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RESEARCH

Enhancement of growth and bioremediation potential of *Chlorella vulgaris* by silicon nanoparticles

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Abstract

The effects of silicon oxide nanoparticles (SNPs) on the growth of *Chlorella vulgaris* and its influence on biodegradation of pyrene (PYR) were evaluated. Addition of SNP to culture media at low concentrations (0.5–5 mg L^{-1}) showed beneficial effects on growth and biomass of *C. vulgaris*. SNP at 1 mg L^{-1} showed 13% enhancement in biomass during 7 days of cultivation of *C. vulgaris*. The total chlorophyll content and PSII activity were highest in 1 mg L^{-1} SNP. We observed the toxicity mitigating aspect of SNPs (1 mg L^{-1}) when algae were grown in a medium containing PYR (5 mg L^{-1}). Biodegradation of PYR by *C. vulgaris* was significantly improved (31%) in the presence of 1 mg L^{-1} SNP in 5 days. The study of enzyme activity demonstrated that catechol 2,3 dioxygenase activity, which has an important role in the biodegradation of PYR, was upregulated in algal cells treated with SNP + PYR, whereas dehydrogenase activity which is also responsible for degradation of organic chemicals, was unaltered. This result proves the role of SNP in PYR biodegradation capacity of *C. vulgaris* through involvement of the dioxygenase pathway. Current findings suggest the possibility of SNP addition to the microalgal media to improve algal growth as well as PYR bioremediation capacity.

Keywords Chlorophyceae · Nanoparticle · Biodegradation · *Chlorella vulgaris* · Pyrene · Silicon nanoparticle (SNP)

Introduction

Microalgae have been recognized as a potential source of biofuels owing to their high lipid content, fast growth rates, and ability to grow in a wide range of environmental conditions (Sharma et al. 2012; Dolganyuk et al. 2020). Additionally, microalgae have the ability to bioremediate organic pollutants in water bodies. Algae can use organic pollutants, such as hydrocarbons and aromatic compounds, as a carbon source for growth (Takáčová et al. 2014; Baghour 2019; Asghari et al. 2020; Ghodrati et al. 2022; Touliabah et al. 2022). This makes them useful for bioremediation as they can remove pollutants from water while also producing biomass that possibly can be used for biofuel production. Some algae can also grow mixotrophically meaning that they can use both organic and inorganic sources of carbon for growth. This flexibility allows them to thrive in a wide range of environmental conditions making them an attractive option for bioremediation (Tomar and Jajoo 2021; Tomar et al. 2022a, b). However, further research is needed to optimize the use of algae for bioremediation and to develop cost-effective and more sustainable methods for this purpose.

Recently, nanoparticles (NPs) are emerging as an effective tool to improve the feasibility of microalgae-based bioremediation of inorganic and organic pollutants (Sendra et al. 2018; Xiong et al. 2020; Ahn et al. 2022). While there has been significant research on the potential toxicity of nanoparticles to microalgae (Sadiq et al. 2011; Sendra et al. 2018; Hassanpour et al. 2020), it is also important to explore the potential benefits of using nanoparticles to improve microalgal growth and enhance their bioremediation capabilities. Several studies have reported that nanoparticles enhance the bioremediation capabilities of microalgae. Xiong et al. (2020) highlighted the potential of nanoparticles, such as cerium oxide, to enhance the microalgal degradation of sulfonamides. Cerium oxide nanoparticles have antioxidant properties and can scavenge free radicals, which explains their ability to enhance the activity of

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the sulfonamide-degrading enzymes in the microalgae. Luo et al. (2018) demonstrated that exposure to titanium dioxide nanoparticles could enhance the bioavailability and methylation of inorganic arsenic in two freshwater algae species. CeO_2 nanoparticles also alleviated the negative effects of erythromycin on the growth and effective quantum yield in *Chlamydomonas reinhardtii* (Sendra et al. 2018). Ahn et al. (2022) reported that presence of aluminum oxide nanoparticles promotes bioremediation of sulfacetamide by the freshwater microalga, *Scenedesmus obliquus*. Aluminum oxide nanoparticles enhanced the removal of heavy metals by *S. obliquus* and have been shown to increase the production of glutathione, an antioxidant that can help to protect the microalgae from the toxic effects of heavy metals (Li et al. 2016). Therefore, NPs can be used to enhance the growth of microalgae by acting as fertilizers or delivery agents for nutrients, such as nitrogen and phosphorus (Vargas-Estrada et al. 2020). Moreover, they can also improve the uptake of organic pollutants by microalgae by increasing their surface area and providing more binding sites for pollutants (Pena et al. 2006; Li et al. 2016).

In this study silicon oxide (SiO_2) nanoparticles (SNPs) were used to investigate their effect on the growth and bioremediation capacity of *C. vulgaris* for the polycyclic aromatic hydrocarbon (PAH), pyrene (PYR). PAHs are known to be resistant to conventional treatment processes such as physical absorption, coagulation and chemical processes (Cobas et al. 2014; Rubio-Clemente et al. 2014; Tomar et al. 2019). Moreover, these processes have several disadvantages such as high cost and environmental pollution (Dong et al. 2015; Diao et al. 2019). Therefore, the application of nanoparticles can be a promising pathway to improve bioremediation efficiency of microalgae for PAHs.

Nanoparticles can affect the growth and photosynthesis of microalgae which depends on many factors. Therefore, in the present study, optimization of SNPs concentration was evaluated by measuring algal growth, biomass yield, and pigments. Later, using optimum concentration of NP, remediation of PYR was carried out and a probable mechanism has been proposed. To our knowledge, this is the first study to investigate the influence of SNPs on the bioremediation of PYR by microalgae. Understanding the mechanisms by which nanoparticles affect microalgae can provide insights into how to optimize their use in other applications, such as wastewater treatment and bioproduction. This research will lead to obtain more sustainable and efficient methods for bioremediation of PAHs, which will have significant benefits for the environment.

Materials and methods

Chlorella vulgaris was obtained from the Phycospectrum Environmental Research Centre, Chennai, India. The cells were cultured in BG11 (Himedia, M1958) medium in an environmental chamber with 16 h daytime cycle with intensity of 100 µmol photons m^{-2} s^{-1} at 25 °C on a shaker (100 rpm). Upon reaching the exponential growth phase the cells were harvested by centrifugation at 2500 ×*g* for 5 min for further experimentation.

Characterization of SiO_2 nanoparticles (SNPs) and suspension preparation

SNP were obtained from Nano Research Lab Jamshedpur, India. According to the manufacturer, the nanoparticles obtained were 99.9% pure. The manufacturer carried out characterization of SNP using UV–Vis spectroscopy, transmission electron microscopy and X-ray diffraction and were found to be nearly spherical and in agglomerated form with particle size in the range of 20–30 nm. SNPs (100 mg L^{-1}) were prepared freshly by dispersing the particles in BG-11 medium using ultrasonication by running three cycles at (400-500W) of 30 s each.

Experimental setup

For each treatment, 3 conical flasks (250 mL), containing 150 mL culture medium were autoclaved. Different concentrations of SNPs (0.5, 1, 2, 5, 10, and 15 mg L^{-1}) were added to the culture medium, while PYR solution was spiked into the flasks from a 10 g L^{-1} stock solution of pyrene (in acetone) to obtain a concentration of 5 mg L^{-1}. Flasks without the addition of PYR and NaCl served as control. The experimental conditions were maintained as mentioned above. After 5th and 7th day of culture triplicate samples of each treatment were collected for analysis.

Determination of growth, biomass productivity and photosynthetic pigments

Microalgae growth was assessed by measuring the optical density (OD) at 680 at 24 h intervals. To determine the dry biomass, 80 mL of culture was dried in pre-weighed centrifuge tube in a hot air oven at 80°C for 4–6 h. The growth rate (day^{-1}) was calculated using the following equation:

$$GR(day^{-1}) = (\ln N_2 - \ln N_0)/(t_2 - t_0)$$

where N_2 is the OD at time t_2 and N_0 is the OD at time t_0 (day 0).

The pigment content was determined according to the method outlined by Dere et al. (1998). In brief, 5 mL of culture was centrifuged at 5000 ×*g* for 5 min. The supernatant was discarded and 5 mL of 99.9% methanol was added to the pellet, mixed properly and incubated at 90ºC for 5 min. The culture was centrifuged at 9000 ×*g* for 5 min and the supernatant was used for pigment estimation.

Determination of PSII reaction center energy distribution parameters

Measurements of the quantum yields of energy conversion in PSII were carried out through saturation pulse technique, using a pulse amplitude modulated device (Dual PAM-100, Walz, Germany) as described by Tomar et al. (2022). The algal sample was dark adapted for 30 min at 23 ± 2°C before measurements and then an induction curve was measured. A weak modulated light (12 µmol photons m^{-2} s^{-1}) was given to get minimal fluorescence (F_0), followed by actinic light (53 µmol photons m^{-2} s^{-1}), and saturating pulse (SP) (6000 µmol photons m^{-2} s^{-1}) to measure maximum fluorescence (F_m). After determination of F_0 and F_m, the induction curve was analysed using Dual PAM-100 software. The induction curve was recorded with SP for 5 min to achieve the steady state of the photosynthetic apparatus and then the actinic light was turned off.

Determination of proline, MDA and total polyphenol content

Proline was extracted using 3% sulfo-salicylic acid and estimated using L-proline as a standard as described in Bates et al. (1973). Briefly, harvested fresh algal biomass was homogenized in 3 mL of 3% sulpho-salicylic acid and then centrifuged at 9000 ×*g* for 10 min. Supernatant (1 mL) was heated with 1 mL of ninhydrin and 1 mL glacial acetic acid at 100ºC for 1 h. Proline was quantified spectrophotometrically at 440 nm by use of a standard curve of L-proline. For the determination of lipid peroxidation, microalgal cells were harvested by centrifugation at 9000 ×*g* for 10 min. The cell pellet was homogenized in 2 mL 10% trichloroacetic acid (TCA) and centrifuged. 1 mL supernatant was mixed with 1 mL of 0.65% thiobarbituric acid (TBA) prepared in 20% TCA solution containing 0.01% BHT and heated at 95ºC for 25 min. After cooling to room temperature the content was centrifuged at 9000 ×*g* for 10 min and the absorbance of the supernatant was read at 532 nm and 600 nm. MDA content was calculated using the following equation (Chokshi et al. 2017):

$$\text{MDA (µmol mg}^{-1}\text{DW)} = 6.45(\text{OD}_{532} - \text{OD}_{600})/\text{DW}$$

Total phenolics were colorimetrically determined as described by Lopez et al. (2011) with minor modifications. Cells were harvested and homogenized in 2 mL 80% methanol. The homogenate was centrifuged at 5000 ×*g* for 10 min. The extract was mixed with Folin–Ciocalteu reagent and distilled water in a 5:1:5 ratio and allowed to stand at room temperature for 10 min. A 1 mL sodium bicarbonate solution (2%) was added to the mixture. After incubation overnight in the dark at room temperature the absorbance was measured at 750 nm. Total phenolics were quantified by calibration curve using gallic acid standard solutions (25–150 µg mL^{-1} in 80% methanol). The results were calculated as gallic acid equivalent (GAE) g^{-1} dry weight of microalgae.

Determination of activity of enzymes

Algal cells harvested and centrifuged at 5000 ×*g* for 5 min. The cell pellet was then washed twice with 5 mL buffer (20 mM sodium-phosphate, pH 7.5) and transferred to 5 mL of extraction buffer. The cells were crushed in liquid nitrogen and centrifuged at 15,000 ×*g* for 25 min at 40 °C. The resulting supernatant was collected and used as the crude extract for the subsequent enzyme activity assays.

Superoxide dismutase (SOD) activity was determined by the reduction rate of nitroblue tetrazolium (NBT) according to Beauchamp and Fridovich (1971). Peroxidase (POD) and catalase (CAT) activities were determined by following the method described by Zhang et al. (2007). Catechol 2,3-dioxygenase activity was determined using the method of Semple and Cain (1996). Dehydrogenase activity was determined as per the method of Xie et al. (2008).

PYR sampling and analysis from the medium

The algal cells were removed from the medium by centrifugation at 1000 ×*g* for 5 min. The supernatant was then extracted using ethyl acetate (HPLC grade, Sigma Aldrich) in a separating funnel, with three extractions performed and further concentrated using a vacuum rotary evaporator. The extract was analyzed spectrophotometrically (250–380 nm) and the percentage reduction was calculated (El-Sheekh et al. 2012).

Statistical analysis

Graphpad Prism 5.01 software was used for data analysis. One-way analysis of variance (ANOVA) followed by Newman-Keuls Multiple Comparison Test was used. Significance was determined at $P < 0.001$ and results expressed as mean values ± SD. All the experiments were done five times in triplicates (biological replicates were 5 and analytical replicates were 3).

Results

Growth response of *C. vulgaris* with SNP

To determine the optimum concentration of SNPs algal growth response induced by different concentrations of SNPs was analyzed (Fig. 1). The growth of *C. vulgaris* was

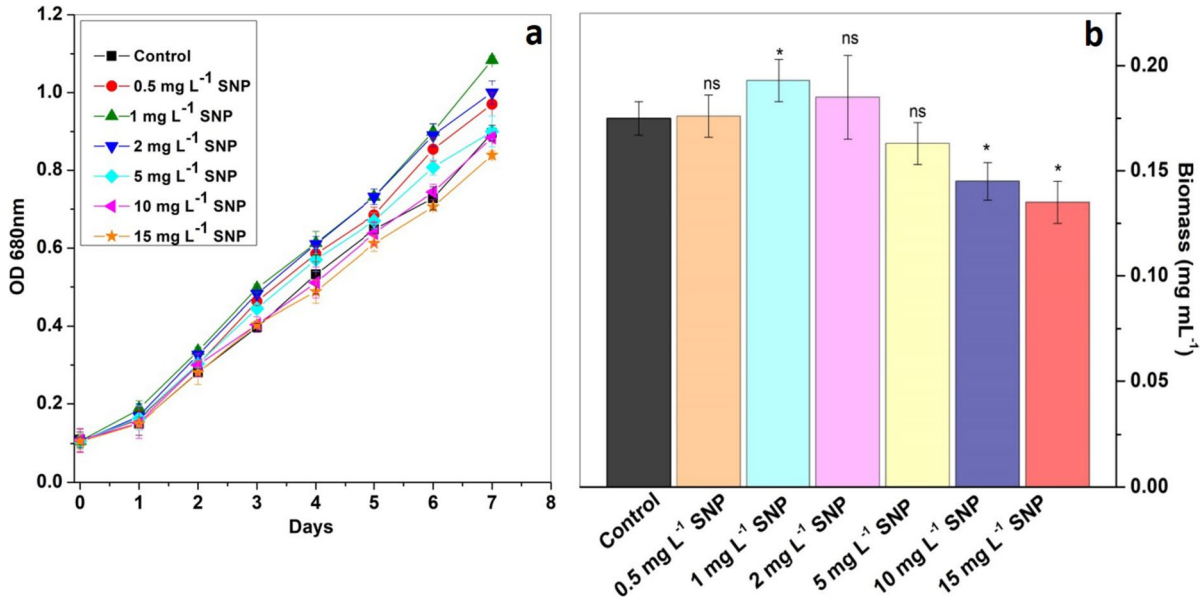

Fig. 1 Effect of different concentrations of SNPs on the growth of *C. vulgaris* (**a**) effect on growth curve from 0–7 days, (**b**) effect on biomass on 7th days of incubation

significantly influenced in a dose-dependent manner after exposure to different concentrations (0.5, 1, 2, 5, 10, and 15 mg L^{-1}) of SNPs for 7 days (Fig. 1a). Increased growth of *C. vulgaris* was observed in 0.5 to 5 mg L^{-1} SNP concentration (0.112 in control and 0.123, 0.139, 0.127, 0.113 OD_{680} day^{-1} in 0.5, 1, 2, 5 mg L^{-1} SNP respectively), however a further increase of SNP concentration, led to a decrease in relative algal growth (0.104 and 0.09 OD_{680} day^{-1} in 10 and 15 mg L^{-1} SNP respectively). The biomass concentration of *C. vulgaris* in the control culture was 0.175 mg mL^{-1} and with 0.5, 1, 2, 5, 10, and 15 mg L^{-1} SNPs it was 0.175, 0.195, 0.185, 0.173 0.145 and 0.135 mg mL^{-1}, respectively, after 7 days of cultivation (Fig. 1b). Following these observations, we excluded concentrations of 2, 10, and 15 mg L^{-1} of SNP from subsequent experiments.

Table 1 depicts the effect of the selected concentrations of SNPs (0.5, 1, and 5 mg L^{-1}) on photosynthetic pigment content of *C. vulgaris*. The highest concentration of total chlorophyll (8.3 µg mg^{-1} DW) was achieved with 1 mg L^{-1} SNP concentration, which is in accordance with the increase in growth rate and biomass of cells. In contrast, 1 mg L^{-1} SNP treated cultures exhibited higher concentrations of total carotenoid content and carotenoid/total chlorophyll ratio as compared to control culture cells. Table 2 shows the information about Chl *a* fluorescence parameters, the Y(II)-quantum yield of PSII, Y(NO)-yield of non-regulated energy dissipation and Y(NPQ)-yield of regulated energy dissipation in algal cells during the course of induction curve. It was found that *C. vulgaris* cell treated with SNP showed a significant increase in Y(II) and ETR(II) (electron transport rate of PSII) and a parallel decrease in Y(NPQ) and Y(NO) (Table 2). The efficiency of linear electron transport rate in PSII (ETRII) showed a prominent increase in SNP inoculated algal cells as compared to control algal cells. On the basis of these results, it is concluded that 1 mg L^{-1} SNP is the optimum concentration for *C. vulgaris* growth, therefore, this concentration was used for impact on PYR toxicity and bioremediation.

Table 1 Effect of SNPs on various photosynthetic pigments (µg mg^{-1} DW) of *C. vulgaris* during 7 days of cultivation. Data represents the mean of three replicates, each consisting of five sets. The values are expressed as mean ± standard deviation (n = 5). Newman-Keuls multiple comparison test was performed with a significance level of $p < 0.001$

Treatments	Chl *a*	Chl *b*	C_{x+c}	Total Chl	C_{x+c} /Total Chl	Chl *a/b*
Control	4.80 ± 0.07	2.70 ± 0.08	0.65 ± 0.03	7.50 ± 0.15	0.086 ± 0.005	1.76 ± 0.02
0.5 mg L^{-1} SNP	4.90 ± 0.04 ^{ns}	2.80 ± 0.06 ns	0.68 ± 0.05 ns	7.70 ± 0.10 ^{ns}	0.088 ± 0.002 ^{ns}	1.75 ± 0.02 ^{ns}
1 mg L^{-1} SNP	5.30 ± 0.03 **	2.98 ± 0.03 **	0.77 ± 0.07 **	8.30 ± 0.08 **	0.092 ± 0.003 *	1.77 ± 0.02 ns
5 mg L^{-1} SNP	4.90 ± 0.03 ^{ns}	2.76 ± 0.04 ^{ns}	0.72 ± 0.05 *	7.60 ± 0.09 ^{ns}	0.093 ± 0.002 *	1.77 ± 0.01 ^{ns}

Table 2 Effect of SNPs on PSII reaction center energy distribution parameters of *C. vulgaris* during 7 days of cultivation**.** Data represents the mean of three replicates, each consisting of five sets. The values are expressed as mean ± standard deviation (n = 5). Newman-Keuls multiple comparison test was performed with a significance level of $p < 0.001$

Parameters	Control	0.5 mg L^{-1} SNP	1 mg L^{-1} SNP	5 mg L^{-1} SNP
ETR (II)	13.0 ± 1.6	14.0 ± 1.0 ^{ns}	15.8 ± 0.7 *	15.5 ± 0.5*
Y(II)	0.539 ± 0.090	0.575 ± 0.025*	0.616 ± 0.028***	0.601 ± 0.017**
Y(NO)	0.283 ± 0.015	0.284 ± 0.025 ^{ns}	0.246 ± 0.016*	0.263 ± 0.024 **
Y(NPQ)	0.176 ± 0.024	0.141 ± 0.017*	0.128 ± 0.015***	0.137 ± 0.028**

Effect of SNP on antioxidant properties of *C. vulgaris* during PYR toxicity

Algal cells have the ability to produce antioxidants during adverse conditions. Proline accumulation is a common response to stress in microalgae. In our study proline content was 45% less in the 1 mg L^{-1} SNP treated cells compared to control cells (Fig. 2). Interestingly, the change in proline content in PYR treated cells was non-significant, moreover, it decreased in SNP + PYR treated cultures (29%) compared to the control. With respect to control cells, total polyphenolic content was unchanged in SNP treatment, however it decreased in PYR treated culture (38%) and in SNP + PYR treatment (37%) (Fig. 2a).

The treatment with SNP led to a decrease in MDA levels by 15% compared to control cells (Fig. 2a). In contrast, treatment with PYR resulted in an increase of 72% in MDA content (Fig. 2a), indicating a higher lipid peroxidation in cells during PYR toxicity. However, in the presence of SNP along with PYR, the increase in MDA content was less compared to PYR treated cells (18%) (Fig. 2a).

The response of antioxidant enzymes in control, SNP, PYR and SNP + PYR treated cells are shown in Fig. 2b. The relative activity of superoxide dismutase (SOD) was not affected by PYR exposure, while the activity of peroxidase (POD) and catalase (CAT) was higher in cells under PYR treatment, suggesting an upregulation of these antioxidant enzymes in response to increased production of reactive oxygen species (ROS). However, the presence of SNP has a positive effect on the cells by increasing the activity of POD enzymes and reducing the MDA content during PYR toxicity.

Effect of SNP on removal efficiency of *C. vulgaris* for PYR

PYR uptake from media by the algal cells was studied with 5 mg L^{-1} concentration by absorption spectra. Figure 3a displays the absorption spectra of PYR extracted in ethyl

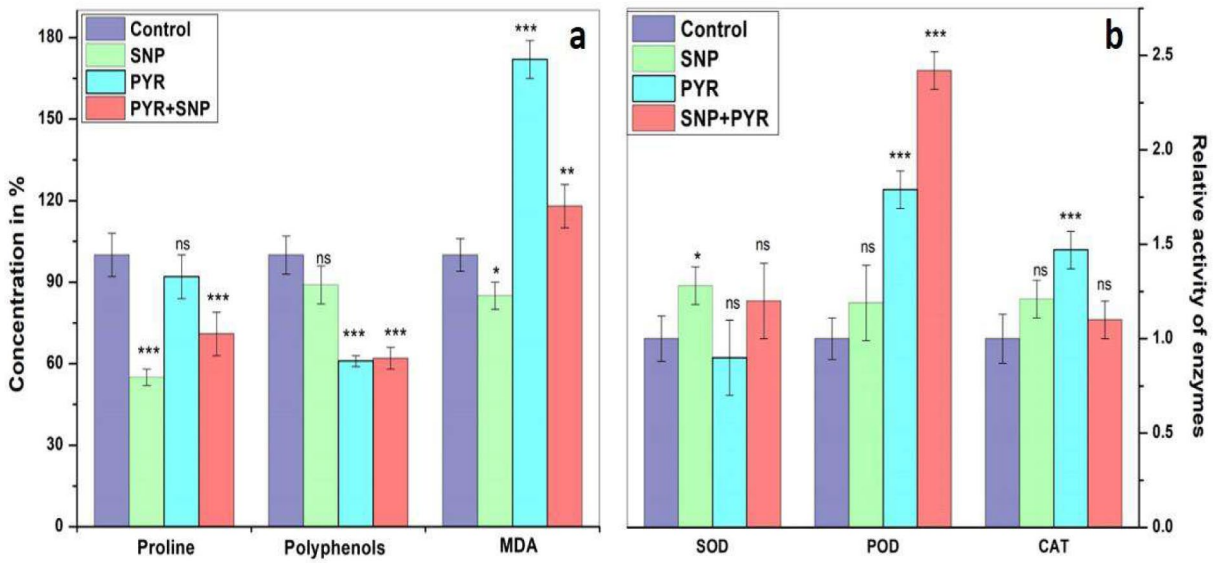

Fig. 2 **a**) Effect of different treatments on proline, polyphenols and MDA content, **b**) Activity of SOD, POD, and CAT in *C. vulgaris* cells after 5 days of cultivation. Error bars represent standard deviation ($n = 5$). Different *, **, ***, indicate significant differences ($p < 0.05$, $p < 0.01$ *and* $p < 0.001$ respectively) between the control and treatments

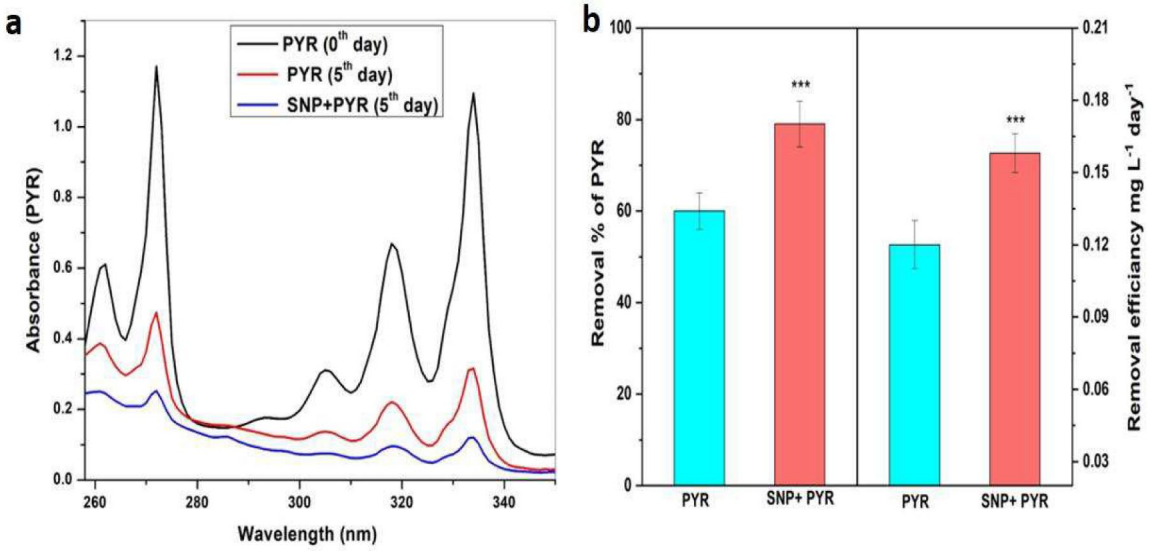

Fig. 3 **a**) Absorption spectrum of PYR extraction from culture media of *C. vulgaris* in PYR and SNP + PYR treatments. **b**) The percentage removal of PYR and removal efficiency of *C. vulgaris* cells after 5th day of incubation in PYR and SNP + PYR treatments. Error bars represent standard deviation ($n = 5$). Different *, **, ***, indicate significant differences ($p < 0.05$, $p < 0.01$ *and* $p < 0.001$ respectively) between the control and treatments

acetate from different treatments of media in the beginning (0th day) and on the 5th day. In our previous study (Tomar et al. 2022), we observed that in media containing the PYR only, there was negligible elimination of PYR. There was 59% removal of PYR observed through algal cells from culture media after 5 days of cultivation (Fig. 3b). Addition of 1 mg L^{-1} SNPs significantly enhanced the removal of PYR, resulting in 80% removal of PYR. Absorbance spectra of PYR in media indicated that when algal cells were grown in the presence of SNP + PYR, the uptake of PYR increased significantly. *Chlorella vulgaris* removal efficiency of PYR was 0.12 mg L^{-1} day^{-1} and 0.158 mg L^{-1} day^{-1} in PYR and SNP + PYR treatments, respectively.

To determine the effect of SNP on PYR degrading enzymes in *C. vulgaris* cells, dehydrogenase and catechol 2,3-dioxygenase (C2,3D) activity was measured. To measure the activity of dehydrogenase in response to different treatments, we used TTC (2,3,5- triphenyl tetrazolium chloride) as an artificial hydrogen acceptor. SNP did not show any significant effect on dehydrogenase activity, however, a drop of 67% and 65% was observed in PYR and SNP + PYR treated cells, respectively (Fig. 4). Crude extract of the enzyme from algal cells was used to examine the activity of C2,3D enzyme. The activity of C2,3D enzyme was not altered in SNP treated cells whereas the relative activity of C2,3D was increased by 110% and 190% in PYR and SNP + PYR treated cells, respectively (Fig. 4). The activity of C2,3D increased rapidly after the addition of SNP under PYR toxicity. The observed increase in enzyme activity coincided with a significant decrease in the concentration of PYR in the media in the presence of SNP.

Discussion

In this study we employed SNPs to enhance the growth and biodegradation efficiency of *C. vulgaris*. We observed that lower concentrations of SNPs (0.5 to 5 mg L^{-1}) positively influenced the growth of the algal cells. However, increasing SNP concentrations beyond this range had detrimental effect on cell growth and biomass, indicating the onset of toxicity. The reduced growth and biomass due to higher concentrations of NPs could be because of the "shading effect" which may limit the light absorption and reduction in photosynthetic productivity (Kang et al. 2014; Ren et al. 2020) or the metal effect which affected cell proliferation, a process that involves the inhibition binding of the metal to sulfhydryl groups (Dauda et al. 2017). Moreover, the adsorption of NPs in higher concentrations potentially resulted in insufficient nutrient uptake by the algal cells, consequently leading to reduced lipid productivity, which could be a reason for the suppression of cell growth leading to a reduction in overall algal biomass (Wang et al. 2016a; He et al. 2017). Thus, the results showed that 10 and 15 mg L^{-1} SNPs in the culture medium had considerable negative effects on *C. vulgaris* cells and 0.5, 1, 2, and 5 mg L^{-1} SNP concentrations were beneficial concentrations for *C. vulgaris* cultivation.

Further, we examined the photosynthetic aspects of *C. vulgaris* cells in response to various concentrations of SNPs.

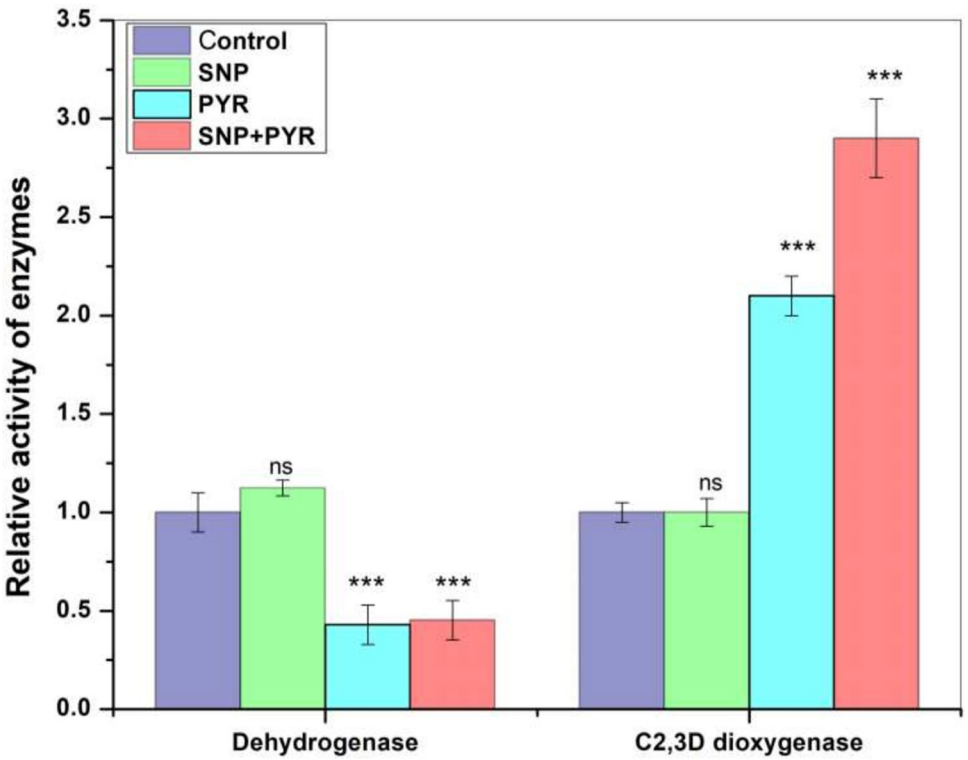

Fig. 4 Effect of different treatments on activity of dehydrogenase and C2,3D dioxygenase in *C. vulgaris* cells. Error bars represent standard deviation ($n = 5$). Different *, **, ***, indicate significant differences ($p < 0.05$, $p < 0.01$ *and* $p < 0.001$ respectively) between the control and treatments

Monitoring photosynthetic pigment content is crucial for assessing the influence of external factors on algal cells. An increased total chlorophyll content and other pigments was observed in the presence of SNPs. Application of SNPs may induce significant shear forces between the cells and NPs, which could potentially be perceived as a nutrient competitor by the cells (Zhang et al. 2013). This phenomenon poses a threat to the cells, as they may rapidly take up nutrients, thereby promoting chlorophyll synthesis in algal cells. Consequently, this could enhance cell proliferation, leading to better biomass production.

Chl *a* fluorescence, as recorded by PAM induction curve is a valuable technique for evaluating the effect of any treatment on PSII activity in intact algal cell cultures. This technique provides insights into the energy distribution parameters of PSII reaction centre, the efficiency of photosynthetic energy conversion and the partitioning of absorbed excitation energy between photochemical and non-photochemical processes (Mathur et al. 2019; Jain and Jajoo 2020; Rai-Kalal et al. 2021a, b; 2022). Y(II), the fraction of energy that is photochemically converted in PSII, increased in all SNPs concentrations, indicating enhanced PSII photochemistry. The remaining fraction, 1-Y(II), reveals the total quantum yield of all "loss" processes, which can be used to evaluate energy dissipation modes within PSII. Induction curve measurements of Y(NPQ) and Y(NO) provide further information about energy optimization mechanisms under specific conditions (Sekulska-Nalewajko et al. 2019) were decreased in SNPs treatments. This indicates that addition of SNP in the growth medium could favour photosynthesis activity of the algae. Exposure to low concentrations of SNP can activate the photosynthetic receptor system, leading to an induction of cell growth. This growth is directly correlated with an increase in the quantum yield efficiency of PSII, as reflected by an increase in F_m. In plants the photosynthetic rate is positively associated with the quantum yield of PSII (YII), which is related to the fraction of active PSII reaction centers (Mathur et al. 2019; Rai-Kalal et al. 2021b). Consistent with this, our results showed that SNP inoculation leads to an increase in Y(II) in correlation with an increase in total Chl content. This suggests that SNP treatment results in an increase in the number of active reaction centers, maximizing light absorption and improving photochemistry performance. Therefore, SNPs could influence metabolic changes in algal cells including photosynthetic reaction. These modifications can lead to changes in the better pigment content of the cells, as well as improvement in their growth and biomass production. Based on cumulative observations, we opted for a concentration of 1 mg L^{-1} SNP for bioremediation of PYR by *C. vulgaris* cells.

In our previous studies we extensively investigated the effects of PYR on microalgal growth patterns, photosynthesis performance, and biochemical characteristics, such as lipid, carbohydrate, and protein (Tomar et al. 2022). We also investigated the effect of light intensities on toxicity of PYR (Tomar et al. 2023). However, there is no information

regarding the impact of SNP on PYR toxicity and its removal by *C. vulgaris*. The removal of PYR (5 mg L^{-1}) by *C. vulgaris* was investigated with 1 mg L^{-1} SNP to explore the potential mechanism of enhanced bioremediation by the cells. Algal cells can protect themselves during periods of stress by altering their biochemical characteristics, and enzymatic and non-enzymatic antioxidant systems. Various types of nanoparticles (NPs) have been shown to provoke these protective responses (Wang et al. 2016b; Chen et al. 2018).

Algal cells produce polyphenols and proline as a mechanism to protect themselves from damage resulting from stress (Chokshi et al. 2017). They serve to maintain chlorophyll levels and cell turgor, thereby protecting photosynthetic activity. In addition to acting as an excellent osmolyte, proline plays three major roles during stress. First, it acts as a metal chelator, second, it serves as an antioxidative defence molecule and lastly, proline functions as a signalling molecule (Meena et al. 2019). In our study proline content remained unchanged in PYR treated cells and there was a significant decrease in polyphenol content. These findings suggest that *C. vulgaris* cells were unable to effectively protect themselves from the toxicity induced by PYR. It is also possible that *C. vulgaris* cells may have produced some degrading enzymes that could act on polyphenols due to their structural similarity with hydrocarbons, resulting in a decrease in polyphenol content. The observed decrease in polyphenol content in PYR-treated cells is concerning as polyphenols play a critical role in protecting cells from damage caused by stress. The lack of protection afforded by polyphenols in PYR-treated cells could contribute to cellular damage and compromise the overall health of the cells. The low polyphenol content in PYR-treated cells may be a possible reason for the increased production of reactive oxygen species (ROS), which in turn led to a significant increase in MDA content (Agarwal et al. 2022). MDA is a lipid peroxidation product that is commonly found to be increased in plants and algae under stress conditions. With PYR cultured cells, the higher levels of ROS had more reactivity and were able to initiate membrane peroxidation, resulting in the liberation of MDA as an end product. In response to PYR toxicity algal cells produce ROS which is one of the reasons for the increase in the activity of antioxidant enzymes in PYR-treated cells. However, in the presence of SNP along with PYR, the increase in MDA content was found to be less compared to PYR-treated cells by promoting the activity of POD enzymes, which are responsible for scavenging H_2O_2 molecules in algal cells. This suggests that SNPs play a role in protecting algal cells from ROS during hydrocarbon toxicity. These findings highlight the potential of SNPs in mitigating the harmful effects of PYR toxicity in algae cells. Therefore, it can be concluded that SNPs play an important role in detoxifying ROS by promoting the activity of POD enzymes.

Interestingly, addition of SNPs significantly enhanced the PYR removal efficiency in *C. vulgaris* cells. The enhanced removal efficiency of PYR in the presence of SNPs might be because of more binding sites for pollutants due to the larger surface area. The altered bioremediation of organic pollutants by NPs in microalgae has been reported previously with some contradictory observations regarding the removal of pollutants (Xiong et al. 2020). It might be possible that NPs help to promote the enzyme activity that is needed to decompose pollutants. To know the effect of SNPs on PYR metabolizing enzymes dehydrogenase (DH) and dioxygenase activity were measured in *C. vulgaris* cells. Dehydrogenase enzymes are involved in a wide range of metabolic processes, including the breakdown of organic compounds, either biotic or xenobiotic. They catalyze the transfer of electrons from a substrate to a cofactor such as NAD^+ or $NADP^+$, resulting in the formation of either NADH or NADPH (Xie et al. 2008). They play a vital role in cell functioning and their dysregulation has been implicated in a range of irregularities in growth, survival, and productivity of algal cells. Based on the results obtained, it can be inferred that the role of dehydrogenase enzymes in PYR metabolism of *C. vulgaris* cells may not be critical or essential. However, dehydrogenase enzymes are critical for the proper functioning of cellular metabolism. A substantial decrease in dehydrogenase activity in *C. vulgaris* cells with PYR treatment indicated a decrease in active algal cells on culture. Algae can use catechol 1,2-dioxygenase (C1,2D) or catechol 2,3-dioxygenase (C2,3D) for the degradation of hydrocarbons (Warshawsky et al. 1995; Semple and Cain 1996). These enzymes catalyze the cleavage of the aromatic ring of catechol compounds, which are intermediates in the degradation of many hydrocarbons. It was previously reported that *C. vulgaris* in fluoranthene toxicity induced meta-pathways and increased expression of C2,3D (Tomar and Jajoo 2021). In the same way activity of C2,3D increased in PYR treated cells. These findings suggest that C2,3D plays a crucial role in the metabolic breakdown of PYR. The activity of C2,3D was further elevated with the addition of SNP during PYR toxicity. These observations provide strong evidence for the effectiveness of using SNP to enhance the bioremediation efficiency of *C. vulgaris* cells by promoting activity of degrading enzymes. However, additional studies are necessary to evaluate the actual application potential of this approach, particularly at larger scales.

Based on these results, we propose a probable mechanism of action of SNPs on microalgal growth and its bioremediation potential for pyrene (Fig. 5). Nanoparticles enhance the growth of microalgae and degradation of pyrene through enzymes such as dehydrogenase and dioxygenase. The process of PYR removal by *C. vulgaris* is proposed in two phases. First, application of SNP in low concentrations (0.5—5 mg L^{-1}) positively altered several characteristics of *C. vulgaris*

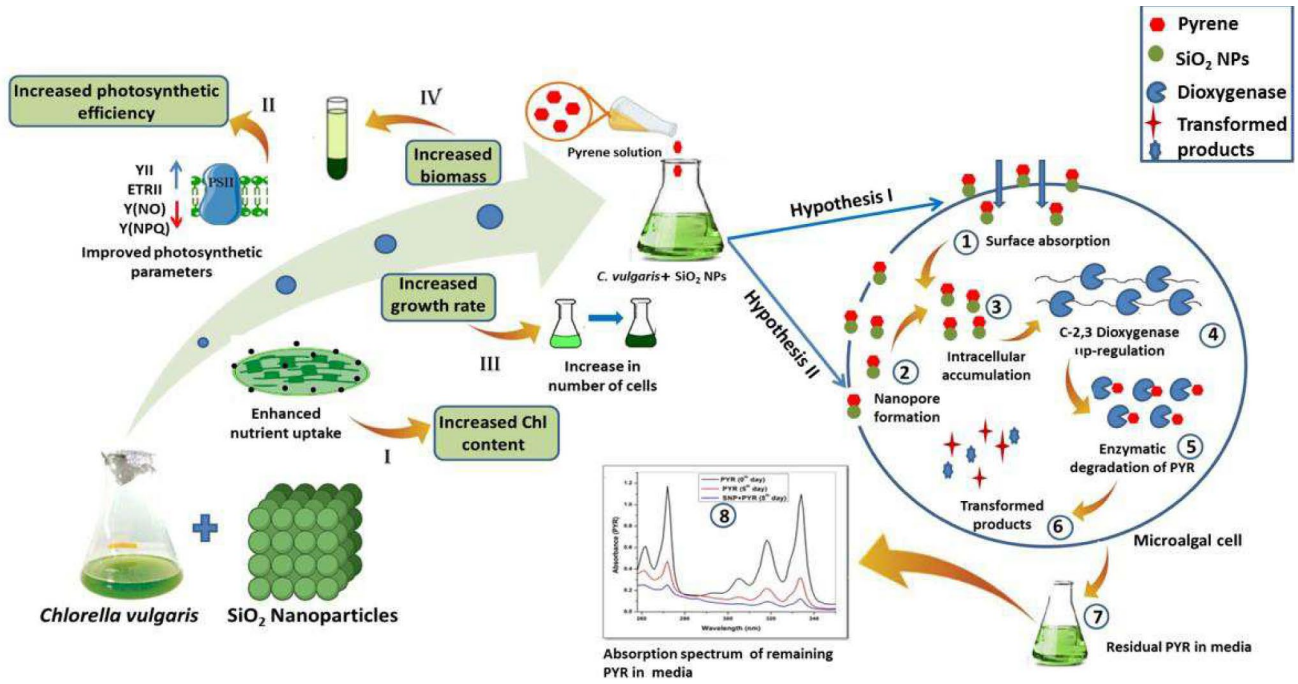

Fig. 5 Schematic representation of degradation of PYR by *C. vulgaris* in the presence of SiO_2 nanoparticles (SNPs)

such as growth rate, photosynthetic pigments, photosynthetic activity and biomass. Presence of nanoparticles may complement nutrient as well as CO_2 absorption efficiency of algal cells thereby increasing photosynthetic efficiency. Increased photosynthetic performance ultimately led to logarithmic growth phase of algal cells followed by an increase in biomass accumulation in presence of SNPs. In the second phase, SNPs promote biodegradation efficiency of *C. vulgaris* for PYR. This phenomenon can be put forward following two hypotheses: firstly, owing to large surface area to volume ratio, NPs increased the rate of adsorption of PYR to their surface resulting in significantly enhanced absorption into the algal cells. Secondly, SNPs could penetrate cell membrane of algal cells to create nanopores, thus enhancing PYR uptake from the medium. Once the pyrene is internalized SNPs further triggered a metabolic shift and promoted activity of C2,3D dioxygenase. As a consequence, more degradation of PYR occurred in the algal cells as evident from the absorption spectra. However, a more comprehensive study of the enzyme expression patterns associated with biodegradation of PAHs by algae in the presence of SNPs.

Conclusion

Silicon oxide nanoparticles (SNPs) have been found to be beneficial in the cultivation of *C. vulgaris*. Results showed that low concentrations (1 mg L^{-1}) of SNPs are more efficient than higher concentrations. Addition of SNPs in the algal culture medium could enhance the activity of PSII and hence improve algal growth and biomass production. SNP addition could also improve absorption efficiency of PYR by the algal cells. This study comprehensively demonstrates that *C. vulgaris* in presence of SNPs was more efficient in PYR removal (31% higher). In addition, SNPs have an impact on dioxygenase activity which is involved in PYR degradation in *C. vulgaris* cells. To enhance the sustainability and eco-friendliness of the bioremediation process it is recommended to focus research efforts on incorporating nanoparticles into wastewater treatment alongside microalgae. This approach can potentially improve the economic viability of the process while also minimizing the environmental impact of PAHs.

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Authors contributions **Rupal Singh Tomar**- Conceptualization, designed and performed the experiments, Data curation, Writing- Original draft preparation. **Prabha Rai-Kalal-** Performed the experiments and writing. **Anjana Jajoo**-Conceptualization, designed the experiments, Supervision, Writing- Reviewing and Editing.

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Data availability Raw data can be provided on request.

Declarations

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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