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A comparison between the effect of zinc oxide and zinc oxide nannoparticles on the growth and some metabolic processes of *Cosmarium sp.*

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Abstract: Bold Basal Media was used to culture the unicellular microalga *Cosmarium sp*. under carefully monitored conditions. In order to aid in microalga culture and extract useful compounds, we looked into biochemical characterizations applying nonmetals. Although the vast majority is cognizant of how harmful metal nanoparticles may be for microalgae, nanotechnology has a significant effect on a lot of other living organisms. The aim of this research is to evaluate the effects of zinc oxides and zinc oxide nannoparticles on *Cosmarium sp.* growth and induce resistance in order to improve the manufacture of high-value microalgae products. Zinc oxides and zinc oxide nannoparticles were added during its growth, with concentrations ranging from 0 to 300 mg/l. Studies comparing the effects of zinc oxide nanoparticles have shown that the treated cells accumulated more lipids and biomass than the non-exposed cells. The polymer's chloroform extract for *Cosmarium sp.* exposed to nannoparticles yielded eighteen distinct biodegradable chemicals, according to the results of GC/ MS analysis. Acetic acid, 2-methylpropyl ester (isobutyl acetate), Heptanol acid, nonanoic acid, and Heptanol acid were the main compounds found. Isobutyl acetyl citrate had the highest molecular weight of 402 and was the most prevalent compound with a total percentage of 29 and molecular weight of 116.08. *Cosmarium sp.* is capable of producing polyhydroxyalkanoate, which is about to be used extensively as a bioplastic, at different levels from zinc oxide nanoparticles.
Keywords: *Cosmarium sp* growth; Zinc oxides nanoparticles; Lipid content; growth rate; Poly hydroxyalkanoate

1. Introduction

In response to environmental stress, microalgae developed a variety of active compounds that they were biochemically and physiologically separated [1]. *Cosmarium sp.* is a freshwater alga that belong to the division of Chlorophyta [2]. An alga is a third-generation biofuel feedstock that is playing an increasingly major part in the global marketplace for renewable energy [3]. Because of its rapidly growing, high linear accumulation, and potential to engage in action to promote carbon neutralizing and microalgae production to reduce greenhouse gas emissions and air pollutants, the creation of biodiesel from plankton has been successful. Due to their capacity to convert nearly all food stock into biomass, microalgae can be thought of as the primary feedstock for the manufacture of biodiesel. Instead of utilizing energy for growth and development, their bodies have the ability to convert and store it so Microalga can be viewed as the main feedstock for biodiesel production [4-5]. Algae have 200 times more biomass per hectare than land-based crops [6]. A significant food source strong in vitamins, minerals, and proteins are microalgae, which in some areas have concentrations higher than those of conventional vegetable and animal protein sources. Metal and metal oxide nanoparticles are greater to their original state when it comes to enhancing the biological function of diverse species [7]. Reactive oxygen species (ROS) have been linked to nanoparticle toxicity to microalgae [8]. Oxidative stress-causing nanoparticles may encourage the growth of algae and the buildup of secondary metabolites [9]. The use of zinc oxide nanoparticles in this work to enhance *Cosmarium sp.* growth at various zinc oxide nanoparticle concentrations was an attractive approach. Protein, carbohydrate, and lipid contents, growth rate, biomass concentration, pigment concentration, and other factors were evaluated in order to assess how well metal nanoparticles worked in the process of producing high-value goods like polyhydroxyalkanoate from *Cosmarium sp.*

2. Material and Methods

2.1 Purification and Growth of *Cosmarium*

Cosmarium sp. was isolated by a streak-plate method from the waste water plant in Sohag, Egypt. Solid and liquid Bold Basel Medium are used to grow the culture, and a light/dark (length of 20ºC a12:12 h, with a cool white fluorescent illumination of Philips TL 40W and 140 µmol photons/m^2/s for about 15 days) incubator is used. Until Chlorophyta isolated single species, each species was grown

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in the same previous condition [10]. *Cosmarium sp.* was grown in a medium containing metal oxide and metal oxide nannoparticles. The effect of zinc oxide and zinc oxide nannoparticles was evaluated by measuring the growth of the microorganism in each media using a different concentration range of 0-300 mg/l.

2.2 Metal Nanoparticle Synthesis and Characterization of Metal Nano Particles

The nanostructures of ZnO are synthesized using chemical vapor deposition (CVD) system, where Zn granules are placed in alumina boat in the center of the tubular furnace at temperature of 900°C for 40 minutes. prior to the experiment an evacuation is carried out for the purpose of reducing the unwanted gas-phase reactions and improving the nanostructure uniformity. Argon and oxygen gases are allowed to flow with rates of 200 sccm and 20 sccm for the purpose of furnace purification and nanostructure growth. At the end of the experiment, the tubular furnace is cooled down to room temperature. The nanostructures' crystallographic structure was analyzed using X-ray diffractometry (Bruker D8 ADVANCE diffractometer) using Cu-Kα radiation (λ = 1.54056 Å). The size of the crystallite was determined using Debye-Scherrer's formula.

2.3 Determination of Specific Growth Rate

The *Cosmarium sp.* (μ) specific growth rate has been determined using the following formula.

$$\mu=\text{Ln}\ (N_t / N_0)/T_t\text{-}T_0$$

where Nt and N0 represent the concentration of dry cell weight (g L^{-1}) at the beginning (T_0) and end (T_t) of the log phase, respectively.[11].

2.4 Determination of Biomass Concentration

The following formula was used to determine the biomass (g L^{-1}) of *Cosmarium sp.* cultivated in the presence of metal nanoparticles by measuring the optical density of samples at 600 nm (OD600) using UV-Vis spectrophotometers.

2.5 Chlorophyll-a Estimation

Centrifuged algae cells isolated overnight with 80% acetone were used to assess the chlorophyll concentration [12]. The extract was centrifuged for five minutes at 3000 x g, and a spectrophotometer was used to measure the amount of chlorophyll in the supernatant using the following equation.

$$\text{Chl (mg/L)} = 8.02 \text{ x OD663} + 20.21 \text{ x OD645}$$

2.6 Carotenoids Estimation

After centrifuging, algae cells were treated with 60% w/w KOH. Ethyl ether was used to extract the mixture after it had been homogenized and heated to 40°C for 40 minutes [13]. The optical density was measured at 444 nm after the solvent was evaporated and then resuspended in acetone. The following formula was used to compute total carotenoids. Total carotenoids were calculated using the below equation.

$$\text{Ct (mg/L)} = 4.32 \times \text{OD444} - 0.0439$$

2.7 Determination of Total Lipids

Algal cells were collected by centrifugation, and they were then dried for lipid content analysis. A one-step extraction process had been used to remove the lipids [14]. Dried algal cells that were ultrasonic and chloroform were mixed with distilled water and methanol (2:1). After 30 minutes at 30°C in a water bath, the mixture was filtered through Whatman No. 1 filter paper. The layer of chloro-purified chloroform was transferred to a different tube holding the NaCl solution after being continuously evaporated to weight in a smoking cap at 60°C. The following formula was used to get the total dry weight lipid content.

Content of lipids (%) = $(m_2 - m_0)/m_1 \times 100$ where m0 represents the weight of the new screw cap tube when it is empty, m1 represents the weight of the dried algal cells, and m2 represents the weight of the new screw cap tube containing the dried lipids. Equation (g L^{-1} d^{-1}) was used to calculate lipid productivity.

Biomass productivity × Lipid content equals lipid productivity.

2.8 Gas Chromatography Mass Spectroscopy (GCMS)

Using an Agilent 5975 GC/MSD system, a gas chromatograph mass spectroscopy equipment was used to analyze the polyhydroxyalkanoate that was isolated from *Cosmorium sp.* column temperature was first maintained at 35 for 1.0 minute, then programmed to 20 at a rate of 25 / minute with holding time 1.0 minute, and finally at a rate of 10 / minute with holding time 2.0 minute. The column oven temperature was set to 60°C. The capillary column measured 30 m _ 0.25 mm (1.0μm film thickness).

2.9 Statistical Analysis

Using SPSS Statistics 21, mean and deviation from the mean data have been calculated for experiments conducted in triplicate. Three replicates (n = 3) of the data were analyzed using a single-way ANOVA, with $p < 0.05$ being considered statistically significant.

3. Results

The corresponding figure (Fig.1) shows results of XRD of the thin films deposited by CVD technique. Clearly, the nucleated species produced polycrystalline phases of Zn and ZnO (JCPDS files card no. 9008522 for Zn and 2107059 for ZnO). The relative peak integrated

Intensity (RPII) of the mixed phases are calculated using equations:

$$RPII_{Zn} = \frac{I_{Zn}}{I_{Zn} + I_{ZnO}}$$

$$RPII_{ZnO} = \frac{I_{ZnO}}{I_{ZnO} + I_{Zn}}$$

That are resulted 69% for Zn and 31% for ZnO, confirming the main prominent of Zn phase. In this work the bi-structure of Zn/ZnO are intentionally synthesized by using short deposition time that are beneficial for water disinfection compared with the single phase of ZnO. Previously, it was reported that the existence of different phases in the structure prevent the electron-hole pair recombination hence enhancing the catalytic performance. Crystallite size (D) is calculated using Debye–Scherer formula:

$$D = \frac{0.9\,\lambda}{\beta\, Cos\theta_c}$$

where β is FWHM and λ is the wavelength of X-ray. The calculated crystallite size was 38.6 nm that confirms the granules size within the limits of nanometer. To inquire into how zinc oxide and zinc oxide nanoparticles behave differently into growth of *Cosmarium sp.* For seven days, *Cosmarium sp.* was grown in the presence of zinc oxide and zinc oxide nanoparticles. The growth rate of *Cosmarium sp.* that were tested and cultured in a solution of of zinc oxide and zinc oxide nanoparticles was determined and shown at figure2,3. The metal oxide and metal oxide nanoparticle concentrations that have been used were 0.0, 50, 100, 150, 200, 250, and 300 mg/L. The test organism was grown in the various concentrations for seven days. The results showed that, although the average rate of growth increased with increasing zinc oxide concentrations (p=0.05), it declined significantly with arising zinc oxide nanoparticle concentrations up to 300 mg/l (Figure 3). Biomass and chlorophyll-a content was assessed when they were cultivated with zinc oxide nanoparticles (fig. 4-7). Zinc oxides' has an inhibitory effects whereas zinc oxide nanoparticles' has an enhancing effects on microalgae. The values of biomass concentration also increased highly significantly up to the level of 250 mgl from metal oxides nanoparticles then they gradually up to the level of 300 mg/l ZnO nannoparticles, (p=0.05), above which a marked reduction in the values of biomass and chlorophyll-a content yield was reached. Biomass concentration had positive correlations with Specific growth rate of *cosmarium sp.* exposed to ZnO nannoparticles (r=0.681-0.989, p= 0.002-0.05), and Chl-a concentrations (r=0.603-0.777, p=0.038-0.045). Fig. 8,9. showed that once metal oxide nanoparticles were found, the lipid content in *Cosmarium sp.* increased. The chloroform extract of the polymer yielded eighteen distinct biodegradable chemicals, as indicated by the GCMS results of analysis. The most common compounds found were acetic acid, 2-methylpropyl ester (isobutyl acetate), which had a total percentage of 29 and a molecular weight of 116.08; other compounds included heptanoic acid, which had a percentage of 15, and nonanoic acid, which had a percentage of 11. The compound tributyl acetyl citrate had the highest molecular weight, which was recorded at 402. According to this research, *Cosmarium sp.* was able to synthesize PHAs, or polyhydroxyalkanoate. PHAs were first widely used in medicine in the 1970s. Thus, in 1974, the first biodegradable Vicryl surgical suture material made of chemically manufactured polymers was introduced to the pharmaceutical industry. A wide range of products (such as biodegradable surgical staples, screws, plates, pins, and cords; bioresorbable suture material and skin staples; wound and burn dressings; membranes for periodontal guided regeneration; surgical mesh endoprostheses; patches for surgical repair of intestinal and pericardial defects; mesh plugs for coloproctological applications and hernioplasty; vascular prosthetic implants; coronary stents; mesh tubes for nerve regeneration; artificial heart valves, and other medical devices) are either in use or in the process of being developed.

PHAs also bring characteristics as directed distribution, farther activity, less toxicity, and enhanced stability to new dosage forms utilized in pharmaceutics.

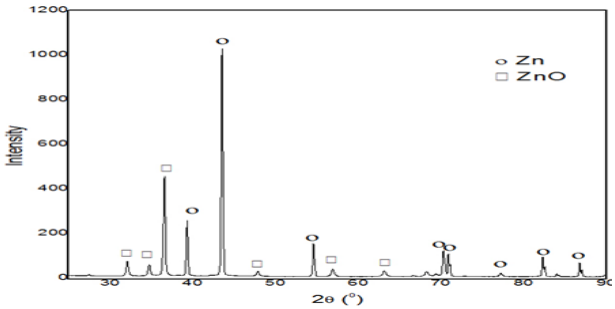

Fig. 1: XRD of Zno thin films.

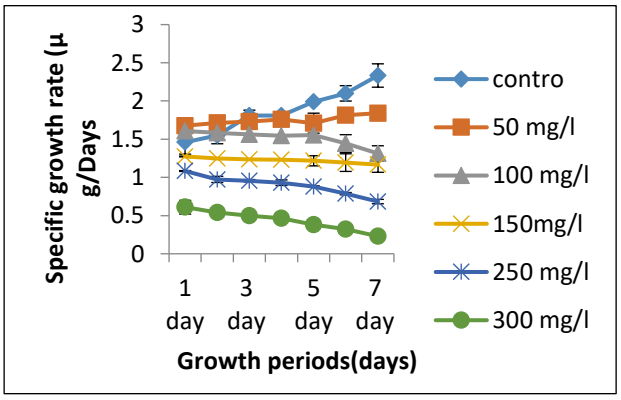

Fig. 2: The average rate of growth of *Cosmarium sp.* in the presence of zinc oxide.

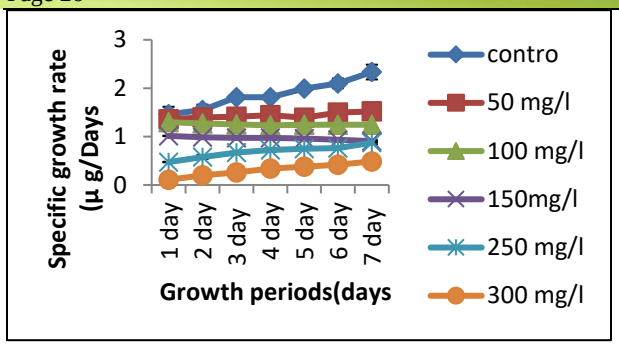

Fig. 3: The average rate of growth of *Cosmarium sp.* in the presence of zinc oxide nanoparticles.

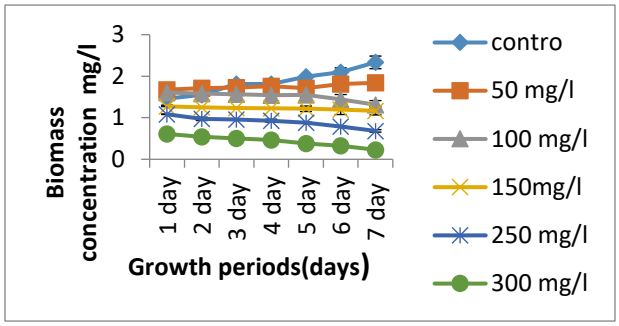

Fig. 4: Biomass concentration of *Cosmarium sp.* in the presence of zinc oxide.

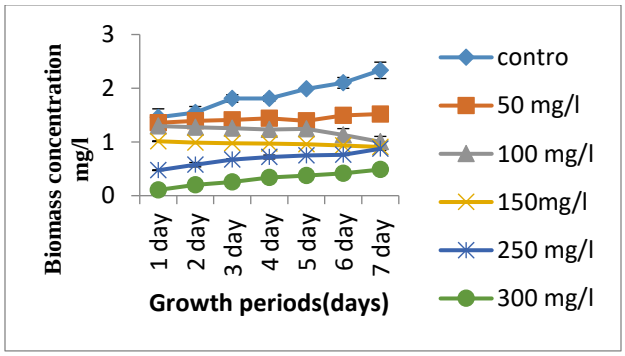

Fig. 5: Biomass concentration of *Cosmarium sp.* in the presence of zinc oxide nanoparticles.

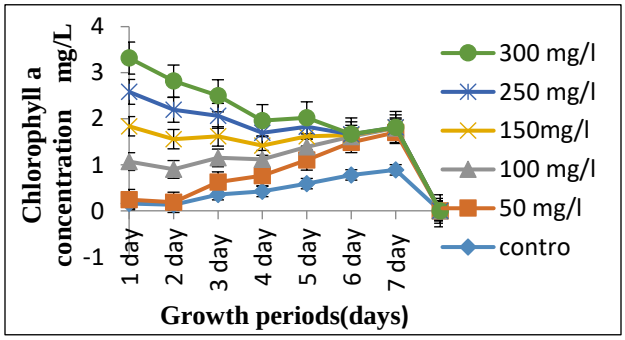

Fig. 6: Chlorophyll a concentration of *Cosmarium sp.* in the presence of zinc oxide.

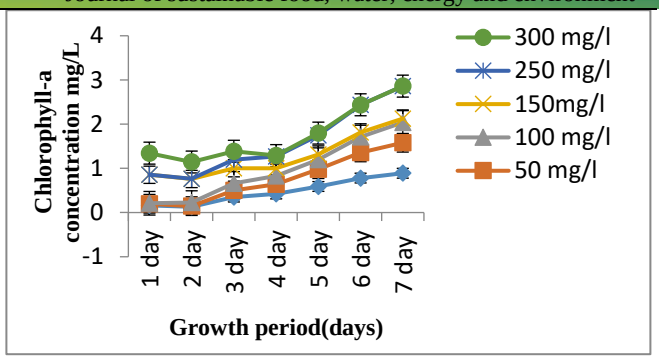

Fig. 7: Chlorophyll a concentration of *Cosmarium sp.* in the presence of zinc oxide nanoparticles.

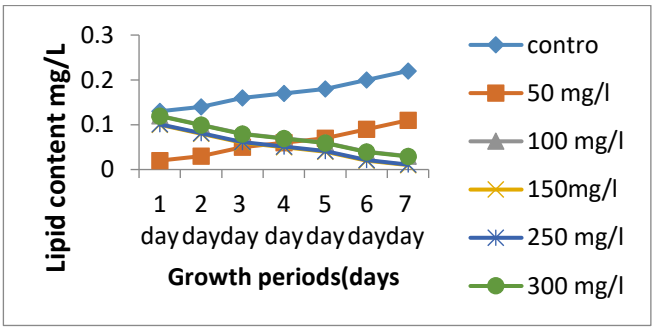

Fig. 8: Lipids content of *Cosmarium sp.* in the presence of zinc oxide.

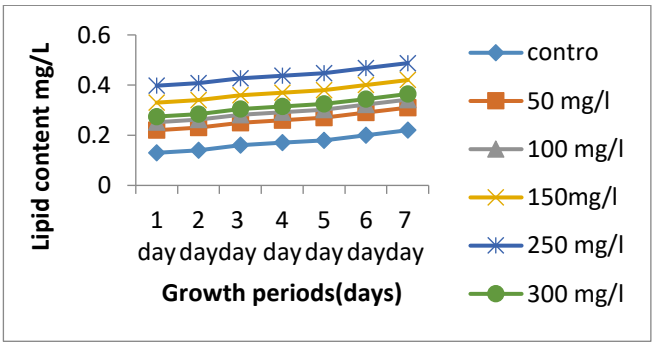

Fig. 9: lipids content of *Cosmarium sp.* in the presence of zinc oxide nannoparticles.

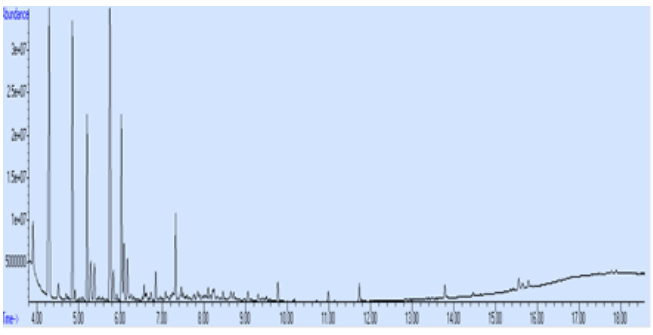

Fig. 10: Chromatogramme of MS peaks and linear retention indices were compared with the published data [15].

4. Discussion

The growth rate and biomass content of *Cosmarium sp.* were affected by concentrations of zinc metal oxides and metal oxide nannoparticles. The study revealed that zinc oxide nanoparticles on microalga had an expanding effect and that rate of growth, biomass, and biochemical characteristics of tested alga produced in the presence of metal oxide had inhibitory effects. The values of biomass concentration increased greatly up to 250 mg/l from metal oxide nanoparticles, then slightly dropped up to 300 mg/l ZnO nanoparticles, ($p=0.05$), after which a considerable decline in biomass production was observed. These results are in agreement with investigations[16], the microalgal cells were subjected to several concentrations of aqueous solutions containing zinc metal for a period of 15 days in order to assess growth inhibition [17].These concentrations of chlorophyll an also increased significantly up to the level of 250 mg/l from metal oxide nannoparticles, then reduced marginally up to the level of 300 mg/l ZnO nannoparticles, ($p=0.05$), above which a significant reduction in biomass yield values was seen. This is also strongly connected with *Cosmarium* growth rate ($r=0.9$), the degree in the reduction of growth and photosynthesis was much more pronounced than in metabolic rate (carbohydrate and protein metabolism) in most cases of *Cosmarium* that were treated with zinc oxides and zinc oxides nannaparticles. There were significant variations in concentration of chlorophyll under the effect of zinc oxides nannoparticles. ($p<0.05$). This is compatible with Navarro, E.; et al.2008 that showed the adsorption of NPs on the surface of algal cells results in a shading effect that affects algal photosynthesis. The shading effect caused by NPs affects the light, pigment and other conditions necessary for photosynthesis, weakening the algae absorption of light and thereby inhibiting the photosynthesis process [18-20].Reduced amounts of copper nanocarboxylates (20 to 40 mg L^{-1}) and selenium nanocarboxylates (0.07 to 0.2 mg L^{-1}) were added, and this promoted the growth of Chlorella and an increase in biomass in addition to the microalgae's chlorophyll content..The lipid content of algal biomass is presented in Figure 5. lipid content was observed increased on the 13th day ZnO nannoparticles exposed and control cultures, respectively It was interesting to note that the total lipid content in MNPS was 0.74 mg L^{-1} while it was just 0.15 mg L^{-1} in the control group. Another remarkable finding is an increase in microalgae lipid content, which has had a beneficial effect on *Cosmarium sp.* biomass and lipid content of 0.76 mg L^{-1}. High lipid yield and biomass productivity are critical for the viability of mass-culturing microalgae for the manufacture of biodiesel [21]. When carbon nanotubes, Fe_2O_3 nanoparticles, and MgO nanoparticles were present in Scenedesmus obliquus, both the neutral and total lipid concentrations increased [22]. The chloroform extract of the polymer yielded eighteen distinct biodegradable chemicals, as indicated by the GCMS analytical findings for *Cosmarium sp.* (Table 1). The melting peak of PHA was found to be 335oC and the total weight loss within this temperature range was 95%. The crystallinity of the PHA had been determined for standard PHB in previous reported [23].

5. Conclusion

The applications of metal nanoparticles to increase *Cosmarium sp.* metal resistance and increase the production of high-value byproducts like biomass, cellular pigments, and lipid from microalgae was assessed in this work. The extracted polymer was identified as PHA by GCMS analysis. The isolated PHA's thermal and mechanical properties were identical to those of regular PHA. As PHA producers, *Cosmarium sp.* has the added advantage of recycling waste carbon dioxide, a greenhouse gas, into plastics that are safe for the environment by employing solar energy. As a result, it's fair to assert that *Cosmarium sp.* can be used to produce PHA on a wider scale.

6. References

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