Evaluation of microalgal-based nanoparticles in the adsorption of heavy metals from wastewater

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Abstract

Bioremediation remains an eco-friendly and inexpensive method employed in getting rid of the toxic compounds like heavy metals present in wastewater. Different microorganisms are being used in either removing or reducing the concentration of heavy metals in wastewater but microalgae have a high affinity for heavy metals. In this study, nanoparticles from microalgae were evaluated for the potential to absorb lead and zinc from pharmaceutical effluent. Silver (AgNPs) and gold nanoparticles (AuNPs) were synthesized from both Nannochloropsis sp (NN) and Chlorella vulgaris (CV). The nanoparticles were introduced into a pharmaceutical effluent and the mixture was allowed to interact for 48 h after which the concentration of zinc and lead was determined using atomic absorption spectrophotometer. The AgNPs of Nannochloropsis sp (NN-AgNPs) and C. vulgaris (CV-AgNPs) had percentage reduction of 70.35 % and 74.62% respectively for zinc. Also gold nanoparticles of Nannochloropsis sp (NN-AuNPs) and C. vulgaris (CV-AuNPs) had percentage reduction of 60.32% and 66.83% respectively for zinc. The concentration of lead was reduced by CV-AgNPs (66.10 %), NN-AgNPs (68.86%), CV-AuNPs (57.41%), and NN-AuNPs (66.53 %). This study has established algal based nanoparticles as adsorbent with affinity for heavy metals and can be adopted for bioremediation of wastewater.

Keywords: Heavy metals, microalgae, nanoparticles, pharmaceutical effluent, adsorption
1.0 Introduction

All living beings all over the world depend on water for survival and for the various developmental processes which has the potential to enhance the economic status and growth of a community. Water is very important because it is highly required for industrial activities and by both rural and urban dwellers. The availability of water has therefore pose a major challenge in the economic development of any country. The contamination of the aquatic environ as a result of industrial activities has resulted in serious environmental concerns. Contaminated water contains complex materials especially heavy metals that have been implicated for their high toxicity, non-biodegradability and ability to bioaccumulate [1]. Heavy metals are released into the environment from various sources; these are agricultural activities, mining industries, metallurgical industries, pharmaceutical industries, chemical plants and domestic wastewater. Some metals (Pb, Zn, Cu, Hg) that are heavy in nature could pose a severe threat to human health when they can be accumulated biologically in the food chain [2]. Heavy metals are known to cause damages to the central nervous functions, mental, lungs, kidneys and other organs [3-5]. Moreover, heavy metals on the contrary do accumulate through the food chain because they are not degradable by microorganisms. Once they are released into the environment, they exert adverse effects on the receiving aquatic environment and other ecological receptors.

Heavy metals are responsible for causing several diseases [6]. It is therefore very important to either reduce or completely remove these unfriendly pollutants from the environment since they are toxic and cannot be degraded by microorganisms [7]. Various industrial activities has caused the issue of heavy metals discharged directly or indirectly into the water bodies thereby increasing the rate of waste pollution, especially in developing countries [8]. Wastewaters obtained from both petrochemical industries and refineries contain heavy metals; their toxic nature has presented them as threat to the environment and human. When emptied into water bodies, it imparts taste and odor to drinking water with even minute concentrations of their chlorinated derivatives and creates an oxygen demand in the river [9]. Several precipitation techniques that are used to remove these metals from industrial effluents include membrane filtration [10-13], ion exchange [14-16], and adsorption [17-20].

Algal biomass as an adsorbent in removing heavy metals from contaminated water bodies has attracted attention [21-23]. They are rich sources of several metabolites and they contain functional groups which take a great part in the attachment of heavy metals to the wall of the
algae [24-26]. Improving the adsorptive potential of algae in the removal of heavy metals can be achieved by employing algal nanoparticles which can enhance the adsorption of heavy metals through a larger surface area.

Chemical, physical and biological techniques are methods through which preparation of metal nanoparticles is usually carried out [8]. Chemical and physical approaches produce toxic materials and harmful to the environment, more expensive and required high pressure [9]. In contrast, cost-effective, sustainable, non-toxic, ability to use without massive equipment and chemical materials are advantages of biological method over former methods [27]. Kappaphycus alvarezi [28] and Diclyota indica [21] have been used as bioagent to synthesize nanoparticles which were used as adsorbent for heavy metal removal. Marine algae such as microalgae used as reducing agent in biosynthesizing nanoparticles can produce various metabolites which include carbonyl groups, phenolics, polysaccharides, proteins, amines, amides, flavonones, alkaloids, and other reducing-agents in the biological extracts [29].

In this study, nanoparticles were synthesized from both Nannochloropsis sp and Chlorella vulgaris and they were evaluated as adsorbents in removing heavy metals (zinc and lead) from wastewater.

2 Materials and methods

2.1 Maintenance and harvesting of microalgal cultures

Nannochloropsis sp and Chlorella vulgaris which were previously isolated from waste water in our laboratory was cultured in BG 11 medium. The algal biomass was harvested on day twenty one through centrifugation at 5000 rpm for 20 min. The biomass of the microalgae was washed with sterile distilled water to remove the culture medium that was present in the cell [30].

2.2 Silver and gold nanoparticles synthesis from microalgae

One gram of algal powder was suspended in 100 ml of distilled water, boiled at 100 °C for 20 min [30]. The cell-free extract of each microalgae was separated from the mixture in a centrifuge at 5000 rpm for 10 min. In order to synthesize both silver and gold nanoparticles from the extract, 90 ml each of 1 mM AgNO₃ and 1 mM gold chloride was added to 10 ml of the extract in separate bottles. The mixtures were incubated and the colour change was monitored until it stabilized [31].
2.3 Characterization of the nanoparticles

UV-visible spectra of samples were recorded using Ultraviolet-visible spectroscopy from 300-500 nm. Morphology of nanoparticles was characterized by both Scanning (SEM) and transmission Electron Microscopes (TEM) to obtain the shape and size of nanoparticles. Fourier-transform infrared spectroscopy (FTIR, Nicolet Avatar, Thermo, US) in the range of 4000-400 cm\(^{-1}\) was used to determine the presence of the various functional groups on the samples.

2.4 Bioadsorption of zinc and lead from pharmaceutical effluent

Twenty millilitres of each nanoparticle (CV-AgNPs, CV-AuNPs, NN-AgNPs, and NN-AuNPs) and the algal biomass of Chlorella vulgaris (CV) and Nannochloropsis sp (NN) was added to 200 ml of the industrial effluent. The mixture was subjected to manual agitation during the experiment. The ability of the nanoparticles and algal biomass to absorb both zinc and lead was determined after 12 h using Atomic Absorption Spectrophotometer. The percentage reduction in concentration of each metal by the nanoparticles was calculated:

\[
\text{Reduction in metal concentration} = \left(\frac{\text{Initial concentration} - \text{Final concentration}}{\text{Initial concentration}}\right) \times 100
\]

3 Results and Discussion

The colour change which is an indicator of nanosynthesis was observed after 20 min of adding silver nitrate to the cell-free extracts of both C. vulgaris and Nannochloropsis sp. There was colour change from pale green to brown which is indicative of silver nanoparticles. The surface plasmon resonance for the AgNPs was obtained with broad absorbance at a wavelength of 426 nm for C. vulgaris (Figure 1a) and 430 nm for Nannochloropsis sp (Figure 1b). These absorption spectra are similar to those reported for AgNPs of Turbinaria conoides, a brown marine alga which had a broad spectrum at 420 nm [32]. The synthesis of AgNPs from both C. vulgaris and C. calcitrans with a strong Plasmon vibration at 436 nm and 420 nm respectively has been reported [33]. Sudha et al. [31] reported a broad peak of 420 nm from AgNPs synthesized from Scenedesmus abundans.
Figure 1. UV-visible spectra of silver nanoparticles synthesized from A (C. vulgaris) and B (Nannochloropsis sp) with colour change in reaction bottles after incubation.

The gold nanoparticles of both C. vulgaris and Nannochloropsis sp was synthesized with change in colour after 2 h of incubating the extract of each microalgae with gold chloride. A purple colour was seen in both bottles and synthesis of gold chloride was further confirmed with UV-visible spectra at 520 nm and 535 nm for C. vulgaris and Nannochloropsis sp respectively (Figures 2a and b). This result is in agreement with previous reports on synthesis of gold nanoparticles [34] where AuNPs was synthesized using Camellia sinensis and Juniperus communis and had absorbance spectra within ranges around 530-540 nm.

The micrographs for silver and gold nanoparticles of C. vulgaris and Nannochloropsis were taken using both scanning electron microscope for silver nanoparticles (CV-AgNPs and NN-AgNPs) and transmission electron microscopy for gold nanoparticles (CV-AuNPs and NN-AuNPs) in order to obtain the shape and size of each nanoparticles. The CV-AgNPs and NN-AgNPs were polydispersed and spherical in shape with size ranging from 27 to 90 nm and 41 to 88 nm respectively (Figures 3a and b). Also CV-AuNPs and NN-AuNPs had spherical polydispersed particles with particle range from 7 to 20 nm and 7 to 26 nm (Figures 3c and d).
Figure 2. UV-visible spectra of gold nanoparticles synthesized from A (C. vulgaris) and B (Nannochloropsis sp) with colour change in reaction bottles after incubation.

The micrographs for silver and gold nanoparticles of C. vulgaris and Nannochloropsis was taken using both scanning electron microscope for silver nanoparticles (CV-AgNPs and NN-AgNPs) and transmission electron microscopy for gold nanoparticles (CV-AuNPs and NN-AuNPs) in order to obtain the shape and size of the nanoparticles. The CV-AgNPs and NN-AgNPs were polydispersed and spherical in shape with size ranging from 27 to 90 nm and 41 to 88 nm respectively (Fig. 3a and b). Also CV-AuNPs and NN-AuNPs had spherical polydispersed particles with particle range from 7 to 20 nm and 7 to 26 nm (Fig 3c and d).

Figure 3. Electron micrographs of A (CV-AgNPs), B (NN-AgNPs), C(CV-AuNPs) and D (NN-AuNPs).
The EDX of the nanoparticles revealed the different elemental composition. The silver nanoparticles had silver as the element with the highest weight composition of 47.82 % for CV-AgNPs and 54 % for NN-AuNPs with a strong signal strength at 3 keV (Figures 4a and b). The weight percentage of gold in the gold nanoparticles was higher than that of the other elements present: CV-AuNPs had gold composition of 96.62%, while 79 % gold was present in NN-AuNPs (Figures 4c and d).

The FTIR spectra showed absorbance for CV-AgNPs at 3272 (O-H of alcohol), 2103 (broad C≡N stretching vibration of amine), and 1636 cm\(^{-1}\) (C=C of olefin) (Figure 5a), CV-AuNPs at 3295 (O-H of alcohol), 2103 (broad C≡N stretching vibration of amine), and 1636 cm\(^{-1}\) (C=C of olefin) (Figure 5b), NN-AgNPs at 3294 (O-H of alcohol), 2104 (broad C≡N stretching vibration of amine), and 1638 cm\(^{-1}\) (C=C of olefin) (Figure 5c), and NN-AuNPs at

![Figure 4](image-url)
3280 (O-H of alcohol), 2106 (broad C≡N stretching vibration of amine), and 1636 cm\(^{-1}\) (C═C of olefin) (Figure 5d). The function groups represented on each nanoparticles include O-H, C≡N, and C═C. FTIR analysis showed the involvement of hydroxyl and unsaturated hydrocarbons in the reduction, stabilization and capping of the metal nanoparticles [35-36].

![Figure 5](image)

**Figure 5.** FTIR spectra of (A) CV-AgNPs, (B) NN-AgNPs, (C) CV-AuNPs, (D) NN-AuNPs

The silver and gold nanoparticles of *C. vulgaris* and *Nannochloropsis* sp were introduced into pharmaceutical effluent and the concentration of each metal was obtained after 12 h of adsorption. The percentage reduction in concentration of zinc by CV-AgNPs, NN-AgNPs, CV and NN were 74.62, 70.35, 46.98 and 48.49 % (Figure 6A) while 66.83, 60.32, 46.98 and 48.49 % were achieved by CV-AuNPs, NN-AuNPs, CV and NN respectively (Figure 6B). Similarly, the adsorption potential of CV-AgNPs (66.10 %), NN-AgNPs (68.86%), CV (30.29%) and NN (55.51%) for the adsorption of lead were obtained (Figure 6C) while CV-AuNPs (57.41%), NN-AgNPs (66.53 %), CV (30.29%) and NN (55.51%) were also recorded (Figure 6D).
The two microalgae (CV and NN) and their nanoparticles were efficient in adsorbing both zinc and gold from the effluent, however, the nanoparticles outperformed the algal biomass with nanoparticles of *C. vulgaris* (74.62 and 66.83 %) having the maximum efficiency for zinc and *Nannochlopsis* sp (68.86 and 66.53%) with the maximum efficiency for lead. The improved performance of both silver and gold nanoparticles compared to their biomass is as a result of the increased surface area of reaction which consequentially increased the binding sites available for the metals to adhere to the cells [37]. The application of both microalgae and their nanoparticles as adsorbent for removing heavy metals from effluent is a prospective technology which is cost effective and environmental friendly. Different strains of microalgae and nanoparticles from different metal oxides have been documented for their activities in reducing the concentration of heavy metals present in effluent [6, 37].

4 Conclusion

Heavy metal remains a major component of industrial effluent which puts both man and its environment in great risk due to their persistence, non-biogeradability and ability to bioaccumulate. Various technologies are being adopted to either remove or reduce the heavy metal concentration to become less toxic. Two microalgae and their nanoparticles were employed in this work as bioadsorbent for the removal of both zinc and lead. The nanoparticles out performed the microalgal biomass as adsorbent owing to increased surface of reaction which led to increased metal adsorption.
Figure 6. Percentage reduction in concentration of lead by (A) CV-AgNPs and NN-AgNPs, (B) CV-AuNPs and NN-AuNPs; zinc by (C) CV-AuNPs and NN-AuNPs, (D) CV-AgNPs and NN-AgNPs.

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