

Review

Bioremediation for the recovery of oil polluted marine environment, opportunities and challenges approaching the Blue Growth



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ABSTRACT

The Blue Growth strategy promises a sustainable use of marine resources for the benefit of the society. However, oil pollution in the marine environment is still a serious issue for human, animal, and environmental health; in addition, it deprives citizens of the potential economic and recreational advantages in the affected areas. Bioremediation, that is the use of bio-resources for the degradation of pollutants, is one of the focal themes on which the Blue Growth aims to. A repertoire of marine-derived bio-products, biomaterials, processes, and services useful for efficient, economic, low impact, treatments for the recovery of oil-polluted areas has been demonstrated in many years of research around the world. Nonetheless, although bioremediation technology is routinely applied in soil, this is not still standardized in the marine environment and the potential market is almost underexploited. This review provides a summary of opportunities for the exploiting and addition of value to research products already validated. Moreover, the review discusses challenges that limit bioremediation in marine environment and actions that can facilitate the conveying of valuable products/processes towards the market.

1. Introduction

The Blue Growth is the long-term strategy designed to support sustainable growth in the marine and maritime sectors (COM, 2014). It is based on the potential of seas and oceans as drivers for the European economy and it is expected to provide economic and social benefits by the exploitation of marine resources. Although environmental safety is a precondition for the Blue Growth strategy, the marine environment is still highly affected by anthropic-derived activities such as untreated wastewater discharge, agriculture, shipping, port activities, aquaculture, offshore oil exploration, consumption of fossil fuels, industrial activities, the most part of which is potentially a source of oil-pollution (Reker et al., 2014; UNESCO-IOC, 2021). Indeed, several efforts on the use of renewable energy have been made, however, the global use of petroleum in the world is predicted to continue at least until 2040 (Conti et al., 2016). On the other hand, there are many reports of accidents occurring during oil extraction, refinery, naval operations, and oil

transport up to nowadays as in the Indian Ocean (Mauritius, July 2020), Gulf of Mexico (Deepwater Horizon oil spill, April 2010, Louisiana November 2023), and Norilsk (Russia June 2020) (Gomez and Sartaj, 2013; Khudur et al., 2015; O'Brien et al., 2004). The best known oil-spills have been caused by oil-tankers or off-shore platforms (Faticanti, 2011), and because of the massive amounts of oil spilled, they generated a huge public and media interest. In parallel, many marine areas also suffer chronic oil-pollution due to continuous discharge of oil (e.g., urban runoff and boat bilge) and oil-polluted sediments which can release hydrocarbons on the surface leading to toxic conditions which in turn affect the inhabiting biota (Brussaard et al., 2016; EPA, 2007). Oil pollution in the marine environment impacts human and animal health, but it has also deleterious effects on biodiversity, marine industries such as fishery, aquaculture, desalination plants, salt production facilities, coastal engineering and shipbuilding, tourism, recreational activities, and cultural/natural goods; finally, the affected regions suffer a significant socio-economic decline also in terms of jobs (ITOPF, 2014).

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Consequently, oil pollution in the marine environment affects a wide range of stakeholder categories (Fig. 1) directly connected to the intervention emergency plan or interested in enterprise business or social affairs as well as for scientific interest as clean-up companies, oil industries, governmental authorities, research institutions, civil society, and non-governmental organizations (NGOs) (Diplock et al., 2010).

The economic loss caused by oil pollution within the overall chronic pollution in marine environment is very difficult to estimate. Instead, despite the great variability on the environmental impact of oil spills due to spill size, season, oil type, currents, and distance from the shores, Montewka et al. (2013) have estimated costs of clean-up operation (oil containment and collection) in case of big disasters, close to 13 million euros for moderate spills and 98 million euros for severe spills. As an example, according to the different treatments used, the costs for soil bioremediation have been estimated to be significantly lower than those related to other remediation technologies, that include transportation, incineration, and final disposal (Nedoroda et al., 2022; Orellana et al., 2022). Most emergency response systems, apart from the use of dispersants to enhance naturally occurring biodegradation processes, do not include environmental restoration actions (O'Brien et al., 2004). Generally, well organised National contingency plans start by an active response to the first alert, of which promptness is essential for success. This is based on the deployment of human, infrastructural (vessels) and equipment resources (EMSA, 2012; Tewari and Sirvaiya, 2015).

Conventional contingency plans refer to clean-up operations such as containment, adsorption and/or collection (Gomez and Sartaj, 2013; O'Brien et al., 2004) but usually lack guidelines for in situ or ex situ treatments, which instead could significantly ameliorate the procedures and are often necessary for environmental restoration (ex. for chronically polluted areas) (Atlas and Hazen, 2011).

However, the European Blue Growth strategy, while encouraging marine and maritime activities, requires a parallel sustainability plan, such as the development of innovative technologies aimed at safeguarding and/or recovering marine areas exposed to risk of pollution (De Vet et al., 2016).

In this view, the present article reports some consideration on the opportunities and gaps on bioremediation in marine environment both in terms of management and research challenges.

2. Bioremediation: principles and current strategies

During the last 20 years, research breakthroughs have broadened perspectives in the use of biological processes for breaking down hazardous pollutants into less toxic or non-toxic substances or to environmental levels below concentration limits established by regulatory authorities (de Lorenzo et al., 2016; Yakimov et al., 2007). Bioremediation strategies present several advantages compared to classical remediation techniques (Fig. 2) (Tekere, 2019). Although an accurate comparison of remediation technology costs in different scenarios is currently difficult (e.g. site-specific geologic, geochemical, and contaminant conditions), bioremediation is considered the most cost-effective remediation technology (Landa-Acuña et al., 2020) with costs that vary from 5 to 300 \$ per cubic meter, according to the different techniques utilized for bioremediation. Physico-thermal treatments and incineration cost about 600 \$ and 2000 \$ per cubic meter, respectively and are thus significantly higher than bioremediation costs (Bianco et al., 2023). Regarding bioremediation, a major price component relies on the dredging, transport, and storage of the sediments, for ex situ bioremediation. By contrast, in situ procedures, generally require minimal equipment and effort since native or specifically designed microbial communities play a major role also favouring the ecosystems resilience (Yakimov et al., 2005, Bagi et al., 2014, Genovese et al., 2014). Moreover, since bioremediation is based on the use of microorganisms or microbial products and does not foresee chemical treatments, it is positively perceived by public opinion compared to other remediation strategies (Prince, 1999; Sharma, 2012). Downsides of the in situ bioremediation rely on the time required for optimal degradation of polycyclic aromatic hydrocarbons (PAHs) that can go up to several months, and on the heavy dependence of environmental factors and oxygen availability (Bianco et al., 2023). Bioremediation strategies can be divided according to the biological nature of the agent used and are generally referred to as bioaugmentation (the addition of hydrocarbons-degraders biomass strengthening the native potential) or biostimulation (the addition of nutrients or other additives in the environment to stimulate indigenous hydrocarbons-degraders) (Tyagi et al., 2011). Microorganisms are the core of both processes (Abatenh et al., 2017). In addition, the recent technologies have allowed scientists to unveil and exploit the peculiarity of hydrocarbon-degrading bacteria for new applications in sectors other than bioremediation; Table 1 shows some examples.

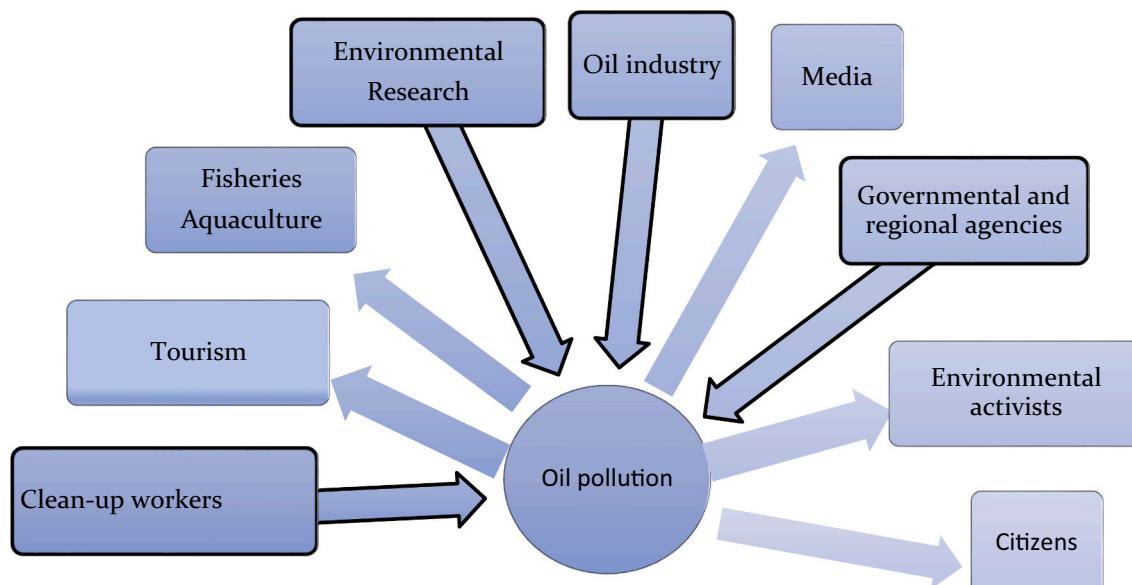


Fig. 1. Stakeholders in the field of oil-pollution in marine environment. The bold block indicate the sectors directly involved in the clean-up operation.

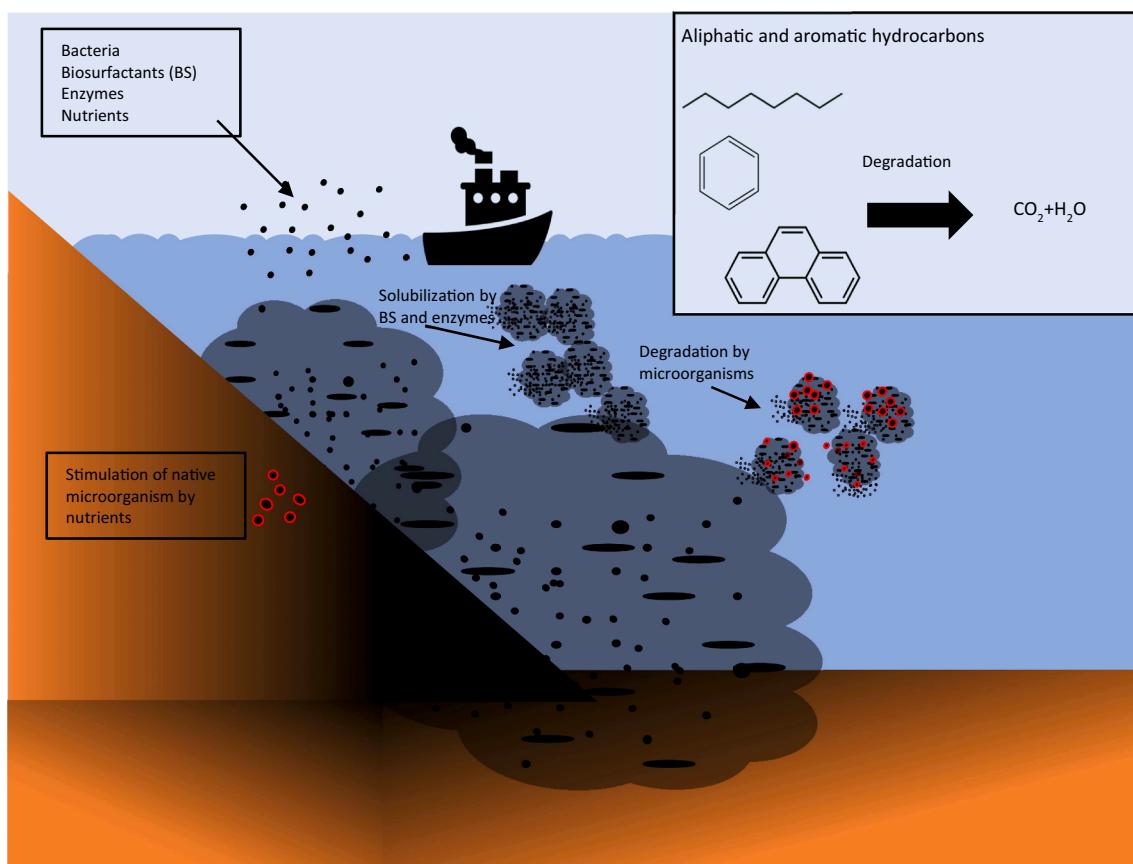


Fig. 2. Examples of marine bioresources applications for bioremediation of hydrocarbons in seawater.

Table 1

Example of marine bioresources relevant for *bioremediation* in marine environment and in other sectors.

	Products	Application in bioremediation field	Application in other sectors	Ref.
Bioaugmentation	Microorganisms: •hydrocarbon-degrading bacteria	Harbors restoration, Wastewater treatments, Bilge water treatments, seawater and marine sediments treatments, produced waters (Refineries). Oil-spill remediation	Biosensors Functional Genes providers	(Kanoh et al., 2008; Kumari et al., 2012; Sevilla et al., 2015)
Biostimulation	Enzymes: •Oxidoreductase, monooxygenase, dioxygenase, laccases, peroxidase Biosurfactants: •Glicolipids, lipopeptides, Fatty acids Siderophores: •Amphifilic siderophores	Pollution control, oxygenation of hydrocarbons, biosensors Oil spill cleanup Bioremediation	Polymer synthesis, textile, food and wood processing, pharmaceutical and chemical, biotransformation Antibacterial, antifungal activity, industrial cleaning, leather, paper and metal industries, textiles, cosmetics, pharmaceutical industry. Plant growth and biocontrol, Optical biosensor, drug-delivery, bio-bleaching of pulps.	(Martínez et al., 2017; Nolan and O'Connor, 2008) (de Cássia et al., 2014; Fakruddin, 2012); (Denaro et al., 2014; Saha et al., 2016)

2.1. Marine resources for bioremediation

2.1.1. Microorganisms

Hydrocarbon-degrading bacteria have been largely studied for their capability to consume hydrocarbons either as specialized microorganisms (the so-called 'hydrocarbonoclastic bacteria' that use hydrocarbons as the sole source of carbon and energy) or generalists (those that co-metabolize hydrocarbons together with other substrates) (McGenity et al., 2012). Those bacteria are targeted by the chemical industry for the high biotechnological potential of their genes (Habe and Omori, 2003; Nyssönen et al., 2009), enzymes (Kadri et al., 2018; Peixoto et al., 2011), siderophores (Denaro et al., 2014; Martinez et al., 2000), and biosurfactants (BS) (Bami et al., 2022).

Among the factors affecting the fate of hydrocarbons in the marine

environment, microbial communities naturally occurring in the hydrocarbon-rich environments have exhibited a determinant role (Liu et al., 2017; Newton et al., 2013). Therefore, the concept of site-specificity is especially relevant for *in situ* bioremediation (Alori et al., 2022). In particular, the exploitation of the natural microbial community already adapted to the site pollution improves the biodegradation performance (Bargiela et al., 2015) and avoids spreading foreign bacterial species. Furthermore, the huge repertoire of skills of marine microorganisms for the degradation of wide range hydrocarbon fractions, both as a single strain or in consortium, was widely demonstrated (Crisafi et al., 2016; Meyer-Cifuentes et al., 2020; Rojas-Vargas et al., 2022). Although these microorganisms are ubiquitous in marine environments (Yakimov et al., 2007), the biogeography of the specific groups is mainly dictated by environmental parameters, as an example

Table 2

Selection of bacterial strains able to degrade hydrocarbons.

Bacterial genus	Hydrocarbons	Reference
<i>Alcanivorax</i> sp.	Aliphatics alkanes up to C32 and branched aliphatic, as isoprenoid hydrocarbons, alkylarenes and alkylcycloalkanes, cycloalkanes	(Yakimov et al., 1998)
<i>Marinobacter</i> sp.	Aliphatics Alkanes C16-C20, heptadecano, tetradecano, dodecylbenzene, phenanthrene, pristine fluoranthene,	(Gauthier et al., 1992)
<i>Halomonas</i> sp.	Aromatics Diesel fuel, alkanes C11-C22	(Melcher et al., 2002)
<i>Novosphingobium</i> sp. alfa	Aromatics Pyrene, benz(a)anthracene, benz(b) fluoranthene, benzo(a) pyrene	(Xu et al., 2018)
<i>Alteromonas</i> sp. g	Aliphatics and Aromatics Naphthalene, phenanthrene	(Teramoto et al., 2013)
<i>Oleiphilus</i> sp. g	Aliphatics aliphatic hydrocarbons C11 —C20, alkanoates alkanoates	(Golyshin et al., 2002)
<i>Oleispira</i> sp. g	Aliphatics aliphatic hydrocarbons C10-C18 and their fatty alcohols and acids, cycloalkanes	(Yakimov et al., 2003)
<i>Pseudoalteromonas</i> sp. g	Aliphatics and aromatic Naphthalene, 1/2 methylnaphthalene, biphenyl, phenanthrene, fluorine linear alkanes (decane, tetradecane and eicosane), branched alkanes (pristane and squalane)	(Liu et al., 2017)
<i>Thalassolituus</i> sp. g	Aliphatics C12–C32 aliphatic hydrocarbons and their oxidized derivatives	(Yakimov et al., 2004)
<i>Neptunomonas</i> sp. g	Aromatics Naphthalene, phenanthrene, 1-methylnaphthalene, 2-methylnaphthalene, 2,6 dimethylnaphthalene	(Hedlund et al., 1999)
<i>Roseobacter</i> sp. a	Aliphatics and Aromatics	(Viggør et al., 2013)
<i>Cycloclasticus</i> sp. g	Aromatics Naphthalene, phenanthrene, methylnaphthalene, fluorene, anthracene, biphenyl, acenaphthene, fluoranthene, pyrene, chrysene, benzo(a) pyrene	(Dyksterhouse et al., 1995)
<i>Eribacter</i> sp. a	Aromatics	(Zhuang et al., 2015)
<i>Oleibacter</i> sp. g	Aliphatic	(Teramoto et al., 2011)
<i>Thalassospira</i> sp. a	Aromatic	(Demanèche et al., 2004)
<i>Sphingomonas</i> sp.	Aromatic	(Hackbusch et al., 2020)
<i>Rhodococcus</i> sp. PC20 a	saturated and aromatic fraction of crude oil	(Fakhrzadegan et al., 2019)
<i>Vibrio</i> sp. strain NW4, <i>g Idiomarina</i> sp. strain BW32, <i>Kangiella</i> sp. strain DP40, <i>Marinobacter</i> sp. strain DW44, <i>Halomonas</i> sp. strain BS53, and <i>Vibrio</i> sp. strain DS35	Alkanes	(Murphy et al., 2021)
<i>Thalassolituus</i> and <i>Oleispira</i>	Alkanes	(Djhahit et al., 2019)
<i>Alcanivorax</i> , <i>Marinobacter</i> , <i>Pseudomonas</i> , <i>Gordonia</i> , <i>Halomonas</i> , <i>Erythrobacter</i> , <i>Brevibacterium</i> , <i>Xanthomarina</i>	Alkanes	
<i>Alcanivorax</i> , <i>Erythrobacter</i> , C1-B045, <i>Alteromonas</i> , <i>Pseudohongiella</i>	Alkanes	
<i>Luteibacter</i> g, <i>Parvibaculum</i> a and a genus belonging to <i>Alcanivoraceae</i>	Alkanes	(Uribe-Flores et al., 2019)
<i>Pseudomonas aeruginosa</i> and <i>Rhodococcus erythropolis</i>	Aliphatic and aromatic hydrocarbons	(Gao et al., 2019)
<i>Gordonia amicalis</i>	Alkanes	(Kumari et al., 2023)
<i>Psychrophilic</i> strains <i>Flavobacterium psychrolimnae</i> LMG22018 and <i>Flavobacterium petrolei</i> sp.	Diesel	(Delegan et al., 2019)
<i>Malikia spinosa</i>	Benzene and pyrene	(Révész et al., 2020a)
<i>Acinetobacter</i> spp.	Aliphatic and aromatic hydrocarbons	(Révész et al., 2020b)
<i>Cupriavidus</i> sp. OPK bet	Crude oil	(French and Terry, 2019)
<i>Pseudomonas stutzeri</i> (<i>P. stutzeri</i>), <i>Bacillus simplex</i> (<i>B. simplex</i>) and <i>Bacillus pumilus</i> (<i>B. pumilus</i>)	Aromatic hydrocarbons	(Veluppillaimani and Muthaiyan, 2019)
<i>Rhodococcus</i>	Aromatic	(Puntus et al., 2019)
<i>Halomonas</i> sp., <i>Vibrio gazogene</i> , <i>Marinobacter hydrocarbonoclasticus</i> SP17	Aliphatic	(Alabresm et al., 2018)
<i>Alteromonas</i> and <i>Thalassospira</i>	Aromatic and aliphatic	(Bacosa et al., 2018)
<i>Streptomyces parvus</i> B7	Crude oil	(Parthipan et al., 2018)
<i>Pseudomonas resinovorans</i> , <i>Plantibacter auratus</i> , <i>Bacillus subtilis</i>	Aromatic and aliphatic	
<i>Stenotrophomonas maltophilia</i> , <i>Microbacterium esteraromaticum</i> and <i>Pseudomonas aeruginosa</i>	Aromatic	(Kumari et al., 2018)
<i>Gordonia amicalis</i>	Aromatic and aliphatic	(Delegan et al., 2019)
<i>Cobetia marina</i> , <i>Rhodococcus soli</i> , <i>Pseudoalteromonas agarivorans</i>	PAH	(Lee et al., 2018a)
<i>Rhodococcus</i> Y2-2		
<i>Brevibacillus</i> sp., <i>Microbacterium oxydans</i> , <i>Methylobacterium persicinum</i> , <i>Achromobacter xylosoxidans</i>	Aromatic	(Godini et al., 2018)

Modified from Wanapaisan et al. (2018).

temperature is one of the main selective factors (Perez Calderon et al., 2019; Xu et al., 2017; Yakimov et al., 2022).

Recent studies have described the distribution of hydrocarbon-degrading bacteria at regional level; as an example, in deep-sea sediments signatures belonging to genera *Pseudomonas*, *Acinetobacter*, *Achromobacter* sp., *Sphingobium* sp. (Tomasino et al., 2021), *Parvibaculum*, *Erythrobacter*, *Alcanivorax*, *Marinobacter*, *Halomonas*, *Colwellia* (Perez Calderon et al., 2019)) were observed. In coastal seawater and sediments located in close proximity to refineries or harbors in temperate regions, gamma-Proteobacteria especially from the genera *Alcanivorax*, *Thalassolituus*, *Cycloclasticus*, *Pseudomonas*, *Oleibacter*, *Marinobacter*,

Colwellia, *Shewanella*, *Oleiphilus* were detected most frequently (Cravo-Laureau and Duran, 2014; Yakimov et al., 2005).

Coastal anoxic sediments showed microbial signatures attributable to the *Desulfosarcina/Desulfococcus* group (Desulfovibacteracee, delta-proteobacteria) as key players of the initial step of anaerobic short-chain and long-chain alkane degradation in marine seep (Acosta-González and Marqués, 2016; Kleindienst et al., 2014).

Signatures belonging to the genera *Cycloclasticus*, *Oceanospirillales*, *Pseudoalteromonas*, *Sulfitobacter*, *Thalassospira* and *Roseobacter* have been detected in oceanic blooms (Hazen et al., 2016). The ecological succession that occurs after an oil-spill in coastal sediment typically

Table 3

Selection of patents based on bacterial hydrocarbons-degrading strains.

Petroleum hydrocarbons components	Bacterial genus	Origin	No. patent (Villela et al., 2019)	Scientific articles
Aliphatics, Aromatics Asphaltenes and resins	<i>Pseudomonas</i> sp.	Soil	114	(Jiménez et al., 2004; van Beilen et al., 2004; Kasai and Harayama, 2004, Wang et al., 2016) Kasai and Harayama, 2004
Aliphatics, Aromatics Asphaltenes and resins	<i>Bacillus</i> sp.	Soil	75	(Borah and Yadav, 2014; Kato et al., 2001; Maddela et al., 2017; Rojas-Avelizapa et al., 2002)
Aliphatics and Aromatics Asphaltenes and resins	<i>Rhodococcus</i> sp.	Soil	60	(Cappelletti et al., 2015; Cejková et al., 2005; Kim et al., 2002; Zampolli et al., 2014)
Aliphatics, Aromatics Asphaltenes	<i>Acinetobacter</i> sp.	Soil	43	(Bihari et al., 2007; Mishra et al., 2001; Throne-Holst et al., 2007)
Aliphatic Aromatics	<i>Arthrobacter</i> sp. <i>Mycobacterium</i> sp.	Soil	37 19	(Ionata et al., 2005; Lee et al., 2018a, 2018b) (Dudhagara et al., 2016; Hennessee and Li, 2016; Johnsen et al., 2006) Zeng et al., 2010
Aliphatic and Aromatics Aromatics	<i>Nocardia</i> sp. <i>Micrococcus</i> sp.	Soil	17 16	(Baek et al., 2006; Haritash and Kaushik, 2016, Le et al., 2010) (John et al., 2012; Mirdamadian et al., 2010)
Aliphatic and Aromatics Aromatics	<i>Alcaligenes</i> <i>Achromobacter</i>	Soil	16 16	(Ashok et al., 1995) (Dave et al., 2014; Hong et al., 2017; Peng et al., 2015)
Aliphatics	<i>Alcanivorax</i> sp.	Marine	11	(Denaro et al., 2014; Kadri et al., 2018; Schneiker et al., 2006; Scoma et al., 2016; Sevilla et al., 2017; Yakimov et al., 2019; Yakimov et al., 1998; Yakimov et al., 2007)
Aliphatics	<i>Marinobacter</i> sp.	Marine	9	(Abed et al., 2014; Branchu et al., 2017; Ennouri et al., 2017; Klein et al., 2010; Mounier et al., 2014)
Aliphatics	<i>Ochrobactrum</i> sp.	Soil	8	(Nozari et al., 2018)
Aromatics	<i>Halomonas</i> sp.	Marine	6	(Mnif et al., 2009)
Aliphatics	<i>Dietzia</i>	Soil	5	(Bodtker et al., 2009; Wang et al., 2011)
Aromatics	<i>Sphingomonas</i> sp.	Soil	5	(Chen et al., 2008; Ley et al., 2004; Luo et al., 2012; Story et al., 2004)
Aliphatics	<i>Brevibacterium</i> sp.	Soil	4	(Pavitran et al., 2004)
Aromatics	<i>Novosphingobium</i> sp.	Marine	4	(Lyu et al., 2014; Yuan et al., 2009)
Aliphatics and Aromatics	<i>Alteromonas</i> sp.	Marine	2	(Gutierrez et al., 2018; Jin et al., 2012; Math et al., 2012)
Aliphatics	<i>Azoarcus</i> sp.	Soil	1	Patent N° WO2008/155302
Aliphatics	<i>Oleiphilus</i> sp.	Marine	1	Golyshin et al., 2002
Aliphatics	<i>Oleispira</i> sp.	Marine	1	(Kube et al., 2013; Yakimov et al., 2003)
Aliphatics	<i>Pseudoalteromonas</i> sp.	Marine	1	(Hedlund and Staley, 2006; Liu et al., 2017)
Aromatics	<i>Cycloclasticus</i> sp.	Marine	1	(Kasai et al., 2002; Wang et al., 2018) Teira et al., 2007 Staley, 2010 Messina et al., 2016 Dyksterhouse et al., 1995
Aliphatics	<i>Thalassolituus</i> sp.	Marine	nf	Dong et al., 2014 Golyshin et al., 2013 Gregson et al., 2018 Shtratnikova et al., 2018 Yakimov et al., 2004
Aromatics	<i>Neptunomonas</i> sp.	Marine	nf	Rong et al., 2016 Hedlund et al., 1999 Díaz-Ramírez et al., 2008
Aliphatics and Aromatics	<i>Roseobacter</i> sp.	Marine	nf	Wang et al., 2016 Prabagaran et al., 2007 Seo et al., 2009

involves autochthonous oil-degraders affiliated to the families Desulfovibraceae, Methylococcaceae, Methylophilaceae, Desulfuromonadaceae, or the genera *Hyphomonas*, *Pseudomonas*, *Marinobacter*, *Methylophaga*, *Sulfurimonas* (Bargiela et al., 2015). The genera *Oleispira*, *Colwellia*, *Shewanella*, *Polaribacter*, *Glaciecola*, *Psychrobacter* and *Pseudoalteromonas* are instead the most representative oil-degraders in polar regions (Martinez-Varela et al., 2022; Peeb et al., 2022). Neethu and colleagues reported two previously unnoticed taxa of oil-degraders, i.e. *Acinetobacter* in sediments and *Methylococcales* in seawater (Neethu et al., 2019). These unprecedented blooms draw attention towards the underexplored hydrocarbon degrading communities in tropical waters. *Oleibacter* sp. was also observed in tropical regions (Teramoto et al., 2011). Table 2 shows a selection of the 100 bacterial genera recognized by Wanapaisan et al. (2018) as hydrocarbon degraders in different environments, especially in marine matrices. They mainly belong to γ and α -Proteobacteria and exhibit specific aptitude in the degradation of several hydrocarbon fractions. Despite the wide repertoire of hydrocarbon-degrading metabolisms showed by marine bacteria, very few patents for marine microbe-driven oil bioremediation in seawater are available. Table 3 shows a selection of patents based on hydrocarbon-degrading bacteria. Villela et al. (2019) highlighted that

most of these patents are based on the use of bacteria (368 out of 500 patents) among which the most used belong to the genera *Pseudomonas*, *Rhodococcus*, *Acinetobacter*, and *Bacillus*. However, the proposed bacteria were not indigenous of contaminated areas and many of them refer to artificial systems and not in the field. The market currently offers some microbial products for hydrocarbon decontamination, such as nutrients or other biostimulant substances. Regarding the use of native bacterial consortia for the degradation of petroleum hydrocarbons in seawater, there are very few patents, one of which is focused on the bioremediation PAH in polar regions¹ (Perdigão et al., 2021).

In addition to bacteria, microalgae have also been occasionally used in oil bioremediation experiments and proposed as suitable candidates (Table 4). For example, *Nannochloropsis* sp., *Tetraselmis* sp., and *Chlorella* sp. were successfully used to remove emulsified oils from the liquid phase in the laboratory (Al-Zuhair et al., 2015; Kuttiyathil et al., 2021) and the potential of *Nannochloropsis* for oil removal has been also demonstrated on oilfield produced water (Ammar et al., 2018). However, since the abovementioned experiments are based on non-axenic

¹ <https://patents.google.com/patent/CA2673576C/en>

microalgal cultures, a direct role of microalgae in hydrocarbon-degrading activities is uncertain and culture-associated bacteria might have been involved in such processes. Microalgae are likely to play an indirect role in hydrocarbon degradation, by providing oxygen to their surrounding environment thus promoting bacteria-driven degradation activities. For example, the oil-tolerant microalga *Scenedesmus obliquus* was found to carry out a complete degradation of alkylcycloalkanes and alkylbenzenes and a partial degradation of PAHs when in co-culture with four oil-degrading bacteria, whereas no degradation was observed in the axenic treatment (Tang et al., 2011). Axenic microalgal cultures can partially degrade selected compound classes such as phenols (Zhang et al., 2022) or phthalates (Vingiani et al., 2022; Zhang et al., 2019) but degradation rates are significantly lower than those observed in experiments based on bacteria or microalgae/bacteria consortia (Yi et al., 2020). The advantage of using microalgae/bacteria consortia rather than bacteria alone for oil bioremediation is linked to the positive influence of microalgae in sustaining and enhancing bacterial growth by supplying organic exudates and oxygen and on the fact that only dissolved nitrogen, phosphorus, and micronutrients are required for culturing such consortia (Denaro et al., 2021).

2.2. Products from marine bacteria

The addition of microorganisms is not always an applicable strategy due to the legal restriction or incompatibility of the microorganisms with the contaminated environment. In many cases, the autochthonous bacteria might be better adapted to cope with hazardous contaminants. The addition of nutrients and other molecules (carbon, nitrogen, or phosphorus sources) to a contaminated environment to stimulate the metabolic activity of endogenous microorganisms is called bio-stimulation (Andreoli et al., 2015; Michel, 2015). Biostimulation is regarded as an effective remediation strategy with the advantage of not altering the taxonomic structure of the bacterial community. The artificial addition of nutrients to the natural system, which enhances its self-cleaning capacity, has lower environmental impact than any other chemical treatments (Crisafi et al., 2016).

2.2.1. Biosurfactants

BS are very important additives for hydrocarbon bioremediation. They are emerging as a clean alternative to chemical dispersants, which are specific molecules able to break oil into readily biodegradable small droplets (Krishnaswamy et al., 2008). BS are gradually replacing their synthetic counterparts in many fields because of their low toxicity, high biodegradability, and tolerance to extreme conditions (Naughton et al., 2019; Olasami and Thring, 2018).

Generally, there is a distinction between high molecular weight polymeric (HMW) BS, and low molecular weight (LMW) BS. HMW BS consist of polysaccharides, proteins, lipopolysaccharides, lipoproteins, or complex mixtures of these compounds (Rosenberg and Ron, 1997). These molecules have generally interesting emulsifying activities. LMW BS are generally classified into groups based on the chemical structures: i) fatty acids and phospholipids that can display interesting activities; ii) lipopeptides, derived from amino acids that possess also interesting bioactivities and are mainly produced by *Bacillus*, *Lactobacillus*, *Streptomyces*, *Pseudomonas*, and *Serratia* strains (Inès and Dhouha, 2015); iii) glycolipids that consist of mono- or oligosaccharides and a lipid moiety, and are well-known for relatively easy large-scale production (Hausmann and Syldatk, 2015).

BS production by many marine bacteria is induced by the presence of available hydrocarbon sources. In fact, BS can significantly increase hydrocarbon bioavailability thus increasing degradation rates (Thavasi R et al., 2014). After the marine oil spill from deep water horizon in the Gulf of Mexico it was observed that marine bacteria able to produce BS became predominant in the area. Many of these strains belonged to the genera *Alteromonas*, *Halomonas*, *Alcanivorax*, *Colwellia*, *Cycloclasticus* and *Pseudoalteromonas* (Mapelli et al., 2017).

The addition of BS to improve oil degradation is defined as “biosurfactant enhanced bioremediation” and it is considered as a promising method to promote the removal rate of petroleum (Ostendorf et al., 2019). Many HMW BS have been reported to be extremely effective in bioremediation. It is the case of Emulsan, the most powerful emulsion stabilizer produced by *Acinetobacter calcoaceticus* RAG-1 that coats these substrates, increases their bioavailability for microbial access, and degrades them by forming minicapsules. Emulsan works better with mixtures or aliphatic and aromatic hydrocarbons (Mujumdar et al., 2019). Ornithine lipids, another type of HMW BS produced by *Myroides* sp. SM1, exhibited interesting emulsifying activity for crude oil in a broad range of pH, temperature, and salinity (Maneerat et al., 2006).

LWM BS can also act as stable emulsifiers. The strain *Halomonas* sp. was shown to produce on its cell surface a mixture of glycolipids that were able to enhance the solubility of hydrocarbons and therefore their biodegradation rates. The emulsion was stable under a wide range of pH and temperatures making the use of these glycolipids suitable for extreme environments. Among glycolipids it was shown that the addition of rhamnolipids, a class of glycolipids produced by many *Pseudomonas* strains, at the concentration of 15 mg/L significantly increased the removal efficiencies of PAHs. Specifically, rhamnolipids caused a significant increase in the degradation of 5- and 6-ring PAHs (Sponza and Gök, 2010). Several glycolipids extracted from *Marinobacter hydrocarbonoclasticus* strain SdK644 were found to be able to solubilize oil significantly better than Tween 80 (Zenati et al., 2018). Another molecule reported as surface active is the lipopeptide produced by *Paenibacillus dendritiformis* CN5 that was able to increase the removal of pyrene by a microbial consortium from increased from 16 % to 67 % at the concentration of 600 mg/L. The authors also observed that further increase in the BS concentration had negative effects on the pyrene degradation (Bezza and Chirwa, 2017). In another report, a phospholipopeptide from a *Marinobacter* species was identified and its emulsion was found to be stable for 30 months in extreme conditions of salinity and temperature and was able to disperse crude oil in artificial marine water (Raddadi et al., 2017).

Although marine BS have great potential for oil bioremediation, a current limitation to their broad use is the slow production yield in the native strains that significantly impacts the production costs. However, the identification of BS biosynthetic pathways that can lead to enhanced production in heterologous hosts, will be crucial for the further exploitation of these molecules in bioremediation.

2.2.2. Enzymes

Enzymes involved in the degradation of hydrocarbons are mainly represented by cytochrome P450s, laccases, hydrolases, oxygenases, dehydrogenases, and lipases, which have shown potential degradation of polymers, aromatic hydrocarbons, halogenated compounds, dyes, detergents, agrochemical compounds (Bhandari et al., 2021). The direct use of enzymes for biocatalysis has the advantage of obtaining faster results in the biodegradation of toxic substances with low impact on the environment and reduced energy consumption, all features that perfectly match the bioeconomy (Pellis et al., 2017). In addition, marine enzymes are adapted to face high salinity, large range of temperature and pH, metal ions, and high incidence of light and pressure (Fernandes et al., 2014). They have revealed peculiar biocatalysts applied in several fields as fine chemicals, pharmaceuticals, and for the feed and food industries (Dalmaso et al., 2015; Duarte et al., 2018) providing competitiveness and efficiency to different industrial processes (Birolli et al., 2019; May and Padgett, 1983), (Table 4). The bacterial enzymatic pathways are usually different for aliphatic or aromatic biodegradation (Abbasian et al., 2015). Aerobic degradation of n-alkanes in bacteria starts with the initial or terminal hydroxylation catalyzed by monooxygenases for short-chain n-alkanes (C2–C4), and AlkB alkane hydroxylase for medium- (C5–C11) or long-chain alkanes (>C12) (van Beilen et al., 2004). *Pseudomonas oleovorans* GPo1 enzymatic complex has been firstly described by van Beilen et al. (1994, 2002) and more

Table 4

List of microalgae able to degrade hydrocarbons.

Microalga	Initial hydrocarbon	Incubation time (days)	Removal efficiency	Reference
<i>Nannochloropsis</i> sp.	Glycerol	7	80 % oil	(Al-Zuhair et al., 2015)
<i>Tetraselmis</i> sp.	Glycerol	7	80 % oil	(Al-Zuhair et al., 2015)
<i>Chlorella</i> sp.	Crude oil	4	50 % TOC	(Kuttiyathil et al., 2021)
<i>Nannochloropsis</i> sp.	Oilfield produced water	20	90 % oil	(Ammar et al., 2018)
<i>Isochrysis galbana</i>	Oilfield produced water	20	80 % oil	(Ammar et al., 2018)
Mixed microalgal biofilm	TDS, TSS, COD	14	33 % TSS, 26 % TDS, 7.9 % COD	(Ugya et al., 2021)
<i>Scenedesmus obliquus</i> and bacteria (<i>Sphingomonas</i> , <i>Burkholderia</i> , <i>Pseudomonas</i> , <i>Pandorea</i>)	Crude oil	7	Almost complete removal of straight chain alkanes, alkylcycloalkanes, alkylbenzene, and PAH.	(Tang et al., 2011)
<i>Chlorella vulgaris</i>	Crude oil	7	71 % Light compounds ^a , 49 % heavy compounds	(Kalhor et al., 2017)
<i>Chlorella vulgaris</i>	Crude oil	14	94 % Light compounds, 88 % heavy compounds	(Kalhor et al., 2017)
<i>Chlorella kessleri</i>	Crude oil	13	Partial degradation of most alkanes	(Hamouda et al., 2016)
<i>Anabaena oryzae</i>	Crude oil	13	Complete degradation of most alkanes	(Hamouda et al., 2016)
<i>Chlorella vulgaris</i> ^b	Crude oil	18	Partial degradation of alkanes and PAHs	(El-Sheekh et al., 2013)
<i>Scenedesmus obliquus</i> ^b	Crude oil	18	Partial degradation of alkanes and PAHs	(El-Sheekh et al., 2013)
<i>Chlorella</i> sp.	Refinery produced water	15	73 % TOC reduction	(Das et al., 2019)

^a Light and heavy compounds of crude oil were discriminated based on their boiling point (350 °C).^b Heterotrophic cultures: carbon sources were supplied to the culture media and microalgae were incubated under dark conditions.

than 400 homologs have been described to date (Moreno and Rojo, 2019). Moreover, C5–C11 *n*-alkanes can be hydroxylated by soluble P450 cytochromes (van Beilen et al., 2003)). As an example, the recombinant cytochrome P450 CYP153A13a of *Alcanivorax borkumensis* showed a strong capability to hydroxylate aromatic compounds with terminal alkyl groups, including diphenyl, naphthalene, ibuprofen methyl ester and phenol derivatives (Otomatsu et al., 2010; van Beilen et al., 2003). As monooxygenase, dioxygenases are multicomponent enzyme systems that introduce molecular oxygen, their substrates are aromatic hydrocarbons and belong to a large family of Rieske non-heme iron oxygenases (Mason and Cammack, 1992). PAH-dihydroxylating dioxygenase genes from marine *Cycloclasticus* sp. strain and expressed in *E. coli* have exhibited efficient bioconversion for a variety of aromatic compounds with wide substrate preferences. Substrates, included substituted naphthalenes, were converted to natural products with one or more prenyl groups possessing anti-microbial, anti-oxidative, anti-inflammatory and anti-cancer activities (Shindo et al., 2007)). Dehydrogenases belong to the family of oxidoreductases, together with the enhancement of alkanes biodegradation processes, the application of dehydrogenase has been described for epoxidation reactions and bioremediation of steroids (Xue et al., 2018). Laccases (benzenediol oxygen oxidoreductases) are multi-copper-containing extracellular enzymes well-known for oxidizing various PAHs, phenols and aromatic amines to form quinones or oligomers, as well as to reduce molecular oxygen to produce water (Dai et al., 2021; Shindo et al., 2007). Wu et al. (2008) used fungal laccase to treat aged PAH-contaminated soils. Their results showed that laccase could degrade 15 priority PAHs (specified by the U.S. Environmental Protection Agency) achieving 60 % of benzo[a]pyrene degradation, already after 24 h. The most attentive biochemical properties of laccases are their stability under various conditions of pH, temperature, organic solvents, and salt concentrations (Arregui et al., 2019). Moreover, bacterial laccases secreted have also been related to numerous other biodegradation processes, including plastic polyethylene (Gollan et al., 2023; Santo et al., 2013; Zhang et al., 2022). Lipases catalyze both the synthesis and degradation of esters. As crude

oil degradation is a continuous process, the intermediate products from the conversion of *n*-alkanes along the pathway would produce the substrate needed for lipase and esterase enzymes to utilize. Hence, this would enable the monitoring of oil biodegradation (Adlan et al., 2020). Moreover, as a perspective, a lipase from the marine halophilic bacterium *Bacillus sonorensis* was tested as additive in the formulation of a detergent; it was also capable of removing corn oil from natural as well as synthetic fabrics dyed with a respective, preferred class of dyes (Nerurkar et al., 2013).

2.3. Bioremediation opportunities for society

Bioremediation can be considered one of the major topics within Marine Biotechnology sector (Hurst et al., 2016)). It is a fruitful field where scientific and industrial sectors can interact and, in this way, it promotes the entering of the research results within a value chain. Indeed, in the field of bioremediation, it is possible to identify products and services with interesting perspectives for environmental restoration and ultimately for society benefits (Table 5). The pipeline of the value chain for the commercialization of products and services is summarized in Fig. 3, also showing inter-sectorial collaborations within each step concerning specific activities during R&D phase, product/service development and the addressing to the market. The phase of product development can match the interest of private company, overall if the final product is targeted to the industry needs (as an example, specially designed microbial consortia and protocols for the biodegradation of specific waste products at specific conditions). In this view, it is noticeable that the use of databases consisting in huge amounts of information about strains and their pool of biodegradation pathways combined with a predictive approach based on *in silico* exploration can significantly reduce time and costs of the two first phases of the value chain. In this case, the value of the product is already captured and ready to be developed (starting from *in vitro* simulation).

Products or processes can be validated on a pilot scale, applying the selected bioremediation treatment, and observing both its efficiency and

environmental impact. The validation of the product/service will be carried out also under the supervision of official governmental authorities responsible for environmental protection and product approval. Technological cluster and network represent an interesting field where the interconnection between products/process suppliers and business are facilitated because of their co-presence in the same structure. At the same time, bridging professional figures are necessary to interweave different sectors and facilitate knowledge and technology transfer. Skills on the legal aspect of academia-industry collaboration (e.g., IP regulation) are also fundamental.

Bioremediation can virtually fit primary, secondary, tertiary, quaternary, and quinary economy sectors because it includes the transformation of raw material in basic products, formulation of special cocktails (BS, nutrients, and bacteria selection), manufacturing (supporting materials and treatments procedures, scale-up), and services (customer bioremediation design and implementation). Moreover, the application of bioremediation techniques requires knowledge and skills for complex processing and handling, technology transfer, as well as research and development (quaternary sector); and quinary sector, services that focus on the creation, re-arrangement, and interpretation of new and existing ideas or technologies (Francocci et al., 2020). Therefore, bioremediation is a very interesting opportunity both in terms of environmental safety and for new job perspectives. Hamilton (2012) describes a complete picture of the careers linked to the bioremediation. The author put in evidence that, due to the complexity of bioremediation processes in the natural environment, a broad range of competences to carry out adequate actions are necessary, e.g., to design, implement, monitor, evaluate the efficiency of the treatment as well as its impact on the environment and finally to make decision. Starting from the planning of the project, finishing to the implementation of the treatment, it is possible to identify several professional profiles: business specialists who work in environmental remediation, experts in regulation issues and cost estimation, or public relations; scientists and engineers for the characterization of the site. Workers in environmental remediation might be employed by companies; by management, scientific and technical consulting firms; for sanitation companies, in manufacturing, at a university, for many private companies, law firms, not-for-profit groups, or government agencies such as the EPA or the National Park Service. Moreover, a multidisciplinary interaction is necessary for the communication between different sectors and competences, including professional figures able to bridge academy and industry interests. Skills for dissemination to a wide audience are becoming more important as it is fundamental that science is properly communicated to the general public.

3. Challenges

Hydrocarbon-degrading bacteria have been described for nearly seventy years. Already in 1946, Zobell reported "Action of

microorganisms on hydrocarbons" describing occurrence, characteristics, biochemical activity and even perspectives for economic benefits. Nevertheless, bioremediation in the marine environment remains a commercial opportunity underexploited to date (Kalogerakis et al., 2015). European countries use in prevalence conventional methods (physical-chemical treatments) already standardized and overall consistent with legislation (Majone et al., 2015). The authorized environmental agencies whilst expressing their interest in new biotechnologies are required to abide by the existing memoranda officially approved. In this view, to strengthen bioremediation as a real opportunity within the blue growth strategy, one of the major challenges is the harmonization among the regulation and legal aspects connected to the intervention strategies against oil pollution and the innovative technologies efficient, cheap, and low impact. As an example, in many countries, the use of autochthonous bacterial consortia is approved for certain soil treatments, but it is not allowed in marine environments and the lack of the field tests reduces drastically the availability of robust data supporting the evaluation of the treatment effectiveness and of its impact on the natural environment (Frascari et al., 2014). Moreover, studies concerning this aspect, put in evidence that regulation on the field of bioremediation regards mainly genetically modified microorganisms (Atlas and Cerniglia, 1995; Bakst, 1991). Indeed, the conventional methods used for the soil bioremediation are today often replaced by advanced techniques, such as the use of genetically modified organisms (GMOs). Genetic engineering techniques can lead to highly efficient microorganisms able to degrade pollutants at higher rates (Yuanfan et al., 2010); moreover, GMOs can be also designed to use toxic bioremediation intermediates. Genetic modification can increase enzyme specificity, by upregulating or downregulating specific metabolic pathways (Basarkar et al., 2022). Petroleum is a complex mixture of different hydrocarbons; therefore, the construction of engineered bacteria capable of degrading various petroleum hydrocarbons could be an important direction for oil bioremediation.

Therefore, potential genetically engineered strains for biodegradation of petroleum have been constructed (Liu et al., 2019). However, the introduction of GMOs into the environment is still a controversial issue that involves risks similar to those related to the introduction of allochthonous species (Wolfenbarger and Phifer, 2000) and there are currently no reports of their use in the marine environment.

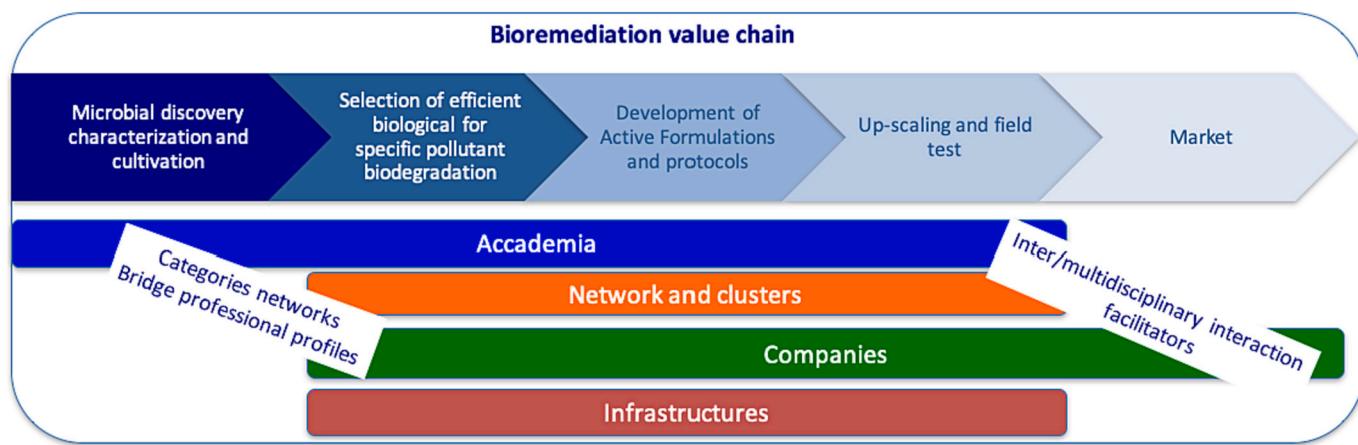
To ensure that this technology is understandable, convincible, and feasible for governmental competent bodies, end users and wide public, it is also important to provide them with robust results and descriptions of the real application perspectives and expected results. Current information indicates that further scientific evidence on the ability of marine microbes to degrade hydrocarbon is needed, their degradation efficiency needs to be demonstrated at larger scales and validated by policymakers for outdoor application.

Molecular tools and high-throughput screening platforms have revealed an extraordinary biodiversity and functional capability inside the marine microbial communities associated to oil polluted areas (Acosta-González and Marqués, 2016; Bargiela et al., 2015). In addition, substantial information on the potential of microbial biocatalyses, BS production, substrates affinity, etc. is now available both for many isolated strains and selected microbial consortia in different conditions, so that we are able to predict fraction, time and rate of biodegradation based on in vitro or at micro/mesoscale simulation studies (Genovese et al., 2014; Nikolaivits et al., 2017; Röling et al., 2002; Xue et al., 2015). Anyway, despite that microorganisms can virtually degrade every organic pollutant (Dunnivant and Anders, 2006), certain hydrocarbons are recalcitrant to the biodegradation (e.g., PAHs), especially in the natural environment, as it was frequently observed in marine sediments. Moreover, since bioremediation is based on natural processes, its duration can be too long to achieve the customer needs.

Also, the cultivability of the hydrocarbon-degraders is a barrier to the implementation; in fact, in spite of the huge biodiversity determined by molecular methods, the availability of isolated strains in pure culture

Table 5
Activities involved in the development of bioremediation procedures.

Activity	Specific actions
Site characterization	Identification of pollutants and selection of autochthonous bacterial degraders
Products manufacturing	Microbial and nutrient formulations for specific treatment
Up-scaling	Biostimulation and bioaugmentation protocols for large scale application
Database generation	Development of inventory of microorganisms and related pathways for specific pollutants
Software development	Modelling, aeration systems, release and remote control of nutrients or formulations
Customized treatment	Ad hoc bioremediation programs and technologies according to environmental features
Training	Education on bioremediation treatments, procedures, protocols and guidelines for product use



Inspired by DG Maritime Affairs and Fisheries (2014). Study in Support of Impact Assessment Work on Blue Biotechnology. Final Report FWC MARE and Hurst D. 2016 Marine Biotechnology strategic roadmap ERANET Marine Biotechnology modified

Fig. 3. Bioremediation value chain and inter-sectorial interest.

is quite limited. This dramatically limits advances in knowledge on biotechnological potential (Genovese et al., 2014).

Moreover, the intervention strategies cannot be generalized, as the bioremediation treatments must be site-specific, depending on the characteristics of the site and should be adapted to the natural perturbations that can occur. It is necessary to combine an effective and real-time monitoring plan to better address the protocols. There are still difficulties in choosing the criteria for evaluating the process. For example, the acceptable levels of a selected contaminant can be uncertain, and the heterogeneity of sediments can cause difficulties in technical evaluation. It is extremely important that proper skills are recruited during the planning of bioremediation treatment in marine environment, including the maintaining of relations between academy and industry.

Products and services deriving from bioremediation that show an economic potential do not have an added value in comparison to products applied in other sectors (e.g., pharmaceuticals). Therefore, investments in this sector are slow and poorly encouraged, including activities aimed at the concept or the development of new products to be conveyed to the market (Caplan, 1993).

Although we are now able to report a huge amount of *in vitro* / *mesoscale* success examples on the use of bioremediation to restore oil-polluted marine samples, the scarceness of the field studies makes the comprehension and the overcoming of its limits in marine environment difficult also preventing advancements on knowledge and innovation.

4. Regulation framework of bioremediation

Although the political debate about the application of bioremediation approaches in oceans and aquatic environments has been occurring for decades, a clear, homogeneous, and standard legislation has not been implemented to date (Francocci et al., 2020). The main reasons behind this worldwide law fragmentation are due to the potential risks associated with the release of microorganisms into the environment. In the U.S. the main control and reference organization dealing with oil spill control is EPA. After one of the most dangerous ecological disasters caused by the Exxon Valdez oil spill in 1989,² which dumped more than 11 million gallons of oil in the Gulf of Alaska, the US revised and implemented its regulations. The first action of EPA was the amendment of Clean Water Act (1977)³ by introducing the Oil Pollution Act⁴

establishing the guidelines in terms of response, liability, and compensation regime in case of oil pollution in U.S. waters. Since then, U.S. EPA became the lead Federal agency for oil spill remediation research and management, promoting the development of innovative technologies and best practices⁵. In this context, bioremediation has been considered as an important instrument to adopt. EPA introduced the Bioremediation Action Committee⁶ (BAC), then subdivided into six subcommittees, including Oil Spill Response and Research subcommittees among others. The major goals are: 1) to scientifically evaluate and demonstrate the safety and effectiveness of bioremediation approaches; 2) to provide necessary information to decision makers to design implementation actions; 3) to prepare guidelines about the use of bioremediation technologies; 4) to promote the inclusion of bioremediation technologies in the National Spill Response Plan⁷. The objective of BAC is to provide guidelines and protocols for decision makers to make quick and defensible decisions about the use of bioremediation technologies and approaches. Furthermore, EPA promoted the National Oil and Hazardous Substances Pollution Contingency Plan (NCP) and the Product Schedule⁸, which include a list of dispersants, chemicals, and other spill mitigating devices and substances that may be used in case of oil spill, nevertheless the quantity and the minimum safety standards to meet for environmental uses. To approve a product, EPA developed a specific process which must be followed (reported in the Subpart J of the NCP⁹), including requesting preauthorization and the compliance with Toxic Substances Control Act (TSCA).¹⁰ The TSCA evaluates the potential risk

⁵ https://clu-in.org/greenremediation/docs/gr_factsheet_biorem_32410.pdf

⁶ <https://nepis.epa.gov/Exe/ZyNET.exe/10002T0G.txt?ZyActionD=ZyDocument&Client=EPA&Index=1995%20Thru%201999&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QFieId=&QFieldYear=&QFieldMonth=&QFieldDay=&UseQField=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5CZYFILES%5CINDEX%20DATA%5C95THRU99%5CTX%5C0000004%5C1002T0G.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1>

⁷ <https://www.epa.gov/emergency-response/national-oil-and-hazardous-substances-pollution-contingency-plan-ncp-overview>

⁸ https://www.epa.gov/system/documents/2022-09/TN_Aug22_508.pdf

⁹ <https://www.epa.gov/emergency-response/national-contingency-plan-subpart-j#:~:text=Subpart%20J%20establishe%20a%20schedule,other%20chemicals%2C%20or%20other%20spill>

¹⁰ <https://www.epa.gov/laws-regulations/summary-toxic-substances-control-act>

² <https://www.epa.gov/emergency-response/exxon-valdez-spill-profile>

³ <https://www.epa.gov/archive/epa/aboutepa/meaning-1977-clean-water-act.html>

⁴ <https://www.epa.gov/laws-regulations/summary-oil-pollution-act>

to human health caused by the substance and includes both chemicals in general, and microorganisms. Currently, however, only companies which aim to directly apply genetically engineered organisms must be subjected to notification and review. The scientific analysis required by EPA include the experimental evaluation of % of oil reduction (alkenes and aromatics) after 7 and 28 days, in comparison with i) a control (natural degradation) and ii) the addition of nutrients (biostimulation). In case of clear benefits, the product can be considered for the NCP list, which is periodically updated and revised by EPA and implemented with technical information. In August 2022, it included a total of 129 products, divided among: 29 Bioremediation Agents (B), 19 Dispersants (D), 18 Miscellaneous Oil Spill Control Agents (M), 2 Surface Collecting Agents (S), 61 Surface Washing Agents (SW). Out of 29 Bioremediation Agents on the list, only 4 declared to not have any microorganisms, while all the other products contain microorganisms, even though the composition is confidential.¹¹ The best strategy is discussed case by case and the bioremediation approach (including the addition of microorganisms) is considered a feasible option.

While in the U.S. EPA represents a point of reference towards the oil pollution management, at European level the political and regulative scenario is more fragmented, with single Nations which mainly follow independent directions. In this context, the European Union is supported by the European Maritime Safety Agency¹² (EMSA), which oversees reducing the risk of maritime accidents, managing marine pollution from ships, and enforcing the pertinent EU legislation. EMSA periodically revise protocols and actions, developing guidelines for EU countries to counteract oil pollution. An effort to establish a common strategy towards oil spill prevention, response and management is realized by signing the Bonn Agreement¹³ (BA). The signatories to the BA are the Governments of Belgium, Denmark, France, Germany, Ireland, United Kingdom, Ireland, Netherlands, Norway, Sweden, Spain and European Union (EMSA). The agreement ensures and regulates mutual cooperation in the avoidance and combating of oil (and other substances) pollution, in particular of the North Sea, one of the most traffic-intensive marine regions of Europe, where most offshore oil and gas extraction activities take place in Europe.¹⁴ Over the years, the BA has adopted several decisions to facilitate joint operations to combat pollution or to put the agreement into practice. These decisions and other practical information are contained in the Bonn Agreement Counter Pollution Manual¹⁵ which is continuously refined. In general, the BA establishes the intervention approaches of each participant Country in regard of the Department involved, policies, simulated exercises, management, equipment, response techniques, etc. Similarly, to the North Sea, the Mediterranean Sea is preserved by the Regional Marine Pollution Emergency Response Centre for the Mediterranean Sea¹⁶ (REMPEC) which assists the Mediterranean coastal States in ratifying, transposing, implementing, and enforcing international maritime conventions, including in relation to oil pollution response. Although the direct application of microorganisms in the sea is not explicitly forbidden by EU laws, their use is limited by the lack of a standardized and recognized laboratory evaluation and risk assessment analysis. The primary response to an oil spill for most Countries is the mechanical recovery, while the use of dispersants represents the second major strategy. However, the procedure varies among the EU countries.¹⁷ In general, a specific authorization from the responsible national administration(s) is

required prior to the actual application of dispersants at sea.¹⁸ These oil control agents are composed of emulsifiers/surfactants and solvents which are sprayed on the polluted area breaking down spilled oil, facilitating diffusion into the water column and reducing acute pollution. Beside most of them have a synthetic/fossil origin, biological dispersants are also used. The UK Government was the first (1980s) to approve the use of dispersants in the sea which is considered the first response mitigation action. This country developed an advanced and structured procedure for the use of dispersants in case of oil spill, including the evaluation of effectiveness and toxicity and established a list of Approved oil spill treatment products¹⁹ (Updated 24 November 2023, accessed on 31-01-2024). The responsible organization is the Marine Management Organization (MMO). Out of 17 approved products, only one (Oil Spill Eater II) is classified as a bioremediation agent, due to its microbial origin and fermentation production process. MMO requires robust scientific evidence of effectiveness to add a new substance to the list. The product efficacy is based on the OECD 306 marine biodegradation tests with modifications.²⁰ The only type of oil spill control agent approved in Ireland is dispersant²¹ following the UK system and approved list. As a matter of fact, if a product is approved in UK there is no further authorization needed for Ireland. In the same way, the use of dispersant in Belgium is only permitted when authorized by Management of the marine environment (MUMM)²² (after consideration of advantages and disadvantages of response options – Net Environmental Benefit Analysis) and follows the rules of BA. In France, dispersants can be used after the product assessment based on standard laboratory tests for efficiency, toxicity, and biodegradability. The list of accepted products is published on Centre of Documentation, Research and Experimentation on Accidental Water Pollution (CEDRE) Website.²³ However, not all countries use dispersants freely. For example, their use represents the last resort and is strictly regulated in Germany, Denmark, Italy, among others.²⁴ EMSA has established a network of stand-by oil spill response vessels through contracts with commercial vessel operators which equipped the ships with selected dispersant services and devices.²⁵

In general, EU countries seem to be conservative and reluctant to directly use microorganisms for bioremediation in open waters, whereas the U.S. government is inclined to apply biological solutions if necessary. In some countries (e.g. Australia) the likelihood of being able to obtain permission from a body such as the EPA to release a microorganism is very small.²⁶ A proper and harmonized risk assessment strategy is necessary to encourage the adoption and commercialization of hydrocarbons-degrading microorganisms. For example, the Canadian EPA has established guidelines for risk assessment and a safety mechanism for the release of microorganisms into the environment. In terms of hazard assessment, this involves the characterization of the microorganism, identifies the potential adverse effects on the environment and/

¹⁸ <https://transport.ec.europa.eu/system/files/2019-01/2018-cost-effective-neess-and-efficiency-of-emsa-oil-pollution-response-services.pdf>

¹⁹ <https://www.gov.uk/government/publications/approved-oil-spill-treatment-products/approved-oil-spill-treatment-products>

²⁰ <https://www.arapha.com/OECD-306.html#:~:text=OECD%20306%20is%20an%20aerobic,addition%20of%20a%20specific%20inoculum.>

²¹ <https://assets.gov.ie/77984/1d06d2ab-9cb0-4bbb-bca4-354762f60e34.pdf>

²² <https://odnature.naturalsciences.be/mumm/en/aerial-surveillance/results>

²³ <https://wwz.cedre.fr/en/About-Cedre>

²⁴ <https://transport.ec.europa.eu/system/files/2019-01/2018-cost-effective-neess-and-efficiency-of-emsa-oil-pollution-response-services.pdf>

²⁵ <https://www.emsa.europa.eu/oil-spill-response.html>

²⁶ <https://www.oecd-ilibrary.org/docserver/9789264213562-12-en.pdf?expires=1702895446&id=id&accname=guest&checksum=972C661F8901ECCD63FB977DF345B08C>

¹¹ https://www.epa.gov/system/files/documents/2022-09/TN_Aug22_508.pdf

¹² <https://www.emsa.europa.eu/>

¹³ <https://www.bonnagreement.org/>

¹⁴ <https://transport.ec.europa.eu/system/files/2019-01/2018-cost-effective-neess-and-efficiency-of-emsa-oil-pollution-response-services.pdf>

¹⁵ <https://www.bonnagreement.org/publications>

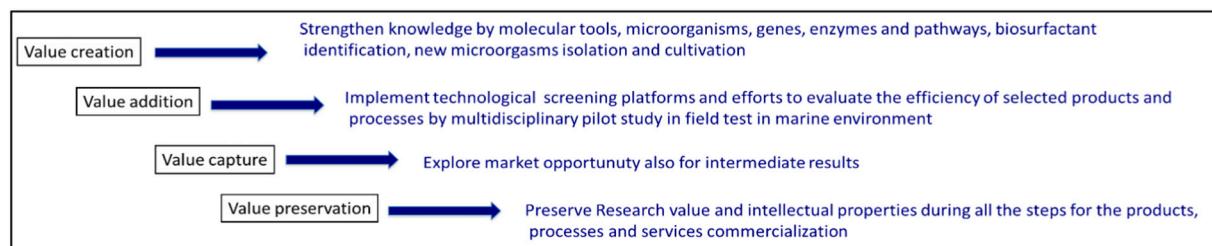
¹⁶ <https://www.rempec.org/en>

¹⁷ <https://safety4sea.com/emsa-overview-of-national-dispersant-testing/>

Table 6

Selection of available products for marine bioremediation.

Product name	Bioremediation agent	Application	Manufacturer	State	Cost
AGROREMED	Microorganisms	Soil	Sarva Bio Remed, LLC	USA	78 \$ per gallon NA
BioWorld BH	Microorganisms	Water and soil	BIOWORLD USA DBA RESTORED ENVIRONMENTAL	USA	78 \$ per gallon NA
HYDROREMED	Microorganisms	Free product or fuel oil in groundwater	Sarva Bio Remed, LLC	USA	78 \$ per gallon NA
MUNOX SR®	Microorganisms	Marine oil spills	BIOWORLD USA DBA RESTORED ENVIRONMENTAL	USA	78 \$ per gallon NA
OPPENHEIMER FORMULA	Microorganisms	Open sea, fresh water, soil, municipal waste treatment	Oppenheimer Bioremediation, INC	USA	125 \$ per 20 pound
SPILLREMED (MARINE)®	Microorganisms	marine open water oil spills and shoreline cleanup	Sarva Bio Remed, LLC	USA	78 \$ per gallon NA
SPILLREMED (INDUSTRIAL)	Microorganisms	Oily wastewater and oil spills under freshwater or reduced salinity conditions.	Sarva Bio Remed, LLC	USA	78 \$ per gallon NA
WHITZORB (AKA of DUALZORB®)	Microorganisms	Contaminated soils	Unotech	Brasil	NA
ACT TERRA FIRMA	Microorganisms/Biological Additive	Soil, water, roadways, rocks, metal, wood and other hard surfaces	American Cleaning Technology	USA	55 \$ per 2,5 pound
BIO-REGEN HYDROCARBON	Microorganisms/Nutrient Additive	Industrial Wastewater; soil and water	3 Tier Technologies	USA	NA
BIOREM-2000 OIL DIGESTER™	Microorganisms, Enzyme Additive, Nutrient Additive	Groundwater, open water and soil	Biorem 2000	USA	NA
MICRO-BLAZE® EMERGENCY LIQUID SPILL CONTROL	Microorganisms, Nutrient Additive, Biological Additive	Organics and hydrocarbons in soil and water	Verde Environmental, Inc	USA	190 \$ per 5 gal
PETROCLEAN™	Microorganisms/Biological Additive	Soil and water	Green Earth Naturally	USA	NA
SOIL RX	Microorganisms, Nutrient Additive	Hydrocarbons in soil and water	3 Tier Technologies	USA	NA
BIO-RECLAIM	Microorganisms, Biological Additive	Fresh/salt-water and soil	Long Chain Reclaim	Canada	NA
WASTE AWAY®	Microorganisms	salt/fresh water	CX International	USA	NA
REMEDIADE™	Nutrient Additive	Fresh, salt or brackish water	GrowMate International	NA	NA
OILGONE S-200C	Nutrient Additive	Sand, soil, fresh and ocean water	Slick Solution	Ireland	NA
VB591™	Nutrient Additive	Soil	BioNutraTech Inc	USA	NA
VB997™	Nutrient Additive	Water	BioNutraTech Inc	USA	NA
ECOBIOCLEAN® 100 COSW	Enzyme Additive, Nutrient Additive	Fresh/salt-water and soil	EcoBioClean	USA	NA
Oil Spill Eater II	Enzyme	Soil and water	OIL SPILL EATER INTERNATIONAL, CORP.	USA	NA

**Fig. 4.** Bioremediation value level and related activities to reach it.

or human health, and predicts the extent and duration of these effects.²⁷

Table 6 shows a selection of available commercial bioremediation agents, and the majority of the companies state that their products are suitable for freshwaters or marine environment. Most of the approved and commercialized bioremediation products are from US. This suggests less stringent controls from the American authorities towards the application of bio-based products, compared with Europe, which on the contrary, requires a more in-depth risk assessment. In order to confirm and demonstrate the effectiveness of their products, some of the reported companies describe on their website the success stories or case studies, however, most of them have been applied on groundwater or

confined water environments. In general, there are three main product formulations which include i) hydrocarbon-degrading microbes (usually in a dry form) without any additional ingredients or additives, ii) a mix of hydrocarbon-degrading microbes (usually in a dry form) with additional growth enhancer ingredients or additives, iii) nutrient additives without any microbes to stimulate the autochthonous microbiome. The latter also includes products based on enzymes which breakdown the pollutants in simpler molecules facilitation the natural biodegradation, as the case of Oil Spill Eater II, approved by EPA, but applied internationally, including in Europe.

5. Conclusion and proposed actions

Environmental bioremediation is an important sector of the blue economy because it is a tool which provides benefits for human, animal, and environmental health and it carries an underexploited economical

²⁷ <https://www.oecd-ilibrary.org/docserver/9789264213562-12-en.pdf?expres=1671624398&id=id&accname=guest&checksum=7FF81A1482A563CAEABC500351A01B3>

potential. Despite the fascinating perspectives, bioremediation in the marine environment, which requires knowledge and skills of a variety of disciplines, is far from being sufficiently implemented, to date. Nonetheless, *in situ* biotechnological approaches have been applied for more than 20 years. An extensive review on successful applications of *in situ* bioremediation on sediment is provided by Fragkou et al. (2021). However, this technique has been poorly applied to seawater mainly because of technical limitations. In general, large scale bioremediation projects are missing due to legislative, environmental, and technological factors. From a scientific point of view, it appears that the combination of different techniques would be pivotal in a successful process. An example could be a pilot bioremediation project that is taking place in Bagnoli Bay (Gulf of Naples, Mediterranean Sea) on an ex-industrial site combining electrokinetic system with bioaugmentation (LIFE SEDREMED). Another key point would be the approval for the utilization of GMOs, that can help to decrease the time required for the biodegradation. BS have already proven their efficacy, but the current production cost make them less suitable for large scale application. The improvement of the BS production yield and therefore cost will finally unlock their potential for bioremediation.

However, this scientific aspect requires different actions from many players. Specifically, we believe that the following actions are required to incentive the utilization of bioremediation on field:

- Promote specific programmes for the follow-up on the knowledge of the most promising cutting-edge biotechnologies, including innovative materials allowing diversified protocols for seawater and sediments, novel/new strains, enzymatic pathways, and related validated procedures.
- Activate interactive knowledge and technology transfer within stakeholders.
- Strengthen the efforts for mesoscale before, and long-term field tests after, in the marine environment according to governmental recommendations.
- Create an inventory of opportunities offered by the milestones reached to date, e.g. microorganisms, the substrates suitable for bioremediation, optimised conditions and robust, clear results already applicable in accurately designed defined plans.
- Provide policymakers with robust advice based on solid scientific knowledge and exert pressure for any technical revision of current regulations.
- Increase the opportunities for the efficient and sustainable use of by-products generated from blue bioeconomy sectors.
- Improve the professional skills, competences, and bridging action for cross sectors interactions.
- Focus on developing products specifically suited for marine environments, using sea-derived microorganisms and additives.
- Harmonize intervention strategies in countries insistent in the same basins (e.g. Mediterranean Sea) as an example for the use of biodispersants.
- Provide public opinion (civil society) with the adequate knowledge and guidelines about safe, health, efficiency, advantages, and limits of the bioremediation treatments in marine environment.

Specific actions can be implemented to reach several value levels (Fig. 4) and it could be also fruitful to support the “already done”, as an example, providing further financing to the most promising projects outcomes.

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CRediT authorship contribution statement

Pietro Tedesco: Writing – review & editing, Writing – original draft, Visualization, Validation, Conceptualization. **Sergio Balzano:** Writing – review & editing, Writing – original draft, Visualization, Validation. **Daniela Coppola:** Writing – review & editing, Writing – original draft, Validation. **Fortunato Palma Esposito:** Writing – review & editing, Writing – original draft. **Donatella de Pascale:** Writing – review & editing, Visualization, Validation, Funding acquisition, Conceptualization. **Renata Denaro:** Writing – review & editing, Writing – original draft, Visualization, Validation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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