to grow in media containing PAH and heavy metals, we selected for the bioaugmentation experiment one strain belonging to the *Aspergillus* genus, which has been previously reported to efficiently degrade hydrocarbons (Wu et al., 2009; Passarini et al., 2011; Mukherjee, 2016).

2.4. Polycyclic aromatic hydrocarbons (PAHs) and heavy metals

PAH determination was carried out by high performance liquid chromatography with fluorometric detection (HPLC PerkinElmer Series 200), according to 3545A EPA method (US EPA, 2007; Beolchini et al., 2010). Nineteen different congeners were analysed including: naphthalene, acenaphthylene, 1-methylnaphthalene, 2- methylnaphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, 7,12-dimethylbenzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(ah)anthracene, benzo(ghi)perylene, indeno (123cd)pyrene.
Heavy-metal content in the sediment was determined after acid digestion, as follows: dried sediment sub-samples were transferred in Teflon boxes, added with 5mL fluoridric acid and 1mL of ‘‘aqua regia” (i.e. $HCl:HNO_3=3:1$) incubated for 90 min at 150 C. At the end of the incubation period, 5 mL of 10% boric acid were added and the obtained extracts were analysed by atomic absorption spectrophotometry and by inductively coupled plasma-atomic emission spectrometry. Metals distribution in different mineralogical fractions were determined by means of a selective extraction procedure, which utilized, sequentially, specific chemical reagents to extract heavy metals associated with different geo-chemical phases (Quevauviller, 1998). Four different fractions are considered: (i) the exchangeable and carbonate bound fractions (hereafter defined as exchangeable fraction), extracted utilizing 0.11M acetic acid, pH 2.8; (ii) iron and manganese oxides fraction (i.e., reducible

fraction), extracted with 0.1M NH_2OH, pH 2; (iii) organic and sulfide fraction (i.e., oxidizable fraction), extracted by hydrogen peroxide 30% and treated with ammonium acetate at pH 2, and (iv) the residual fraction, that remains in the residual solid, is determined by difference with the total metal content. Based on the ratio between the concentration of each pollutant in the sediment and that indicated by the sediment quality guideline (Ratio to Reference), the chemical hazard (LOE-1) of the sediment has been assessed. The reference limits used in the present study were those indicated by the SQG-L2 of the Italian decree for determining quality class and management options for dredged marine sediments (Ministro dell'Ambiente e della Tutela del Territorio e del Mare, 2016). The Ratio to Reference (RTR) was corrected by a factor (w) which depend on the typology of chemicals (i.e. non priority w=1, priority w=1.1, priority and hazardous pollutants w=1.3). In the calculation of the specific Hazard Quotient (HQ_C), an average RTRw is obtained for all of the parameters with RTR ≤1 (i.e. values below the SQG), while for those with RTR >1, the RTRw are individually added into the summation Σ:

$$HQc = \frac{\sum_{j-1}^{N} \mathrm{RT\ R}w\ (\mathrm{j})\mathrm{RT\ R}(\mathrm{j}) \leq \mathbf{1}}{N} + \sum_{k-1}^{M} RT\ Rw\ (k)RTR(k) > 1$$

The values of HQ_C are assigned to one of six classes of chemical hazard, absent, negligible, slight, moderate, major and severe depending on the number, typology and magnitude of exceeding chemicals (Regoli et al., 2019).

2.5. Environmental sustainability assessment

To assess the environmental sustainability in terms of carbon footprint of three possible management scenarios of the contaminated sediments from the Bagnoli-Coroglio area, we used the LCA tool, in compliance with the LCA ISO standard 14040 and 14044:2006 (ISO 14044:2006 *Environmental management - Life cycle assessment - Requirements and guidelines*, 2006, UNI EN ISO 14040: 2006. *Environmental management - life cycle assessment - principles and framework.*, 2006). In particular, the environmental sustainability of the most effective bioremediation strategy (i.e., biostimulation approach, see results for details) was compared to conventional management practices of the contaminated sediments (Fig. 1). Based on the extent of contamination of the study area, we considered 100,000 tons of sediments as representative functional unit for such assessment. The GaBi software-System and Database for Life Cycle Engineering (compilation 7.3.3.153; DB version 6.115) were used to obtain data on energy and raw material production processes (i.e., phosphate, ammonium) and the quantification of the environmental loads for the three scenarios. The Product Environmental Footprint (PEF) method was selected for the carbon footprint assessment. The considered system boundaries do not take into account the sediment transportation after dredging, since it is common to the three different scenarios identified on the basis of three different management options of the dredged sediments. More specifically, scenario 1 included the disposal in landfill site, after a preliminary stabilization step of the sediment. A 45km distance from the site was assumed and a representative truck with a maximum payload of 24.7 tons was considered to estimate the CO_2-equivalent emissions. Scenario 2 considered sediment transportation far 10km by a bulk carrier shore (with a payload of 124 tons) to a confined aquatic disposal site located in the Naples port. Scenario 3 was the scale-up of the biostimulation approach investigated in the present study. In this scenario the dredged sediment was treated in a slurry bioreactor, installed on-site in the Bagnoli-

Coroglio area. The demand for both energy and raw material production processes were considered for the assessment, assuming an operating time of 4 weeks. At the end of the bioremediation process, the sediment was assumed to be used as building/filling material in the Naples port far 10km from the Bagnoli-Coroglio area.

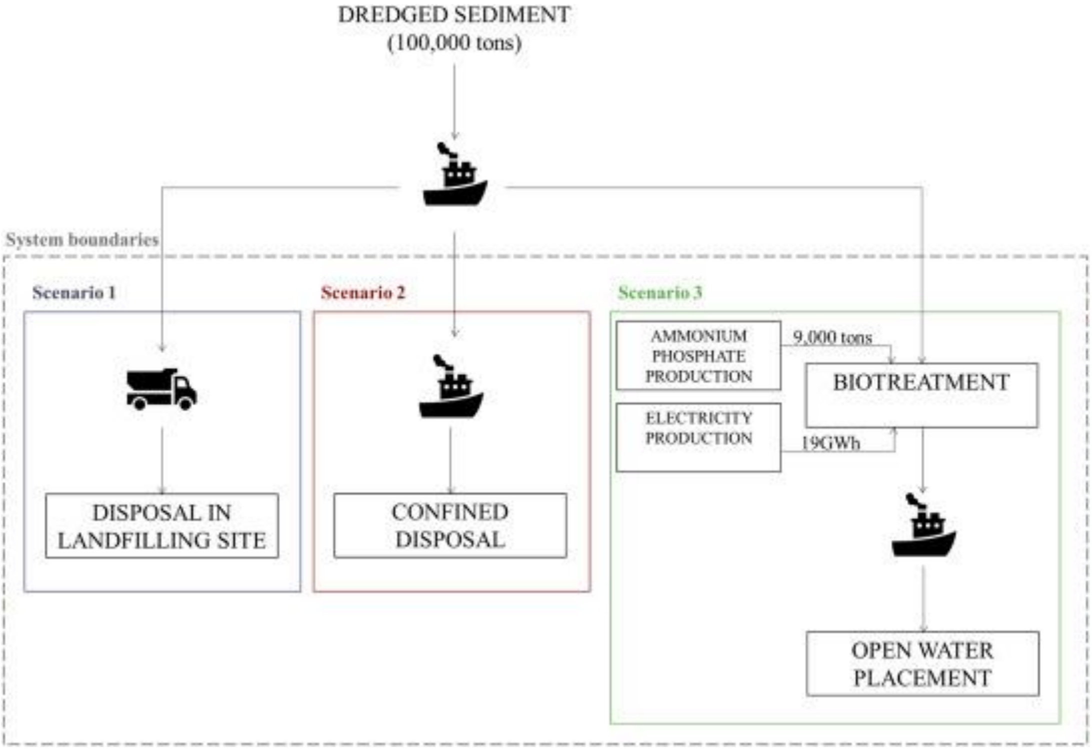

Fig. 1.System boundaries considered for the carbon footprint assessment. Functional unit: 100,000 tons of dredged sediment.

3.Results and discussion

Sediments collected in the present study for the bioremediation experiments were characterized by high concentrations of both PAHs and heavy metals (Table S1in the supplementary material), confirming previous findings obtained in the Bagnoli-Coroglio bay (Romano et al., 2008; Mercogliano et al., 2016; Qu et al., 2018). PAHs were largely dominated by high-molecular weight (HMW) compounds (accounting for >90% of the total PAH concentrations) with concentrations largely exceeding the threshold levels established by the Italian law for the management of contaminated marine sediments (Table S1). Overall, on the basis of the chemical hazard quotient, the sediment used for bioremediation experiments was classified as severely contaminated.

Inorganic nutrient addition, by stimulating microbial growth, has been repeatedly reported as an effective strategy for enhancing biodegradation of hydrocarbons in contaminated seawater and sediments (Swannell et al., 1996; Kasai et al., 2002; Head et al., 2006; Aspray et al., 2008; Nikolopoulou and Kalogerakis, 2008; Beolchini et al., 2010; Rodríguez-Blanco et al., 2010; Dell'Anno et al., 2012). In the present study, we observed both in the untreated and nutrient-treated sediments significant temporal changes of the prokaryotic abundance (ANOVA $p<0.01$; Fig. 2a), but with a different pattern. Indeed, after 1 week of incubation, the prokaryotic abundance was significantly higher in the untreated than in the nutrient-treated sediments, whereas afterwards, till the end of the experiment, the opposite was observed (ANOVA, $p < 0.01$). These results suggest that the stimulation of prokaryotic assemblages due to nutrient addition can occur with a certain time lag.

We also argue that sediment stirring *per se*, by enhancing the contact rates between prokaryotes and organic matter adsorbed to sediment particles and oxygen diffusion, could be an effective way to stimulate prokaryotic assemblages.

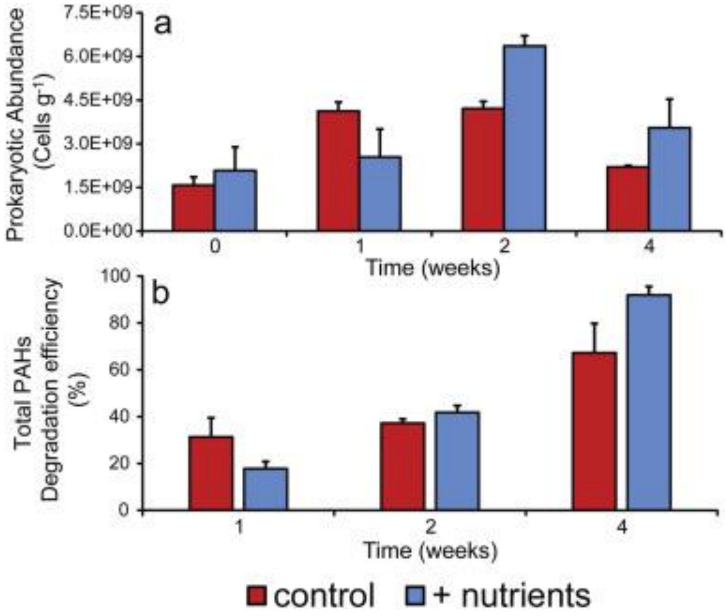

Fig. 2.Temporal changes of prokaryotic abundances (a) and total PAH concentrations (b) during bioremediation experiment of contaminated sediments with and without nutrient addition.

Temporal changes of prokaryotic abundances in untreated and nutrient-treated sediments were associated with changes of biodegradation yields of PAHs (Fig. 2b). After 1 week of incubation, the biodegradation yield of total PAHs was higher in untreated (ca. 31%) than in nutrient-treated sediments (ca. 18%), whereas after 4 weeks, nutrient addition resulted in a significant higher biodegradation yield (>90% vs 67%; $p<0.01$).

We did not observe significant changes of fungal biomass from the beginning (time 0) to the end (after 4 weeks) of the biostimulation experiment (data not shown), suggesting that inorganic nutrient addition alone was not able to efficiently stimulate fungal growth. Thus, fungi had likely a minor role in the PAH degradation observed during such experiment.

All of the PAH congeners at the end of the biostimulation experiments reached concentrations lower than the threshold limits established by the Italian law, except for the anthracene (572ng g^{-1}) and benzo(a)pyrene (130ng g^{-1}) (Table S1). These findings suggest that biotreatments based on inorganic nutrient supply could be an effective *ex situ* strategy to significantly reduce in a relatively short time PAH contamination of marine sediments, possibly leading to alternative management options compared to landfill disposal.

Based on PAH concentrations determined at each time interval during biostimulation experiment, we assessed the degradation rates of PAHs assuming a first order degradation kinetic. The degradation rate of total PAHs was approximately 1.3-fold faster in the experiments with nutrients when compared to the control (Fig. 3a). Similar results were observed for degradation kinetics of high-molecular weight PAHs, which accounted for the large majority of PAHs present in the sediments (Fig. 3b).

Conversely, the degradation rate of low-molecular weight PAHs was 1.7 times faster in the untreated sediments when compared to those enriched with inorganic nutrients (Fig. 3c).

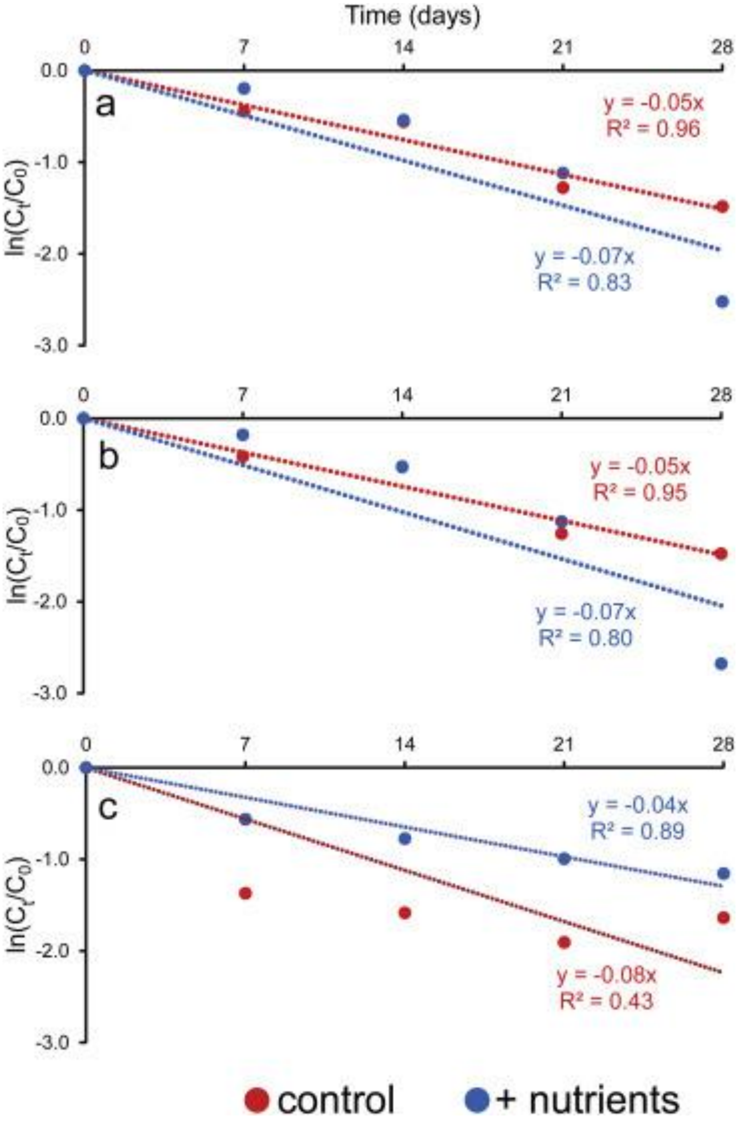

Fig. 3. Degradation kinetics of total PAHs (a), total high molecular weight PAHs (b), and total low molecular weight PAHs (c) in sediments with and without nutrient addition.

Previous studies have reported a high potential of prokaryotes to degrade both low- and high-molecular weight PAHs (Déziel et al., 1996; Ghosal et al., 2016; Perfumo et al., 2006), but information on degradation rates of the different congeners following bioremediation of chronically contaminated marine sediments is still largely lacking. In the present study, we found that the 7, 12 dimethylbenz(a)anthracene and fluoranthene, which were the dominant congeners of the PAH pool, displayed different degradation rates in the untreated and nutrient-treated sediments (Fig. 4a and b). Indeed, the degradation rate of 7, 12 dimethylbenz(a)anthracene in the sediments supplied with nutrients was 1.5-fold faster than that in untreated ones (Fig. 4a). The effect of nutrient addition was even more evident for fluoranthene, with a degradation rate 3-fold faster than in untreated sediments (Fig. 4b). Such differences suggest that nutrient addition, by selecting specific benthic prokaryotic taxa (Dell'Anno et al., 2012), may speed up the preferential biodegradation of specific PAH congeners.

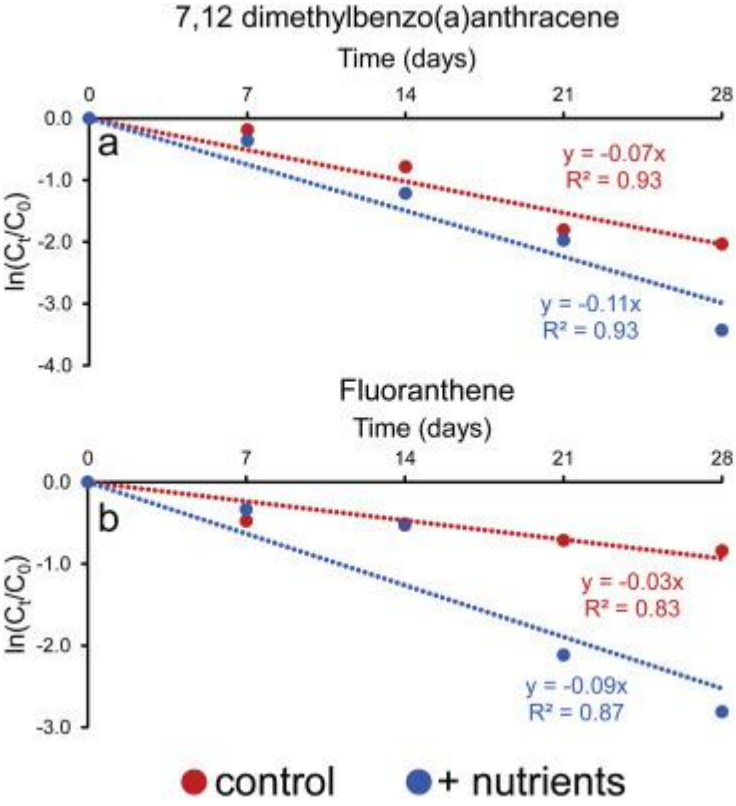

Fig. 4.Degradation kinetics of 7, 12 dimethylbenzo(a)anthracene (a) and fluoranthene (b) in sediments with and without nutrient addition.

Kinetic models can help to explain the factors influencing biodegradation rates of contaminants in the sediments and can ultimately be used to predict timescales and effects of biotreatments (Beolchini et al., 2010, 2014; Deary et al., 2016). In the present study, we applied a semi-empirical kinetic model, previously used for total hydrocarbon biodegradation in the sediments (Beolchini et al., 2010), to fit experimental data describing temporal changes of prokaryotic abundances (N, expressed as the number of cells g^{-1} dry sediment) and total PAH concentrations (C, expressed as µg PAH g^{-1} dry sediment).

In particular, the following semi-empirical equations have been fitted to experimental data:

$$\frac{dC}{dt} = kN(1-\beta N) + k0\int_0^t N(t)dt \text{...}(2)$$

$$\frac{dC}{dt} = \frac{1}{Y}\frac{dN}{dt} \text{...}(3)$$

integrated with the initial conditions of $N_{(0)} = N_0$, $C_{(0)} = C_0$, $N_{(t)}$ and $C_{(t)}$ at t time.

The microbial growth rate dN/dt was mathematically described by a modified version of the empirical logistic equation, 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). The contaminant degradation rate dC/dt was supposed to be associated to the microbial growth through a yield factor, Y (i.e. ratio between number of prokaryotic cells and PAH concentrations), hypothesizing that the K_0 term was not relevant for contaminant biodegradation. The estimated values for the different parameters and the regression coefficients are reported in Table 1. The relatively high values of the regression coefficients confirm the suitability of the equations to mathematically describe the temporal changes of prokaryotic abundance and contaminant concentrations in the experimental conditions investigated in the present study. It can be observed that the prokaryotic growth kinetic constant (k) in the control was 3-fold higher than in the nutrient amended treatment. These results are justified by the faster prokaryotic growth observed in the untreated sediments during the first week of incubation. Nevertheless, the estimated value of the inhibition/limiting factor (β) for the untreated sediments was about 3-fold higher than that of the nutrient enriched ones, indicating a positive effect of the nutrient addition for sustaining prokaryotic growth over a longer time scale.

Table 1. Parameters (*k*, *k₀*, *β* and *Y*) estimated by non linear regression analyses in the sediments treated with and without inorganic nutrients. k = kinetic constant; k_0 = decrease of microbial abundance after the stationary phase (due to either a bottom-up control – nutrient limitation or top down control due to predation); β = inhibition/limiting factor; Y = yield factor. Regression coefficients (R^2) are also reported.

Experimental system	***k*** **(d^{-1})**	***k₀*** **(d^{-2})**	***B*** **(10^{-9} cells)**	***Y*****(10^9 cells μg^{-1} PAH)**	**R^2**	
					PAH concentration	**Prokaryotic abundance**
Without nutrient	0.61	−0.011	0.21	0.99	0.97	0.98
With nutrient	0.21	−0.009	0.08	0.98	0.99	0.90

On the basis of kinetic model outputs and assuming a carbon content of prokaryotes equal to 20 fg C $cell^{-1}$(Lee and Fuhrman, 1987), we estimated that the degradation of PAH (converted into C equivalent considering a C content equal to ca. 95%) supplied a very similar fraction of the organic C needed to sustain prokaryotic biomass production in the untreated and in the nutrient-treated sediments (in both experimental conditions ca. 9%). These findings suggest that inorganic nutrient addition, despite it does not influence the relative consumption of PAHs over other C sources, can increase the efficiency of PAH degradation by stimulating co-metabolic processes (Ghosal et al., 2016).

Besides prokaryotes, also fungi can be effective for the bioremediation of hydrocarbon-contaminated sediments (Passarini et al., 2011; Ghosal et al., 2016). In the present study, we investigated, as an alternative to the biostimulation strategies based on nutrient addition, the performance of the bioaugmentation approach based on *Aspergillus* sp. addition (previously isolated and identified by molecular tools from Bagnoli sediments) for the removal of PAHs. In our experiment, the total fungal biomass exponentially increased over time both in the controls and in the sediments added with *Aspergillus* sp. (ANOVA $p < 0.01$; Fig. 5a). However, despite in the sediments added with fungi the total fungal biomass after 4 weeks reached values ca. 2 times higher than in the controls (ANOVA $p < 0.01$), *Aspergillus* sp. addition did not significantly increase PAHs degradation (Fig. 5b) when compared to untreated sediments (60±9% vs. 52±9%). These findings suggest that the addition of a single microbial taxon may not be able to further enhance the degradation potential of the autochthonous microbial assemblage present in historically contaminated sediments. Further tests based on the use of microbial consortia (i.e., different fungi and bacteria taxa) are required to improve the biodegradation efficiency of PAHs in contaminated marine sediments (Mikesková et al., 2012). Recent studies highlighted that biodegradation processes of sediments contaminated with hydrocarbons can be speeded up by microbial fuel cell-based strategies employing solid-state electrodes (Cruz Viggi et al., 2015, 2017; Kronenberg et al., 2017; Nastro et al., 2019). By applying this approach to Bagnoli sediments, we observed, after 6 weeks of treatment, biodegradation yields of 60% and 55% for high molecular and low molecular weight PAHs, respectively (Fig. 6). These values are relatively high when compared to those reported in the literature, especially for high molecular weight contaminants which are known to be more recalcitrant to degradation (Kronenberg et al., 2017). For instance, Nastro et al. (2019), using a single chamber electrochemical configuration for the treatment of sediments collected in the same area of the present study, reported after 4 weeks degradation rates of total high molecular weight PAHs of ca. 10%. In this experiment, the degradation efficiency of indenol [1,2,3-c,d] pyrene, which is one of the most hazardous PAH (US EPA, 2008), was much lower when compared to that of the present investigation (ca. 65%). The highest degradation efficiency of PAHs, observed in our study, cannot be explained by the longer duration of our treatments (6 vs. 4 weeks), since the degradation yield was higher even after only 1 week. Such differences can be associated with the higher ratio between the surface area of the anode electrode and the quantity of treated sediment we used (SA/TS, 0.12 cm^2/g vs. 0.06 cm^2/g). This, indeed, can increase contaminant oxidation, due to an increased contact between the "electroactive" bacteria and the electrode (Zhou et al., 2016). The high biodegradation efficiency observed in our experiment was further confirmed by the higher degradation yields of pyrene and benzo(a)pyrene (65% and 70% respectively) when compared with the values reported for other sediment samples (20–25%; Yan et al., 2015).

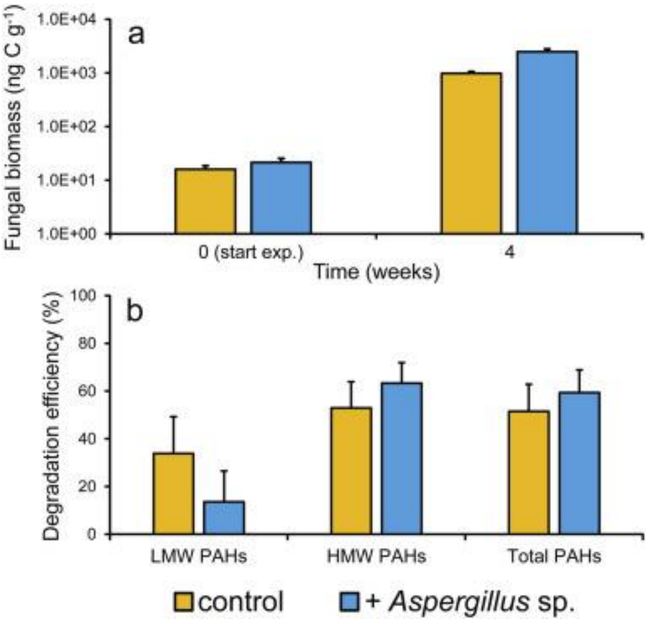

Fig. 5.Changes of fungal biomass at the beginning and at the end of the bioaugmentation experiment (a) and degradation yields of total, low molecular weight (LMW) and high molecular weight (HMW) PAHs at the end of the bioaugmentation experiment (b). The y axis of Fig. 5a is in log scale.

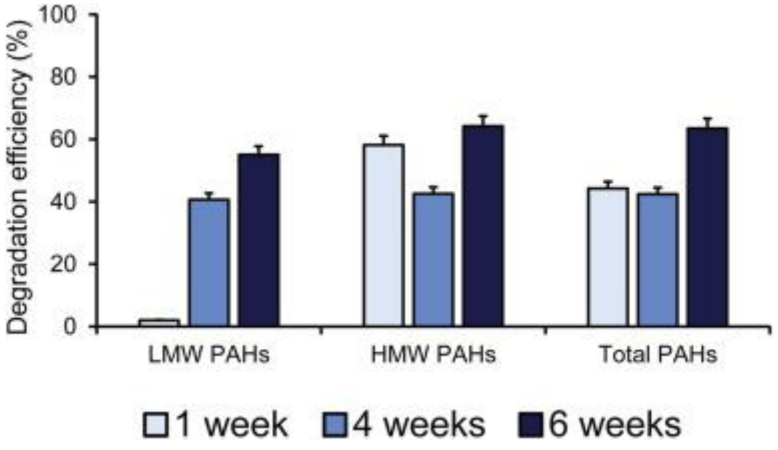

Fig. 6. Temporal changes of degradation efficiency of total, low molecular weight (LMW) and high molecular weight (HMW) PAHs during the bio-electrochemical experiment.

Our results provide additional support to the suitability of bio-electrochemical strategy for the remediation of marine sediments highly contaminated by PAHs. However, further studies are needed for a better understanding of biodegradation mechanisms employing microbial fuel cell-based strategy.

Increasing evidence suggests that biotreatments based on nutrient addition (both organic and inorganic) of contaminated sediments can induce significant changes in metal partitioning and mobility (Dell'Anno et al., 2009; Rocchetti et al., 2012; Fonti et al., 2015), but such an effect due to either bioaugmentation or biolectrochemical stimulation still needs to be investigated.
We found major changes in Pb and As concentrations in the sediments after *Aspergillus* sp. addition (Table 2). Indeed, these two elements at the end of the experiments decreased in the solid phase by up to 35% and 45%, respectively, suggesting a leaching effect not only chemically-mediated, but also biologically-mediated by *Aspergillus* sp. (Horeh et al., 2016). At the same time, Cd concentrations increased 2.5-fold in the exchangeable fraction, concomitantly with a in the reducible fraction (ca. 77%; Table 2). The Cu associated to the oxidizable fraction decrease decreased in favour of an increase in the residual phase. For the other elements investigated (Hg, Mn and V), no marked variations were observed.

Table 2. Comparison of metal concentrations (expressed as µg g^{-1}) in the different geochemical fractions of sediment at the beginning and at the end of the experiment with and without fungal addition and at the end of the bio-electrochemical experiment. Mean values and standard deviations (±) are reported. na = not available.

Metals ($\mu g\ g^{-1}$)	**Fraction**	**Time (0)**	**Without fungi (after 4 weeks)**	**With *Aspergillus* sp. (after 4 weeks)**	**Bio-electrochemical (after 6 weeks)**
As	Exchangeable	12.8 ± 0.4	9.5 ± 5.3	5.7 ± 0.5	4.9 ± 2.7
	Reducible	4.4 ± 0.1	3.8 ± 0.8	5.8 ± 1.8	4.1 ± 0.6
	Oxidizable	0.7 ± 0.2	1.0 ± 0.5	1.1 ± 0.5	0.5 ± 0.8
	Residual	192.5 ± 3.5	129.0 ± 0.01	108.0 ± 0.01	53.0 ± 4.2
Hg	Exchangeable	0.0048 ± 0.01	0.0051 ± 0.01	0.0054 ± 0.01	na
	Reducible	0.0048 ± 0.01	0.0051 ± 0.01	0.0054 ± 0.01	na
	Oxidizable	0.0048 ± 0.01	0.0051 ± 0.01	0.0054 ± 0.01	na
	Residual	1.1 ± 0.15	1.1 ± 0.01	0.7 ± 0.4	na
Pb	Exchangeable	255.1 ± 78.9	189.1 ± 16.6	91.3 ± 37.3	94.9 ± 48.6
	Reducible	403.8 ± 13.2	477.1 ± 84.8	284.4 ± 84.4	274.6 ± 144.8
	Oxidizable	115.5 ± 6.6	91.9 ± 36.4	99.0 ± 36.4	175.5 ± 136.6
	Residual	48.7 ± 2.9	35.6 ± 8.3	69.5 ± 33.5	185.5 ± 9.2
Mn	Exchangeable	727.42 ± 15.2	677.3 ± 65.0	475.9 ± 100.6	772.1 ± 68.5
	Reducible	51.8 ± 5.7	70.6 ± 8.3	56.5 ± 10.8	74.7 ± 20.1
	Oxidizable	44.9 ± 4.3	47.4 ± 13.5	38.0 ± 2.7	52.3 ± 21.7
	Residual	1028.5 ± 59.5	1044.0 ± 322.0	1245.5 ± 235.5	871.0 ± 45.3
V	Exchangeable	17.8 ± 0.4	8.4 ± 3.6	10.1 ± 2.2	18.9 ± 0.5
	Reducible	7.6 ± 0.1	12.5 ± 8.6	8.3 ± 2.8	7.5 ± 1.3
	Oxidizable	5.4 ± 0.3	2.8 ± 0.3	4.2 ± 0.3	2.9 ± 0.7
	Residual	85.8 ± 2.1	97.8 ± 41.2	89.2 ± 12.8	27.9 ± 5.8
Cd	Exchangeable	1.3 ± 0.4	3.6 ± 0.01	4.6 ± 1.9	na
	Reducible	3.7 ± 0.04	1.8 ± 0.2	0.9 ± 0.4	na
	Oxidizable	1.5 ± 0.04	1.1 ± 0.6	2.2 ± 0.5	na
	Residual	2.4 ± 0.05	2.9 ± 1.3	2.9 ± 0.8	na
Cu	Exchangeable	1.1 ± 0.8	13.3 ± 11.5	15.6 ± 2.4	0.02 ± 0.01
	Reducible	8.9 ± 4.3	4.4 ± 0.03	3.8 ± 0.5	6.9 ± 4.2
	Oxidizable	76.6 ± 34.8	23.3 ± 13.4	41.2 ± 2.7	90.7 ± 4.5
	Residual	42.5 ± 2.5	62.5 ± 27.0	67.5 ± 0.5	41.0 ± 2.8

Major changes for As and Pb were also observed after the bioelectrochemical remediation experiment. Indeed, a large fraction of As was mobilised to the water phase (ca. 70%; Table 2), whereas Pb mobility was reduced as documented by the increase of its concentration in the residual fraction. These results indicate that both bioaugmentation and bioelectrochemical stimulation can induce not only changes in the partitioning of metals among the different geochemical fractions of the sediments, but also their solubilization. Such aspect can be important from one hand for metal removal through biotechnological strategies (Abbas et al., 2017; Wu et al., 2017) and from the other for the ecotoxicological risk that may arise, especially if bioremediation approaches are applied *in situ*.

The assessment of the carbon footprint highlighted environmental benefits (estimated in terms of CO_2-equivalents) of the biotreatment of sediments based on inorganic nutrient addition compared to their disposal in landfilling sites (Fig. 7). Almost all of the emissions of the scenario based on the biotreatment (scenario 3) was due to the electricity consumption (60%) and the nutrient production (39%), with a minimum effect of stream transportation. In the perspective of a further improvement of the sustainability of bioremediation, future strategies could significantly decrease electric power demand by conversion of the slurry-reactor approach into a heap-leaching treatment (Fonti et al., 2016). Furthermore, using a waste flow rich in both nitrogen and phosphorous, instead of inorganic nutrient addition could contribute to further increase bioremediation sustainability. In this case, a double benefit can be achieved due to a lower carbon footprint coupled with wastewater recycling, thus pursuing circular economy principles (Geissdoerfer et al., 2017).

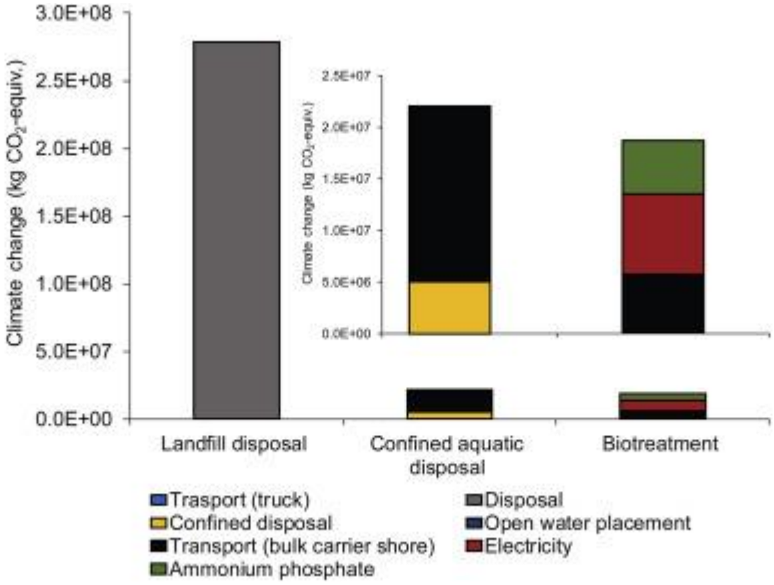

Fig. 7. Comparison among carbon footprint of the three considered scenarios (disposal in landfill site, confined aquatic disposal and biotreatment). The smaller panel on the right allows to better appreciate differences between scenarios.

For the confined aquatic disposal option (scenario 2), transport by bulk carrier to the shore mainly contributed (>70%) to the total emissions. This contribution could even increase, in case of insufficient capacity of the disposal site with consequent increase in the transportation distance. The disposal of contaminated sediments in landfilling sites (scenario 1) represents the worst option, with a significant impact caused by the large volume of sediments, containing hazardous compounds (Fig. 7).

Overall, our results indicate that all the treatments investigated in the present study can significantly reduce PAH concentrations in historically contaminated sediments of the Bagnoli Coroglio bay in relatively short time interval and that biostimulation based on inorganic nutrient addition can be the most effective strategy. Nonetheless, biotreatments can induce important changes in the partitioning of metals, including their dissolution, and this should be carefully taken into account for environmental risk assessment. Our findings also suggest that *ex situ* biotreatments of contaminated sediments can be convenient in terms of carbon footprint if compared to current management options, such as the disposal in landfilling sites and/or in confined aquatic facilities, but integration with other strategies for metal removal (e.g. through bioleaching) is needed for sediment safe re-use. Overall results presented here provide new insights into the development of effective and eco-sustainable bioremediation approaches for the reclamation of highly contaminated marine sediments.

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CHAPTER 3

Ozonation and Bioremediation of PAH contaminated sediments from Bagnoli site, Naples Italy

This chapter is a version of a paper with the same title under preparation for submission in the journal Applied Sciences, Special Issue "Microbial and Plant-Assisted Bioremediation for Eco-Sustainable Environment Journal”

Introduction

The contamination of marine sediments with polycyclic aromatic hydrocarbons is a major environmental pollution problem. PAHs are a group of complex organic contaminants that can occur naturally in the environment following natural events such as volcanic eruptions or forest fires(Abdel-Shafy and Mansour 2016; Bandowe et al. 2014). However, their high concentrations result mainly from industrial activities, waste discharges and past oil spills (Marini and Frapiccini, 2013). They are highly heterogenic and there are over a hundred different of these compounds containing two or more fused benzene rings (Kumar et al. 2014; C.-W. Chen et al. 2016). PAH are lipophilic compounds and can easily bind to the organic fraction in sediments. Also due to their very low water solubility and complex structure, they are difficult to degrade and when released in the aquatic environment they often end up accumulating for long-term in the sediments (Maletić et al. 2019). Several studies have reported the toxicity and persistency of PAHs contaminants in sediments with high concentrations recorded years and even decades after the event leading to the pollution has occurred. It was observed that a year after the deep water horizon (DWH) oil spill, heavy fractions of crude oil could still be found on the sea shores of the Gulf of Mexico (Mapelli et al. 2017). Also, sediments from the salt marsh of Wild Harbour in Massachusetts, USA were collected in 2002 for petroleum hydrocarbon analysis following the West Falmouth oil spill that occurred in 1969, results indicated that over thirty years after the spill, specifically between 6-28cm depths, petroleum compounds had concentrations similar to those recorded shortly after the spill while above 6cm depth, there was no trace of petroleum residue (Christopher M. Reddy et al. 2002). The former industrial area of Bagnoli in Naples, Italy constitute another contaminated zone as a result of heavy industrial activity in the area for almost a century. PAH and toxic metals concentrations have remained persistently high although all industries in the area were dismantled between the years 1990- 2000 (Qu et al., 2018). This accumulation in sediments constitutes a threat to the survival of the biota in the contaminated ecosystems due to their detrimental effects and carcinogenic properties (Ferrarese, Andreottola, and Oprea 2008). PAH toxicity on animals was evidenced with tumours, reproductive disorders and various complications developed after their exposition to various PAHs under laboratory conditions (Abdel-Shafy and Mansour 2016; Masih et al. 2010)

Several factors such as temperature, lack of oxygen, sediment characteristics, organic matter content or salinity have been reported to enhance PAH accumulation in sediments (Marini and Frapiccini 2013; Wang, Zhang, and Chen 2001) According to Marini and Frapiccini (2013), salinity constitutes one major factor among these, especially for high molecular weight PAHs in marine sediments. In this study, the persistency of PAH in various marine sediments with salinity of 20 and 37 was investigated and results showed longer PAH persistency in sediments with salinity 37. Specifically, the PAH half-life was increased by 28%, 40%, 53% and 34% for the two and three benzene ring PAHs, four rings, five rings and six rings respectively.

The high concentrations, complex structures and low bioavailability of PAH compound can become major hinderances to the success of bioremediation especially when dealing with long-term contaminated sediments. Physico-chemical methods can be necessary pre-treatment options for partial breakdown and increased bioavailability of contaminants prior to the bioremediation step (Chen et al. 2016; Samsami et al. 2011). It is generally accepted that methods such as chemical oxidation are more effective to partially degrade the most recalcitrant PAHs into less complex fractions although they can be more economically and environmentally costly and involve the use of chemical agents in processes that do not exclude possibilities of secondary pollution. (Rulkens 2005)(Fulekar 2017). Various chemical oxidation agents have been proposed for sediment remediation among which ozone treatment. Although several studies have shown the detrimental effect of ozone treatments on microbial life and biota(Anderson et al. 2012; Tabla-Hernandez et al. 2020), it was also suggested that these detrimental effects were short term and could help attenuate the toxic effects of the organic contaminant targeted by the ozone (Anderson et al. 2012). Also in the longterm ozone treatment could achieve contaminants degradation into more biodegradable products and equally provide oxygen to the sediment environment which generally is anoxic (Agarwal and Liu 2015)

In this study, bioremediation experiments were carried out on contaminated sediments from the Bagnoli site using organic nutrients, precisely a nutrient rich supercompost and activated sludge from a municipal waste treatment plant. Rhamnolipid biosurfactant was equally applied and each of these biotreatments were applied on ozone pre-treated and non-pre-treated sediments. The effects of ozone on PAH concentrations was investigated as well as effects on the microbial population, pH and redox potential in the sediments during a 28days experiment

2. Materials and Methods

2.1 Sediment Sampling and Study Area

The sediments used for the present study were collected from the Bagnoli-Coroglio port area in Naples, Italy. The sediment were sampled at 0-50cm depth using a vibrocorer and kept at 4°C prior to the beginning of experiments. Sub-samples were immediately analysed for the determination of the initial PAH concentrations and the total prokaryote count. Table 1 reports the main PAH contaminants determined in the sediment sample. The characterization showed a high molecular weight (HMW) PAH predominance with a HMW and low molecular weight (LMW) ratio greater than 20.

Table 1:

LMW PAH (µg/kg dry sediment)	
Naphthalene	0.11 ± 0.01
Acenaphthylene	390 ± 30
1-methylnaphthalene	0.11 ± 0.01
2-metilnaftalene	0.11 ± 0.01
Acenaphthene	100 ± 10
Fluorene	530 ± 40
Phenanthrene	0.011 ± 0.001
Anthracene	4900 ± 400
Total LMW PAH	5900 ± 400
HMW PAH (µg/kg dry sediment)	
Fluoranthene	36000 ± 3000
Pyrene	11000 ± 1000
Benzo(a)anthracene	9400 ± 700
Chrysene	7500 ± 500

7,12-dimethylbenzo (a) anthracene	33000 ± 2000
Benzo (b) fluoranthene	8000 ± 600
Benzo (k) fluoranthene	6100 ± 400
Benzo (a) pyrene	4400 ± 300
Dibenzo (ah) anthracene	1300 ± 100
Benzo (ghi) perylene	2100 ± 100
Indeno (123cd) pyrene	270 ± 20
Total HMW PAH	120000 ± 10000
Total PAH	130000 ± 10000

2.2 Ozonation Pretreatment

Sediments were ozonated using a Fischer ozone generator (Fischer America Inc, Texas, USA) producing pure O3 at a constant flow rate of 40L/hr (equivalent to 8g/hr of ozone). The sediments were ozonated in batches of 50g of sediments containing 200ml of SW for a total of 3 hours and 8minutes to achieve a dosage of O.5g of ozone per gram of wet sediment. Ozonation experiments were entirely carried out under a chemical fumehood.

2.3 Bioremediation Experiments

The various bioremediation agents investigated were activated sludge, a nutrient rich supercompost and rhamnolipid biosurfactants, each treatment were administered individually with and without an ozone pre-treatment as presented in table 2.

Table 2: Experimental Plan

Group 1-AS	50g Sediment + 200ml SW+ Activated Sludge	Group1 O3-AS	50g Sediment + 200ml SW + O3+ Activated Sludge
Group 2-SC	50g Sediment + 200ml SW + Supercompost (SC)	Group2-O3-SC	50g Sediment + 200ml SW + O3 + Supercompost (SC)
Group 3-B	50g Sediment + 200ml SW + Biosurfactants (BS)	Group3-O3-B	50g Sediment + 200ml SW + O3 + Biosurfactants (BS)

The activated sludge was collected from a municipal waste treatment plant while the supercompost was a product from vermicomposting. The Rhamnolipid biosurfactants were purchased from AGAE Technologies LLC, Oregon, USA.

2.4 Microbial Count

The microbial growth and PAH degradation was monitored weekly and bi-monthly respectively during the 28 days of treatment. Total prokaryote abundance was measured by direct count using epifluorescence microscopy following the protocol described by Danovaro et al., (2002).

2.5 PAH Analyses

Gas Chromatography/ Mass Spectrometry Operation Conditions Analysis

PAHs from the Sediment samples were extracted by ultrasonication using 0.5g of the lyophilised samples in 10ml of hexane/acetone solvent (1:1). This procedure was repeated three times to ensure a complete PAH extraction. The supernatants was filtered and 0.5_µl were analysed (without being concentrated because of the suspected high concentration of PAH) by GC/MS

The sample extracts were analysed with an Agilent 6890 N Gas Chromatograph (Agilent Technologies, Burwood, Australia) coupled with a Pegasus 15756 TOF mass spectrometer (Pegasus, LECO, St Joseph, MI, USA) operating in electron ionization mode (EI: 70 eV) The capillary column was a DB-5MS UI high resolution GC column with 25m*0.25mm *0.25 µm film thickness with helium as carrier gas(at a flow rate of 1ml/min).). The injector was set on splitless mode at 270°C as well as the ion source while the transfer line heater temperature was at 300°C. The oven temperature was programmed from 70°C for 2mins, then increased to 200°C for 5 mins at a rate of 30°C/min and finally increased to 300°C for 15mins at a rate of 5°C/min. The data collected from the total ion chromatograms (TIC) was analysed using the automated data processing software ChromaTOF$^{\mathrm{TM}}$ (LECO, St. Joseph, MI, USA).

The 16 congeners analysed were: naphthalene, acenaphtene, fluorene, fenanthrene, anthracene, fluoranthene, benzo(a)anthracene, benzo(a)pirene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perilene, crisene, dibenzo(a,h)anthracene, indeno and pirene.

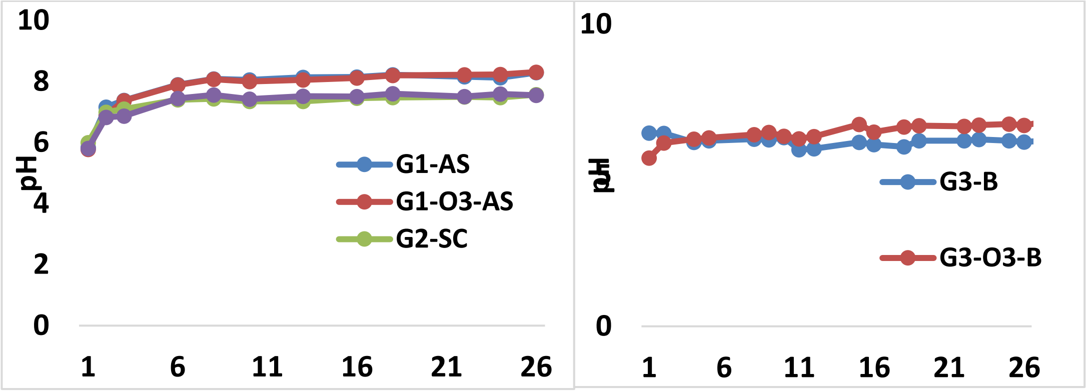
pH and Redox Measurement

pH and redox were measured daily during the experiments using respectively a pH meter

3. RESULTS

pH and Redox Results

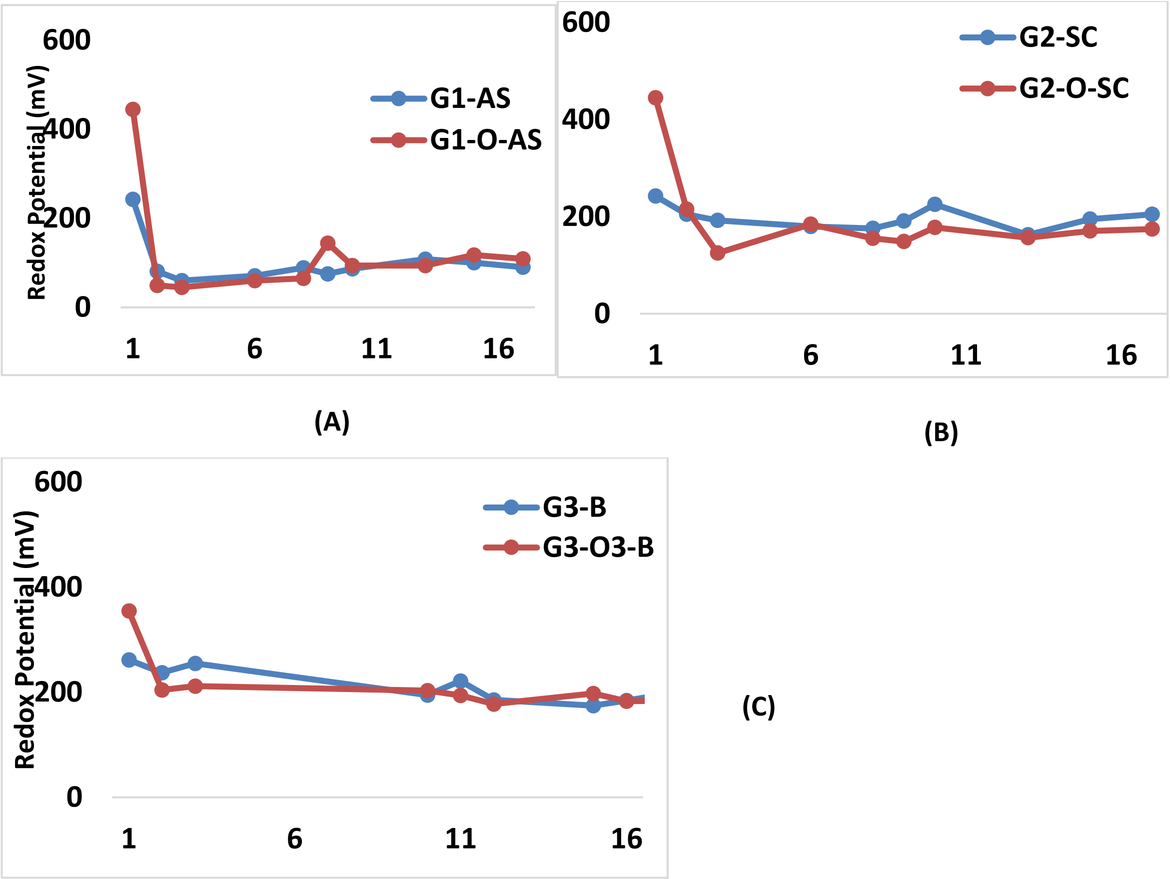
The pH and redox measured every day of the experiments are presented in figures 1 and 2 below. It was generally observed that ozonation had a very short-term effect on both parameters in the various experiments. The values recorded immediately after ozonation were lower for pH (especially in the experiment with biosurfactants) and much higher for redox, however after two to three days there was no considerable differences between ozonated and non ozonated replicates.



(A)

(B)

Figure 1: pHresults in Bioremediation Experiments: (A)G1-AS: Experiment with Activated sludge, G1-O3-AS: Experiment with Ozonation pretreatment and Activated sludge, (B)G2-SC: Experiment with Supercompost, G2-O3-SC: Experiment with Ozonation pretreatment and Supercompost, G3-B: Experiment with Biosurfactants, G3-O3-B: Experiment with Ozonation pretreatment and Biosurfactants



(A)

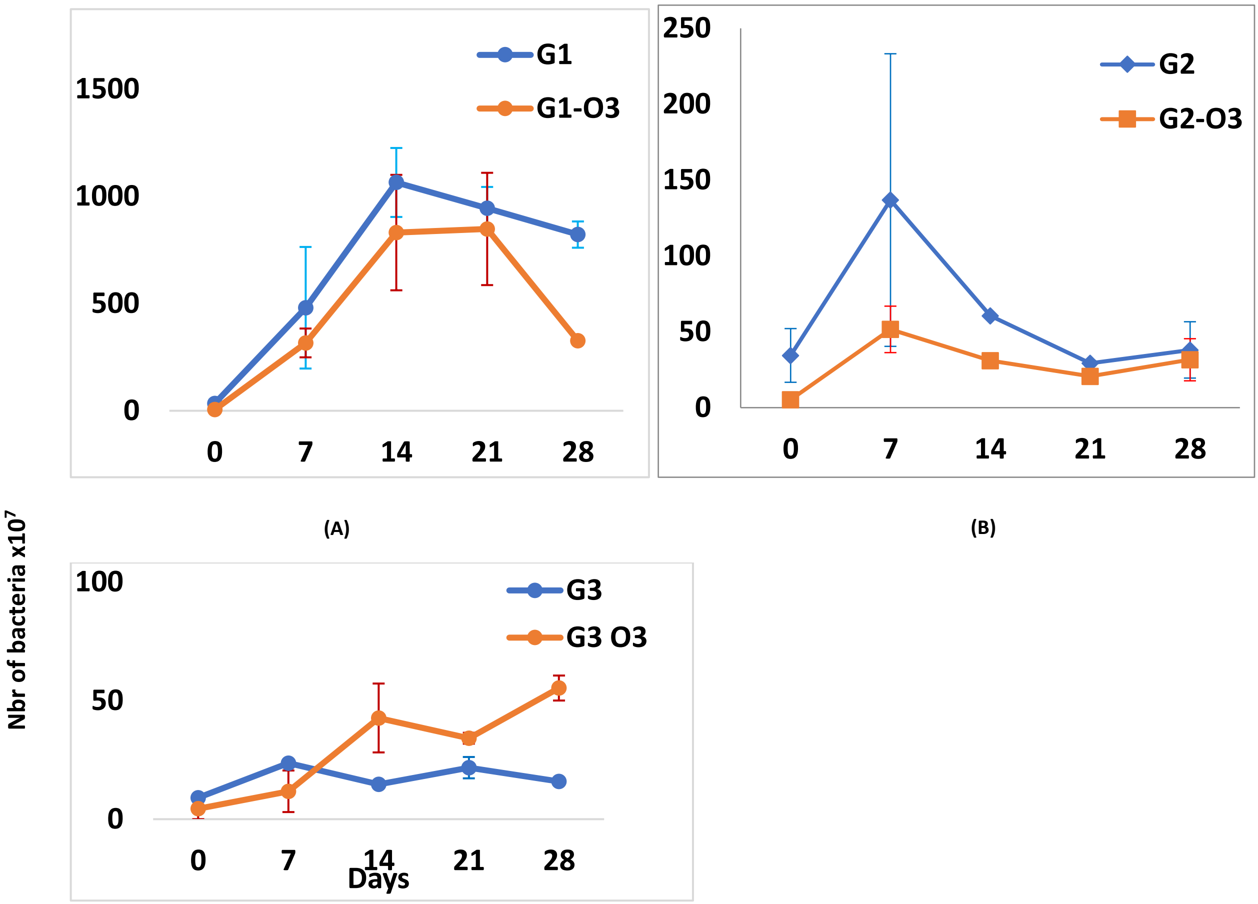
(B)

(C)

Figure 2: Redox Potential Results in Bioremediation Experiments: (A)G1-AS: Experiment with addition Activated sludge, G1-O3-AS: Experiment with Ozonation pretreatment and Activated sludge, (B)G2-SC: Experiment with Supercompost, G2-O3-SC: Experiment with Ozonation pretreatment and Supercompost, (C):G3-B: Experiment with Biosurfactants, G2-O3-SC: Experiment with Ozonation pretreatment and Supercompost.

Microbial growth

The microbial growth observed during the different experiments is illustrated in figures 3. Ozonation initially altered the microbial population. However, by the end of the experiment with the Supercompost nutrient, the microbial population was relatively similar in both ozonated and non-ozonated experiments. In the experiment with Activated sludge the microbial population remained higher in the non-ozonated sediments while with the sediments treated with biosurfactants (G3), the microbial population was over twice higher in ozone treated sediments by the end of the 28 days.



(A)

(B)

(C)

Figure 3: Microbial Growth Curve during Experiments: (A)G1-AS: Experiment with addition Activated sludge, G1-O3-AS: Experiment with Ozonation pretreatment and Activated sludge, (B)G2-SC: Experiment with Supercompost, G2-O3-SC: Experiment with Ozonation pretreatment and Supercompost, (C):G3-B: Experiment with Biosurfactants, G3-O3-B: Experiment with Ozonation pretreatment and biosurfactants.

PAH degradation efficiencies are presented in figure 4 below. There was no considerable changes in PAH concentrations in sediments before and after over 3 hours of ozonation and best results in terms of PAH degradation at the end of the experiments were less than 10% degradation and obtained in the sediments treated with supercompost

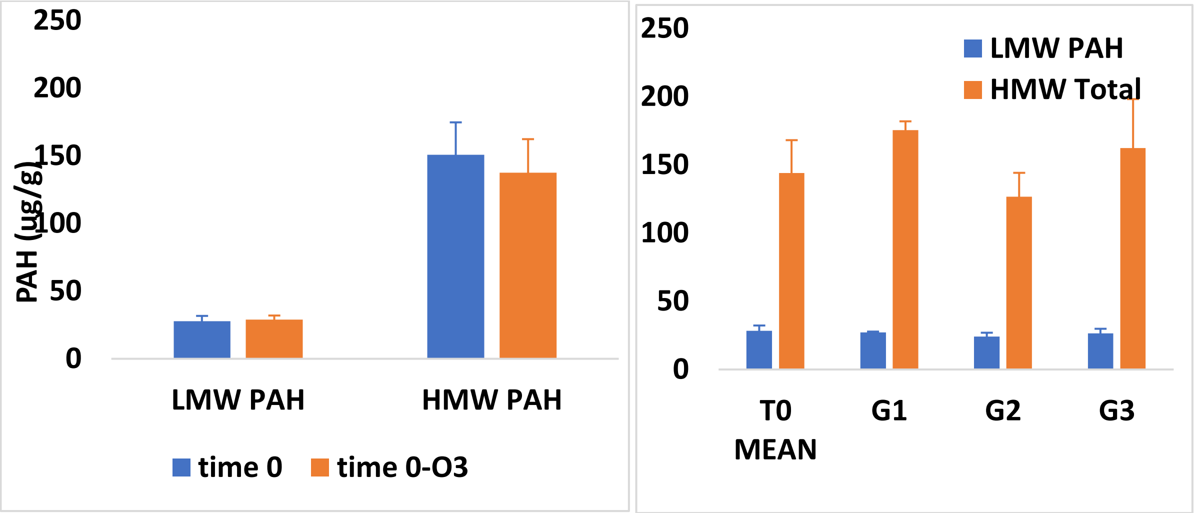


Figure 4: PAH Degradation Efficiency during Experiments (A)G1: All Experiments with addition Activated sludge, (B)G2: All Experiments with Supercompost, (C):G3: All Experiments with Biosurfactants.

4. Discussion and Conclusion

The Ozonation experiment was carried out for over 3 hours at at flow rate of 8g of O3 per hours did not yield any significant difference in terms of PAH degradation.in this experiment, the moisture content in sediments was over 40% and some studies have suggested that high moisture levels can lower the efficiency of ozone treatments in solid matrices (Mahony et al. 2006). Also, in some cases of high and long-term contamination, a minimum dosage of ozone per hour is required to effectively degrade the recalcitrant PAHs. Below that minimum ozone dosage per time, the ozonation process could be inefficient not withstanding its duration(Filho et al., 2011) Hence it is probable that the Bagnoli sediments require a minimum dosage higher than the 8g of Ozone/hr administered in this study and/or a lower moisture level. . As for the nutrients, higher quantities of the Supercompost were required for this experiment to yield optimum results. This is also confirmed by the decline in the microbial population after only 7 days. Similarly, the rhamnolipids added in the experiment seemed to be in lower than optimum concentration, coupled with the fact that the sediments contain other organic compounds more readily available to the microbial population especially after ozonation and addition of biosurfactants Bioaugmentation on another hand seemed to be non effective probably due to some antagonistic effect between the indigenous population and the allochthones. Similar results were obtained by (Leunen et al., 2002) in a series of bioremediation experiments on PAH contaminated sediments where bioaugmentation did not yield any positive results while considerable degradation was observed with biostimulation. Ozonation as pre-treatment in combination with biostimulation methods constitute a promising approach for PAH degradation in aged sediments. Also, a rich organic nutrient such as the Supercompost proposed in this study represents a more sustainable alternative to inorganic nutrients commonly used. This Supercompost might require to be processed into a liquid form to be easily assimilated by the microbial remediation agents. Furthermore, ozone treatment needs to be administered in the right minimal doses per time to ensure recalcitrant PAH breakdown but also in doses low enough to limit the damages to the biota. Consistent monitoring of the microbial population as well as pH and redox potential values can help as indicators on the efficiency of the treatment and recovery rates of the sediment ecosystems.

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CHAPTER 4

Electrokinetic Remediation of PAH and Toxic Metal Contaminated Sediments

This chapter was also included in a deliverable of the MSCA-RISE project "e.THROUGH - Thinking rough towards sustainability.

Introduction

Marine ecosystems are threatened by various contaminants among which heavy metals which tend to accumulate especially in the bottom sediments (Fonti et al., 2015; Lofrano et al., 2016). Generally, metals such as Fe, Cu, Co, Mn and Zn can be found naturally in the environment and at low concentrations, they constitute vital micro-nutrients for plants, animals and humans. On another hand, others like Cd, Pb, Hg and Ni, qualified as non-essential can be detrimental even at low concentrations (Carnevali et al., 2020).

The rapid industrialisation and urbanisation of the last century has resulted in large quantities of industrial and anthropogenic waste discharges in aquatic environments. At threshold concentrations, these metals become detrimental to living organisms. They are even more toxic as they cannot be biodegraded and hence are persistent especially in solid matrices such as sediments.(Akcil et al., 2015; Beolchini et al., 2017; Owolabi and Hekeu, 2015).Various studies have demonstrated the detrimental effect of heavy metals on marine biota. Notably, studies on benthic foraminifera, a highly abundant marine protozoan commonly used as environmental bioindicators, have shown lower diversity as well as fewer and/ or deformed species in metal contaminated sediments.(Caruso et al., 2011; Le Cadre and Debenay, 2006). Metals in the environment are absorbed by primary producers and are thereby introduced into the food chain with potential harmful effects on the entire biota (Javed, 2005; Sakan et al., 2009)

The remediation of metal contaminated sediments is a critical issue closely related to another major challenge which is the appropriate treatment and disposal or ideally, the beneficial reuse of dredged sediments often contaminated with metals (Akcil et al., 2015). According to SedNet estimates, about 100 – 200 million cubic metres of contaminated sediments are produced yearly in Europe alone as a result of dredging activities. The valuable use of these sediments have been proposed for purposes such as construction materials or for agricultural purposes as co-composted materials for growth of ornamental plants for example (Mattei et al., 2017; Paipai, 2003). However, this is often hindered by their high metal content among other factors (Bortone et al., 2004).

Several remediation approaches have been proposed for metal contaminated sediments. On one hand, biotechnological methods which constitute more economic and environmentally friendly options have yielded promising results in terms of metal biostabilisation or biomobilisation using various microbial strains such as Fe-reducing and/ or Fe/S oxidizing bacteria (Beolchini et al., 2017; Rocchetti et al., 2014) or biosurfactants such as rhamnolipids reported to enhance metal mobility in sediments (Dahrazma and Mulligan, 2007; Peng et al., 2009). Sediment Microbial Fuel Cell (SMFC) systems is a more recent approach equally yielding promising results in terms of metal immobilisation. This method relies on electro-active bacteria which have the ability to transfer electrons directly to inert anode electrode(s) introduced in the anoxic sediments. The cathode electrode(s) placed in the aqueous oxic phase are connected to the anode via an electric circuit connected to a multimeter. This system achieves both metal immobilisation and power generation simultaneously (Abbas et al., 2017). However, generally, the efficiency of biotechnological approaches can be hindered due to excessively high initial contaminant concentrations and hence the application of an appropriate physico-chemical pre-treatment to considerably reduce these concentrations might be a necessary preliminary step step (Chen et al., 2016).

Physico-chemical methods on another hand, consist of methods such as sediment washing with various metal leaching or chelating agents (acids, EDTA, surfactants), metal immobilisation methods using stabilising agents such as lime, clays, phosphate and various iron rich materials such as red mud and beringite which bind with the metals and reduce their bioavailability. Thermal treatments with adequate air pollution control equipment were also proposed for metals like Cd, Hg and As which can evaporate at 800°C. Electrokinetic remediation involves the application of low intensity current in the sediments via two oppositely charged electrodes (anode and cathode) inserted at different poles in the sediments and creating an electric migration of metals. There is possibility for recovery of positively charged metals at the cathode.(Akcil et al., 2015; Mulligan et al., 2001; Peng et al., 2009). Electrokinetic remediation presents the advantage of efficiently mobilising metals as well as other inorganic salts all together in a single stage and works efficiently on fine grained low permeable matrices. Also this method has demonstrated good applicability on the field scale (Pazos et al., 2013; Villen-Guzman et al., 2015).

Various factors such as sediment morphology and the general physico-chemical characteristics can greatly influence the outcome of a treatment method coupled with the fact that metals accumulate in various forms in the different sediments' fractions (exchangeable, acid-reduction, oxidisable-organic and residual). Hence, assessing the feasibility of different remediation strategies is critical to determine a most adequate remediation approach and according to SedNet, there can never be a generic solution but rather, sediment remediation remains site specific. (Bortone et al., 2004; Mohsen et al., 2014). In the present study, the efficiency of an electrokinetic remediation approach was investigated on metal contaminated sediments from the Bagnoli industrial area in Naples, Italy. This method is generally accepted as efficient for metal mobilisation and potential recovery in contaminated samples(Mao et al., 2019). The objective was specifically to observe the mobilisation of metals especially Pb in the sediments under application of a constant low current. Also the effect of this method on PAH concentrations was also measured although no enhancement agent was used.

1. Materials and Methods

2.1 Sediment Sampling and Characterisation

The sediments were collected in the Bagnoli former industrial Area in Naples, Italy using a vibrocorer from surface to approximately 2m depth. The sediment core was transported to the lab and stored at -4°C prior to the experiment.

Various physico-chemical parameters were measured in the sediments including pH, moisture, bulk density and total carbonates. The initial concentration of various metals was also analysed by AAS.

2.2 Metal Leaching Preliminary Experiments

Prior to the beginning of the electrokinetic remediation (EKR) experiment, the choice of a more appropriate agent was determined by carrying out leaching experiments on the contaminated sediments using a strong and a weak acid (nitric and acetic acid respectively) at pH 4. 2.5ml of each of the 2 acids was added to 0.5g of sediments and kept in a shaking incubator for 24hrs at room temp. (to mimic the conditions during EKR). The experiment was carried out in triplicates and at the end, the metal concentrations in each samples was determined by AAS

Similar leaching experiments were further carried using HNO3 at pH 1 since no metal leaching was observed at pH 4 for both acids.

2.3 Electrokinetic Remediation Experiment

The experiment was carried out as described by (Villen-Guzman et al., 2015) using methacrylate columns of 9cm length and 8cm diameter. The sediments were mixed till saturation with a solution of 10^{-3}M of NaNO3, to enhance the sediment conductivity. The sediments was in duplicates with each column containing approximately 700g of saturated sediments. Both columns were connected in series to ensure a similar current flow (maintained at 100mA) between the two.

The experimental set up was as illustrated in figure 1 below consisting of the anode compartment on the left, the cathode compartment on the right and the sediments between separated from both electrolyte by a filter membrane (pore dimensions).

The anolyte and catholyte liquid were contained in external glass containers connected to their respective electrolyte compartments. Both anolyte and catholyte consisted of a solution of 10-3M NaNO3 which was continuously recirculated in and out of the respective compartments using a pump system. The pH at the anode was maintained at pH 1 while at the cathode the pH was 4.

An automatic pH control system (Vacoquing, Spain) consisted of pH probes dipped into the external electrolytes also connected with a burrette filled with 3M NaOH (at the anode) and 3M HNO3 (at the cathode) solution for pH adjustment. The system was connected to a computer and set up so as to maintain pH at the anode >1, otherwise, the burrette valve opened automatically to release NaOH until pH was restaured to around 1. Similarly at the cathode, the pH automatically adjusted with NaOH when >4.

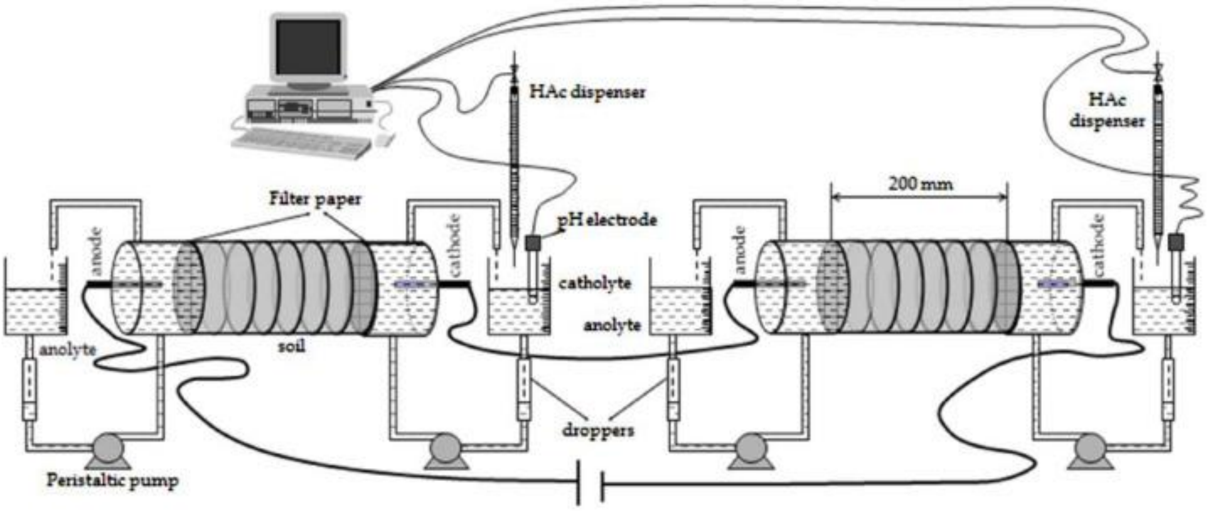
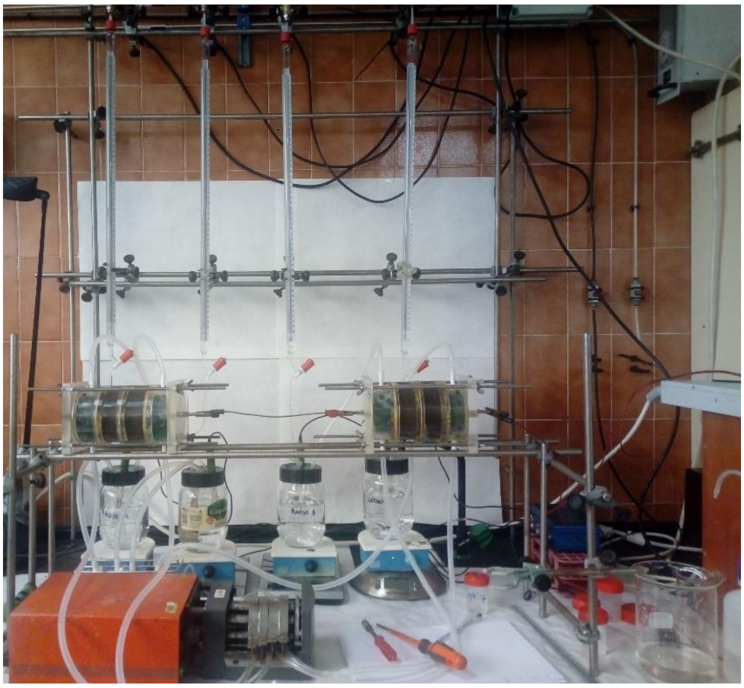


Figure 1. Electrokinetic Remediation System (Source: Villen et al., 2015)

The experiment was carried out for 10 days and samples were collected daily from the external anode and cathode compartments for metal analyses. At the end of the experiment, sediments in the columns were divided into 4 slices and samples were taken from each slice for metal content and speciation analyses. The pH in each sediment portion was also determined.



Picture 1. Electrokinetic Remediation Experiment Set-up in duplicates

2.4 Metal Speciation Analyses

The sequential extraction protocol used for metal speciation analyses was as described by Rauret et al. (2000). In this experiment, 0.5g of sediments was used in 20ml of each extracting solutions. Duplicate samples were collected and analysed for each sediment portion.

2.5 PAH Analyses

Following the EKR experiment, approximately 4g of sediments was collected from each of the 4 portions of sediments and stored at -20 for 24hours. The frozen samples were then lyophilized during 24hrs in a CHRIST ALPHA 1–4 lyophilizer (B. Braun Biotech)) under 75mbar of vacuum pressure.

PAHs from the Sediment samples were extracted by ultrasonication using 0.5g of the lyophilised samples in 10ml of hexane/acetone solvent (1:1). This procedure was repeated three times to ensure a complete PAH extraction. The supernatants was filtered through….. filters and 0.5 µl were analysed (without being concentrated because of the suspected high concentration of PAH)by GC/MS

GC/MS Conditions

The sample extracts were analysed with an Agilent 6890 N Gas Chromatograph (Agilent Technologies, Burwood, Australia) coupled with a Pegasus 15756 TOF mass spectrometer (Pegasus, LECO, St Joseph, MI, USA) operating in electron ionization mode (EI: 70 eV) The capillary column was a DB-5MS UI high resolution GC column with 25m*0.25mm *0.25 µm film thickness with helium as carrier gas(at a flow rate of 1ml/min).). The injector was set on splitless mode at 270°C as well as the ion source while the transferline heater temperature was at 300°C. The oven temperature was programmed from 70°C for 2mins, then increased to 200°C for 5 mins at a rate of 30°C/min and finally increased to 300°C for 15mins at a rate of 5°C/min. The data collected from the total ion chromatograms (TIC) was analysed using the automated data processing software ChromaTOF^{TM} (LECO, St. Joseph, MI, USA).

2. Results and Discussion

Sediment Characterisation and Metal content

The initial metal concentration and characteristics of the sediments are presented in table 1.

Table 1: Characterisation and Initial metal concentration of Contaminated sediments

Sediment Characterisation		
pH	6.51	
Humidity	8.56%	
Total Carbonates	Not detected	
Bulk Density	2.92g/cm3	
Initial Metal Concentrations		
Metal	Rep 1 (µg/g)	Rep 2 (µg/g)
Mn	842.83	828.98
Fe	131115.51	132835.34
Co	19.96	16.53
Cu	39.00	37.95
Zn	1310.69	1188.21
Ga	5.87	5.89
As	100.42	64.28
Pb	510.96	476.75

Pb and Fe Leaching at pH 4 and pH 1

As observed in the results in tables 2, there was neither Pb nor Fe leaching at pH 4 and the pH tended to increase to the original sediment pH of 6, however, at pH 1 using HNO3, considerable metal leaching was achieved.

Table 2: Pb and Fe Leaching at pH 4

Metal Analysis Results for Leaching Experiments			
At pH 4			
Sample	pH	Pb (mg/L)	Fe (mg/L)
HNO3-Blank	4.06	0.13	0
HNO3-1	4.06	0.12	0.244
HNO3-2	5.17	0.14	0.069
HNO3-3	5.39	0.16	0.394
HAc-Blank	3.84	0.22	0
HAc-1	5.24	0.2	0.045
HAc-2	5.54	0.22	0.009
HAc-3	5.76	0.23	0.064
At pH 1			
HNO3-4	1.1	7.68	141.7
HNO3-5	1.12	6.96	117.98
HNO3-Blank	0.85	0.3	0

Based on these results, the pH was set automatically during the experiment. At the anode pH was set at 1 and at 4 at the cathode.

Pb Mobilisation Profile during the EKR

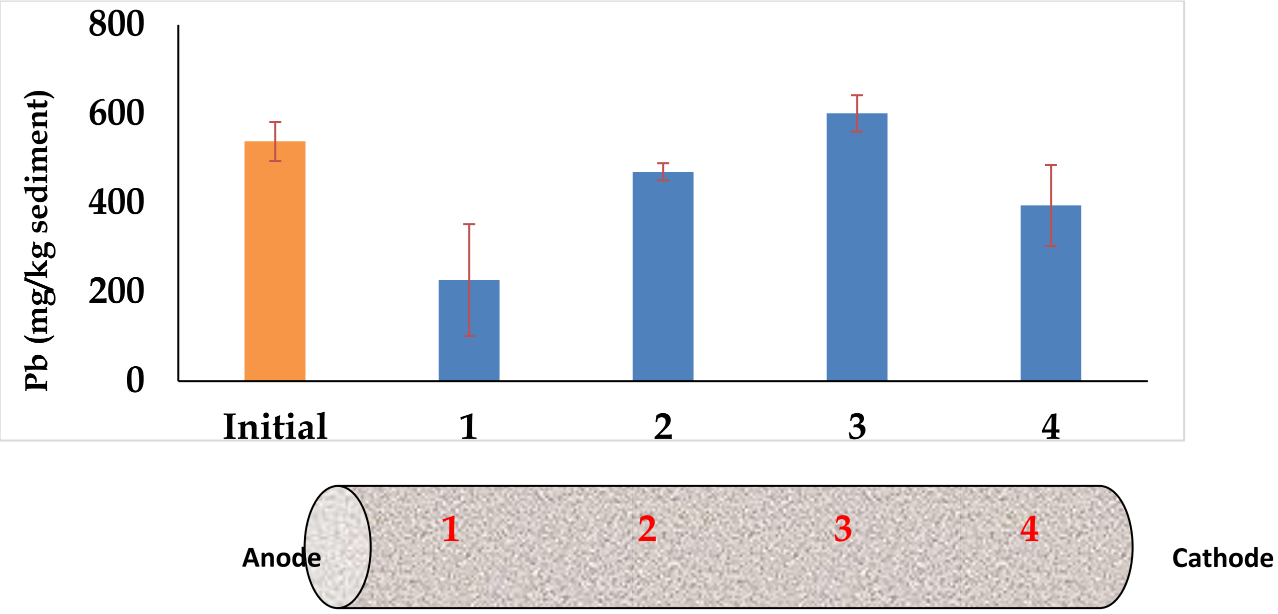


Figure 2: Pb Mobilisation during EKR Experiment

Figure 2 presents Pb concentrations in the initial sediments and in the various sediment portions from anode to cathode following the EKR. It can be observed, as expected that the highest mobilisation was obtained closest to the anode. This experiment was carried out for a period of 10 days which was insufficient to achieve a complete migration. However considerable metal mobility for Pb was obtained showing that EKR constitutes a good option for metal removal from these sediments and mass balance for Pb in the sediments and at the electrodes as against initial Pb concentrations was approximately 80% at the end of the EKR. Also, considerable Iron accumulation was obtained at the cathode as observed in picture 2.

Picture 2: Cathode compartment dismantled after the EKR Experiment (Considerable Fe accumulation at the cathode can be observed)

Metal Mobility Profile

The sequential extraction profile for Pb in each portion of the treated sediment as well as in the initial sediments is presented in figure 3. Pb accumulation was highest in the reducible form in the sediments and this could be justified by the high Fe concentration (over 14000 ppm) in the sediments. The concentrations in the other fractions are very low and in residual fraction it seems to remain relatively stable.

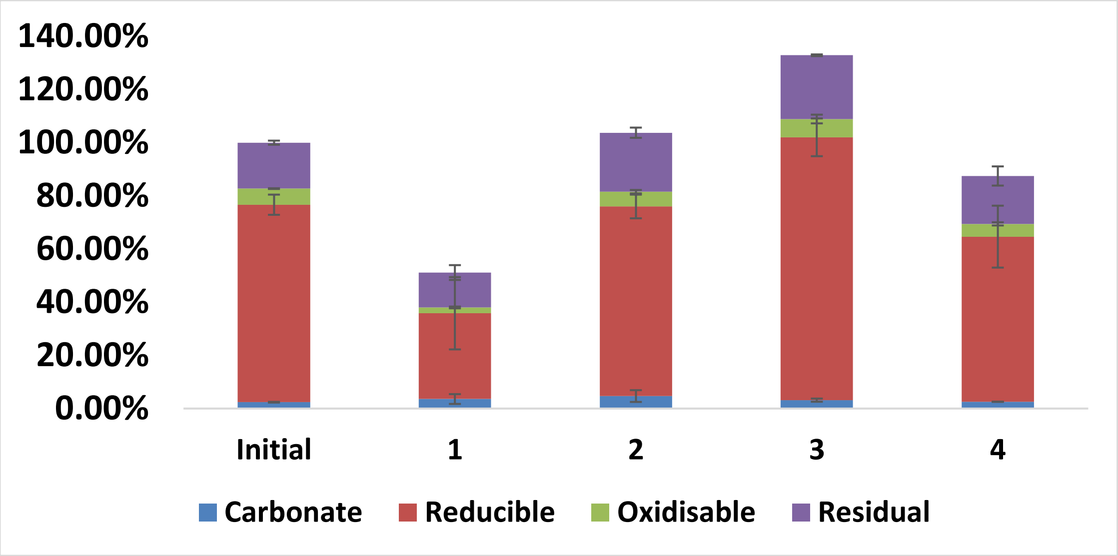
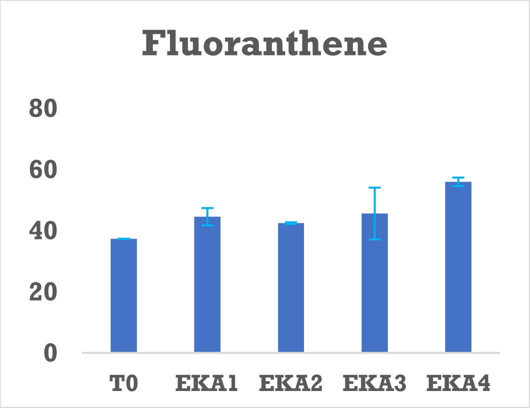
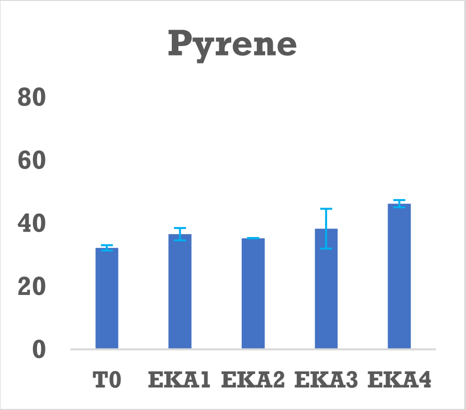


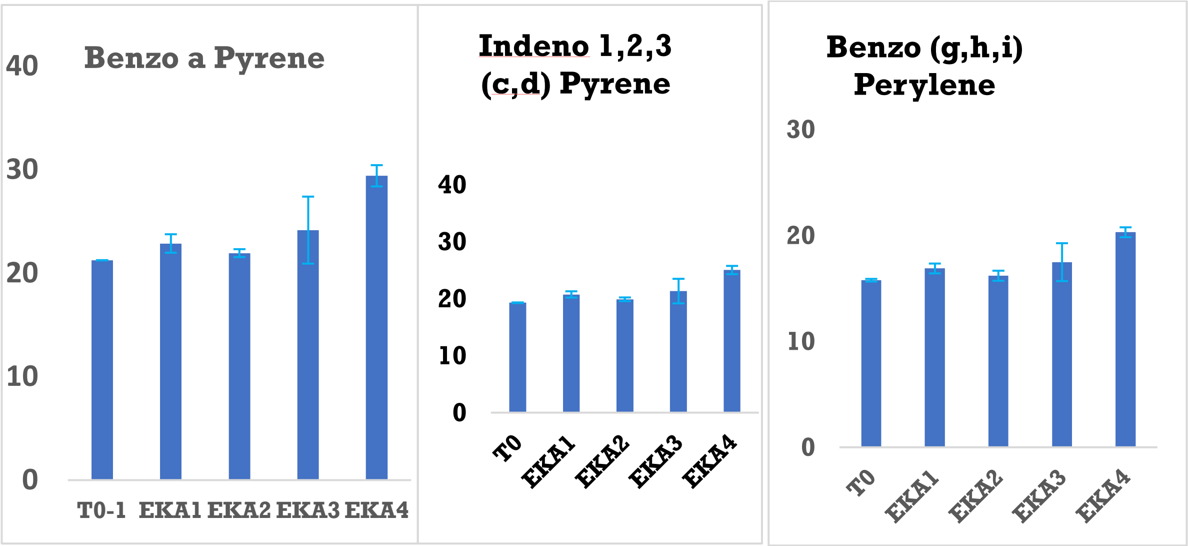
Figure 3: Pb Partitioning Profile in Initial Sediments and in each portion of treated Sediments

PAH Mobility Profile during the EKR

PAH are known to be strongly recalcitrant and hydrophobic compounds. However, in the present study despite no addition of enhancing agents, there seemed to be some PAH mobilisation towards the cathode. This was observed essentially with HMW PAH such as fluoranthene, pyrene, benzo(a)pyrene, benzo(a)anthracene, indeno (1,2,3) c,d pyrene.







Figures 3a, b, c, d, e: PAH Mobilisation during EKR Experiment

Conclusion

This study confirms that EKR is a promising approach for metal mobilisation and specifically for the contaminated sediments used for this work. In this study, Over 50% of the lead was mobilised within 10 days in the first portion of sediments closest to the anode alongside other metals such as iron which could be identified by a characteristics reddish colour at the cathode. Fe is known as a binding agent to various inorganic (mostly other metals) as well as organic contaminants (including PAHs) in sediments due to its strong reductive and absorption properties (Pardo et al., 2016; Sherman and Randall, 2003). It has been proposed in various studies, as a remediation agent for contaminant immobilisation in soils and sediments (Cundy et al., 2008; von der Heyden and Roychoudhury, 2015). Hence, it is most likely that various other contaminants including PAHs are bound to the highly concentrated iron in these sediments where Fe concentration was over 130 g/kg. Results of Pb partitioning analysis seem to confirm this hypothesis since over 60% of the Pb was found accumulated in the reducible Fe oxide fraction of the sediments. In other words, one efficient approach in the treatment of these sediments might be by targeting the Iron since iron mobilisation could induce the mobilisation of various other contaminants bound to it, among which Pb and most likely as indicated in this study. However, due to the high level of contamination in the sediments, to achieve complete metal mobilisation towards the cathode would require several weeks of constant current application.

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FINAL CONCLUSION

During these three years of research work, various remediation approaches were investigated for PAH/Toxic metal removal in the highly contaminated Bagnoli sediments. These include essentially bioremediation methods with a lesser extend of physico-chemical process to achieve lower environmental cost, social acceptability, and technical feasibility on the field. The goal althrough was to investigate on how the sediments could be remediated in a most sustainable, efficient and practical way with potential beneficial re-use of the treated sediments. Assessing the feasibility of different remediation strategies is critical to determine a most adequate remediation approach since every contaminated site has unique characteristics and there can not be a generic remediation solution. This is even more relevant especially when dealing with scenarios such as that of the Bagnoli sediment, which have been heavily contaminated with recalcitrant compounds such as PAHs and toxic metals for over 100 years.making them particularly complex to remediate Nevertheless, a number of methods investigated in this study have shown to be promising approaches for the Bagnoli sediment remediation and further experiments are necessary to determine their optimum application conditions. Also, investigating these methods at pilot scale is necessary to further validate their efficiency and also help overcome the problem associated with the high heterogenicity of the sediments.