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*Review*

# Unravelling Diatoms' Potential for the Bioremediation of Oil Hydrocarbons in Marine Environments

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**Abstract:** The search for practical solutions to alleviate the destructive impact of petroleum hydrocarbons in marine environments is contributing to the implementation of prospecting strategies for indigenous microorganisms with biodegradative and bioremediation potential. The levels of petroleum contamination entering the marine environment each year have been estimated at around 1.3 million tonnes, a figure that is expected to increase by 1.9% annually over the next decade. The recent interest in decarbonizing our energy system and accelerating the clean energy transition has created a demand for greener technologies and strategies to find innovative, sustainable, and cost-effective treatments for the marine environment. Diatoms (Bacillariophyta) are one of the most diverse and successful taxa in coastal–marine environments and are a relatively untapped pool of biodiversity for biotechnological applications. Recent reports have revealed the significant presence of diatoms associated with oil spills and petroleum hydrocarbon degradation. Most diatoms can secrete substantial amounts of exopolysaccharides (EPSs) into their environment, which can act as biosurfactants that, in addition to oxygen and other enzymes produced by diatoms, create suitable conditions to enhance hydrocarbon solubility and degradation into less toxic compounds in seawater. Recent reports on the biodegradation of aliphatic and aromatic hydrocarbons by diatoms are indicative of the potential of these taxa to achieve success in the bioremediation of hydrocarbons in marine environments. This review highlights the main attributes and roles that diatoms could play in integrated strategies for biodegradation and bioremediation of petroleum hydrocarbon pollutants and as such represent a green, eco-friendly, and sustainable contribution to mitigate damage to biodiversity and value chains of marine ecosystems.

**Keywords:** diatoms; hydrocarbons; bioremediation; biodegradation; marine; oil pollution; oil spill



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## 1. Introduction

In the present era of unsustainable fossil fuel use, lifestyle and society are still reliant on petroleum and other petrochemicals as energy sources and for other needs. For more than 100 years, oil has powered global economic growth and prosperity, but has also negatively impacted and degraded environmental conditions, threatening not only the biosphere but also humans' own quality of life.

In general, petroleum hydrocarbons represent 50–98% of crude oil volume in marine habitats either from oil spills (other than those derived from natural seeps) or other minor releases. They are mainly classed as alkanes, cycloalkanes, mono-aromatic, and polycyclic aromatic hydrocarbons [1]. The range concentrations of total petroleum hydrocarbon concentration are variable and may depend on the source, composition, and season. Hence, in seawater, the average level of total hydrocarbons has been reported in the range from 2 to 80 mg/L [2]. Petroleum hydrocarbons are highly toxic and dangerous pollutants that produce carcinogenic, mutagenic, and teratogenic effects in humans and on the biodiversity of marine ecosystems and their soluble fractions can bioaccumulate at different scales in the marine food web [2]. Similarly, pollution by crude oil composed mainly of PAHs affects coastal resources (seafood, fisheries, and aquaculture) represents a harmful and serious human health concern and is a major threat to the resilience and sustainability of marine ecosystems [1]. Moreover, a substantial portion of the spilled oil can be long-lasting in marine environments and negatively impact fragile marine wildlife.

The increasing pollution resulting from oil-related activities in the twentieth century demands integrated and sustainable solutions for the development of circular bioeconomies, even if peak oil production has probably already occurred [3]. For the last five decades, and continuing to the present day, the devastating effects of the discharge of large amounts of petroleum into the marine environment because of spillages represent approximately eight million tons globally, causing immense damage to the planet that can remain in coastal marine environments for decades [4,5]. Chronic pollution, as well as marine oil spills, exploration activities, and other large-scale industrial processes such as oil refining, are considered real and potential sources of environmental pollution in coastal and marine environments.

It is expected that the burden of these pollutants will increase many folds in the following decade. The recent interest in decarbonizing our energy system and accelerating the clean energy transition has created a demand for greener technologies and strategies for finding innovative and sustainable treatments for the marine environment, which is the largest ecosystem in the world. The paradigm shift toward sustainable growth is a priority, and biomass may be a prospective solution for resolving the challenges presented by the implementation of renewable energy [6] and pollution mitigation. Thus, the marine microbiome must play a key role in the bioeconomy. However, among the existing techniques used for clean-up activities, microbial biodegradation and bioremediation have gained acceptance because of their low cost, eco-friendly basis, and sustainability. Although bacteria have been studied for a long time as the main degraders of petroleum hydrocarbons in the marine environment, unfortunately, the bacterial biomass thus produced does not generate additional value-added attributes. In this regard, the bioprospection and screening of photosynthetic microorganisms for the purposes of potential applications in the biodegradation and remediation of petroleum hydrocarbons appears promising.

Increasing interest in marine bioremediation technologies based on microalgae and their derived biomass through phytoremediation may represent a viable shift in solutions toward several novel innovations related to green biotechnology for a bio-based circular economy. Due to the immensity of the marine biosphere, and the lack of strategies related to the capabilities of different species with respect to the biodegradation and bioremediation of pollutants, most microbiomes remain unexplored. Coastal and marine environments often experience stress from pollution generated by petroleum, but little is known regarding the role of phytoplankton, in general, and diatom cells, in particular, on the biodegradation of petroleum-derived hydrocarbons. Diatoms and their metabolites are a renewable energy source and are affordable resources for eco-sustainability that may represent novel hydrocarbon degraders for use in bioremediation initiatives. Diatoms are a dominant and important group of eukaryotic microalgae, inhabiting all contemporary oceans at the global scale because of their evolution over the last nearly 200 million years [7]. Diatoms are one-celled or colonial members of microalgae in phytoplankton belonging to the algal phylum Bacillariophyta, class Bacillariophyceae, which are considered the dominant group of microalgae in phytoplankton. Conventionally, diatoms have been classified as centric (Centrales), which are radially symmetric, and ennales, which are bilaterally symmetric and named pennate diatoms [7,8].

Diatoms are the only organism on the planet to have cell walls composed of transparent, opaline silica consisting of two interlocking symmetrical valves, in addition to its uniqueness among marine algae in performing C4 photosynthesis. Diatoms, siliceous algae, are the most dominant and widespread group of eukaryotes on Earth and are responsible for 20% of carbon fixation through photosynthesis [9,10]. They are abundant in oceanic, coastal, and freshwater habitats, and occur in terrestrial environments in soils, ice, rocks, and even in some plants [11]. Diatoms are responsible for a large fraction of the organic carbon buried in oceans and have been recognized among the microalgae that play a relevant role in the formation of crude oil deposits in coastal and marine environments [10,12,13]. According to a previous study [14], petroleum hydrocarbons in crude oil are natural deposits derived from aquatic algae, occurring between 180 and 85 million years ago. The natural presence of petroleum hydrocarbons in marine environments for millions of years has allowed microorganisms (bacteria, archaea, and fungi) to evolve the capacity to use hydrocarbons as sources of carbon and energy for growth. In addition, the almost universal presence of diatoms in oil spills is comparable to the capacity of bacteria to not only survive but also to use such compounds as an energy source [15].

Diatoms produce large quantities of extracellular polymeric substances (EPSs) in order to build up an environment in which the organisms are embedded in biofilms [16]. Such metabolites produced by diatoms have been proposed as effective agents for reducing the interfacial tension between oil and water (thus enhancing emulsion formation), which would improve hydrocarbon solubility and bioavailability and may contribute to the biodegradation processes of hydrocarbon pollutants [17,18]. This is particularly significant considering that the ability of most other microorganisms to degrade carbon, including short and longer-chain alkanes (<C10 and C20–C40) and some polycyclic aromatic hydrocarbons (PAHs), is not sufficient to ensure the removal of all oil components from marine environments [19,20].

Most petroleum hydrocarbons are biodegradable under aerobic conditions, but a minor component has been considered unbiodegradable to date [21]. Recently, Zehnle and co-workers [22] reported that when archaea of the genus *Candidatus alkanophaga,* isolated from the Guaymas Basin hydrothermal vent, were grown under anoxic conditions and enriched with the enzyme alkyl-coenzyme M reductase (Acrs), they exhibited an ability to degrade mid-chain petroleum n-alkanes (C5-14) at 70 °C using oil-rich deep seafloor sediments (2000 m) from the Guaymas Basin (Gulf of California, Mexico). In these cultures, the archaea activate the alkanes harboring Acrs and successfully oxidize the alkyl groups to $CO_2$. In diatoms, the biodegradation feasibility of petroleum hydrocarbons can be attributed, among other factors, to whether the molecule is composed of lower-molecular-weight hydrocarbons, which undergo more biodegradation than heavy crudes. The aerobic biodegradation process in the case of treatment of PAHs is reliant on the presence of oxygen during the insertion of dioxygenase enzymes, which has been reported in diatoms [23] and is considered a good way of achieving successful biodegradation [24]. Under anaerobic conditions, the biodegradation of petroleum hydrocarbons can also proceed, but the process is much slower [14,24].

Among Eukarya, diatoms have been reported to grow on or transform hydrocarbons [25]. There is only a limited amount of research available on the biotechnological potentials of diatoms to perform the biodegradation and bioremediation of petroleum hydrocarbons in marine environments (Table 1).

Among the studies on the biodegradation and bioremediation of petroleum hydrocarbons, adsorption, metabolism, transformation, accumulation, and cometabolism have all been reported to occur in diatoms [19,25]. Nevertheless, to the best of our knowledge, the present review is one of the first to analyze some crucial, but often overlooked, factors influencing the biodegradation and or removal effects of diatoms based on their phenotypic plasticity, properties, and peculiar metabolism, which give them a promising potential for the bioremediation of petroleum hydrocarbons in the marine ecosystem.

In this review, the existing literature on the action of diatoms on petroleum hydrocarbon bioremediation for a number of aromatic and alkane contaminants and structurally related chemicals was analyzed. The petroleum hydrocarbon background from diatoms was highlighted, and their advantages and potentialities, also supporting this premise, were also emphasized.

**Table 1.** Eukaryotic microalgae that degrade petroleum hydrocarbons (modified from Prince [25]).

| Genera | Substrata | References |
|---|---|---|
| Phylum Chlorophyta | | |
| *Chlamydomonas reinhardii* | Phenanthrene | [26] |
| *Chlorella vulgaris* | Naphthalene | [27] |
| | Nonadecane | [28] |
| *Ankistrodesmus capricornutum* | Benzo(a)pyrene | [29] |
| *Prototheca zopfii* | n-alkanes and PAHs | [30] |
| *Dunaliella salina* | Naphthalene | [31] |
| *Scenedesmus acutus* | Benzo(a)pyrene | [29] |
| Phylum Heterokontophyta | | |
| Class Bacillariophyta | | |
| *Navicula* sp. | Naphthalene | [32] |
| *Achnanthes minutissima* | Alkanes | [33] |
| *Cyclotella caspia* | Fluoranthene | [34] |
| *Cylindrotheca* sp. | Naphthalene | [35] |
| *Synedra* sp., *Amphora* sp. | Naphthalene | [32] |
| *Skeletonema costatum* | Fluoranthene | [36] |
| *Nitzschia* sp. | Phenanthrene | |
| *Nitzschia* sp., *Navicula* sp. | Naphthenic acid | [37] |
| Diatom BD1IITG | Phenol | [38] |
| *Thalassiosira* sp. | Phenol | [39] |
| *Nitzchia* sp. and Bacteria | Benzo(a)pyrene | [40] |
| *Genera Marivita*, *Erythrobacter*, *Alcaligenes* | Fluoranthene | |
| *Cylindrotheca* sp., *Amphora* sp. | Naphthalene | [35] |
| *Navicula* sp., *Nitzschia* sp., | Naphthalene | [32] |

Most of the existing reviews on the microbial biodegradation and bioremediation of petroleum hydrocarbons have focused on bacteria, fungi, cyanobacteria, and eukaryotic microalgae in soil and marine environments. Compared with bacteria and other eukaryotic microalgae, diatoms offer advantages over eukaryotic microalgae for the biodegradation of petroleum hydrocarbons, based mainly on their intrinsic evolution associated with petroleum hydrocarbons in the marine environment. In addition, diatoms' wide applications in oil exploration and use as bioindicators of water quality, their ubiquitous presence in all marine photic zones contaminated by petroleum hydrocarbons, and their recognized ability to produce and metabolize some petroleum hydrocarbons make them ideal candidates for petroleum bioremediation investigation in coastal and marine environments.

In this review, we used the Web of Science database to search for keywords including petroleum hydrocarbons, diatoms, marine environments, biodegradation, bioremediation, oil contamination, treatments, oil pollution, and oil spills to screen and collect publications from the past 20 years related to this topic.

## 2. Diatom Microalgae: Attributes and Potential for the Biodegradation and Bioremediation of Petroleum Hydrocarbons

Although hydrocarbon-degrading microorganisms, mainly bacteria, are ubiquitous in marine ecosystems [41,42], the biodegrading role of autochthonous microbial communities has been considered the ultimate destination of most of the petroleum hydrocarbons that enter the marine environment [14,25,42]. Bioremediation using bacteria has been an effective treatment of oil hydrocarbons; however, the biomass produced by this process lacks any significant profitable value. Moreover, several studies have demonstrated the potential of select microalgae and diatom species in the removal of petroleum hydrocarbons, which can be optimized by the potential action of algal–bacterial consortia [43]. In this case, diatom characteristics have been envisaged as a potential biofuel feedstock that can be combined for emulsified oil bioremediation [44], which can represent renewable energy production using bioremediation. Over the last few decades' investigations have shown that in situ and ex situ oil biodegradation and remediation initiatives can be successful when algal enhancement and development conditions are provided. Firstly, to achieve efficient bioremediation, once the oil separates from the seawater surface, microorganisms act on the emulsified fractions, which are more difficult to remove and pose a significant threat to the marine environment. Therefore, diatoms and their possible combination with hydrocarbonoclastic bacteria could play an important role in the biodegradation and bioremediation of the components of petroleum hydrocarbons in the marine environment.

Diatoms are a major group of eukaryotic microalgae that have been reported to be associated with oil in the sea [18,19] as well as other representative groups of dinoflagellates and cyanobacteria. More recently, the sequenced genomes of *Cyclotella cryptica* have provided valuable information on their metabolism and elevated lipid productivity, while in *Seminavis robusta*, remarkable insights into its evolutionary and ecological success in heterogeneous environments have been revealed [45]. Both these observations support their contributions to biogeochemical oil accumulation. Diatom genomes encode all the conventional enzymes of the Calvin–Benson cycle, which is responsible for carbon fixation [45], primary productivity, and ecosystem stability, as well as the complex evolution of their plastids.

It is generally known that algae can eliminate organic pollutants through two natural processes related to the species itself, viz., bioaccumulation, in which certain organic and inorganic substances are taken up and/or stored inside the cells. Biodegradation is the other process, in which organic material is transformed and decomposed into simpler substances such as carbon dioxide and water. This mechanism is carried out mainly via enzymes that are produced by living algae cells in the presence of a carbon source.

Traditionally, bacteria have been regarded as efficient bioremediators of petroleum hydrocarbons, and recently, microalgae have emerged as a promising alternative because of a bacterial impediment to breakdown and remove all hydrocarbon pollutants. This is why diatoms can be considered a major group of microalgae that may be preferred for that purpose due to bacterial constraints such as the release of carbon dioxide into the atmosphere and their increasing metabolic waste material, which increases costs, in addition to other limitations.

Among the biodegradable structural group, n-alkanes are the most susceptible to biodegradation, except for C20–C40, which are hydrophobic solids and are not easily degraded [46]. Another major group of petroleum hydrocarbons are polycyclic aromatic hydrocarbons (PAHs), which are partially recalcitrant to biodegradation.

Contrary to the widely accepted use of bacteria for the biodegradation of petroleum hydrocarbons, in certain cases where bacteria are not able to biodegrade oil hydrocarbons, some eukaryotic microalgae and diatoms can use these hydrocarbons as the carbon and source of energy in their metabolism [47]. Similarly, the challenges and opportunities of algal-based biodegradation and bioremediation initiatives for petroleum hydrocarbons indicate several advantages, including being simple, effective, non-invasive, less disruptive, cost-effective, safer, and eco-friendly, compared with other technologies [43,48]. Typical remediation technologies are costly, generate secondary pollution, and are not recommended for natural systems because they damage native biodiversity [48,49]. Therefore, contrary to these unsustainable technologies, carrying out more eco-sustainable methods is essential [49]. Moreover, bioremediation by microalgae (diatoms) is currently widely accepted due to the ability of algae to grow at a faster rate than terrestrial plants using solar energy. The possibility of their cultivation on nonarable lands and in marine environments or seawater media has the advantage of lower requirements for freshwater and land and offers the possibility to carry out large-scale cultivation using indigenous diatom species in situ and ex situ conditions (in photobioreactors) with limited impact on the environment.

Bioremediation is therefore considered an accessible and effective technology to eliminate oil pollution from contaminated environments and mitigate the damage caused by oil spills, in addition to the possible use of wastewater for its different modes of nutrition and fertilization [50,51]. For instance, *Navicula accomoda*, *Navicula synedra*, and *Nitzschia palea* have been reported to be good indicators or biomarkers of heavily polluted zones and for the recovery of resilience where other species cannot occur [52].

These phototrophic indigenous microorganisms have been underused thus far in the bioremediation of hydrocarbon-contaminated sites. Such applications are beginning to be considered, although other microorganisms, such as bacteria [53], have been unsuccessful in the biodegradation and bioremediation of all hydrocarbons. Despite the importance of diatoms, studies examining their role in hydrocarbon biodegradation are limited [54]. The degradation of various aromatic compounds in impacted marine environments is of great interest and is actually part of a worldwide initiative that includes the sustainability goal agenda of the United Nations Organization [55–57]. Hence, the search for efficient and applicable systems requires prospecting, isolation, and the characterization of autochthonous microorganisms with potential for bioremediation [58].

Other attributes of diatoms to be considered include their availability to mass culture and omics data, genetic manipulation techniques, and concomitant production of value-added products [59,60]. Moreover, the limitations regarding the effectiveness of bacteria in certain specific hydrocarbon components and environments have led to new taxa and species being explored with the capabilities to perform oil biodegradation and bioremediation. Among Eukaryotic microalgae, therefore, diatoms have characteristics that allow them to be considered potential microorganisms for such purposes, an idea that remains only sparsely studied (Figure 1).

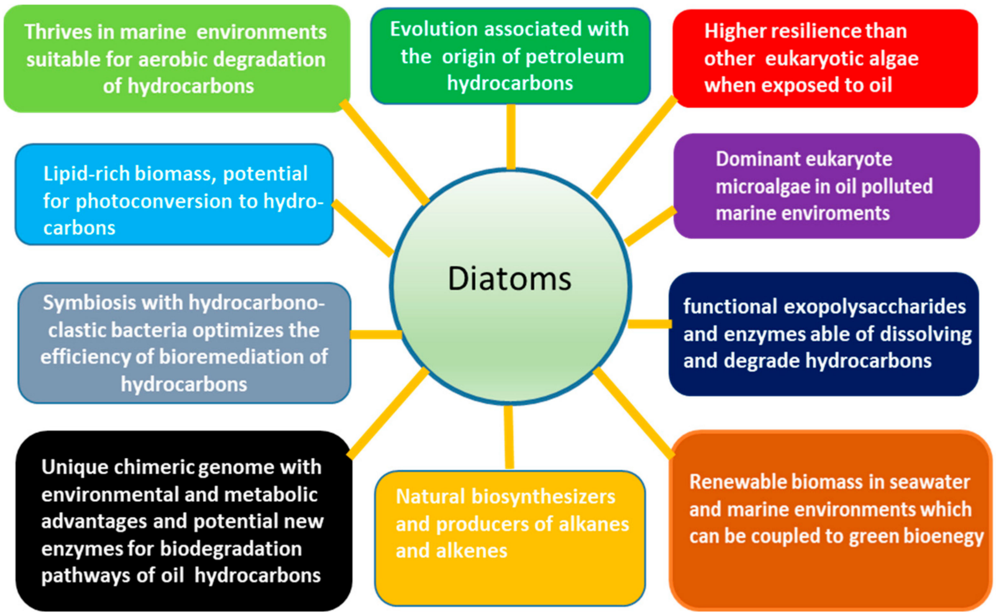


**Figure 1.** Attributes of diatoms for the biodegradation and bioremediation of petroleum hydrocarbons in the marine environment.

## 3. Diatom Exopolysaccharides—Role as Biosurfactants and Biodegraders of Oil

Marine ecosystems represent the largest aquatic ecosystems in which marine microbiomes thrive and flourish. In addition to the characteristics required for marine microorganisms to succeed in challenging environmental conditions, such as at extremes of salinity, temperature, pH, nutrient fluctuations, increased pressure, and UV exposure [61,62], they are also characterized by exopolysaccharide production under such conditions.

Marine microorganisms have been characterized as producing polysaccharides, which are the most abundant carbohydrates in nature, with high molecular weight biopolymers (10 to 1000 kDa) exhibiting diverse structures in addition to many physico-chemical properties [63]. These molecules are preferred because of their biodegradability and low toxicity, acting as surface-active compounds called biosurfactants (BSs), which are among the amphiphilic biomolecules that possess both hydrophilic and hydrophobic moieties. These natural surfactant-like polysaccharides are promising for use in remediation technologies as an alternative to synthetic surfactants [64,65]. Among the polyanionic functional groups forming the structural backbone of EPSs, the interface solubilization of hydrocarbons is included. Because of their singular structural and functional characteristics, EPSs are anticipated to be applicable in the remediation of hydrocarbons from contaminated wastewater, metals, crude oil, and hydrocarbons from polluted waters, mines, and oil spills [66]. Polysaccharides from microalgae can be categorized as intracellular, structural, and extracellular glycans [63]. Recently, due to the production and renewable characteristics of these metabolites, they have been regarded as strong candidates to replace the synthetic surfactants widely used in oil spills, which are unsustainable because they produce secondary pollution and toxic effects, along with their resistance to biodegradation [61]. Biosurfactants (BSs) are preferable metabolites because of their environmental compatibility and wide acceptance due to their low toxicity and surface functionality, and mainly because of their high biodegradability and ease of production using renewable and low-cost substrate [67–69].

In eukaryotic microalgae (diatoms), EPSs are secreted through diatom frustule, viz., raphe (in pennate diatoms), axillary pores, or fissures (in centric diatoms) to the environment where EPSs continue through a self-building process [70–72]. Diatoms play a key role in the marine carbon cycle, accounting for approximately 40% of photosynthetic inorganic carbon fixation in oceans [18,73], thus leading to high productivity. This process is considered the main contribution to carbon in marine environments, while at the same time, diatoms secrete an abundance of organic carbon in the form of exudates such as EPSs [74] and transparent exopolymeric particles into the ocean [75] in order to meet their inherent basic functional needs. EPSs in Bacillariophyta are 95% polysaccharides and may consist of neutral sugars, uronic acids, sulfonated sugars, or ketal-linked pyruvate groups [16,76].

Although exopolysaccharide-producing diatoms are a neglected resource, several authors have reported that EPS production by diatoms is a phenomenon that is based strictly on oxygenic photosynthesis and on atmospheric $CO_2$ fixation, which is dependent on light and nutrient availability [77]. According to [78], EPS production in diatoms is achieved with light in active photosynthetic conditions, combined with low nutrient stress situations. Diatoms, which are part of the phytoplankton responsible for about 40% of marine primary productivity, also contribute to the ocean biological carbon pump, exhibiting EPS exudates, which are partially constituted of transparent exopolymeric particles [79,80]. EPS exudates, when mixed with debris, cells, microorganisms, and organic materials, form marine snow, which is considered ubiquitous throughout the marine environment and is mostly involved in the transport of carbon to the seafloor [80–82].

The role of EPSs in the natural degradation and attenuation of crude oil in the sea was evidenced after the catastrophe of the Deepwater Horizon Oil Spill in the Gulf of Mexico and the Macondo Prospect 2010 spill. The spilled oil was combined with marine oil snow harboring abundant EPSs and diatoms, which, after sedimentation and flocculent accumulation, contributed to the sinking of oil [18]. During this process, most of the hydrocarbons were consumed by the indigenous microbiome, and diatoms exerted an important role as degraders and consumers around the oil fractions of the marine oil snow during its transport to the seafloor. This process represented about 14% of the degradation of the total oil released in the Gulf of Mexico by the DWH [80,83].

This scenario represents a way of bioremediation in which diatoms and other microorganism producers and degraders of extracellular polymeric substances associated with marine oil snow [84] played a significant role in bioremediation. Passow and Stout [85] reported that levels of degraded oil in marine oil snow were observed when analyzing a sediment trap after the MC-252 well was caped. The result evidenced a highly degraded oil at residual concentrations of 1.45 mg fresh oil/$m^2$/d, which represented a median percentage degradation of 85% in the marine oil snow constituted of diatoms, EPS, fecal pellets, minerals, and organic matter.

Recently, studies on the biosynthesis of carbohydrates in *Phaedactylum tricornutum* by Yang et al. [86] corroborated the notion that Chrysolaminarin, formed mainly of β-1,3-glucan with β-1,6-branched chains (molecular weight at 10 kDa), is the main form of polysaccharide reserve in this diatom, emphasizing the role of the PtTGS1 gene in the Chrysolaminarin synthesis and carbon deviation pathways. Kamalanathan et al. [87] reported the effective role of Chrysolaminarin as a carbon reserve and, consequently, in the growth and survival of *Thalassiosira pseudonana* in the presence of oil.

Currently, with advancements in metagenomics, new genes involved in the secretion of biosurfactants [88,89] have been discovered. A deep and concise analysis is required to better characterize and unravel the metabolic pathways and different nodes involved in sugar carbon shunt contributing to extracellular polysaccharides' biosynthetic pathway from central metabolism [88].

## 4. Diatoms—Origin and Producers of Isoprenoids, Alkanes, and Alkenes

Historically, geologists have often claimed that an important fraction of crude oil may have originated from diatoms, a claim that could be supported by the proven ability of diatoms to make oil [13]. Although most hydrocarbons found in the environment occur in crude oil, many oil hydrocarbons may have originated from algae in ancient oceans [90]. This claim remains controversial because explorations have mainly been undertaken in the land and coastal–marine areas of the world, not in deep sea areas. Moreover, evidence from many oil basins around the world suggests that they can be attributed to diatomaceous deposits [10]. Although the sources of organic compounds such as highly branched isoprenoid (HBI) alkenes were initially hypothesized to originate from diatoms, Rowland and Robson 1990 [91], and more recent studies, recognized diatoms as an important source of many classes of hydrocarbons, including alkanes and alkenes [92]. Alkanes constitute up to 35% of crude oil petroleum and its derivatives. Usually, straight-chain alkanes are typically the most abundant and do not contain ring structures, which is why they are the more readily degraded.

n-Alkanes are saturated hydrocarbons, i.e., formed of hydrogen and carbon with single covalent bonds between both atoms, while alkenes are hydrocarbons with at least a double bond. Short-chain alkanes are aliphatic hydrocarbons and are the most easily degraded, followed by alkanes with side chains or branched alkanes and then aromatic hydrocarbons with stable ring structures. Although alkanes have been reported in groundwater, rivers, lakes, oceans, and soil [46], they may also occur in aquatic organisms and sediments (estuarine, marine, and riverine). Alkanes with chains of up to 44 carbon atoms are susceptible to microbial degradation; however, only a limited number of species can use Cl–C4 and C5–C9 alkanes because of their toxicity [93,94]. Alkane and alkene hydrocarbons of various chain lengths are biotechnologically important because they are major components of diesel (C12–C20), jet fuels (C5–C16), and gasoline (C5–C9) [95].

In marine environments, diatoms are the main sources of sterols, mainly C28 sterols, and are less abundant in C27–C29 sterols [10]. The presence of highly branched isoprenoids (HBIs) in diatoms has been used as an effective environmental biological marker, for example, alkenes can be considered a useful indicator of petroleum deposits [10,92]. For instance, the ratio of steranes (28 and 29 carbon atoms) [96], 24-norcholestanes [97], and the highly branched isoprenoid (HBI) alkenes [98] detected in diatoms are also found in many oil fields around the world [99].

The diatom genera *Rhizosolenia*, *Haslea*, *Navicula*, and *Pleurosigma* and their ancestors are thought to be the major sources of a group of C25 highly branched isoprenoid (HBI) alkanes and thiophenes [98,100]. It was later recognized that different growth conditions determine the amount and type of HBI produced in diatoms. Studies on microalgae alkane and alkene biosynthesis in eukaryotic algae are scarce. In the 1970s, Lee et al. and Lee and Loeblich [101,102] reported the presence of a very long-chain alkene, a C21 hexaene in certain species of diatoms and other algal species. The diatom *Synedra acus* subs. *radians* evidenced the accumulation of polycyclic aromatic hydrocarbons (PAHs) during its cultivation with crude oil hydrocarbons [103]. The results corroborated the role of this diatom in the accumulation of PAHs in lipid bodies when cultivated in the presence of crude oil or light oil, exerting a selective accumulation of C12–C18 alkanes. In principle, the transmembrane transport of PAHs in this diatom is induced by the insertion of n-alkanes into the membrane lipid bilayer. This process of bioaccumulation may contribute to the mitigation and removal of hydrophobic petroleum hydrocarbons from aquatic systems.

Diatoms and microalgae exhibit remarkably promising feedstock to replace non-renewable sources of biofuel production and energy. This fact is based on the high lipid content of algae, which is considered a major feedstock of petroleum, where lipids have remained stable over millennia [104,105].

Under special culture conditions, mainly when exposed to nutrient deprivation, diatoms can accumulate and store high amounts of triacylglycerols, as evidenced by the substantial portion of the cells' volume often occupied by spherical organelles, called oil bodies, or lipid droplets. The diameter ranges of these lipid oils observed near the chloroplast is 100–200 μm [106], suggesting that they are synthesized within the chloroplasts in some diatoms [13,106]. This particularity has attracted significant attention because of the unique combination of genes involved and the evolutionary history involving secondary endosymbiosis. One evidence of the biosynthesis and production of alkanes and alkenes by chlorophyte microalgae, including diatoms, was corroborated by Sorigue et al. [95]. In principle, microalgae synthesize hydrocarbons from long-chain fatty acids induced by a light-dependent pathway. In *Chlamydomonas reinhardtii*, the loss of the carboxyl group of a C18 monounsaturated fatty acid gives rise to the alkene heptadecene (7-heptadecene) as C17-heptadecene. It was suggested that this isomer was formed by decarboxylation of cis-vaccenic acid as a product of the deuterated palmitic acid that yielded stearic acid (D31-18:0), oleic and cis-vaccenic (D29-18:1) acids, and D29-heptadecene. In essence, the amount of 7-heptadecene is dependent on the growth phase of the algae and temperature, and it rigorously depends on light and not on photosynthesis since photosystem II was not blocked by the assayed inhibitor [95]. In other chlorophytes, viz., *Chlorella variabilis* NC64A and *Nannochloropsis* species, and in the diatom *Phaeodactylum tricornutum*, around 80% alkenes were detected. This diatom accumulated C21 hexaene. Thus, a new light-dependent pathway was evidenced across the algal ability to transform C16 and C18 fatty acids into alkanes and alkenes [95].

The interaction between oil bodies and associated organelles (chloroplast, mitochondria, and endoplasmic reticulum) during biogenesis and degradation suggests their dynamic response to environmental changes [107]. Similarly, the presence of lipid droplets in diatoms represents a vestigial reminiscence of an ancient organelle conserved among all eukaryotes [13,108], which have been optimized by certain diatoms during their evolution. All these oily backgrounds indicate diatoms' inherent properties as biochemical synthesizers of oils, a mechanism considered regarding the ability of diatoms to remove oil derivatives such as petroleum hydrocarbons [13,109]. Studies on the pennate genome revealed that there have been many lateral gene transfers from bacteria into the diatoms [109]. In addition, it is recognized that several classes of hydrocarbons including alkanes and alkenes are synthesized by diatoms [92]. Cerniglia and co-workers [17,32] reported that some marine microalgae, including diatoms, can produce enzymes that degrade harmful organic compounds from petroleum hydrocarbons into less toxic compounds, thus contributing to the removal oil hydrocarbons. This ability to synthesize hydrocarbons was recently reported by Harada et al. [110] in the microalga *Dicrateria rotunda* from the western Arctic Ocean, which was able to biosynthesize a group of saturated hydrocarbons (n-alkanes) from $C_{10}H_{22}$ to $C_{38}H_{78}$. These compounds were grouped as petrol (C10–C15), diesel oils (C16–C20), and fuel oils (C21–C38).

The feasibility of using diatoms, a fatty acid-rich species, to promote the synthesis of alkanes and alkenes hydrocarbons from fatty acids precursors is particularly attractive because it combines a high biomass yield and a robust capability to biosynthesize and accumulate fatty acids, which may lead to hydrocarbon biosynthesis that may represent a hydrocarbon removal strategy. Hence, it makes sense that certain diatoms may contain the biochemical mechanisms and strategies, as well as the metabolic pathways, involved in petroleum breakdown, in addition to their exopolysaccharides, oxygen, and degradative enzymes.

## 5. Marine Diatoms—Sustainable and Renewable Remediation of Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs are highly recalcitrant, carcinogenic, and a mutagenic class of organic compounds that can be persistent in some coastal and marine environments, and their hydrophobicity and low water solubility are a challenge for their sustainable biodegradation and mitigation [5]. PAHs are ubiquitous in the environment, and they typically originate from biogenic and geochemical as well as anthropogenic sources. The last-mentioned source contributes to the incomplete combustion of organic constituents (coal, wood, diesel) and represents an important origin of environmental pollution [111]. PAHs are acutely toxic and suspected carcinogens to human health due to their conformational structures [112]. Their genotoxicity, which depends on activation by mammalian enzymes [111], and environmental persistence exhibit a direct increase in their molecular structure and size, which consists of two or more fused benzene rings that can expand to four or five. The available studies on the potential of diatoms for the bioremediation of PAHs (Table 2) have been carried out mainly on anthracene, benzo(a)pyrene, fluoranthene, phenanthrene, and pyrene. These pollutants are considered in the US EPA's list of 16 PAHs as priority pollutants and in the EU's 15 + 1 list, which are mainly based on toxicity, abundance, lethality to life, and environmental quality.

**Table 2.** Main remarks reported in studies on the role of diatoms in the bioremediation of petroleum hydrocarbons.

| Diatoms | Remarks | References |
|---|---|---|
| *Navicula* sp., *Nitzschia* sp. *Synedra* sp. | Oxidation with 6–9 mg/L of naphthalene at 6 and 12 °C | [35] |
| *Cylindrotheca* sp., *Amphora* sp. | Oxidation of 78 µM naphthalene at 30 °C | [32] |
| *Nitzschia* sp. | Absorption and metabolic degradation of phenanthrene (53%) | [36] |
| *Skeletonema costatum* | Absorption and metabolic degradation of phenanthrene (16%) | |
| *Navicula* sp. 1 and 2, *Nitzschia* sp. | Naphthenic acids *cis*- and *trans isomers*, uptake and degradation at 5.5 mg/L | [37] |
| *Thalassiosira* sp. | Growth and degradation in 0.25 mM phenol and 1 mM benzoate | [39] |
| *Achnanthes minutissima* | n-Alkanes and isoprenoids biodegradation | [33] |
| Diatom BD1IITG | Phenol degradation in the range 50–100 mg/L in refinery waste-water. Highest degradation rate and production of biosurfactant with emulsifying activity at 100 mg/L | [38] |
| *Cyclotella caspia* | Biodegradation of fluoranthene (86%) | [34] |
| *Nitzchia* sp. and Bacteria *Genera Marivita*, *Erythrobacter Alcaligenes* | Biodegradation of benzo(a)pyrene (79%) and fluoranthene (82%) by co-metabolic synergy | [40] |

Among the main pollutants released from oil spills, PAHs represent one of the major components [113,114]. Ben-Othman et al. [114] reported that the common representative of these highly toxic PAHs is naphthalene and its alkylated derivates (89% of the total PAH concentrations), which are commonly found in the oil water-soluble fraction.

Biological transformation remains a prevalent and sustainable alternative to decrease the amount of PAH in the environment. In general, PAHs are weakly biodegradable by bacteria, which is why the expected potential of algae and diatoms remains to be more extensively studied. Recently, diatoms have been attracting attention since they are considered to be one of the most important entry points of contaminants in marine food webs as well as for their association and evolution with oil hydrocarbons. The main factors involved in the bioremediation of PAHs are temperature, oxygen, and pH. Temperature plays an important role in the solubility of PAHs, a condition that increases the bioavailability of PAH molecules for biodegradation [115]. Several authors have indicated that aerobic hydrocarbon biodegradation is most likely the main mechanism of attenuation of crude oil in the marine environment, in which diatoms participate mainly in zones of high hydrocarbon-degrading activity [1,32].

Under such a scenario, diatoms can contribute to the solubilization and enhanced biodegradation of short- and longer-chain alkanes and polycyclic aromatic hydrocarbons (PAHs). Thus, oxygen is the most important element in the biodegradation of hydrocarbon. In principle, the oxygenase content of photoautotrophic microalgae enables the biodegradation of hydrocarbons into metabolites, which can then be metabolized [5,116,117].

In microorganisms, monoxygenases and dioxygenases incorporate two oxygen atoms in the substrate to form diodioxithanes, which are then oxidized into cisdihydrodiols and then into dihydroxy products. When diatoms and microorganisms take up PAHs, aerobic metabolism is switched on by the insertion of two oxygen atoms to produce either cisdihydrodiols or phenols [118,119].

The combined effect of reduced temperature and elevated salinity was observed to result in an increase in EPS mannose and fucose production in the sea ice diatom *Fragilariopsis cylindrus*, which, in principle, is a strategy for helping polar ice diatoms to overcome the cold saline conditions prevailing in sea ice [120]. For example, under high-salinity conditions, *Nitzschia frustulum* increases its gel thickness while also increasing its production of rhamnose and xylose sugars [121]. The transcriptomic analysis carried out by Liang et al. [71] indicated that differentially expressed genes could suggest an increase in the budget of the photosynthate and chemical constitution of aggregates of *Odontella aurita* related to exudates as a response to oil exposure.

Cerniglia et al. [17,32] reported that *Navicula* sp., *Nitzschia* sp., and *Synedra* sp. from the Kachmark Bay region of Alaska were able to metabolize the aromatic hydrocarbon naphthalene at 6–12 °C, oxidizing naphthalene to ethyl acetate-soluble and water metabolites. The amount of naphthalene oxidized to recoverable products ranged from 0.7 to 1.4%. The data suggested that cold-adapted microalgae may prove to be more metabolically active, although this may have been due to their slow growth rates [122]. In experiments at the scale of microcosms at 5 °C, the dominant Arctic diatom *Fragilariopsis cylindrus* and its associated microbiome may play a relevant function in the formation of oil-related aggregates [122]. A role was observed for this diatom in the biodegradation of naphthalene, and the corresponding oil accumulation in the aggregates was observed mainly in the presence of this diatom, which supports its consideration in biodegradation and bioremediation initiatives as an alternative to other unsustainable approaches prohibited by the laws stipulated in the Arctic treaty [43,122]. This information is also valuable because of the availability of hydrocarbon pollutants in cold environments and the fact that harsh conditions are more persistent than in temperate regions [43]. Moreover, according to these results, as well as other findings by Steele and coworkers [77], marked differences can be observed in exopolysaccharide production depending on the taxon and strain, as well as environmental conditions, in the production of tensio active molecules with the aim of degrading petroleum pollutants.

Cerniglia and Heitkamp [123] reported that the initial ring oxidation plays an important role as the rate-limiting step in the biodegradation of PAHs. Diatoms and some eukaryotic algae have the enzymatic ability to oxidize PAHs ranging from naphthalene to benzo(a)pyrene [124]. In the case of *Selanestrum capricornutum*, benzo(a)pyrene is incorporated via the dioxygenase pathway; similarly, diatoms can metabolize naphthene via the dioxygenase pathway [34]. Studies by [125] indicated that the metabolism of phenanthrene and pyrene in diatoms was carried out mainly by the enzyme-oxidation process converting PAHs to less or non-toxic forms of compounds. The complementary biotransformation of organic pollutants, such as 2,4-dichlorophenol by the diatom *Skeletonema costatum*, was confirmed by Yang et al. [86], which was effective for the mitigation of this pollutant.

Headley et al. [37] also reported the successful role of diatoms in the degradation of petroleum naphthenic acid, in which the diatoms *Navicula1*, *Navicula2*, and *Nitzschia* sp. were effective in the uptake of naphthenic acid, which was assumed to mainly be degraded by *Navicula2* at a level of 5.5 mg/L over a period of two weeks. The authors assigned differences in the phytodegradation of naphthalenic acid by transport mechanisms in diatoms and the structure of naphthenic acids. It is accepted that bacteria cannot biodegrade naphthenic acid, thus raising expectations regarding the potential of algae with diatoms as a sustainable source of renewable biomass for the degradation of this organic molecule. Ben Othman et al. [114] studied PAH toxicity and phylogenetic phytoplankton group distribution, mainly between Chlorophyceae and diatoms regardless of the PAH compound. Their correlation analysis of the harmful concentration to 5% of the tested species was 1.21 and 39.7 µg/L for chlorophyceae and diatoms, respectively. In their study, although diatoms proved to be more tolerant than chlorophytes, it was concluded that the comparative survey of PAHs must be further extended by testing other species' taxa sensitivity to PAHs in order to draw any conclusive results.

DNA microarray-based transcriptomics was also applied to investigate the effect of sub-lethal concentrations of the PAH benzo(a)pyrene (BaP) on *T. pseudonana* [126], and the results indicated that genes involved in lipid biosynthesis and catabolism were up- and down-regulated, respectively. These results suggest the activation of the membrane repair mechanism to restore the membrane organization affected by the incorporation of hydrophobic BaP [126]. It would be highly interesting in future works to assess the effect, tolerance, and optimal environmental conditions for the removal or mitigation of specific PAHs in diatom species to obtain information on the possible roles of this type of pollutant in coastal and marine environments.

The effect of the PAH benzo(a)pyrene on *T. pseudonana* was corroborated using DNA-based transcriptomics, in which gene regulation associated with BaP degradation was related to the cell cycle and putative enzymes [127]. Studies on the pennate genome revealed that there have been many lateral gene transfers from bacteria into the diatoms [109]. Thus, it makes sense that Bacillariophyta may contain metabolic pathways involved in petroleum breakdown, in addition to their exopolysaccharides, oxygen, and degradative enzymes. Moreover, the process of exopolysaccharide production occurs under aerobic conditions and thus requires oxygen for successful completion. This relationship gives a clue as to the association between exopolysaccharides and the degradation of hydrocarbons. Previous applications of diatoms in biosynthesis, biodegradation, and biomineralization are gathered in Figure 2. Moreover, reports published recently [23] have also shown that the marine diatom *Thalassiosira* sp. OUC2 can produce enzymes and oxygenases that degrade harmful organic compounds from petroleum hydrocarbons into less toxic compounds, indicating that this diatom may thus be suitable for the remediation of marine environments contaminated by p-xylene.

Previously, Cerniglia et al. [17,34] revealed the ability of diatoms to carry out naphthalene oxidation under photoautotrophic conditions (Table 1), but more studies in this field are needed in order to diversify the limited number of PAHs that can be biodegraded by diatoms and expand our knowledge in this area. Despite the toxicity of PAHs, some marine diatoms, such as *Skeletonema costatum* and *Nitzschia* sp. have shown the ability to degrade phenanthrene (PHE) and fluoranthene (FLA), as reported by [19,20,128], who indicated that *Skeletonema costatum* can stimulate the mineralization rates of PAHs by passively concentrating these pollutants in the marine water column, as was observed in members of *Nitzschia* sp., which are highly efficient degraders of phenanthrene (PHE) and fluoranthene (FLA) [17,32].

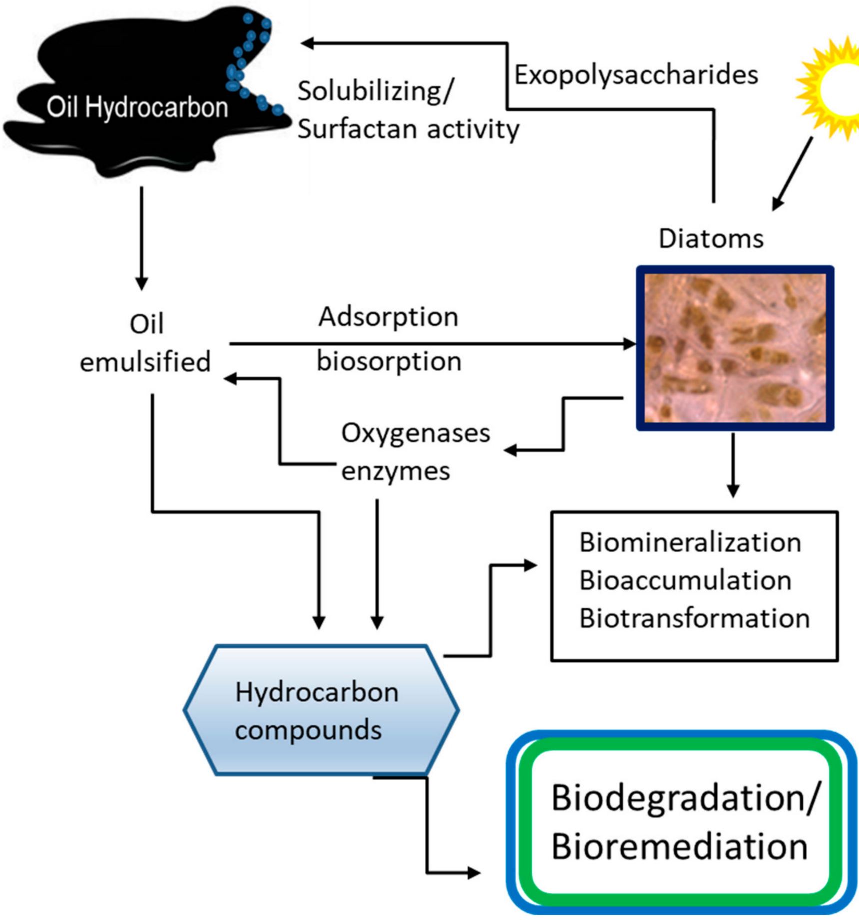


**Figure 2.** Schematic representation of the role of diatoms in the main mechanisms and processes involved in petroleum biodegradation and bioremediation.

Hong et al. [36] also studied the accumulation and biodegradation of two polycyclic aromatic hydrocarbons (PAHs), phenanthrene (PHE) and fluoranthene (FLA), by the diatom species *Skeletonema costatum* and *Nitzschia* sp., isolated from a mangrove estuary ecosystem. The results obtained indicated that *S. costatum* possessed greater accumulation and degradation abilities and tolerances to PHE and FLA than those of *Nitzschia* sp. However, both diatom species exhibited slower degradation of FLA, which may indicate that it is a more toxic and recalcitrant aromatic compound than PHE. However, for the practical purposes of bioremediation, both species demonstrated higher removal efficiency of the mixture of both aromatics compared with the efficiency for PHE and FLA individually, suggesting a synergistic effect when using the two diatoms, where the presence of one PAH stimulated the degradation effect of the other. Genzer et al. [18] pointed out that PAH toxicity in algae is primarily dependent on the species under study, the dose, and the exposure time, as was exemplified when authors discussed the 2010 Deepwater Horizon Oil Spill in the Gulf of Mexico, in which the huge oil volumes also impacted the phytoplankton community. The released oil was reported to be associated with large phytoplankton aggregates mainly dominated by chain-forming diatoms [71,83,85]. Among the diatom species reported in the vicinity of the Deepwater Horizon Oil Spill (station 29.10°N, 87.88°W), in August 2010, were the following dominant eukaryotic members: *Thalassionema nitzschioides* (90%), *Pseudo-Nitzschia* sp., (4%), *Dactyliosolen fragilissimus* (3%), *Leptocylindrus danicus* (2%), and *Cyclotella* sp. (1%). An analysis performed at the station located at 28.64°N, 87.88°W, on the 8th of September 2010, reported that *Pseudo-nitzschia* sp. (50%) was the dominant species, followed by *Thalassionema nitzschioides*, *Leptocylindrus minimus*, *Dactyliosolen fragilissimus*, *Chaetoceros* spp., and *Eucampia* spp., which were all about 7% [18,87].

Although some phytoplankton exhibit resilience when exposed to oil, diatoms have generally been reported as being more resilient than green algae [18,129]. In particular, diatoms are more sensitive to low concentrations of PAHs (<8 ppb) [130]. A remarkable feature is an increase in the numbers of four taxa (*Pseudo-nitzschia*, *Amphidinium*, *Amphidinium*, *Thalassiosira*, and *Chaetoceros*) in the presence of crude oil Corexit solutions, supporting the notion that *Pseudo-nitzschia* spp. may become more abundant during oil spills [18].

The conceptual diagram for the role of diatoms and the main mechanisms and factors involved in the biodegradation and bioremediation of petroleum hydrocarbons in the marine environment is exemplified in Figure 2.

## 6. Diatom–Bacterial Synergy in the Removal of Petroleum Hydrocarbons

Bacteria and eukaryotic phytoplankton such as diatoms have coexisted for a long time. Hydrocarbon-degrader bacteria have been demonstrated to co-occur with phytoplankton [131,132]. In fact, the association between phytoplankton dominated by diatoms and bacteria is the most common interaction occurring in oil spills. During the interactive process, oxygen released by algal photosynthesis is likely to be very important in activating hydrocarbon degradation and acting as an electron acceptor in aerobic respiration [118,131].

The identification and isolation of different hydrocarbonoclastic bacteria from diverse algal cultures [132,133] supports the conclusion of [134] that phytoplankton cells represent a "biotope" for hydrocarbon-degrading bacteria. This could explain the capacity of certain species of phytoplankton, viz., diatoms, to adsorb and produce hydrocarbons [89], which may be indicative of the presence of hydrocarbon-degrading bacteria on diatom cells [134]. Specifically, stimulations upon exposure to oil have been observed in cultures of the diatom *Skeletonema*, in which the oil-stimulating effect induced changes in the bacterial community [89] commonly associated with phytoplankton and dominated by hydrocarbon degraders such as *Piscirickettsiaceae* (including *Methylophaga*) followed by *Arenibacter*, *Marinobacter*, and *Polycyclovorans algicola* [134].

Diatoms have also previously been shown to secrete C8 and C11 hydrocarbons as pheromones [135], which might explain the presence of associated *Alcanivorax* members. It is expected that a well-designed microbial consortium with hydrocarbonoclastic bacteria and complementary catabolic pathways of functional diatoms may contribute to the degradation and bioremediation of hydrocarbons in polluted coastal marine environments. Gutierrez et al. [19] attributed the biodegradation of petroleum hydrocarbon functions by bacteria and diatoms to the ability of each taxon to contribute to the shared functions of bioremediation. In the case of diatoms associated with oiled tidal biofilms, aerobic hydrocarbon biodegradation has been reported to be the most likely primary mechanism of attenuation of crude oil in the early weeks of an oil spill [134]. At the end of the process, the *Nitzschia* strain PLAT907 (EU346643) provides photosynthetically derived oxygen [119] and terminal electron acceptors for alkane degradation. This process is enhanced by the increase in $CO_2$ concentration produced by the heterotrophs.

Recently, co-metabolism has been suggested and, in some cases, validated in order to harness the simultaneous capabilities of microorganisms to achieve successful biodegradation, mainly of recalcitrant high-molecular-weight PAHs [136]. For instance, bacteria carry out the oxidation of aromatic hydrocarbons via the incorporation of dioxygenases into the substrate, initially leading to cis-dihydrodiols [124,137]. Therefore, two oxygen atoms are directed to the aromatic nucleus [136,138], and catechols are formed via the oxidation of cis-dihydrodiols [14]. Aromatic rings are cleaved by other dioxygenases, and subsequently, fumaric, pyruvic, and acetic acids and aldehydes are formed [139]. Therefore, co-oxidation and co-metabolism are effective and feasible processes for achieving the biodegradation of petroleum hydrocarbons, which can be enhanced by the action of surface-active compounds (surfactants) produced by exopolysaccharides of diatom cells in environments containing petroleum hydrocarbons. This process makes hydrocarbons more water soluble and available for degradation by cells.

Diatoms are known to produce exopolymeric substances, which are enhanced by bacterial action [87] in the surrounding seawater. Diatoms' significant production of polysaccharides can provide a favorable organic carbon source for bacteria. Increased production of EPS was observed following the 2010 Deepwater Horizon oil spill, which initially impacted the phytoplankton community in the Gulf of Mexico. Polysaccharides make up a major fraction of the EPSs, with Chrysolaminarin acting as a carbon source reported to contribute to the recovery of *T. pseudonana* in the presence of oil [87]. Hence, the synthesis of chrysolaminarin plays a relevant function in the growth and survival of *T. pseudonana* in the presence of oil, and its inhibition can influence the composition and activity of the surrounding bacterial community [87].

During the process of biodegradation at the oil–water interface, exopolysaccharides released by the diatoms act as an emulsifier, breaking the oil into several small fractions that are subsequently attacked by heterotrophs [140]. Therefore, the degradation of complex organic molecules by consortia of algae containing diatoms and other biodegrading microbes is more effective in the removal of hydrocarbons than algal systems alone [141]. Bacillariophyta, for example, may provide dissolved oxygen to bacteria, which in turn can supply $CO_2$, vitamins, nutrients, enzymes, and iron to diatoms via hydrocarbons [1,130], indicating mutualistic interactions between both taxa for the degradation of oil hydrocarbons (Figure 3).

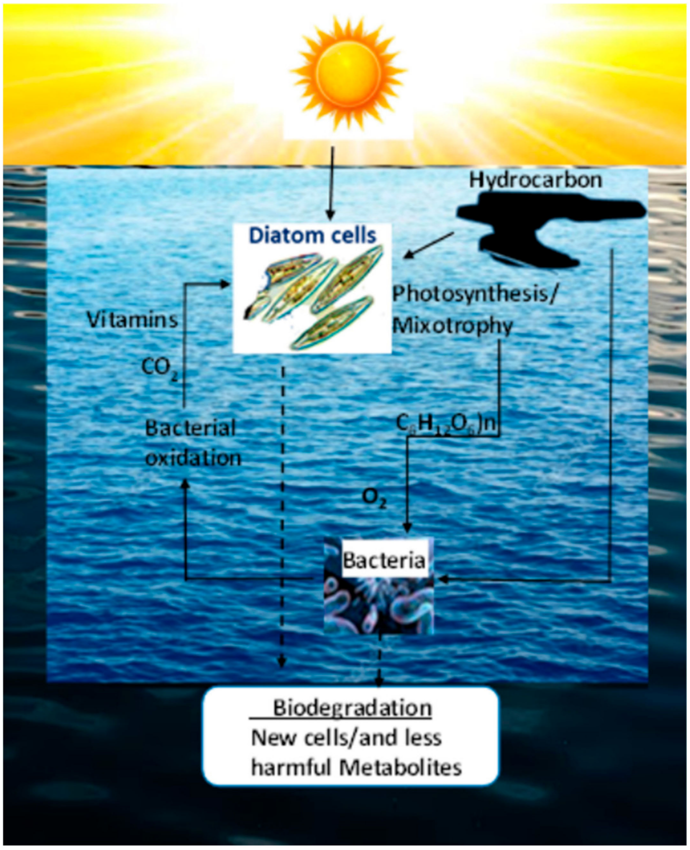


**Figure 3.** Diagrammatic representation of the synergy between diatoms and bacteria during the biodegradation of petroleum hydrocarbon emulsions (adapted from [142]).

In diatoms, EPSs may contribute to hydrocarbon degradation by emulsifying hydrocarbons [1,143]. Moreover, high concentrations of hydrocarbons such as PAHs may contribute to the enhancement of the association between microalgae and bacteria. For instance, the *Navicula phyllepta*-bound EPS has a predominant role during the first stages of biofilm development [143]. The diatoms *S. costatum* and *Nitzschia* sp. can accumulate and decompose two polycyclic hydrocarbons simultaneously [144]. Diatoms also play an important role in supplying organic carbon for bacteria during the production process of polysaccharides. This was evidenced by an analysis of the impact of the 2010 Deepwater Horizon Oil Spill in the Gulf of Mexico, in which the enormous amounts of oil seriously impacted the phytoplankton community through the production of polysaccharides via exopolymeric substances [87].

Modern omics approaches may contribute to an increased understanding of biodegradation and bioremediation initiatives that are based on the ability of diatom genes and their respective repertoires of biochemical pathways, in addition to their environmental and sustainability advantages. The pyrosequencing analysis and qPCR reported by [134] revealed a marked transition in the diatom-associated bacterial community.

This possibility cannot be ruled out, although more studies are needed on the effects of the ecology and interrelationships between specific groups of phytoplankton, diatoms, and bacteria on hydrocarbon genesis and the biodegradation of petroleum in oceans and marine environments.

## 7. Environmental Conditions Affecting Diatoms' Petroleum Hydrocarbon Biodegradation and Bioremediation Activities

Unlike laboratory research, no field trial has been carried out to test the effectiveness of specific diatoms in the biodegradation and bioremediation of petroleum hydrocarbons in the field, including polyaromatic hydrocarbons. This is largely because such trials are difficult, expensive, and unpredictable. This is partially due to the logistical difficulties involved in conducting controlled open-sea trials [145]. Despite their important role and the need for such field trials, the only evidence from such studies is recorded after an oil spill in relatively different time periods. This is the case of oceanographic research cruises that have reported diatoms associated with oil spills and as biomarkers of petroleum hydrocarbon reserves. However, the results of experiments with diatoms exposed to polyaromatic hydrocarbons under controlled conditions using, in some cases, seawater from oil hydrocarbon-contaminated seas [19,36–38,40] have conclusively shown that diatoms' presence enhances the biodegradation of polycyclic aromatic hydrocarbons in contaminated shorelines and seas. Nevertheless, most of the environmental factors that influence microorganisms, mainly eukaryotic microalgae in the sea, also influence the effectiveness of diatoms' biodegradation and bioremediation rate of polyaromatic hydrocarbons in the laboratory, except sea and shoreline energy, such as currents and waves, and seawater pressure [145,146]. Among the environmental parameters, nutrient silicates, nitrogen, phosphorous, and potassium are important for the successful thriving of diatoms in the sea. Other equally important parameters are the level of dissolved oxygen, temperature, exposure to sunlight (photo-oxidation), salinity, and the species of diatom (size, pennate, or centric), sole or in consortium inhabiting the marine habitat as well as the type and concentration of aromatic hydrocarbon present.

## 8. Future Perspectives

Future research is needed to derive an effective petroleum hydrocarbon bioremediation strategy that integrates indigenous diatoms into the process to confirm their effectiveness under field conditions, preferably directly after an oil spill. The possibility of the assessment of nutrient concentrations, mainly silicates, before and after an oil spill may also be considered to expand our knowledge on this topic. The type of diatom species, oil composition and concentration, exposure time to oil components, and environmental status, as well as the prevailing environmental conditions, must be considered for the optimum degradation of such petroleum hydrocarbons by diatoms. Although our knowledge of diatom biosynthesis and hydrocarbon degradation pathways remains patchy with some gaps, their elucidation may further improve our understanding of species-specific capabilities. Advances in the diversity of genomic and metabolic features within different diatom species, particularly, the most performing strains, may suggest multiple possibilities for the manipulation of their enzymes and pathways to redirect and enhance selected processes toward the optimization of petroleum hydrocarbon degradation pathways. Hence, green and sustainable processes that can contribute to the bioremediation of petroleum hydrocarbons in marine environments may be coupled with the co-production of green bioenergy from renewable diatom biomass. Such a perspective represents a significant interest in industrial applications and can be a useful part of the bioenergy value chain to achieve circular economic concepts that contribute to resolving a problem of worldwide interest

## 9. Conclusions

Bioremediation is a highly effective, low-cost, environmentally safe, versatile, and attractive greenway to mitigate and remove petroleum hydrocarbon pollution from terrestrial and marine environments. There are many microorganisms, including eukaryotic microalgae, which show the ability to degrade and metabolize petroleum hydrocarbons, although the results so far remain controversial as there are many gaps that require elucidation. Diatoms may be a unique substitute for existing conventional biological and microalgal methods and open new avenues for the biodegradation and bioremediation of petroleum hydrocarbons for biodiversity-rich environments, accomplishing processes that specific bacteria cannot carry out during the degradation of petroleum hydrocarbons since no single bacterial strain has the metabolic capacity to degrade all the compounds present in crude oil.

For a long time, the evolution of diatoms has been closely associated with the origin of petroleum hydrocarbons in addition to producing hydrocarbons themselves and accumulating intracellular lipid oil bodies and some oils. Diatoms have also been associated with major and minor oil spills in the marine environment. The dominant omnipresence of diatoms in oil-polluted marine environments, and the available early and recent literature on the tolerance and ability to biodegrade petroleum hydrocarbons, are indicative of their degradation potential, support this premise, and place diatoms as unique compared with other microorganisms and eukaryotic microalgae. This fact is comparable to the capacity of bacteria to survive and use such compounds as a strategic and important energy source for growth. Therefore, diatom efficiency can be optimized by the association with hydrocarbonoclastic bacteria, which can complement each other in a consortium through co-metabolic interactions. This synergic collaboration may lead to more effective degradation and bioremediation processes. Diatoms also are rich producers of functional exopolysaccharides and enzymes (oxygenases) capable of dissolving and degrading hydrocarbons as a response to damage through the liberation of free radicals.

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