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Original Research Paper

Utilization of Organic Selenium Nanoparticles to Inhibit Algal Growth

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ABSTRACT

Algae are the most predominant members of photosynthetic eukaryotic forms, which form a major component of global aquatic ecosystem in India. Tannery industries around Erode and Tirupur in Tamil Nadu face the problem of algal growth in reverse osmosis unit due to the presence of chemicals such as ammonium, ferrous salts, sulphate and nitrates of other metals. The refuse after tannery treatment causes eutrophication of aquatic ecosystems, which is a major problem in this surrounding area. Selenium nanoparticles can have significant adverse effects on growth and morphology of the filamentous green algae in a dose-dependent manner. In the present study, our objective is to expose organic nanoparticles with various concentrations to the algal cells and inhibit the same. The bacterial strain *Lactobacillus* sp., found in milk, was used for the synthesis of nanoparticles and in the pH range of 7-8 and temperature ranging from 30°C-35°C could effectively inhibit the algal cells. Characterization studies were performed including UV-Vis Spectrophotometer and SEM micrograph to confirm the presence of nanoparticles. The production of dimethyl selenide by algal cells becomes toxic to the algae themselves and thus causing death. The technology can be readily implemented in industries.

INTRODUCTION

Nanoparticles have been found in a varying range of applications in the past decade. They are more penetrable, easy vectors and are less toxic in nature (Péter Eszenyi et al. 2011). They have unique optical, electrical and magnetic properties. The release of nanoparticles into the environment can be dangerous and toxic too in some cases and the study of the toxicity is known as nanotoxicology (Dash et al. 2012). The inherent antimicrobial action of nano-selenium has not been studied before in a commercial scale. According to Péter Eszenyi et al. (2011) selenium nano-particles are very good antioxidant and less toxic than other selenium forms. They do not trigger liver injury and less accumulated in body than other forms. The production of nanoparticles organically using *Lactobacillus* sp. has been employed before. The biological preparation of nanoparticles is employed to get the nanoparticles of desired size (100-500nm). The effect of selenium salts on the growth of marine algae has been studied before and the study showed positive deteriorative effects on its growth (Yu et al. 2007). Dash (et al. 2012) proved that selenium nanoparticles have been found to be less toxic than selenium salts in the environment.

Algae form an integral part of the environment. The algae in the study have been isolated from tannery water and were cultured in BG11 medium. The algae form a major problem in tannery industries by adhering to the tank surfaces. This inversely affects the BOD and COD levels of water discharged into lakes and rivers. The ultimate effects of algal growth are eutrophication. Tannery water being a source of ferrous, potassium and ammonium salts (Sarkar 1981), favours the growth of the algae. The study to evaluate the effect of selenium nanoparticles on algal growth was conducted and analysed. The increase in death was verified using various methods and confirmed. The possible mechanism for the algal death due to selenium nanoparticles has been referred and verified. The biochemical pathway followed by the selenium nanoparticles in the algal cell is also illustrated.

MATERIALS AND METHODS

Collection and cultivation of algae: Algal cells were collected from the tannery water and subjected to washing using running distilled water to remove unwanted epiphytes and associated debris. The cultivation of the algal cells was done in BG11 medium and tannery water was supplemented to it in the ratio 10:1. This was done to increase the growth rate using tannery nutrients. The culture was maintained at 25±1°C with an illumination of 14.8 dyne.cm^2 from a white fluorescent tube light for 19 hours per day.

Synthesis of selenium nanoparticles: Selenium nanoparticles were synthesized as described earlier with a little modification (Dash et al. 2012). 100 mL of pure milk was taken and sterilized at 120°C for 30 minutes. The selenium source used is the sodium selenite (Na_2SeO_3) in pow-

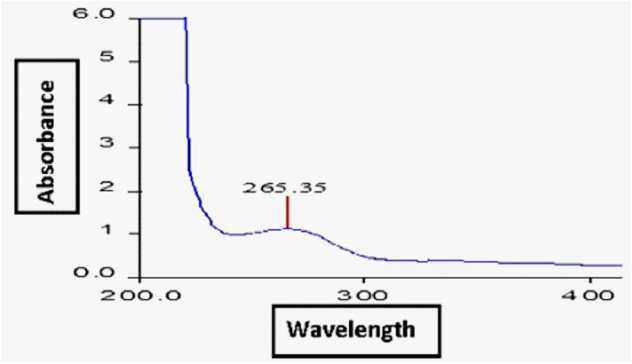

Fig. 1: The Fig. shows the absorbance maximum between 200 to 400nm. SeNPs shows an absorbance maximum at 265nm (Parisa Jafari Fesharaki et al. 2010).

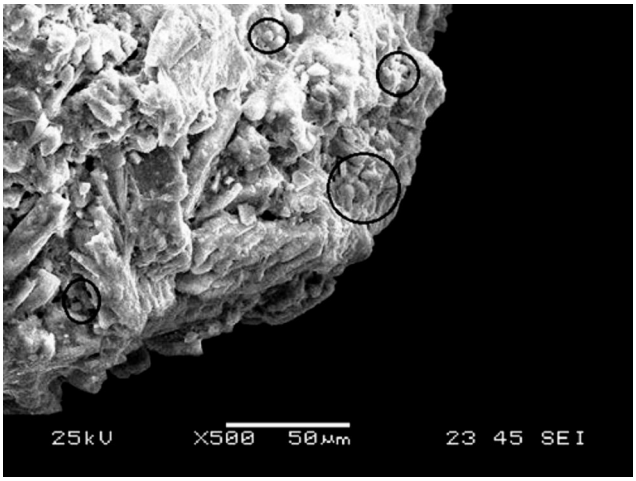

Fig. 2: The selenium nanoparticles synthesized by the *Lactobacillus* sp. at a range of (100-500nm).

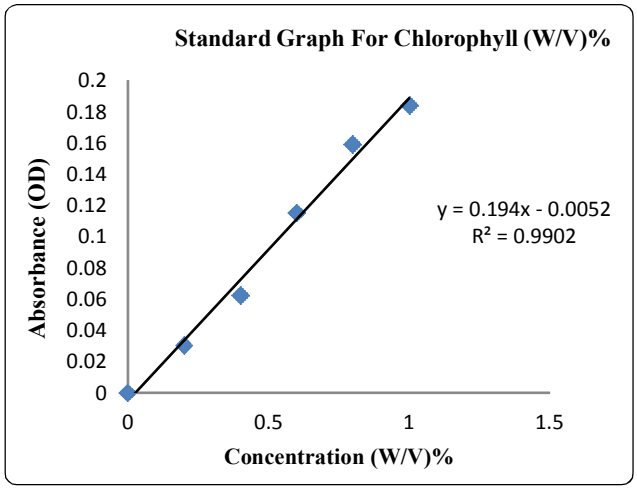

Fig. 3: Standard graph for the estimation of chlorophyll concentration in the algal cells.

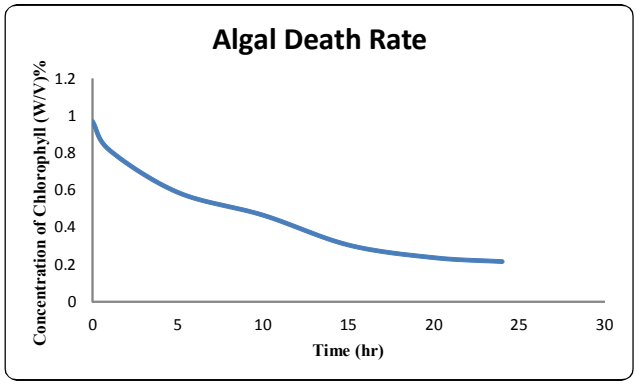

Fig. 4: Graphical analysis of variation in the chlorophyll concentration with respect to time. A linear decrease is observed until the 24th hour.

dered form. The ratio of medium to source is given as 100:0.1. The pH is adjusted since the *Lactobacillus* sp. are pH dependant (usually 7-8) and changes may destroy the strain. After adding the source to the medium in a conical flask, the flask is placed in an orbital shaker at 35°C and 140 rpm. The conditions were maintained for 36-48 hours and at the end of it, a deep pink or red colour broth culture was obtained. This is due to the production of intracellular selenium nanoparticles. The extraction procedure was started by centrifugation of the culture at 10,000 rpm for 5 minutes. The debris was collected and subjected to acid hydrolysis. This is done because the cell wall of *Lactobacillus* sp. is very resistant and hard to break (Anjali Dash et al. 2012). The media was added with 1:1.5 ratio of 37% HCl and is further subjected to orbital shaker at 35°C at 140 rpm. At the end of the fifth day, the sample was centrifuged again at 10.000 rpm and the supernatant was collected. The supernatant was diluted with water until the pH scale returned to 7. The sample was sonicated for 10 minutes with an amplification of 60% to break the cohesive spheres. The samples were stored at 4°C.

Table 1: Variation in the concentration of the viable algal cells with respect to time after treatment with SeNPs.

Sample	Time (hr)	Absorbance (OD)	Concentration (W/V)%
S_0	0	0.183	0.9701
S_1	1	0.153	0.8155
S_2	5	0.109	0.5887
S_3	10	0.0852	0.4660
S_4	15	0.0541	0.3056
S_5	20	0.0409	0.2376
S_6	24	0.0368	0.2165

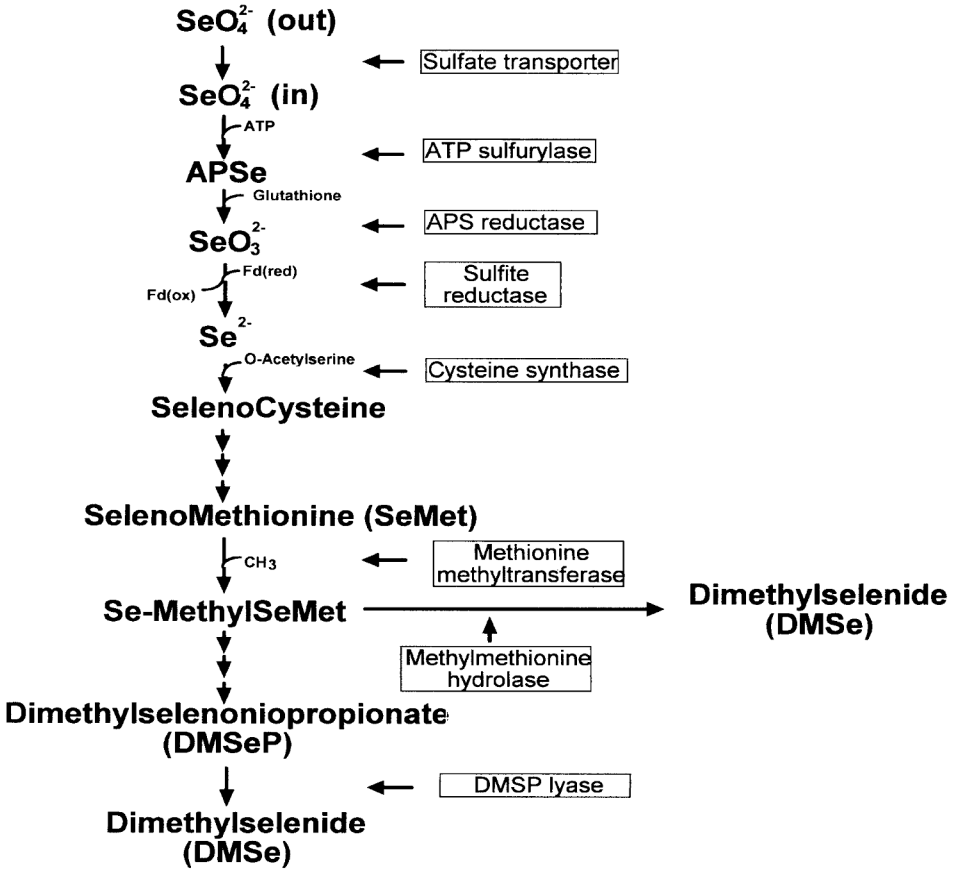

Fig. 5: The biochemical pathway for the production of DMSe is illustrated above (Neumann et al. 2003).

RESULTS

Characterization of Se NPs: To verify the presence of selenium ions, neutralized sample was scanned in UV-Vis spectrometer (Perkin Elmer Lambda 35) using quartz cuvettes from 200-700nm. A peak was observed at 265.5 nm (Fig. 1) confirming the presence of selenium in the samples. The size and morphology of the nanoparticles were later done using scanning electron microscope (500x) by drying the samples in a piece of cover slip and crushing it for further analysis in SEM (Fig. 2).

Growth and inhibition: The algae grown in BG11 were separated and 1 g was again separated from it. From the separated algal cells, 0.5 g of the algae (S_0) was subjected to chlorophyll extraction using acetone as solvent. A standard graph of chlorophyll content of various concentrations of algae was derived from 6 samples with varying concentrations of algae from 0 to 1 g (Fig. 3). Varying concentrations of algae were inoculated in 100 mL of BG11 with 10 mL supplement of tannery water and labelled S_0 to S_6. The algae were subjected to 15 mL of the sample containing SeNPs each. The algal cells (S_0-S_6) were then maintained at 35°C for 48 hours under 24 hour illumination. The algal cells were visually confirmed for death and the chlorophyll was extracted using acetone as solvent. The absorbance values for the samples were taken at 625nm (Dash et al. 2012). The acetone extracts of S_0 showed an absorbance of 0.183 and that of S_6 showed 0.0368. A graphical analysis of the absorbance and the time showed a linear decrease in the chlorophyll content with respect to time (Fig. 4). The decrease in the chlorophyll content ultimately represents the number of viable algal cells in the culture. The difference in OD value, thus, confirms the decline in algal growth due to SeNPs.

DISCUSSION

The SeNPs cause death in the algal cells due to the production of dimethyl selenide, through a biochemical pathway observed in algal cells (Fig. 5) (Neumann et al. 2003). When the concentration of Se increases, it forms SeO_4^{2-} and the pathway leads to the formation of DMSe which potentially

becomes toxic to the algae in large amounts and since the surface to volume ratio of the algal cells is high, it accumulates the DMSe in large amounts ultimately causing death. The conversion of SeNPs in an organic system to selinate has been studied before (Péter Eszenyi et al. 2011). Thus, the application of nano selenium particles for the death of algal cells is found viable. The toxicity of the nano Se particles is also less effective and Se can be recovered back by carbon disulphide precipitation (Parisa Jafari Fesharaki et al. 2010) or by reverse osmosis, thus, leaving the threat of selenium nanotoxicity covered. The pathway has to be further analysed for dose dependent studies of SeNPs. Studies on the various dosage forms of SeNPs to confirm algal death and LD_{50} can be analysed. This will allow the application of nanoparticles in the industrial real time basis, thus, providing benefits to mankind and nature.

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