Biosynthesis of Au(0) from Au(III) via biosorption and bioreduction using brown marine alga Turbinaria conoides

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In the present study, the potential of a new brown marine alga, Turbinaria conoides, in biosorption and subsequent bioreduction of Au(III) was explored. The biosorption process was found to be rapid and completed within 60 min of contact. Also, the solution pH strongly affected Au(III) biosorption by T. conoides, with maximum uptake of 34.5 mg/g being observed at pH 2 according to the Langmuir model. Biosorption mechanism was proposed to involve electrostatic interactions between gold anions and algal functional groups, which implies that when virgin T. conoides was exposed to gold solution, AuCl₄⁻ anion binds to positively charged functional groups, such as amino groups (NH₂), on the algal surface. After 60 min, T. conoides reduced Au(III) to gold nanoparticles. Hydroxyl groups present in the brown algal polysaccharides were involved in the bioreduction of Au(III) to Au(0). The field emission scanning electron micrographs showed uniformly scattered nanoparticles with sporadic aggregation on the surface of T. conoides. XRD diffraction patterns of gold-loaded T. conoides also confirmed that the Au(III) bound on the cell wall of the biomass had been reduced to Au(0). The UV–vis spectra results indicated that the reaction solution had an absorption maximum at about 540 nm attributable to the surface plasmon resonance band of the gold nanoparticles.

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1. Introduction

Gold is a precious metal, generally valued by mankind throughout history. The unique chemical and physical properties offered by this precious metal are increasingly being sought for use in a growing number of industrial and medical applications. One area that has seen significant growth is the use of gold in electronics. Despite attempts to replace gold in the electronics industry, its demand has continued to grow. The effluents from these scrap industries often contain noble metals such as gold. Due to high market prices, recovery of gold from these effluents is of great interest. Some traditional methods can be used for gold recovery and these include cyanide leaching, precipitation and filtration, electrochemical treatments, reverse osmosis, ion exchange resins and evaporations [1].

In recent years, biosorption has been reported to be an effective and economical process for the removal and recovery of different metals [2,3]. It is based on the passive retention of metals by active sites or functional groups present in the biomass. It has numerous distinct advantages over other conventional methods including low-cost, environmental-benign, operation over wide range of pH and temperature and biomass regeneration, etc. Among different types of biosorbents, marine algae (seaweeds) have distinct advantages due to their high metal uptake capacity, low cost and macroscopic structure [4]. Among divisions of marine algae, brown algae were found to be an excellent biosorbent for different heavy metal ions. Recently, brown algae also performed well in sequestration of few precious metals [1,5].

Earlier researchers identified that some microalgae were able to isolate gold from aqueous solutions and also observed possible reduction of Au(III) to Au(0) [6]. In recent years, biosorption of gold using higher plant biomass materials has started appearing in the literature [7,8]. Brown seaweeds such as Sargassum biomass showed a similar high affinity towards gold ions in aqueous solutions, with the capability of producing colloidal Au(0) [5,9]. Consequently, there has been considerable interest in developing biosynthesis methods for the preparation of gold nanoparticles as an alternative to physical and chemical methods. Literature review of previous studies revealed that few articles were published on gold uptake and biosorption compared to the great number of articles on biosorption for other metals [10] and none of the studies used the alga Turbinaria conoides. Also, most of the biosynthesis studies on gold nanoparticles [8,9] focused on bioreduction phase only and ignored the important biosorption phase of the process.

The objective of the present study is to explore the biosynthesis of Au(0) from Au(III) using a new brown marine alga T. conoides using a combination of biosorption and subsequent bioreduction
processes. *T. conoides* is a very common brown alga found throughout the Pacific and Indian Ocean and is known for its rigidity.

2. Materials and methods

2.1. Preparation of seaweed and stock gold solution

*T. conoides*, a brown marine alga, was collected from the beaches of Mandapam region (Tamilnadu, India). The biomass was extensively washed with deionized (DI) water, until the pH of wash solution was equal to DI water, and subsequently sun-dried. The dried biomass was then ground in a blender then sieved to prepare particles in the average size of 0.75 mm. Stock solutions containing 100 mg/L of initial gold concentration were prepared from hydrochloroauric acid (HAuCl₄), obtained as analytical grade from Sigma–Aldrich. The pH of gold solutions was adjusted using 0.1 M HCl and 0.1 M NaOH.

2.2. Experimental procedure

The rate of Au biosorption (effect of pH, sorption isotherm and kinetics) and its subsequent bioreduction were evaluated using batch experiments. The sorption pH edge experiments were performed with 0.02 g of dry *T. conoides* biomass and 10 mL of 100 mg/L gold solution in 15 mL Falcon bottles (high density polyethylene). The bottles were agitated in a rotary shaker at 200 rpm until biosorption equilibrium was reached. During agitation, the pH of the solution was controlled by adding 0.1 M HCl or NaOH. After 60 min, the reaction mixture was filtered through a 0.45 μm PTFE membrane filter and analyzed for Au concentration using inductively coupled plasma-atomic emission spectrometer (ICP-AES; PerkinElmer Optima 3000 DV). Biosorption isotherms were conducted in a similar manner except that the solution pH was fixed and gold concentration was varied from 10 to 100 mg/L. Kinetic experiments were also performed in a similar manner except that the samples were withdrawn at regular intervals to study the equilibrium attainment.

To confirm bioreduction of gold, biosorption experiments were extended beyond equilibrium time and samples of filtrate were collected at different time intervals. The samples at different time intervals over a period of 48 h were analyzed in UV–vis spectrophotometer (Hitachi U2800, Japan). For the analysis, 0.1 mL of the sample was taken in a cuvette and was diluted to 2 mL with deionized water. The UV–visible spectra of the resulting diluents were collected at different time intervals. The samples at different time intervals were also performed in a similar manner except that the samples were withdrawn at regular intervals to study the equilibrium attainment.

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2.3. Analytical procedure

After recovery, the biomass that had reacted with gold solutions was dried and stored for later observation by scanning electron microscopy (SEM), field emission scanning electron microscopy (FESEM), energy dispersive X-ray spectroscopy (EDX) and X-ray powder diffraction (XRD). For microscopy analysis, dried samples of virgin and gold-exposed *T. conoides* were dried, coated with thin layer of platinum and analyzed by SEM equipped with EDX (JEOL, JSM-5600 LV) and FESEM (JEOL, JSM-6700F). FE-SEM observations were performed on a JEOL JSM-6700F instrument (Japan). XRD was conducted in Shimaizu LabX XRD-6000.

2.4. Biosorption isotherm and kinetic models

Two equilibrium models were used to fit the Au(III) biosorption isotherm experimental data, as follows:

Langmuir model: $$Q = \frac{Q_{\text{max}} bC_f}{1 + bC_f}$$

Freundlich model: $$Q = K_{F}C_{f}^{1/n}$$

where $Q_{\text{max}}$ is the maximum Au(III) uptake (mg/g), $b$ the Langmuir equilibrium constant (L/mg), $K_F$ the Freundlich constant (mg/g) $(L/mg)^{1/n}$ and $n$ the Freundlich exponent.

The experimental biosorption kinetic data were modeled using the pseudo-first and second order kinetics, which can be expressed in their non-linear forms, as follows:

Pseudo-first order model: $$Q_t = Q_{e} - k_1 t$$

Pseudo-second order model: $$Q_t = \frac{Q_{e}^2 b t}{1 + bQ_t}$$

where $Q_p$ is the amount of Au sorbed at equilibrium (mg/g), $Q_t$ the amount of Au sorbed at time $t$ (mg/g), $k_1$ the pseudo-first order rate constant (min⁻¹) and $k_2$ the pseudo-second order rate constant (g/mg min). All the model parameters were evaluated by non-linear regression using Sigma Plot (version 4.0, SPSS, USA) software. The average percentage error between the experimental and predicted values is calculated using:

$$\varepsilon(\%) = \frac{\sum_{i=1}^{n} (Q_{\exp} - Q_{\text{cal}}/Q_{\exp}) \times 100}{N}$$

where $Q_{\exp}$ and $Q_{\text{cal}}$ represent experimental and calculated Au uptake values, respectively, and $N$ is the number of measurements. All experiments were done in duplicates and the data presented are the average values of two experiments.

3. Results and discussion

Biosorption of Au(III) from aqueous solution by the brown seaweed, *T. conoides* was strongly dependent upon of solution pH (Fig. 1). Experiments were performed under a wide pH range of 2–10 at a fixed initial Au concentration of 100 mg/L. The results indicated that relatively strong acidic pH ranges enhanced biosorption potential of *T. conoides* towards Au(III) ions. It is worth noting that after 60 min of agitation, the color of solution changed to purple indicating the possible bioreduction of gold [11]. Possible mechanisms of gold biosorption and subsequent bioreduction are explained below.

Brown algae mainly consist of alginic acid, which constitutes 10–40% of the dry weight of algae [4]. The alginic acids are linear carboxylated copolymers constituted by different proportions of 1,4-linked β-D-mannuronic acid (M-block) and α-L-guluronic acid (G-block). Among the different functional groups, carboxyl groups are abundant. Several investigators reported that these functional...
groups play an important role in metal biosorption at different pH conditions [12,13]. Other functional groups present in the brown seaweed cell wall are: amino, sulfonate, phosphonate and hydroxyl groups. In solution, gold exists as $\text{AuCl}_4^{-}$ and therefore, the first stage involves biosorption of $\text{AuCl}_4^{-}$ anion to positively charged functional groups, such as amino groups ($\text{NH}_2$), on the algal surface. Results indicated that the biosorption potential of $T. \text{conoides}$ decreased sharply when the proton concentration in solution was lowered (Fig. 1). The reason for this trend might be that $\text{AuCl}_4^{-}$ has a lower solubility and is thus biosorbable at a lower pH [14]. On the other hand, the lower pH leads to higher concentrations of protons, which neutralize the overall negative charge of $T. \text{conoides}$. This change in turn makes binding groups carry more positive charges at a low pH and strengthens $\text{AuCl}_4^{-}$ biosorption by means of electrostatic attractions. To be precise, the interaction between amine groups (RN) and gold anions ($A^{-}$) is electrostatic, involving ion pairing (RN-$\text{H}^{+}\cdot\cdot\cdot A^{-}$).

The scanning electron micrograph (SEM) of virgin $T. \text{conoides}$ revealed important information on the surface morphology (Figure AM1 in Supplementary Data). Surface protuberance and microstructures were observed, which is thought to be due to calcium and other salt crystalloid deposition. After Au binding, the surface of $T. \text{conoides}$ appeared flattened in comparison to the raw sample (Figure AM2 in Supplementary Data). No further significant morphological changes were apparent in the SEM images. In EDX analysis, strong Ca peaks were observed in virgin $T. \text{conoides}$ (Fig. 2a). Peaks for Na, K, and Mg were also recorded in EDX spectra. On observing the EDX spectra of Au-exposed $T. \text{conoides}$ (Fig. 2b), it became very clear that Na, K and Mg peaks were absent and new peaks of biosorbed Au were present. This supports our earlier explanation that when virgin $T. \text{conoides}$ is exposed to Au solution at acidic pH, $\text{H}^{+}$ ions may replace some of the alkali and alkaline earth metals naturally present in the cell wall through ion-exchange mechanism.

An isotherm pertaining to the biosorption of Au onto $T. \text{conoides}$ at pH 2 is presented in Fig. 3. A critical analysis of the shape of the isotherm revealed that it was favorable and can be classified as “L-shaped” without a strict plateau. This means that the ratio between the Au concentration in the solution and that sorbed onto
the biomass decreased with increase in the Au concentration, providing a concave curve without a strict plateau. The experimental Au isotherm was tested using the Langmuir and Freundlich models. Initially, the Langmuir model [15] was applied to the present system, with the assumptions that adsorption sites are identical, each site retains one molecule of the given compound and all sites are energetically and sterically independent of the adsorbed quantity. Although these assumptions are not completely valid for the biosorption system, the model was able to describe the isotherm data with high \( r^2 \) (0.994) and low % error (3.67%) values. A maximum gold biosorption capacity of 34.5 mg/g was observed at pH 2. The Langmuir affinity constant \( b \), which characterizes the initial slope of the isotherm, was recorded as 0.61 L/mg. A high \( Q_{max} \) and steep initial isotherm slope (i.e., high \( b \)) are desirable for a good biosorbent. The Freundlich isotherm [16] was originally empirical in nature, but was later interpreted as sorption to heterogeneous surfaces or surfaces supporting sites of varied affinities. It is assumed that the stronger binding sites are occupied first and that the binding strength decreases with the increasing degree of site occupation. The Freundlich constants \((K_F \) and \( n)\) were recorded as 14.1 L/g and 3.61, respectively. High K_F and 1/n values indicate that the binding capacity reached its highest value, and the affinity between the biosorbent and Au was also high. Compared to the Langmuir model, the Freundlich model resulted in a slightly inferior description of the experimental Au isotherm, as relatively low correlation coefficient (0.984) and high % error value (8.3%) were observed.

As discussed earlier, the biosorption process lasted for about 1 h and after that bioreduction (appearance of purple color) apparently started. Thus, kinetic studies were focused to determine the rate of biosorption. Fig. 4 shows the time profile of gold biosorption onto \( T. \ conoides \). These results revealed that it was a rapid process with more than 97% of total gold biosorption occurred within 30 min, followed by relatively slow equilibrium attainment in 60 min. Among the two kinetic models examined for the present data, pseudo-first order model better predicted the experimental equilibrium Au uptake, which was 34.0 mg/g compared to predicted uptake of 34.7 mg/g. This agreement also resulted in a high correlation coefficient (0.998) and low % error (0.13%) values. The first-order rate constant was determined as 0.19 min\(^{-1}\). In contrast, the pseudo-second order model over-predicted the uptake values, with the model predicting uptake as 37.9 mg/g. Also, the model resulted in a relatively less correlation coefficient (0.994) and high % error (0.97%) values, with the second-order rate constant of 0.0072 g/mg min.

The second stage, which started 1 h after \( T. \ conoides \) contacted with HAuCl\(_4\) solution, involved reduction of Au(III) to Au(0) on the surface of \( T. \ conoides \). During the second stage (bioreduction), the color of solution slowly became purple and its intensity increased as the time progressed. Many researchers associated this color change to gold reduction and the presence of gold nanoparticles [1,7,14]. The mechanism of Au(III) reduction most likely involved oxidation of biomass groups such as hydroxyl [17]. Hydroxyl groups (OH) are very abundant in polysaccharides of the brown algal cell wall. Mata et al. [1] confirmed the participation of hydroxyl groups during reduction of Au(III) to Au(0) by Fucus vesiculosus. Algal pigments such as fucoxanthins, a kind of carotenoid rich in hydroxyl groups, could also have participated in the gold reduction. These pigments have good reductive properties while AuCl\(_4^-\) is a very strong oxidizing agent and could thus aid in reduction of Au(III) to Au(0). A sharp decrease in pH during the experimental run also indicated that protons were released during gold reduction, which is in agreement with the reaction proposed by Kuyucak and Volesky [17]:

\[
\text{AuCl}_4^- + 3\text{R-OH} \rightarrow \text{Au(O)} + 3\text{R=O} + 3\text{H}^+ + 4\text{Cl}^-
\]

(6)

Reaction (6) indicates that reduction of Au(III) to Au(0) occurs through oxidation of hydroxyl to carbonyl groups.

The presence of elemental gold was also confirmed by FESEM (Fig. 5). Uniformly scattered nanoparticles with sporadic aggre-gation were found at the surface of \( T. \ conoides \). An advantage of biosynthesis of gold nanoparticles using dead algal biomass is that they can be obtained at extracellular level and in large quantities making its purification easier as compared to other methods where they are entrapped inside a polymer or living biomass [1,18,19]. On the surface of the biomaterial, gold nanoparticles ranging from 20 to 80 nm were observed. The gold nanoparticles that are found adhered to the surface of the biomaterial can be recovered through sonication as described by Sathishkumar et al. [20]. The UV–vis spectra (Fig. 6) results indicated that the reaction solution had an absorption maximum at about 540 nm, which is attributable to the surface plasmon resonance (SPR) band of the gold nanoparticles. An UV–vis spectrum is one of the important techniques that can be used to ascertain the formation of metal nanoparticles, provided surface plasmon resonance exists for the metal. It is interesting to note that the colorless/straw color solution transformed to reddish purple after 60 min of reaction, which indicates rapid bioreduction of gold. The spectra were continuously monitored and the results indicated the intensity increased as the time progressed and reached constant value around 48 h.
Electrostatic interaction. The gold anions AuCl₄⁻ biosorption of Au(III) ions onto the cell wall of algal biomass via bioreduction. The biosorption phase, which lasted for 60 min, resulted in T. conoides biomass.

4. Conclusions

Metallic gold was also detected by XRD analysis of Au-exposed T. conoides (Fig. 7). The gold nanoparticles formed on the surface of T. conoides have shown clear peaks of cubic phases (ICPDS No. 03-0921) at 38.21 (1 1 1), 44.43 (2 0 0), 64.64 (2 2 0) and 77.21 (3 1 1). The slight shift in the peak positions may be due to the presence of some strain in the crystal structure which is a characteristic of nanocrystallites synthesized through bio-route [20,21]. The bottom area of the peaks is broad, which indirectly represents the smaller size of the nanoparticles. Thus the XRD pattern provides strong evidence in favor of the UV–vis spectra and FESEM images for the presence of gold nanocrystals.

4. Conclusions

The interaction of the new brown seaweed T. conoides with Au(III) ions was studied. The results suggest that there are two phases in the interaction between the seaweed and Au(III), a fast biosorption phase followed by a relatively slow bioreduction process. The biosorption phase, which lasted for 60 min, resulted in biosorption of Au(III) ions onto the cell wall of algal biomass via electrostatic interaction. The gold anions AuCl₄⁻ anion binds to positively charged functional groups, such as amino groups (NH₂), on the algal surface. Among two isotherm models, Langmuir model better described gold isotherm data, with a maximum uptake of 34.5 mg/g at pH 2. The bioreduction phase involved reduction of Au(III) to Au(0), thereby producing gold nanoparticles. The formation of gold nanoparticles was confirmed by FESEM, XRD and UV-spectroscopy. Thus, the biosynthesis of gold nanoparticles by T. conoides is effective, rapid, nutrient independent and does not use toxic chemicals. This biosynthesis method has the potential to be an alternative process for gold recovery from dilute hydrometallurgical solutions for the production of reduced gold nanoparticles.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.cej.2010.12.027.

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