

Review - Agriculture, Agribusiness and Biotechnology

Potential Biotechnological Applications of Cyanobacterial Exopolysaccharides

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HIGHLIGHTS

- Illustration of various important fields of biotechnology where cyanobacterial exopolysaccharides are potentially useful.
- Discussion of research gaps and new opportunities to make the biomaterial suitable for industrial uses.

Abstract: The cyanobacterial exopolysaccharides (EPSs) are considered as one of the important group of biopolymers having significant ecological, industrial, and biotechnological importance. Cyanobacteria are regarded as a very abundant source of structurally diverse, high molecular weight polysaccharides having variable composition and roles according to the organisms and the environmental conditions in which they are produced. Due to their structural complexity, versatility and valuable biological properties, they are now emerging as high-value compounds. They are possessing exceptional properties and thus are being widely explored for various applications like in food and pharmaceutical industries, in bioremediation for removal of heavy metals, for soil conditioning, as biopolymers, bioadhesives, and biofloculants. However, poor understanding of their complex structural properties, lack of concrete information regarding the genes encoding the proteins involved in the EPS biosynthetic pathways, their process of production and about the associated factors controlling their structural stability, strongly limits their commercialization and applications in the various fields of biotechnology. Owing to the above context, the present review is aimed to organize the available information on applications of cyanobacterial EPSs in the field of biotechnology and to identify the research gaps for improved industrial utilization and commercialization of these biomaterials.

Keywords: cyanobacteria; exopolysaccharides; biotechnological applications; biofloculants; bioadhesives; bioremediation.

INTRODUCTION

Natural polysaccharides of microbial origin have been widely explored by researchers in recent decades due to their possible physical, chemical, functional and industrial applications. Their biodegradability, abundant availability, versatility, and wide possible applications in variable fields are other additional advantages making them suitable candidates for research. In the present scenario, cellulosic polysaccharides are gaining special attention as cellulose is an extremely abundant and extensively recyclable polysaccharidic material. Currently, microbial cellulose is widely being explored for the formation of varied 3D nanostructures, electronic, and energy devices, enzyme immobilization, flexible electronics and modeling of microfibrils [1]. On the other hand, owing the advantage of cellulosic biomass utilization for manufacturing fine chemicals and fuels new technologies are rapidly emerging. Metal-organic frameworks derived solid catalysts are currently being explored for efficient conversion of lignocellulosic biomass into biofuels [2,3].

In recent decades cyanobacterial EPSs have gained increasing scientific attention due to their easy renewability, massive/hyperproduction, rheological features, and variable possible applications in the different fields of biotechnology. Cyanobacteria belong to a group of photosynthetic, cosmopolitan prokaryotic microorganisms and for a long time, they have been known for their potential ability to produce copious amounts of EPSs. The production of EPS in these groups of organisms is considerably related to their capability to survive in unfavorable conditions. Their synthesis contributes to a structurally-stable and hydrated microenvironment, which provides chemical/physical protection against biotic and abiotic stress factors. Cyanobacteria are the choice of organisms for the production of industrially valuable EPSs due to their structural versatility, low nutrient requirements, and the ease of the manipulation of the culture conditions to improve the yields by controlling the growth conditions. However, the production of EPSs in cyanobacteria is highly affected by various nutritional and environmental parameters to a species-specific level which makes the process of production complex and difficult to understand [4].

The structural complexity of cyanobacterial EPS is due to the presence of various structural components in different quantities and configurations. They are majorly heteropolysaccharidic in nature containing a small amount of some non-carbohydrate substituents like peptides, DNA, fatty acids along with a variable amount of uronic acids (mainly glucuronic and galacturonic), pyruvic acid, and O-methyl, O-acetyl, and sulfate groups [5]. Similarly, their species-specific production and involvement of various environmental and nutrients conditions like high salt conditions, high temperature, rate of dilution, phase of growth, presence of some ions like Mg, K, Ca, glucose, citrate, and ethylenediaminetetraacetic acid (EDTA) have a significant impact on their production which make their biosynthesis very complex [6]. Their production favors the microbial association with varying levels of complexity and form biofilms where they play key protective and structural roles [4]. Cyanobacterial EPSs have many potential applications and are used as bioflocculant, bioadhesives, soil conditioners, biopolymers, biofilms formation, food supplements, in bioremediation, and also useful as medicinally important bioactive compounds [6].

Although cyanobacterial EPSs are considerable for their ecological, physiological, and industrial significances but their synthesis is a complex and minimally understood process. Very little information is available related to the genetics of EPS synthesis, assembly of polymers, its excretion, and factors affecting the yield, stability of the material, and rate of production [7]. All these factors limit their commercialization and applications in various fields of biotechnology, resulting in the unavailability of any value-added commercialized product in the market currently. The present review is aimed to organize the available information on applications of cyanobacterial EPSs in the field of biotechnology and identify the research gaps to open the hidden treasure of these unique compounds having the potential to be explored widely for the development of new value-added products.

BIOTECHNOLOGICAL APPLICATIONS

Bioflocculant

The process of contact, adhesion, clumping together of fine dispersed organic particles mediated by microbes which creates large flocs and their faster and complete settling by gravity without the use of any metal is termed as bioflocculation. Despite of efficient flocculation ability and low cost of artificial organic and inorganic polymeric flocculants, their usage is of great concern due to some undesirable, hazardous health effects allied with them. Moreover, synthetic polymeric flocculants are also considered as the origin of environmental problems because of their non-biodegradable nature [8]. Owing to this perilous nature of

organic and inorganic synthetic flocculants, microbial flocculants are gaining considerable interest as they are non-toxic and eco-friendly. Microbial bioflocculants are kind of extracellular biopolymeric materials secreted by microorganisms at their specific growth period. They are considered superior to chemical flocculants due to their non-toxicity and biodegradability [9]. Microbial bioflocculants are made up of glycoproteins, polysaccharides, proteins, cellulose, lipids, and nucleic acids. It is observed that freshwater cyanobacteria like species of *Phormidium*, *Microcoleus* sp., *Anabaenopsis circularis* have the ability to release sulfated heteropolysaccharidic extracellular flocculants into the medium which helps in flocculating particles of clay from water [10]. Extracellular flocculant produced by *Anabaena* species has the immediate power of flocculation with high thermal resistance [11]. *Anabaena* species strain J-1 and *Anabaena circularis* 6720 secretes extracellular flocculants in surroundings and these are used for clarification of turbid lakes and other water bodies [12]. Khangembam and coauthors. [13] reported bioflocculant EPS production by 10 cyanobacterial strains during their photoautotrophic growth. Tiwari and coauthors. [14] found maximum EPS bioflocculant production by *Nostoc* sp. BTA97 and *Anabaena* sp. BTA990 during their stationary phase, at high pH (8) and with low nitrogenase activity. A positive correlation was found between the uronic acid content of EPS and their flocculation activity. The produced flocculants were anionic and were able to bind cationic dyes like Alcian Blue.

Biopolymeric properties

Biopolymers are complex macromolecules, suitable and sustainable alternatives of synthetic polymers due to their non-toxicity, biodegradability, flexibility, active functionality, and renewability. Currently, biopolymers have a high-value market because of their extensive utilization in various industries like cosmetics, food, pharmaceutical, electronics, and biomedicine [15]. Plants and macroalgae originated exopolysaccharides have traditionally been known for their potential applications in food, pharmaceutical, medicine, textile, cosmetic, paper, and oil industries whereas microbial polysaccharides have significant commercial values due to their high productivity, easy renewability, and low cost. Their complex structure and variable physicochemical properties confer their versatility and wide range of applications in different sectors [16]. Commercial production of bacterial polysaccharide through heterotrophic fermentation requires the addition of organic substrates which makes their production very costly. Whereas, photoautotrophic metabolism of cyanobacteria allows low-cost production of polysaccharidic polymers and they can be used as cell factories for biopolymer production without any addition of carbon feedstocks. Cyanobacterial EPSs consist of industrially valuable distinct features like strong anionic nature, presence of uronic acids, sulfate groups, structural complexity, and amphiphilic behavior [17]. Released polysaccharide (RPS) produced by *Cyanospira capsulate* and *A. halophytica* GRO2 show xanthan like physical properties in aqueous solutions [18, 19]. The rheological properties of EPSs produced by the cyanobacterium *Lyngbya stagnina* have been studied by Jindal and coauthors. [20]. Shear-thinning pseudoplastic behavior of EPS of *L. stagnina* is similar to commercial gum Xanthan which is widely useful in the food industry. These pseudoplastic properties are important to provide mouth feeling, flavor releasing and suspending properties to the food products. Aqueous dispersions of EPS of *L. stagnina* showed non-Newtonian, pseudoplastic behavior, and its viscosity was found to be quite stable with time. Pectin, a plant polysaccharide commonly used as a gelling agent, requires some chemical modifications to be used for certain applications. Monosaccharide composition of capsular polysaccharide obtained from *Microcystis flos-aquae* C3-40 shows similarity with plant pectin and its harvesting by washing with deionized water is a simpler procedure than the extraction of pectin from plant tissue [21]. Alkimir 110 is widely used as a stabilizer in the food industry. Released polysaccharidic dispersions of *Anabaena* sp. ATCC 33047 shows Alkimir 110 like viscosity and/or shear-thinning properties and thus suitably useful for industrial exploitation [22]. Cyanoflan a released polysaccharide of *Cyanothece* sp. CCY 0110 consists of a highly branched chemical structure with a large number of sugar residues. It has high molecular mass fractions (above 1 MDa) and an entangled structure with 71% carbohydrate content, 11% of sulfated residues, and 4% protein of dry weight. It shows high apparent viscosity in aqueous solutions and high emulsifying activity making it potentially useful as an emulsifying/thickening agent in food or cosmetic industries [23]. These studies are deliberating promising properties of cyanobacterial EPSs and suggesting their industrial exploitations as emulsifiers, stabilizers, or thickening agents [10]. The carboxylate groups present in most cyanobacterial exopolysaccharides can provide additional advantages and might be used for linking to natural or synthetic polymers and can produce new polysaccharides with special and more advantageous physical properties. A biopolymer polyhydroxybutyrate (PHB) is a kind of polyester and accumulated by several cyanobacteria such when there are abundant carbon source and inadequate nitrogen and phosphorus. PHB is eco-friendly, biodegradable, and biocompatible and has the potential to

replace the hazardous petrochemical plastics [24]. Coelho and coauthors. [25] studied PHB production by *Spirulina* sp. LEB 18 under different nutritional conditions and observed that higher concentration of carbon source increased biopolymer accumulation. Ansari and Fatma, [26] screened 23 cyanobacterial strains for PHB production and the highest production was observed in *Nostoc muscorum* NCCU- 442. The produced PHB polymer is biodegradable and shows comparable physical and mechanical properties with petrochemical plastic. PHB production from cyanobacteria is commercially more attractive as they can utilize carbon sources from industrial effluents (CO₂ and wastewater). Besides this, the development of open systems for the cultivation of cyanobacteria is comparatively easy to construct and operate and also an alternative that may decrease production cost.

Bioactive Compounds

A bioactive compound is a substance that has a biological activity for modulating metabolic processes for better health conditions. Cyanobacteria considered a prolific originator of biologically active compounds for biotechnological industries. Consistent research started to be unveiled antiviral, antioxidant, anticoagulant, antimicrobial, immunomodulatory, and anticancerous activities of cyanobacterial EPS and opened various new opportunities for their use in biomedical applications and they have been identified as a new source of bioactive compounds [27]. The establishment of the relationship between the structure of cyanobacterial biopolymers and their biological activities is difficult to understand due to the unavailability of their detailed structural information. However, the available findings suggest that the anionic charge and presence of sulfate groups are contributing to their significant antiviral activity [28]. Virus inhibitory effects are presumably due to contact inhibition of the virus with the target membrane either by impairing the virus-cell attachment or the direct charges interactions between the negatively charged polymer and positively charged virus surface. Thus their antiviral activity largely depends upon their number of negative charges and the molecular weight of the polymer. Sulfated polysaccharides have been known for their antiviral activity and inhibit penetration of the virus into host cells and also prevent the activity of various retroviral reverse transcriptases [29]. The sulfated polymer isolated from *Arthrospira platensis* is known as calcium spirulan having antiviral activity and chelate calcium ions which is helpful to retain its molecular conformation [30]. EPSs produced by *Gloeocapsa* sp. *Gacheva* 2007/R-06/1 and *Synechocystis* sp. show antimicrobial activity against a broad spectrum of the most common food-borne pathogens [31]. Different extracts of exopolysaccharide obtained by *Arthrospira platensis* having antimicrobial activity against Gram-positive as well as Gram-negative bacteria. All these different extracts showed different antimicrobial activity, presumably due to the presence of different components which have differential solubility in the solvents used for extract preparations [32]. Bhatnagar and coauthors. [33] reported the antibacterial activity of exopolymers extracted from four desert cyanobacteria, *Tolypothrix tenuis*, and three species of *Anabaena* against common wound pathogens including *E. coli*, *S. aureus*, *P. aeruginosa* and *B. licheniformis*. Studies showed a positive correlation between the sulfate groups and the anti-oxidative potential of cyanobacterial polymers [34]. Similar correlations were demonstrated with anticoagulant and immunomodulatory activities of polymers [35]. Hydrophilic EPSs having anionic groups on the surface are useful to form fibrin network and further stabilize the blood clot in bleeding wounds [33]. Studies also have been conducted to demonstrate the antitumor activity of sulfated cyanobacterial EPS. Calcium spirulan also known for its antitumor activity and prevent metastasis [36]. Antitumor activity displayed by EPS isolated from *Aphanothece halophytica*, *Nostoc sphaeroides*, *Aphanizomenon flosaquae*, and *Synechocystis* sp. due to induction of apoptosis in tumor cells [37, 38]. Success in optimizing the yield of polymers and efficiency to re-engineer their composition and structure for specific applications can open new possibilities for their wide and varied use.

Soil Conditioners

Microbial polysaccharides play important role in soil aggregation they bind soil particles into micro aggregates and have the potential to alter the physical, chemical, and biological properties of soil. Soil aggregation by microbial polysaccharides results in microbial mineralization, prevents soil erosion by wind and water, and forms microbial degradation products. Cyanobacteria ubiquitously present in all terrestrial habitats, their potential to produce copious amounts of EPSs and fixation of atmospheric nitrogen highly influence the soil structure and fertility. EPS secretion increases soil carbon content, EPS soil matrix also acts as a repository for nutrients, hydrophilic nature of these polymers increases water uptake, and retention ability of soils [39]. All these together improve soil properties and enable it to release more nutrients for seed germination as well as for plant growth which facilitate the succession of biocrust and plant communities [40].

Inoculation of soil with nitrogen-fixing cyanobacterium *Nostoc muscorum* had improved the physical, chemical, and biological properties of soil and also improved soil's seedlings germination ability. EPS secretion by *N. muscorum* subsequently increased soil aggregation by an average of 18%, soil carbon and nitrogen content increased by ~60% and more than 100% respectively. Microbial population was increased as well as the emergence of lettuce seedlings was also increased by more than 52% when compared with synthetic polyacrylamide soil conditioners [41]. A study on the post-harvest rice cultivation soil showed that soil organic matter and stabilization of soil aggregates increased after inoculation with *Tolypothrix tenuis* and *N. muscorum* [42]. Cyanobacteria are ubiquitous in nature and due to their potential ability of microbial mat or crust formation they are considered as primary colonizers of bare soils. For stabilization of desert dunes in China, studies conducted with *Oscillatoriales species*, *Microcoleus vaginatus*, and a mixture of *Oscillatoriales* and *Nostocales species*, with *M. vaginatus* in higher proportions were used. The findings of these studies revealed that biocrust formation majorly affected by the selected cyanobacterial strain and inherent soil properties [43, 44]. Macromolecular and chemical features of the produced cyanobacterial polysaccharides have been examined in induced biocrusts to access the role of polysaccharidic matrix in the successful development of biocrust, its performance and effects on soil properties were also studied by Chen and coauthors. [45]. Addition of the capsular polysaccharide of *Nostoc muscorum* to the soil increased the amount of water-stable aggregates, either by gluing soil particles or by stimulating the soil community to produce more EPSs [4]. Microcosm study was conducted by Mugnai and coauthors. [46] to assess the capability of *Leptolyngbya ohadii* to form biocrust. The authors reported biocrust formation by the studied organism after 15 days of incubation having a similar thickness and physical stability present in typical natural biocrusts. Characteristics of biocrust formation primarily depended upon the ability of the organism to produce EPS. Chamizo and coauthors. [47] studied the role of soil types in macro-molecular distribution and monosaccharidic composition of the polysaccharidic matrix produced by two cyanobacterial species *Phormidium ambiguum* (non N-fixing) and *Scytonema javanicum* (N-fixing). It affects biocrust development, soil structure, and nutrient cycling. Studies on other cyanobacterial strains *Oscillatoria* and *Schizothrix delicatissima* AMPL0116 showed positive effects of EPS secreted by these strains on soil permeability and soil particle aggregations [48].

Heavy Metals Removal

Heavy metal toxicity in surface and groundwater poses a serious public health concern due to their possible health threats. Currently practiced techniques for heavy metals removal from water are precipitation, coagulation, complexation, solvent extraction, and ion-exchange techniques. All these techniques have their own merits and demerits. Their large scale utility is very limited due to their high cost and inefficiency of removal of heavy metals when these contaminants are present at trace concentrations [49]. Biosorption is a cost-effective, emerging process having excellent potential for overcoming the limitations of conventional technologies. Although a large number of studies have been conducted to exploit microbial biomass as a cost-effective biosorbent however the presence of a large number of negative charges on the external cell layers of cyanobacterial EPSs making them very promising chelating agents for the removal of positively charged heavy metal ions [50]. An increasing number of studies have been published in recent years exploring the role of cyanobacterial EPS and their use in metal biosorption. Large numbers of cyanobacterial strains are known to produce copious amount of EPSs which are used for removal and recovery of heavy metals. The presence of large numbers of biosorption sites in the form of anionic groups (carbonyl, carboxyl, hydroxyl, and sulfate) and comparatively high surface to volume ratio in cyanobacterial EPSs are making them excellent biosorbent materials. Carboxyl and hydroxyl groups present on cyanobacterial capsular polysaccharides (CPS) enable them to chelate positively charged metal ions. The CPS of *Chroococcus parisi* can remove Cu, Cd, and Zn from water [10]. Reports are available on metal biosorption by capsulated *Microcystis*. This cyanobacterial strain is known for eutrophication in lakes, ponds, and reservoirs worldwide and causes water-bloom. During the extensive growth of *Microcystis* in water bodies, large amounts of slime are produced which strongly chelate cations due to the presence of negatively charged galactouronic acid groups in slime. The organism has a high binding affinity for Cd^{2+} , Ni^{2+} , Fe^{3+} , Cu^{2+} , and Zn^{2+} . The heavy metals biosorption ability of *M. aeruginosa* is pH-dependent. It has the highest affinity towards copper, followed by nickel and zinc [21]. Similarly, CPS of *Phormidium laminosum* has a fast metal binding mechanism for iron, cadmium, copper, zinc, and nickel [10]. A study by Asthana and coauthors. [51] reported species specific Ni adsorption ability in *Aphanothece* sp. and *Rivularia* sp. The presence of sulfate groups in EPS of green algae *Chlorella stigmatophora* can bind and remove both zinc and cadmium. High molecular weight polysaccharide produced by *Anabaena spiroides* secrete EPS which efficiently binds with manganese,

copper, lead, and mercury and thus reduces the concentration of these heavy metals in the solution [5]. Presence of specific binding sites for removal of Cu^{2+} on the sheath of unicellular cyanobacterium *Gloeotheca* sp. PCC 6909 and also on the released polysaccharide of its sheathless mutant *Gloeotheca* sp. CCY 9612 was reported by Micheletti and coauthors. [52]. The EPS of *Nostoc linckia* has the potential for biosorption of cobalt and chromium ions which depends on pH, and initial concentration of these metals. Cyanobacterial EPSs exhibit the complex and different compositional properties which are attributed to play a significant and decisive role in the metal-binding capacity of polymers [53].

Bioadhesives and Biofilms Formation

The EPSs of cyanobacteria can adhere to the solid surface and thus functions as bioadhesives. Adherence ability is facilitated by the hydrophobic nature of the polysaccharides as well as the surface used for adhesion. These polysaccharides comprise deoxy-sugars like rhamnose and fucose, peptides, and ester-linked acetyl groups which confer their contemporary hydrophobic nature as well as affect their rheological, emulsifying properties and adherence capability on the solid surfaces [4]. The adhesive nature of the polymeric substances produced by cyanobacteria is important in biofilm formation. Biofilms form a complex multi-layered matrix which first provides adhesion, whereby cells are kept attached to the surface and also with each other. Within these biofilms nutrients are concentrated, stabilized phototrophic biofilms protect the organisms against UV irradiation, drought, predation, host immune defenses, extremes of temperature, pH, salinity, and pollutants. Thus the EPSs of biofilms support the living communities to thrive in a wide variety of habitats [54]. Substrate adherence ability in EPSs of *Phormidium* sp. strain J1 and *A. circularis* 6720 has been also reported [4]. Authors explained the role of the hydrophobic nature of EPS in adhesion they found a detachment of cells and colonization of new surfaces by organisms during their stationary phase of growth. Emulycan, a polymeric extracellular emulsifying substance produced by *Phormidium* sp. strain J1 at the stationary phase of growth. Emulycan contains sugar moieties, proteins, and fatty acids and can mask the hydrophobicity of the EPS. It is attributed that emulycan production is the reason for cell detachment in older cultures of *Phormidium* [55]. Gantar and coauthors. [56] reported that the bioadhesive ability of cyanobacterial EPS is useful in the association of N_2 fixing cyanobacteria with agronomically important plants. Yang and coauthors. [57] studied the role of EPS produced by *Microcystis aeruginosa* in heteroaggregation between cells of the organism and CeO nanoparticles. The findings of the study revealed that bound and soluble EPSs of *M. aeruginosa* can reduce the toxicity of nanoparticles and by increasing the stability of nanoparticles, EPSs could aggravate their adverse effects in the environment. EPS produced by *Cyanothece epiphytica* comprised sulfated sugars with different functional groups. It was found as a good hydrophobic dispersant, an excellent emulsifier as well as a flocculant. It is potentially useful as a cost-effective, eco-friendly bio-lubricant, with characteristics that are better than the conventional lubricant grease [58].

Cyanobacterial EPSs make complex microbial associations known as "microbial mats". These mats are formed by groups of microbes present on a solid surface that is exposed to air or water. Cyanobacterial mats, when formed on surface of underground water, helps to reduce the rate of effluent deposition in the ground. *Phormidium autumnale* is one of the dominant species of cyanobacteria present in microbial mats and *Microcoleus chthonoplastes* is other very common species [10]. Marine benthic cyanobacteria *Calothrix*, *Dermocarpa*, *Plectonema*, *Phormidium*, and *Xenococcus* can produce acidic polysaccharides. These EPSs play important role in cell motility, act as antidesiccant, enable to attach on both toxic and non-toxic surfaces, and thus consider as pioneer communities in an ecosystem [59]. *Microcoleus vaginatus* is considered a suitable example of pioneer cyanobacteria, a prevalent species in arid soil systems. The addition of CPS of *Nostoc muscorum* in the soil increased the amount of water-stable aggregates either due to their gluing ability or to stimulate the soil community for more production of EPSs. The complex matrix in the marine environment produced by EPSs of cyanobacteria and diatoms provides stability to mudflat sediments against erosion and also supports organic matter and nutrient enrichment [60]. Seven biofilm-forming cyanobacterial strains *Trichormus variabilis*, *Anabaena augstumalis*, *Synechocystis aquatilis*, *Calothrix* sp., *Nostoc* sp., and *Phormidium autumnale* were isolated from an Italian wastewater treatment plant. Authors suggested their role in biomass production using a biofilm-based culture system which can further utilized for the production of valuable by-products, biofuel and also can be used as an economically viable option for biomass production at an industrial scale [61]. Oxygenic photosynthesis and biofuel production was studied in axenic cultures of *Synechocystis* sp. strain PCC 6803 by Allen and coauthors. [62]. On the other hand study by Kumar and coauthors. [63] reported that the EPS produced by *Oscillatoria* sp. and *Phormidium* sp. which showed biofilm inhibition in infection-causing bacteria *Pseudomonas aeruginosa* PA14. The cyanobacterial EPS produced by these organisms interrupted the production of elastase and rhamnolipid molecules in the

Pseudomonas thus inhibited biofilm formation and can be potentially useful as antibiofilm agents. The promising facts of bioadhesive ability of cyanobacterial EPS are considerable for industrial and ecological utility but on the contrary, bioadhesion makes them pioneering fouling organisms responsible for serious economic losses on ships and various equipment placed in the marine environment. Biofouling by cyanobacterial EPSs results in detoxication of antifouling paint enhanced corrosion of metal surfaces, their conditioning, and further by the colonization of various organisms [59]. Antifouling technology can be used as a prevention strategy for slime layer formation.

Bioremediation

Bioremediation is a process in which naturally occurring or deliberately introduced microorganisms and their enzymes are used for the degradation of pollutants into non-toxic substances. The use of oxygen-evolving photosynthetic cyanobacteria for bioremediation is a cost-effective and eco-friendly approach to environment management. These organisms are efficiently useful for the bioremediation of several gaseous, solid, and liquid recalcitrant pollutants including natural and xenobiotic originated like heavy metals, crude oil, pesticides, antibiotics, carbon dioxide, nitrogen, phosphorous, phenanthrene, naphthalene, phenol, catechol, detergents, etc. [64]. Using the atmospheric CO₂ and solar energy cyanobacteria can fix globally about 25 Gt a⁻¹ of carbon into energy-dense biomass. Cyanobacteria EPS plays an important role in bioremediation, 25% of their total biomass is contributed by EPSs. Cyanobacterial EPS remove and degrade environmental toxicants and pollutants by various means [65]. The slime nature of EPS is useful to establish a symbiotic association of cyanobacteria with other organisms. The developed consortia of microorganisms are potentially useful in the bioremediation of affected soils or aquatic systems [66]. Extreme environmental conditions accelerate EPS production as a defense mechanism in cyanobacteria and such habitats are widely occupied by usar soils. These types of soils are unproductive, impermeable, and hard due to the presence of high salinity and pH. EPS producing cyanobacteria exhibit salt tolerance and useful in usar soil reclamation. *Anabaena torulosa*, *Nostoc*, *Calothrix*, *Plectonema*, *Lyngbya*, *Microcoleus*, *Oscillatoria*, and *Aphanocapsa* are some of the examples useful in soil salinity reduction [67]. *Cyanothece* sp., *Nodularia* sp., *Nostoc* sp., *Oscillatoria* sp., and *Synechococcus* sp., showed biodegradation and biosorption of pollutants discharged by industrial effluents. Bioremediation of wastewaters and oil-contaminated sediments by consortia of cyanobacteria and chemotrophic bacteria have been studied by Abed and Köster [68]. The authors reported that the consortium of *Oscillatoria-Gammaproteobacteria* is useful to degrade phenanthrene, dibenzothiophene, pristine, and n-octadecane like hydrocarbons. Similarly, Sánchez and coauthors. [69] reported atmospheric nitrogen fixation and degradation of aliphatic heterocyclic organo-sulfur compounds and hydrocarbons such as alkylated monocyclic and polycyclic aromatic compounds by the consortia of *Microcoleus chthonoplastes* with organotrophic bacteria. An artificially designed biofilm consortium using cyanobacteria and hydrocarbon-degrading bacteria has been developed for cleaning up of crude oil contamination in seawater samples [70]. Similar studies were also conducted with consortium of *Anabaena oryzae* and *Chlorella kessleri* [66]. The role of cyanobacterial EPSs in heavy metal removal has already been discussed. The anionic nature of polysaccharides makes them suitable biosorbent, capable to remove cationic heavy metals from water bodies. Reduction in the excess amount of nitrate and phosphate from agricultural fields is also possible by these polysaccharides [71]. Consortia of *Aphanocapsa* sp. BDU 16, *Oscillatoria* sp. BDU 30501 and *Halobacterium* US 101 has been studied for calcium and chloride reduction in wastewater up to the level that can support the survival of fishes [72]. Removal of phenol and melanoidin from the effluents of the distillery is reported by consortia of *Phormidium valderianum* BDU 30501 and *Oscillatoria boryana* BDU 92181 [73]. Cyanobacterial EPS is an effective biosorbent useful in the removal of organic pollutants such as dyes and pesticides [74]. Entrapment of cyanobacterial cell mass in natural or synthetic polymers is useful in industrial wastewater treatment [75]. The bioflocculation ability of EPS, extracted from *Phormidium persicinum* is useful in wastewater treatment [76]. EPS production ability of cyanobacteria enhances their potential to adsorb and remove nutrients from the waste, rich in nitrogenous and phosphorous compounds. Thus they are extensively useful in the stabilization of ponds as well as tertiary treatment of sewage. Bioaccumulation ability of EPS is useful to remove organochlorine and organophosphorus insecticides [68]. *Oscillatoria*, *Synechococcus*, *Nodularia*, *Nostoc*, *Anabaena*, and *Microcystis* reported to have a break down ability of lindane residues [77]. EPS producing cyanobacterial strains *Anabaena* sp., *Lyngya* sp., *Microcystis* sp., *Nostoc* sp., and *Spirulina* sp. can remove glyphosate herbicide from contaminated soil [78]. The bioadhesive nature of cyanobacterial EPSs making them primary colonizers of bare soil types thus their application with bacteria, mosses, algae, lichens, and fungi form biological soil crusts in semiarid and arid areas of various geographical regions and can reverse the process

of desertification [39]. Thus, cyanobacterial EPSs are useful to remove various contaminants from the soil as well as water.

Food and Pharmaceutical Industry

The structural complexity of cyanobacterial EPSs is making them useful for industrial applications. In the food industries, they use in the preparation of jelly, cakes, dressings, sauces, gravy, beverages, ice-creams, candy, etc. EPSs can be used as viscosifying agents, stabilizers, emulsifiers, and gelling agents, in the food industry. They are useful to provide the texture of food products and are thus widely utilized in the development of new products mainly in the manufacturing of fermented milk [79]. The EPS of *Anabaena* sp. exhibit an advantageous flow property, known as pseudoplasticity, its mixture with other materials is useful in commercial applications. *Anabaena* EPS dispersions possess viscosity similar to commercially available food-grade polysaccharides and very near to xanthan gum. It is useful as a stabilizer in the food industry. EPS of *Anabaena* has more mitigation to temperature, pH, and salt concentration than the other commercially available food-grade gums [80]. Cultivated *Cyanospira capsulate* possessed the capability to release a xanthan-like polysaccharide with quite stable molecular and rheological properties [10]. *Spirulina* the blue-green algae, popularly known for its extraordinary nutraceutical and pharmaceutical applications. It is the most nutritious food source due to the presence of good-quality proteins, carotenoids, vitamins, fatty acids, and minerals. The organism is commercially valuable and cultivated in mass for its high-value phycobilin pigments useful for coloring of foodstuffs [81]. The two most important species *Arthrospira maxima* and *Arthrospira platensis* are commercially cultivated worldwide for nutritional purposes. Genus *Arthrospira* can secrete a specific sulfated EPS known as spirulan, a phycocyanin co-product, useful in the food industry [82].

Similarly, in pharmaceutical industries cyanobacterial EPSs are widely exploited for their antiviral, anti-tumor, antibacterial, wound healing, antifungal, antioxidant, immunomodulatory, anticoagulant activities and also useful in the coating of capsules and tablets [83]. Rheological properties of cyanobacterial EPS are concentration-dependent and they form viscous solutions to gels. Such dispersions can be used as texturizing agents, carriers, or active protectors and enhancers. Sulfated cyanobacterial polysaccharides are known to inhibit the transcriptase activities of viruses and interfere in the absorption and penetration of the viruses into host cells [84]. Costa and coauthors. [85] reported antioxidant and anticoagulant activities of cyanobacterial EPSs. They are also known to be immunostimulatory and can induce the production of oxidants and antioxidant enzymes in hepatocytes. Bhatnagar and coauthors. [33] reported the antibacterial activity of EPSs extracted from four desert cyanobacteria including *Tolypothrix tenuis* and three species of *Anabaena*. Antibacterial activity of EPSs was evaluated against important wound infectious agents *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC 25923), and *Bacillus licheniformis* (ATCC 14580). These polymers were also characterized by their antiradical and Fe²⁺ chelating activity. They had antioxidant activities against O₂^{•-}, H₂O₂, OH[•], and NO[•]. A strong correlation of sulfate content of polysaccharides against superoxide and nitric oxide radicals scavenging activity was reported. Their H₂O₂ scavenging activity and reducing power ability were related to the presence of phenolics in the preparation. Iron chelation had a strong bearing upon the overall reducing power and superoxide control [34]. The anticoagulant activity of spirulan is reported by *in vitro* and *in vivo* assessments. Spirulan possesses antithrombotic activity, it directly decreases the activity of thrombin and factor X activate the procoagulant proteins that can be used for the prevention of thrombus formation, and partial lysis of thrombus [86]. On the contrary, there is a single report till date mentioning thrombogenic activity of sulphated cyanobacterial EPSs. Exopolymer from three *Anabaena* species and *T. tenuis* showed a reduction in activated partial thromboplastin time and prothrombin time by 16-41% and 12-65% respectively. The blood clot formed by the cyanobacterial exopolymers was heavier vis a vis glass (positive control) and thus was thrombogenic. The polysaccharide of *Aphanothece sacrum* reported to have anti-inflammatory activity [87]. An *in vitro* study by Gudmundsdottir and coauthors. [35] revealed exopolysaccharide of some cyanobacteria had effects in the modulation of the immune system.

CONCLUSION AND FUTURE PERSPECTIVES

Fundamental studies related to the structural and functional diversity of cyanobacterial EPSs are relatively very few. Very little is known about the structure, physicochemical properties, and biosynthesis of cyanobacterial EPSs, creating many knowledge gaps in this field. Thus, research is urgently required on these aspects, not only to satisfy the need for fundamental knowledge but also to explore this unique group of organisms for their wide utilization in various fields of biotechnology. The currently available knowledge

indicate that cyanobacterial EPSs are potentially useful as biofloculants, soil conditioners, in bioremediation of heavy metals and other pollutants, in food and pharmaceutical industries. The produced polysaccharides possess quite stable molecular and rheological properties but further extensive research is required for the establishment of their practical applicability in the various fields. On the other hand in the absence of concrete information about the genes responsible for the EPS production, biosynthetic pathways, and controlling factors limits their further applications in various emerging fields of biotechnology. Thus, further studies are required to understand their biosynthetic pathways at the molecular level. Similarly, very less is known about the correlation of cyanobacterial biomass and EPS production. Further research in this direction can help us to identify the economically viable options of biomass production at the industrial scale. The spent biomass after EPS production can be utilized as a valuable commodity that could be used for the production of other value added products and biofuels. Further, the identification of integrative methodologies of production and extraction of cyanobacterial EPSs can make this bioprocess more attractive for industrial use due to increased productivity and efficiencies of the material. The structural complexity of these polysaccharides offers certain advantages, however further detailed studies of their structure, rheological properties, molecular and rheological stability concerning environmental factors are essentially required for the establishment of their potential use as a new source of natural biopolymers. Cyanobacterial EPSs play a significant role in soil neogenesis. There is an urgent need to identify the novel techniques of combined applications of cyanobacterial EPS with soil fixing chemicals for the formation of stable biocrusts to combat desertification. As a final concluding remark, to carry out a multi-product strategy when we study the use of cyanobacterial EPSs can be reasonably appropriate, which will further help to open the hidden treasure of these biomaterials.

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