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**EFFECT OF ZINC OXIDE AND RUTHENIUM NANOPARTICLES ON
THE GROWTH OF *CHLAMYDOMONAS REINHARDTII* AND
*ASTERARCYS QUADRICELLULARE***

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ABSTRACT

Algae are one of the major contributors that provide solutions to some of the current global problems such as food insecurity, several ailments in humans, energy crisis etc. Specifically, they are considered as the best source for biofuels including biohydrogen which are generate through alternate pathway in photosynthesis. Recently, nanoparticles are in use for large scale production of bioproducts including biofuel generation. In the present investigation, both zinc oxide (ZnO) and ruthenium (Ru) nanoparticles were used along with tris-acetate-phosphate (TAP) medium to know their effect on algal growth and biomass accumulation. Both the compounds are useful as catalyst or electrocatalyst in generation of hydrogen besides many functions. Hence, the present screening was carried out under *in vitro* conditions using green algal species such as *Chlamydomonas reinhardtii* and *Asterarcys quadricellulare*. The improvement of growth and total chlorophyll contents were more in cultures grown in 10 µg/L of nano-ZnO when compared to other concentrations in both the species. Higher doses of nano-ZnO reduced the

algal growth and chlorophyll accumulation. In addition, augmented growth and total chlorophyll contents were noticed in *C. reinhardtii* cultures grown in 500 µg/L of nano-Ru and for *A. quadricellulare* it was 1000 µg/L. Present results may be helpful for biohydrogen generation from algal species and also for future research.

Keywords: *Chlamydomonas, Asterarcys, Nano-ZnO, Nano-Ru, Growth, Chlorophylls*

INTRODUCTION

Algae are very beneficial in various fields and often used as food, fodder, nutraceutical, medicine, secondary metabolite and biofuel sources. To be more specific, algae are the main source of non-renewable fuels such as bioethanol, biodiesel, biohydrogen etc. [1]. Both edible and non-edible advanced plants were used for first and second generation fuels, but at present algae are the main sources. Algae possess both unicellular and multicellular organisms and are divided into freshwater and marine groups. Depending on the pigment contents, algae are again classified into green, blue-green, red, brown etc. [2]. Algae can harvest solar energy and capture CO₂ by converting it into biofuel in the process of photosynthesis. Alterations in steps involved in photosynthesis may lead to enhancement of biohydrogen instead of regular carbohydrate production [3]. Several agencies worldwide are sponsoring funds to make algal fuel production on a large scale which is commercially viable.

Though algae are useful for biofuel production, biomass is the main factor for efficient production. In general, biomass is

developed from various chemical and biological sources from the environment which is renewable. Moreover, biomass offers other significant environmental and consumer benefits, such as assuring food security and greenness, protecting air quality and offering the most dependable renewable fuels [4]. Enhancement of biomass production is one of the challenging issues to get sufficient bioproducts in the recent times. Specifically, both organic and inorganic elements play major role in biomass accumulation. In *in vitro* conditions, the composition of culture medium is the key factor for the successful establishment of optimal conditions for high biomass production in any photosynthetic organism including algae. Addition of elements, chemical compounds and metals including all the types of nanomaterials alters the photosynthetic pathway in algal species [5]. In contrast, accumulation of excess elements and chemical compounds (even essential) causes the production of reactive oxygen species (ROS) in most of the biological organisms including algae in turn suppress the growth and development.

In recent times, nanomaterials are in use for various industrial applications particularly energy sector. Nanoparticles have unique physico-chemical properties such as high surface zone and high reactivity with different morphology. These are available as nanoparticles, nanowires, nanotubes, nanorods etc. [6]. In algae, the rate of hydrogen production depends on the photolysis of water i.e. proton (H^+) and electron (e^-) sources at photosystem II (PSII) in photosynthesis. Later, these protons are reduced in the absence of oxygen through active hydrogenase in an alternate pathway of photosynthesis without proceeding for regular carbon fixation (CBB cycle) under stress condition [7]. Hence, the photolysis of water is a crucial stage in photosynthesis which is to be focused for sustainable H_2 production from algae. Some of the nanoparticles such as Ru, Pd, Pt, TiO_2 , Pt-Ru, ZnO, CuO etc., function as catalysts or electrocatalysts and involved in the hydrogen production [8-9]. For example, nano-titanium dioxide or titania (nano- TiO_2) particles exhibit photocatalytic activity and mostly used for H_2 generation from water-splitting mechanism [10].

Addition of nanomaterials alters the growth and development of algal species [11]. In extent, several nanomaterials altered the biomass in several algal species including

advanced plant species [12]. Certain nanoparticles were screened in both *in vitro* and *in vivo* experiments to know their role in algal metabolism [13]. Recently, our laboratory tested the reduced-graphene oxide supported platinum-ruthenium nanoparticles (nano-Pt-Ru/RGO) using green alga *Chlorococcum minutum* and witnessed improved biomass [5]. Nanosize metal oxide and catalysts are recently manufactured at industrial levels which have tremendous applications in water treatment, medicine, cosmetics, energy and engineering sectors [14-15]. Chemically, both ZnO and Ru are working as catalyst or electrocatalyst and involved in hydrogen generation besides many other functions. It is assumed that both ZnO and Ru nanoparticles are involved in algal growth and development particularly in water-splitting process at PSII in photosynthesis. Hence in this study, nano-ruthenium and nano-zinc oxide were used to know their role on the growth and chlorophyll accumulation capacity of model alga *Chlamydomonas reinhardtii* and *Asterarcys quadricellulare*. Both are green algal species with unicellular nature [16]. Moreover, both the species belong to class chlorophyceae and habituated in freshwater condition. To the best of our knowledge, for the first time we designed to screen these nanoparticles using algal

cultures for the improvement of biomass in turn biofuels.

MATERIALS AND METHODS

Green algal samples such as wild type *Chlamydomonas reinhardtii* and *Asterarcys quadricellulare* (MN179327) were collected from University of Madras, India. Later, regular preservation protocols were applied using liquid nitrogen depending on the algal species for future usage.

It is important and must to do washing of glassware (Borosil, India) with detergent solution properly to avoid unnecessary contamination in *in vitro* experiments. Further, washed glassware were rinsed with distilled water and kept in a hot air oven (Kemi, K04.3, Ernakulam, India). Tris-

acetate-phosphate (TAP) media (Fisher Scientific and Hi-Media, India) along with ZnO and Ru nanoparticles were prepared and the pH was adjusted to 7.0 using pH meter (Elico Limited, India). Individually, zinc oxide and ruthenium were used in TAP medium with different concentrations for *in vitro* algal cultures (**Table 1**). Total 5.0 ml of medium was dispensed into 30.0 ml serum vials and were autoclaved (Inlab Equipment, Madras, India) at 121°C and 15 lbs for 20 min. Three fourth of empty space is to be left to ensure healthy algal growth. Inoculation of both the algal samples was carried out in a laminar air flow chamber (Hitech products, Chennai, India).

Table 1: Different concentrations of nanoparticles in TAP medium

S. No.	TAP medium with nanoparticles	Concentrations ($\mu\text{g/L}$)
1	Zinc oxide (ZnO)	0, 10, 20, 40, 80, 160 and 320
2	Ruthenium (Ru)	0, 250, 500 and 1000

After inoculation, all the cultures were kept in an orbital shaker (Pelican equipment, Rotek-Les, India) with 140 rpm speed for algal growth in continuous light (107.02 cd) conditions. Various set of experiments along with ZnO and Ru nanoparticles were carried out under *in vitro* conditions. Hemocytometer was used for cell count and to understand the growth phase/stage condition of both the algal cultures using the methods of Paramesh *et al.* [3] and

Varaprasad *et al.* [2]. In the current study, chlorophyll contents were estimated using modified Arnon's [17] and Varaprasad *et al.* [18] methods. One gram of algal material of treated samples along with controls were ground with a mortar and pestle using 80% acetone. Later, the homogenate was centrifuged at 5000 rpm for 5 min. Further, supernatant was saved and the residue was re-extracted with 80% acetone up to the formation of pale yellow residue. Then the

supernatant final volume was made up to 100 ml with 80% acetone and absorbance values were read at 645 and 663 nm in a UV-Vis spectrophotometer (Shimadzu UV-1800, India). Total chlorophyll content was estimated by using standard Arnon's formula and expressed in mg/L fresh weight basis. All the experiments were carried out using minimum of three replicates and experiments were conducted thrice. The statistical work has been performed using excel program in computer for various parameters.

RESULTS AND DISCUSSION

At present, nanoparticles are widely used on a large scale in biological experiments due to their unique reactive nature. In the present work, TAP salts along with various concentrations of ZnO and Ru nanoparticle supplements were screened using *C. reinhardtii* and *A. quadricellulare* cultures. Both the species belong to freshwater group and the basic idea to design this experiment is to know the ZnO and Ru resistance level, algal growth pattern and chlorophyll accumulation. All the obtained results were statistically analyzed and the data were documented below.

Effect of nano-ZnO on algal growth and accumulation of chlorophylls

In the current work, nano-zinc oxide was used in various concentrations (0, 10, 20, 40, 80, 160 and 320 µg/L) along with TAP medium to know its effect on algal growth and formation of chlorophyll pigments. Both the species were grown well at optimal concentration in nano-ZnO treatments. Best growth in both the algal species was observed in TAP medium with 10 µg/L nano-ZnO (**Figure 1**). Particularly, improved total chlorophylls in both *C. reinhardtii* (43.63 mg/L) and *A. quadricellulare* (44.71 mg/L) was noticed in TAP medium with 10 µg/L of zinc oxide nanoparticles. Higher doses of nano-ZnO reduced the growth and chlorophyll content. Clear suppression of growth and development of both the algal species was noticed at 160 and 320 µg/L of nano-ZnO (**Figure 1**). Higher concentration of nano-ZnO exhibits the toxic nature and reduces the chlorophyll accumulation. Similarly, toxicity of ZnO nanoparticle was compared in both *Chlorella* and *Scenedesmus* species and earlier one proved to be nine times more sensitive than later [19]. Franklin *et al.* [20] studied the toxic effect of ZnO nanoparticle using *Scenedesmus* species.

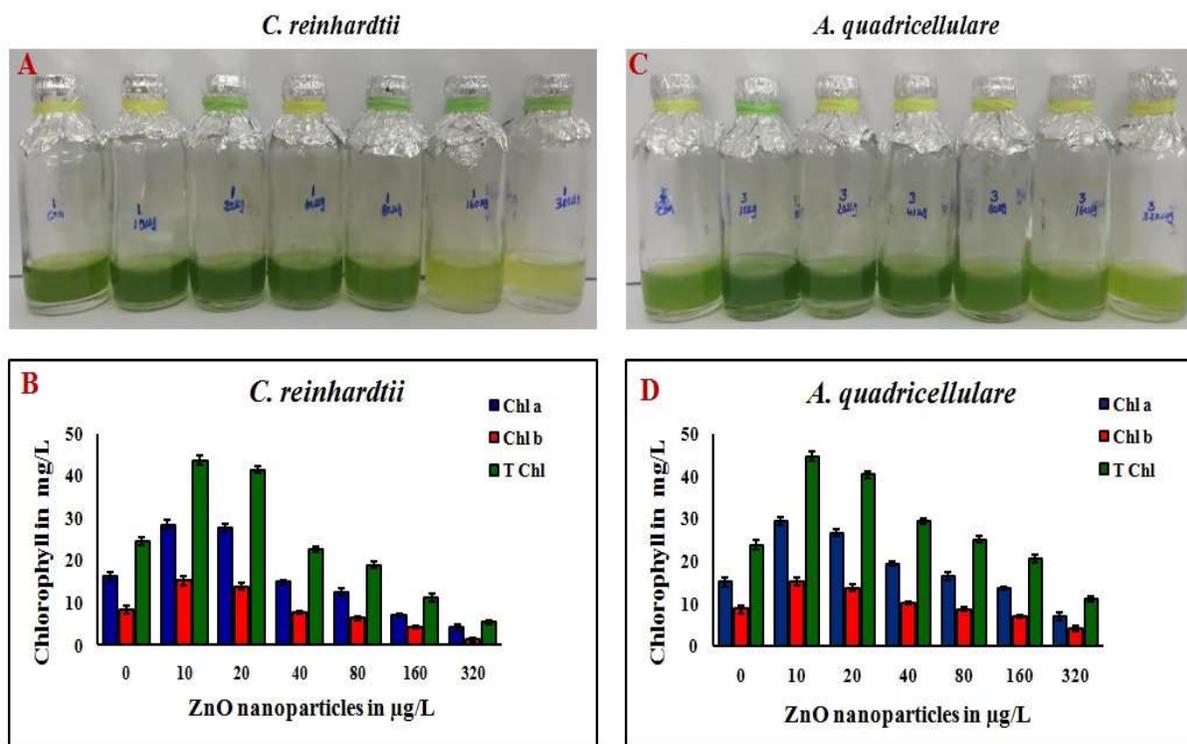


Figure-1: Effect of nano ZnO on growth and chlorophyll accumulation in algal species (Chl a-chlorophyll a, Chl b-chlorophyll b and T Chl-Total chlorophyll)

Natasha *et al.* [21] conducted the comparable toxicity studies using ZnO, bulk ZnO and ZnCl₂ nanoparticles using *Pseudokirchneriella subcapitata* and mentioned the importance of solubility of the particles. Aruoja *et al.* [22] also studied the toxic effect of ZnO nanoparticle in *Pseudokirchneriella subcapitata*. Djearmane [23] proved the nano-ZnO toxicity with *Spirulina platensis*. As per the present results, 10 µg/L nano-ZnO is safe and suitable for algal cultures for biomass production. Future work may answer the level of biofuel production with this catalyst.

Effect of nano-Ru on algal growth and accumulation of chlorophylls

Nano-ruthenium was also screened using different concentrations (0, 250, 500 and 1000 µg/L) in TAP medium for the estimation of growth and chlorophyll content in both the algal species (Figure 2). Out of three concentrations used better growth and high total chlorophyll content (33.84 mg/L) was noticed at 500 µg/L of nano-Ru in *C. reinhardtii* and further high doses reduced the growth and development including chlorophyll content. In contrast, better growth and high chlorophyll content (45.13 mg/L) was noticed at 1000 µg/L of nano-Ru in *A. quadricellulare* compared to other concentrations. This data indicates the

differential response of algal species with nano-ruthenium.

To date, effect of ruthenium nanoparticles was not studied with algal species, but was synthesized from marine alga *Dictyota dichotoma* [24]. It is used as catalyst and involved in redox processes and electron transfer process. Our laboratory used platinum-ruthenium nanoparticles along with reduced-graphene oxide (nano-Pt-Ru/RGO)

for *Chlorococcum minutum* cultures and proved that these nanoparticles were involved in electron transport system of photosynthesis. Also, improvement of algal biomass was noticed in low concentration of nano-Pt-Ru/RGO [5]. Overall, total chlorophyll contents were more in *A. quadricellulare* when compared to model alga *C. reinhardtii*. This data indicates that the nanoparticle resistance will also depend on the species.

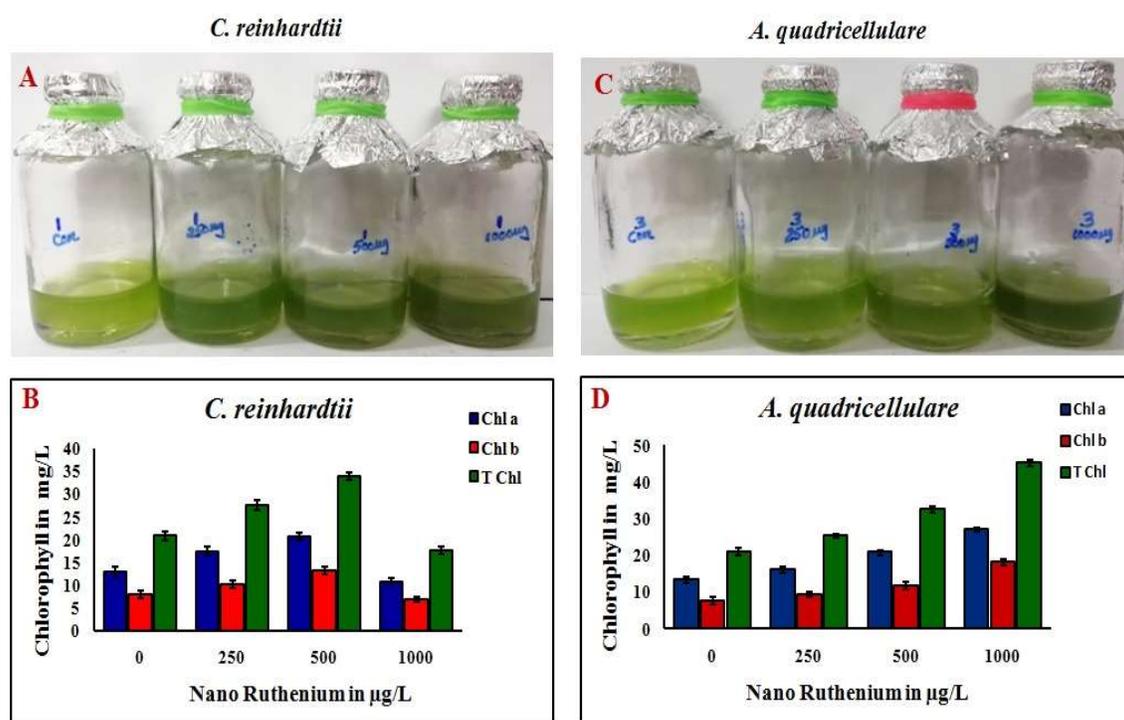


Figure 2: Effect of nano-ruthenium on growth and chlorophyll accumulation in algal species (Chl a-chlorophyll a, Chl b-chlorophyll b and T Chl-Total chlorophyll)

CONCLUSIONS

The continuous consumption of non-renewable fossil fuels urges us to focus on renewable energy sources such as biofuels including biohydrogen, bioethanol, biodiesel etc., from biological organisms including

algae. Before that improvement of biomass is essential process to get optimal levels of fuels. In the current work, both ZnO and Ru nanoparticles were screened to know the algal growth and accumulation of chlorophylls. Enhanced total chlorophyll content was

observed in *C. reinhardtii* (43.63 mg/L) and *A. quadricellulare* (44.71 mg/L) cultures grown in TAP medium with 10 µg/L zinc oxide nanoparticles. Augmented total chlorophylls were noticed in *C. reinhardtii* (33.84 mg/L) at 500 µg/L of nano-ruthenium along with TAP medium and for *A. quadricellulare* (45.13 mg/L) it was 1000 µg/L. Higher doses of nanoparticles inhibit the growth and reduced the total chlorophyll content. On the whole, total chlorophyll content was more in *A. quadricellulare* when compared to *C. reinhardtii*.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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