Review Paper: Algae and microalgae mediated synthesis of iron nanoparticles and their applications

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ABSTRACT

Iron nanoparticles (FeNPs) have attracted increasing attention due to their unique properties and great potential for various applications. Physical and chemical methods are conventionally used for synthesis of FeNPs. These methods employ toxic chemicals and organic solvents and usually are performed at harsh conditions. Hence, there is an increasing demand to develop sustainable methods for synthesis of FeNPs. Biosynthesis is emerging as a green approach to alleviate the environmental and economic disadvantages of the traditional techniques. Since now, various organisms such as bacteria, fungi, and plants have been used for FeNPs fabrication with no or limited attention to algae and microalgae. While, these organisms are wealthy sources of bioactive compounds which can be used in biosynthesis of FeNPs in a non-toxic, cost-effective, ecofriendly, and facile manner. The present work summarizes algae and microalgae as potential biofactories for the synthesis of iron-based nanoparticles and their potential applications in biomedicine and environmental remediation.

1. Introduction

Nanoparticles are small particles in the range of 1 to 100 nm in size. They act as a bridge between bulk materials and atomic or molecular structures [1] . Due to the small size and high surface volume ratio of nanoparticles, their characteristics are different from their bulk counterparts. These properties make nanoparticles effective in many fields of science and technology [2]. In recent years, iron nanoparticles (FeNPs) have gained a significant interest due to their unique physico-chemical and biological properties and variety in chemical structure and properties. These particles are available in different forms including zero valent iron, iron oxides, iron oxide-hydroxides, and iron hydroxides [3]. FeNPs with biocompatible coatings are FDA approved for application in food and pharmaceutical industries. Due to increase in industrial and technical demands for

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FeNPs various approaches for the synthesis of these useful nanoparticles were developed[4-8]. These approaches can be divided into two main categories, known as top-down and bottom-up. Top-down approaches employ the physical and chemical means to reduce the size of bulk materials. These methods are not preferred, because large amount of materials and high energy are needed. Also, prepared particles are not usually in fine quality. In bottom-up approaches atoms or molecules are assembled into nanostructures [9]. Classically, wide variety of physical and chemical methods are used for synthesis of metal nanoparticles. The physical method comprise of ball milling [10, 11], laser ablation [12], pyrolysis [13], electric discharge [14], gas phase deposition and aerosol [15]. Physical methods are not capable to control the size of particles in the nanometer range and also require high temperatures and pressures making them uneconomic. The main chemical methods which are developed for the synthesis of FeNPs are coprecipitation [5, 16, 17], microemulsion [18], sol-gel [19, 20], sonochemical [21], hydrothermal [22], flow injection [23], electrospray [24], hydrolysis and thermolysis [25]. Many of these processes employ very active and hazardous reducing, capping and stabilizing agents such as hydrazine [26], sodium borohydride [27] potassium bitartrate [28] and methoxy polyethylene glycol [29]. These chemicals are environmentally toxic and also restrict the medical and biological applications of synthesized nanoparticles [11, 30]. Hence, there is an essential need to develop sustainable techniques for the synthesis of biocompatible FeNPs. To resolve these problems, researchers are moving toward green synthesis of nanoparticles. The green technologies usually happen at ambient condition while using renewable and natural sources instead of chemical reagents and organic solvents [6-8]. As a result, this technology is an environmental friendly, clean, biocompatible, non-toxic, cost effective, and economic process [8]. In the green synthesis approaches bioactive compounds from biologic sources were used instead of chemical reagents. These bioactive compounds can act as reducing and stabilizing agents for biosynthesis of nanoparticles with diverse morphologies and chemical formulas [31-33]. Since now, green synthesis of FeNPs are performed by different organisms such as bacteria [6, 34], fungi [35], and plants [7, 8]. But, less attention was paid to the potential role of algae and microalgae [36, 37]. These organisms are widely used for the synthesis of silver nanoparticles (AgNPs) [38], while there are a few reports on the synthesis of iron-based nanoparticles [39]. Algae and microalgae cells are rich in lipids, polysaccharides, minerals, polyunsaturated fatty acids, vitamins, polyphenols, and secondary metabolites [40]. These organisms are also reach in bioactive compounds effective against bacteria [41], cancer [42, 43], allergy [44], diabetes [45], obesity [46], and inflammatory diseases [47]. It has been shown that aquatic organisms are full of biochemical molecules with hydroxyl, carboxyl, and amino functionalities which can act as both reducing and capping agent to provide a powerful coating on the metal nanoparticles [48-50]. Algae and microalgae can be considered as a source of bioactive compounds for the green synthesis of FeNPs due to their availability, safety and efficacy [39]. The present work is aiming to discuss the biogenic fabrication of iron-based nanoparticles from algae and microalgae and their possible applications.

2. Algae-mediated synthesis of FeNPs

Algae or seaweeds are classified into brown algae (Phaeophyceae), red algae (Rhrodophyceae), and green algae (Chlorophyceae) according to cell wall chemistry, presence or absence of flagella, nature of the chlorophyll and pigments [51]. Brown algae are the most predominant species of seaweeds employed for FeNPs synthesis. Until now, Sargassum muticum, Sargassun acinarium, Padina pavonica, and Dictyota dicotoma were used for biosynthesis of FeNPs [52-54]. Kappaphycus alvarezii was the only red algae that was reported for this purpose [55]. Algal aqueous extract solution is commonly used for the algae mediated synthesis of nanoparticles. For this purpose, algae specimens are freeze-dried or shine dried then grounded to fine powder. The powder is boiled in deionized water for about 15 min. The extract is cooled at room temperature, filtered and used in the biosynthesis reaction. Ferric ions or a mixture of ferric and ferrous ions is used as iron precursor. After addition of iron salt solution to algal extract, color of the reaction mixture is rapidly changed to black or dark brown which is an indicative of FeNPs formation [53, 54, 56]. This procedure is schematically illustrated in Fig. 1. Since now, algae mediated biosynthesized FeNPs have been reported to be in the range of 10 nm to 50 nm in diameter. This difference can be due to variations in the content of bioactive molecules from different algal species. The biggest reported algae derived FeNPs were obtained from *D. dicotoma*. Particles were in the size of 40-50 nm having a cubic shape. Despite of the large size, biosynthesized nanoparticles were stable even after three months of storage indicating that the nanoparticles were properly coated with biological molecules. This biologic coating provides stable dispersity in aqueous solutions and prevents particles from aggregation [53]. In fact, algae contain a wide variety of biochemicals such as proteins, carbohydrates, lipids, pigments, vitamins, phenols, polyphenols, flavonoids, alkaloids, fucoxanthins, and diterpenoids which can act as both reducing and stabilizing agent [52, 54, 55, 57-61]. Algae mediated synthesis of FeNPs has some drawbacks. The main disadvantage is that algae derived FeNPs are mostly magnetite (Fe_3O_4) nanoparticles and there are rare reports for production of other iron-based nanoparticles. Limitation in the particles morphology is another disadvantage. As depicted in Table 1, cubic and spherical nanoparticles are the only reported forms to date. It has been determined that certain functional groups and amino acid residues in biological compound can play a shape controlling role [62]. By modification of these effective groups it is possible to manipulate the shape of prepared particles [62]. As another alternative, iron nanoparticles which were synthesized by chemical processes can be coated with algal biomolecules. Silva and coworker have coated magnetic nanoparticles with fucan polysaccharide from Sargassum symosum [63]. Naked and fucan-coated Fe_3O_4 nanoparticles were 10 nm in size and fucan coating had no adverse effect toward increasing the particle size. Both particles showed same XRD patterns which indicate that the process of coating and functionalization dose not degrade structure of the core magnetite. Fucan-coating can reduce particles interactions and aggregations and hence increase particles colloidal stability. Interestingly, reduction in the interactions between particles limits the disorders of the spins on the surface of nanoparticles and increase the magnetization [63].

Figure 1. Algae mediated synthesis of FeNPs in schematic illustration

Table 1. Characteristic features of algae and microalgae mediated synthesized FeNPs

Phylum	Genus/Species	FeNPs	Size(nm)	Shape	Synthesis	Reference
Algae	*Sargassum muticum*	Fe_3O_4	17-25	Cubic	-	(52)
	Padina pavonica	Fe_3O_4	10-19.5	Spherical	-	(54)
	Sargassum acinarium	Fe_3O_4	21.6-27.4	Spherical	-	(54)
	Dictyota dicotoma	Fe	40-50	Cubic	-	(53)
	Kappaphycus alvarezii	Fe_3O_4	11-20	Spherical	-	(55)
Microalgae	*Chloroccum sp*	Fe^0	20-50	Spherical	Intracellular, Extracellular	(64)
	Chlorella sp	Fe	5-50	Spherical	-	(66)
	Euglena gracilis	2-Lines ferrihydrite	0.6-1	Spherical	Intracellular	(68)
	Klebsormidium flaccidum	β-FeOOH	100	Nanorod	Intracellular	(36)
Cyanobacteria	*Anabaena flos-aquae*	β-FeOOH	100	Nanorod	Intracellular	(36, 80)
	Calothrix pulvinata	β-FeOOH	100	Nanorod	Intracellular	(36)
	Not indicated	Fe_3O_4/AC composite	325	-	-	(65)

3. Microalgae-mediated synthesis of FeNPs

Biosynthesis of iron-based nanoparticles using microalgae can be performed by using dried biomass (Fig. 2) or living cells (Fig. 3) [36, 64-66]. When using algal biomass, nanoparticles form by adhesion of iron ions on the cell surface followed by reduction into the FeNPs. The carbonyl and amine groups of carbohydrates and glycoproteins present in algal cell wall actively reduce iron ions into nanoparticles [66]. However, in the living cell approaches, iron salt solution is added directly into the culture of living microalgae cells and they can continue to photosynthesis, metabolism and proliferation. The cells uptake iron cations and transport it into the cells through an intracellular process leading to the formation of FeNPs in the cytoplasm [67, 68]. Whenever living cells were used for the synthesis of FeNPs, chosen iron salt has an immense impact on the viability of cells and the formation of nanoparticles. By adding $FeCl_3$ as iron precursor into the living culture of microalgae, the pH of culture medium decreases due to progressive formation of HCl. This phenomenon can reduce pH of the medium to about 4 which results to a progressive decrease in photosynthetic activities and eventually cell death [36]. Meanwhile, the cells are resistance to an equimolar solution of ferrous and ferric ions. Also, iron ions complex can increase the photosynthetic activity of the cells between 5 and 15 days [36]. Since now biosynthesis of FeNPs has been carried out by some photosynthetic microorganisms such as Chlorococcum sp, Chlorella sp, Euglena gracilis, Klebsormidium flaccidum, Anabaena flos-aquae, and Calothrix pulvinata [36, 65, 67, 68]. FeNPs fabrication by using microalgae and cyanobacteria has some advantages over algae mediated synthesis. In this regards, photosynthetic microbial cells are capable to biosynthesize small FeNPs with about 5 nm diameter [66]. Similar findings were also reported for other metal nanoparticles which employed microalgae cells [38]. Preparing ultra small FeNPs with a huge surface area is a great advantage to be used in catalytic application and contaminant remediation [66, 69]. In addition to ultra-small nanoparticles, iron microparticles were reported to be synthesized by using microalgae [68]. Microalgae are capable to synthesize a wide variety

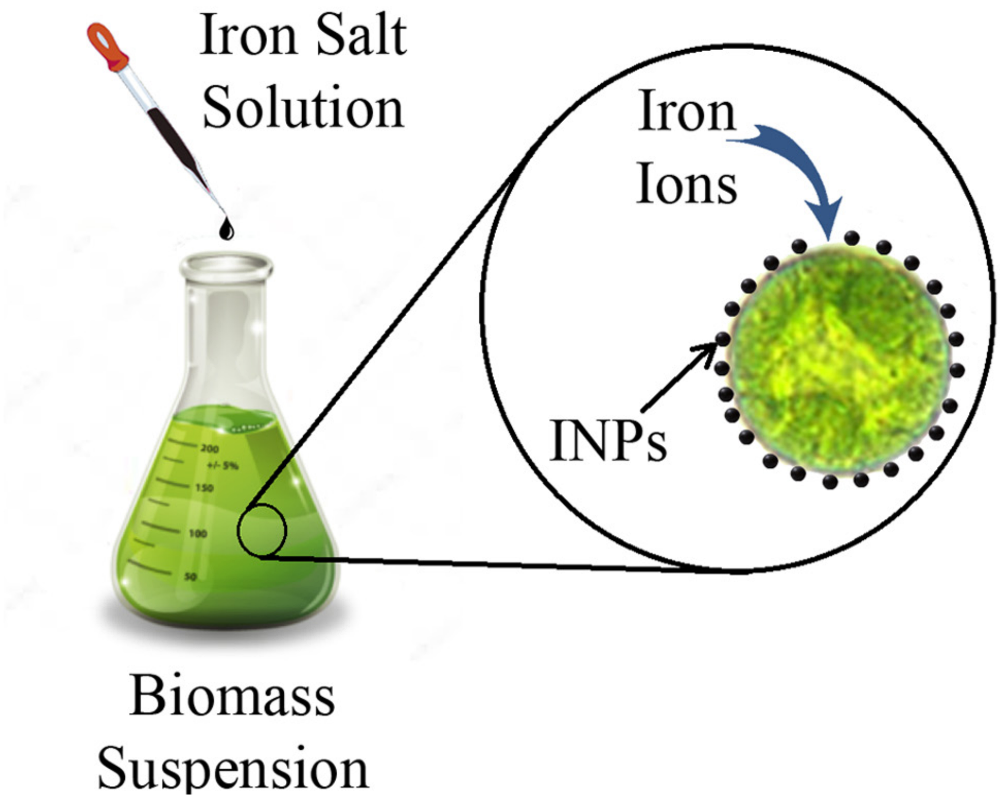

Figure 2. Extracellular biosynthesis of FeNPs by using microalgae dried biomass

of iron-based nanoparticles from zero valent to iron oxides and iron oxide hydroxide nanoparticles [36, 66, 68]. Despite these advantages, biosynthesis of iron-based nanoparticles using microalgae is conducted in longer time as compared to algae-mediated synthesis. FeNPs were reported to be synthesized in just one hour when using algae. However, reaction time for microbial cells mediated synthesis is about 24 to 48 h and this time can be longer while using living cells [65]. Cultivation of microalgae and cyanobacteria is another matter. These cells are not fast growing and usually a long period of incubation and illumination is required to provide enough biomass for biosynthesis purposes. In addition, the media which are commonly used for cultivation of photosynthetic microorganisms are salty solutions with no facile preparation protocol [38, 42].

4. Applications of algae and microalgae mediated synthesized FeNPs

Anticancer activity

Cancer is among the main cause of mortality worldwide and results in serious health problems. Currently, researchers have focused on developing methods to control and treat cancer. Among developed and applied techniques, nanotechnology and treatment of tumors by nanoparticles have gained an extensive attention. Decrease in drug resistance and drug dosage, high efficiency diagnosis, sensitive and intelligent targeting, unique magnetic properties, ease of functionalization, biocompatibility, and biodegradability are the factors that make FeNPs one of the first candidates for cancer therapy. Algae and microalgae mediated synthesized FeNPs are not exceptional and investigations in order to apply these particles for cancer treatment are undergone. Namvar and colleagues (2014) have evaluated in vitro anticancer activity of magnetic iron oxide nanoparticles synthesized via seaweed extract on several human cell lines [70]. The examined particles inhibited proliferation of cancer cell lines in a time- and dose-dependent manner. After 72 hours of treatment, inhibitory concentration 50 (IC50) of Fe_3O_4 magnetic nanoparticles on $HepG_2$, MCF-7, HeLa, and Jurkat cell lines were 23.83±1.1, 18.75±2.1, 12.5±1.7, and 6.4± 2.3 µg/ml, respectively [71]. In another study, same research group

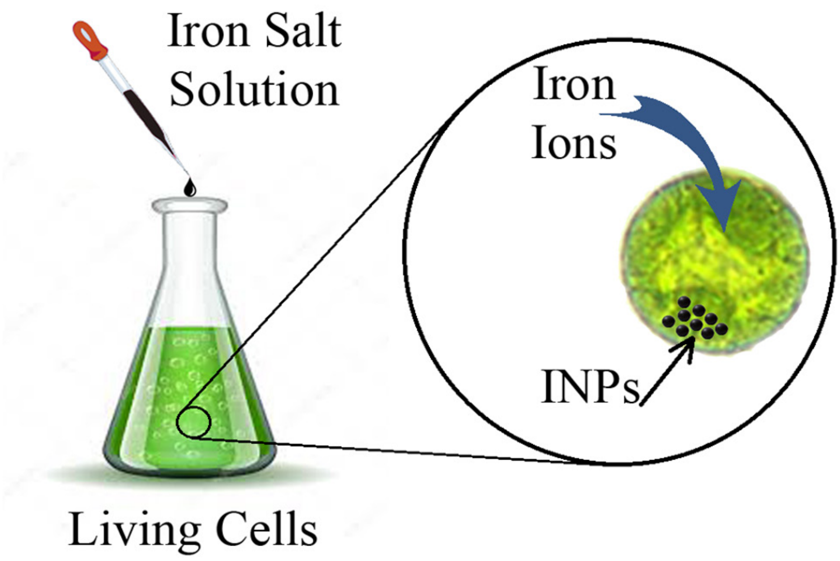

Figure 3. Intracellular biosynthesis of FeNPs by using microalgae living cells

showed apoptotic effect of magnetic iron oxide nanoparticles on A2780cp ovarian cancer cells which show superior resistance to chemotherapy. Induced apoptosis in ovarian cancer cell line was observed in a time- and dose-dependent manner [70].

5. Antibacterial activity

Infectious diseases are considered as a serious problem for public health worldwide due to emergence of resistant strains. Hence, there is a need to develop new generation of antimicrobial agents. So far, several studies have been conducted on the toxicity of AgNPs against microbial cells but very little is known about the toxicity of iron oxide nanoparticles [4, 5, 16, 17, 72-76]. Impacts of FeNPs on the microorganisms are depending on physicochemical properties of nanoparticles and species of microorganisms. In some studies, FeNPs show inhibitory effect on microorganisms while in other studies iron-based nanoparticles can promote the growth of bacterial cells [4, 5, 16, 17, 72-76]. Usually at low concentrations, FeNPs can act as exogenous iron source for microbial cells and promote their growth. However, at high concentrations the antimicrobial effects are appeared [73, 75]. Similar findings were also reported for mammalian cell lines [77]. FeNPs also can improve the efficiency of antibiotics against microorganisms. It has been shown that use of FeNPs as nanocarrier for antibiotics can increase the sensitivity of microorganisms [53, 76, 78]. Algae derived FeNPs were also applied for antimicrobial purposes. Antimicrobial activities of FeNPs synthesized by *Dictyota dicotoma* and nanoparticles in conjugation with antibiotics have been evaluated against Enterococcus hirae, Pseudomonas aeruginosa and Escherichia coli. The results revealed that nanoparticles in conjugation with penicillin G and amoxicillin have higher antibacterial effects [53].

6. Remediation

FeNPs are widely used for the remediation of contaminants due to their small size, high surface area and magnetic property. Easy separation of adsorbents from the system is possible by magnetic property of iron-based nanomaterials. Moreover, these particles can be reused several times which reduces their production costs [79]. The potential of algae mediated synthesized FeNPs were also examined for removal of heavy metals. It has been shown that magnetite nanoparticles which were synthesized by Padina pavonica and Sargassum acinarium and were entrapped in calcium alginate beads were capable for Pb adsorption. It is interesting that particles which derived from different algae have different affinity for Pb adsorption. The biosynthesized nanoparticles from *P.* pavonica showed higher capacity for Pb adsorption (91%) while that of *S.* acinarium had a capacity of (78%) after 75 min [54]. Subramaniyam *et al.* evaluated the potential of phycosynthesized iron nanoparticles in remediation of Cr (VI). The results of their study showed that iron nanoparticles reduced 92 % of 4 mg/L Cr (VI) to Cr (II). They also indicated a dose-dependent relationship between nanoparticle concentrations and removal percentage of Cr (VI) so that increasing the nanoparticle concentration elevated the Cr (VI) removal percentage [64]. FeNPs from cyanobacteria were also employed for pollutant removal from aqueous environments. Fe_3O_4/activated carbon (Fe_3O_4/AC) composites were synthesized by using cyanobacterial cells and were used for methylene blue removal from aqueous solution. Maximum capacity of the prepared composite for dye adsorption was evaluated to be 207.9 mg/g [65].

7. Conclusion

The biogenic synthesis of iron nanoparticles has attracted considerable attention due to their unique properties, which makes them highly desirable for application in wide range of industries. Algae and microalgae offer a non-toxic, cost-effective, ecofriendly, facile, and one-pot approach for the synthesis of iron nanoparticles. Where polysaccharides, proteins and other bioactive compounds present in algae and microalgae can act as both reducing and stabilizing agent during the biosynthesis process. To date, various organisms such as bacteria, fungi, and plants have been used for fabrication of FeNPs with no or very limited attention to algae and microalgae. Thus, there is a significant need for further studies to explore different algae for FeNPs production and evaluate their potential use in various applications.

Ethical Considerations

Compliance with ethical guidelines

There was no animal or human study in this work.

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Authors contributions

Conceptualization: A. Ebrahiminezhad, S.-M. Taghizadeh
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Writing: S.-M. Taghizadeh, A. Taherpour, A. Ebrahiminezhad
Review & Editing: A. Ebrahiminezhad
Visualization: S.-M. Taghizadeh
Supervision: A. Ebrahiminezhad

Conflict of interest

The authors declared no conflict of interest.

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