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Biofertilizer for Bioremediation

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ABSTRACT

Industrial revolution is the mother of environmental pollution. This affects the quality of life and ecosystem. To reduce the environmental pollution, various attempts are being taken in different ways. Among these attempts bioremediation is one of the good remedial techniques. In this technique microorganisms are used to reduce the toxicity of harmful substances from contaminate environments. Bioremediation solves the problem in the field of solid and liquid wastes by the process of detoxification. It is an attractive clean-up technology due to its cost effectiveness and environmental-friendly nature. Biofertilizer is a large population of a specific or a group of beneficial microorganisms incorporated aseptically into sterile carrier materials. Microorganisms perform several important processes such as cycling of nutrients and degradation of various compounds. In sustainable agriculture, algae, bacteria and fungi are being utilized as biofertilizers. Plant growth promoting bacteria (PGPB) plays important role in soil fertility and can be used as biofertilizers that stop deteriorations of soil caused by excessive application of chemical fertilizers. Different microbes were utilized in degradation of different environmental contaminants like hydrocarbons, heavy metals, and toxic substances. In recent years researchers are carried out to study the effect of bioremediation by using biofertilizer in reducing the environmental hazards.

Keywords: Microorganisms, Biofertilizer, Bioremediation, Biodegradation.

13.1 INTRODUCTION

Biofertilizer use is the best modern tools for sustainable agriculture. Biofertilizer is an important component of integrated nutrient management in agricultural system. It is a cost effective and renewable source of plant nutrients to supplement the chemical fertilizers. The commercial history of biofertilizers began by Nobbe and Hiltner, a laboratory culture of *Rhizobia* in 1895, followed by the discovery of *Azotobacter* and then the blue green algae and a host of other microorganisms. In India the first study on legume *Rhizobium* symbiosis was conducted by N.V. Joshi and the first commercial production started in middle of 1950s.

Biofertilizers are the most advanced biotechnology tool to support developing organic agriculture, sustainable agriculture, green agriculture, and non-polluting agriculture. This bioorganic fertilizer can increase the output, improve the quality in agriculture. In recent years the biofertilizer has been widely used with excellent results in agriculture all over the world. It is well known that continuous and excess use of chemical fertilizers and pesticides have caused a detrimental effect to our environment and health. The alternate sources of environment-friendly plant nutrients are the immediate need for the recent times. Biofertilizers has become a hope for the future world as far as economical and environmental viewpoints concerned. Especially in developing countries like India it can solve the problem of high cost of chemical fertilizers and help in saving the economy of the country [1, 2]. The disposal of bio-digested slurry after biogas production is a major concern for the environment [3-8]. It contains considerable amount of plant nutrients and helps to improve crop production, also preventing adverse environmental impacts of waste disposal [9, 10]. The application of biodigested slurry in agricultural field increases the soil fertility by fixing atmospheric nitrogen, solubilize soil phosphates and produces plant growth substances in the soil. The role and importance of biofertilizers in sustainable crop production has been reviewed by several authors [11-13]. The degradation of organophosphorus insecticides, chlorpyrifos, chlorpyrifos – methyl, cyanophos and malathion in mineral salts media were studied by several researchers [14-18]. The effect of additional biofertilizers, individually or combined with organic amendments, on chlorpyrifos and cyanophos degrading activity in soil were investigated. *Paenibacillus polymyxa* (*Bacillus polymyxa*) and *Azospirillum lipoferum* (Beijerinck) were found to degrade the organophosphorus insecticides, chlorpyrifos, chlorpyrifos — methyl, cyanophos and malathion in mineral salts media as a carbon and phosphorus source. *Bacillus polymyxa* appeared to be more effective than *Azospirillum lipoferum* in degrading all the tested organophosphate pesticides in mineral salts media.

There is a continuous interest in algal based waste stabilization pond systems that are inexpensive and are known for their ability to achieve good removal of pathogens and organic pollutants all over the world [19]. Cyanobacteria and microalgae plays an important role by supplying molecular oxygen to heterotrophic partners and thus support the initial steps of degradation [20]. Nutrient removal with aid of algae compares favourably with other conventional technologies [21-23]. It also found that some cyanobacteria and algae might remove xenobiotics from the environment by sorption, transformation and degradation [24]. Several attempts have been made to explore the efficiency of microalgae for metal removal [25-27]. Increasing the light intensity can lead to a higher microalgal activity and an increased removal of nutrients from wastewater [28].

Phycoremediation is the process of employing algae for improving water quality which can fix carbon dioxide by photosynthesis and remove excess nutrients effectively at minimal cost [29-31]. The use of algae in purification of wastewater and to eliminate the nutrients was studied widely [32, 33]. Additionally, these photosynthetic microorganisms are also useful in bioremediation applications and as nitrogen-fixing biofertilizers [34-36]. Anabaena is capable of evolving hydrogen through the process of indirect biophotolysis of water, using nitrogenase as a catalyst. Hydrogen stands a promising alternative to fossil fuels, being renewable, eco-friendly and efficiently [37]. Photobiological hydrogen production by cyanobacteria is an ideal process, owing to their simple nutritional requirements from air, water, mineral salts, and light as the main energy source [38, 39]. There are several reports concluding that the metal-binding capacities of several biomasses including marine algae, fungi, and yeast are very high [40-44].

13.2 MICROBES IN BIOFERTILIZER PRODUCTION

Biofertilizers are products containing living cells of different types of microorganisms which, applied to seed, plant surface or soil, colonize the rhizosphere or the interior of the plant and promotes growth by converting nutritionally important elements (nitrogen, phosphorus) from unavailable to available form through biological process such as nitrogen fixation and solubilization of rock minerals [45]. Beneficial microorganisms in biofertilizers accelerate and improve plant growth and protect plants from pests and diseases [46]. There is important role of soil microorganisms in sustainable development of agriculture [47-48]. Organisms that are commonly used as biofertilizers component are nitrogen (N) fixers, potassium (K) solubilizer and phosphorus (P) solubilizer, or with the combination of molds or fungi. Most of the bacteria included in biofertilizer have close relationship with plant roots. Rhizobium has symbiotic interaction with legume roots, and Rhizobacteria inhabit on root surface or in rhizosphere soil.

13.2.1 Nitrogen-Fixing Microorganism

Nitrogen is one of the major important nutrients essential for plant growth. Nitrogen fixation in soil by different microorganism is a complex biochemical process by which atmospheric elemental nitrogen (unavailable to plants) is transformed into organic nitrogenous compound, which is available to plants. Different species of bacteria, some actinomycities and blue-green algae are agriculturally important Nitrogen fixers. The value of nitrogen-fixing legumes in improving and higher yield of legumes and other crops can be achieved by the application of biofertilizers [49]. Nitrogen-fixing bacteria (NFB) that function transform inert atmospheric N_2 to organic compounds usable for plants [50-51]. Nitrogen fixer organism are used in biofertilizer as a living fertilizer composed of microbial inoculants or groups of microorganisms which are able to fix atmospheric nitrogen. They are grouped into free-living bacteria (Azotobacter and Azospirillium) and the blue-green algae and symbionts such as Rhizobium, Frankia and Azolla [52]. The N_2-fixing bacteria associated with non-legumes includes species of *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Acetobacter*, *Azomonas*, *Beijerinckia*, *Bacillus*, *Clostridium*, *Enterobacter*, *Erwinia*, *Derxia*, *Desulfovibrio*, *Corynebacterium*, *campylobacter*, *Herbaspirillum*, *Klebsiella*, *Lignobacter*, *Mycobacterium*, *Rhodospirillum*, *Rhodo-pseudomonas*, *Xanthobacter*, *Mycobacterium* and *Methylosinus* [53].

13.2.1.1 Symbiotic nitrogen-fixing microorganism for leguminous plants

Symbiotic bacteria infecting plant roots, numerous taxa of less intimately associated N_2-fixing bacteria can be considered for improvement of crop yield. Examples of such bacteria include *Acetobacter diazotrophicus* and *Herbaspirillum* spp. associated with sugar cane, sorghum, and maize [54-56], *Azoarcus* spp. associated with kallar grass *(Leptochloa fusca)* [57], and *Alcaligenes, Azospirillum*, *Bacillus, Enterobacter, Herbaspirillum, Klebsiella, Pseudomonas,* and *Rhizobium* associated with rice and maize [58]. The genus *Azospirillum* colonizes a great variety of annual and perennial plants, many of which have never been reported to be colonized by N_2-fixing bacteria. Accordingly, *Azospirillum* possesses a great potential as a general root colonizer, whose use is not limited by host specificity [59]. Several studies indicate that *Azospirillum* can increase the growth of various crops, include sunflower, carrot, oak, sugar beet, tomato, eggplant, pepper, and cotton in addition to wheat and rice [59, 60].

Some species of Rhizobium bacteria forms nodule by colonizing in the roots of different leguminous plants. They derive carbohydrates (energy) and water from the root tissues of the host plants, which enables them to directly fix atmospheric nitrogen for the production of amino acids and protein to make cell materials. In return, the host plant received the mentioned nitrogenous compounds synthesized by the bacteria for their own growth and development.

13.2.1.2 Free-living or non-symbiotic nitrogen-fixing microorganism

Many free-living bacteria also fix atmospheric N_2 like *Azotobacter, Beijerinckia,* and *Clostridium.* In favourable condition nodulating bacterial symbionts (e.g., *Frankia*) of plant roots can also fix N_2 without symbiotic association (free-living) with their plant host [61]. More interestingly, it has been found that *Frankia* can occur and possibly fixes atmospheric N_2 in the rhizosphere of non host plants. *Frankia* has been recorded in the rhizosphere of *Betula pendula* and in soil where actinorhizal plants were not present [61, 62]. Certain free-living microorganisms exist in soil and water is able to fix atmospheric nitrogen without symbiosis. These organisms are mainly bacteria (i.e., *Azotobacter, Clostridium, Verxia*, *Azospirillum,* etc.). Among the free-living nitrogen-fixing bacteria, Azotobacter is most important. They colonize in rhizosphere and derived their energy by oxidation of soil organic matter and fix atmospheric nitrogen into the soil and subsequently increase the soil fertility.

13.2.1.3 Blue green algae and azolla

N-fixing blue-green algae (BGA) or cyanobacteria and *Azolla* (free floating water-borne fern), have been shown to be the most important in maintaining and improving the productivity of rice fields [63]. It has been demonstrated that the N fertility of soil is sustained better under flooded conditions than under dryland conditions [64]. Favourable conditions for biological N_2 fixation by such BGA is considered to be one of the reasons for the relatively stable yield of rice under flooded condition. Unlike chemical N fertilizers, BGA and *Azolla* neither contaminate the environment nor consume the photosynthates of rice plants [65]. The importance of N_2- fixing BGA was first recognized by De [66, 67], who attributed the self-maintenance of the N status of tropical rice-field soils to the growth of N_2-fixing BGA. Similarly, the fertilizing value of *Azolla* in rice fields is well-known and has been utilized over year old in China and Vietnam [68, 69]. The plant-available N of rice soils is increased considerably by the growth of N_2-fixing BGA and *Azolla* [70-80]. BGA liberate extracellular organic compounds and photosynthetic O_2 during their growth, while *Azolla* prevent a rise in the pH, reduce water temperature, curb NH_3 volatilization and suppress weeds; and both of them contribute biomass.

The symbiotic association of Anabaena and Azolla produces 40-60 tonnes organic matter and at the same time they are capable of fixing 100 to 150 kg of atmospheric nitrogen per hectare per year. In India, their use as biofertilizer is yet to be popularized and their location specific and thermo-sensitive cultures are not easily available in the market. Another difficulty in mass production of these biofertilizer is costly.

13.2.2 Phosphate Solubilizing Microorganisms

Phosphorus is one of the least mobile elements in soil. In most soils (especially in acid soil), soluble phosphatic fertilizers locked in the soil by reacting with calcium, ferrous and aluminium and converted into insoluble phosphate. Phosphorus is the second most limiting plant nutrient after nitrogen [81]. Total P content in soil is usually high, but most of this soil P pool is not in forms available for plant uptake. Bacteria that can mobilize P from unavailable soil pools and increase P availability to plants are of great importance. Most predominant phosphorus-solubilizing bacteria (PSB) belong to the genera *Bacillus* and *Pseudomonas* [82]. The fixed phosphorus in the soil can be solubilized by phosphate solubilizing bacteria, which have the capacity to convert inorganic unavailable phosphorus to available for the plants. Bacteria are more effective in phosphorus solubilization than fungi [83]. Among the whole microbial population in soil, phosphate solubilizing bacteria (PSB) constitute 1 to 50%, while phosphorus solubilizing fungi (PSF) are only 0.1 to 0.5% in P solubilization potential [84].

Microorganisms involved in phosphorus acquisition include mycorrhizal fungi and phosphate solubilizing microorganism (PSMs) [85]. Among the soil bacterial communities, ectorhizospheric strains from *Pseudomonas* and *Bacilli*, and endosymbiotic rhizobia have been described as effective phosphate solubilizers [86]. Strains from bacterial genera *Pseudomonas*, *Bacillus*, *Rhizobium* and *Enterobacter* along with *Penicillium* and *Aspergillus* fungi are the most powerful P solubilizers [87]. *Bacillus megaterium*, *B. circulans*, *B. subtilis*, *B. polymyxa, B. sircalmous, Pseudomonas striata*, and *Enterobacter* could be referred as the most important strains for phosphate solubilizer [88, 89]. A nemato fungus *Arthrobotrys oligospora* also has the ability to solubilize the phosphate rocks [90].

13.2.3 Potassium Accumulating Organism

Potassium is one of the important nutrients for plants. There are a number of microorganisms which can also accumulate potassium and thus help in enhancing soil fertility. Some microorganisms in the soil are able to solubilize unavailable forms of K-bearing minerals, such as micas, iolite and orthoclase, by excreting organic acids which either directly dissolves rock K or chelating silicon ions to bring the K into solution [91,92]. These microorganisms are commonly known as potassium solubilizing bacteria (KSB) or potassium dissolving bacteria or silicate dissolving bacteria. Some research has been made about the use of potassium dissolving bacteria, known as "biological potassium biofertilizer (BPF)", particularly in China and South Korea to investigate the bio-activation of soil K-reserves so as to alleviate the shortage of K-fertilizer. It was shown that KSB increased K availability in soils and increased mineral uptake by plant [93, 94]. Therefore, application of KSB holds a promising approach for increasing K availability in soils.

13.2.4 Sulphur Solubilizing Organism

Sulphur is generally regarded as trace element in majority of crop plants. But this is one of the major elements in oilseed crops, some important vegetables (onion, oat, cauliflower, etc.) and in some spices (ginger, garlic, etc.). Sulphur is essential for biochemical synthesis of some important glycosides, pungent compound and disease resistant properties. Deficiency of sulphur in agricultural soil could be corrected using *Azotobacter pasturianam* as boifertilizer.

It is essential for all plants and is indispensable for the oxidation of pyrite can be coupled to solubilization growth and metabolism. Khandkar et al., observed that the nodule in blackgram was increased due to sulphur application [95]. Sulphur is involved in the formation of nitrogenase enzyme and is known to promote nitrogen fixation in legumes [96]. Sulphur is involved in the formation of chlorophyll [97]. Hanesklaus and Schnug reported that sulphur is associated with production of crops of superior nutritional and market quality [98]. The sulphur application increased the total chlorophyll content of greengram [99]. Sulphur plays a dominant role in improving the quality of pulses [100].

13.2.5 Mycorrhiza

Mycorrhizal fungi form mutualistic symbiosis with a vast majority of land plants [101]. Possibly more than 80% of all land plants form mycorrhizal symbiosis. The extent of mycorrhizal symbioses emphasizes the ancient evolutionary history and potential importance of fungal symbioses for plant production and physiology. The association between plants and their root-colonizing mycorrhizal fungi is a functional symbiosis in which the mycorrhizal fungus is obligately or facultatively dependent on host photosynthates and energy. Mycorrhizal fungi may also improve soil quality by having a direct influence on soil aggregation and therefore aeration and water dynamics [102]. An interesting potential application for mycorrhizal fungi is their ability to allow plant access to nutrient sources generally unavailable to the host plants. For example, crop plants may be able to use insoluble sources of P when inoculated with mycorrhizal fungi but not in the absence of inoculation [101].

13.2.6 Cellulose Decomposing Inoculants

Cellulases are especially common in soil and plant inhabiting fungi. Many fungi in the Ascomycotina and Basidiomycotina are able to digest cellulose. The necessary enzymes are less common in members of the Zygomycotina. Presence of the cellulase enzymes in moist soil may indicate release of glucose dimers available for absorption by all microbes in the near vicinity which indicating a continuing and community-based process of cellulose degradation by microbes. Cellulose is the most abundant constituent of plant residues. It has been reported that cellulose decay would be enhanced by nitrogen addition due to the increased cellobiases [103,104]. *Serpula lacrimans*, a cellulose degrading fungus, produced more mycelium and faster decay of cellulose under nitrogen fertilization [105].

Excess doses of manure or sludge fertilizers can harm mesofauna because of toxicity (e.g., anhydrous ammonia) and high osmotic pressure create due to salt accumulation [106], or heavy metal accumulation [107]. The repellent nature of ammonium can affect soil invertebrates adversely [108]. The potential of toxic effects can be decreased by applying composted manure or sludge [109]. The time period for material is composted prior to incorporating it into soil must be considered. For example, the plant-pathogenic fungus *Rhizoctonia solani* may cause damping-off disease in soil when fresh or immature compost material of high content of cellulose is added. However, in aged compost, cellulose is degraded and the biocontrol fungus *Trichoderma* spp. can grow and parasitise the pathogen effectively. Many soil-borne fungal species like *Aspergillus, Penicillium, Tricoderma, Chaetomium,* etc. acts as activator in the decomposition process of plant bodies containing cellulose or lignin.

13.3 CONSTRAINTS AND SUCCESS OF BIOFERTILIZER TECHNOLOGY

Though the biofertilizer technology is a low cost, eco-friendly technology, several constraints limit the application or implementation of the technology. The constraints may be environmental, technological, infrastructural, financial, human resources, unawareness, quality, marketing, etc.

13.3.1 Technological Constraints

- Use of improper, less efficient strains for production.
- Lack of qualified technical personnel in production units.
- Unavailability of good quality carrier material or use of different carrier materials by different producers without knowing the quality of the materials.
- Production of poor quality inoculants without understanding the basic microbiological techniques.
- Short shelf life of inoculants.

13.3.2 Infrastructural Constraints

- Non-availability of suitable facilities for production.
- Lack of essential equipments, power supply, etc.
- Space availability for laboratory, production, storage, etc.
- Lack of facility for cold storage of inoculant packets.
- Financial constraints.
- Non-availability of sufficient funds and problems in getting bank loans.
- Less return by sale of products in smaller production units.

13.3.3 Environmental Constraints

- Seasonal demand for biofertilizers.
- Simultaneous cropping operations and short span of sowing/planting in a particular locality.

- Soil characteristics like salinity, acidity, drought, waterlogging, etc.

13.3.4 Human Resources and Quality Constraints

- Lack of technically qualified staff in the production units.
- Lack of suitable training on the production techniques.
- Ignorance on the quality of the product by the manufacturer.
- Non-availability of quality specifications and quick quality control methods.
- No regulation or act on the quality of the products.
- Awareness on the technology.
- Unawareness on the benefits of the technology.
- Problem in the adoption of the technology by the farmers due to different methods of inoculation.
- No visual difference in the crop growth immediately as that of inorganic fertilizers.

13.3.5 Awareness on the Technology

- Unawareness on the benefits of the technology.
- Problem in the adoption of the technology by the farmers due to different methods of inoculation.
- No visual difference in the crop growth immediately as that of inorganic fertilizers.
- Unawareness on the damages caused on the ecosystem by continuous application of inorganic fertilizer.

13.3.6 Marketing Constraints

- Non-availability of right inoculant at the right place in right time.
- Lack of retain outlets or the market network for the producers.

13.4 BIOREMEDIATION PROCESS: AN OVERVIEW

Bioremediation is defined as a process whereby hazardous wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities [110]. This uses living organisms, especially plants and microorganisms, to reduce, eliminate, transform, and detoxify the unwanted products present in soils, sediments, water, and air. Phytoremediation technology, one of its many approaches, uses plants as filters for accumulating, immobilizing, and transforming the contaminants to less harmful form [111]. More specifically, it is the utilization of vascular plants, algae, and fungi to control, breakdown, remove wastes, or to encourage degradation of contaminants in the rhizosphere [112]. Phytoremediation has recently become a tangible alternative to the traditional methodology in restoring the polluted sites [113].

Bioremediation technologies currently used in many countries are mostly directed to the remediation of oil spillages on land and include in-situ biotreatment of contamination, for example, the addition of bacterial fertilizers, mineral, and organic nutrients to the oil-contaminated soil [114-116]. However, these technologies are not acceptable for the treatment of oil wastes, as the high concentration of toxic contaminants and anaerobic conditions in the pit content prevent the development of an active oil-oxidizing microbial consortium.

Rhodococcus biosurfactants have been used for the bioremediation of oil-contaminated agricultural soils after an accidental oil spill [117, 118]. The application of composting systems enhanced by nutrient addition, bulking with straw and inoculation of Rhodococcus-biosurfactant complexes provided a 57% decrease in oil contamination during a 3-month treatment. An ex situ biotechnology developed to employing a *Rhodococcus* biosurfactant-based biofertilizer for the decontamination of heavily oil-polluted soil [119].

13.5 BIOFERTILIZER TECHNOLOGY IN BIOREMEDIATION

Success or failure of bioremediation depends on several factors such as the competitive ability of the bioremedial agents and biotic factors such as soil moisture, pH, and temperature [120, 121. Inoculated biofertilizers (Phosphoren, Microbien, Cerealin and *Azospirillum*) may act as potential agent for soil inoculation to bioremediate pesticides contaminated soil [122]. Successful removal of pesticides by the addition of bacteria (bioaugmentation) has been reported for many compounds including, coumaphos (ethoprophos, dicofol and malathion) [123-126].

13.5.1 Use of Microorganisms in Bioremediation

Plant growth-promoting bacteria (PGPB) are mainly used to improve agricultural yields along with solving environmental problems [127]. A number of microorganisms (bacteria and fungi) have been used in soil inoculations intended to improve the supply of nutrients to crop plants, to stimulate plant growth, to control or inhibit the activity of plant pathogens and to improve soil structure (Table 13.1). Other more recent, objectives for the introduction of microorganisms into soil are the mineralization of organic pollutants [121]. Phosphoren, micrebien, cerealin and azospirillum are the biofertilizers (derived from the living biomass of different microbial species) produced by general organization belongs to the Egyptian Ministry of Agriculture as a mixed inoculums are beneficially applied to many fields and horticultural crops [128].

Table 13.1 Plant growth promoting bacteria (PGPB) applied in bioremediation strategies

Characteristics of PGPB	*Microorganism*	*Plants associated*	*References*
Nitrogen fixation			
Freely associated bacteria	*Azotobacter chroococcum*	*Brassica juncea*	Wu et al., (2006) [129]
Symbiotic bacteria	*Sinorhizobium meliloti*	*Medicago truncatula*	Bianco and Defez (2009) [130]

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Enhanced the nodule numbers, seed yield, grain protein, root and shoot	*Bradyrhizobium* sp. (vigna)	Greengram (*Vigna radiate*)	Wain et al., (2007) [131]
Phosphate mobilization			
Inorganic P source	*Pseudomonas aeruginosa*	*Vigna mungo*	Ganesan (2008) [132]
Organic P source	*Bacillus amyloliquefaciens*	*Zea mays*	Idriss et al., (2002) [133]
Phosphate solublizing bacteria	*Pseudomonas putida, Azospirilium, Azotobacter*	Artichoke (*Cynara scolymus)*	Jahanian et al., (2012) [134]
Siderophore release			
Hydroxamates	*Streptomyces acidiscabies*	*Cicer arietinum*	Dimkpa et al., (2008) [135]
Phenol catecholates	*Rhizobium* sp.	*Sesbania procumbens*	Sridevi et al., (2008) [136]
Carboxylates	*Pseudomonas fluorescens*	*Arachis hypogaea*	Dey et al., (2004) [137]
Salicylic acid	*Arthrobacter oxidans*	*Pinus* sp.	Barriuso et al., (2008) [138]
Stimulated plant growth, reduced Cd uptake	*Pseudomonas aeruginosa*	Indian mustard and pumpkin	Sinha and Mukherjee (2008) [139]
Auxin production			
Indole acetic acid	*Enterobacter chloacae*	*Oryza sativa*	Mehnaz et. al., (2001) [140]
Gibberellin	*Bacillus pumilus*	*Alnus glutinosa*	Gutierrez-Man˜ero et al., (2001) [141]
Influence on metal toxicity			
Increased Ni accumulation	*Bacillus subtilis*	*Brassica juncea*	Zaidi et al., (2006) [142]
Increased Cd accumulation	*Xanthomonas* sp.	*Brassica napus*	Sheng and Xia (2006) [143]
Reduction of Cr(VI) to Cr(III)	*Ochrobactrum intermedium*	*Helianthus annuus*	Faisal and Hasnain (2005) [144]
Stimulated plant growth, facilitated soil Pb mobilization, enhanced Pb accumulation	*Bacillus edaphicus*	Indian mustard (*Brassica juncea*)	Sheng et al., (2008) [145]

13.5.1.1 Bioremediation by microalgae

Algae are universally known as playing a very important role in natural water purification process [24, 146]. Thus, the use of microalgae for removal of nutrients from different wastes has been described by a number of authors [21, 147-156].

Industrialization has led to increased emission of pollutants into ecosystems. Metal pollutants can easily enter the food chain if heavy metal-contaminated soils are used for production of food crops. Farm productivity has decreased in toxic metal polluted areas [157]. Accumulation of toxic metals, e.g., Hg, Cu, Cd, Cr and Zn in humans has several consequences such as growth and developmental abnormalities, carcinogenesis, neuromuscular control defects, mental retardation, renal malfunction and wide range of other illnesses. Elevated levels of such metal ions are generally toxic and cause major damage to cell [158]. Conventional technologies, such as ion exchange or lime precipitation, are often ineffective and/or expensive, particularly for the removal of heavy metal ions at low concentrations (below 50 mg/L). Furthermore, Most of these techniques are based on physical displacement or chemical replacement, generating yet another problem in the form of toxic sludge, the disposal of which adds a further burden on the techno-economic feasibility of the treatment process. In view of this, the development of new techniques is necessary to meet the environmental standards at affordable costs. They can provide solutions to the twin challenges of energy security and environmental pollution. They have great potential for the removal of excess nitrogen and phosphorus from wastewater including the farm runoff. They can capture carbon dioxide in the flue gas from coal fired power plants thereby reducing greenhouse gas and also producing algal biomass, which can be converted into biofuel *Chlorella, Scenedesmus* and *Spirulina* are the most widely used algae for nutrient removal.

The ability of algae to absorb metals has been recognized for many years [159]. In natural environments, algae play a major role in controlling metal concentration in lakes and oceans [160,161]. Algae possesses the ability to take up toxic heavy metals from the environment, resulting in higher concentrations than those in the surrounding water [140, 162].

13.5.1.2 Bioremediation by N_2-fixing bacteria

N_2 fixation is an energy-consuming process and has been widely observed in symbiotic, associative and free living bacteria. This bacterial physiological feature plays a tremendous role in ecological sustenance, world agriculture and global nitrogen cycle. Nitrogen availability can limit microbial growth and affect ecosystem activity [163]. Although N_2 fixation is widely distributed among Bacteria and Archaea [164], this significant physiological feature has not been revealed in reductively dechlorinating bacteria.

Nitrogen is essential to microorganism metabolism and it is necessary to biosynthesis of amino acids, protein and nucleic acids [165-167]. The soil rich in nitrogen has a good metabolic activity and good microbial biomass. Therefore, its presence in the soil for a good bioremediation process is necessary. However, some authors claim that biodegradation in negligible amounts of nitrogen is possible, but the efficiency is lower [168-170].The literature quotes different bacterial genera, which have the capacity to degrade hydrocarbons in poor concentration of nitrogen [169]. The genera *Pseudomonas* [168, 171], *Agrobacterium, Alcaligenes* [172], *Arthrobacter* [173], *Azotobacter* [174] have the capacity to fix nitrogen in soil with deficiency of nitrogen. The effects of bio-stimulation on petroleum hydrocarbon degradation have been investigated in different conditions [175, 176]. However, the effects of nitrogen affect the biodegradation of hydrocarbon classes, i.e. aliphatic, aromatic and polar hydrocarbons have not been completely studied in the Patagonian soil, which has a poor concentration of nitrogen and it is an important oil production area.

Nitrogen and phosphorus are the nutrients that most frequently limit bioremediation [177]. The presence of petroleum products in the soil can widen the C:N ratio, therefore limiting available nitrogen for degradation processes [178]. In addition, microbes able to metabolize hydrocarbons will quickly immobilize the mineral nitrogen that is available, leaving unfavourable conditions for other microorganisms and growing plants [179]. The absence of sufficient nitrogen in the soil will, in turn, slow the degradation process resulting from microbial metabolism [177]. Therefore, adding nutrients in the form of either organic or inorganic fertilizers can stimulate contaminant degradation [180]. Free-living nitrogen-fixing bacteria can fix atmospheric nitrogen into a more usable form such as ammonia. Thus, the bioaugmentation of hydrocarbon polluted soil with free-living nitrogen-fixing bacteria may indirectly contribute to soil nitrogen by releasing nitrogenous biomass [181]. This study was undertaken to isolate hydrocarbon-degrading free-living, nitrogen-fixing bacteria from the soil and to determine their potential in bioremediation of hydrocarbon polluted soil.

13.5.2 Role of Azolla in Phytoremediation of Heavy Metals

Among various water pollutants, heavy metals are of major concern because of their persistent and bioaccumulative nature [182-185]. Water is an indispensable part for the sustenance of mankind and the increasing awareness about the environment; especially aquatic ecosystems have attracted the attention of researchers worldwide. A definite need exists to develop a low cost and eco-friendly technology to remove pollutants particularly heavy metals, thereby improving water quality. Phytoremediation offers an attractive alternative. Among these, Azolla, a free-floating, fast growing, and nitrogen-fixing pteridophyte seems to be an excellent candidate for removal, disposal, and recovery of heavy metals from the polluted aquatic ecosystems [186, 187].

Both living and dead biomass of Azolla have been exploited for the removal of heavy metals from industrial effluents and sewage water [188-191]. Bioaccumulation potential of different species of Azolla for various heavy metals. Azolla has great possibility of use in bioremediation of wastewaters and soils. There are three main aspects of use of Azolla in bioremediation. These areas are:

(a) For treatment of wastewaters rich in heavy metal pollutants.
(b) Treatment of domestic sewage effluents which are rich in N and P wastes.
(c) Bioremediation of saline soils.

A study in China stated tolerance of four *Azolla* species to Cu, Mn, Fe, Zn, Mo, Co, Cd, etc. under laboratory conditions and found that concentration capacity of *Azolla* for metals affected its growth only slightly without any detrimental effect or not at all [192].

13.6 CASE STUDIES ON BIOREMEDIATION WITH BIOFERTILIZER

13.6.1 Bioremediation of Sewage Wastewater Using Selective Algae for Manure Production

Phycoremediation is the process of employing algae for removing excess nutrient load from wastewater and subsequently diminish the pollution load. It is an alternative technology of treating sewage wastewater compare to conventional treatment process in economical and sustainable way. IARI gives the effort to phycoremediate sewage wastewater with different microalgae, viz., *Chlorella minutissima*, *Scendesmus* spp. & BGA (Nostoc) and their consortium. Algae were very effective in reduction of BOD_5, COD, NO_3^-, NH_4, $PO4_3^-$ and TDS in sewage wastewater. Further, it has been observed that Chlorella was having best phycoremediation potential as well as manure production among all three microalgae and even better than consortium. Among the potential uses of algal biomass from such systems is its use as a slow release fertilizer. After 20 days microalgae were harvested using muslin cloth and fresh and dry weigh were determined. The maximum biomass was observed in *Scendesmus* spp. and *Chlorella minutissima* while percentage of nitrogen and phosphorus was highest in *Chlorella minutissima*. So *Chlorella minutissima* has the best manurial potential. The algae in nutrient-rich sewage wastewater offers a new option of applying algae to manage the nutrient load and after phycoremediation the biomass itself can be utilized for manure application in agriculture, serving the dual roles of nutrient reduction and valuable manure feedstock production [193].

13.6.2 Bioremediation of Olive Oil Mill Wastewater: Chemical Alterations Induced by *Azotobacter vinelandii*

An environmentally friendly bioremediation system of olive oil mill wastewater (OMWW) is studied with respect to its physicochemical characteristics and degradation efficiency on major characteristic constituents. The method exploits the biochemical versatility of the dinitrogen fixing bacterium *Azotobacter vinelandii* (strain A) to grow in OMWW at the expense of its constituents and to transform it into an organic liquid fertilizer. The system eliminates the phytotoxic principles from OMWW and concomitantly enriches it with an agriculturally beneficial microbial consortium along with useful metabolites of the latter. The end product, branded "biofertilizer", is used as soil conditioner and liquid organic fertilizer. Growth of *A. vinelandii* in OMWW results in the decline of content of most of the compounds associated with phytotoxicity, and this is confirmed by the assessment of degradation yields. In parallel, during the process several other compounds noncommittally undergo degradation and biotransformation. More specifically, the biofertilization system is capable of achieving removal yields as high as 90 and 96% after 3 and 7 days of treatment, respectively [194].

13.6.3 Biofertilizers for Bioremediation of Pesticide Contaminated Soil

Biofertilizers by the General Organization of Agriculture Fund, Ministry of Agriculture, Egypt (*Phosphoren, Microbien, Cerealin* and *Azospirillum*) to degrade five selected pesticides representing different classes including organophosphate, carbamate and chlorinated organic compounds. There were differences in rates of biotransformation, suggesting the selective induction of certain metabolic enzymes. Inoculation of soil incorporated with malathion, fenamiphos, carbaryl, aldicarb and dieldrin, resulted in 80-90% removal of malathion and fenamiphos within 8 days, carbaryl and aldicarb within 11-15 days respectively. Dieldrin removal occurred slowly within 2 months. These data suggest that biofertilizers may act as potential candidates for soil inoculation to bioremediate pesticide contaminated soil. The production of CO_2 (soil respiration) was stimulated by some pesticides. In samples with microbien, about 2 times higher CO_2 production was measured [195].

13.6.4 Bioremediation of Petroleum Pollutants

Bioremediation tries to raise the rates of degradation found naturally to significantly higher rates. The two general approaches that have been tested for the bioremediation of marine oil spills are the application of fertilizer to enhance the abilities of the indigenous hydrocarbon-utilizing bacteria and the addition of naturally occurring adapted microbial hydrocarbon-degraders by seeding. Bioremediation, accomplished by the application of fertilizer to enhance the abilities of the indigenous hydrocarbon-utilizing bacteria, was successfully applied for the treatment of the Alaskan oil spill in Prince William Sound, Alaska [196].

13.6.5 Cyanobacterial Consortia for Bioremediation Purposes

Many studies have reported the ability of cyanobacteria to oxidize oil components and other complex organic compounds such as surfactants and herbicides [22, 197-199]. Among these cyanobacteria were the nonaxenic cultures of *Microcoleus chtonoplastes* and *Phormidium corium*, degrading n-alkane [200], *Oscillatoria* sp. and *Agmenellum quadruplicatum* oxidizing naphthalene to 1-naphthol [201, 202], *Oscillatoria* sp. strain JCM that oxidized biphenyl to 4-hydroxybiphenyl [203] and *Agmenellum quadruplicatum* that metabolized phenanthrene into trans-9,10-dihydroxy-9,10-dihydrophenanthrene and 1-methoxy-phenenthrene [204]. Cyanobacteria those are responsible for the degradation of these compounds but the associated aerobic organotrophic bacteria [205-207]. Presence of cyanobacteria alongside with the aerobic organotrophs facilitated the degradation process and both groups constituted idea consortia for degradation of petroleum and other complex organic compounds [206].

13.6.6 Potential Use of Cyanobacteria Species in Phycoremediation of Municipal Wastewater

The treatment of municipal wastewater becomes effective by using microalgae which are superior as the wide range of toxic and other wastes can be reduced with them.

Locally available blue-green algae such as *Oscillatoria limosa* and *Nostoc commune* have ability to remove of various nutrients to prevent further deterioration of water quality. The phycoremediation experiments were conducted at Department of Environmental Sciences, University of Pune, India using randomized complete block design with three replications of each treatment. The results of present investigation clearly indicated that both the algal species, viz., *Oscillatoria limosa* and *Nostoc commune* are highly efficient for removal of NO_3^{-2}, PO_4^{-2}, SO_4^{-2}, Cl^- and for reducing EC values. The average reduction was between 84 to 98%. The pollutant removal efficiency was increased with decreasing concentration of wastewater. Amongst the selected algae *Oscillatoria limosa* was the best candidate as compare to *Nostoc commune*. It was concluded that the cyanophycea members would be the best options for phycoremediation [208].

13.6.7 Bioremediation of Wastewater by Using Microalgae

Autotrophs play an important role in remediation of wastewater particularly domestic waste through it photosynthetic ability. Dominant and pollution tolerant algae such as *C. vulgaris* and *S. quadricauda* cultures in BBM isolated form sewage wastewater treatment plant Bopodi from Pune city and used for the treatment of the wastewater. To study the role of microalgae in wastewater, the following protocols were used, (i) Wastewater treated with culture of *C. vulgaris* and *S. quadricauda*; and (ii) Wastewater treated without culture of *C. vulgaris* and *S. quadricauda* (Control). Samples were periodically (every 5th day) analyzed for physico-chemical parameters such as pH, phosphate, nitrate, BOD and COD using standard methods. *C. vulgaris* showed the best removal capacity of nitrate and COD while *S. quadricauda* shows BOD and phosphate reduction [209].

13.6.8 Microalgae for Bioremediation of Distillery Effluent

Distillery effluents, also referred to as spent wash/stillage/slop/vinasses, are one of the most environmentally aggressive industrial effluents. With the development of economies and resultant growth of distillery industries, large volume of spent wash is produced which is likely to cause extensive soil and water pollution due to the presence of high amount of organic matter and dark brown coloured recalcitrant compounds. There have been many isolated studies for treatment of distillery effluents and related compounds using microalgae.

For treatment of wastewaters native microalgal strains are a favourable alternative to the traditional wastewaters treatment systems. A consortium of *Oscillatoria, Lyngbya* and *Synechocystis* decolorized melanoidin by 98% by absorption followed subsequently by degradation of the organic compounds. The microalgal strains like *Anabaena cylindrica, Phormidium foveolarum, P. valderianum, Synechococcus, Ankistrodesmus braunii and Scenedesmus quadricauda* have been reported instrumental in degradation of phenol and its derivatives, whereas the performances of *Phormidium ambiguum*, *Chroococcus minutus, Oscillatoria*, and *Anabaena azollae* were found satisfactory for degradation of lignin. *Phormidium ambiguum* and *Chroococcus minutus* were found to reduce lignin by over 73.0% from the pulp and paper mill wastes in 5 days; whereas *Phormidium, Oscillatoria*, and *Anabaena azollae* were able to degrade lignin by 89% and hemicellulose by 92% from coir waste [210].

The role of cyanobacteria in distilleries effluent was studied in Kanchipuram, Tamilnadu, India. Totally 12 species of cyanobacteria belonging to 6 genera were identified. *Nostoc muscorum* was found to be the most dominated genus in this effluent (heterocyst organism). The inoculation of cyanobacteria *Nostoc muscarum* resulted in removal of various chemicals as well as nutrients such as nitrogen, ammonia and phosphorus from the effluent and could be potentially employed for the treatment of distilleries effluent [211].

13.7 FUTURE SCOPE FOR BIOREMEDIATION BY BIOFERTILIZER PRODUCING ORGANISMS

Bioremediation using biofertilizer producing microorganisms is though a very new concept, still it is really advancing field of research. Different types of interactions like biotransformation, biosorption and binding of heavy metals with extracellular or intracellular organelles are effectively used and scientifically manipulated to perform with higher effectiveness. Designing of different types of bioreactors, within which microbes can accumulate or adsorb metals or radionuclides under controlled systems, is also being modernized. Several strains having the capacity of promoting plant growth and reducing environmental toxicants. However, more studies are needed to be done on application fields, so that we can better understand the effect of natural factors and of co-toxicants present in concerned environment. Apart from choosing and developing such strains there remains a considerable amount of work required to be done on effective and easy desorbtion of toxic contaminants from environment and regeneration of the biomass for further use. To attract more usage of biosorbent technology using plant growth promoting microorganism (PGPM), certain strategies have to be formulated and promoted in agricultural and environmental sectors. The education, awareness and biotechnological research in this area increased our crop production and decrease the environmental contaminants. With so much to be done in this rapidly growing field of biotechnology, hope that future promises us a better and healthier environment.

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