Nutrient and Tetracycline Removal From Simulated Biogas Slurry and Biogas Upgrading by Microalgae Cultivation Under Different Carbon Nanotubes Concentration

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Abstract

The present study aimed to determine the effects of multi-walled carbon nanotubes (MWCNTs) concentrations (0, 0.1, 1, 5, 10 mg·L^{-1}) on tetracycline (TC) and biogas slurry nutrients removal by microalgae *Chlorella vulgaris* cultivation. Treatments with 1 mg·L^{-1} MWCNTs yielded maximum dry weight and cells quantity of 0.81 ± 0.008 g·L^{-1} and 5.83×10^7 cell·L^{-1}, respectively. The results of chlorophyll a were consistent with rapid fluorescence induction kinetics (OJIP-test), indicating that moderate MWCNTs concentration could enhance microalgal photosynthesis. Maximum chemical oxygen demand (COD), total phosphorus (TP), total nitrogen (TN), tetracycline (TC), and CO_2 removal efficiencies were 90.43 ± 5.15%, 78.12 ± 4.33%, 77.07 ± 4.12%, 89.64 ± 3.08%, and 64.26 ± 0.71%, respectively when treated with 1 mg·L^{-1} MWCNTs. Of the five MWCNTs concentrations set in this study, the optimal concentration was 1 mg·L^{-1} for nutrient and CO_2 removal efficiencies. These results indicated that moderate MWCNTs concentrations would promote tetracycline and nutrients removal by enhancing *Chlorella vulgaris* photosynthesis activity.

1 Introduction

Antibiotics are widely adopted for infectious disease treatment, protecting human health, and promoting animal growth (Liu et al., 2019). In recent decades, antibiotic-induced pollution has emerged as a global environmental and health problem. According to 2018 WHO (World Health Organization) reports, antibiotic resistance has been escalated as one of the severe threats to current global health, food safety, and development. Tetracycline antibiotics (TCs) are a group of broad-spectrum antibiotics and animal veterinary drugs, of which tetracycline (TC) is most commonly used (Dai et al., 2019). Accumulating evidence suggests that *Chlorella vulgaris* was often used as an ecological indicator to evaluate the aquatic toxicity of TC (Chen et al., 2019). Moreover, TC could significantly inhibit the growth and physiological processes of freshwater algae, including primary photochemistry and antioxidant system (Yang et al., 2013). It was reported that the 96 h-EC_{50} values of TC on *Chlorella vulgaris* cells were 7.73 mg·L^{-1} (Xu et al., 2019). After 2 days of treatment, the removal rates of cephalosporins antibiotics by *Chlorella* were 62.4–85.4% (Guo & Chen, 2015). Daneshvar et al. investigated the removal of TC from water by microalgal biomasses and found that the maximum adsorption capacities of freshwater microalgae *Scenedesmus quadricauda* and marine water microalgae *Tetraselmis suecica* were 295.34 and 56.25 mg·L^{-1}, respectively (Daneshvar et al., 2018). The microalgae could remove the antibiotics based on the antibiotics lipophilic properties, and the functions in microalgal adsorption and accumulation were existential (Chen et al., 2020a). Besides, microalgae are characterized by their unique properties, such as the biological purification of biogas slurry and biogas. These unicellular photoautotrophic microorganisms account for 40% of the global photosynthesis and reduce atmospheric greenhouse gase concentrations by utilizing CO_2 (Vaz et al., 2019; Zhu et al., 2014). Therefore, CO_2 absorption from biogas through microalgae photosynthesis might be an effective technology to upgrade biogas due to high CO_2 fixation capacity, rapid growth rates, and strong adaptability of microalgae to the environment (Zhang et al., 2020a).

As one of the most universal manufacturing materials, carbon nanotubes (CNTs) have been widely used in the fields of electronics, medicine, and nanobiotechnology (Sun et al., 2020a). The manufactured carbon nanotubes are of two types: one is Multi-walled carbon nanotubes (MWCNTs) and the other is Single-walled Carbon nanotubes (SWCNTs) (Sohn et al., 2015). The global CNTs market is expected to increase from 2.26 Billion USD to 5.64 Billion USD by 2020 at a compound annual growth rate of 20.1% (Pikula et al., 2018). In contrast, MWCNTs exposure can lead to reductions in algal growth and cell viability (Pereira et al., 2014). MWCNTs exert toxic effects on cellular metabolic activity, cell components, and plasma membrane of filamentous green microalgae (Munk et al., 2017). Marine algae *Heterosigma akashiwo* had no toxic effects at CNTs doses of 1 and 10 $mg·L^{-1}$, while 100 $mg·L^{-1}$ exerted both acute and chronic toxic effects (Pikula et al., 2018). It has been reported that the CNTs level higher than 40 $mg·L^{-1}$ could be fatal to freshwater algae within a few days, such as *Chlorella vulgaris* (Long et al., 2012; Sohn et al., 2015). It was demonstrated that toxic effects caused by the carbon nanotubes are strongly correlated with the contents of heavy metal impurities in the nanoparticles (Pikula et al., 2020). The maximum growth inhibition ratio (IR) of CNTs on the microalgae was 58% at 96 h under 200 $mg·L^{-1}$ CNTs treatment, whereas microalgae growth inhibition was far lower than nano-Cu (Zhang et al., 2018). Another study has reported that the photosynthetic current obtained by immobilizing *Chlorella* cells with multiwall carbon nanotubes (MWCNTs) was 3-folds higher than the normal *Chlorella* cells (You et al., 2019). The CNTs could potentially influence the behavior of other coexisting pollutants due to their unique structure and properties, thereby altering their toxicity to aquatic organisms and enhancing microalgal photosynthesis to some extent (Sun et al., 2020a). Moreover, CNTs are good adsorbents of organic and inorganic compounds (Long et al., 2012), and can remove antibiotics by adsorption. Zhuang et al. found that the antibiotics removal efficiencies (initial sulfamethazine concentration: 20 $mg·L^{-1}$) of MWCNTs on deionized water and tap water were 96.03% and 95.30%, respectively, but 64.46% for effluent (Zhuang et al., 2020). Yu et al. reported that the optimal adsorption capacities on antibiotic TC were 1530 $\pm$ 51.9 $mg·g^{-1}$ (Yu et al., 2016).

In this study, biogas slurry and biogas was simulated those of pig farm, so adding amount of tetracycline. Therefore, the study focused on the effects of low MWCNTs concentrations (0, 0.1, 1, 5, 10 $mg·L^{-1}$) on tetracycline (TC), biogas, and biogas slurry nutrients removal by microalgae *Chlorella vulgaris* cultivation. Moreover, the microalgal biomass, cell quantity, chlorophyll a content, photosynthetic performance, intracellular carbonic anhydrase activity, and nutrients, TC, and CO_2 removal efficiency were investigated to determine the effects of CNTs on the purification of biogas slurry and biogas through algae growth and photosynthesis. Besides, this research combines nanomaterials with biological science and offers a new perspective for purifying biogas slurry and biogas by analyzing the photosynthetic parameters from OJIP test.

2 Materials And Methods

2.1 Microalgae strains and chemical treatments

Chlorella vulgaris (FACHB-8) was purchased from Freshwater Algae Culture Collection at Institute of Hydrobiology, China. The tetracycline (TC, CAS NO: 60-54-8) was obtained from MACKLIN, Shanghai, China. Short-Carboxylic Multi-walled MWCNTs (OD: 10 ~ 20 nm, purity: > 95 wt%, length: 0.5 ~ 2 μm) were carboxylic carbon nanotubes, purchased from XFNANO, Jiangsu, China.

Chlorella vulgaris cells were propagated in BG11 medium before the experiment, and the medium was changed every 7 days. Later, the microalgae cells were collected by centrifugation, and then resuspended in simulated biogas slurry to ensure the microalgae cell concentration at the logarithmic phase. The simulated biogas slurry components were glucose (0.8 $g \cdot L^{-1}$), urea (0.752 $g \cdot L^{-1}$), $NaH_2PO_4 \cdot 2H_2O$ (0.203 $g \cdot L^{-1}$), KH_2PO_4 (0.0203 $g \cdot L^{-1}$), $CaCl_2$ (0.004 $g \cdot L^{-1}$), $MgSO_4$ (0.002 $g \cdot L^{-1}$), and Tetracycline (10 $mg \cdot L^{-1}$). The initial pH, chemical oxygen demand (COD), total nitrogen (TN), and total phosphorus (TP) were 6.87 ± 0.46, 1424.51 ± 40.13 $mg \cdot L^{-1}$, 330.34 ± 10.12 $mg \cdot L^{-1}$, and 42.72 ± 1.68 $mg \cdot L^{-1}$, respectively.

2.2 Experimental apparatus and conditions

The photobioreactor consisted of two 16.8 L glass jars filled with 2.8 L of simulated biogas slurry and 14 L of biogas, as reported in the previous study (Zhao et al., 2019). In this study, the initial cell concentration of *Chlorella vulgaris* was maintained at 1.5×10^6 cells mL^{-1}. MWCNTs were dispersed in deionized water by sonication at 300 W for 10 minutes. Different MWCNTs concentrations of 0, 0.1, 1, 5, 10 $mg \cdot L^{-1}$ were obtained by adding 0, 10 μL, 100 μL, 500 μL, and 1 mL of MWCNTs solutions (1 $g \cdot L^{-1}$) into 100 mL of simulated biogas slurry. Later, the culture was cultivated using MWCNTs for different treatments, and 0 $mg \cdot L^{-1}$ MWCNTs concentration was considered as the control group. The microalgae cells were propagated with 200 $\mu mol \cdot m^{-2} \cdot s^{-2}$ light for 9 d at 25℃ under 12 h light/12 h dark light conditions in an illuminated incubator shaker (HZ-9310KBG, Shanghai, China). The biogas slurry and biogas were sampled at 1th, 3th, 5th, 7th, and 9th days to measure all the indicators.

2.3 Evaluation methods

2.3.1 Estimation of *Chlorella vulgaris* growth

The microalgal cell quantity was estimated by comparing the optical density (OD) of algae at 680 nm to establish a linear relationship between them.

Dry weight ($g\ L^{-1}$) is one of the simplest but intuitive indicators to judge the microalgae growth. 10 mL of algal liquid were taken into the centrifuge tube and centrifuged at 10,000 rpm for 10 minutes to remove the clarified supernatant. Then, the centrifuge tube was placed in an oven at 105 °C to dry until the weight did not change. The weight difference between the front and back of the centrifuge tube was the dry weight of 10 mL of algal fluid.

2.3.2 Measurement of chlorophyll a (Chl-a)

10 mL of algal liquid was centrifuged (XZ21K-T, Hunan, China) at 10000 rpm for 10 min, and the supernatant was discarded. The obtained precipitate was dissolved in 10 mL of acetone with 90% volume fraction and placed in a dark environment at 4 °C for 24 h. After 24 h, the supernatant was collected by centrifugation at 8,000 g for 15 min to measure Chl-a concentration. The absorbance of the supernatant was determined by ultraviolet spectrophotometry at 630 nm, 645 nm, 663 nm, and 750 nm using 90% aqueous acetone solution as blank. Chl-a concentration ($\mu g \cdot L^{-1}$) was calculated according to Eq. (1):

$$\mathrm{CHl-a} = \left(11.64 \times (OD_{663} - OD_{750}) - 2.16 \times (OD_{645} - OD_{750}) + 0.10 \times (OD_{630} - OD_{750})\right) \cdot V \qquad \text{Eq. (1)}$$

where Chl-a represents the concentration of chlorophyll a ($\mu g \cdot L^{-1}$); OD_{630}, OD_{645}, OD_{663}, and OD_{750} are the measured absorbance values of the sample at the corresponding wavelengths; V is the sample volume (L) (Ji et al., 2018).

2.3.3 OJIP test

Chlorophyll a fluorescence transient (OJIP) was measured by Aquapen (Photon Systems Instruments, AP-C 100, Brno, Czech Republic) to study the effects of different MWCNTs concentrations on *Chlorella vulgaris* photosynthesis. After the algae solution was treated in the dark at 25 °C for 10 min, the fluorescence parameters (F_v/F_m, PI_{ABS}, Ψ_o, Φ_{EO}, and Φ_{DO}) were obtained by Aqua Pen.

2.3.4 Physicochemical analysis

Chlorella vulgaris was centrifuged at 10,000 rpm for 10 min in low-temperature centrifugation and filtered with 0.45 μm membrane filter for concentration determination of tetracycline and nutrients. The COD, TN, and TP concentrations in biogas slurry were measured according to the standard method (APHA, 2005). Pollutants removal efficiency (%) was calculated according to Eq. (2):

$$\text{Removal efficiency}(\%) = \frac{C_I - C_F}{C_I} \times 100 \qquad \text{Eq. (2)}$$

where C_I and C_F are the initial and final concentrations of target index, respectively ($mg \cdot L^{-1}$).

The initial biogas included CH_4 (60.32 ± 3.26, v/v), CO_2 (36.17 ± 1.97, v/v), H_2O (3.04 ± 0.23, v/v), and O_2 (0.47 ± 0.04, v/v). Biogas was collected by a syringe during microalgae culture using a gas analyzer (GA94, ONUEE Co., Ltd., China) for component analysis. The CO_2 removal efficiency was also calculated by Eq. (2).

2.3.5 Determination of tetracycline in simulated biogas slurry

Chlorella vulgaris (5 mL) was centrifuged and filtered for physicochemical analysis, and then the microalgae filtrate was injected into the EC-C18 column (4.6×100 mm, 2.7 μm) supporting the Liquid Chromatography System. Mobile phase A was trifluoroacetic acid (1/1000, V/V), and the mobile phase B was acetonitrile. The ratio of phase A to phase B was 85:15. The flow rate was set to 1 $mL \cdot min^{-1}$, the detection wavelength to 357 nm, the column temperature to 35 °C, the injection volume to 20 μL, and the injection time to 15 min. The tetracycline concentration had a good correlation with the peak area; hence, it was quantitatively analyzed by the peak area. Tetracycline removal efficiency (%) was calculated, according to Eq. (2).

2.3.6 Determination of intracellular carbonic anhydrase activity

The carbonic anhydrase activity was measured with a pH electrode, as described in the previous study (Shen et al., 2020).

2.3.7 TEM measurement

10 mL samples were centrifuged at 10000 rpm for 10 minutes, and then fixed by 2.5% glutaraldehyde. Algal cell sections were made using a microtome (Leica RM2235, Germany), followed by staining with uranyl acetate and lead citrate for 20 min. Finally, the sections were observed using via transmission electron microscopy (TEM, HT7800, Hitachi, Japan).

2.4 Statistical analysis

In this study, all the experiments were carried out in triplicate and the values were expressed as mean ± standard deviation (SD). All statistical analyses were performed using SPSS 19.0. Duncan's multiple range test was used to separate means. Significance was set at $p < 0.05$.

3 Results And Discussion

3.1 Microstructure

CNT like structures were found in TEM slides of MWCNT exposed microalgae. TEM images showed that, not only the surface of *Chlorella vulgaris* out cell wall was coated with carbon nanotubes, but also MWCNT could be observed inside the cells (Fig. 1). Figure 1 (a) and Fig. 1 (b) displayed the ultrastructure of microalgae under 1 $mg \cdot L^{-1}$ and 10 $mg \cdot L^{-1}$ MWCNTs, respectively. MWCNTs are tubular carbon structures, that could enter cells through holes in the plasma membrane and then make changes in gene expression (Martinez-Ballesta et al., 2020), rather than via endocytosis (Serag et al., 2011). It has demonstrated that multi-walled carbon nanotubes (MWCNTs) could be directly interacted with photosynthetic materials like thylakoids, increasing charge transfer (Dewi et al., 2015). When the microalgae cells are treated with low concentration of MWCNTs (1 $mg \cdot L^{-1}$), cells structure is not affected, very complete (Fig. 1 (a)). However, structure of cells is damaged, and the plasmolysis occurs while the concentration of MWCNTs exceed 10 mg·L^{-1} (Fig. 1 (b)), which indicated that high concentration of MWCNTs is not conducive to the growth of microalgae.

3.2 Effect of MWCNTs concentration on microalgae growth

In this study, the microalgal dry weight (0.81 ± 0.008 g·L^{-1}) and cell-quantity (5.834×10^{7} cells mL^{-1} on 7th day) obtained from 1 mg·L^{-1} MWCNTs treatment were measured to determine the effects of MWCNTs on *Chlorella vulgaris* growth (Qian et al., 2018). The result indicated that MWCNTs concentration significantly affected microalgal growth (Table 1).

Table 1
Dry weight, pollutants and CO_2 mean removal efficiency (Res) of microalgae under different MWCNTs concentrations. Note: Different superscript letters indicate a significant difference at p < 0.05 (Duncan's multiple range tests) across different carbon nanotube concentrations.

MWCNTs concentration (mg L^{-1})	**Dry weight (g L^{-1})**	**Tetracycline-Res (%)**	**COD-Res (%)**	**TN-Res (%)**	**TP-Res (%)**	**CO_2-Res (%)**
0	0.65^{b} ± 0.009	62.98^{b} ± 2.36	69.23^{b} ± 3.10	57.52 ^{bc}±3.28	60.93 ^{b}±2.84	49.91 ^{bc}±1.91
0.1	0.70^{b} ± 0.015	70.56 ^{a}±3.08	72.28 ^{b}±4.03	60.55 ^{ab}±3.79	62.76 ^{ab}±3.18	53.32 ^{ab}±1.85
1	0.81^{a} ± 0.008	75.36 ^{a}±2.86	78.20 ^{a}±3.36	65.95 ^{a}±3.46	66.77 ^{a}±3.71	57.27 ^{a}±2.02
5	0.53^{c} ± 0.009	59.03^{bc} ± 2.45	64.45 ^{bc}±4.13	53.92^{c} ± 2.95	56.33 ^{bc}±4.29	47.34 ^{c}±2.18
10	0.46^{c} ± 0.012	55.58 ^{c}±2.03	60.21 ^{c}±3.58	51.34^{c} ± 2.50	51.52 ^{c}±3.92	39.61 ^{d}±1.79

Note: Values with different superscript letters indicate a significant difference at $p < 0.05$ according to the Duncan's multiple range tests.

Microalgal dry weight and cell-quantity gradually increased and then decreased with the increase of MWCNTs concentration (Fig. 2). These two indicators represented the microalgae biomass and *Chlorella vulgaris* growth rate. Statistical analysis results indicated that the biomass was significantly affected by MWCNTs concentration between the treatment and control group (p < 0.05) (Table 1). This result was consistent with the results of Munk et al. reporting MWCNTs at 1 mg·L^{-1} could enhance the microalgal growth (Munk et al., 2017). MWCNTs at low concentrations could promote metabolite production by changing the ROS generation (Rahmani et al., 2020). However, the microalgal cell-quantity obtained under 5 and 10 mg·L^{-1} MWCNTs were 5.018×10^{7} cells·mL^{-1} and 4.807×10^{7} cells mL^{-1} on 7th day, respectively, which were worse than the control group. The same result was observed for microalgae dry weight. It was obviously observed that, the biogas slurry adding 5 and 10 mg·L^{-1} MWCNTs presented darker color, which affected the effect of photosynthesis and microalgae growth state. However, the MWCNTs concentrations in this study were not too high to toxic *Chlorella vulgaris*. Thus, different MWCNTs concentrations were likely to mediate *Chlorella vulgaris* growth by promoting metabolism at 1 $mg·L^{-1}$, and inhibiting at 5 and 10 $mg·L^{-1}$. Therefore, a moderate MWCNTs concentration of 1 $mg·L^{-1}$ was found to be highly suitable for *Chlorella vulgaris* growth.

3.3 Effect of MWCNTs concentration on chlorophyll *a* of microalgae

Chlorophyll a (Chl-a) content is an efficient indicator of microalgal stress (Jia et al., 2018) and *Chlorella vulgaris* growth (Sun et al., 2020b). During the experiment, *Chlorella vulgaris* Chl-a contents accumulated gradually due to the rapid growth rate and peaked on the 7th day. Similar to the growth trend, *Chlorella vulgaris* obtained the highest Chl-a content, reaching 143.73 ± 3.11 $μg·L^{-1}$ on the 7th day under 1 $mg·L^{-1}$ MWCNTs. Besides, on day 7, Chl-a contents at 0, 0.1, 5, and 10 $mg·L^{-1}$ MWCNTs were 105.77 ± 2.23 $μg·L^{-1}$, 112.24 ± 2.83 $μg·L^{-1}$, 88.23 ± 2.82 $μg·L^{-1}$, and 75.67 ± 2.77 $μg·L^{-1}$, respectively. This indicated that microalgal growth was poor under the environmental stress of 5 and 10 $mg·L^{-1}$ MWCNTs. Moreover, the intracellular physiological metabolism, Chl-a synthesis in algal cells, and photosynthesis were seriously damaged/disturbed. In this study, the optimal MWCNTs concentration for *Chlorella vulgaris* Chl-a content was 1 $mg·L^{-1}$. A relatively high concentration of MWCNTs would decrease microalgal Chl-a content. Accumulating studies have reported that Chl-a content of freshwater diatoms increased by MWCNTs treatment at lower concentrations (Jia et al., 2018) as MWCNTs might have attacked certain pigments converting them into Chl-a.

3.4 Effects of MWCNTs concentration on photosynthetic performance

In this study, OJIP (Open-JIP test) was employed to measure the microalgal photosynthetic performance, such as chlorophyll a fluorescence. OJIP test is the widely adopted method to study plant photosynthesis and stress (Bates et al., 2019). After treatment under dark mode for ten minutes, the algal culture was excited to visible light to obtain the fluorescence parameters, including F_v/F_m, PI_{ABS}, $Ψ_o$, $Φ_{EO}$, and $Φ_{DO}$. These five parameters represent the maximum quantum yield of PSII, performance index based on the absorption of light energy, the efficiency of electron transport, the maximum yield of electron transport, and the quantum yield of energy dissipation of the non-photochemical processes of PSII, respectively (Ji et al., 2018; Sun et al., 2020b). In particular, the PI_{ABS} varied significantly, suggesting consistency with the results of (Sun et al., 2020a). The photosynthetic performance was affected by MWCNTs concentrations. For instance, F_v/F_m, PI_{ABS}, $Ψ_o$, and $Φ_{EO}$ were the highest, and $Φ_{DO}$ was the lowest at 1 $mg·L^{-1}$ MWCNTs. These results suggested that 1 $mg·L^{-1}$ MWCNTs promoted light absorption, electron transfer efficiency, maximum electron transport yield, and reduced energy dissipation in *Chlorella vulgaris* (Xu et al., 2020). F_v/F_m and PI_{ABS} increased with the increase of MWCNTs concentration from 0 to 1 $mg·L^{-1}$, but these two values of high MWCNTs concentrations (5 and 10 $mg·L^{-1}$) were lower than the control group, revealing that the MWCNTs could only promote PSII photosynthesis of microalgae within a certain concentration range. High CNTs concentrations are toxic to microalgae in various ways. The toxicological mechanisms of CNTs exposed to *Chlorella* sp. were due to the combined effects of oxidative stress, agglomeration, and physical interactions (Long et al., 2012). This study result was consistent with the previous study on MWCNTS promoting PSII, but the optimal MWCNTs concentration was different (Sun et al., 2020a). There may be two reasons for the differences, the different microalgae species and the addition of toxic heavy metals in that article which were toxic to microalgae. Three heavy metals, copper (Cu), cadmium (Cd) and zinc (Zn) were have a crucial role in the effects of microalgae growth process.

Table 2
Fluorescence data of microalgae, obtained via OJIP test on 7th day.

MWCNTs concentration ($mg\ L^{-1}$)	FV/FM	PIABS	ΨO	ΦEo	ΦDo
0	$0.628^{b} \pm 0.002$	$3.811^{c} \pm 0.018$	$0.749\ ^{ab}\pm0.005$	$0.484\ ^{b}\pm0.016$	$0.332\ ^{b}\pm0.002$
0.1	$0.667\ ^{a}\pm0.005$	$4.801^{b} \pm 0.017$	$0.757\ ^{ab}\pm0.007$	$0.505\ ^{a}\pm0.003$	$0.318\ ^{c}\pm0.004$
1	$0.682\ ^{a}\pm0.005$	$5.449\ ^{a}\pm0.012$	$0.789\ ^{a}\pm0.006$	$0.520\ ^{a}\pm0.001$	$0.311\ ^{c}\pm0.005$
5	$0.604\ ^{b}\pm0.009$	$2.984^{d} \pm 0.019$	$0.705\ ^{c}\pm0.003$	$0.472\ ^{b}\pm0.005$	$0.351\ ^{a}\pm0.009$
10	$0.579^{c} \pm 0.003$	$2.798\ ^{d}\pm0.007$	$0.669^{c} \pm 0.003$	$0.468\ ^{b}\pm0.005$	$0.368\ ^{a}\pm0.003$

3.5 Effects of MWCNTs concentration on nutrient and tetracycline removal from biogas slurry

The concentration of COD, TP, TN, and tetracycline were evaluated from simulated biogas slurry by *Chlorella vulgaris* under different MWCNTs concentrations (Fig. 4a, b, c, and d respectively). The removal efficiencies of simulated biogas slurry, COD, TN, TP, and tetracycline are summarized in Table 1. The initial COD concentration was 1424.51 ± 40.13 $mg{\cdot}L^{-1}$. During the cultivation period, COD concentration decreased from 723.08 ± 37.43 $mg{\cdot}L^{-1}$ to 136.33 ± 27.02 $mg{\cdot}L^{-1}$ under 1 $mg{\cdot}L^{-1}$ MWCNTs treatment, while it decreased from 812.11 ± 38.02 $mg{\cdot}L^{-1}$ to 282.34 ± 39.37 $mg{\cdot}L^{-1}$, 796.87 ± 26.14 $mg{\cdot}L^{-1}$ to 227.78 ± 20.39 $mg{\cdot}L^{-1}$, 876.50 ± 31.47 $mg{\cdot}L^{-1}$ to 305.70 ± 19.75 $mg{\cdot}L^{-1}$, 903.28 ± 36.58 $mg{\cdot}L^{-1}$ to 354.85 ± 20.26 $mg{\cdot}L^{-1}$, respectively under 0, 0.1, 5, 10 $mg{\cdot}L^{-1}$ MWCNTs treatments (Fig. 4a). Meanwhile, it was found that *Chlorella vulgaris* under 1 $mg{\cdot}L^{-1}$ MWCNTs exhibited 78.20% mean COD removal efficiency value, while 0, 0.1, 5, 10 $mg{\cdot}L^{-1}$ yielded 69.23%, 72.28 %, 64.45% and 60.21% (Table 1). These results revealed that 1 $mg{\cdot}L^{-1}$ MWCNTs concentration was superior to 0.1, 5, 10 $mg{\cdot}L^{-1}$ in terms of COD removal by *Chlorella vulgaris* cultivation. The values obtained in this study were consistent with the study reporting higher COD removal efficiencies than 64.0% (Zhang et al., 2020b), except 10 $mg \cdot L^{-1}$ MWCNTs treatment, might attributing to the different simulated biogas slurry formula.

The effects of different concentrations of MWCNTs on TP removal were also investigated (Fig. 4b). During the cultivation period (until the 7th day), the TP content declined and then tended to be unchanged towards the 8th to 9th days, which was consistent with the trends of COD and TN. Under 1 $mg \cdot L^{-1}$ MWCNTs concentration, TP removal efficiency of microalgae in simulated biogas slurry was the highest, and the TP concentration ranged from 23.08 ± 1.51 $mg \cdot L^{-1}$ to 9.35 ± 1.71 $mg \cdot L^{-1}$. Similarly, the concentration ranges for 0, 0.1, 5, 10 $mg \cdot L^{-1}$ MWCNTs were from 24.14 ± 1.12 $mg \cdot L^{-1}$ to 11.96 ± 1.85 $mg \cdot L^{-1}$, 23.72 ± 1.41 $mg \cdot L^{-1}$ to 11.34 ± 1.81 $mg \cdot L^{-1}$, 24.76 ± 1.75 $mg \cdot L^{-1}$ to 14.82 ± 1.29 $mg \cdot L^{-1}$, 26.05 ± 1.03 $mg \cdot L^{-1}$ to 17.34 ± 1.92 $mg \cdot L^{-1}$. The mean TP removal efficiency values were also calculated (Table 1), and the highest value reached 66.77 ± 3.71% under 1 $mg \cdot L^{-1}$ MWCNTs, while the mean values of 0, 0.1, 5, and 10 $mg \cdot L^{-1}$ were 60.93 ± 2.84%, 62.76 ± 3.18%, 56.33 ± 4.29%, and 55.52 ± 3.92%, respectively.

The TN removal was investigated to assess nitrogen removal efficiency under different MWCNTs concentrations, and the results are summarized in Fig. 4c. The initial TN concentration was 330.34 ± 10.12 $mg \cdot L^{-1}$. Following 9 days of incubation experiments, TN concentration ranged from 172.97 ± 4.34 $mg \cdot L^{-1}$ to 75.75 ± 3.19 $mg \cdot L^{-1}$ under 1 $mg \cdot L^{-1}$ MWCNTs concentration, while it ranged from 193.51 ± 6.31 $mg \cdot L^{-1}$ to 101.98±8.28 $mg \cdot L^{-1}$ in the control group, from 184.46±7.18 $mg \cdot L^{-1}$ to 97.81±3.93 $mg \cdot L^{-1}$ at 0.1 $mg \cdot L^{-1}$, from 191.37±4.17 $mg \cdot L^{-1}$ to 121.73±3.52 $mg \cdot L^{-1}$ at 5 $mg \cdot L^{-1}$, from 195.16±3.28 $mg \cdot L^{-1}$ to 130.55±4.10 $mg \cdot L^{-1}$ at 10 $mg \cdot L^{-1}$. As summarized in Table 1, the mean TN removal efficiency was also higher at 1 $mg \cdot L^{-1}$ than any other MWCNTs concentrations during the period. Compared to the control groups and other MWCNTs concentrations, the microalgae at 1 $mg \cdot L^{-1}$ MWCNTs converted more inorganic nitrogen to organic nitrogen in biogas slurry. The maximum mean removal efficiency of 1 $mg \cdot L^{-1}$ MWCNTs (65.95% removal efficiency) was consistent with that obtained from piggery wastewater in photobioreactor using *Desmodesmus* sp. (65.3% removal efficiency) (Chen et al., 2020b).

Tetracycline is one of the most widely adopted broad-spectrum antibiotics and animal veterinary drugs worldwide (Dai et al., 2019). In recent years, the rapid development of the aquaculture industry has led to an increase in the types and content of residual antibiotics in the water environment. In this study, tetracycline was added as a part of the simulated biogas slurry, and the tetracycline content was measured by Liquid Chromatography System. The initial concentration of tetracycline was 10 $mg \cdot L^{-1}$. Following 9 days of incubation experiments, the concentration of tetracycline under 1 $mg \cdot L^{-1}$ MWCNTs decreased from 5.11 ± 0.35 $mg \cdot L^{-1}$ to 1.04 ± 0.04 $mg \cdot L^{-1}$, which was lower than the control group and other three groups. Similarly, the mean removal value of tetracycline under 1 $mg \cdot L^{-1}$ MWCNTs was 75.36 ± 2.86%, which was 12.38%, 4.80%, 16.33%, and 19.78% higher than that of microalgae under 0, 0.1, 5, and 10 $mg \cdot L^{-1}$ MWCNTs, respectively. The tetracycline-REs in this study were far below the values of 93% on 4th day and 99% on 7th day (Norvill et al., 2017). The differences might be due to the initial tetracycline concentrations and algae species. 10 $mg \cdot L^{-1}$ of TC is a high concentration for the natural environment and also exists in the biogas slurry. It is necessary to purify the TC concentrations as it affects the microalgal growth, affecting the antibiotic removal efficiency.

In general, the tendencies of MWCNTs to remove nutrients and tetracycline from the biogas slurry is consistent. In this study, the optimal concentration of MWCNTs for nutrients and tetracycline was 1 $mg \cdot L^{-1}$. The removal efficiency of nutrients and tetracycline for the control group was always higher than 5 and 10 $mg \cdot L^{-1}$ groups, indicating that MWCNTs could only improve the removal efficiency of nutrients and tetracycline within a certain concentration range.

3.6 Effects of MWCNTs concentration on CO_2 removal from biogas

Microalgae could absorb CO_2 rapidly (Ronan et al., 2020) and convert it into organic compounds (Zhao et al., 2019), increasing the economic value. The CO_2-Res and CO_2 concentration changes in different MWCNTs concentration treatments are summarized in Table 1 and Fig. 5a. The optimal MWCNTs concentration for removing CO_2 from biogas was 1 $mg \cdot L^{-1}$ in this study. The increasing trend was consistent with Chl-a of *Chlorella vulgaris* under different MWCNTs concentrations. Higher Chl-a content was correlated with greater CO_2 removal efficiency and more rapid growth rate (Shen et al., 2020).

Carbonic anhydrase (CA) activity represents the CO_2 fixation ability, and periplasmic CA enhances CO_2 uptake ability from external HCO_3^- (Basu et al., 2014). After the one-week of cultivation, microalgal CA activity decreased, indicating the availability of free CO_2 in the culture medium. These were as follows: 1 $mg \cdot L^{-1}$ MWCNTs (22.38 ± 0.22 EU) > 0.1 $mg \cdot L^{-1}$ MWCNTs (18.74 ± 0.33 EU) > control group (15.91 ± 0.21 EU) > 5 $mg \cdot L^{-1}$ MWCNTs (13.74 ± 0.19 EU) > 10 $mg \cdot L^{-1}$MWCNTs (7.89 ± 0.27 EU) (Fig. 5b). The maximum CA activity of *Chlorella vulgaris* was found under 1 $mg \cdot L^{-1}$ MWCNTs treatment and the optimal MWCNTs concentration range for CA activity was 0.1-1 $mg \cdot L^{-1}$, which was highly consistent with the microalgal dry weight and cell quantity. The results indicated that *Chlorella vulgaris* under 1 $mg \cdot L^{-1}$ MWCNTs treatment was more effective in enhancing CO_2 fixation and microalgal bicarbonate transport, corresponding to the Chl-a content, growth rate, and mean daily biomass productivity (Xu et al., 2020).

4. Conclusions

In this study, the effects of different MWCNTs concentrations on the purification of simulated biogas slurry and biogas with tetracycline by freshwater microalgae *Chlorella vulgaris* were discussed. A moderate MWCNTs concentration (1 $mg \cdot L^{-1}$) enhanced the PSII photosynthesis, the growth rate, the nutrients (including COD, TP, TN, CO_2) and tetracycline removal efficiencies, Chlorophyll *a* content, and CA activity. This study will provide a better understanding of MWCNTs mechanisms on *Chlorella vulgaris* and new insight into the combination of nanomaterials and biological purification.

Declarations

Ethical approval and consent to participate

Not applicable

Consent to publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Authors contributions

Li Sun: Formal analysis, Writing-Original draft preparation; Chunzhi Zhao: Data Curation, Software; Shiqing Sun: Methodology, Investigation; Changwei Hu: Methodology; Yongjun Zhao: Supervision, Conceptualization, Methodology; Juan Liu: Conceptualization, Writing-Review & Editing.

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Figures

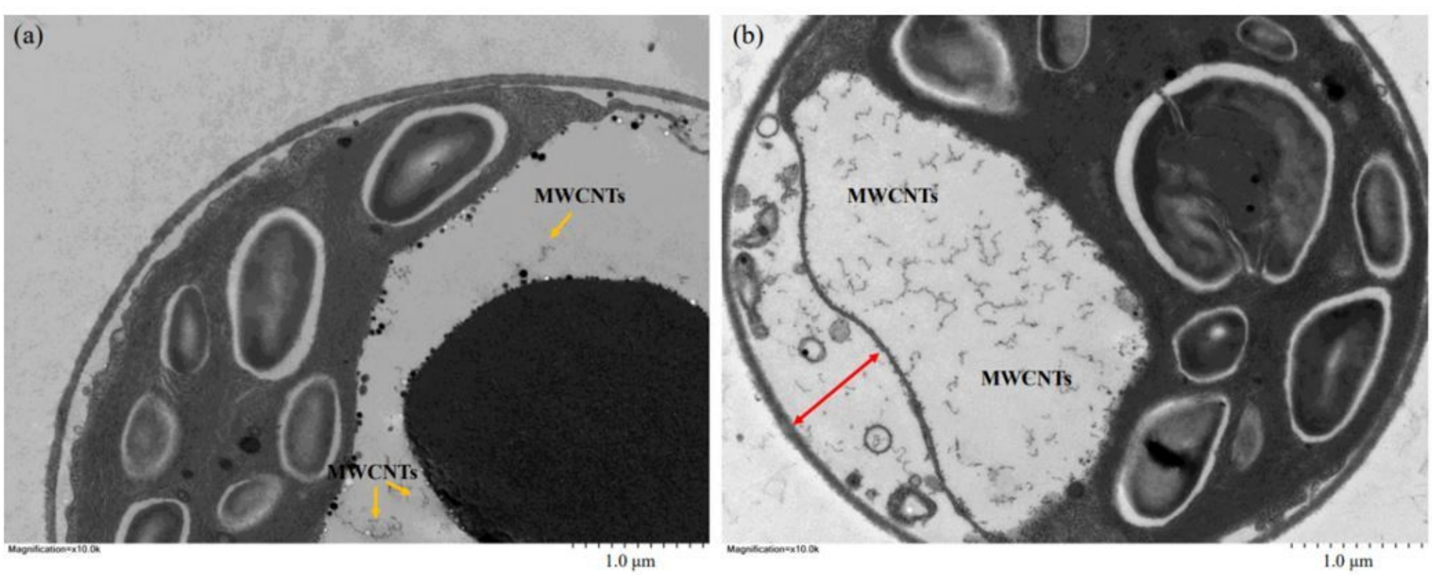

Figure 1

TEM image of Chlorella vulgaris treated different concentration of MWCNTs. (a) 1 mg·L-1; (b) 10 mg·L-1

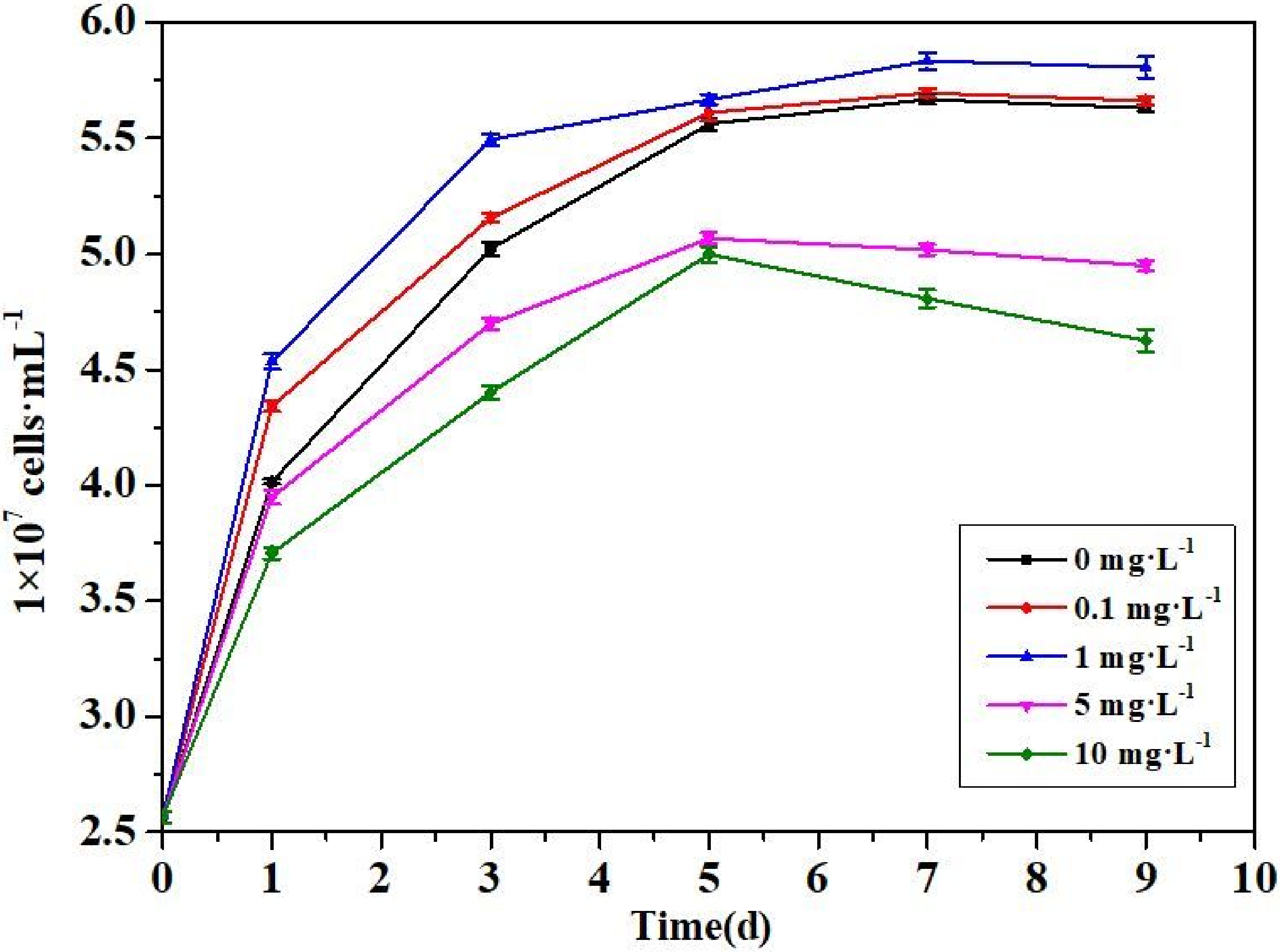

Figure 2

The cells quantity of microalgae under different concentration of MWCNTs

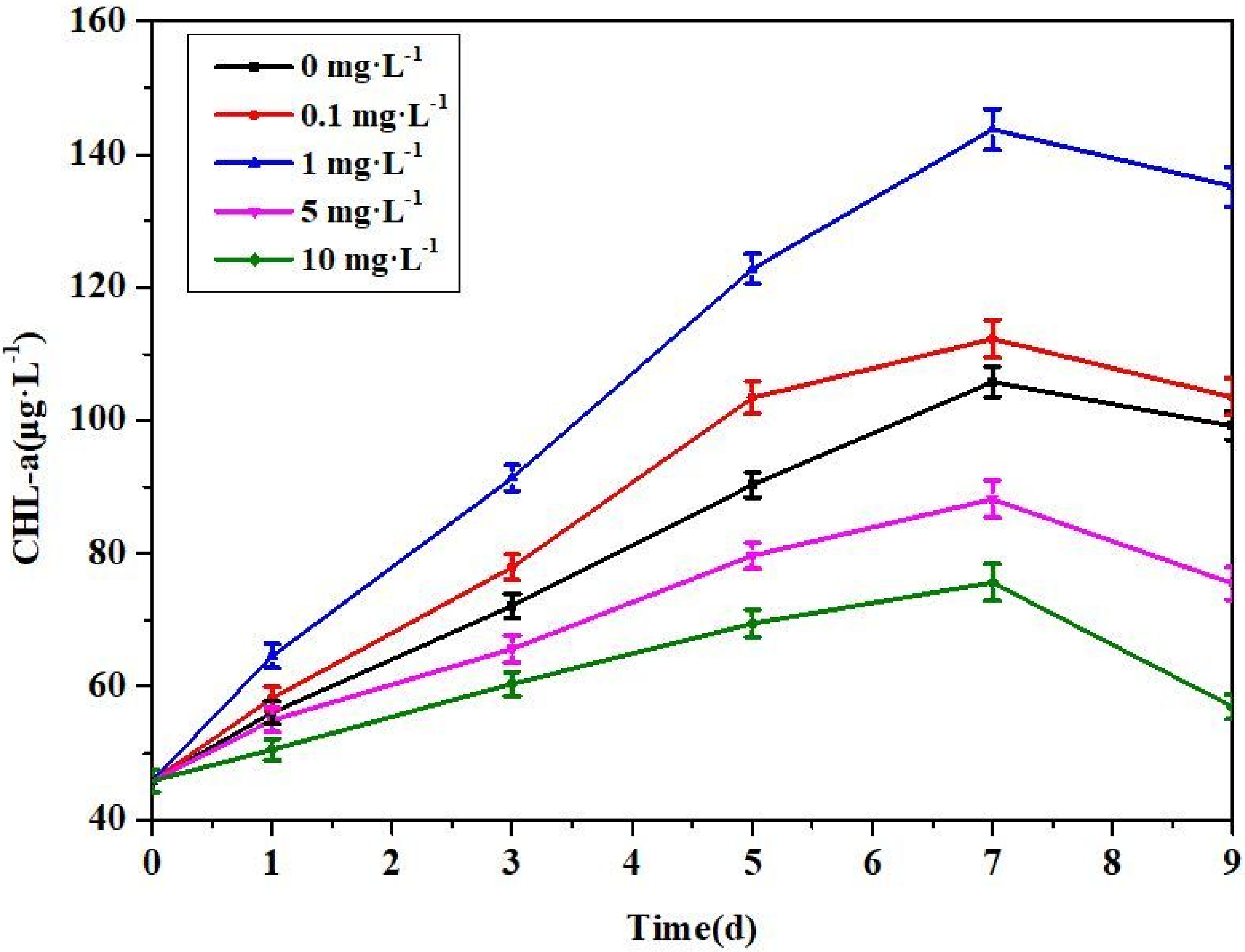

Figure 3

Chlorophyll a content of microalgae cultivated under different MWCNTs concentration

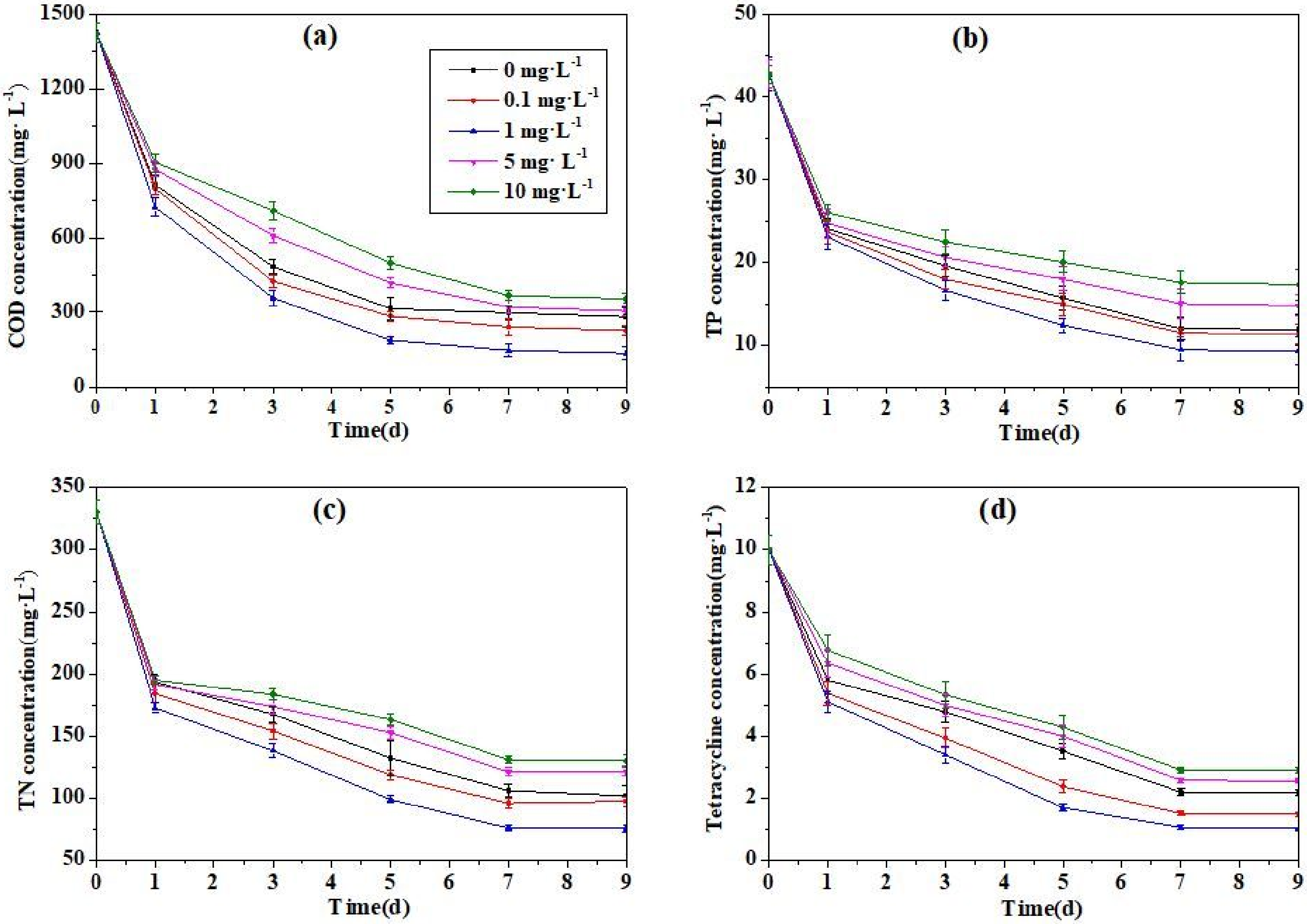

Figure 4

Nutrients concentration under different MWCNTs concentration by microalgae cultivation for 1d, 3 d, 5d, 7 d, and 9 d. (a) COD, (b) TP, (c) TN and (d) tetracycline

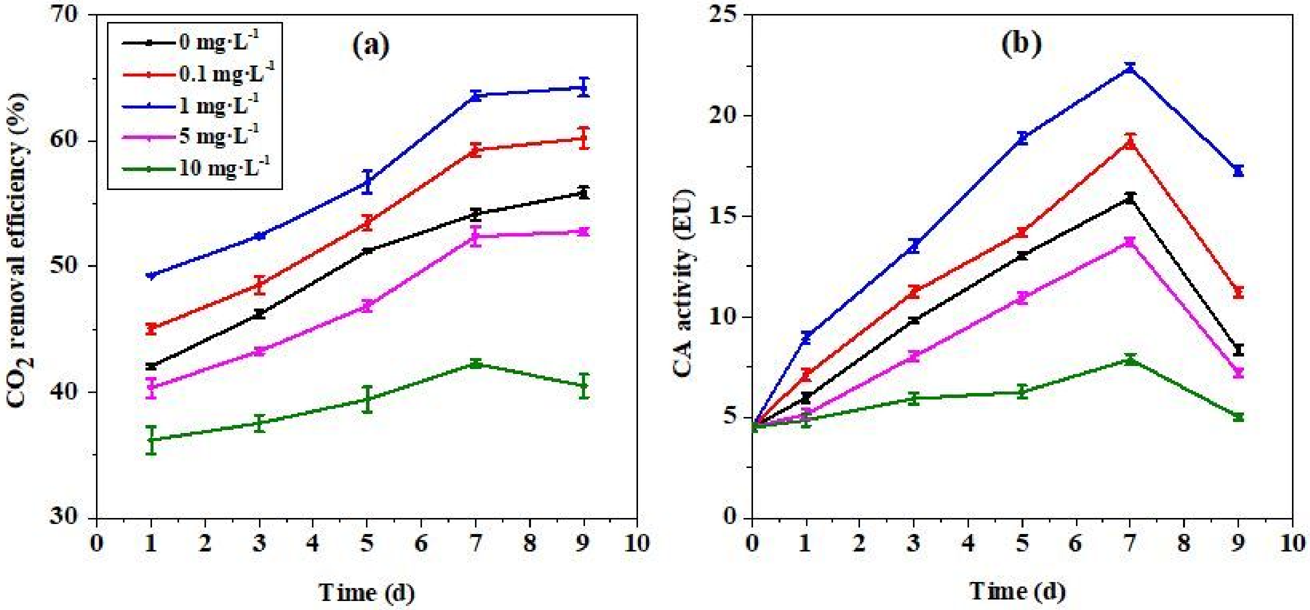

Figure 5

Effects of different MWCNTs concentrations on CO2 removal efficiencies and CA activity of microalgae for 1d, 3 d, 7 d, and 9 d. (a) CO2, (b) CA activity.