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Perspective

Genetically Engineered Organisms: Possibilities and Challenges of Heavy Metal Removal and Nanoparticle Synthesis

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Abstract: Heavy metal removal using genetically engineered organisms (GEOs) offer more cost and energy-efficient, safer, greener, and environmentally-friendly opportunities as opposed to conventional strategies requiring hazardous or toxic chemicals, complex processes, and high pressure/temperature. Additionally, GEOs exhibited superior potentials for biosynthesis of nanoparticles with significant capabilities in bioreduction of heavy metal ions that get accumulated as nanocrystals of various shapes/dimensions. In this context, GEO-aided nanoparticle assembly and the related reaction conditions should be optimized. Such strategies encompassing biosynthesized nanoparticle conforming to the green chemistry precepts help minimize the deployment of toxic precursors and capitalize on the safety and sustainability of the ensuing nanoparticle. Different GEOs with improved uptake and appropriation of heavy metal ions potentials have been examined for bioreduction and biorecovery appliances, but effective implementation to industrial-scale practices is nearly absent. In this perspective, the recent developments in heavy metal removal and nanoparticle biosynthesis using GEOs are deliberated, focusing on important challenges and future directions.

Keywords: heavy metals; biorecovery; biosynthesis; genetically engineered organisms; bioreduction; sustainable technologies

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1. Introduction

The application of genetically engineered organisms (GEOs), with their great potentials for heavy metal (HM) removal and biosynthesis of nanomaterials, is an attractive field of science based on green chemistry principles and clean technologies [1–3]. Various nanomaterials and nanoarchitectures can be prepared utilizing GEOs containing stabilizing, reducing, and capping agents. Bio-inspired synthesis of nanomaterials using GEOs exhibited several advantages of simplicity, cost-effectiveness, and environmentally-benign features in comparison with the conventional methods consisting of toxic/hazardous agents and laborious processes [4–10]. In this context, GEOs are able to execute the biorecovery and bioreduction of HM ions, offering excellent opportunities for HM removal. These biofactories with great potentials for the metal ion bioreduction are capable of accumulating them as nanocrystals with well-organized and controllable size/morphology, but only after optimizing their bioaccumulation/biotransformation, biosynthesis capabilities, and reaction conditions (e.g., temperature, pH, substrate/biomass concentration, enzymatic procedures, and cellular activity) [11,12]. Application of organisms for green synthesis and sustainable removal of HMs is rapidly developing due to their unique intrinsic properties and advantages [13,14]. For instance, *Bacillus megatherium* was applied for the synthesis of gold (Au) nanoparticles (NPs); the reaction time and dodecanethiol (the capping agent) were reported as the crucial factors for regulating the size and morphology of the NPs [15].

Nanomaterial production capacity of organisms can be extended via the detailed cellular re-programming, offering the ease of handling and downstream processing via the application of GEOs for eco-friendly synthesis of NPs at ambient temperature and pressure [16]. However, their up-scalability and industrial appliances with an exact stance towards their possible toxicity and biosafety issues are vital and should be analytically evaluated. Finding the related nanoparticle synthesis pathways and HM removal mechanisms, as well as responsible enzymes/proteins and metabolic pathways, need to be given high priority in research to obtain engineered systems with tuned characteristics for specific purposes, especially via clean and sustainable technologies. In this context, various nanotechnological advances have been inspired by nature, and consequently researchers are looking for designing smart nature-inspired systems with various promising environmental potentials. For bioremediation and nanoparticle synthesis purposes, there are still concerns about the commercialization and large-scale applicability of organisms. Thus, investigations should be focused on the engineering organisms with high capabilities and standards of industrialization to obtain detailed optimization strategies/techniques, surface functionalization of the prepared NPs, and analytical/characterization processes. To identify the responsible genes for NP synthesis, several investigations have been considered to use gene silencing processes and identify the expected genes for synthesis. The introduction of candidate gene clusters into the other organisms for the verification of their capabilities to initiate NP production has been performed [17,18]. These strategies are vital for understanding the related mechanisms of NP formation and controlling their nucleation and growth. Herein, the deployment of GEOs with their unique potentials in HM removal and NP formation has been deliberated, focusing on current advances, important challenges, and future perspectives.

2. GEOs in Removal of Heavy Metals

Biological remediation is an environmentally benign and low-cost strategy that can be deployed for cleaning up the complex industrial tannery effluent containing HMs, which is a critical threat for contamination of ecosystem [19–21]. Notably, the toxicity of metals is a critical concern because of their bioaccumulation in nature and non-biodegradability. GEOs have been deployed for the bioremediation of HMs with the advantages of eco-friendliness, cost-effectiveness, simplicity, and up-scalability (Tables 1 and 2). Several factors can influence the efficiency of bioremediation using GEOs, including reaction conditions, chemical composition of HMs, redox potential, nutritional status, among others [22]. The chelation, biotransformation, oxidative stress response, metal regulation and transportation, and engineering of cell surfaces can play vital roles in HM remediation and NP synthesis by these organisms [20]. In phytoremediation by genetically modified plants, the selection of suitable plant species, interactions between plants and microorganisms, translocation processes, mechanisms of tolerance, characteristics of metals, and environmental conditions have significant effects [23–26]. Further, molecular modifications have been deployed for displaying metal-binding proteins at the cell surfaces through the overexpression of genes or introduction of exogenous DNA to produce transgenic algae with high selectivity and efficacy for HM adsorption; however, these modifications are variable and more elaborative studies are necessary for clarifying the underlying mechanisms and solving possible limitations or challenges [27]. On the other hand, significant HM concentrations and poor competitiveness may restrict the application of these organisms, but the efficacy can be enhanced by improving their bioreduction and bioaccumulation potentials [28]. In this context, active transportation of metal ions (efflux), extracellular barriers, intracellular or extracellular appropriation, and metal ions bioreduction capability should be considered [29]. Some important aspects regarding the sorption sites, configuration of microbial cell walls, and ionization of chemical entities on the cell walls can affect the bioremediation by GEOs. Microbes have shown several mechanisms for interacting and surviving in toxic metal environment such as extrusion, biotransformation, enzymatic processes, exopolysaccharide formation, and metallothioneins production [30]. As an example, bacteria exhibited

metal resistance and detoxification potentials with ion exchange, surface complexation, precipitation, redox procedure, and electrostatic interaction in reaction to metals in the environment [31]. Notably, bacterial HM resistance includes methylation/demethylation, metal chelators formation (e.g., metallothioneins and bio-surfactants), metal efflux pumps, extracellular/intracellular metal appropriation, elimination by permeability barriers, metal ligand destruction, metal-organic complexation, and metal oxidation [32].

The detection of metal-binding peptides responsible for capturing HMs has been paid attention to by researchers. As an example, cadB encoding a metal-binding protein with cadmium (Cd) (II) and zinc (Zn) (II) or pbrT and pbrD encoding proteins with binding and uptake ability for lead (Pb) (II) have been recognized. Additionally, copM encoding a binding protein for copper (Cu) (II), as well as metallothioneins with cysteine and sulfhydryl groups for binding with HMs, have been exploited in several investigations [33–35]. Additionally, some genes encoding enzymatic transformations have been reported, such as aoxA/aoxB encoding arsenite oxidase or arsC encoding the cytoplasmic arsenic (As) (V) reductase for the altering arsenite into arsenate [36]. Mercury and arsenic transporter genes have been investigated, in addition to the genes encoding regulatory proteins (e.g., aoxS and aoxR) [37,38]. Modifications in enzymes, regulation or control of biological pathways, developments in affinity sensors, post-release monitoring of GEOs, and application of molecular tools (such as rational designing, direct evolution, saturation mutagenesis, metabolic engineering, and whole-transcriptome profiling) are crucial aspects in constructing GEOs for the removal of pollutants. In addition, risk assessments, pathogenesis, adverse environmental and health effects, and biosafety issues should be considered [39–41].

Table 1. Some selected GEOs investigated for the removal of HMs.

GEOs	Removal Efficiency	HMs	Refs.
Escherichia coli (MT2 and MT3)	212 and 250 mg L^{-1}	Cd(II)	[42]
E. coli (Jm109)	10.11 mg/g	Ni(II)	[43]
E. coli (Jm109)	90 %	Hg(II)	[44]
E. coli (Jm109)	96%	Hg(II)	[45]
E. coli (Jm109)	98%	As(III)	[46]
Saccharomyces cerevisiae (W303)	27.1 ± 0.46 nmol mg^{-1}	Zn(II)	[47]
Pseudomonas putida (X4)	90%	Cd(II)	[48]
Rhodopseudomonas palustris	77.58 mg g^{-1}	Hg(II)	[49]
E. coli (pBLP1)	526 µmol g^{-1}	Pb(II)	[50]
E. coli (BL21)	7.59 mg As/g dry cells	As(III)	[51]
E. coli	99%	Hg(II)	[52]

Cd: Cadmium; Ni: Nickel; Hg: Mercury; As: Arsenic; Zn: Zinc; Pb: Lead.

Table 2. Some important transgenic plants applied for the removal of HMs.

Plants	HMs	Genes	Refs.
Nicotiana tabacum	As	AtACR2	[53]
Oryza sativa	Cu and Cd	ricMT	[54]
Brassica napus	Zn and Cu	OsMyb4	[55]
Sedum plumbizincicola	Cd	SpHMA1	[56]
Arabidopsis thaliana	Cd	MAN3	[57]
Brassica juncea	Pb	AtACBP1 AtACBP4	[58]
A. thaliana	Cd	YSL	[59]
N. tabacum	As and Cd	OsMTP1	[60]
A. thaliana	Cd	PCs1	[61]

As: Arsenic; Cu: Copper; Cd: Cadmium; Zn: Zinc; Pb: Lead.

Bacteria can bind to metal cations because of the negative change on their cell surfaces owning to the anionic structures [62]. In one study, *Tetragenococcus halophilus* and *Halomonas elongata* have shown great potentials for removing HMs in the examined medium; pH and incubation time could significantly affect the metal removal capacity of these microbes [63].

By genetically engineering bacteria, some of their capabilities such as metal-chelating proteins, metal stress tolerance, metal bioaccumulation of HM, and overexpression of peptides can be improved [64,65]. As an example, after genetically engineering of HM-tolerant *Ralstonia eutropha*, the metallothioneins were overexpressed on the cell surfaces, and the inoculation of Cd^{2+}-polluted soil with this GEOs could significantly reduce the toxic effects of HMs on the growth of tobacco plant [66]. Further, after genetically engineering of *Escherichia coli* JM109, metallothioneins and merT-merP protein were expressed in this bacterium to increase its mercury bioaccumulation ability, providing excellent opportunities for treating contaminated water [45].

In another approach, magnetic NPs were combined with metal binding proteins to improve the removal Pb and Cd from solutions. *E. coli* cells were engineered to express metallothioneins on the surface of cells. These cell surface structures also comprised histidine tags, which enabled the cells to form a complex with chemically modified magnetic NPs. The cells and magnetic NPs would precipitate for easy isolation and removal of the metal bound cells. Genetically engineered *E. coli* cells were obtained via the introduction of a de novo synthetic HM-capturing gene to encode a protein SynHMB consisting of a six-histidine tag, two cysteine-rich peptides, and a metallothionein arrangement in addition to the synthetic type VI secretory system (T6SS) cluster of *Pseudomonas putida*, providing the synthetic cells (SynEc2) with significant capability of showing the HM-capturing SynHMB on their cell surfaces (Figure 1) [67]. The synthetic bacterial cells and magnetic NPs were co-accumulated to produce biotic/abiotic complexes showing self-evolving characteristics, because of the surface exposure of six-histidine tag on the synthetic bacteria and carboxylic functionalities on magnetic NPs@SiO_2-polyethylenimine-diethylenetriaminepentaacetic acid. Consequently, the prepared complexes could remove Cd^{2+} and Pb^{2+} with high removal efficiency (>90%) and recyclability by artificial magnetic fields [67].

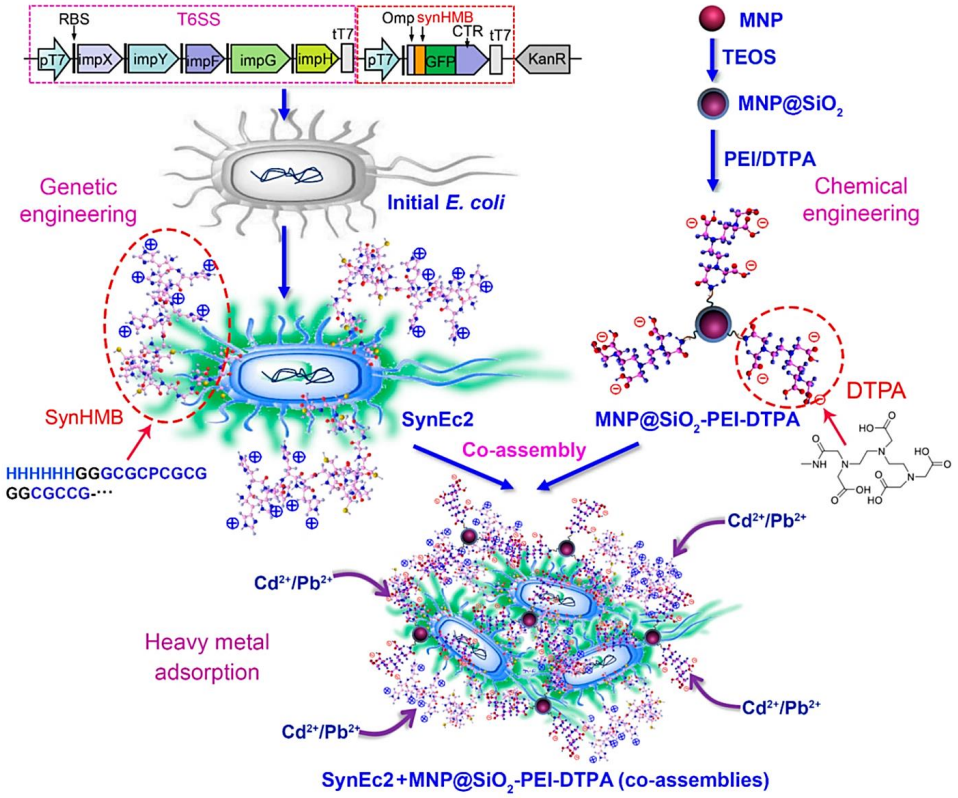

Figure 1. The preparative process of genetically engineered bacterial cells from *E. coli* to obtain co-assembles of magnetic NPs@SiO_2-polyethylenimine (PEI)-diethylenetriaminepentaacetic acid (DTPA) for efficient removal of HMs. MNP: Magnetic NPs; TEOS: Tetraethyl orthosilicate. SiO_2: Silicon dioxide. Adapted from Ref. [67] with permission. Copyright 2020 American Chemical Society.

3. GEOs in NP Synthesis

Biosystems with unique intrinsic capabilities have shown attractive applicability in nanoparticle synthesis. In this context, with developments in genetic tools and technologies, various GEOs can be reportedly deployed as biofactories with great potentials for NP fabrication and HM removal [68]. However, several challenging issues regarding the polydispersity of NPs, limited investigations in up-scalable production, lack of details pertaining to the underlying mechanisms, commercialization, and optimization conditions are still lingering. The related metabolic pathways or the role of proteins/enzymes for NP synthesis need to be systematically evaluated [18,69]. Additionally, industrial scale challenges with a focus on yield of production and monodispersity, as well as the cellular metabolism and product recovery optimization, are essential aspects. The improvement of strains with controllable NP synthesis and HM removal ought to be given the priority in such explorations. Notably, understanding the signal transduction, formation of stress-related proteins, stress perception, and transcriptional activation of stress-responsive target genes have to be considered [1,70]. In one study, genetically engineered *Pichia pastoris* strain that overexpressed metal-resistant variant of cytochrome b5 reductase enzyme was studied for the biosorption and eco-friendly synthesis of silver (Ag) and selenium (Se) NPs ranging from 70 to 180 nm [71]. After 24 h incubation, the max level of recombinant enzyme expression could be obtained ~31 IU ml^{-1} in the intercellular fluid. Additionally, the recombinant biomass capacity for the biosorption of Ag and Se in examined aqueous solutions was ~163.90 and 63.71 mg g^{-1}, respectively. The produced NPs of spherical shape are crystalline and well-dispersed in nature [71]. Recombinant *E. coli* could be applied for synthesizing a variety of nanostructures via the deployment of the strain co-expressing metallothioneins (metal-binding proteins) as well as phytochelatin synthase that produces phytochelatins (metal-binding peptides) [72]. Besides, *E. coli* JM109 bacteria were genetically engineered to generate phytochelatins (as capping agents) for intracellularly fabricating CdS quantum dot nanocrystals (~3–4 nm); the size of the semiconductor nanocrystals was tuned by adjusting the amount of phytochelatins [73].

Silver-resistant *Morganella morganii*, gram-negative bacteria, were applied for the biosynthesis of Cu NPs. Consequently, Cu^{2+} ions were reduced within the bacterial cells and strong link between the Ag and Cu resistance machinery of bacteria could be detected regarding the metal ions bioreduction [74]. Furthermore, *Magnetospirillum gryphiswaldens* magnetotactic bacterium was applied for synthesizing Fe_3O_4 NPs (~50 nm) inside the self-assembled magnetosomes (membranous structures present in magnetotactic bacteria) [75]. Magnetosome biomineralization pathway was moved from *M. gryphiswaldense* for heterologous expression into *Rhodospirillum rubrum* (as another synthetic host) via the incorporation of mamGFDC, mamAB, mms6, and mamXY genes to produce magnetite NPs (~24 nm) encircled by protein shells. [76]. It was established that mamO gene played an important role in the synthesis of magnetic NPs in magnetotactic bacteria [77]. Besides, chalcogenide nanostructures were synthesized after transferring reductase genes originating from *Shewanella* sp. ANA-3 and *Salmonella enterica* serovar Typhimurium into *E. coli* as a heterologous host [78]. The initial materials were processed by redox enzymes, and arsenic sulfide nanomaterials were formed after the nucleation started by the cellular components (Figure 2). Rapid culture, cost-effectiveness, and simplicity are important advantages, providing genetically engineered strains expressing metal reductases suitable for synthesizing various nanomaterials [78].

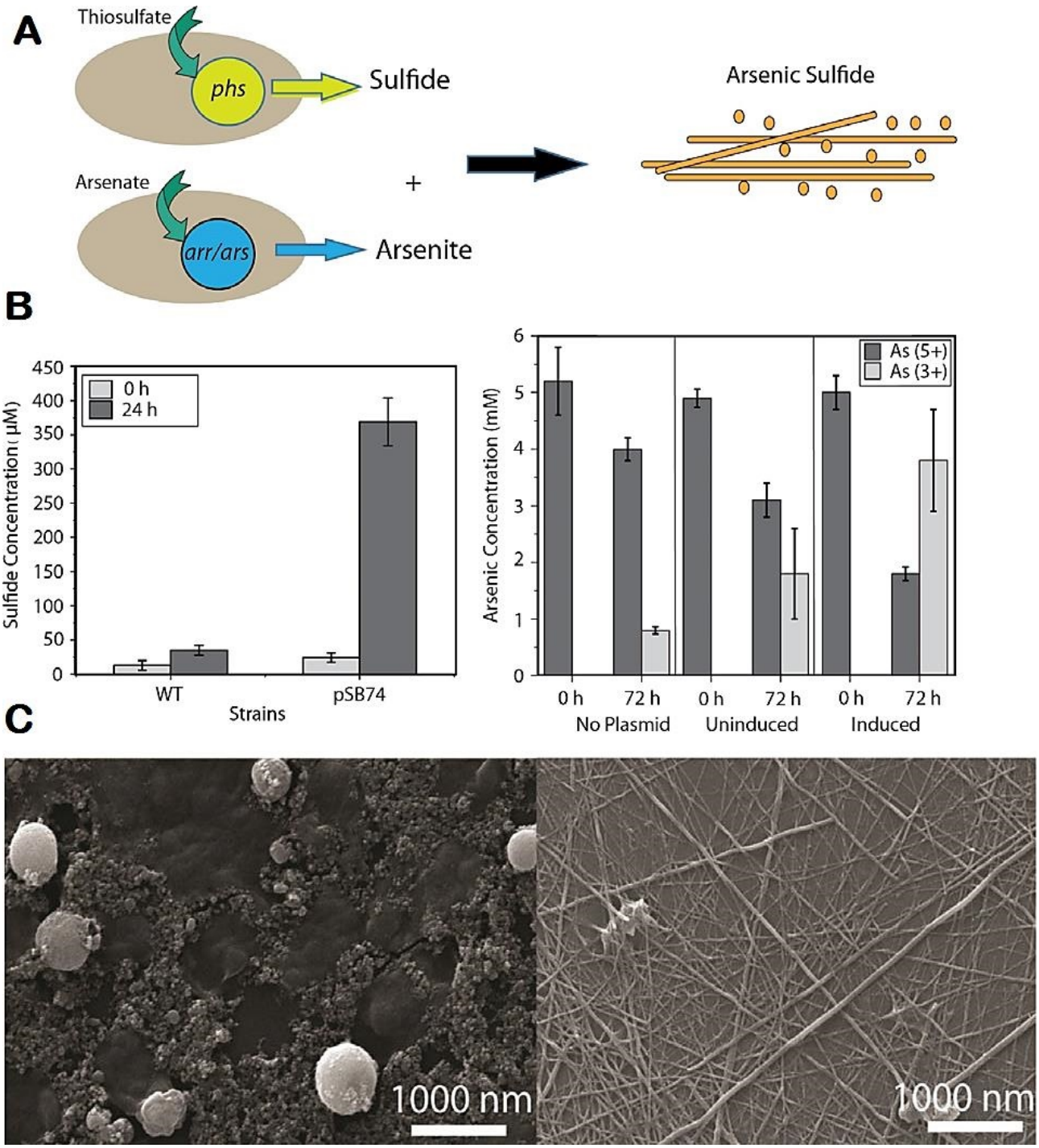

Figure 2. (**A**,**B**) Genetically modified *E. coli* strains applied for the bioreduction of thiosulfate into sulfide in addition to the arsenate into arsenite to synthesize arsenic sulfide chalcogenide nanomaterials (**C**). Adapted from Ref. [78] with permission. Copyright 2018 John Wiley & Sons Ltd. (CC BY 4.0).

Genetically engineered *Thalassiosira pseudonana* micro-algae were applied for the attachment of IgG binding domain on biosilica for cancer targeting appliances. Accordingly, chemotherapy drug-loaded liposomes were affixed to the IgG biosilica complexes to target cancerous cells [79]. Besides, plasmids originated from Bacillus host could be deployed as scaffolds for producing Ag NPs (~20–30 nm) at room temperature [80]. The phosphate backbone of DNA was negatively charged and could fasten to the positively charged metal

ions via the associated electrostatic interaction. After the photo-irradiation by UV light, the NP nucleation was initiated on plasmid scaffolds as reducing agents, showing these plasmids as suitable templates for NP synthesis [80]. The fusion of glutamates on the N-terminus of the capsids of P8 of M13 bacteriophages was performed to produce barium titanate ($BaTiO_3$) NPs (~50–100 nm) after the incubation with barium (Ba) and titanium (Ti) glycolates. The production of NPs with perovskite crystal structures and viral fibrous morphologies have been reported via the electrostatic interaction and hydrogen bonding [81]. Genetically engineered tobacco mosaic viruses were employed for surface-displaying a characterized peptide with strong metal ion binding and reducing capacity. Unlike wild type tobacco mosaic viruses, these constructs led to the formation of isolated Au NPs with stability and crystallinity (~10–40 nm) [82].

4. Conclusions and Future Directions

A variety of GEOs have been introduced by applying recombinant DNA or RNA strategies, showing great potential for the elimination or remediation of HMs and for the fabrication of nanomaterials. Rapid culture, high yield of production, monodispersity, simplicity, and formation of well-organized NPs are important advantages by deployment of these biofactories. However, finding the underlying mechanisms and identifying responsible agents ought to be undertaken; important mechanisms of HM uptake such as cell surface adsorption, bioaccumulation, surface complexation, electrostatic interactions, precipitation, and ion exchange need to be profoundly investigated. Bioremediation using organisms has exhibited cost-effectiveness and simplicity advantages for treating environmental HM contaminations. However, the pathways for HM removal using these organisms such as bioaccumulation, bioleaching, biotransformation, biosorption, and biomineralization still must be analytically addressed. Additionally, the selection of suitable host, growth rate, recombinant strains, biochemical activities, and replication processes are crucial aspects that need to be considered for optimized NP production and efficient HM removal. Notably, designing cost-effective processes for acquiring recombinant strains is imperative for environmental appliances, particularly on an industrial scale. The specific recognition of related biomolecules with excellent stabilization and reduction capabilities, as well as microbial growth parameters and optimization conditions, should be systematically analyzed, especially for additional movement from laboratory to industrial stages.

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