THE GREEN SYNTHESIS AND CHARACTERIZATION OF ZERO VALENT IRON NANOPARTICLES USING AZOLLA AND BLUE GREEN ALGAL SYSTEMS

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ABSTRACT

Iron nanoparticles (nZVI) were synthesized by photosynthetic systems such as Azolla, the water fern and Blue green alga (Westiellopsis). No additional polymers or capping agents were used as reducers in the synthesis of nZVI. The zero-valent iron nanoparticles were entrapped or capped by polyols (functional compounds) present in the biological materials. The morphology, size and structure of green synthesized iron nanoparticles using Azolla and Blue Green algae (A- nZVI & BGA- nZVI) were confirmed by Particle Size Analyzer (PSA), UV-visible spectroscopy (UV-vis), Fourier Transform Infrared spectroscopy (FT-IR), Raman spectroscopy, Scanning Electron Microscope (SEM) and energy-Dispersive X-Ray Analysis (EDAX). The nanoparticles agglomerated clusters were ranging from 70-430 nm and due to biologic coating, the green nanoparticles possessed good stability. Since green synthesized nZVI emerged as potential ecofriendly nanomaterials in the field of environmental remediation, pharmaceutical and industrial level, these A-nZVI and BGA-nZVI indicate enhanced potential to serve as green nanomaterial in various applications.

KEYWORDS: Green Synthesis, Nano Zero Valent Iron Nanoparticles, Ecofriendly, Azolla, Blue Green Algae & Characterization

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INTRODUCTION

A nano particle can be defined as a microscopic particle that has at least one dimension less than 100 nanometers in size (Thakkar *et al.*, 2010). There are two approaches for synthesis of metallic nanoparticles: the “bottom-up” approach and the “top-down” approach. Bottom-up (self-assembly) refers to the construction of a structure atom-by-atom, molecule-by-molecule, or cluster by-cluster. In case of top-down approach, a suitable starting material is reduced in size using physical (Eg: mechanical) or chemical means. These two methods employ toxic chemicals as reducing agents, organic solvents or non-biodegradable stabilizing agents and are therefore potentially dangerous to the environment and biological systems. Moreover, most of these conventional methods entail defective surface formations, high manufacturing and energy requirement but low production and also quite expensive (Njagi *et al.*, 2010). Many authors reported that metal nanoparticles had been produced in novel way using wide range of biological sources using plant materials and microorganism (bacteria, fungi, algae, yeast and viruses). At present, nano zero valent iron (nZVI) is considered to be highly efficient reductant with wide applications in remediation of contaminated site and aquifers (Mystrioti *et al.*, 2014). Due to its small particle size, large specific area and intrinsic reactivity, they are employed in groundwater treatment, site remediation (Shi *et al.*, 2011) including organic contaminants (O’Carroll *et al.*, 2012), toxic metals and dyes (Venkateswarlu *et al.*, 2013).

The green synthesized nZVI overcomes the conventional drawbacks such as aggregation, high reactivity when exposed to air, loss of magnetism and cannot be used in biomedical application (Herlekar *et al.*, 2014). Plant extracts used by researchers in production of Iron nano particles(nZVI) were green tea, oolong tea, sorghum bran, Eucalyptus, Pomegranate, Plantain peel, Banana peel, Mulberry, Cherry, Vine leaves, *Dodonaeaviscosa*, *Tridaxprocumbens*, grape marc, *Terminaliacheluba*, *Urticadioica*, Rose and many more. The polyol components present in the plant extract are responsible for the reduction of iron ions whereas water soluble heterocyclic components stabilize the nanoparticles formed (Pattanayak *et al.*, 2013). *Azolla*, the aquatic macrophyte is fast growing, free floating, high biomass has ability to tolerate wide range of pollutants and accumulation of heavy metal contamination. Hence both living and dead *Azolla* biomass have been exploited for phytoremediation of industrial effluents and sewage water (Sood *et al.*, 2011). Blue green algae, a natural carbon sequester is also employed in heavy metal remediation (Parameswari *et al.*, 2009) and nutrient removal in waste water treatment. In this work, nZVI were green synthesized using phototrophically abundant *Azollamicrophylla* and *Westielloopsis* found in paddy field by reduction of Ferric compound.

MATERIALS AND METHODS

Reagents and Chemicals

Azollamicrophylla and *Westielloopsis* (Blue green algae) biomasses were obtained from *Azolla* germ plasm and Culture collection center, Department of Rice, TNAU, Coimbatore respectively. Ferric chloride anhydrous ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 98%), used as precursor of analytical grade, was obtained from HiMedia laboratories and Millipore water was used in the preparation of all reagents.

Methodology

Preparation of Green Extracts from *Azolla* and Algae

Azollamicrophylla pure culture was collected and grown in tanks for biomass multiplication. After 10 days, the four fold increase in biomass was observed and the fronds were weighed and separated using sieve and washed twice using fresh water. *Westielloopsis* mats were soaked in fresh water for 12 hours to remove the impurities and washed several times using fresh water. Both *Azolla* and BGA masses were washed twice with Millipore water prior to extraction.

Both the samples were crushed using pestle and mortar and 10 g of crushed samples were added with 100 ml of Millipore water (1:10) and boiled at 70°C for 30 minutes (Table 1). Both the samples were cooled to room temperature, filtered twice using Whatman no.1 and the extracts of *Azolla* and *Westielloopsis* were refrigerated till further use.

Table 1: Preparation of Green Extracts

Product	Boiling Time (mins)	Mass of Leaf : Vol. of Water (gm) (ml)	Ratio
Azolla	30	10:100	1:10
BGA	30	10:100	1:10

Green Synthesis of Iron Nanoparticles

Ferric chloride solutions of different concentration were prepared as precursor and *Azolla* and *Westielloopsis* extracts were used as reducers. The precursor prepared in different concentrations (0.001M, 0.01M, 0.1M, 0.5M, 1M) were loaded in burette and added in drops into the beaker containing *Azolla* and *Westielloopsis* extracts in different ratio (1:1, 1:1.5, 1:2) under magnetic stirring. The temperature of about $55-60^\circ\text{C}$ was adjusted during stirring. The slow release

of FeCl_3 prevents the quick nanoparticle agglomeration than direct pouring method. All the series of suspensions prepared were tightly closed and kept under room temperature for 24 hours undisturbed. Various parameters including precursor and bio extract concentration, precursor to bioextract ratio, reaction time, reaction temperature are deciding factors in nanoparticles synthesis that varies with different plants in green synthesis (Ebrahimezhad *et al.*, 2016). Hence series of trial study would determine the optimal state of each parameter towards best result.

RESULT AND DISCUSSIONS

Characterization of Iron Nanoparticles

Particle size and stability of A-nZVI and BGA-nZVI were analysed using HORIBA-SZ-100 Nano Particle Size Analyzer instrument that works based on the principle of Dynamic Light Scattering. The dark color was a result of surface plasmon excitation vibrations in the Fe nanoparticles and this was confirmed by band formation in UV-vis spectroscopic analysis (SPECORD PLUS, made in Germany) and the results coincided with previous works (Patanayak *et al.*, 2013). The biomolecules responsible for reduction of precursor or the functional groups present in reducer were identified through FTIR analysis (Jasco FTIR 6800 Class 1 Laser product). The structural fingerprint of the molecules was analysed using Raman Spectroscopy which relies on the Raman or inelastic scattering. The morphological characterization of samples were analysed using Scanning Electron Microscope (FEI Quanta 250) and the composition of chemicals for the elemental analysis was characterized using EDAX. The pH was determined using Digital pH meter.

Particle Size and Stability of A-nZVI and BGA-nZVI

The reduction of Fe^{2+} ions employed in formation of iron nanoparticles was visually observed by appearance of dark brown and brown precipitate in Azolla and BGA suspensions respectively (Figure 1). The immediate color change from orange to dark brown in Azolla samples and yellow to brown in BGA observed visually (Chen *et al.*, 2013). The particle size and stability reports indicated the agglomeration of particles. As reported by Muthukumar and Matheswaran, biogenic coating reduces the agglomeration rate and they measured the size of biogenic produced nanoparticles to be from 58 to 530nm. The suspensions of Azolla and BGA nanoparticles distributed between 77.8 nm to 432 nm and the nanoparticles settled completely at the bottom of container within 1 to 2 hours of synthesis. Cloud type agglomeration was observed in both iron nanoparticles on storage which was in line with cherry based green synthesis (Machado *et al.*, 2015).

Figure 1: Appearance of Dark Brown Precipitate in Different Concentrations (0.001M, 0.01M, 0.1M, 0.5M, 1M) of Precursor a) Azolla- nZVI and b) BGA-nZVI

The stability of A-nZVI and BGA-nZVI synthesized at different concentrations ranged between 40.6 to 0.5 mv. This variation is because of phytochemicals, phenolic compounds, amino groups in various plants. As reported earlier *Hordeumvulgare*, *Amaranthusspinosus*, green tea, *Rosa sinensis*, Hibiscus, coffee, *Cassia fistula*, *Cinnamomumzeylanicum*, *Terminaliachebula* acted as reducing as well as capping agents engulfing different

phytochemicals (Herlekar *et al.*, 2014).

pH Analysis

The pH of the suspensions after adding precursor was found to be acidic which confirmed the formation of nZVI which was similar to the result when Neem extract was used as reducer (Pattanayak *et al.*, 2013). The pH reduced in all series of suspensions after adding ferric chloride solution (Table 2).

Table 2: pH and Color Change

Product	Extract			After Adding Extract to Precursor		
	pH	Color	Color Intensity	pH	Color	Color Intensity
Azolla	6.9	Orange	++	2.3	Brown	+++
BGA	7.4	Yellow	+	2.3	Brown	++

Result: + = Light color ++ = Dark color +++ = Very Dark color

UV-Vis Spectrometry of A-nZVI and BGA-nZVI

The immediate color change from orange to dark brown and yellow to brown in A-nZVI and BGA-nZVI respectively is due to surface plasma excitation vibrations in the Fe nanoparticles indicating the reduction of Fe^{3+} to Fe^0 . The appearance of absorption bands in range between 280 nm to 310 nm due to complexation of Fe salts and capping of Fe by phenolic compounds which were similar with characteristic UV-vis spectrometry results (Figure 2)

Figure 2: UV-Vis Spectrometry Band Distribution- a) A-nZVI b) BGA-nZVI

FTIR Analysis

The FTIR spectrum of A-nZVI and BGA-nZVI were observed in the range between 400 and 4000 cm^{-1} . This provided the direct evidence for formation of nZVI and confirms the presence of organic ligands on its surface. The presence of amines, aldehydes, polyphenols are responsible for reduction and capping of nZVI (Huang *et al.*, 2013). As shown in figure 3, in the range between wave number 3847-3202 cm^{-1} in nZVI corresponds to polyphenols, it proved the presence of phenolic compounds in nZVI which reduced Fe^{3+} to Fe^0 . The increased number of phenolic OH group enhanced the antioxidant capacity. The peak in the region between 1605-1612 cm^{-1} corresponds to resonance absorption of C=C in alkene groups belonging to non-saturated hydrocarbon family. Another peak observed in the range of 635-660 cm^{-1} with C-H bending indicates aromatic compounds of alkanes. The predominant peaks and stretches in both nZVI are more or less similar and they are in line with other green synthesized nZVI.

Figure 3: FT-IR Band Distribution-Blue and Green
Line Represents A-nZVI and BGA-nZVI Resp

Raman Spectroscopy

The formation of zero valent nZVI confirmed by series of characteristic peaks appeared in 190.6,322.6,569,692.2 and 1354.7 cm^{-1} in case of A-nZVI and 188,322.6,654.5,797.8 and 1593.9 cm^{-1} corresponding to the Fe-O symmetrical bend and Fe-O-Fe symmetrical and asymmetrical stretches of magnetite (Venkateswarlu *et al.*, 2015) are shown in Figure 4. The appearance of other characteristic peaks 1742.3 and 927.1 cm^{-1} in A-NZVI and 1742.5 and 927.1 cm^{-1} in BGA-nZVI corresponding to the stretching vibrations of phenolic compounds in green extracts and capping of nZVI.

Figure 4: Raman Spectroscopy – Band Distribution Representing
Compounds in a) A-nZVI b) BGA-nZVI

SEM-EDAX Analysis

The dipole-dipole interaction of isolated iron nanoparticles caused the aggregation of iron nanoparticles. The Figure 5 shows the size and morphology of A-nZVI and BGA-nZVI respectively. The surface irregular spherical particles indicate the chain structure which showed no difference from chemically synthesized nZVI (Ye Kuang *et al.*, 2013). The polydispersed spherical particles attributed to presence of polyphenols in Azolla and BGA extract coat on the surface of nZVI. The elemental composition of nZVI synthesized using Azolla and *Westiellopsis* were determined by EDAX analysis. The atomic percentage of iron (10.03% in A-nZVI and 8.67% in BGA-nZVI) dominated after carbon and oxygen percentages as indicated in Figure 5. c & d. Other mere percentage of Sodium and Magnesium are due to extract constituents.

Figure 5: SEM Images of a) A-nZVI, b) A-nZVI and EDAX Graphs of c) A-nZVI, d) A-nZVI Representing the Percentage of Fe in Both nZVI

CONCLUSIONS

There are plenty of flora in the biodiversity rich world which could be involved in production of green nanoparticles. *Azolla* and BGA are fast growing photosynthetic systems that are abundant and easily available. Hence the nano particles synthesized using these systems would be cost effective and eco-friendly when compared to other plant materials previously used such as green tea, Eucalyptus, Pomegranate, Banana peel, Mulberry, Cherry, Vine leaves. Green synthesized iron nano particles using *Azolla/Westiellopsis* with an average size of 80 nm has potential paybacks in environmental pollution abatements, biomedical and pharmaceutical fields.

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