Growth kinetics of the algae Scenedesmus sp., and Chlorella sp. and its nanoparticle synthesis, collected from southern coasts of Chennai, India

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ABSTRACT

The microalgal samples were collected from rock ponds in kovalam and isolated using plating techniques and identified in Krishnamurthy Institute of Algology, Chennai, Tamil Nadu, India. Algal cells were grown in Bold’s Basal Medium (BBM) and cultured for 14 days in 16:8 h in 24°C, after 14 days algal cells were pelleted by centrifugation. The growth Kinetics of the algae Scenedesmus sp., and Chlorella sp. were studied and the generation time was calculated. The bioreduction of Ag⁺ ions in solution was monitored using UV-visible spectroscopy from zero reading and double distilled water was used as blank. The samples were withdrawn at various time intervals and the absorbance was measured. The silver nanoparticles were synthesized using extracts of algal samples was confirmed by color changes and was characterized by UV-visible spectrophotometer; the UV-visible spectra showed a broad peak located at 450nm for silver nanoparticles. The nanoparticles were also characterized by Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD) and Fourier Transmission Infra-Red Spectroscopy (FTIR) analysis.

Key words: Scenedesmus sp., Chlorella sp., Silver Nanoparticles, Growth Kinetics

INTRODUCTION

Algae are an extremely diverse group of organisms that make up the lower phylogenetic echelons of the plant kingdom. A precise definition of this group is elusive; they share many obvious characteristics with higher (land) plants, whereas their distinguishing features from other plant groups are varied and more subtle [2]. Most of the algae are photosynthetic like higher plants. Algae perform roughly 50% of the photosynthesis on this planet and thus are instrumental in supporting the biosphere.

The “green algae” is the most diverse group of algae, with more than 7000 species growing in a variety of habitats. The “green algae” is a paraphyletic group because it excludes the Plantae. Like the plants, the green algae contain two forms of chlorophyll, which they use to capture light energy to fuel the manufacture of sugars, but unlike plants they are primarily aquatic. They each have their own unique qualities. Chlorella is a major component of phytoplankton, which is a very small free floating aquatic plant found in plankton. Chlorella is a popular food supplement, especially in Japan and is sold as a nutritional supplement in the United States and Canada. There are several species of Chlorella, but the most commonly found in supplements are Chlorella vulgaris and Chlorella pyrenoidosa. Scenedesmus is a microalgae genus commonly used in heavy metal removal experiments. It has proven removal capacity for U6+ [11], Cu2+, Cd2+ [8] and Zn2+ [1; 9; 3].

A Nanomaterial may be defined as any material (insulator, conductor or semiconductor), which has been controllably synthesized on the size range of roughly 1 to 100 nm. At this size and dimensional range, essentially
any material will exhibit different properties from those it would as an atomic cluster or as the larger bulk materials. The Ag NPs (Silver Nanoparticles) are reported to be nontoxic to human and most effective against bacteria, viruses, and other eukaryotic microorganisms at very low concentration and without any side effects [5]. Only one gram of AgNPs is known to impart antibacterial properties to hundreds of square meters of substrate material [6]. Some of these procedures are involved using toxic solvents or high-energy consumption. This leads to a growing awareness of the need for developing clean, nontoxic and environmentally friendly procedures, [7] 

Production of metallic nanoparticles can be achieved through chemical, physical or biological methods. Now-a-days biological synthesis of metallic nanoparticles is gaining importance, as it is reliable and eco-friendly. There is an increasing need to develop green synthesis method must consisting of environmentally acceptable solvent system, eco-friendly reducing and capping agents [10], high-yield, low cost, non-toxic and environmentally benign procedures [6], for synthesis of metallic silver nanoparticles.

**MATERIALS AND METHODS**

**Culture Collection and Isolation:**
The cultures samples were collected from the southern coast of Tamil Nadu, India and the cultures *Scenedesmus* sp. and *Chlorella* sp. isolated using the plating techniques; it was made axenic by antibiotic treatment following the method of Droop [4].

**Growth conditions:**
Bold Basal Medium (BBM) was used for culturing of the algae. Cultures were grown at 24 ± 1°C in a thermostatically controlled room illuminated with cool white fluorescent tubes (Philips 40 W) providing an irradiance of 50 µE/m²/s in a 12h: 12h light/dark regime.

**Growth kinetics:**
Photometric readings were taken at 690 nm for 30 days using UV – Spectrophotometer for growth curve measurement. Growth kinetics was measured for the algae *Scenedesmus* sp. *chorella* sp. using the formula: 

\[
G.T = \frac{\log \text{ of } OD \text{ fi} - \log \text{ of } OD \text{ Di} \times 3.322}{T}
\]

**Mass culture preparation:**
The 2.5 litres of media were prepared in Erlenmeyer flasks, BBM (Bold Basal Medium) in Erlenmeyer flasks.

**Nanoparticle Synthesis:**
Algal cells were grown in suitable medium. After 14 days medium was removed by centrifugation at 10,000 rpm for 15 minutes. Pellet was used in the experiment; 2 ml of pellet was added to 12.5ml of AgNO₃ solution and made up to 50ml with double distilled water. The bioreduction of Ag⁺ ions in solution was monitored using UV-Vis spectroscopy. The samples were withdrawn at various time intervals and the absorbance was measured at 450 nm OD.

**Characterization of Silver nanoparticles:**
The reduction of pure Ag⁺ ions and biosynthesized silver nanoparticles were first analyzed by using UV- vis spectrophotometer. The silver nanoparticles were characterized using FT-IR, Scanning Electron Microscopic (SEM) analysis was done using ZIEES SEM instrument and the structure of synthesized nanoparticles was synthesized by X-Ray Diffractometer.
RESULTS

Growth Curve:

The growth curve was calculated for *Scenedesmus* sp. and *Chlorella* sp. using HITACHI U-2900 Spectrophotometer for 30 days. (Fig. 1)

Mass Cultivation:

A. Mass culture of *Scenedesmus* sp.  B. Mass culture of *Chlorella* sp.

The mass culturing of the cultures in Erlenmeyer flasks were shown in this Fig. 2

Nanoparticle Synthesis:

A. Flasks showing color change for *Scenedesmus* sp.  B. Flasks showing color change for *Chlorella* sp.

The color change to brownish is shown in Fig. 3 which indicates the formation of silver Nanoparticles.
FT-IR:

A. Spectrum for *Scenedesmus* sp.

![FT-IR Spectrum for Scenedesmus sp.](image1)

B. Spectrum for *Chlorella* sp.

![FT-IR Spectrum for Chlorella sp.](image2)

The spectrum for FT-IR is shown in the Fig. 4 which shows the presence of Silver Nano particles.

**Table 1. Shows the spectrum for *Scenedesmus* sp.**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Wavenumber (cm(^{-1}))</th>
<th>Molecular Motion</th>
<th>Functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3419.5</td>
<td>N-H Stretch</td>
<td>Hetero cyclic Amine</td>
</tr>
<tr>
<td>2</td>
<td>2854.7</td>
<td>C-H stretch</td>
<td>Thiois</td>
</tr>
<tr>
<td>3</td>
<td>1650.1</td>
<td>N-H bend</td>
<td>Secondary Amine</td>
</tr>
<tr>
<td>4</td>
<td>1357.3</td>
<td>C=H=C stretch</td>
<td>Aromatic ring</td>
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<tr>
<td>5</td>
<td>1431.9</td>
<td>C=H=C stretch</td>
<td>Aromatic ring</td>
</tr>
<tr>
<td>6</td>
<td>1381.3</td>
<td>C-H (asymmetric/symmetric bend)</td>
<td>Dimethyl or isomethyl</td>
</tr>
<tr>
<td>7</td>
<td>1054</td>
<td>C-H in plane bend</td>
<td>Aromatic</td>
</tr>
</tbody>
</table>

**Table 2. Shows the spectrum for *Chlorella* sp.**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Wavenumber (cm(^{-1}))</th>
<th>Molecular Motion</th>
<th>Functional group</th>
</tr>
</thead>
<tbody>
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<td>Hetero cyclic Amine</td>
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<td>2</td>
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<td>2374.9</td>
<td>C=C</td>
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<td>N-H bend</td>
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<td>Aromatic ring</td>
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<td>8</td>
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<td>C-H in plane bend</td>
<td>Aromatic</td>
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<tr>
<td>9</td>
<td>1043.9</td>
<td>C-H in plane bend</td>
<td>Aromatic</td>
</tr>
</tbody>
</table>

XRD:

A. *Scenedesmus* sp.

![XRD Spectrum for Scenedesmus sp.](image3)

B. *Chlorella* sp.

![XRD Spectrum for Chlorella sp.](image4)

Available online at www.scholarsresearchlibrary.com
XRD data of Ag NPs for the algae Scenedesmus sp. and Chlorella sp. displayed strong Bragg reflections corresponding to the (111), (200), (220), and (311) reflection planes of fcc metallic silver. (Fig.5)

Scanning Electron Microscopy:
The Fig.6 shows the magnified view of algal assisted silver nanoparticles with the spherical shape in algae. Scenedesmus sp. and Chlorella sp. extracts and average size of the nanoparticle were 36 nm and 34 nm respectively.

DISCUSSION
The present work deals with the synthesis and characterization of silver nanoparticles from the Algae extract of Scenedesmus sp. and Chlorella sp. The brownish color shows the formation the nanoparticles, which is the first indication of the synthesis. The color will occur due to the reduction of silver-to-silver nanoparticles which was further confirmed by the UV spectrophotometer analysis and the characteristic peak was observed for the silver nanoparticles at the 450 nm range. This FTIR spectrum supports the presence of proteins in the synthesis of silver nanoparticles. The XRD patterns shows four characteristic peaks in the whole spectrum. The XRD pattern shows some of the unassigned peaks may be due to the fewer biomolecules of stabilizing agents are enzymes or proteins in the algal extract. The mean particles diameter of silver nanoparticles was calculated from the XRD pattern using Scherrer equation. The calculated average particles size of silver nanoparticles was found to be 36 nm and 34 nm respectively.

Acknowledgement
We thank The Principal and Head, Plant Biotechnology Department, Presidency College (Autonomous), Chennai 05 and Department of Water and Waste Water Technology, Durban University of Technology, Durban, South Africa. The authors expressed sincere thanks for their kind support and encouragement.

REFERENCES