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REMOVAL OF CRYSTAL VIOLET FROM WATER BY IRON OXIDE NANOPARTICLES LOADED GREEN ALGA

Upasna	(M.Sc. student), Department of Botany and Microbiology, Hemvati Nandan Bahuguna Garhwal University, S.R.T Campus, Badshahithaul, Uttarakhand-249199, India,
Abhinav Kamal Yadav	(M.Sc. student), Department of Botany and Microbiology, Hemvati Nandan Bahuguna Garhwal University, S.R.T Campus, Badshahithaul, Uttarakhand-249199, India
Rahul Bose	(Researcher), Department of Botany, University of Calcutta, 35 Ballygunge Circular Road, Kolkata, west Bengal-700019, India
Piya Roychoudhury*	(Assistant Professor), Department of Botany and Microbiology, Hemvati Nandan Bahuguna Garhwal University, S.R.T Campus, Badshahithaul, Uttarakhand-249199, India *Corresponding Author

ABSTRACT In this present communication, green filamentous alga, *Oedogonium capillare* has been collected from Badshahithaul, Uttarakhand, India. The green alga has been exploited to fabricate spindle shaped iron oxide nanoparticles (IONPs) against 10 mM ferric chloride solution. The biofabricated IONPs have been characterized by UV-Vis spectroscopy, Scanning electron microscopy (SEM) and Energy-dispersive X-ray analysis (EDAX). The changes of pigment content in *O. capillare* specially Chlorophyll a, Chlorophyll b and carotenoids during production of IONPs have been analyzed and it was observed the amount of all studied pigments decreased after 72h of reaction. The crystal violet (CV) removal capacity of metal free and IONPs loaded *O. capillare* has been checked by spectroscopic method. Control and IONPs loaded filaments of *O. capillare* showed 65% and 83% of CV removal within 20 min of exposure in aqueous CV solution respectively.

KEYWORDS : Algae, Biofabrication, Iron Oxide Nanoparticles, Crystal Violet

INTRODUCTION

Crystal Violet (CV) is very harmful dye if mishandled. This dye is commonly used in industries for staining purposes and laboratories for research work. Large amount of ingestion of this harmful dye may lead to toxicity and resulting in severe health effect viz. skin, eye and respiratory irritation [1].

Nowadays scientists are avoiding to use chemical processes to remove any kind of toxic substances from water as these processes are not always environment friendly and sometimes contaminate aquatic food chain with other undesired chemicals.

Bioreagents are commonly used for removal of dyes from water due to their effectiveness, cost efficiency, easy availability and biocompatibility.

Algae are most commonly used bioreagents for removal of any toxic chemical from water as they are mainly aquatic and they are having high surface area which help them to absorb a large amount of pollutants. Researchers used a number of algae like *Ulva lactuca* [2], *Chlamydomonas variabilis* [3], *Sargassum duplicatum* [4], *Chlamydomonas moewusii [5], Gracilaria* sp. [6], *Halamphora* sp. [7], *Nanofrustulum* sp. [8] for elimination of aqueous hazardous dye like methylene blue (MB).

Likewise, various cyanobacteria viz. *Nostoc sp., Microcystis aeruginosa, Oscillatoria geminate and algae like Chlorella vulgaris and Scenedesmus sp [9], Hormophysa triquetra [10] have also been evaluated for their CV adsorption capacity.*

Besides, algae are known as popular reducing agent for metal ion reduction and consequent biogenic nanoparticles production. Algae showed efficacy in synthesis of various metal nanoparticles like gold [11], silver [12], copper [13], zinc [14], titanium [15], palladium [16], platinum [17], iron [18]. In this study O. *capillare* has been used first time as bioreagent for phycogenic IONPs production.

Researchers have already exploited many green algae like *Chlorococcum sp* [19], *Scenedesmus ovalturnu* [20], *Spirogyra* sp. [21], *Aegagropila linnaei* [22]; red algae viz. *Pterocladia capillacea,* [23], *Kappaphycus* sp. [24]; brown algae such as *Dictyota dicotoma* [25], *Colpomenia sinuosa* [26], *Sargassum muticum* [27], and some blue green algae like *Arthrospira platensis, Leptolyngbya valderiana* [28], *Calothrix* sp. [29] for production of IONPs. However, no report is available regarding biogenic production of IONPs by *O. capillare*.

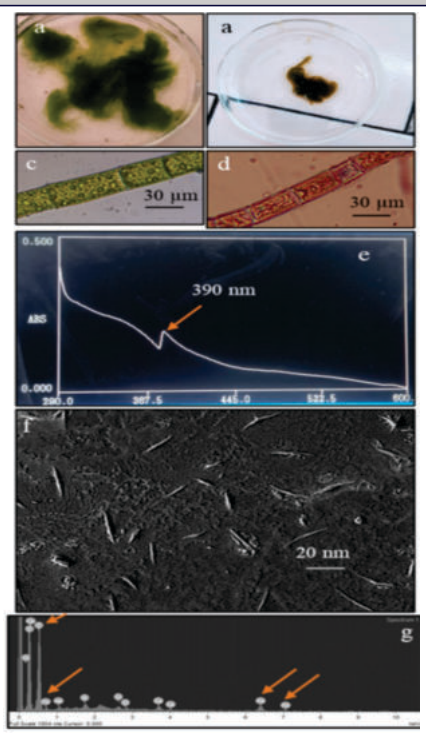

Fig 1: showing control biomass (a), IONPs loaded biomass (b), control filament (c) and IONPs loaded filaments of *O. capillare;* UV-VIS spectroscopy extracted IONPs (e), SEM image of synthesized spindle shaped IONPs (f); EDAX of IONPs (g)

IONPs are well known for their excellent photocatalytic activity. IONPs are potential for toxic dye degradation because of their small size, high surface area, magnetic property and they have already been utilized by researchers to remove MB [30] and CV [31] from water.

IONPs synthesized by *Chlorella vulgaris* showed 96.21% CV removal from 10 mg/L CV solution (pH- 8.98) within 90 min of reaction [31]. Amla seed mediated produced IONPs showed efficacy in aqueous MB removal [30]. In this study IONPs loaded and IONPs free *O. capillare*

have been exposed to aqueous solution of CV solution to understand the dye elimination capability of *O. capillare*. from water.

MATERIAL AND METHODS:

Collection, isolation, identification and cultivation of algal species

The algal sample, O. *capillare* was collected from Badshahithaul, Tehri-Garhwal, Uttarakhand, India (30.33^0 N, 78.4071^0 E). The collected sample was left overnight in betadine solution to remove surface contaminants and thoroughly washed with double distilled water. The filaments of O. *capillare* was isolated using compound microscope (Biocraft scientific systems PVT. LTD, Agra, Uttar Pradesh, India). The species of O. *capillare* was identified following the literature of Jitendra & Anand (2016) [32] and Marta & Beata (2015) [33]. The identified species was cultured in Bold basal medium (BBM) formulated by Nichols & Bold 1965 [34] for one month.

Biosynthesis of IONPs by *O. capillare*

Following the protocol of Roychoudhury et al. 2023 [18] the biosynthesis of IONPs by *O. capillare* was performed. The healthy growing biomass of *O. capillare* from exponential phase (400 mg FW) was exposed to 10 mM 400 mL ferric chloride ($FeCl_3$, MW:162.2, CDH) solution. The experimental set was maintained at room temperature in dark condition for 7 days.

Analysis of morphological changes and pigment content (Chlorophyll a, Chlorophyll b and carotenoids) in control and metal treated *O. capillare*

Associated morphological changes in metal treated *O. capillare* was documented on day 1-7 of reaction using compound microscope (Biocraft scientific systems PVT. LTD, Agra, Uttar Pradesh, India). Changes in pigment content especially Chlorophyll (Chl a & Chl b) and carotenoids in Fe^{3+} treated *O. capillare* was measured following protocol of Arnon 1949 [35] and Carreto 1977 [36] respectively.

The metal treated biomass was collected at 1, 24, 72 h of reaction. The collected biomass was crushed in 70% acetone and absorbance was measured at 663, 645 and 450 nm using UV-Vis spectrophotometer (2375 spectrophotometer, Electronics India, Haryana, India) for Chl a, Chl b and carotenoids respectively. All the experiments were performed in triplicates. A control set was maintained for each experiment.

Extraction, purification and characterization of IONPs:

The IONPs loaded *O. capillare* was sonicated with 7.5 mM sodium citrate ($Na_3C_6H_5O_7$, MW:258.07, CDH) solution using sonicator (Digital Ultrasonic sonicator, Model -LMUC-3, Labman, Tamil Nadu, India) for 30 mins and after that it was centrifuged (Refrigerated Centrifuge, Elite Research, Haryana, India) at 3000 rpm for 5 mins. The extracted solution was characterized by UV-Vis spectrophotometer (2375 spectrophotometer, Electronics India, Haryana, India), Scanning Electron Microscopy with EDAX (Zeiss, Germany). To understand the maximum absorbance of IONPs, extracted nanosuspension was subjected for wavelength (300-900 nm) scanning in UV-Vis spectroscopy.

The size, shape and surface morphology of the synthesized particles were analyzed by SEM. To prepare SEM sample few drops of extracted nanosuspension were dried on glass coverslip properly. Then the coverslip was coated with 10 nm thick gold layer. Then the coverslip was mounted on aluminium stub and observed at various magnifications. EDAX study was performed using the same sample and same instrument to confirm elemental composition of synthesized particles.

Removal of CV using control and IONPs loaded *O. capillare*

The control (400 mg) and IONPs loaded biomass (400 mg) was exposed to 10 ppm CV solution separately. The reaction mixture was kept under light in a continuous stirring condition using a magnetic stirrer (Elite magnetic stirrer, Haryana, India) for 1 h. The maximum absorbance of violet colored CV solution at 590 nm was recorded at 5, 10, 15, 20, 30, 60 min of reaction respectively. The experiment was performed in triplicates. A control set of colored dye without any bioreagent was also maintained in the same reaction conditions.

The amount of dye removal from water was calculated by this equation

% of dye removal= $(C_0 - C) \times 100/C_0$

[C_0 = initial conc. and C = final conc. of dye. Concentration of dye were determined by standard curve.]

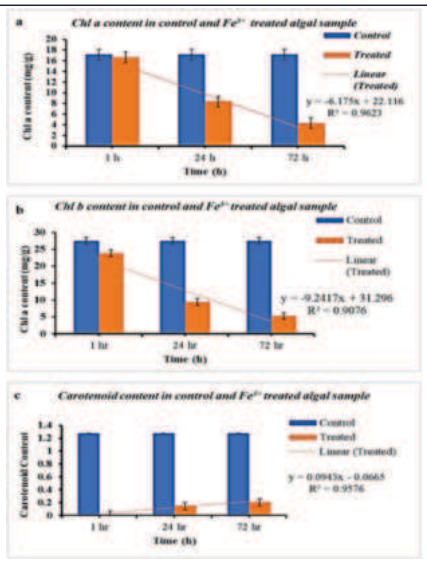

Fig 2: Showing variation in pigment content in control and Fe^{3+} treated *O. capillare*

RESULTS AND DISCUSSIONS:

In this communication collected algal sample has been identified as *O. capillare*. The filaments of *O. capillare* were unbranched with many celled and composed of tapered apical, vegetative and basal cells. Cap cells were observed with rings, nucleus and with reticulate chloroplasts. Each cell of filament was 37-56 µm wide and 30-102 µm long with 6, 8 or 10 discoid pyrenoids, which were 3-5µm in diameter. One nucleus was visible in the central part of the cell (6-10 µm). Initially, the capability of *O. capillare* in reduction of Fe^{3+} and subsequent fabrication of IONPs was confirmed by visual observation. The identified green colored filamentous alga turned brown in color after 3 days of exposure in 10 mM FeCl3 solution (Fig 1). After 7 days of reaction the external solution also turned dark brown in color. After 7 days of reaction no further color change was observed. As it is well known that IONPs are dark brown in color [18], therefore appearance of brown color in filaments and in external solution primarily confirmed fabrication of IONPs at intra and extra cellular level. The synthesis of IONPs was again confirmed by UV-Vis spectroscopy, SEM and EDAX study. In UV-Vis spectroscopy the extracted brown suspension showed maximum absorbance at 390 nm (Fig 1). The biogenic IONPs synthesized by higher plant showed maximum absorbance in UV-Vis spectroscopy at 390 nm [37]. So, it can be said that synthesized particles by *O. capillare* are pure IONPs. The purity of the particles another time confirmed by EDAX. In EDAX analysis the appearance of both peak of Fe and O from single particle, confirmed the production of IONPs (Fig 1). The shapes of the particles confirmed by SEM. The synthesized partciles are spindle shaped with 20-60 nm length and 10-30 nm width (Fig 1). The phycogenic IONPs by *Arthrospira platensis* [38], *Pseudostaurosira trainorii* [18] showed production of spindle shaped particles against 10 mM $Fecl_3$ solution. In this study also, it was observed *O. capillare* mediated particles are spindle shaped with variable size range.

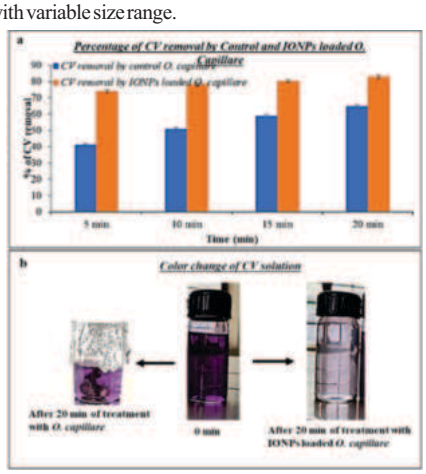

Fig 3: showing percentage of CV removal by metal free and metal loaded *O. capillare* with time (a), color change of CV solution due to treatment with control and IONPs loaded *O. capillare* (b).

During synthesis of IONPs, the green color of *O. capillare* gradually vanished. The disappearance of green color indicates loss of chlorophyll due to Fe^{3+} stress. The chl a and b content in Fe^{3+} treated *O. capillare* decreased with reaction time. In control sample chla content was 17.16 mg/ g and after 1, 24, 72 h of reaction it became 16.64, 8.35 and 4.29 mg/g respectively (Fig 2a). Similarly, the chl b content also decreased while increasing reaction time. Chl b content in *O. capillare* at 0 min of reaction was 27.48 mg/g and it reduced to 23.75, 9.4, 5.27 mg/g at 1, 24, 72 h of reaction respectively (Fig 2b). The carotenoid content decreased initially and then increased upto 72 h of reaction and then again decreased. Initial carotenoid content was 1.27 mg/g which was suddenly decreased after 1 h of reaction and the value became 0.016 mg/g. After that the carotenoid content increased little bit upto 72h of reaction. The carotenoid content in Fe treated *O. capillare* was 0. 14 and 0.20 mg/g at 3h, 72 h respectively (Fig 2c). The disappearance of pigment content in algae during nanoparticle synthesis due to metal stress is very common and observed by scientists in *Pseudostraurosira trainorii* [18] and *Nanofrustulum shiloi* [39] during IONPs and gold nanoparticles synthesis.

Both metal free and metal loaded *O. capillare fil*aments showed positive response in elimination of CV from water (Fig 3). Control biomass showed 65% of CV removal within 20 min of exposure whereas IONPs loaded filaments showed 83% of CV removal. Cyanobacteria and algae both are potential bioreagent for removal of toxic substances from water because of their high surface area and high adsorption capacity [9-10]. However, dye removal capacity of *O. capillare* increased due to presence of IONPs. IONPs have been identified by researchers as potential photocatalytic agent for dye degradation [30-31]. Here, the whole experiment has been performed under light and CV solution treated with IONPs loaded *O. capillare* showed maximum removal of CV from water. Therefore, it can be concluded that IONPs loaded *O. capillare* showed combined dye adsorption and degradation efficiency.

CONCLUSIONS:

It can be concluded that *O. capillare* is an efficient bioreagent for rapid biofabrication of spindle shaped IONPs. The filamentous green alga also showed efficacy in elimination of CV from water. The aquatic, easily available algae can be utilized in future for removal of any hazardous dyes from water due to their high adsorption capacity. Additionally, IONPs loaded *Oedogonium* filaments showed more efficiency in CV removal from water than control filaments as IONPs are well known for photodegradation of dyes. It can be hypothesized that presence of IONPs in algal biomass showed combined dye adsorption and degradation property which could be exploited in future for preparation of cost effective, biocompatible water-filter.

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