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Enhancing microalgae-based bioremediation technologies with carbon-coated zero valent iron nanoparticles

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ABSTRACT

This study investigated the influence of the addition of carbon-coated zero-valent nanoparticles to enhance CO_2 removal efficiency, contributing to improved microalgae growth and biogas upgrading. NPs were studied in different formats - raw suspension (Raw-NPs) and dried format (D-NPs)-, along with the liquid fraction of the raw suspension (SP) on the performance of *Chlorella sorokiniana* for biogas upgrading and domestic wastewater treatment. The supplementation of SP at increasing concentrations during biogas upgrading resulted in higher CO_2 levels and microalgae inhibition. The addition of dried NPs enhanced microalgae growth but did not foster CO_2 absorption or biomethane quality regardless of their concentration (CO_2: 0.3–0.8 %; CH_4 69–73 %). Similarly, higher microalgae growth and photosynthetic activities were recorded at increasing dried NP concentrations during domestic wastewater treatment. The addition of D-NPs resulted in removal efficiencies of 74 %, 49 %, and 90 % for IC, TN and ammonium, respectively.

1. Introduction

Domestic wastewater treatment (WWT) is nowadays mandatory to guarantee the safe disposal of wastewater and preventing environmental contamination. In most developed countries, WWT is typically conducted using activated sludge processes, which rely on the action of heterotrophic and nitrifying bacteria in a series of interconnected aerobic, anoxic, and anaerobic processes. While this technology is effective at removing carbon, nitrogen, and phosphorous from domestic wastewater, activated sludge processes still exhibit significant drawbacks such as their high energy consumption, high CO_2 footprint and nutrient loss [1].

In this context, anaerobic digestion has also played a key role in the sanitation of both domestic and industrial wastewaters [2,3]. However, this method often exhibits a poor nutrient removal performance, which requires additional and costly post-treatment steps to achieve an effective nutrient destruction [4]. The biogas generated during the anaerobic digestion of the biodegradable organic matter present in wastewaters represents a promising and eco-friendly source of energy, with multiple potential applications such as heat and power generation or substitute of natural gas (for injection into gas grids or use as vehicle fuel) [5–7]. The composition of biogas is highly dependent on the substrate and type of anaerobic digester used [8,9], and typically consists of 55–75 % methane (CH_4) and 30–40 % carbon dioxide (CO_2). Minor contaminants such as nitrogen (0–3 %), oxygen (0–1 %), hydrogen sulfide (0–10.000 ppm_v), ammonia (0–10.000 ppm_v), and trace levels of halogenated compounds and volatile organic compounds, are also present in raw biogas [9–11]. The presence of contaminants in raw biogas restricts its utilization, requiring their removal with efficiencies as a function of the final use of biogas [12,13]. Multiple physical and chemical methods for upgrading biogas are nowadays commercially available, such as water scrubbing, membrane separation and pressure swing adsorption [6,14]. However, these technologies are associated with a high energy consumption and significant environmental impacts [15].

In this context, the adoption of biological approaches such as photosynthetic biogas upgrading has gained an increasing attention due to its economic and environmental benefits. This technology, based on the solar-driven CO_2 fixation by microalgae, offers a cost-effective and environmentally friendly option for the removal of CO_2 and H_2S from biogas [14,16,17]. This process is also characterized by an efficient nutrient assimilation and effective removal of pathogens, facilitated by the high pH, temperatures and O_2 concentrations mediated by photosynthesis [1,18]. Photosynthetic biogas upgrading in algal-bacterial photobioreactors has demonstrated to be a feasible technology,

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reaching CO_2 removals up to 98.6 % at pilot and demo scale [10,11,19–22]. Nevertheless, this technology presents several challenges and limitations, including the low CO_2 mass transfer to the culture medium, high sensitivity of biomethane quality to variations in the liquid and gas flow rates, pH and alkalinity, and environmental factors affecting photosynthetic activity [10,11,16,23]. In this context, novel operational strategies are required in order to enhance the biofixation of CO_2 from biogas.

In the field of CO_2 capture, nanoporous materials have recently gained increasing interest due to their unique properties such as high reactivity, abundance of active sites, adsorption capacity, and a high surface area to volume ratio [24–26]. Among the various nanomaterials available for CO_2 capture, metal oxide nanoparticles (NPs) and nanoporous carbons have been recently tested during photosynthetic biogas upgrading with promising results. These NPs not only exhibit potential to enhance CO_2 biofixation during photosynthetic biogas upgrading, but also could enhance nutrient recovery during domestic WWT based on the symbiotic interactions between microalgae and bacteria [26–28]. However, the effect of metal oxide NPs on microalgae metabolism has been a subject of controversy, with most studies primarily addressing their toxicity, specifically highlighting the toxic effects induced by NPs composed of Ag, ZnO, TiO_2 and CuO [24,29]. The use of carbon-coated zero-valent NPs has shown promising results during photosynthetic biogas upgrading but their impact on alga-bacterial systems treating domestic wastewater has not been explored [27]. For instance, the addition of CACOI NPs at a concentration of 70 mg·L^{-1} supported a higher CO_2 consumption compared to lower NP concentrations and controls in a study by Vargas-Estrada et al. [28]. This effect was observed when cultivating a consortium of microalgae and cyanobacteria in batch enclosed photobioreactors. Finally, one of the main limitations of carbon-coated zero-valent NPs is the cost associated with NPs drying during their manufacture process, which poses the question of the potential of the raw NPs suspension.

In this context, this work evaluated the potential of carbon-coated zero-valent NPs on *Chlorella sorokiniana* cultures devoted to biogas upgrading and domestic wastewater treatment. The NPs will be tested in three formats: i) the liquid fraction from the raw NP suspension, ii) the raw NPs suspension, and iii) the dried NPs, in order to gain a comprehensive understanding of their mechanisms on algal metabolism.

2. Materials and methods

2.1. Nanoparticles suspensions

Carbon-coated zero-valent iron (ZVI) (7.26 % of Fe, wt%) NPs were kindly donated by CALPECH (Spain) and will be referred as NPs. NPs (containing 8.68 % wt of Fe) exhibited a BET surface area of 27.3 m^2 g^{-1}, a pore volume of 0.28 cm^3 g^{-1} and an average pore diameter of 41.5 nm (mesoporous material according to the IUPAC classification). Further details about the properties of the dried NPs are described elsewhere [28].

Two different formats of NPs were used in this work: NPs in suspension in the aqueous solution remaining during manufacture (hereinafter named Raw-NPs), which were provided at a concentration of 3.5 g NPs·L^{-1}, and dried NPs (D-NPs). The Raw-NPs were centrifuged (10,000 rpm 4 °C, 10 min) and the supernatant was then filtered (0.2 μm size pore filter) to obtain the liquid fraction (from now on referred as SP).

2.2. Microalgae culture, biogas and culture media

The microalgae used in this study was *C. sorokiniana* CCAP 211/8 k, which was originally purchased from the Culture Collection of Algae and Protozoa (Cambridge, UK). *C. sorokiniana* was stored at 4 °C on sterile agar plates in SK medium enriched with glucose (3.125 g·L^{-1}), peptone (0.0625 g·L^{-1}) and yeast extract (0.0625 g·L^{-1}) according to [30,31]. The inoculum of *C. sorokiniana* was grown at 25 °C in enriched SK medium under continuous illumination (900 μE m^{-2} s^{-1}) and magnetic stirring at 200 rpm. When the culture reached exponential growth, the volatile suspended solids (VSS) concentration was measured in order to set the initial microalgae concentration in the batch assays. *C. sorokiniana* was not adapted to high irradiance conditions. The fresh aerobic activated sludge used in the domestic wastewater bioremediation assays was obtained from Valladolid WWTP (Spain).

In order to investigate the impact of NPs on *C. sorokiniana* metabolism during biogas upgrading, a synthetic biogas mixture consisting of 30 % CO_2 and 70 % CH_4 (Carburos Metalicos, Spain) was employed. Similarly, an inert gas such as helium (Linde, Spain) was used to replace the photobioreactor headspace during the domestic wastewater bioremediation assays.

Two synthetic mineral salt media were used in the biogas upgrading and bioremediation assays. The biogas upgrading assays employed a mineral salt medium enriched with carbonates, following the recipe described elsewhere [32]. On the other hand, the bioremediation assay used synthetic urban wastewater (SWW), following the procedure outlined by Toledo et al. [33].

2.3. Experimental set-up and operational conditions

Two different experimental tests series were performed in batch mode in order to evaluate the influence of NPs on microalgae-based biogas upgrading and domestic wastewater bioremediation. Batch assays were conducted in 1.2 L gas-tight glass photobioreactors with the raw NPs suspension (Raw-NPs), the centrifuged and filtered supernatant (SP), and dried NPs (D-NPs) at equivalent final NPs concentrations of 70, 140 and 280 mg L^{-1}.

The photobioreactors used for biogas upgrading contained 0.3 L of mineral salt medium rich in carbonates and those used for domestic wastewater bioremediation contained 0.3 L of SWW. The bottles were then supplied with the corresponding NPs suspension (Raw-NPs, D-NPs or SP) and concentration, biomass inoculum and synthetic gas in the headspace (0.9 L). The photobioreactors devoted to biogas upgrading were inoculated with *C. sorokiniana* at an initial concentration of 600 mg VSS L^{-1}, while the photobioreactors used during domestic SWW treatment were inoculated with 600 mg VSS L^{-1} of total biomass (50 % *C. sorokiniana* and 50 % aerobic activated sludge). The photobioreactors were closed with butyl septa and plastic caps, and initially flushed with helium for 10 min using inlet and outlet needles to remove the air atmosphere. Synthetic biogas was also flushed for 10 min applying the same process in the photobioreactors devoted to biogas upgrading. After 1 h of incubation at ambient conditions (200 rpm, 25 ± 2 °C) to allow gas-liquid equilibrium, the gas composition of the headspace was determined. Then, the bottles were incubated at ambient conditions under continuous magnetic stirring and continuous illumination (900 μE m^{-2} s^{-1}) using visible LED lights (PHILLIPS, Spain).

In the test series devoted to biogas upgrading, three batch assays were conducted in duplicate: i) abiotic test (No biomass) with sterile synthetic medium supplemented with Raw-NPs, SP, and D-NPs at an equivalent NPs concentration of 140 mg L^{-1} (in the case of SP addition, same volume of supernatant corresponding to 140 mg NP L^{-1} was added); ii) *C. sorokiniana* and Raw-NPs at 70, 140 and 280 mg L^{-1}; and iii) *C. sorokiniana* with D-NPs at 70, 140 and 280 mg L^{-1}. The selection of this NP concentration was based on previous studies carried out in our laboratory [28]. A set of control photobioreactors containing only *C. sorokiniana* and synthetic mineral salt medium was conducted in all biotic series. Biotic treatments with SP were omitted based on the results obtained from the biotic experiments with raw NPs suspension (see Section 3.2).

In the domestic wastewater bioremediation assays, only D-NPs were evaluated based on the results obtained in biogas upgrading tests. Different concentrations were run under an He headspace in duplicate using SWW with *C. sorokiniana* and activated sludge, supplemented with

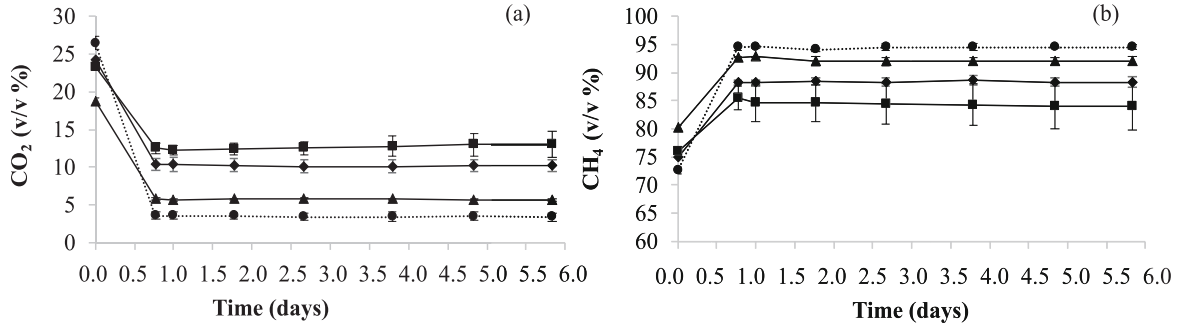

Fig. 1. Time course of the headspace concentrations of CO_2 (a), CH_4 (b), during biogas upgrading abiotic tests supplemented with culture medium (●), the liquid fraction from NP preparation (◆), dried solid NPs (▲) and the raw suspension of NPs (■).

D-NPs at 70, 140 and 280 mg L^{-1}. A control test containing only *C. sorokiniana* + activated sludge in SWW was also performed.

TSS and VSS concentrations, and pH, were determined at the beginning and end of each test. An aliquot of 5 mL of culture broth was daily extracted from each photobioreactor under sterile conditions using a 5 mL syringe in order to measure optical density at 550 nm (OD_{550}), and dissolved fractions of inorganic carbon (IC), total organic carbon (TOC), and total nitrogen (TN) concentrations. Additionally, $N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $P\text{-}PO_4^{3-}$, $S\text{-}SO_4^{2-}$ and $N\text{-}NH_4^+$ concentrations were measured in SWW bioremediation tests.

2.4. Analytical procedures

The gas composition (CH_4, CO_2, and O_2) in the photobioreactor headspace was determined by GC-TCD (Bruker) according to [34]. The pH was recorded using a pHmeter SensION™ + PH3 pHmeter, (HACH, Spain). IC, TOC and TN concentrations were quantified using a Shimadzu TOC-VCSH analyzer (Japan) equipped with a TNM-1 chemiluminescence module. $N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $P\text{-}PO_4^{3-}$, $S\text{-}SO_4^{2-}$ concentrations were quantified by HPLC-IC (Waters 432, conductivity detector, USA). $N\text{-}NH_4^+$ concentration was quantified using the Nessler analytical method in a spectrophotometer SpectroStar Nano (BGM Labtech) at 425 nm. Microalgae growth in biotic tests was daily determined by OD_{550} (SpectroStar Nano (BGM Labtech)) [35]. TSS and VSS concentrations were measured according to standard methods [36].

2.5. Statistical analysis

The results are presented as mean values ± standard deviation. An analysis of variance (ANOVA) followed by Tukey's test considering $\alpha = 0.05$ was performed to assess the influence of NPs on microalgae culture using SPSS statistics software.

3. Results and discussion

3.1. Influence of NPs conditions on biogas composition under abiotic conditions

After a 6-day incubation period, significant changes in the headspace CO_2 content were observed among the different conditions tested (Fig. 1a). The concentration of CO_2 after 1 h of incubation accounted for 26.5 % in the control tests without NPs, 24 % in assays supplemented with supernatant, 23 % in the photobioreactors supplied with Raw-NPs, and 18.8 % in tests supplemented with dried NPs. The CO_2 concentrations remained constant under all tested conditions from day 1 onwards. In the control abiotic tests, CO_2 concentration significantly decreased to 3.4 ± 0.5 % as a result of the high pH and buffer capacity of the mineral salt medium. This finding is aligned with previous studies by Marin et al.

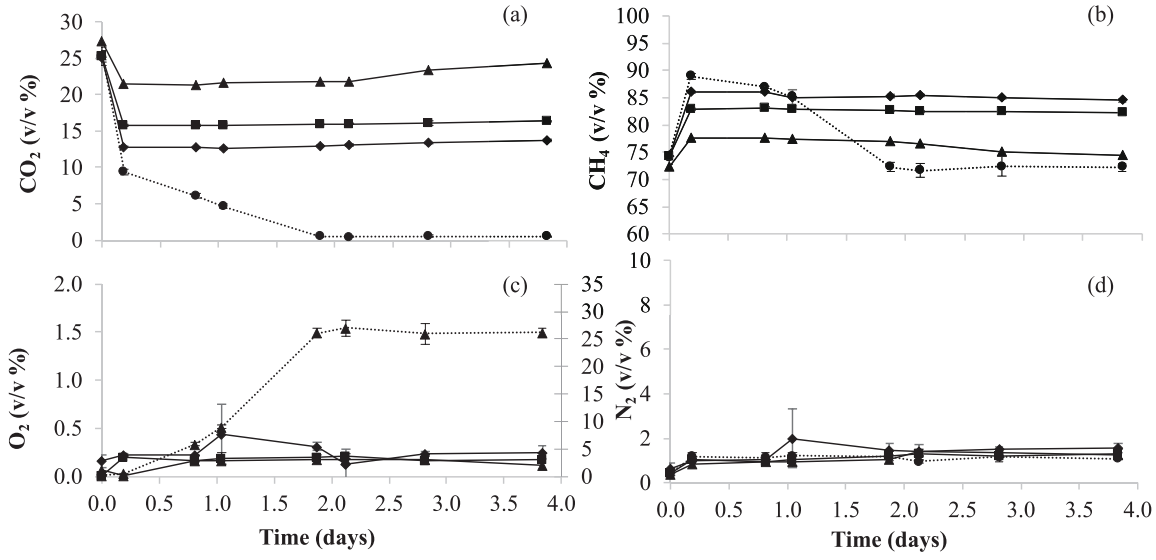

Fig. 2. Time course of the headspace concentration of CO_2 (a), CH_4 (b), O_2 (c) and N_2 (d) in assays devoted to biogas upgrading with *C. sorokiniana* and different concentrations of NPs supplied in the raw suspension at 70 mg·L^{-1} (◆), 140 mg·L^{-1} (■) and 280 mg·L^{-1} (▲). Control assays with mineral salt medium were also conducted (●). Note: In Figure (c), the right axis corresponds to control levels, while the left axis corresponds to the other treatments. Dual axes have different scales in this specific figure.

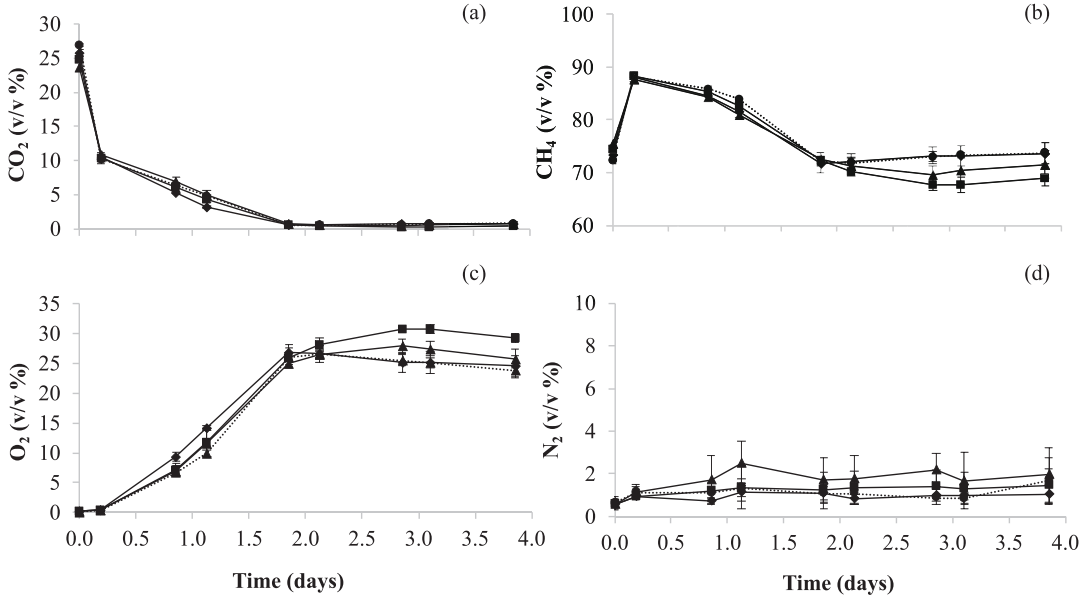

Fig. 3. Time course of the headspace concentration of CO_2 (a), CH_4 (b), O_2 (c) and N_2 (d) in assays devoted to biogas upgrading with *C. sorokiniana* and dried NPs concentrations of 70 mg·L^{-1} (◆); 140 mg·L^{-1} (■); 280 mg·L^{-1} (▲). Control assays with mineral salt medium were also conducted (●).

[11] and Mendez et al. [10], who demonstrated the key role of high inorganic carbon (IC) concentrations and pH in promoting CO_2 absorption. The assays conducted with dried NPs experienced a decrease in CO_2 content to 5.7 ± 0.2 %, which can be explained by the slightly lower final pH (8.33 ± 0.01) compared to the control tests (8.57 ± 0.09).

Interestingly, the addition of supernatant to the culture medium resulted in CO_2 levels of 10.2 ± 0.8 %, which agree with the lower pH of the broth (8.05 ± 0.06). This higher CO_2 content suggests the presence of acidic compounds in the supernatant of the NPs broth, resulting in a less favorable scenario of biogas upgrading in terms of CO_2 mass transfer. Finally, the supplementation of raw NPs suspension induced final CO_2 contents of 13.0 ± 1.7 % and a final pH value of 7.91 ± 0.10. Indeed, no significant differences in CO_2 levels were recorded in the tests supplied with supernatant or raw NPs suspension.

The CH_4 content in the headspace of the bottles increased during the first hour of incubation (Fig. 1b), as a result of the pH mediated CO_2 absorption. More specifically, the CH_4 contents after 1 h of incubation accounted for 72.5 % in the control assays, 76 % in assays with supernatant, 76 % in the tests with raw NPs suspension, and 80.2 % in the assays with D-NPs. As a result of the gradual dissolution of CO_2 in the liquid medium, the CH_4 content increased up to 94.5 ± 0.4 % by the end of the abiotic tests conducted with culture medium alone. Previous studies have proposed various mechanisms through which NPs induce effects that enhance the transfer of CO_2 transfer to the liquid phase, including shuttle effects, gas bubble breaking effects, or hydrodynamic effects [37]. This reduction in CO_2 levels in the headspace results in an increase in CH_4.

When dried NPs were added to the culture medium, a slightly lower CH_4 content of 92.1 ± 0.8 % was recorded by the end of the abiotic experiment. The acidification caused by the addition of supernatant to the medium resulted in CH_4 concentrations of 87 %. This reduced CH_4 levels correspond to the higher CO_2 levels observed in these conditions and indicates the shift in gas composition favoring CO_2 desorption. Likewise, in SP-NPs bottles, the biogas composition displayed a CH_4 concentration of 84.0 ± 4.2 %, supporting the influence of the compounds dissolved in the SP on reduced CH_4 upgrading due to increased CO_2 desorption, as mentioned above.

3.2. *Influence of Raw-NPs suspension dosage on C. sorokiniana growth and CO_2 removal*

In the tests conducted with Raw-NPs, the initial CO_2 concentrations (Fig. 2a) remained relatively similar at 25–27 % after 1 h of incubation. However, these headspace concentrations experienced varying levels of reduction after 4 h of incubation depending on the Raw-NPs dosage, and these CO_2 concentrations remained stable throughout the remaining incubation period. More specifically, the CO_2 levels at the end of the experiment averaged 24.3 ± 0.0 %, 16.4 ± 0.05 % and 13.7 ± 0.1 % in the assays with NP concentrations of 280 mg·L^{-1}, 140 mg·L^{-1}, and 70 mg·L^{-1}, respectively. The CO_2 concentration in the control tests accounted for 9.4 ± 0.4 % after 4 h of incubation, and gradually decreased to 0.5 ± 0.1 % by day 2.

The headspace methane concentrations were inversely correlated to the above-described CO_2 concentrations during the first 4 h of experiment (Fig. 2b). Thus, the methane content increased from 72 to 74 % up to 77.0 %, 82.9 ± 0.2 %, 85.9 ± 0.02 % and 88.9 ± 0.5 % in the assays supplied with NPs 280 mg·L^{-1}, 140 mg·L^{-1}, 70 mg·L^{-1} and 0 mg·L^{-1}, respectively. Interestingly, these concentrations remained almost stable throughout the rest of the incubation period for all tests, except for the control with mineral salt medium. The CH_4 content in the control tests from day 1 onwards decreased to 72.3 ± 0.8 %, concomitantly with CO_2 content, as a result of the increase in photosynthetic O_2 content.

A similar pattern was observed for N_2 content in the headspace regardless of the NPs concentrations tested. Thus, N_2 concentrations increased from approximately 0.4–0.6 % to final concentrations of 1.1 ± 0.03 %, 1.2 ± 0.2 %, 1.3 ± 0.2 % and 1.6 ± 0.2 % in the photobioreactors supplied with 0, 280, 140 and 70 mg NPs L^{-1}, respectively (Fig. 2d). These variations did not show any significant differences ($p > 0.05$). In the presence of raw NPs suspension, the O_2 content increased from 0.1 % to 0.15–0.25 %, with no significant difference among different NPs concentrations, whereas the control tests showed a remarkable increase in O_2 content up to 26 ± 0.8 % in <2 days of incubation. This significant increase in O_2 content was attributed to the active photosynthetic metabolism of *C. sorokiniana*, while the low O_2 levels recorded in tests supplied with Raw-NP suspension suggested a severe inhibition of microalgal metabolism. Thus, it can be concluded that the fluctuations in biogas composition observed in bottles with NPs were exclusively linked to the influences of both the NPs and the

Table 1
Initial and final pH and optical density in abiotic and biotic tests with varying NPs concentrations during biogas upgrading.

		pH						OD_{550}					
		Initial			Final			Initial			Final		
Abiotic test													
CM +	CM	9.42	±	0.02	8.57	±	0.09	[a]			[a]		
	SP	9.25	±	0.01	8.05	±	0.06	[a]			[a]		
	Raw-NPs 140	9.19	±	0.05	7.91	±	0.10	[a]			[a]		
	D-NPs 140	9.47	±	0.04	8.33	±	0.01	[a]			[a]		
Biotic test with *C. sorokiniana*													
CM +	CM	9.47	±	0.01	9.16	±	0.04	0.3	±	0.05	4.1	±	0.2
	Raw-NPs 70	9.33	±	0.02	7.83	±	0.01	2.7	±	0.04	2.92	±	0.24
	Raw-NPs 140	9.15	±	0.03	7.71	±	0.01	6.46	±	0.06	6.42	±	0.23
	Raw-NPs 280	8.68	±	0.05	7.43	±	0.02	13.02	±	0.04	11	±	0.22
CM +	CM	9.42	±	0.04	8.97	±	0.08	0.4	±	0.01	3.9	±	0.3
	D-NPs 70	9.41	±	0.06	9.04	±	0.11	0.6	±	0.03	3.7	±	0.3
	D-NPs 140	9.40	±	0.03	9.28	±	0.04	0.8	±	0.01	6.2	±	0.2
	D-NPs 280	9.35	±	0.03	9.06	±	0.07	1.04	±	0.01	6.03	±	0.21

[a] Parameter not evaluated in this condition.

medium, similar to those observed in abiotic test.

Similarly, no significant increase in optical density was observed for any of the Raw-NPs dosages evaluated, which confirmed the above-mentioned inhibition. However, a noticeable increase in optical density was observed in the control tests after one day of incubation. It is worth noting that the bottles containing Raw-NPs suspension exhibited a brownish coloration, which likely hindered the penetration of light into the algal cultures and indirectly photosynthetic activity. Finally, the analysis of VSS revealed a significant increase in biomass concentration in control bottles from 80 $mg{\cdot}L^{-1}$ to 430 $mg{\cdot}L^{-1}$, corresponding to the growth of *C. sorokiniana*. In contrast, the VSS concentrations in the tests with raw NPs suspension averaged 64, 84, and 98 $mg{\cdot}L^{-1}$ at the beginning of the assays conducted with 70, 140, and 280 mg $NPs{\cdot}L^{-1}$, respectively, and decreased to 19, 34, and 69 $mg{\cdot}L^{-1}$ by the end of the incubation period. This decrease can be attributed to cell lysis as a result microbial inhibition.

Given the results obtained in this assay, microalgal growth inhibition could be attributed to the limited light penetration due to the dark coloration of SP. However, the lack of data on the SP composition also raises the possibility of growth inhibition due to potential toxicity from its components. Further research is needed to explore these aspects in greater detail.

3.3. *Influence of D-NPs concentrations on C. sorokiniana growth and CO_2 removal*

The concentration of CO_2 (Fig. 3a) exhibited a sharp decrease during the first hours of incubation ($p < 0.05$), from 24 to 27 % to 10–11 % for all conditions tested. Subsequently, the CO_2 concentration continued to decline at similar rates, reaching minimum values by the second day and remaining constant until the end of the incubation period. No significant differences were observed among the different NPs concentrations assessed ($p > 0.05$), exhibiting CO_2 values from 0.3 to 0.8 % at the end of the assay. The remarkable potential of NPs lies in their carbon coating, which renders them highly effective for CO_2 capture [38,39]. The exact mechanism of interaction between these NPs and CO_2 is still not well understood. However, it has been hypothesized that NPs can temporarily capture CO_2 molecules before releasing them into the aqueous medium containing microalgae cells [40].

CH_4 concentration exhibited a similar pattern throughout the incubation period under all conditions tested (Fig. 3b). Hence, the CH_4 content initially ranged from 72 to 75 %, increased to 87–88 % within the first 4 h and decreased to 81–84 % after one day of incubation. This reduction in CH_4 content continued until the second day, reaching values of 72–73 %. Towards the end of the incubation period, slight variations in the final methane concentration were observed among the different conditions. More specifically, a slight increase to 73.7 ± 2.0 % and 73.7 ± 2 % was observed in the control and the test supplied with 70 $mg{\cdot}L^{-1}$ of dried-NPs, respectively, while the methane content decreased to 69.0 ± 1.6 % and 71.6 ± 2.7 %, respectively, in tests supplied with 140 and 280 $mg{\cdot}L^{-1}$. The O_2 content (Fig. 3c) increased in all photobioreactors from an initial value of 0.11–0.19 % up to 25–27 % of O_2 by day 2 of incubation. Then, slight variations were observed among conditions, following the trend observed in CH_4 measurements. More specifically, the control and tests with 70 $mg{\cdot}L^{-1}$ dried NPs showed a slight decrease to 23.8 ± 1.2 % and 24.6 ± 1.8 % of O_2, respectively, while the tests conducted with 140 and 280 $mg{\cdot}L^{-1}$ showed an increase to 29.2 ± 0.8 % and 25.8 ± 1.5 %, respectively. The increase in O_2 concentration occurred concomitantly with the reduction of CH_4 concentration, similarly to what was observed in previous controls. Thus, it was observed that D-NPs did not inhibit photosynthetic O_2 production and *C. sorokiniana* growth at the tested concentrations. Besides, N_2 levels slightly increased from 0.5 % up to 1.0 ± 0.5, 1.4 ± 0.8, 1.7 ± 1.1 and 2.0 ± 1.3 % in the assays conducted at 70 $mg{\cdot}L^{-1}$, 140 $mg{\cdot}L^{-1}$, 0 $mg{\cdot}L^{-1}$ and 280 $mg{\cdot}L^{-1}$, respectively (Fig. 3d).

The optical density of the culture broths (Table 1) revealed significant differences on the concentration employed. Notable differences were observed between treatments at 140 and 280 $mg{\cdot}L^{-1}$ compared to the control and the test conducted with 70 $mg{\cdot}L^{-1}$ ($p < 0.05$). These results are well correlated with the observed increase in O_2 and decrease in CO_2 concentrations. Thus, it can be hypothesized that CO_2 was effectively absorbed by the solid NPs, which stimulated the primary metabolism of microalgae. However, it is noteworthy that higher concentrations of D-NPs promoted microalgae growth up to a certain threshold, beyond which no further enhancement was observed. In this context, a recent study conducted by Vargas-Estrada et al. [28] also demonstrated similar patterns when investigating the effects of increasing NPs concentrations, specifically up to 70 $mg{\cdot}L^{-1}$, on a consortium of microalgae and cyanobacteria, with increased CO_2 consumption upon increasing NPs concentrations, along with an enhanced biomass productivity and carbohydrates production. These findings support that higher NPs concentrations can effectively stimulate microalgae metabolism and promote enhanced bioprocesses.

In terms of pH (Table 1), the addition of D-NPs resulted in a slight decrease, with a significant effect observed at the highest concentration of 280 $mg{\cdot}L^{-1}$ D-NPs ($p < 0.05$). However, there were no significant differences in pH between the initial and final measurements for any of the treatments. The presence of IC in the culture media may have played a crucial role maintaining the buffer capacity of the culture broth [10], potentially preventing excessive pH shifts and contributing to the

Table 2
Initial and final pH and optical density during domestic wastewater bioremediation tests with varying dried NPs concentrations.

	pH						OD_{550}					
	Initial			Final			Initial			Final		
Biotic test with *C. sorokiniana* addition												
CM	8.49	±	0.03	11.96	±	0.01	0.63	±	0.06	3.96	±	0.5
D-NPs 70	8.52	±	0.08	11.58	±	0.42	0.68	±	0.01	3.4	±	0.7
D-NPs 140	8.54	±	0.05	11.92	±	0.02	0.72	±	0.05	5.12	±	0.01
D-NPs 280	8.37	±	0.05	11.98	±	0.01	0.95	±	0.01	5.21	±	0.2

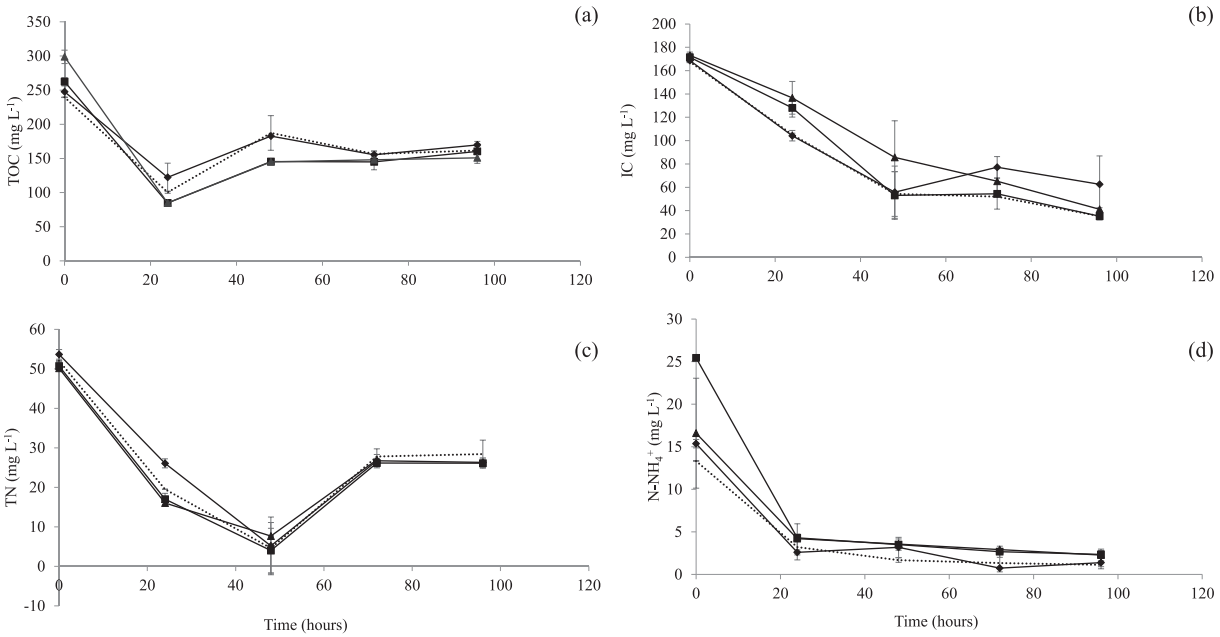

Fig. 4. Time course of dissolved concentrations of TOC (a), IC (b), TN (c) and ammonium ($N\text{-}NH_4^+$) (d) in bioremediation tests with dried NPs concentrations of 70 $mg{\cdot}L^{-1}$ (◆); 140 $mg{\cdot}L^{-1}$ (■); 280 $mg{\cdot}L^{-1}$ (▲). Control assays with synthetic wastewater were also conducted (—).

overall stability of the system.

3.4. Influence of D-NPs concentration on microalgae-based domestic wastewater treatment

In the bioremediation test, where *C. sorokiniana* and activated sludge were inoculated, all tested conditions exhibited microbial growth. A slight pH decrease ($p < 0.05$) and a slight increase in optical density were observed when increasing the concentrations of dried NPs at the beginning of the experiment. Interestingly, the final pH values for all experimental conditions increased to 11.6–12.0, showing no significant differences with NPs concentration. This suggested an effective utilization of IC mediated by photosynthesis, leading to the accumulation of OH^- ions in the medium. Indeed, the SWW experienced a significant reduction in IC concentration compared to the previous biogas upgrading tests, where pH was constant. In contrast, culture absorbance exhibited a significant increase under all tested conditions, concomitantly with an increase in the final VSS concentration, with the highest microbial growth observed in bottles supplemented with 140 and 280 $mg{\cdot}L^{-1}$ of D-NPs (Table 2). It has been suggested that the NPs cause shuttle and hydrodynamic effects, leading to improved transfer of CO_2 to the liquid phase, thus supporting CO_2 availability for microalgal growth.

The time course of TOC, IC, TN and ammonium concentrations did not exhibit significant differences among the conditions tested (Fig. 4). Interestingly, a slight increase in TOC concentrations was observed at increasing concentrations of D-NPs at the beginning of the experiment likely due to soluble carbon present in the NPs. The TOC concentrations decreased regardless of the conditions evaluated. The final average TOC concentrations ranged from 160.6 ± 9.3 $mg{\cdot}L^{-1}$ to 129.3 ± 28.6 3 $mg{\cdot}L^{-1}$, respectively, with no significant differences observed at the NPs concentrations tested. The reduction corresponded to a TOC removal efficiency of 33 % for the control, 32 % for the test supplied with 70 $mg{\cdot}L^{-1}$ D-NPs, 39 % for the test with 140 $mg{\cdot}L^{-1}$ D-NPs, and 50 % for the assays conducted with 280 $mg{\cdot}L^{-1}$ of D-NPs. Hence, the addition of NPs did not seem to influence the reduction of TOC concentration, despite their carbon coating. It is worth noting that although activated carbon has been reported to effectively eliminate soluble organic compounds from wastewater [41], it did not show a significant impact on the decrease of TOC concentration in this particular study. Similar findings were reported by Vargas-Estrada et al. [37], who observed no significant decrease in TOC concentration in a study using carbon-coated NPs in an open-pond photobioreactor system for microalga-bacteria biogas upgrading with artificial centrate as culture medium. The authors suggested that this lack of effect could be attributed to the utilization of NPs coated with non-activated mesoporous carbon [42].

On the other hand, there were no significant differences in IC concentrations among the tested groups by the end of the experimental period. The initial IC concentrations was 170 ± 2.9 $mg{\cdot}L^{-1}$, which decreased to 45.6 ± 15.2 $mg{\cdot}L^{-1}$, resulting in an average IC removal efficiency of 74.4 ± 7.8 %. This finding can be explained by the utilization of IC in photosynthesis, thus enhancing microalgae growth and increasing the pH.

TN removal efficiency of 49.1 ± 2.8 %, resulting in final concentrations of 26.3 ± 2.2 $mg{\cdot}L^{-1}$, were observed in all tests. However, TN concentrations rapidly decreased to 5.4 ± 1.6 mg L^{-1} after 48 h of incubation, and gradually increased up to 26.3 ± 2.2 $mg{\cdot}L^{-1}$. This

phenomenon could be explained by the rapid NH_4^+ assimilation mediated by algal growth and an active NH_3 stripping induced by the increase in pH during the first two days of experiment. Part of the ammonium stripped out to the headspace was likely re-absorbed and biologically oxidized, contributing to the observed increase in TN concentrations (Fig. 4c). The final ammonium removal efficiencies accounted for 90.0 ± 2.4 %, regardless of the NPs dosage. These results also suggested that all tested concentrations exhibited similar removal efficiencies, and no inhibition was observed due to the addition of D-NPs. The effective utilization of ammonium observed could be attributed to the active growth of *C. sorokiniana*, a green microalga that prefers NH_4^+ as primary nitrogen source since its uptake and assimilation consumes less energy than alternative oxidized nitrogen forms [43]. Additionally, the nitrification process, converting N-NH_4^+ into N-NO_3^- and N-NO_2^- contributed to ammonia consumption. In our specific case, there were no significant variations observed in the concentrations of NO_2^- and NO_3^- in the culture broth among the different treatments. Indeed, negligible concentrations of NO_2^- and NO_3^- were detected, and therefore, the consumption of ammonium was primarily attributed to the utilization by microalgae.

4. Conclusions

The impact of three NP conditions and concentrations on *C. sorokiniana* performance for biogas upgrading and nutrient removal was herein investigated. The supplementation of Raw-NPs concentrations to *C. sorokiniana* cultures supported a higher CO_2 removal, while the supernatant hindered microalgal growth at all concentrations tested. Conversely, the addition of dried NPs during biogas upgrading enhanced microalgae growth up to a limited threshold of 140 mg L^{-1}. Similarly, the addition of dried NPs showed removal efficiencies for IC, TN and ammonium that accounted for 74 ± 8 %, 49 ± 3 % and 90 ± 2 %, respectively. This study confirmed the beneficial impact of low dosages of carbon-coated zero valent iron nanoparticles on environmental applications of microalgae.

CRediT authorship contribution statement

Lara Méndez: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Raul Muñoz:** Funding acquisition, Project administration, Supervision, Writing – review & editing, Formal analysis, Conceptualization.

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

The authors are unable or have chosen not to specify which data has been used.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2024.103448.

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