

Impact of silica oxide and functionalized silica oxide nanoparticles on growth of *Chlorella vulgaris* and its physicochemical properties

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ABSTRACT

In this study, silica oxide nanoparticles (SiO₂ NPs) and methyl-functionalized SiO₂ NPs were compared for their effects on the growth of *Chlorella vulgaris*, production of biomass, and lipid content. The synthesised nanoparticles were characterised by using techniques such as FTIR, XRD, UV-Vis, FESEM with EDX and TEM. The synthesised nanoparticles were used to enhance the gas-liquid interface areas, which increase the concentration of dissolved carbon dioxide in the growth medium in order to increase the accumulation of lipid content in *C. vulgaris* cultures. The most effective way to improve the gas-liquid interface area was to use SiO₂ NPs at a concentration of 0.3 wt%. The hydrophobic functional group methyl was added to the hydrophilic surfaces of SiO₂ NPs. Methyl-functionalized SiO₂ NPs improved the gas-liquid interface area more effectively than unmodified silica nanoparticles. The concentration of dissolved CO₂ was 316.66 mg L⁻¹ with methyl-functionalized SiO₂ NPs. During microalgae culture, methyl-functionalized SiO₂ NPs at a concentration of 0.3 wt% considerably improved the production of dry biomass, lipid, protein, carbohydrate, and chlorophyll contents as compared to the control.

1. Introduction

Ever since industrialization evolved about 250 years ago, the "coal-based economy" was established, followed by the "fossil fuel-based economy" in the following 50 years. A rectilinear economic model of "taking, producing, and disposing" was followed, which is mainly based on the presumptions of plentiful and inexpensive environmental resources on earth [8]. Furthermore, excessive use of fossil fuels causes significant economic and environmental consequences [19]. The release of greenhouse gases has a worldwide concern and is one of the important reasons for the change in climate leading to the global warming effect [29]. Due to dependence on conventional energy supplies, the energy crisis has become a serious global problem in recent years [9]. The search for alternative energy sources that can replace coal, petroleum, and natural gas from the current energy system has been triggered by concerns about the depletion of fossil fuel reserves, energy security issues, as well as the effects of global warming caused by rising CO₂ levels in the atmosphere [7,32]. As a direct consequence of this, there is an

ever-increasing level of interest in renewable forms of energy. The production of bioenergy from microalgae, third-generation biofuels, has attracted greater attention since it can create bioenergy, such as biodiesel by utilising CO₂, a greenhouse gas, as a raw material. The biodiesel made from microalgae has a lot of potential as a cleaner energy fuel due to its high economic viability and energy efficiency [39]. Microalgae convert sunlight into energy and store atmospheric CO₂ through photosynthesis in the form of glucose which is further converted into triglycerides. Triglycerides are readily transformed into FAMES (fatty acid methyl esters), a source of biodiesel, through transesterification [12].

According to Thirugnanasambantham et al., [30], *C. vulgaris* is an ideal applicant for biodiesel production on the basis of dry biomass production, lipid content, and quality of lipids. Moreover, the most common fatty acids of *C. vulgaris* are palmitic acid, stearic acid, oleic acid, linoleic acid, and linalenic acid. In microalgae the lipid production can reach up to 58% under stress conditions [14]. The lipid content of different microalgal species *Botryococcus braunii*, *Chlorella emersonii*,

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Chlorella vulgaris, *Chlorococcum* sp., *Dunaliella salina*, and *Nannochloropsis* sp. is 33.6%, 5.0%, 24.0%, 20.0%, 23.1% and 20.0% respectively, (in % dry cell weight). The economic feasibility of large-scale biodiesel production from microalgae faces numerous challenges, including biomass cultivation, harvesting of microalgal cells, and lipid extraction. The biomass cultivation stage is one of the main restrictions [10]. Biomass cultivation is a critical step in storing significant amounts of lipid in microalgal cells. Silica oxide nanoparticles (SiO₂ NPs) are emerging as promising substitutes for improving microalgal lipid production. The addition of SiO₂ NPs to microalgal cultures can boost light conversion and increase CO₂ absorption yield [43]. Nanoparticles may be toxic to microalgae but research has shown that the toxicity depends upon the nanoparticles' nature (like nanoparticle size, oxidation state, functional group, and crystal structure), concentration, growing conditions, and microalgae species [33,34]. In growth media, the concentration of dissolved CO₂ was raised, which increased the production of lipids and biomass. SiO₂ NPs have been used by researchers to boost the production of biomass and lipid in microalgae [20]. Nanoparticle adhesion at the gas-liquid interface increases the mass transfer coefficient and dissolved CO₂ concentration in media [2]. According to Kim and Lee [15], the main mechanisms responsible for the increase in mass transfer coefficient driven by nanoparticle adherence to the gas-liquid boundary layer are changes in the specific gas-liquid contact area. The present study emphasis on synthesis and characterization of SiO₂ and SiO₂-CH₃ NPs and examined their impacts on the dissolved CO₂ concentration in growth media, their effect on *Chlorella vulgaris* growth, and physicochemical properties of microalgae (like biomass production, lipid content, protein, total chlorophyll, and total carbohydrate).

2. Materials and methodology

2.1. Experiment methods

2.1.1. Microalgae cultivation

The microalgal strain *C. vulgaris* was kindly provided by Bioenergy Lab, Central University of Jammu, Jammu and Kashmir, India. Each experiment was carried out in an incubator with an initial pH of 7, a temperature of 27 °C, and a light intensity of 3000–4000 lux from a cool white LED. *C. vulgaris* was grown in a conical flask (capacity 500 mL) having 250 mL of BG-11 media. The flasks were incubated at 120 RPM for 12 days. Conical flasks were fitted with cotton plugs to avoid contamination while yet enabling air to circulate freely inside the flasks. In order to determine the effect of NPs on microalgal growth and its physicochemical parameters, the NPs of SiO₂ and SiO₂-CH₃ were added into growth media in a range of 0.1–0.5 wt%.

2.1.2. Silica oxide nanoparticle synthesis and surface modification

The SiO₂ nanoparticles were synthesised using a technique known as the sol-gel method. Tetraethylorthosilicate (TEOS) (6 mL) was mixed with 300 mL of ethanol in a beaker for one hour on a magnetic stirrer. After thoroughly mixing the ingredients, 60 mL of NH₄OH was added to the mixture, which was then stirred for another two hours. Centrifuging the SiO₂ nanoparticle precipitates at 3000 rpm for 15 min, the particles were washed with an ethanol solution in order to remove any contaminants that may have been present. The obtained NPs were washed three times before being collected, then dried at a temperature of 80 °C. In order to introduce methyl groups onto the surfaces of the SiO₂ nanoparticles (NPs), take 1 g of SiO₂ nanoparticles and dissolve them in a solution that has 20 mL of distilled water, 380 mL of ethanol, and 40 mL of triethoxymethylsilane. The mixture was stirred for 12 h and centrifuged. After that, the precipitates that had formed as a result were washed with an ethanol solution three times. The SiO₂-CH₃ nanoparticles formed as precipitates were dried and stored for further use. The synthesis process of SiO₂ NPs and their modification were described by Kim et al., [16] and Jeon et al., [12].

2.1.3. Measurement of dissolved CO₂ in growth media

The dissolved CO₂ concentration in growth media was calculated by the described method given by Ma and Yung [18]. A 250 mL bottle containing 200 mL of deionized water was deoxygenated using nitrogen gas, and when the internal pressure reached 121.6 kPa, pure carbon dioxide gas was supplied. A magnetic stirrer was used to mix the mixture for 45 min at 300 RPM. The difference between the gas phase's initial and final pressures was used to calculate the dissolved CO₂ concentration.

2.1.4. Microalgae growth and dry biomass

The algal growth rate was measured by UV-Vis spectrophotometer. The optical density (OD) of the cultured microalgae was used as a parameter for measuring the growth rate of the microalgae. UV absorbance was measured between 200 and 800 nm to determine the best wavelength for *C. vulgaris*. At 683 nm, the peak absorbance was observed, which is utilized to calculate cell density [40]. The microalgal samples' dry biomass was determined by measuring the change in weight of a predetermined volume of culture after being filtered through Whatman GF/C filters and having the filter paper dried in an oven at 105 °C for a period of 12 h. The starting and ending weights of the filter paper were measured with the use of an analytical balance in the laboratory. The weight of the dry biomass was measured in erg L⁻¹. The specific growth rate in the exponential phase was calculated using Guillard's (1973) relationship [38].

$$\mu = \ln \left(\frac{X_2/X_1}{(T_2 - T_1)} \right) \quad (1)$$

X1 and X2 represent the OD at time T1 and T2, respectively.

Dry biomass (B) was calculated: -

$$B = \frac{B_1 - B_0}{T_1 - T_0} \quad (2)$$

B₁ and B₀ are the dry biomass means at times T₁ and T₀, respectively.

2.1.5. Lipid content estimation

In order to obtain an algal biomass pellet, 50 mL of the algal culture was centrifuged for 10 min at a speed of 5000 RPM. After that, the algal biomass was combined with 2 mL of methanol and 1.5 mL of chloroform, and it was incubated at a temperature of 25 °C for 24 h. After vortexing for 2 min, 1.5 mL chloroform was added, and the mixture was stirred for 1 min. The combination was strongly shaken for a total of 2 min after the addition of 1.8 mL of distilled water. After that, it was centrifuged for 10 min at a speed of 5000 RPM, and then the bottom lipid layer was carefully extracted using Eppendroff micropipettes in a glass centrifuge tube that had been cleaned and dried beforehand. The chloroform phase was brought to a temperature of 70 °C and then allowed to evaporate in a water bath. The residual residue was dried for an additional 30 min at 104 °C. The lipid content was determined by using the dry weight ratio of extracted lipid to biomass, as stated by Kirrolia et al. [17]. Using equation no. 3, the following yield of lipid extraction was calculated in terms of percent weight-to-weight (% w/w):

$$\text{Lipid extraction yield(dcw\%)} = \frac{\text{Weight of extracted oil}}{\text{Weight of biomass}} \times 100 \quad (3)$$

2.1.6. Protein estimation

This method (described by [5]) was utilised in order to derive the total amount of protein. 10 mL homogenised algal sample was placed in a centrifuge tube and centrifuged at a speed of 5000 RPM for a period of 5 min. The pellet was subjected to a treatment with 0.1 N of NaOH and hydrolyzed for 30 min in a water bath and heated to 60 °C before the supernatant was collected and the precipitate was centrifuged once again. Immediately following the collection 0.5 mL of the hydrolyzed sample, 0.5 mL of the reagent designated as "A" was poured. After being heated for 10 min in a bath of boiling water, the tubes were cooled by

passing them under running water. Then, 2.5 mL of reagent (B) was added to each tube, and the tubes were allowed to sit at room temperature for 10 min. After adding 0.5 mL of reagent C the tubes were allowed to remain at ambient temperature for 15 min. The absorbance of the blue colour was measured at 660 nm and compared to a blank. The amount of protein was measured using a standard calibration curve that was constructed from bovine serum albumin. Protein concentration was determined utilising equation no. 4, which was based on the standard curve of BSA.

Analytical reagents:

A) 50 mL of 0.1 N NaOH solution and 50 mL of 2% sodium carbonate solution were mixed (in 100 mL distilled water).

B) A mixture of 100 mL of 2.37% sodium potassium tartrate solution and 10 mL of 1.56% copper sulphate solution was used.

C) Analytical reagent (C) was made by combining 100 mL of (A) with 2 mL of (B). [5].

$$Y = (0.1097)_X - (0.0005), R^2 = 0.9889 \quad (4)$$

Where y is concentration of glucose, x is optical density.

2.1.7. Carbohydrate estimation

Calculations of total carbohydrates were carried out by the use of the anthrone reagent method. The concentration of carbohydrates was calculated by using a standard curve of graded concentration of glucose [27].

2.1.8. Chlorophyll estimation

In order to calculate chlorophyll, an algal culture was centrifuged for 10 min at 5000 RPM, washed with distilled water, and then incubated in a water bath for 30 min at 60 °C. The pellets were twice extracted with 95% methanol, followed by a 10-min centrifugation period at 5000 RPM, using the hot extraction approach. Houmard and de Marsac [11] estimated the total chlorophyll content of algae using spectrophotometry at 650 and 665 nanometers. The chlorophyll content, expressed in $\mu\text{g mL}^{-1}$, was calculated using equation no. 5 in the list below.

$$\text{Total chlorophyll } (\mu\text{g mL}^{-1}) = 2.55 \times 10^{-2} (\text{OD}_{650}) + 0.4 \times 10^{-2} (\text{OD}_{665}) \times 10^3 \quad (5)$$

2.1.9. Direct transesterification

After placing 1 g of freeze-dried algal biomass in a test tube, 3.4 mL of methanol and 0.6 mL of sulfuric acid were added to the mixture. In addition to that, 4 mL of chloroform was added to the mixture after that the mixture was heated at a temperature of 90 °C for 40 min. After the reaction was complete, the tubes were allowed to cool at room temperature. About 2 mL of distilled water was added to the tube, and it was stirred for a total of 45 s and allowed for the two phases of the tubes to become distinct. According to Kirrolia et al. (2015), the tubes were centrifuged for an additional 8 min at 10,000 RPM if it was essential to speed up further phase separation.

2.2. Analytical method

The chemicals used in this study are of analytical grade quality from Himedia laboratories Pvt. Ltd. The UV-Vis spectrophotometer (Varian Cary-5000) is used for measuring the optical density (OD) of the growth media at 680 nm. The morphologies and elemental analyses of the SiO_2 and $\text{SiO}_2\text{-CH}_3$ NPs were observed by FE-SEM with an EDX (7610 F Plus/ JEOL) model, and the internal structure was analysed by TEM (JEOL JEM 2100 PLUS). The functional group composition of the nanoparticles were evaluated using a FTIR spectrophotometer (Fourier Transform infrared spectroscopy) model (Perkin Elmer FTIR System – Spectrum BX), using a standard optical system with KBr powder, with the spectral range used for the collection of data being 500–4000 cm^{-1} . The crystal

structures of SiO_2 and $\text{SiO}_2\text{-CH}_3$ nanoparticles were analysed using the X-Ray Diffraction (XRD) model (Benchtop Miniflex- II, Rigaku, Japan) spectrum between 10° and 80° (2 θ) at 27 °C. The colloidal stability of SiO_2 and $\text{SiO}_2\text{-CH}_3$ nanoparticles was analysed using Zeta potential (Zetasizer Nano Series Nano-ZS90). UV-Vis, FE-SEM with EDX, FTIR, and XRD are analysed in CIL lab of GJUS&T, University, Hisar and TEM from CIL lab of Central University of Punjab.

3. Results and discussion

3.1. Characterization of the synthesized nanoparticles

3.1.1. FTIR (Fourier Transform Infrared Spectroscopy)

FTIR is generally used for describing functional groups presented in the sample, and the spectra of synthesised SiO_2 and $\text{SiO}_2\text{-CH}_3$ NPs are shown in Fig. 1. The SiO_2 and $\text{SiO}_2\text{-CH}_3$ nanoparticles spectra show peaks at 459.61 cm^{-1} and 459.42 cm^{-1} which show stretching vibrations of the Si-O bond. In the SiO_2 and $\text{SiO}_2\text{-CH}_3$ spectra, peaks at 798.49 cm^{-1} –1798.58 cm^{-1} describe the stretching of the C-C bond with sp^2 hybridization, and the presence of the trisubstituted alkene group. The small peaks of the SiO_2 and $\text{SiO}_2\text{-CH}_3$ spectra were detected at 1093.88 cm^{-1} and 1093.51 cm^{-1} , which show the presence of the alkoxy group. Furthermore, Shokri et al. [28] discovered that the strong bands at 1093, 459, and 798 were associated with asymmetric and symmetric Si-O-Si stretching vibration bodings. In the $\text{SiO}_2\text{-CH}_3$ nanoparticles spectrum, an additional peak was detected at 1402.25 cm^{-1} which describes the stretching of the C-H bond with sp^3 hybridization. In the SiO_2 and $\text{SiO}_2\text{-CH}_3$ nanoparticles, sharp peaks were observed at 1636.09 cm^{-1} and 1636.81 cm^{-1} which describe the C=H double bond with sp^2 hybridization and the N-H bond stronger in amides than amines. In the SiO_2 nanoparticles' spectra, peak was detected at 2946.76 cm^{-1} which describes the stretching of the C-H bond. The wide attributed adsorption in SiO_2 was observed at 3347.49 cm^{-1} , indicating the presence of O-H bond stretching with the alcohol group. According to Jiao et al. [13], finding the broad peak from 3000 to 3700 cm^{-1} was assigned to the presence of the O-H group. The $\text{SiO}_2\text{-CH}_3$ nanoparticles spectra at 3452.03 cm^{-1} revealed widely attributed adsorption, describing primary NH_2 with two stronger bonds in amides and secondary N-H bonding with weaker amines. A similar analysis for silica oxide nanoparticles was found by Petreanu et al. [24].

3.1.2. XRD (X-ray diffraction)

The XRD spectra of SiO_2 and $\text{SiO}_2\text{-CH}_3$ nanoparticles were shown in Fig. 2, which shows the characteristic peak of SiO_2 and $\text{SiO}_2\text{-CH}_3$ nanoparticles. The characteristic peak of SiO_2 NPs is observed at 22.32 on (2 θ). This XRD spectrum of SiO_2 nanoparticles shows their crystalline nature. The characteristic peak of $\text{SiO}_2\text{-CH}_3$ nanoparticle on (2 θ) is 22.32. Similar peaks for silica nanoparticles were identified by Usgodaarachchi et al. [31]. The XRD spectrum of $\text{SiO}_2\text{-CH}_3$ nanoparticles shows a less crystalline nature than SiO_2 nanoparticles. This XRD spectrum shows the formation of different crystal types of SiO_2 and $\text{SiO}_2\text{-CH}_3$ nanoparticles. According to Zhang et al. [42] the crystalline peaks in silica were observed at 2 θ of 22.6 and present study also shows similar results.

3.1.3. UV-Vis (Ultraviolet/Visible Spectroscopy)

The UV-Vis spectra of SiO_2 and $\text{SiO}_2\text{-CH}_3$ nanoparticles were shown in Fig. 3. The peaks of SiO_2 were detected at 290 cm^{-1} with an absorbance of 0.38, while the peak of $\text{SiO}_2\text{-CH}_3$ nanoparticles was observed at 295 cm^{-1} with an absorbance of 1.8. These peaks are very similar to the near-band emission peak of silica oxide nanoparticles, which was mainly initiated by the recombination of excitons [3]. According to Ren et al. [26], the intensity of the absorption spectrum of pure SiO_2 nanoparticles continuously decreases without any major peaks being observed.

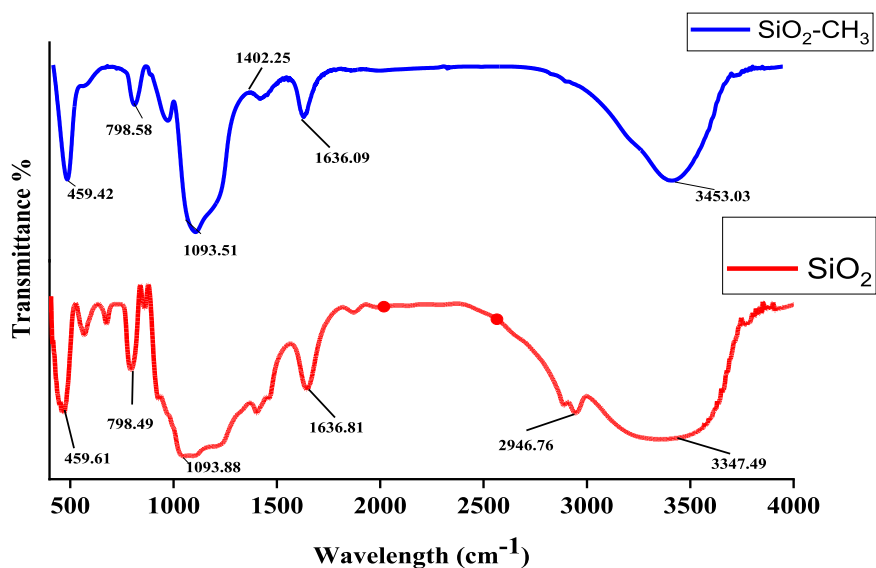


Fig. 1. FTIR Spectrum of synthesized SiO₂ and SiO₂-CH₃ Nanoparticles.

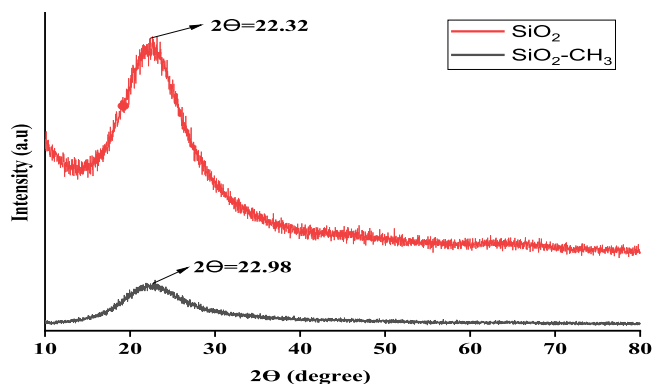


Fig. 2. XRD pattern of synthesized SiO₂ and SiO₂-CH₃ Nanoparticles.

3.1.4. FESEM (Field Emission Scanning Electron Microscopy) and TEM (Transmission Electron Microscopy)

The FE-SEM image of SiO₂ and SiO₂-CH₃ NPs was shown in Fig. 4, and Fig. 5. shows the TEM image of SiO₂ and SiO₂-CH₃ NPs. The sample consists of an irregular distribution of nanoparticles about 200 nm in diameter as shown in Fig. 5. The distribution of the particles was wide, and very small particle sizes were obtained. These nanoparticles are

found in fine shapes with adequate pore diameters. The shape of the SiO₂ and SiO₂-CH₃ nanoparticles was almost spherical, and these nanoparticles were not greatly changed by the substitution of the methyl group.

The elemental analysis of the synthesised SiO₂ and SiO₂-CH₃ nanoparticles, shown in Table 1, was performed by SEM-EDX. The EDX shows the amounts of silica, oxygen, and carbon in SiO₂ and SiO₂-CH₃ nanoparticles. The surface elemental composition of the SiO₂ nanoparticles was as follows: the weight of carbon was 25.5%, the weight of oxygen was 59.1%, and the weight of silica was 15.4%. In the SiO₂-CH₃ nanoparticles, the surface elemental composition was as follows: the weight of carbon was 29.7%, the weight of oxygen was 57.8%, and the weight of silica was 12.5%. The SiO₂-CH₃ NPs showed a low percentage of Si and O and a high percentage of 'C' as compared to the SiO₂ NPs because the surface alteration of the SiO₂ nanoparticles with CH₃ groups was confirmed when some of the Si-O bonds and OH groups were substituted with CH₃ groups. Similar results are found by Jeon et al. [12].

3.1.5. Zeta potential and particle size analyzer (PSA)

Zeta potential indicates that synthesised SiO₂ and SiO₂-CH₃ nanoparticles have a mean zeta potential value of − 23.1 millivolt and − 31.4 millivolt, respectively. The negative charge is observed on the surfaces of synthesised SiO₂ and SiO₂-CH₃ nanoparticles. Zhao et al. (2018) also investigated the zeta potential of silica oxide nanoparticles. The present

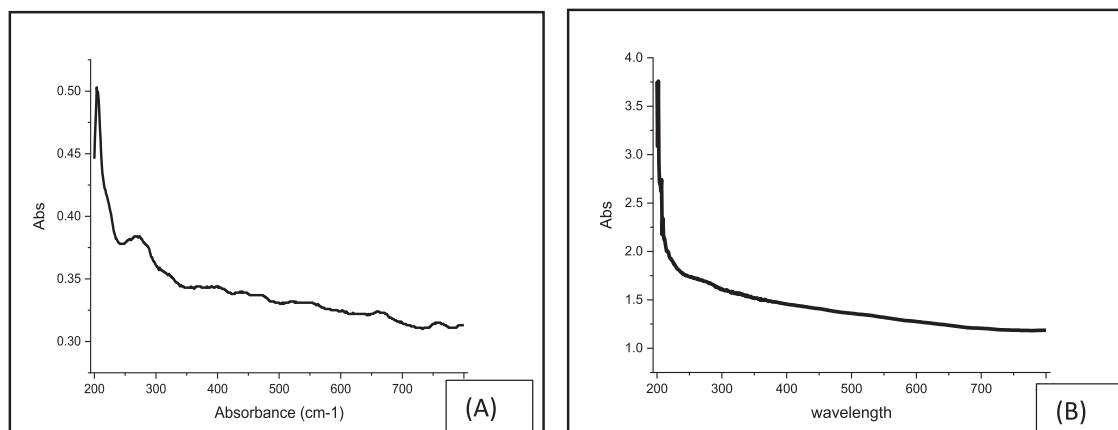


Fig. 3. UV-Vis spectrum of synthesized SiO₂ and SiO₂-CH₃ NPs.

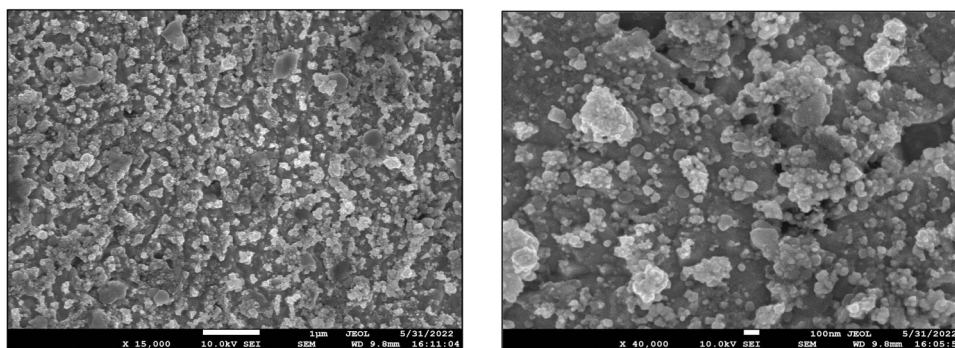


Fig. 4. FE-SEM image of Synthesized SiO_2 and $\text{SiO}_2\text{-CH}_3$ Nanoparticles.

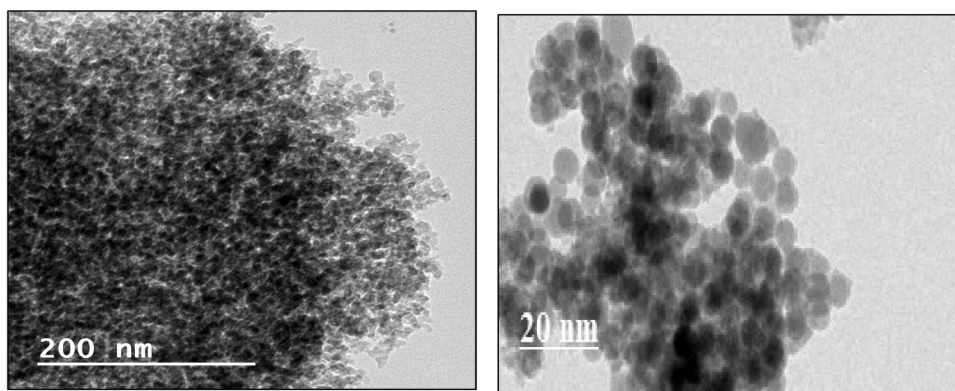


Fig. 5. TEM image of synthesized $\text{SiO}_2\text{-CH}_3$ Nanoparticles.

Table 1
Elemental analysis of SiO_2 and $\text{SiO}_2\text{-CH}_3$ Nanoparticles (FESEM).

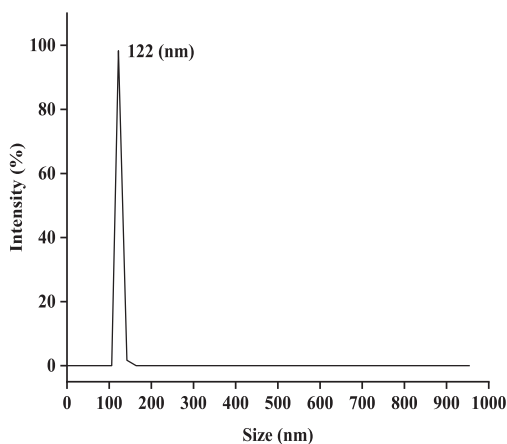
Elements	Weight (%)	
	SiO_2	$\text{SiO}_2\text{-CH}_3$
C	25.5	29.7
O	59.1	57.8
Silica	15.4	12.4

study also shows similar results. The synthesised SiO_2 nanoparticles have moderate stability, but $\text{SiO}_2\text{-CH}_3$ nanoparticles have greater stability than synthesised SiO_2 nanoparticles. Corredor et. al. [6] also

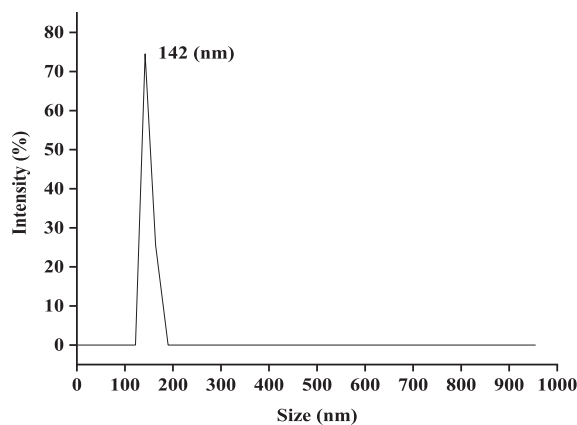
observed similar results. [6]. The particle size analyzer is used for determining the average size of the synthesised SiO_2 NPs. The PSA image of SiO_2 and $\text{SiO}_2\text{-CH}_3$ nanoparticles was shown in Fig. 6. The PSA values of synthesised SiO_2 and $\text{SiO}_2\text{-CH}_3$ nanoparticles are 122 nm and 142 nm, respectively.

3.2. Effect of SiO_2 NPs on growth parameters of *Chlorella vulgaris*

The effects of SiO_2 NP weight percent on various growth parameters of *C. vulgaris* are depicted in Table 2. The maximum concentrations of dissolved CO_2 and dry biomass, with SiO_2 NPs were 292.33 mg L^{-1} , 1.465 g L^{-1} respectively, while the maximum amounts of lipid and



(a) PSA image of SiO_2 Nanoparticles.



(b) PSA image of $\text{SiO}_2\text{-CH}_3$ Nanoparticles.

Fig. 6. Particle Size analyser image of Synthesized SiO_2 and $\text{SiO}_2\text{-CH}_3$ Nanoparticles.

protein were 12.876 % and 0.043% respectively. In comparison to the control, the lipid synthesis increases by 14.53% at 0.3 wt% SiO₂ NPs. According to current findings, 0.3 wt percent of SiO₂ NPs in growth media can store enough lipids to serve as a potential source of biodiesel. According to research by Jeon et al. [12], the dry cell weight increased significantly throughout the culture. In experiments using SiO₂ nanoparticles, the dry cell weight at day 10 was 1.33 g L⁻¹, whereas the dry cell weight in the treatment without nanoparticles was 0.48 g L⁻¹. The dry cell weight was increased by 177 by adding SiO₂ nanoparticles. This research demonstrated that the addition of nanoparticles increased CO₂ mass transfer, which in turn promoted *C. vulgaris* cell growth.

3.3. Effect of modified SiO₂-CH₃ NPs on growth parameters of *C. vulgaris*

The information in Table 3 illustrates how SiO₂ NPs with a methyl functional group weight percentage affect various microalgae development parameters. At a concentration of 0.3% SiO₂-CH₃ NPs in growth media on the 12th day of cultivation, the greatest amount of dissolved CO₂ and all growth metrics are reached. At 0.3% SiO₂-CH₃ NPs, lipid production increased by 15.44%, and biomass output increased by 31.4% when compared to the control.

According to Kim et al. [16], the dry weight of the microalgae produced by the 0.3 wt% methyl-functionalized silica nanoparticles was 0.4541 ± 0.61108 g L⁻¹. When silica nanoparticles functionalized with methyl were compared to the control, the dry weight of the microalgal cells increased by 34.5% due to improved gas-liquid mass transfer.

3.4. The effect of SiO₂ NPs on dissolved CO₂ concentration

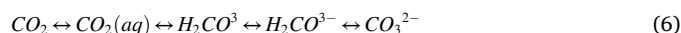
As shown in Fig. 7, adding SiO₂ and SiO₂-CH₃ NPs significantly increased the amount of dissolved CO₂ in growth media. SiO₂ was less effective than SiO₂-CH₃ at increasing the concentration of dissolved CO₂. The mass transfer coefficient and the amount of dissolved CO₂ in media are both improved by SiO₂ NPs adhering to the gas-liquid interface [25]. SiO₂-CH₃ nanoparticles' hydrophobic surfaces are helpful for changes at the particular gas-liquid interface area [37]. In this study, the hydrophilic surfaces of SiO₂ nanoparticles were modified to form hydrophobic functional groups (CH₃), which increased the mass transfer coefficient and more effectively dissolved CO₂. The mass transfer coefficient and the amount of dissolved CO₂ in media are both improved by SiO₂ NPs adhering to the gas-liquid interface [25]. SiO₂-CH₃ nanoparticles' hydrophobic surfaces are helpful for changes at the particular gas-liquid interface area [37]. Ahn et al. [2] investigate the effect of magnetic cobalt ferrite/silica nanoparticles (MSNs) and methyl-MSNs on CO₂ dissolution in water. The findings are consistent with our studies, which found that methyl-functionalized nanoparticles improved gas solubility in water more than bare nanoparticles.

To ascertain how nanoparticle concentration affects dissolved CO₂ concentration, different doses of nanoparticles (0.1, 0.2, 0.3, 0.4, and 0.5 wt%) were used. The concentration of dissolved CO₂ increased considerably when the concentration of SiO₂-CH₃ increased up to 0.3 wt %, but not beyond that. The dissolved CO₂ concentration increased by 24.57% at 0.3 wt% SiO₂-CH₃ concentration compared to the control

experiment without nanoparticles (254.20 mg L⁻¹). When the concentration of nanoparticles is increased from 0.3 wt% to 0.4 wt%, the dissolved concentration of CO₂ tends to decrease. This is likely due to the fact that nanoparticles have a large surface area to volume ratio, which can lead to increased adsorption of CO₂ molecules on the surface of the nanoparticles. As a result, less CO₂ remains in the solution phase, leading to a lower dissolved concentration. After introducing SiO₂ and SiO₂-CH₃, the content of dissolved CO₂ dramatically increased. SiO₂ was less effective at raising the content of dissolved CO₂ compared to SiO₂-CH₃ NPs. SiO₂-CH₃ was more effective in increasing nanoparticle adherence to the gas-liquid interface due to its hydrophobic (methyl group) surfaces. This explains why SiO₂-CH₃ was more successful in promoting CO₂ dissolution in water [41].

3.5. Impact of nanoparticles on *C. vulgaris* growth and biomass

The biomass of *C. vulgaris* at day 12 of culture with SiO₂ and SiO₂-CH₃ NPs at different concentrations ranging from 0.1 to 0.5 wt% was shown in Fig. 8. Fig. 9 depicts the optical density (683 nm) of *C. vulgaris* at various SiO₂ and CH₃-SiO₂ NP concentrations. Biomass was significantly greater in cultures containing 0.3 wt% NPs than in those containing 0.1 wt% NPs. When the concentration of nanoparticles exceeds 0.3 wt%, the concentration of microalgal biomass tends to be inhibited. This could be due to several factors, including reduced light penetration and increased turbidity of the solution, which can limit the availability of light for photosynthesis. In addition, the presence of nanoparticles may interfere with the uptake of nutrients and other essential components by the microalgae, leading to increased growth and biomass production. And low dissolution of CO₂ in growth media results in insufficient carbon source supply, and often limits microalgae growth. The SiO₂ NPs enhanced the transfer coefficient and dissolved CO₂ concentration in growth media. The theoretical studies show that microalgae have a high potential for fixing CO₂, the weak dissolution and diffusion of CO₂ in water prevent algae from effectively using the CO₂ molecule to create organic macromolecules [35]. The following equilibrium equation represents the CO₂ dissolution during microalgae cultivation:



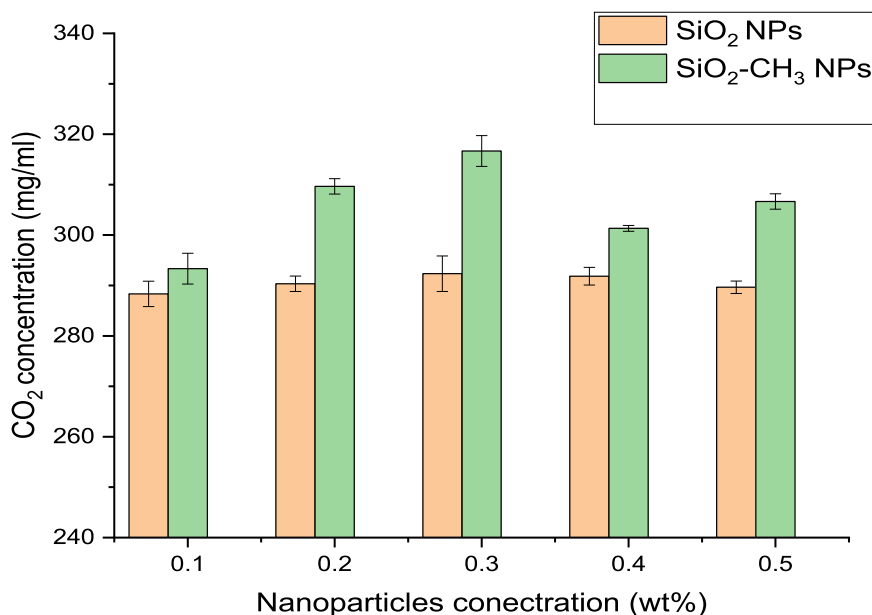
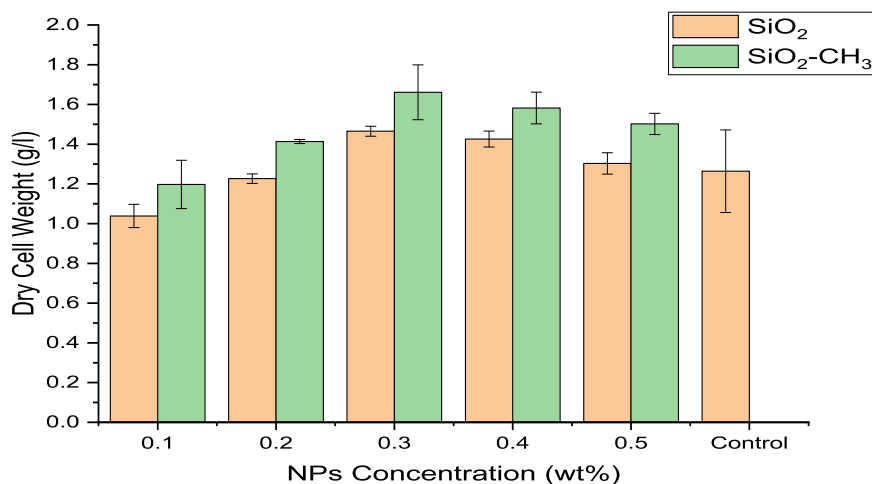
The carbon source species that are preferentially absorbed by the metabolism of microalgae are HCO₃⁻ and CO₂. The increasing HCO₃⁻ concentration promotes carboxysome deposits, which activate the photosynthetic Calvin cycle. The Reactive oxygen species (ROS) damage to PSII is decreased by the rise in HCO₃⁻ concentration, which also provides microalgae with a direct carbon supply [33]. Increased CO₂ fixation by SiO₂ NPs provides rich nutrition to microalgae, resulting in increased biomass productivity throughout logarithmic growth and the accumulation of value-added products. The biomass weights in cultures containing SiO₂-CH₃ were greater than those in cultures containing SiO₂. Similar findings were reported in earlier research by Ahn et al. [2]. The addition of 0.3 wt% SiO₂-CH₃ generated many superior outcomes (dry cell weight was 1.66 g L⁻¹), in comparison to the controls, which produced 1.264 g L⁻¹. Jeon et al. [12] investigated the performance of

Table 2
Effect of different concentration of SiO₂ NPs on biomass and biochemical parameters of *C.vulgaris*.

Biomass and biochemical parameters	Different concentration of SiO ₂ NPs (wt%)					
	Control	0.1	0.2	0.3	0.4	0.5
Dissolved CO ₂ (mg L ⁻¹)	254.20 ± 3.421	288.33 ± 2.51	290.33 ± 1.527	292.33 ± 3.511	291.833 ± 1.755	289 ± 1.229
Biomass (g L ⁻¹)	1.264 ± 0.207	1.038 ± 0.058	1.226 ± 0.024	1.465 ± 0.025	1.426 ± 0.040	1.303 ± 0.053
Lipid (% dry)	11.242 ± 0.028	11.376 ± 0.48	11.635 ± 0.192	12.876 ± 0.530	12.205 ± 0.162	11.849 ± 0.804
Protein (mg mL ⁻¹)	0.025 ± 0.001	0.028 ± 0.001	0.033 ± 0.033	0.043 ± 0.004	0.036 ± 0.005	0.026 ± 0.002
Total chlorophyll (µg mL ⁻¹)	9.33 ± 0.130	9.66 ± 0.150	10.16 ± 0.0529	11.72 ± 0.091	13.76 ± 0.282	11.610 ± 0.200
Total carbohydrate (mg mL ⁻¹)	0.035 ± 0.0015	0.030 ± 0.002	0.030 ± 0.002	0.032 ± 0.003	0.044 ± 0.002	0.032 ± 0.003

Table 3Effect of different concentrations of CH₃-SiO₂ NPs on biomass and biochemical parameters of *C.vulgaris*.

Biomass and biochemical parameters	Different concentration of SiO ₂ -CH ₃ NPs (wt%)					
	Control	0.1	0.2	0.3	0.4	0.5
Dissolved CO ₂ (mg L ⁻¹)	254.20 ± 3.421	293.33 ± 3.050	309.66 ± 1.527	316.66 ± 3.05	301.33 ± 5.77	306.66 ± 1.527
Biomass (g L ⁻¹)	1.264 ± 0.207	1.197 ± 0.121	1.413 ± 0.010	1.661 ± 0.137	1.582 ± 0.080	1.303 ± 0.053
Lipid (% dry)	11.242 ± 0.028	11.467 ± 0.096	12.142 ± 0.126	12.978 ± 0.362	12.404 ± 0.160	12.192 ± 0.295
Protein (mg mL ⁻¹)	0.025 ± 0.002	0.025 ± 0.001	0.033 ± 0.002	0.043 ± 0.004	0.036 ± 0.005	0.026 ± 0.002
Total chlorophyll (µg mL ⁻¹)	9.33 ± 0.130	9.72 ± 0.145	10.24 ± 0.030	13.693 ± 0.553	11.37 ± 0.402	11.846 ± 0.400
Total carbohydrate (mg mL ⁻¹)	0.035 ± 0.003	0.031 ± 0.002	0.35 ± 0.001	0.046 ± 0.002	0.034 ± 0.003	0.032 ± 0.003

**Fig. 7.** Effects of NPs Concentration on dissolved CO₂ Concentrations.**Fig. 8.** Effects of NPs addition on *C. vulgaris* biomass production.

C. vulgaris for 10 days in fermentation flasks without nanoparticles (control), but with SiO₂ and SiO₂-CH₃ nanoparticles, the findings were consistent with present studies. In Table No. 4, comparative data illustrate the effect of different nanoparticles on different microalgae with our study.

3.6. Impact of nanoparticles on *Chlorella vulgaris* lipids and FAME generation

C. vulgaris was grown with SiO₂ and SiO₂-CH₃ nanoparticles as well as without nanoparticles (control) for 12 days in flasks. Lipid concentrations in cultures with 0.1 wt percent SiO₂ and SiO₂-CH₃ NPs were 11.376% and 11.467%, respectively; they were not statistically different

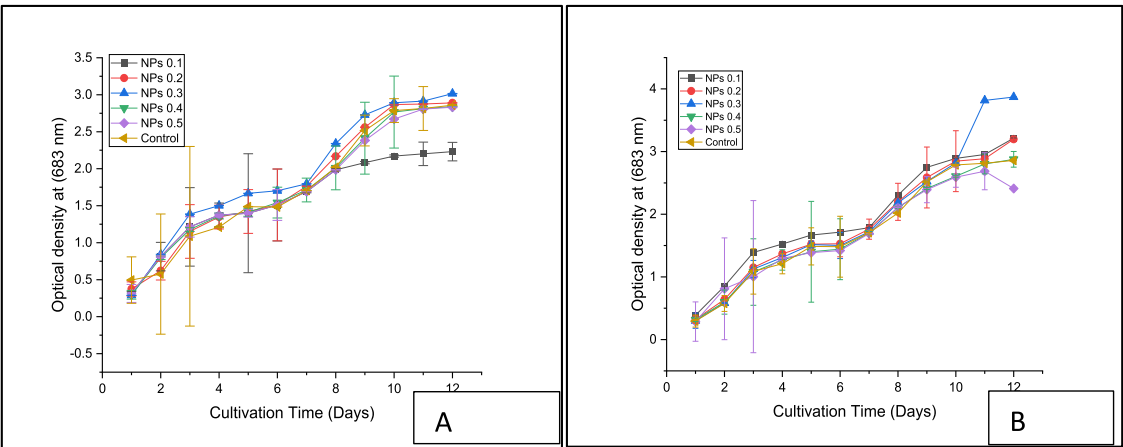


Fig. 9. Optical density (683 nm) of *C. vulgaris* at different concentrations of (A) SiO₂ and (B) CH₃- SiO₂ NPs.

Table 4
Comparative analysis the effects of nanoparticles on the growth of different microalgae species.

Microalgae Species	Nanoparticles		Performances		Ref.
	Types of NPs	Concentration (mg L ⁻¹)	Improved target	Efficiency (%)	
<i>Nanochloropsis limnetica</i>	nZVI	5.1	Lipid	37.14	[23]
<i>Microcystis aeruginosa</i>	TiO ₂	0.8	Growth rate	~50	[21]
<i>Chlorella vulgaris</i>	SiO ₂	0.2 wt%	Cell weight	177	[12]
	SiO ₂ - CH ₃	0.2 wt%	Cell weight	210	[12]
	TiO ₂	100	FAME	10	[21]
<i>Scenedesmus obliquus</i>	MgO	40	Lipid	18.5	[21]
	CNTs	5	Lipid	8.9	[21]
	Fe ₂ O ₃	< 20	Growth rate	10	[21]
	Fe ₂ O ₃	5	Lipid	39.6	[21]
<i>Chlorella vulgaris</i>	SiO ₂	0.3 wt%	Biomass	31.41	Present Study
		0.3 wt%	Lipid	15.44	Present Study
	SiO ₂ - CH ₃	0.3 wt%	Biomass	15.91	Present Study
		0.3 wt%	Lipid	14.53	Present Study

from the control (11.24%). The addition of 0.3 wt percent SiO₂ and SiO₂-CH₃ NPs resulted in a significant increase in the lipid content, which increase to 14.53% and 15.44%, respectively as compared to control. Similar results from previous research have been reported [4]. The dry weight and lipid content of *C. vulgaris* increased as the initial glucose concentration increased. Microalgae could have grown by utilising the abundant carbon source [1]. Lipids taken out of *C. vulgaris* were transformed into methyl fatty acid esters (FAMES). FAME and cell growth enhancement are possible causes of environmental stress resulting from the nanoparticles. According to Wang et al. [36], NPs have the ability to produce intracellular reactive oxygen species (ROS), including superoxide, hydroxyl, and hydrogen peroxide, which cause oxidative stress in cells and cause an imbalance between oxidative reduction processes in microalgal cells. Increased ROS levels trigger a series of cellular responses, such as the increased activity of lipid biosynthesis pathways. ROS may stimulate specific transcription factors and enzymes involved in lipid synthesis, such as acetyl-CoA carboxylase and fatty acid synthase [33]. When microalgal cells are exposed to NPs that induce oxidative stress, this can stimulate the mechanism for the accumulation of intracellular biochemical molecules [21,22]. Nanoparticle-induced oxidative and toxic stressors which promote lipid and biodiesel production during *C. vulgaris* cultivation [12].

4. Conclusion

In *C. vulgaris* cultures, researchers have tried to increase cell growth and lipid synthesis by increasing CO₂ mass transfer with the use of SiO₂ nanoparticles. A greater mass transfer rate and microalgal growth were observed as a consequence of treatment with methyl-silica oxide

nanoparticles rather than bare silica oxide nanoparticles. The hydrophobic functional groups (methyl) were added to the hydrophilic surfaces of SiO₂ nanoparticles to create hydrophobicity, which led to a more efficient rise in the mass transfer coefficient and dissolved CO₂ concentration in the growth media. The addition of silica oxide nanoparticles increased the specific gas-liquid interface area and the concentration of dissolved CO₂ in the growth media. Accordingly, the dry biomass, growth rate, lipid, and FAME productivity were enhanced in SiO₂-CH₃ nanoparticles.

Declaration of Competing Interest

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.

Data Availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.scenv.2023.100029.

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