Review Paper:

Trends in application of Microbial Consortium for Bioremediation- a mini review

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Abstract

Bioremediation has established itself as a promising tool for the treatment of environmental pollutants. Several new approaches that stem from bioremediation have found their application in industries as well. One such approach is the use of consortia. In the past decade, there have been numerous pieces of research on different types of consortia in different types of microbes with the ultimate aim to improve the bioremediation process. Microbial consortia provide us the tools that we can engineer with the help of metabolic and synthetic biology techniques to bioremediate complex and mixed types of pollutants.

The current trends in research appear to be shifting towards mixed consortia especially microalgae-mixed consortia. In this review, we tried to touch upon the process, success and application of microbial consortia for bioremediation. There are challenges involved as well i.e. the efficiency of the process and poor study design. However, positive efforts in this direction could lead to large-scale employment of this technology in the bioremediation of industrial pollutants and wastewater. This will reduce the burden on conventional techniques used for pollutant remediation and will lead to a better and more sustainable future by reducing the overall carbon footprint.

Keywords: Bacterial Consortium, Synthetic Consortia, Fungal Consortium, Mixed Consortium.

Introduction

Microorganisms are the oldest inhabitants of the earth and are an integral part of every ecosystem of the world. These microscopic organisms vary drastically in terms of morphology and diversity. Most of the biosphere is comprised of these organisms[21,25,70,71]. They co-inhabit the ecosystem with other higher-order organisms in a balanced way. This balance is naturally maintained by the ecological relationship among the inhabitants of a particular ecosystem[30,73].

The complex species interaction web is the focal point of the composition, structure and function of any community of microbes. However, the complete understanding of this web is quite complex and challenging. This can somewhat be attributed to the lack of availability of tools efficient enough to disentangle these complex interactions. The interactions are of two basic types i.e. cooperative or non-cooperative. Cooperative interactions are considered positive interactions which include symbiosis, commensalism and mutualism.

Non-cooperative interactions are considered negative interactions which include Amensalism, parasitism and predation[32]. All of these interactions are present together in all the ecosystems among different orders of organisms coexisting in a particular ecological niche altogether driving the cycle of life. These unique interactions are capitalized for various biotechnological applications.

The artificial co-cultures designed for bioremediation are outranked by natural consortia in terms of performance. Compared to single strains, consortia offer a variety of advantages such as reduction in metabolic burden, easy optimization of processes and multitasking through the division of labor[44,52,61,77]. Synthetic microbial communities offer a robust alternative that is easily programmable for a spectrum of complex tasks, therefore enabling novel and versatile applications in several fields.

Microbial consortium (MC) can be classified based on microbial origin and level of human intervention in the context of bioremediation into natural microbial consortia (NMC), synthetic microbial consortia (SMC) and artificial microbial consortia (AMC). NMC's are generally made up of microbes from one source. On the other hand, AMC comprises of microbes taken from multiple sources. A subclass of AMC is also known as SMC and here the microbes from multiple sources are genetically engineered and combined in a defined composition to accomplish predefined tasks.

A microbial consortium can also be classified into single-kingdom consortia which comprise of members from one microbial kingdom or cross-kingdom consortia which comprise of members from different kingdoms. It has been observed that cross-kingdom consortia are more viable and fit for bioremediation purposes, given stringent conditions and environmental factors at the site of interest[43].

Our focus is to highlight the trend and generate a comprehensive summary of various reported applications and success achieved in bioremediation. Cross-kingdom interactions offer added benefits to the consortia. As in the case of bacterial-fungal consortia in the bioremediation of soil, fungus due to its filamentous and rapid colonizing nature offers an ideal "highway" for the bacterial cells to get in contact with contaminants in far-reaching and distant places in soil. The phenomenon of phenanthrene removal by the bacteria and the corresponding levels of contaminant indicated by the nematode parameters has been studied in detail. It is also proposed that a few of these nematodes may assist biodegradation as well.

Microbial consortia with the formation of various synergistic population-level structures like microbial mats, biofilms and stromatolites enable themselves to withstand a variety of environmental conditions. Microbial consortia are reportedly more robust to environmental changes given they are comprised of multiple strains with a variety of functions.[33,44] Microbial consortia are regarded as a promising approach to bioremediation, sustainable energy, high-value product formation and lignocellulose utilization by numerous researchers and studies[57,61,67,78]. There have been several types of research recently exploring the potential of microbial consortia, the mechanism of cell-to-cell communication, statistical modeling of microbial consortia and synthetic pathway distribution among different microorganisms comprising a consortium[40,57,67,75,78]. These studies have added new dimensions to the complexity and functionality of artificial microbial consortia and the science of synthetic biology associated with them.

There are two approaches to designing and engineering synthetic microbial consortia, which are a top-down method and a bottom-up method. The top-down approach involves re-engineering naturally occurring microbial consortia using various omics analyses to tune the consortium as per the need. The bottom-up approach involves designing metabolic pathways and circuits, introducing genetic element modules following the basic engineering principles and constructing an artificial consortium with higher efficiency, controllability and stability. In a consortium, the involved microorganisms create a novel microenvironment that leads to the activation of some silent metabolic pathways which are normally not utilized, granting several desirable properties to the consortia.

Microbial consortia are applied in the field of therapeutics and agriculture. Over the last decade, there has been active research on the microbial consortia making it an important frontier of the second wave of synthetic biology but in-depth research is lacking[7,51]. The low efficiency of artificial microbial consortia is a challenge for further applications. The efforts are being made using several advanced approaches to formulate a highly efficient and stable microbial consortium.

Bacterial Consortium for Bioremediation

The bacterial potential for bioremediation is well-known and extensively studied[50]. Oil spills and petroleum-related pollution are serious environmental and health hazards. Their remediation is of global concern and an active field of research. Bacterial consortia are actively employed towards this goal. Recently a synthetic bacterial consortium using pathway optimization approaches was constructed for efficient degradation of these pollutants. Consortia is comprised of three petroleum degrading strains namely *Achromobacter sp. P3, Sphingobium sp.* P10 and *Rhizobium sp.* and this synthetic consortium displayed a 34.8% higher degradation than the original bacterial community i.e. PDM[13].

Microorganisms can metabolize toxic compounds (such as polycyclic aromatic hydrocarbons, nitroaromatic pesticides and polychlorinated biphenyls) and convert them into carbon and energy. Some of the species can mineralize pollutants to H_2O and CO_2[16,17,22,47,68,72].

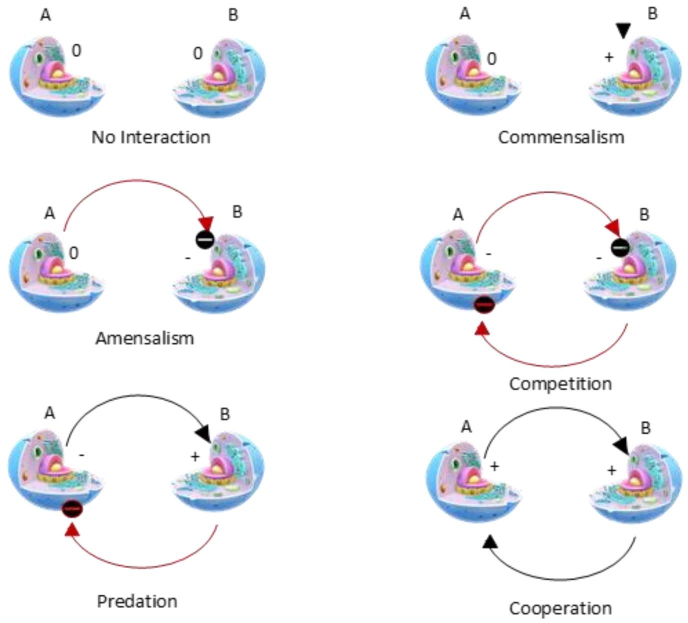

Figure 1: Interactions of the microbial strains in the consortium[5]

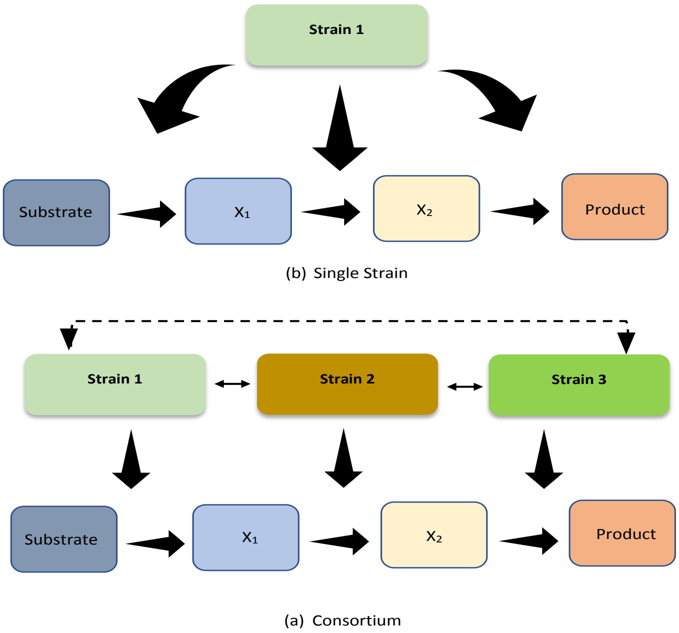

Figure 2: Processing of complex reactions by single strain and consortium[7]

Table 1
Bacterial Consortium in the degradation of various compounds.

Bacterial consortium	Compound	Removal efficiency
Microbial consortium ZSY[27]	Dyes (Metanil Yellow G)	Metanil Yellow G: 93.39%
Microbial Consortium ZW1[26]	Dyes (Methanil Yellow G)	Methanil Yellow G: 93.3%
Mycobacterium spp. PO1 and PO2, Novosphin-gobium pentaromativorans PY1, Ochrobactrum sp. PW1 and Bacillus sp. FW1[77]	Pyrene	Three-fold higher degradation rate for pyrene than the individual degrader
Arthrobacter sp. DNS10, Bacillus subtilis DNS4 and Variovorax sp. DNS12, Arthrobacter sp. DNS9[85]	Atrazine	Removed 100% of atrazine at initial concentration of 100 mg/L, faster than single species.
Rhodococcus sp., Acinetobacter sp. and Pseudomonas sp[84]	PAHs	100% degradation of Fl and Phe in sediment-free liquid medium after 4 weeks of growth.

Table 2
Fungal consortium for bioremediation of pollutants and its efficiency.

Fungal consortium	Compound	Removal efficiency
A. fumigatus * *A. flavus* * *A. fumigatus*[69]	Heavy metal (Cd, Cr)	Cd: 82.21%, Cr: 81.255
Fungal consortium[12]	Pesticide (Chlorpyrifos)	Chlorpyrifos
Microbial consortium SR[81]	Dyes (Crystal Violet, Cresol Red, CBB G250)	Crystal Violet: 63%, Cresol Red: 93%, CBB G250:96%
Trametes hirsuta BYL-3, *Trametes versicolor* BYL-7 and *Trametes hirsuta* BYL-8[76]	Lignin	lignin: 39.7%
P. oxalicum SAR-3, A. niger SAR-6 and A. flavus SAB-3[59]	azo dyes (Acid Red 183, Direct Blue 15 and Direct Red 75)	100% at an initial concentration (200–400 mg L-1)
A. lentulus, *A. terreus* and *R. oryzae*[48]	metals [Cr^{6+} and Cu^{2+}]	100% Cr^{6+} and 81.60% Cu^{2+}
A. lentulus, *A. terreus* and *R. oryzae*[48]	dyes [AB and PO]	98.0% AB and 100.0% PO
Cladosporeum perangustum, *Penicillium commune*, *Paecilomyces lilacinus* and *Fusarium equiseti*[63]	Heavy metal (Cr, Cr (VI) and Pb)	Cr (VI) (100%), Total Cr (99.92%), Total Pb (95.91%)

Table 3
Mixed consortium for the degradation of pollutants

Combinations	Consortium	Degradation compound	Removal efficiency
Microalgae-Bacteria	Microalgae-Bacteria[65]	sulfamethoxazole	54.34%
	Chlorella vulgaris and *Rhizobium sp.*[19]	Organic Nutrients	60.8% (127.0 mg L-l), Total nitrogen: 69.1% (21.7 mg L-l) Total phosphate: 98.9% (0.07 mg L-l)
	Scenedesmus acuminatus Filamentous bacteria[42]	Organic Nutrients	COD: 93.0% (982.0 mg L-l) TDN: 88.0% (52.0 mg L-l) Total phosphate: 69.0% (17.0 mg L-l) NH4 ?-N removal 88.0% (31.0 mg L-l)
	Chlorella sp. Beijerinckia fluminensis[3]	Organic Nutrients	COD: 76.7% (740 mg L-l) Total nitrogen: 78.7% (20.5 mg L-l) Total phosphate: 74.8% (7.4 mg L-l)
	Navicula sp. Comamonadaceae and *itrosomonadaceae, ammonia oxidizing bacteria*[45]	Organic Nutrients	COD: 95% (600 mg. L-l) NH4-N removal: [99% (50 mg. L-l) Total phosphate: 31.0–42.0%
	Tetraselmis chuii and Nannochloropsis gaditana Algal pond bacteria[1]	CO_2	89.0–97.0%
	Chlorella vulgaris Mixed anaerobic sludge collected from the bottom of septic tank[79]	CO_2	190.9 ± 8.6 mg L-1d-1
	Chlorella vulgaris Nitrifier-enriched activated sludge from municipal wastewater treatment plant[60]	CO_2	90% (156 mg)
	Chlorella sorokiniana DBWC2, Chlorella sp. DBWC7 Klebsiella pneumoniae ORWB1, Acinetobacter calcoaceticus ORWB3[23]	COD, N_2	N2: 99.95 %Total COD: 95.16 %
	Chlorella vulgaris MACC360 Native bacteria from sludge (beer brewing factory)[64]	Organic Nutrients	TP and TN: ~75 %, respectively
Fungal-microalgae	*Aspergillus sp. C. vulgaris*[80]	Organic Nutrients	COD:70.7%, TN:67.1%, NH_4-N: 94.7%, TP: 88.4%
	Penicillium sp. Chlorella sp.[10]	Organic Nutrients	COD: 46%, TN: 13%, NH_4-N: 6%, TP: 53%
	G. lucidum C. vulgaris[86]	Organic Nutrients and CO_2	COD: 92.2%, TN: 89.8%, TP: 90.3%, CO_2: 73.3%
Fungi-Bacteria	*M. luteus, R. equi A. niger strain*[53]	COD, Oil and grease, sulphate	COD: 78.7%, oil and grease: 82.6%, sulphate: 89.7%
	Fungi-Bacteria[55]	Organic Nutrients	COD: 81.9-93%

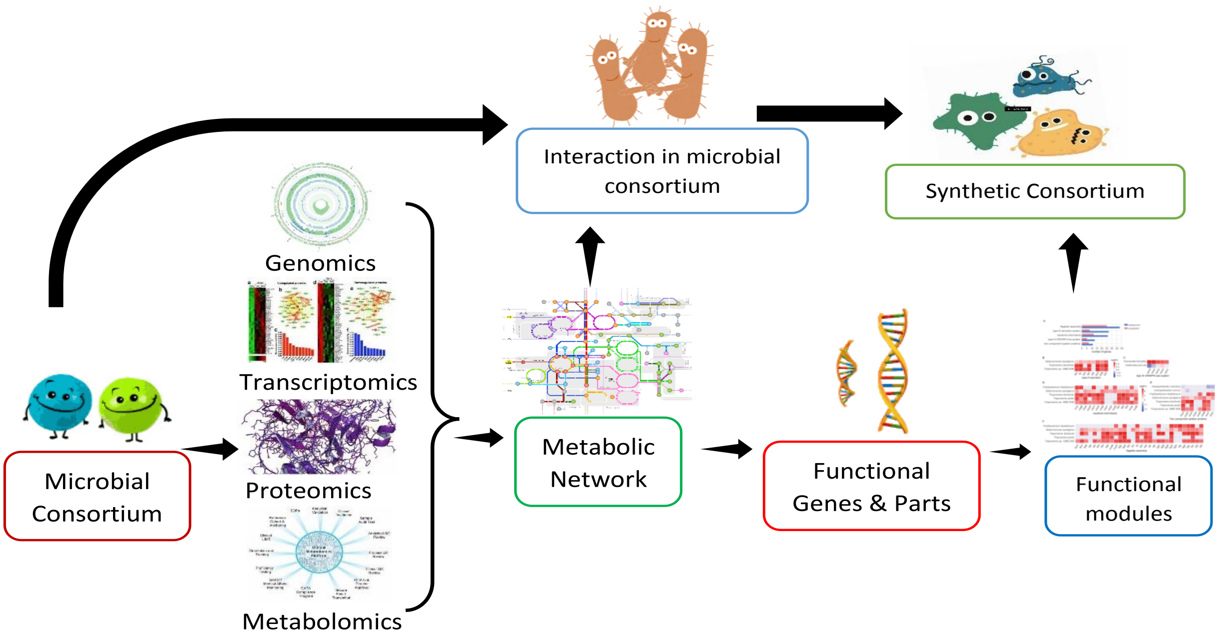

Figure 3: The molecular mechanism of interactions between microorganisms in microbial consortia[67]

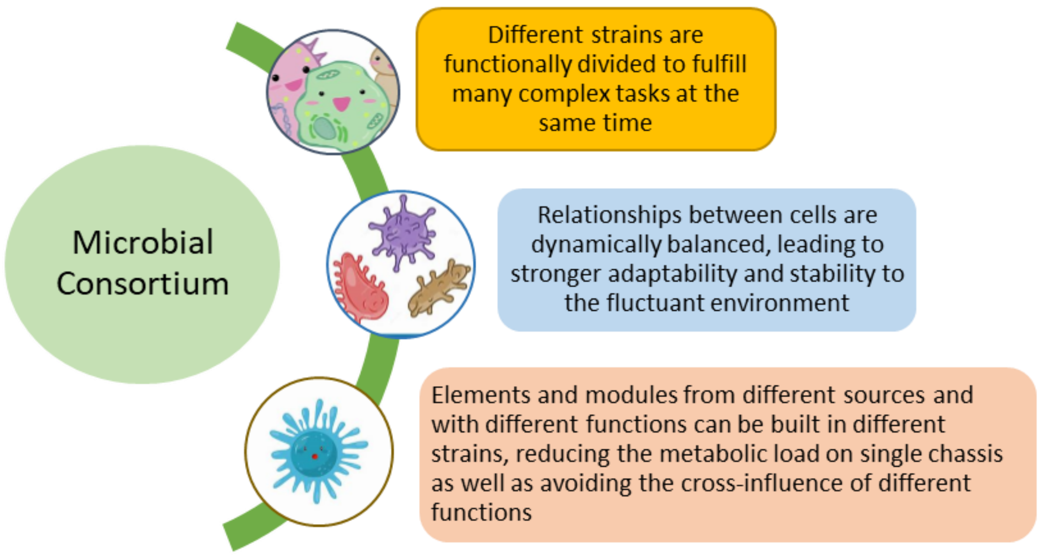

Figure 4: Three advantages of taking microbial consortia as the research object to engineer specific routes

Moreover, the biodegradation capability of microorganisms is sometimes due to their formulation in a culturing media and not possessed by an individual species. According to several studies, the capability of one species for bioremediation falls short of what a consortium offers. Therefore, the use of a consortium for bioremediation is highly recommended.

Fungal Consortium for Bioremediation

Fungal microbes are well known for their bioremediation potential. Fungi decompose biomass with the help of several enzymes like lipase, amylase, nuclease etc. Fungi are also used for environmental bioremediation and biotransformation of organic waste materials[2]. Fungi can metabolize environmental chemicals to meet its nutrient requirements. They are also used for the bioremediation of organic and metal contaminants from wastewater, soil and air in a cost-effective and eco-friendly way. They can grow in soil matrix along with water bodies and are resistant to different climatic changes. The survivability in diverse environmental conditions along with its ability to produce and utilize an arsenal of enzymes makes it a suitable candidate for bioremediation. Some of the applications of fungal bioremediation include mycofiltration, treatment of colored effluents, pesticide treatment and heavy metal treatment. The use of fungi for bioremediation is a rapidly growing field of active research given its capability to transform a wide variety of hazardous materials using an array of enzymes[18].

The use of consortia of fungi is a suitable alternative to overcome some problems associated with the use of

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individual pure strains[9]. The use of consortia enables the bioremediation of mixed substrates, since different fungi have a different set of functional enzymes, overall reducing the cost and energy required for the process allowing the consortia a better chance of survival in native bioremediation sites[44]. White rot fungi consortia are very well studied and their lack of specificity of enzymes enables them to make changes to the molecular structures of industrial, wastewater and soil pollutants[15].

Apart from the numerous research studies about the improved bioremediation capabilities of mixed consortia, few studies have demonstrated a lower performance of co-cultures compared to monocultures. Lower degradation of polychlorinated biphenyls was obtained with co-culture of *Pleurotus pulmonarius/ Trametes sanguinea* than axenic trials of *P. pulmonarius*. The detoxification of organochlorine compound was 23% higher in monoculture of *Trametes hirsuta* than white-rot fungal consortia comprising of *T. hirsute, T. versicolor* and soil fungi. Several other research studies showed mixed results. This proves that it is quite challenging to implement a rational approach to designing fungal consortia, monitoring the experiments and testing the bioremediation capability. Despite all the challenges, the growth in active research in this domain in recent years gives hope for a brighter future for the application of fungal consortia for bioremediation purposes.

Microalgae Consortium for Bioremediation

Microalgae are eukaryotic unicellular organisms with sizes ranging from 0.001 mm to 2 mm in diameter. They include species such as diatoms, green flagellates and dinoflagellates[28]. Microalgae have managed to grab the attention of researchers in recent years as an alternative feedstock for an application such as bioenergy production[24]. Growing microalgae in wastewater allows for the bioremediation of nutrient-rich wastewater and at the same time leads to a high biomass yield in an energy-efficient process. Numeral studies have highlighted the economic and environmental benefits of utilizing microalgae as alternative feedstock. This increasing awareness has allowed industrial applications apart from laboratory research[39,54,58]. Microalgae absorbs CO_2 and utilizes it as a carbon source reducing the overall carbon footprint of the process[49].

Microalgae growth occurs in three modes namely phototrophic, heterotrophic and mixotrophic conditions. Sunlight and CO_2 are primary sources of carbon and energy in case of phototrophic conditions. In heterotrophic conditions, microalgae utilize organic carbon substrates like glucose, glycerol and acetate for energy and carbon sources. Some strains are capable of using mixotrophic mode i.e. a combination of phototrophic and heterotrophic conditions for their growth, either simultaneously or sequentially[41,46].

For the production of biomass, microalgae cells uptake heavy metal and other organic matter with the help of different physiological and biological methods and utilize it as a nutrient source to regulate their metabolism[8,24]. The bioremediation capabilities can be improved by immobilization, consortia formation and designing nanocomposite materials inspired by microalgae. Microalgae consortia are favored for the bioremediation of heavy metals, organic matter and carbon dioxide fixation. The use of microalgae consortia has proved to be sustainable and economical for tertiary WW treatment. It also promises several benefits which in turn improve the economics of biofuel production using microalgae[62].

Mixed Consortium for Bioremediation

As the name signifies, the consortium formulated by combining organisms from different taxonomic ranks due to their synergistic effects is considered a mixed consortium. Mixed consortia are often superior given that a wide variety of functionality is covered and flaws of the organism from one kingdom can be covered by the other. The most studied among the mixed consortium are bacterial-fungal mixed consortia, fungi-microalgae consortia and bacteria-microalgae consortium. These mixed consortia are generally utilized for wastewater bioremediation and CO_2 fixation.

Few studies document the use of defined fungi-bacteria mixed consortia as an alternative in slaughterhouse wastewater treatment[14,20]. Defined mixed bacteria-fungi consortia have been utilized mostly in the biodegradation of recalcitrant pollutants such as textile effluents, dyes and chlorobenzene. These mixed consortia were shown to achieve higher biodegradation rates than axenic cultures[11,37].

Recently, there has been a growing interest in the capabilities of fungi-assisted microalgal bio-flocculation which allows the harvesting of microalgae along with the bioremediation of wastewater. Fungi-microalgae mixed consortiums have a complementary effect on pollutant removal from wastewater and promise excellent prospects for environmental and economic benefits. Co-culture with filamentous fungus also provides an ingenious way of harvesting microalgae as fungal pellets[38].

Apart from fungus-microalgae consortia, bacteria also work well with microalgae forming a symbiotic and mutualistic relationship that demonstrates advantages in terms of energy, economy and environment[83]. As it is known that microalgae have excellent carbon dioxide fixation and biomass production capabilities, its growth is aided by the presence of heterotrophic bacteria which supply carbon dioxide, ammonia and nitrates[35]. These interactions also help the microalgae-bacterial consortia by recovering the energy system involved and reducing the requirement of additional energy sources needed for cultivation.

The grown biomass can be further utilized in the production of biofuel which ensures the supply of bioenergy while treating wastewater. Bacteria also gain from the relationship as the presence of microalgae improves the system's tolerance toward antibiotics. Bioremediation primarily happens via surface adsorption facilitated by covalent bonding and various molecular interactions[34].

The flue gases from industries that are rich in CO_2 and their bioremediation can be facilitated by the mixed bacterial-microalgal consortia. It is believed that in the future, the microalgal-bacteria consortia will be implemented for wastewater bioremediation, bioenergy production and carbon dioxide fixation, in turn promoting advancement in the life science sectors and sustainability[36].

Attached microalgal-bacteria consortia, in an aquatic ecosystem, can grow on several solid substrates and due to high biodiversity allow complex interspecies interactions to occur within the consortium. There has been significant growth in our understanding of the attached microalgal-bacteria consortia, which is evident from the emergence of photobioreactors as a promising technology for the bioremediation of surface water, high in nutrient content. There are still several technological, ecological and engineering challenges accompanied by this technology for a widespread process scale-up.

Conclusion and Future Prospectives

Microbial bioremediation is known to be the most suitable, eco-friendly, harmless and accepted form of remediation for various types of environmental pollutants. In the past few decades, this field is gaining momentum and a lot of research interests leading to few industrial applications as well. Conventional bioremediation faces some challenges which include the understanding of the microbial ecology, selection of a suitable strain, types, site of contamination and environmental and hostile field factors. These limitations are to some extent eliminated with the introduction of consortia-based approaches and metabolic engineering techniques. This is the reason why artificially engineered consortia are slowly taking the central stage in the field of microbial remediation. They promise immense future potential and sustainability. With the advancement of genetics and genomics, metabolic engineering and synthetic biology fields in the current decade, the search for new and efficient consortia for different types of applications is underway. The information-driven construction of metabolic networks leading to silent pathways discovery and optimization has led to highly advanced research in the field of bioremediation. The various types of microbial consortia i.e. bacterial, fungal, microalgae and mixed have seen tremendous growth and research with the trend shifting more towards the mixed consortia and their applications. The technique survives a diverse range of climate and environmental stresses, with the continuous growth in the fields of genomics and synthetic biology, we will see ever-increasing applications of such techniques for bioremediation in the coming future. These consortia-based approaches can further be improved to increase the process efficiency, to enable a widespread acceptance in industries as well.

Understanding the limitations of consortia-based approaches and eliminating them will evolve the way we bio-remediate environmental pollutants. Further studies are needed to study the mechanisms in detail and to increase the process efficiency. This will in turn help us build our knowledge and eliminate the limitations of current microbial consortia for bioremediation.

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