Global Implications of the Nitrogen Cycle

Edited by

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CHAPTER 4

THE ROLE OF NITROGEN-FIXING BACTERIA IN NITROGEN-LIMITING MARINE WATERS

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Abstract

Crude oil spills are a major threat to the marine flora and fauna. Oil spills like the Exxon Valdez in Alaska and the Deepwater Horizon in the Gulf of Mexico have resulted in devastating environmental and socio-economic losses. Oil spills persist in the ocean for a long time due to their recalcitrant nature and low bioavailability. Therefore, there is a pressing need to develop non-invasive, cost-effective, and eco-friendly methods for the removal of oil spills. Bioremediation is favoured, as chemical and physical methods are either costly or contribute to pollution and hence, inefficient. Marine biologists consider nitrogen limitation to be a crucial factor for regulating natural oil degradation by indigenous microorganisms. Biostimulation is a common strategy for the bioremediation of oil spills, wherein external nutrients like nitrogen are added to kick-start the growth of indigenous hydrocarbon-degrading microorganisms. But this method is costly and the nutrients are continuously lost or diluted by the strong ocean currents at the site of application and thus, need to be regularly replenished. Bioaugmentation is another approach for oil spill management involving the use of effective

exogenous pre-selected oil-degrading microorganisms. However, the long term *in situ* survival of these exogenous microbes becomes difficult due to their competition with indigenous microbes or their inability to acclimatise to the prevailing environmental conditions. They may also be washed away by the strong water currents, or they may not degrade oil effectively due to nitrogen limitation. The isolation of indigenous marine oil-degrading nitrogen-fixing bacteria and their application to oil-polluted sites may compensate for the strong limitation in the bioavailable nitrogen in marine ecosystems, rendering a more effective oil remediation strategy. Also, nitrogen-fixing marine bacteria possessing enzymes like carragenase, agarase, alginate lyase, cellulase, protease, and amylase can be productively used in the treatment of seaweed waste dumped in the marine environment. This chapter focuses on the role of nitrogen-fixing bacteria in the bioremediation of oil and seaweed waste in nitrogen-limiting marine waters.

Introduction

Crude oil is a fossil fuel formed by the decomposition of animal, plant, phytoplankton, and zooplankton remains over millions of years, combined with mud/sediments, at naturally high temperatures and pressure. It is a black, unrefined petroleum product, composed of complex aliphatic, aromatic, polycyclic aromatic hydrocarbons, and heavy metals (Bagbya et al. 2016, E9-E18). It can be refined to produce usable products, such as gasoline, diesel, and various forms of petrochemicals. Drilling is a common method employed by petroleum companies to extract the crude oil. Crude oil pollution is mostly caused by accidents of crude oil transporting tankers, barges, cargo ships, pipelines, refineries, and deep-sea drilling machines or via natural seepage.

Crude oil pollution in the marine environment is a major cause of diseases and mortality in sensitive marine organisms. It causes changes in trophic structure and hinders normal growth, reproduction, and larval settlement (Bagbya et al. 2016, E9-E18; Cruz et al. 2017, 41-66). The effect of oil spills on different life forms of the marine environment is summarised in Table 4-1. Oil spills have a blanketing effect on phytoplankton, macroalgae, and zooplankton with lethal effects on their various vital metabolic processes. Bivalve molluscs tend to accumulate petroleum hydrocarbons to higher concentrations and are consequently, adversely affected. When these bivalves are eaten by humans, it ultimately affects human health. Corals are highly sensitive and easily bleached by oil pollution. Seabirds are vulnerable to oil spills. Their plumage helps to trap warm air and provide buoyancy and insulation. But when their feathers are coated with oil, the seawater comes in contact with their skin resulting in the loss of body heat. Mangroves are also susceptible to oil contamination. When the oil coats the stilt roots or pneumatophores, their lenticels get blocked, thereby preventing gaseous exchange. Thus, oil pollution causes major imbalances in the marine ecosystem and, therefore, necessitates clean-up (Saadoun 2015, 75-103).

Table 4-1: The effect of oil spills on the different marine biota

S. No.	Marine species affected	Effect on Marine species
1.	Phytoplankton and Macroalgae	Reduced photosynthesis due to reduced sunlight penetration (blanket effect by oil slicks)
2.	Phytoplankton and Zooplankton	Displacement of lipids in the plasma membrane and inhibition of the TCA cycle and oxidative phosphorylation in mitochondria, leading to death
3.	Bivalve molluscs	Negative influence on respiration and reproduction
4.	Corals	Coral bleaching induced by the blanket effect
5.	Sea birds	Loss of body heat, hypothermia, hindered flight caused by excessive oil in the plumage and death due to suffocation

There are physical and chemical methods employed to clean up oil spills. Amongst the physical methods, skimmers are commonly employed to skim the layer of oil from the surface of the water. High-pressure hot-water is used to remove oil off beaches. Some of the oil is burned away. But this further leads to the damage of the natural ecosystem since hot water and pressure are detrimental to both the aquatic and the beach biota. The burning of oil also contributes to air pollution (the smoke leads to hydrocarbon pneumonia). Moreover, skimmers are unable to entirely clear the oil spilled over the water. Various chemicals, including surfactants, are also applied to remove oil pollution but their addition contributes to marine toxicity (Atlas and Hazen 2011, 6709-6715). Therefore, alternative, non-toxic, non-invasive, eco-friendly and cost-effective bioremediation strategies, involving natural microbial processes to clean up harmful chemicals and oil spills in the marine environment, play a significant role. Due to such long-term advantages, oil spill management by bioremediation has become the key focus of scientists across the globe, when it comes to pollution abatement and environment preservation. (Atlas and Hazen 2011, 6709-6715; Pasumarthi and Mutnuri 2016, 1-158; Cruz et al. 2017, 41-66).

Seaweeds are a rich source of chemicals including agar, carrageenan, and alginate, which find wide applications in food, feed, microbiology, medical, pharmaceutical, cosmetic, and fertiliser industries (Ficko-Blean et al. 2017). *Gelidium* and *Gracilaria* seaweeds are sourced to make agar which is widely used for its jellifying properties (Chi, Chang, and Hong 2012, 917-930; Song et al. 2015, 275-281). Anthropogenic pollution of marine ecosystems with inorganic nutrients further contributes to seaweed growth. The extensive growth of seaweeds has resulted in seaweed waste pollution in marine waters, severely affecting the marine biota (Suyasa and Dwijani 2015, 059-062). Thus, there is an increasing need to remove seaweed waste from marine waters by bioremediation processes to improve the health of the marine ecosystems.

Nitrogen is a limiting nutrient in marine environments. The biodegradation of oil by microorganisms in the ocean is hampered due to nitrogen limitation (Atlas and Hazen 2011, 6709-6715). Therefore, in this chapter, we will focus on the advantages of using nitrogen-fixing microorganisms capable of degrading oil in oil spill management. Furthermore, we will explore the potential of nitrogen-fixing marine bacteria possessing carragenase, agarase, alginate lyase, cellulase, protease, and amylase enzymes to treat seaweed waste dumped in marine waters.

Oil pollution in marine environments

The various sources of oil pollution in the oceans include petroleum industries, oil fields, petroleum reservoirs, leakages, spills, accidents, combustion of fossil fuels, and natural oil seepages (Cruz et al. 2017, 41-66). Some of the major incidents of oil pollution that occurred in the marine environment are listed in Table 4-2. Pollutants accumulate in the deep-sea sediments over time due to their adsorption to suspended particles and their subsequent sedimentation (Louvado et al. 2015, 312-328).

Some of the most devastating oil pollution disasters in human history include the Exxon Valdez, the Arabian Gulf, and the Deepwater Horizon oil spills (Atlas and Hazen 2011, 6709-6715).

Table 4-2: The major oil spills of the world

S. No.	Oil Spill, the year it occurred	Place and degree of oil spill	Cause	Marine species affected
1.	Deepwater Horizon, 2010	The Gulf of Mexico, United States; 205 million gallons	Explosion of the Deepwater drilling unit of British petroleum	Marine mammals, corals, sea birds, sea turtles, dolphins
2.	Kolva River, 1994	Russia; 84 million gallons	Corroded pipeline causing the dam to collapse	Fish
3.	Arabian Gulf oil spill, 1991	Arabian Gulf, Kuwait; 380-520 million gallons	Crude oil spilled in Arabian Gulf	Shellfish, zooplankton, phytoplankton
4.	Exxon Valdez, 1989	Alaska, USA; 10.8 million gallons	Oil tanker hit Bligh Reef	Seals, fish, shellfish, turtles, sea birds, phytoplankton, zooplankton
5.	Ixtoc 1, 1980	Mexico; 140 million gallons	Explosion at the Ixtoc 1 platform	Phytoplankton, fish

The Exxon Valdez oil spill

The Exxon Valdez oil spill took place on March 24, 1989, when Exxon Valdez, an oil tanker hit Bligh Reef at Prince William Sound, Alaska, USA, spilling around 11 million US gallons of crude oil over a 2100 km coastline (Rabalais et al. 2018, 98-107). The ocean surface covering about

28,000 km^2 area was affected due to the oil spill. The most affected marine organisms included seals, fish, shellfish, turtles, seabirds, seaweeds, phytoplanktons, and zooplanktons (Cruz et al. 2017, 41-66). It was one of the worst environmental disasters caused by humans at the time. Even after extensive clean-up strategies rallied across 18 years, the National Oceanic and Atmospheric Administration (NOAA) found that, in the year 2007, more than 26 thousand US gallons of oil remained in the sandy soil of the contaminated shoreline (Lubic 2009). The United States passed the oil pollution act of 1990 to prevent oil spills after the Exxon Valdez oil spill disaster.

The Arabian Gulf oil spill

During the Gulf War (1990-1991), approximately 1.6 million m^3 crude oil was spilled in the Arabian Gulf on purpose, causing detrimental effects to the ecosystems in the marshland and mud tidal flats in the vicinity. The pollution persisted for almost a decade (Bejarano and Michel 2010, 1561-1569). The coastline from southern Kuwait to Abu Ali Island (770 km) was severely smothered killing marine animals, shellfish, zooplankton, and phytoplankton (Saadoun 2015, 75-103).

The Deepwater Horizon oil spill

On April 10, 2010, the world witnessed the biggest ever oil spill disaster, with the explosion of the Deepwater Horizon offshore drilling unit of British Petroleum in the Gulf of Mexico. The catastrophe resulted in the death of 11 crew members and a subsequent spill of around 4.9 million barrels (210 million gallons) of oil (Arora and Lodhia 2016, 1287-1297; Bagbya et al. 2016, E9-E18; Rabalais et al. 2018, 98-107). Staggering numbers of marine mammals, corals, seabirds, and sea turtles were killed due to this devastating oil spill. By November 2010, the death of 6,104 birds, 609 sea turtles, and 100 dolphins was documented in the spill affected areas. The NOAA estimated 67 more dead dolphins (in the year 2011) in the area affected by the spill and 35 of them were newborns.

Strategies employed for oil spill biodegradation

Several factors affect the biodegradation of oil including both abiotic (temperature, pH, salinity, the physical state of the hydrocarbons, and oxygen availability) and biotic (nutrient availability, microbial diversity, microbial degradation capacity, hydrocarbanoclastic capabilities, and catabolic genes) factors. The three types of commonly bioremediation strategies employed include a) Natural attenuation; b) Biostimulation; and c) Bioaugmentation (Pasumarthi and Mutnuri 2016, 1-158).

Natural attenuation

Natural attenuation is the ability of the indigenous bacteria to degrade oil. The effectiveness of this method depends upon all the factors mentioned above. The degradation of oil usually takes place at a very slow rate (Pasumarthi and Mutnuri 2016, 1-158).

Biostimulation

The degradation of many organic pollutants in the ocean is mainly limited due to the unavailability of essential growth elements, such as nitrogen, phosphorus, iron, and dissolved oxygen. Biostimulation is a tactic of bioremediation in which indigenous microorganisms are stimulated to degrade the oil in the ocean by providing nutrients and suitable growth conditions (Atlas and Hazen 2011, 6709-6715). One of the most effective strategies employed for the bioremediation of oil spills is to spray nitrogen and phosphorus-containing fertilisers at the site. The fertilisers serve as effective nutrient sources for the indigenous microbes, stimulating their growth and facilitating oil degradation. The ideal C/N/P ratio for bioremediation is 100:10:1. (Nikolopoulou and Kalogerakis 2009, 802-807). Biostimulation was used for the bioremediation of both the Exxon Valdez and the Deepwater Horizon spills. However, the addition of exogenous nutrients must be controlled to prevent the proliferation of harmful algal blooms which often lead to eutrophication (Macaulay and Rees 2014, 9-37; Nikolopoulou and Kalogerakis 2009, 802-807). Biostimulation is also costly and the nutrients have to be regularly added due to their loss on dilution by strong ocean currents (Tyagi, Fonseca, and Carvalho 2011, 231-41).

Bioaugmentation

Crude oil is a mix of hydrocarbon-containing compounds. Among the indigenous microorganisms in the marine environment, some may degrade aliphatic hydrocarbons; some may only degrade aromatic hydrocarbons, while some other microorganisms may have the capability to degrade both aliphatics and aromatics. In bioaugmentation, pre-selected oil-degrading microorganisms, tested for their potential to effectively degrade oil, are introduced (by a process called seeding) at the contaminated site. Indigenous microorganisms degrade oil at a very slow rate. Therefore, bioaugmentation favours both the rate and the extent of oil spill biodegradation (Zhu et al. 2004). It is normally done in combination with the monitoring and development of suitable growth conditions. Ideally, seed organisms must degrade most of the crude oil components, must be genetically stable, must possess high growth and enzymatic activity in the oil-contaminated environment, must be able to compete with indigenous microorganisms, must be non-pathogenic, must not lose viability during storage, and must not produce toxic metabolites. Mostly, seed microorganisms are obtained from previous oil spill contaminated sites by enrichment culture techniques (Nikolopoulou, Pasadakis, and Kalogerakis 2013, 165-173). However, in some cases, seed microorganisms are of little benefit over the indigenous microorganisms. The exogenous microbes may be washed away by strong water currents and may not even effectively degrade oil *in situ* due to nutrient limitation and other factors. Furthermore, seed microbes often compete with the indigenous microorganisms for food and space, which may further cause havoc to the ecological balance of the system (Tyagi, Fonseca, and Carvalho 2011, 231-41).

Nitrogen limitation

According to Liebig's 'Law of the Minimum', the most deficient nutrient also limits growth (Bristow et al. 2017; Smith 1984, 1149-1160). The Redfield ratio is the ratio of carbon, nitrogen, and phosphorus (106:16:1), found in phytoplankton and also throughout the deep oceans. This ratio was later revised to 117:14:1 (Redfield 1958, 205-222). According to Redfield, ocean productivity is limited by phosphorus and nitrogen (Redfield 1958, 205-222). Ryther and Dustan (1971) concluded that nitrogen is the main limiting factor for marine productivity (Thomas 1970, 380-385; Goldman et al. 1979, 210-215) and nitrogen loss in the ocean is responsible for the decrease in the average ocean productivity. The main processes responsible for the loss of nitrogen from the ocean are denitrification and anammox (anaerobic ammonia oxidation). Nitrogen lost during these processes in the ocean is replaced by nitrogen fixation. It is this balance between nitrogen fixation and nitrogen loss that regulates the net availability of nitrogen in the ocean and thus, the net marine productivity (Bristow et al. 2017).

In open oceans, there exists a high rate of phytoplankton primary productivity which invariably causes the formation of oxygen minimum zones (OMZ) like those found in the Arabian Sea and the Bay of Bengal (Ulloa et al. 2012, 15996-16003; Bristow et al. 2017; Aldunate et al. 2018). Excessive primary productivity creates high respiration rates in the open seawater column resulting in oxygen depletion between 200-1500 m water depths. These OMZ are characterised by high surface productivity and thus, strong oxygen depletion via the degradation of the sinking organic matter at mid-depth (200-1500 m), exacerbated by the limited oxygen replenishment (Kalvelage et al. 2015). Also, due to the warming of the ocean waters, there is a decrease in the solubility of oxygen. The increasing stratification of the ocean column prevents the mixing of water which forms OMZ at depths of about 200 to 1,500 m. Furthermore, according to researchers, these OMZ are rapidly expanding (Ulloa et al. 2012, 15996-16003; Bristow et al. 2017; Aldunate et al. 2018).

Due to the poor availability of oxygen in these OMZ, the most favourable electron acceptor is nitrate. Nitrate reduction to atmospheric nitrogen by denitrification and the anaerobic oxidation of ammonium to atmospheric nitrogen via anammox cause the overall depletion of nitrogen in OMZ (Zehr 2009, 4575-4576; Bristow et al. 2017). Aldunate et al. (2018) reported that in the OMZ off central Chile, oxygen concentrations modulate bacterial community structure in the coastal upwelling waters. Beman and Corolan (2013) also reported that deoxygenation in the OMZ changes bacterial diversity and community structure. Oxygen depletion and nitrogen limitation in marine waters have a significant influence on carbon cycling (Shaffer, Olsen, and Pedersen 2009, 105-109; Meiyappan et al. 2015, 1524-1548). Oil spills like the Exxon Valdez and the Deepwater Horizon in oceans result in hypoxia (dissolved oxygen level ≤ 2 mg/L) due to the low dissolution of oxygen in the water and lead to OMZ and even dead zones (Rabalais et al. 2018, 98-107).

Initially, it was thought that the upwelling of dissolved inorganic nitrogen (DIN) from deep marine waters is relatively more than the amount of nitrogen fixation in marine waters. But in recent years, this view has changed and it is evident from the research work that marine nitrogen fixation is a very important process contributing the same amount of nitrogen input as DIN to surface waters (Sohm, Webb, and Capone 2011, 499-508). Biological nitrogen fixation in the ocean is an important factor for marine productivity and also greatly influences the global carbon cycle. Nitrogen fixers in marine waters have a considerable competitive benefit wherever nitrogen is limiting, and the activity of nitrogen fixers could reverse nitrogen limitation in these waters (Vitousek and Howarth 1991, 87-115). The limitation of nitrogen fixers due to limitations of other inorganic nutrients, such as phosphorus, molybdenum, and iron in the ocean, may ultimately be responsible for the reduction in the net primary productivity (Delmont et al. 2018, 804-813), and may also affect the rate of natural waste degradation (Vitousek and Howarth 1991, 87-115). Cyanobacteria like *Synecococcus* and *Trichodesmium* contribute greatly towards marine nitrogen fixation (Spiller and Shanmugam 1987, 5379-5384) and provide around half of the required nitrogen for primary production (Sanudo-Wilhelmy et al. 2001, 66-69). Earlier, it was believed that only cyanobacteria contributed towards nitrogen fixation in marine waters. Very recently, for the first time, metagenomic studies revealed that along with cyanobacteria, microbial populations belonging to Planctomycetes and Proteobacteria were among the members carrying out nitrogen fixation and contributing towards the oceans' primary productivity (Delmont et al. 2018, 804-813). *Vibrio diazotrophicus*, expressing the *nifH* gene, has been found to be abundant in the OMZ (Cheung et al. 2016, 380-391). The *nifH* transcription data for non-cyanobacterial diazotrophs (NCDs) and nitrogen fixation rates in the absence of diazotrophic cyanobacteria have been detected in marine pelagic environments (Moisander et al. 2017). A recent study on metagenomics, using catalytic *nifH* and *nifD* genes from a wide range of heterotrophic bacterial diazotrophs (HBDs) from open ocean surface waters, provided evidence that such non-cyanobacterial nitrogen fixers belong to lineages within the Proteobacteria and the Planctomycetes, and contribute widely to the fixed nitrogen. These were closely related to the genera *Desulfovibrio*, *Pseudomonas,* and *Azotobacter* (Delmont et al. 2018, 804-813). Delmont et al. (2018) also reported that the discovered diazotrophs were widespread and abundant in the large surface waters of the global oceans (Pacific Ocean and the Atlantic Ocean). Therefore, free diazotrophic bacterial nitrogen fixers with the ability to degrade crude oil components may play an important role in bioremediation studies.

Role of nitrogen fixers in oil degradation

A free-living, nitrogen-fixing bacterial strain of *Azotobacter chroococcum* was isolated from the Tuticorin harbour polluted with crude oil and was found to have a strong potential for the degradation of up to 58% crude oil. The *Azotobacter chroococcum* culture was capable of emulsifying crude oil, diesel, kerosene, naphthalene, anthracene, and xylene via the production of biosurfactants. Bacteria belonging to the genus *Azotobacter* naturally do not possess the ability to breakdown crude oil and are known to pick up the degradative plasmids from the environment via horizontal gene transfer (HGT) (Thavasi et al. 2006, 401-408). The relationship between nitrogen fixation and hydrocarbon degradation was investigated by Taketani et al. (2009) in a mangrove sediment mesocosm. Piehler et al. (1999) observed that the addition of particulate organic carbon (POC), to a diesel polluted marine system, stimulated the capacity of the indigenous marine nitrogen-fixing microbial consortia to degrade petroleum hydrocarbons. The *nifH* gene diversity studies showed the presence of nitrogen-fixing bacteria associated with an extensive range of taxa, containing members of the Alpha-Proteobacteria, Beta-Proteobacteria, Gamma-Proteobacteria, and Firmicutes in oil spill affected mangrove systems located along the coastline of São Paulo, Brasil and proved the involvement of indigenous nitrogen-fixing bacteria in crude oil degradation (Dias et al. 2012, 7960-7967). Three free-living heterotrophic nitrogen fixers *Acinetobacter junii, Achromobacter sp.*, and *Alcaligenes faecalis* were isolated from the crude oil contaminated soil in the vicinity of the Digboi crude oil refinery of Assam, India (Mazumdar and Deka 2013, 69-76). Recently, heterotrophic nitrogen-fixing bacteria *Raoultella ornithinolytica* strain BAL286, *Pseudomonas stutzeri* strain BAL361 (Gamma-Proteobacteria), and *Rhodopseudomonas palustris* strain BAL398, (Alpha-Proteobacterium) containing aromatic hydrocarbon metabolising genes were isolated from estuarine surface waters, of which, *R. ornithinolytica* was previously also isolated from oil-contaminated soil. It was found that nitrogen fixation (via the nitrogenase gene complex) was clustered in distinct regions of the genome in all three organisms that acquired the trait through HGT (Bentzon-Tilia et al. 2015, 1-12). Thus, it is possible that the indigenous microorganisms originally possessing the ability to degrade aromatic hydrocarbons may later acquire the nitrogen-fixing ability through HGT. A reverse situation wherein, the indigenous bacteria capable of nitrogen fixation may acquire the capacity to degrade oil via HGT can also occur. However, this needs to be confirmed by further research. Toccalino et al. (1993) examined nutrient limitations during hydrocarbon degradation (alkane) in sandy soil and found that nitrogen was initially a limiting nutrient but nitrogen limitation was overcome by biological nitrogen fixation. Two bacterial nitrogen-fixing soil isolates, *Agrobacterium* sp. and *Alcaligenes* sp., were found to possess the potential to degrade petroleum aromatic hydrocarbons (benzene, toluene, and xylene) (Prantera et al. 2002, 85-89).

Through these studies it is clear that nitrogen-fixing hydrocarbon-degrading bacteria can not only render nitrogen available, but can also act as important tools to improve the efficiency of bioremediation strategies. Therefore, bioprospecting for nitrogen-fixing, oil-degrading marine and terrestrial bacteria can find significant use in the bioremediation of environmental sites polluted by oil spills.

Nitrogen fixers in seaweed biodegradation

In recent years, seaweed waste has increased enormously. The extensive growth of seaweeds owing to anthropogenic pollution of aquatic ecosystems with inorganic nutrients viz. nitrate, iron, and phosphorus, is a major concern. Furthermore, the mass culturing of seaweeds for industrial applications, such as food, cosmetics, and media preparation in Microbiology and Biotechnology research generates abundant seaweed waste (Tang et al. 2009, 38-43). Ultimately, industrial waste containing algal components is directly discharged into the marine environment leading to an increased BOD. This has a detrimental impact on marine life. Therefore, sustainable seaweed waste management using marine bacteria capable of fixing nitrogen and producing enzymes for the complete degradation of seaweed is significant.

The major organic components of seaweed include carbohydrates and proteins which may be as high as 44% of the dry matter of the seaweed. The storage polysaccharides include starch and glycogen whereas the structural polysaccharides (cell wall constituents of various groups of macroalgae) include cellulose, xylan, mannan, carrageenans, agar, and alginate (Misurkova 2012, 173-192). Therefore, microorganisms producing enzymes, such as cellulase, alginate lyase, agarase, carrageenase, amylase, and protease could prove extremely important to the complete microbial degradation of seaweed.

Naik et al. (2018) isolated marine *Ulva lactuca* (edible green alga commonly called sea lettuce belonging to the family Ulvaceae) associated *Vibrio brasiliensis* strain DM1, *Bacillus subtilis* strain DM5, and *Pseudomonas aeruginosa* strain DM15 capable of producing multiple polysaccharide-degrading enzymes viz. agarase, carrageenase, cellulase, protease, and amylase. These marine bacterial isolates were also found to degrade seaweed (*Sargassum*) waste efficiently in seawater-based media with *Sargassum* powder as the sole source of carbon. All three bacterial isolates were free-living nitrogen fixers.

In 1986, agar-degrading nitrogen-fixing bacteria were isolated from seawater and eelgrass bed sediments of Kanagawa, Japan (Shieh, Simidu, and Maruyama 1988, 1821-1825). Furthermore, nitrogen fixation associated with the decomposition phase of the Giant Kelp *Macrocystis pyrifera* (brown algae) was reported by Hamersley et al. (2015). It was observed that the nitrogen-fixing activity was more on the surface of *Macrocystis pyrifera* in the decomposing phase than on the surface of actively growing *Macrocystis pyrifera* in the living phase (Hamersley et al. 2015, 57-63), which confirmed the role of nitrogen-fixing bacteria in the decomposition of algal waste.

Marine seaweeds harbour several associated heterotrophic bacteria which play a crucial role in their survival processes. Seaweed-bacterial relationships primarily thrive on the production of organic matter (food) by the photosynthetic seaweed which is also utilised by the associated bacteria. Additionally, marine seaweed associated bacteria provide CO_2 and minerals to the seaweed (Comba-González et al. 2016; Singh and Reddy 2016). Some associated bacteria provide auxins, siderophores, and fix nitrogen, increasing the cell division and growth of the seaweed (Comba-González et al. 2016; Singh and Reddy 2016). However, when the seaweeds die, these associated bacteria possess enzymes, such as agarase, carrageenase, alginate lyase, cellulase, protease, and amylase and thus, actively participate in the decomposition of the algal waste and contribute to the cycling of nitrogen and carbon. Therefore, such seaweed associated, nitrogen-fixing bacteria, exhibit great potential and can find significant applications in the bioremediation of marine sites polluted with algal waste instead of biostimulation strategies.

Concluding remarks

Nitrogen limitation in the ocean is considerably responsible for the decrease in microbial activity and ocean productivity. In addition to several biotic and abiotic factors, nitrogen limitation also affects the degradation of oil by indigenous bacteria. The isolation of indigenous marine oleophilic, nitrogen-fixing bacteria and applying them to oil-polluted sites can compensate for the strong limitation in available nitrogen in marine environments and degrade oil more effectively. Furthermore, nitrogen-fixing marine bacteria possessing enzymes, such as carragenase, agarase, alginate lyase, cellulase, protease, and amylase can be applied in the sustainable treatment of seaweed waste accumulating exorbitantly in marine waters.

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