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MICROBIOLOGY

Potential role of *Spirulina (Arthrospira)* platensis biomass for removal of TiO_2NPs -MG hybrid nanocomposite produced after wastewater treatment by TiO_2 nanoparticles

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Abstract: Biosynthesis of titanium dioxide nanoparticles (TiO_2NPs) by *Sphingomonas paucimobilis* B34 bacteria was successfully achieved and followed by UV-Vis spectroscopy. The nanoparticles were characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM) and fourier transform infrared spectroscopy (FTIR) techniques. The biosynthesized TiO_2NPs were spherical in shape with an average particle size of 15.6 nm. These TiO_2NPs were used as nono-catalyst for removing of malachite green (MG) dye (at 10^3 mol/L) from wastewater solution. As indicated by the results, the biosynthesized TiO_2NPs represented a capable approach for MG removal with up to 83 % efficiency. The removal process was found to follow a pseudo-first-order kinetics. Furthermore, the developed TiO_2NPs-MG hybrid nanocomposite was efficiently removed from the medium by using *Spirulina platensis* cyanobacterial biomass after wastewater treatment. *S. platensis* biomass was able to remove up to 89.43 % of the hybrid nanocomposite by a biosorption process. The resultant water effluent, after TiO_2NPs-MG removal, showed no toxicity towards *Vigna radiate* L. seedlings implying its safety for agriculture purposes. According to the obtained results, *S. platensis* living biomass could play a dual re-cycling role, as natural biosorbent for removing both nanoparticles and dye (TiO_2NPs-MG hybrid nano-composite) from solution after wastewater treatment for healthier environmental management.

Key words: *Sphingomonas paucimobilis* B34, Malachite green (MG), Cytotoxicity, *Vigna radiate* L. seedlings.

INTRODUCTION

Nanomaterials have dimensions range between 1–100 nm. In recent daily life of humans, the nanoparticles play important role through numerous commercial and biomedical applications (Dahoumane et al. 2017). Synthesis of nanoparticles has been established by several physical and chemical methods. Synthesis of nanoparticles via biological methods have induced nanoparticles of unique shape and dependent physico-chemical properties, which receive potential applications in biotechnology (Mira et al. 2015). Nanoparticles synthesis (either extracellularly or intracellularly) *via* the biological route using microorganisms (such as bacteria, fungi and microalgae), enzymes and/or plant extracts have been considered nontoxic and ecofriendly alternative to the chemical and physical methods (Song et al. 2016).

Synthetic dyes are present in industrial wastewater effluents, discharged and coated other materials into the aqueous and non-aqueous ecosystems (de Souza et al. 2008). For

the removal of these dyes, the ordinary biological treatments applied for wastewater have become less efficient because these dyes resist the degradation by microorganism. Additionally, the physico-chemical methods used for decontamination of wastewater have been also ineffective at higher effluent concentrations (Nirmohi & Agnihotri 2016). For these reasons, the nanoparticles have been implemented for treatment of wastewater, because they have high specific surface area which increases the dye removal reactivity (Ismail et al. 2020, Salem et al. 2018). Titanium dioxide (TiO_2) is one of well-known semiconductor that is used for the removal of several toxic organic materials through adsorption and photocatalysis routes. It has chemical stability, strong oxidizing power, biocompatibility, low environmental toxicity and cost effectiveness. These advantages render it highly efficient in degradation of organic contaminants (Abdel-Messih et al. 2013, Gupta et al. 2012). TiO_2 in the form of nanoparticles (TiO_2NPs) is now receiving wide applications in many daily life fields. It has been used in environmental cleanup systems technologies by photo-oxidation and photo-degradation of toxic dyes (Farzana & Meenakshi 2014). However, the safety of not readily soluble nanoparticles in the treated wastewater should be investigated before being addressed for further applications.

On the other hand, the high growth rate, less cultivation time, and high biomass productivity of microalgae and cyanobacteria (formerly, blue green algae) have made them potential organisms capable for elimination of heavy metals, removal of dyes from industrial effluents (Abd Ellatif et al. 2020), pollutants bioremediations, and production of commercially important metabolites (Abed et al. 2009). *S. platensis* cyanobacterium is known as a good source for protein, polysaccharides, lipids, minerals and vitamins (Mahdieh et al. 2012). These metabolites compose many functional and charged groups for dye binding capacity of *S. platensis* (Ismail et al. 2020). This cyanobacterium is commonly available and could be cultivated for biosorption of heavy metals such as cadmium, copper, lead and nickel (Seker et al. 2008).

This paper reports results on the ability of *Sphingomonas paucimobilis* bacterial strain for the biosynthesis of TiO_2NPs and further application of these nanoparticles for malachite green dye removal from wastewater solution. The study also aimed to explore the ability of *Spirulina platensis* as living biomass to eliminate the generated TiO_2NPs-MG hybrid nanocomposite from the medium after treatment of wastewater. The safety of the resultant effluents, after TiO_2NPs-MG removal, was also investigated for application in agriculture purposes.

MATERIALS AND METHODS

Titanium dioxide (TiO_2) powder 99.0% and malachite green dye (MG) were analytical grade of Sigma Aldrich products and used as received.

Cultivation and identification of *Sphingomonas paucimobilis* and *Spirulina platensis*

S. paucimobilis and *S. platensis* were isolated from a local wastewater plant. For *S. paucimobilis*, the isolate was cultivated and purified by sub-culturing on nutrient agar media according to the standard microbiological methods and incubated for 24 h at 35°C. The bacterial isolate was then identified by biochemical tests via strip system BioMereux on Vitek 2C and was further recognized by Sigma Scientific Service Company via sequencing of 16S rRNA gene (Das et al. 2014). The isolate was evidently identified as *Sphingomonas paucimobilis* strain B34.

On the other hand, *Spirulina* was cultivated in modified Zarrouk's medium (Zarrouk 1966) and was identified as *Spirulina (Arthrospira) platensis* (Gomont) according to morphological keys described by Desikachary (1959) and Prescott (1962). The cyanobacterium was grown in a 1L batch culture vessel under 45 µ mole photon m^{-2} s^{-1} intensity of fluorescent light at 27°C and supplemented with a dry air pump of air (97%) / CO_2 (3%) to ventilate the culture and accelerate growth. The culture was grown to reach the end of the exponential phase (17 days) then homogenized for 10 min. to obtain a uniform biomass. The culture concentration was estimated using Sedgewick-rafter cell counter.

Biosynthesis of TiO_2NPs

In a preliminary experiment, nutrient broth bacterial cultures of *Bacillus paralicheniformis*, *Bacillus pumilus* and *Sphingomonas paucimobilis* were isolated from a local wastewater treatment vicinity. These isolates were further examined and identified according to the standard microbiological methods in the Bacteriology Lab, Botany Department, Faculty of Science, Tanta University, Egypt. Afterward, the isolates were investigated for their ability to biosynthesize TiO_2NPs. The culture of *S. paucimobilis* B34 (grown for 36 h.) was the most producing strain, so it was used to biosynthesize TiO_2NPs according to the following steps. A known culture volume (25 ml) was diluted to 75 ml with sterile distilled water and the culture was allowed to grow for 24 h. Aliquot of $TiO(OH)_2$ solution (20 ml, 0.025 M) was added to the culture medium and the mixture was heated at 60°C for 10–20 min. on a steam bath until white material deposition started to appear, indicating the transformation of TiO_2 to nanoparticles. After the culture solution was cooled (12–48 h), distinct coalescent white clusters as TiO_2NPs were notably deposited at the bottom of the flask (Kirthi et al. 2011) which were then collected by centrifugation at 5000 rpm for 15 minutes. The same procedures were adopted for *S. platensis* culture to investigate its ability to produce TiO_2NPs.

Characterization of TiO_2NPs

The formation of the biosynthesized TiO_2NPs was followed thoroughly by recording its absorbance using BEAM UV/VIS-2700 spectrophotometer. The reduction of TiO_2 at 350-360 nm was monitored by measuring the UV-Vis spectra of the solution at regular intervals using 2 ml aliquot of the sample (Gaikwad et al. 2008). The size of nanoparticles was determined using X-ray diffraction analysis (XDR) (GNR APD-2000 PRO generator, diffract meter, one line reactor) with CuKα radiation line of λ=1.5405 A over a wide range of 2Θ Bragg angles (20-80°). The particles size was determined from the Debye Scherer's equation:

$$S = 0.9\lambda/\beta \cos \theta \quad (1)$$

where, S is the crystallite size. β is the full width of half maximum (FWHM) of a specific phase in radians, λ is the wavelength of incident rays (λ= 1.54A), θ is the Bragg's angle (the center angle of the peak in radian). The morphological feature of the particles was investigated by transmission electron microscope (TEM) model Joel, 100SX, Japan, with AMT digital camera. FT-IR spectra were recorded in a range of 4000–420 cm^{-1}, with 42 consecutive scans at 2 cm^{-1} resolution using FT-IR instrument model TENSOR27. The average number of atoms per nanoparticle of nanoTiO_2 and the surface area per gram of nanoparticles were determined as described in Liu et al. (2007).

Application of the biosynthesized TiO_2NPs for MG removal

MG is a cationic dye which is being used in many industries as a coloring agent (Kumar et

al. 2005). Its removal from aqueous solutions by the biosynthesized TiO_2NPs were performed following the batch mode technique using three Erlenmeyer flasks. The first flask contained 2 ml of TiO_2NPs solution, the second flask contained *S. paucimobilis* B34 culture suspension, and the third flask contained bacterial suspension mixed with TiO_2NPs solution in equal volumes. For each of these flasks, 1 ml of MG dye solution was added then the volume was completed to 20 ml by adding deionized H_2O. The initial concentrations of solutions were fixed at $1x10^{-3}$ mol/L for MG, 4.1 $x10^{-9}$ mol/L for TiO_2NPs solution and at 3.2 $x10^4$ CFU/ L for bacterial suspension. The reaction was studied at 30°C for 3h at pH 7 and 100 rpm agitation speed, and all experiments were carried out in triplicates. The absorbance of MG was recorded at λmax 617 nm by using UV-Vis spectrophotometer. The dye removal percentage (%) was expressed by equation (2) as:

$$\text{Removal percentage}(\%) = \frac{C_O - C_t}{C_O} \text{ X } 100 \quad (2)$$

where, C_o and C_t denote the initial concentration of the dye and its concentration after time t. The reaction kinetics of dye removal process was studied by applying the pseudo-first order and pseudo-second order kinetic models, respectively as shown by equations (3) and (4), respectively:

$$\ln\left(\frac{C_0}{C_0 - C_t}\right) = k_1 t \quad (3)$$

$$1/C_t = 1/C_0 + k_2 t \quad (4)$$

where, k_1 and k_2 are the rate constants of pseudo-first order (min^{-1}) and pseudo-second order ($Lmg^{-1}min^{-1}$), respectively (Salem et al. 2018). The correlation coefficients of the kinetic equations were estimated from fitting the obtained results to the tested two models by determination of nonlinear regression coefficients (R^2) and standard deviation (SD).

Factors affecting MG removal by TiO_2NPs

Effect of TiO_2NPs and bacterial suspension concentrations

The removal rate of MG was studied at fixed concentrations of both MG (2.5×10^{-5} mol/L) and bacterial suspension (3.2×10^4 CFU/L) while serial concentrations of TiO_2NPs were tested in the range of 1.0 - 4.1×10^{-10} mol/L. Furthermore, the change of MG removal rate was investigated at variable concentrations of bacterial suspension from 32 to 8 $x10^3$ CFU/L while keeping constant the concentrations of MG (2.5×10^{-5} mol/L) and of TiO_2NPxs (4.1×10^{-10} mol/L). Likewise, mixed solutions of the bacterial suspension and its TiO_2NPs solutions were tested for MG removal at serial concentrations of 32×10^3 CFU/L + 4.1×10^{-10} mol/L; 24×10^3 CFU/L + 3.1×10^{-10} mol/L; 16×10^3 CFU/L + 2.1×10^{-10} mol/L; and 8×10^3 CFU/L + 1.0×10^{-10} mol/L. All samples were analyzed spectrophotometrically at λmax of 617 nm for MG. All experiments were done in triplicates and the results were expressed as means ± standard deviation (SD).

Effect of MG concentration

A series of MG concentrations was prepared by dilution of the stock solution (1×10^{-3} mol/L). The effect of the initial concentration of MG on its removal rate was investigated at a serial dilution range of 5 to 1.5×10^{-5} mol/L, while keeping the concentration of TiO_2NPs and bacterial suspension solutions constant at 4.1×10^{-10} mol/L and 3.2×10^4 CFU/L, respectively. The decrease in MG absorbance and the removal percentage were determined as described.

Removal of TiO_2NPs-MG hybrid nanocomposite using *Spirulina platensis* biomass

After removing of MG from solutions using the bacterial biosynthesized TiO_2NPs, the ability of *S. platensis* biomass to eliminate the adsorbed TiO_2NPs-MG hybrid nanocomposite from water solution was studied. A batch experiment was conducted using three conical flasks: the first one contained 20 ml of *S. paucimobilis* B34 TiO_2NPs; the second contained 20 ml of *S. platensis* living culture and the third flask contained a mixture of 20 ml of TiO_2NPs + *S. platensis* culture, in equal volumes. The MG solution (10 ml) was then added to each flask and completed to a constant volume (100 ml) with distilled H_2O. All treated samples were analyzed for MG removal spectrophotometrically. The initial concentrations were fixed at 1×10^{-3} mol/L for MG, 4.1×10^{-9} mol/L for TiO_2NPs, and at 3×10^6 cells/L for *S. platensis* culture. The elimination kinetics of TiO_2NPs-MG hybrid nanocomposite was monitored at 0 time, 24 and 48 h, respectively. All experiments were repeated in triplicates.

TEM image of TiO_2NPs-MG hybrid nanocomposite and *Spirulina platensis* interaction

The transmission electron microscope (TEM) images were taken to explore the biosorption of TiO_2NPs-MG hybrid nanocomposite on *S. platensis* cells formed for the above three batch treatments. Fixation of the samples for 2 h in 2.5 % buffered glutaraldehyde in 0.1 M phosphate buffer solution (PBS) was carried out at pH 7.4 and 4°C. Then, the solutions in the flasks were washed three times with PBS (10 min. each) and post fixated in 1% Osmic acid for 30 min. The samples were then washed three times with PBS (10 min. each), followed by addition of ascending series of diluted ethyl alcohol and absolute alcohol (30, 50, 70, and 90 %) for 30 min. Dehydration of the samples was followed by infiltration with acetone for 1 h. For TEM imaging, the samples were embedded in Araldite 502 resin. The plastic molds were cut in the LEICA Ultracut UCT ultra-microtome and stained with 1% toluidine blue. After examination of the semi-thin sections, the ultra-thin sections of each sample were cut and stained with uranyl acetate. At this stage, samples were counter stained with lead citrate, examined and then photographed using JEOL-JEM-100 SX electron microscope, Japan.

Cytotoxicity assay

The toxicity of the resulting effluents after MG removal by using the previous three treatments (bacterial TiO_2NPs, *S. platensis* living culture and the mixture of both) was investigated on the germination of *Vigna radiate* L. seeds (Tiquia et al. 1996). Dried soil samples (120 g) were dispended in each of 15 boxes, saturated with distilled H_2O and left for two days. After centrifugation of TiO_2NPs-MG hybrid nanocomposite, 20 ml from each resultant effluent was added to each box. Additional 20 ml each of distilled H_2O and MG solution were added to the soil and assigned as +ve and -ve controls, respectively. Then, six seeds of *V. radiate* were sown in each box and allowed to grow for 2 weeks at 28±2°C under constant moisture content. The experiment was performed in completely randomized triplicates. The germination percentage and morphological criteria were recorded for the seedlings after 15 days of growth (Ismail et al. 2020).

RESULTS AND DISCUSSION

Biosynthesis of TiO_2NPs

TiO_2NPs biosyntheszed by *S. paucimobilis* B34 culture was evident with the lapse of 12–48 h as coalescent white aggregated clusters at the bottom of the culture flasks (Figure 1a).

This was confirmed by the appearance of a Plasmon resonance peak at 335 nm using UV-Vis spectrophotometer (Figure 1b). In TEM images, small TiO_2NPs were observed scattered in the extracellular material around *S. paucimobilis* B34 cells (Figure 2). On the contrary, *S. platensis* culture had no ability to biosynthesize TiO_2NPs since the absorbance peak was shifted to 210 nm and no white aggregates were formed (Figure 4).

Characterization of TiO_2NPs

The XRD patterns of the bacterial TiO_2NPs indicated the presence of intense diffraction peaks for NPs at the planes 101, 003, 004, 200, 105, 211, 204, 116 and 215 as illustrated in Supplementary Material - Figure S1. The corresponding Bragg's angles (2θ) were 25.23°(anatase form), 29.65° (rutile form), 37.20° (rutile form), 48.50°(rutile form), 55.34° (anatase form), 63.34° (anatase form), 65.26° (rutile form), 70.89° (anatase form) and 75.52°(anatase form) for TiO_2NPs. The peaks were identified by comparison with JCPDs file (No. 84-1286) according to 2θ angles values which confirmed the bio-synthesized TiO_2NPs. The main peak at 2θ of 25.82° matched with (101) anatase crystallographic plane structure which dominated the formation of TiO_2NPs. The size of the nanocrystals was determined from the Debye Scherer's equation (1), was found to be 15.55 nm (Figure S1). These results were in agreement with those obtained by Allam et al. (2019), Taran et al. (2018). Several explanations have been reported to describe the exact mechanism that leads to the extracellular formation of nanoparticles by microbial cells. The metal reduction by microorganisms' reductase enzymes was the most accepted mechanism (Kalimuthu et al. 2008). These extracellular enzymes have high redox activity as an electrons shuttle for metal reduction. According to Baker & Tatum (1998) the protienaceous surface layer (S-layer) of *Bacillus sp*. can play a key role in bacteria-metal interaction. Kirthi et al. (2011) stated that the formation of amide linkages between the *Bacillus subtilis* bacterial proteins and the TiO_2 were made during the reaction period giving rise to TiO_2NPs. Presence of biomolecules like peptides or carbohydrates produced by *Bacillus mycoides,* as part of their cell envelope, can support TiO_2 nanoparticles synthesis (Órdenes-Aenishanslins et al. 2014). These biomolecules can boost nucleation of the nanoparticles, and/or performing as capping and stabilizing mediators. The same mechanism may be proposed for the biosynthesis of TiO_2NPs by the presently studied *S. paucimobilis* B34.

Regarding the TEM results, the TiO_2NPs were spherical with an average particles size of 15.6 nm. They were dispersed in an extracellular matrix that surrounds the cells of *S. paucimobilis* B34 (Figure 2 a, b). As reported by Azeredo & Oliveira (2000), *S. paucimobilis* B34 strains have the ability to form exo-polymeric material outside their cells in the medium, which is mainly composed of polysaccharides, proteins, DNA and humic substances.

The FTIR of *S. paucimobilis* B34 TiO_2NPs showed a shift in the absorption band due to the binding of the amino group (NH), C-N group hydroxyl group (OH) and alkyl (CH) groups which were present either intracellular or extracellular of the bacterial suspensions during the formation of TiO_2NPs (Figure S2 and Supplementary Material -Table SI). It was reported that the amines linkages of proteins bind strongly with the metal, so that the proteins of the bacterial peptidoglycan layer may possibly form a coat covering the metal nanoparticles to prevent its agglomeration and stabilize the particles in the medium (Kirthi et al. 2011, Taran et al. 2018).

Furthermore, the molar concentration of TiO_2NPs and their specific surface areas (SSA) in

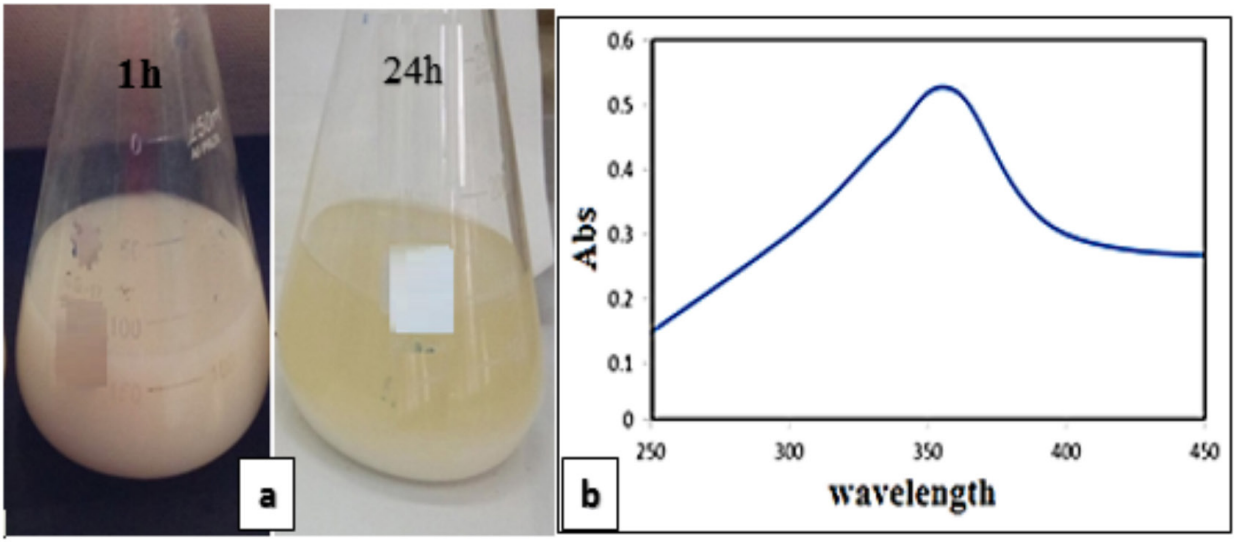

Figure 1. (a) Photo of *S. paucimobilis* cultures with 0.025 mol/L TiO_2 solution after 1 h and 24 h. (b) UV-Vis. absorption spectra of TiO_2NPs produced by *S. paucimobilis* after 24 h incubation period.

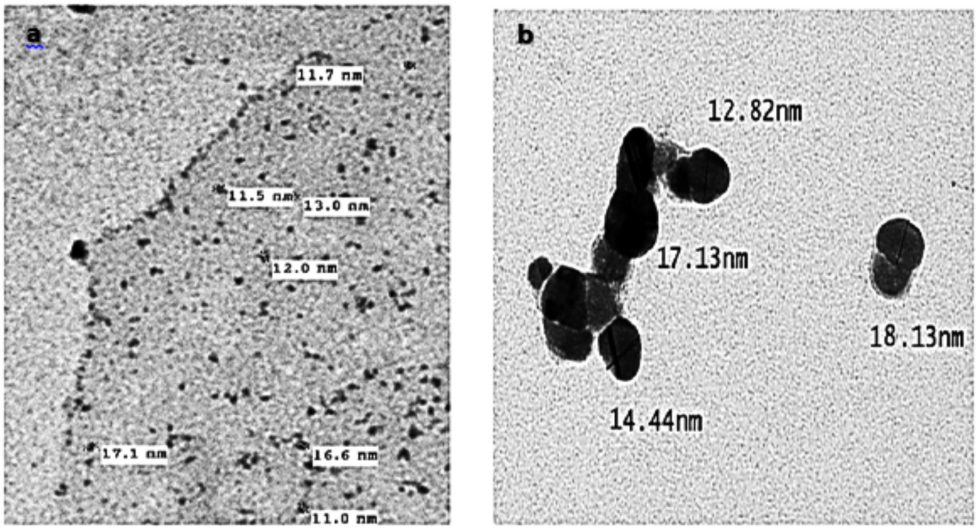

Figure 2. *S. paucimobilis* containing titanium nanoparticles (TiO_2NPs), a) at 100nm and b) at 500nm.

the initial solutions of *S. paucimobilis* B34 were calculated as described in a previous work by Allam et al. (2019). The molar concentration of TiO_2NPs was 4.1 x 10^{-8} (mol/L), the SSA was 67.71 (m^2/g) and its average diameter (D) was 15.55 ± 0.2 nm.

Removal of MG from wastewater using TiO_2NPs

Effect of TiO_2NPs concentration

Figure 3a represents the effect of variable concentrations of TiO_2NPs (1.0 - 4.1 x 10^{-10} mol/L) biosynthesized by *S. paucimobilis* B34 on the removal of MG from wastewater. The MG removal efficiency was found to be 70, 72, 74 and 83 % by increasing TiO_2NPs concentrations, respectively. As biosorbent, the removal efficiency recorded 42, 46, 50 and 56 % due the presence of increased concentrations of the bacterial suspension alone. A lower efficiency of 52, 65, 69 and 76 % was established when using the mixture of bacterial

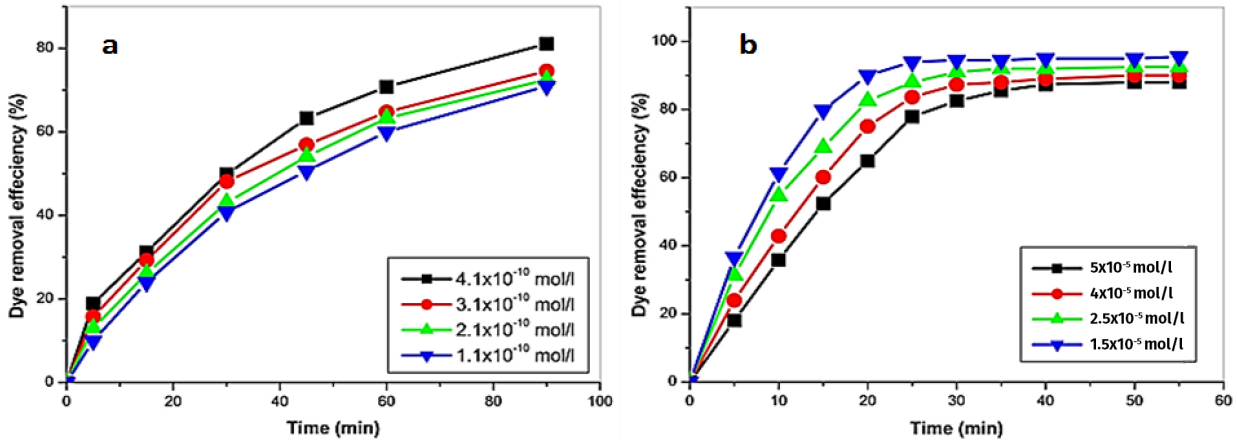

Figure 3. (a) Efficiency (%) of *S. paucimobilis* TiO_2NPs concentrations on MG (fixed at $2.5x10^{-5}$ mol/L) removal at 30 °C, (b) Efficiency (%) of MG concentrations on the removal process using *S. paucimobilis* TiO_2NPs (fixed at $4.1x10^{-10}$ mol/L).

suspension + TiO_2NPs as biosorbent. In general, as the concentration of TiO_2NPs increases, the removal efficiency of MG from solutions increases as well. The kinetics of biosorption is normally a significant parameter to describe how the adsorbate species (pollutant) interacts with the biosorbent material (nanoparticles) in the medium (Dotto et al. 2012). According to the obtained R^2 (regression coefficient) and k_{01} (rate constant) values, the removal process was fitted to pseudo first order kinetics as illustrated in Figure S3 (a, b, c and d) whereby *S. paucimobilis* B34 TiO_2NPs treatment recorded the best removal results (k_{01} =0.0123 min^{-1}) compared to the other two biosorbent treatments (Table SII). A glance to Table SII shows that the coefficient (R^2) values of pseudo-first order kinetics were higher than those of pseudo-second order values for all the tested three adsorbents types. This indicated that MG molecules have migrated from the bulk solution to the TiO_2NPs surface mainly with first-order kinetics behavior. The increase in the removal rate as a function of TiO_2NPs concentration may be attributed to the large surface area of these nanoparticles that accommodates more biosorption sites that interact with the MG molecules and giving rise to higher biosorption capacity. In contrast, at higher MG concentrations, the molecules are not quantitatively adsorbed onto the TiO_2NPs due to a saturation factor that leads to lowering of removal rate (Figure S3a). These results are consistent with the result reported by Allam et al. (2019), Hassan et al. (2017). According to Jyoti & Singh (2016) this biological methods for synthesis and application of nanoparticles offer an efficient, economic and eco-friendly approach that does not need any special conditions.

Effect of MG concentration

Figure 3b displays the effect of variable MG concentration on its removal efficiency (%) in the presence of constant concentration of TiO_2NPs (4.1×10^{-10} mol/L). The removal efficiency was 87, 89, 92 and 95 % at 5×10^{-5}, 4 x1 0^{-5}, 2.5×10^{-5} and $1.5x10^{-5}$ mol/L, respectively of MG serial concentrations. For the bacterial suspension treatment as biosorbent, the removal efficiency recorded 56, 63, 73, and 78 %, while the efficiency was 72, 76, 81 and 89 % for the mixture treatment (bacterial suspension+TiO_2NPs) at the same previous MG concentrations, respectively. It was observed that the removal efficiency decreased as the MG concentration increased. In addition,

the reaction exhibited first-order kinetics within the tested concentration range of MG (Figure S4 a, c). The decrease in the second-order rate constant with increasing MG concentration was given in Figure S4 (b, d). The values of the rate constants k_{o1} and k_{o2} at different MG concentrations were presented in Table SIII. The removal process was tailored well with pseudo-first order model as supported by R^2 values. Inspection of Table SIII reveals that *S. paucimobilis* B34 TiO_2NPs were more efficient as biosorbent for MG removal followed by the mixture (*S. paucimobilis* B34 culture suspension + TiO_2NPs) and then the culture of *S. paucimobilis* B34. Similar results were obtained by Allam et al. (2019) when used AgNPs biosynthesized by some bacterial stains cell free extract for MG removal.

Removal of TiO_2NPs–MG hybrid nanocomposite by *Spirulina platensis* biomass

As mentioned above, *S. platensis* was unable to biosynthesize the TiO_2NPs. Alternatively, when adding TiO_2 solution (white suspension) was added to *S. platensis* culture and left for 24 h, it was observed that *S. platensis* cells deposited the TiO_2 molecules at the bottom of the flask and the solution turned clear (Figure 4a). As shown in Figure 4b, the UV-Vis peak of TiO_2 was shifted to the range of 200 to 250 nm with no formation of white clustered nanoparticles. The peak intensity declined rapidly with increasing the incubation time to 48 h with *S. platensis* culture implying the chelation of the titanium metal by the cyanobacterial biomass and the solution turned clear. This observation was therefore, applied to investigate the capability of *S. platensis* culture to remove TiO_2NPs-MG hybrid nanocomposite from the solutions after MG has been removed by *S. paucimobilis* B34 TiO_2NPs. Inspection of Figure 5 (a and b), it was found that the removal efficiency reached 95.2, 75, and 89.43 % when TiO_2NPs, *S. platensis* living biomass and a mixture of both were respectively added to MG in solutions and incubated for 48 h. Although, the removal efficiency of MG using TiO_2NPs biosynthesized by *S. paucimobilis* B34 was higher than the efficiency caused by the mixture of *S. platensis* biomass + TiO_2NPs, yet it was environmentally feasible since applying *S. platensis* biomass enabled the removal of both TiO_2NPs-MG hybrid nanocomposite in a single step and still retain an efficiency of 89.43 %.

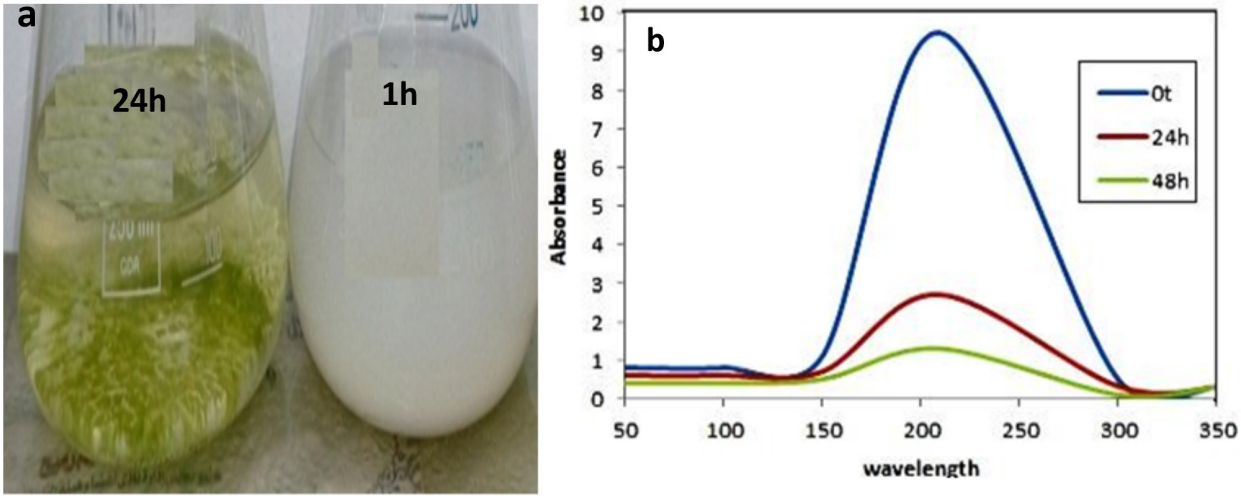

Figure 4. (a) Interactions between TiO_2NPs and *S. platensis* biomass after 1h and 24 h incubation periods, (b) UV-Vis absorption spectrum of TiO_2 after adding *S. platensis* culture after 0 h, 24 h and 48 h of incubation time.

The type of interaction between *S. platensis* cells and TiO_2NPs-MG nanocomposite was examined using TEM at 0, 1, and 24 h as depicted in Figure 6 (a, b and c). Initially at 0 time, no interaction between *S. platensis* and TiO_2NPs-MG hybrid nanocomposite was occurred (Figure 6a). After 24 h, increased association of nanocomposite with the cell surface was observed (Figure 6b). And at 48 h, TiO_2NPs-MG hybrid nanocomposite agglomerated on the cell surface and ruptured the cell wall causing entrance of particles into the cell with increasing incubation time (Figure 6c). Thus, a biosorption process may be responsible for the capability of *S. platensis* biomass to remove the TiO_2NPs-MG hybrid nanocomposite from the medium in a safe and dual goal manner. Similar results were reported by Ismail et al. (2020), Mahdieh et al. (2012). In his study, Dotto et al. (2012) reported that the removal of synthetic dyes occurred through physical biosorption means onto *S. platensis* microparticles while a chemosorption process was conducted with nanoparticles. In addition, the exposure of microalgae cells to various metals and nanoparticles induces cell oxidative stress that trigger the synthesis of bio-products like pigments, peptides, phytohormones, and polyphenols into their cultures as chelating and/or antioxidant agents (Miazek et al. 2015). According to Sendra et al. (2017), *Chlamydomonas reinhardtii* and *Phaeodactylum tricornutum* microalgae released exopolymeric substances as a defense mechanism into their culture medium that contains TiO_2NPs and this by boosted agglomeration and settling down of these nanoparticles. These findings justified the results of the present study by which *S. platensis* cells have removed the TiO_2NPs-MG hybrid nanocomposite. However, many studies reported that microalgae and cyanobacteria can biosynthesize nanoparticles (Dahoumane et al. 2017); yet negative effects of nanoparticles were also reported towards numerous freshwater and marine microalgae (Miazek et al. 2015). The inhibitory effects of nanoparticles were depending on their size, metal ions, composition of growth medium and age of culture suspension.

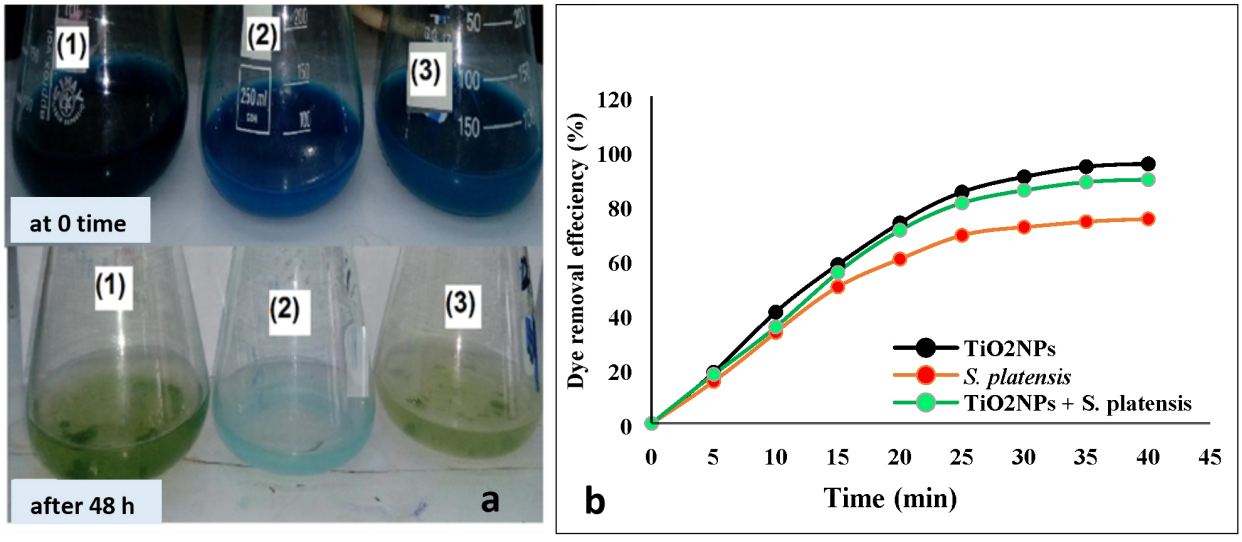

Figure 5. (a) Photo of the three treatments: (1) *S. platensis* culture suspension with MG, (2) *S. paucimobilis* TiO_2NPs with MG, and (3) *S. platensis* + TiO_2NPs with MG at 0 time and after 48 h. **(b)** Efficiency (%) of different biosorbent treatments on MG removal at constant concentration of $1x10^3$ mol/L, at 30°C after 48 h incubation period.

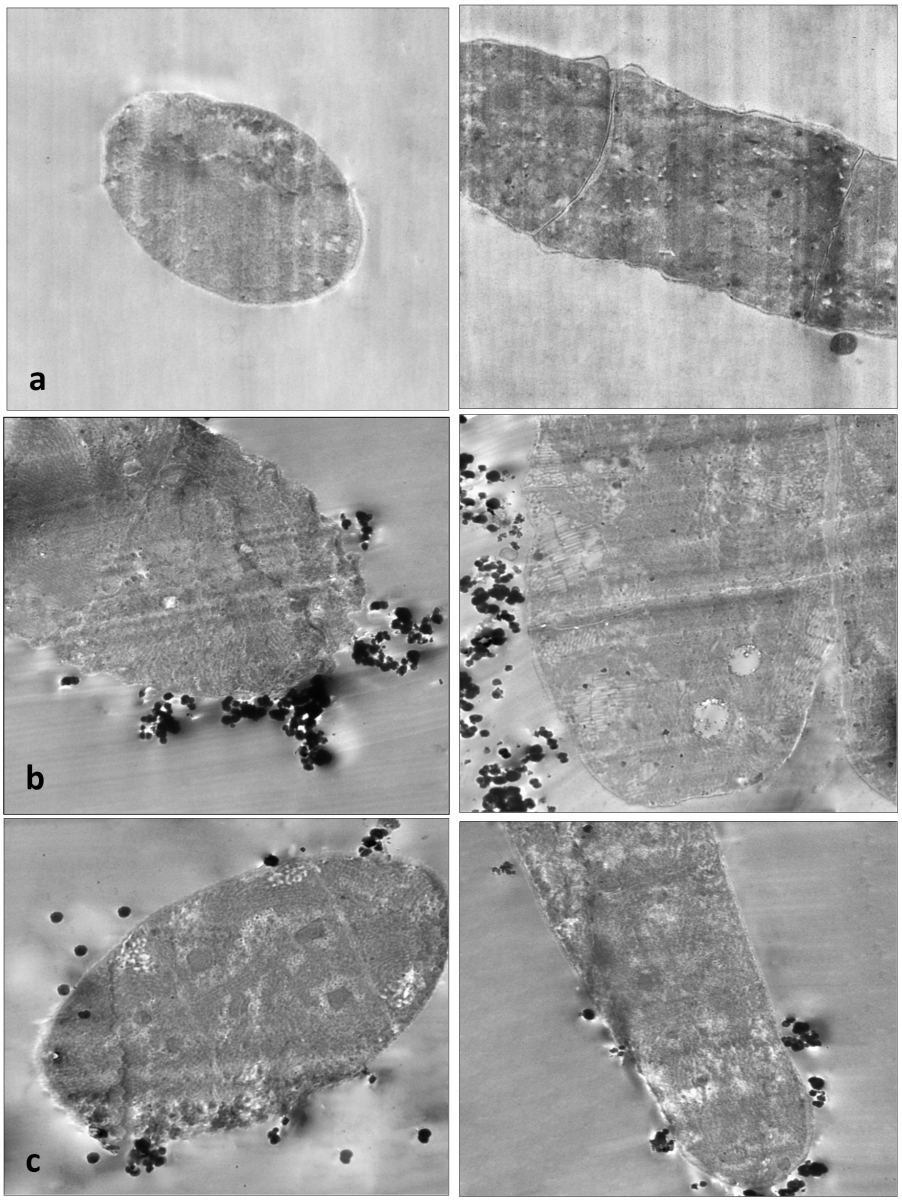

Figure 6. TEM images of cross (lift) and longitudinal (right) sections of *S. platensis* with a high degree of attached TiO_2NPs– MG at **a)** 0 h, **b)** 24 and **c)** 48 hrs.

In some cases, metal ions can also stimulate cyanobacteria and microalgae growth when released from nanoparticles into the medium (Pádrová et al. 2015).

Cytotoxicity assay of treated wastewater effluents

The cytotoxicity test performed on the seeds of *V. radiata* showed that MG solution (as -ve control) was toxic since its treated seeds exhibited only 40 % of germination (Table I). The seeds treated with water effluent produced after removal of MG from wastewater solutions with TiO_2NPs treatment (T1), exhibited a germination percentage of 67 %. Seeds treated with water effluent recovered from the treatment of wastewater with *S. platensis* living culture (T2) exhibited 80 % of germination. While the seeds treated with water effluent produced after MG removal by using TiO_2NPs + *S. platensis*

Table I. Germination percentage, shoot and root lengths of *V. radiate* seedlings treated with water effluents produced after MG removal using different biosorbent treatments*.

Parameters	Water	MG	Treatment 1	Treatment 2	Treatment 3
Germination %	100	40	67	80	93
Shoot (cm)	5^{a}±0.5	2.0^{b}±0.3	4.5^{a}±0.2	8.0^{b}±0.3	9.2^{c}±0.3
Root (cm)	4^{a}±0.3	1.5^{b}±0.2	3.5^{a}±0.2	6.2^{c}±0.5	7.3^{c}±0.5

*** Values are mean of three replicates ± SD. Different superlative letters on the same row indicated significant difference at *P*≤0.05.**

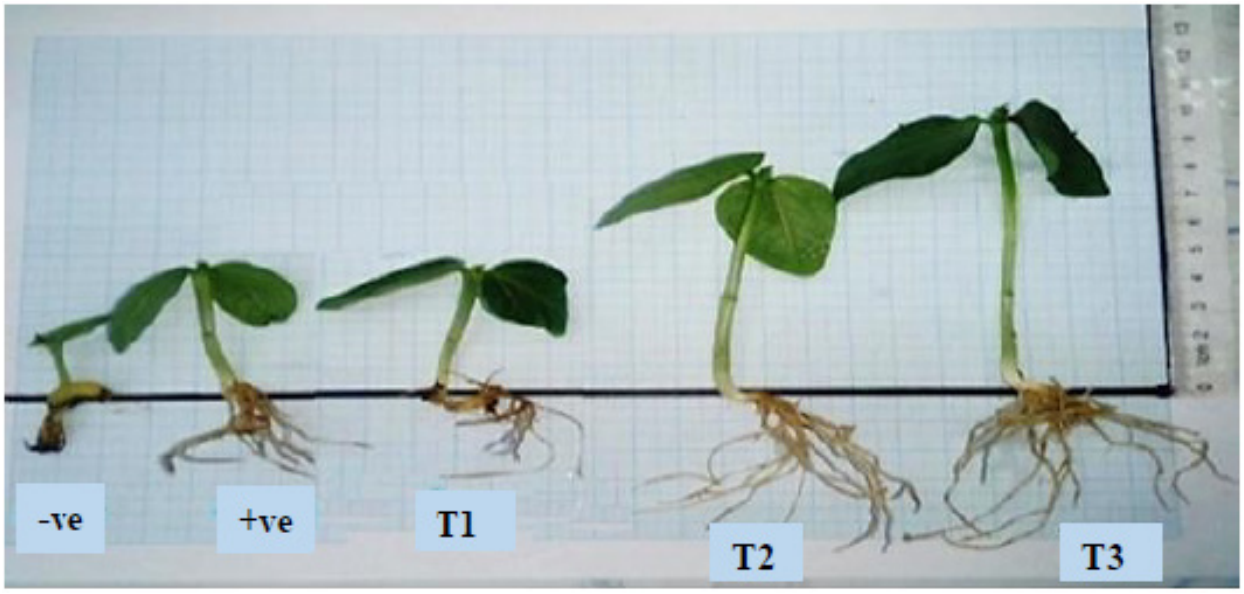

Figure 7. Cytotoxicity test for the water effluents produced after MG removal using different biosorbent treatments on *V. radiate* seedlings; -ve control (MG); +ve control (H_2O), Treatment 1= *S. paucimobilis* TiO_2NPs biosorbent, Treatment 2= *S. platensis* culture biosorbent, and Treatment 3= *S. platensis* culture +TiO_2NPs biosorbent.

living culture treatment (T3) exhibited 93 % of germination, indicating the safety of these effluents for agriculture irrigation. It is worth mentioned that the germination percentage and the morphological criteria of the seedlings were the best for the seeds exposed to treatment 3 particularly if compared with TiO_2NPs treatment. Figure 7 illustrates that roots of the seedlings germinated in treatment 3 were larger in number and longer in length into the soil indicating safe germination process (Tiquia & Tam 1998, Tiquia et al. 1996). The leaves were also greener in color and larger in surface area than those observed in the other treatments. In spite of the germination percentage, the measured values of shoot and root lengths were of insignificant difference from the water treatment (as +ve control). Although the TiO_2NPs were more efficient in MG removal from wastewater (Figure 5b), nevertheless the resulting effluent was less safe than the effluent recovered from treating wastewater with *S. platensis* biomass + TiO_2NPs for removing MG. Therefore, it is concluded that *S. platensis* biomass can safely play a dual role;

first, to eliminate the developed TiO_2NPs-MG hybrid nanocomposite as biosorbent from the aqueous medium after wastewater treatment and second, the resultant effluent will be nontoxic for agricultural applications.

CONCLUSION

In this study, *S. paucimobilis* B34 living culture was able to biosynthesize TiO_2NPs with appropriate small size. The developed TiO_2NPs could be effectively applied to remove MG from wastewater effluents with an efficiency up to 83 %. As a tool for safe environmental recycling, *S. platensis* living cyanobacterial cells were successfully used as biosorbent to remove the formed TiO_2NPs-MG hybrid nanocomposites from the recovered wastewater effluent with an efficiency of 89.43 % after treatment. The produced wastewater effluents proved nontoxic as reflected by the results of germination percentage and the morphological criteria of *V. radiata* L. seedlings. The obtained results indicated that biological nanotechnology of *S. platensis* biomass is an ecofriendly, low cost and effective route for decontamination approaches of wastewater to be reused in agriculture purposes. However, further experiments should be continued to assess the effect of these solutions on plants and soil biochemical composition and to evaluate the accumulation effect of the nano-metals in such ecosystems.

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SUPPLEMENTARY MATERIAL

Figures S1, S2, S3, S4
Tables SI, SII, SIII

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