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Research article

Enhancing microalgae-based biofuels production, wastewater treatment and bio-products generation by synergistic effect of iron and zinc addition to real municipal wastewater

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ABSTRACT

The feasibility of microalgae-based biofuel production is still unclear due to the high cost and energy consumption. In order to be competitive with traditional fuels, the price per unit biofuel produced should be reduced by improving microalgal cells quality for higher biofuels productivity as well as enhancing microalgae other advantages such as wastewater treatment (WWT) and CO_2 bio-fixation. In this research, the synergistic effect of iron (Fe) and zinc (Zn) addition to municipal wastewater (MWW) on *Chlorella sorokiniana Pa.91* performance was investigated in terms of biomass productivity, WWT, bio-products generation and biofuel quality. According to the result, maximum biomass concentration of 1.1, 1.49, 1.36 and 1.9 mg/L were achieved after 10 days *C. sorokiniana* cultivation in MWW before and after addition of Fe (9 mg/L), Zn (1 mg/L) and combined Zn/Fe (6/0.5 mg/L), respectively. It was observed that the nutrients uptake ability of microalgal cells improved by pre-treatment with Fe/Zn, as the mass balance of COD, NH_4 and PO_4^{3-} increased from 104.5, 13.6 and 2.9 to 111.6, 16.6 and 3.78 mg per 1 g *C. sorokiniana*, respectively. By adding Fe/Zn, the lipid content increased from 25 to 33 % CDW, while, no significant changes were observed in the protein and carbohydrate content. The results revealed that the fatty acid composition (i.e. SFA and MUFA content) and biofuel quality of *C. sorokiniana* remarkability enhanced after Fe/Zn supplementation. Overall, our finding suggested that MWW enrichment with combined Fe and Zn at an appropriate dosage is a promising approach for improving microalgae performance in particular increasing biofuel production quantity and quality.

1. Introduction

In the face of accelerating climate change and the imperative to curb greenhouse gas emissions, the world is shifting from fossil fuels to renewable energy sources (Jathar et al., 2023). Consequently, the request for sustainable alternatives such as biofuels has intensified (D' Silva et al., 2021). It is estimated the global biofuel demand increased to more than $ 50 billion by 2026 (Ahn et al., 2022). Among the different biofuel options, microalgae-based biofuels have gained considerable attention due to their inherent advantages and remarkable potential (Wang and Lan, 2011). Unlike the traditional biofuel crops, microalgae cultivation presents several distinct advantages, including CO_2 bio-fixation through photosynthesis and wastewater treatment by consuming nutrients from the medium while simultaneously producing high value bioproducts (Lutzu and Dunford, 2018). However, the microalgae based biofuel production has not yet been commercialized due to relatively high cost of microalgae cultivation in artificial medium and low biomass amount.

Microalgae cultivation in domestic or municipal wastewater (MWW) might be a promising approach for enhancing the environmental and economic sustainability of microalgal technologies due to its multi-achievement of wastewater treatment and biomass production (Lutzu et al., 2021). In line with that, several researchers have focused on growing microalgae in MWW (Nishshanka et al., 2023). For example, Purba et al. (2022) investigated the growth rate of different microalgae spices in MWW and could achieve maximum biomass dry weight of 0.8 g/L after 12 days. Zhou et al. (2017) cultivated *Spirulina platensis* in actual MWW and reported maximum biomass concentration of 0.81 g/L after 12 days which could remove TN up to 79%. Generally, the maximum biomass concentrations reported from microalgae cultivation

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in MWW were less than 1 g/L which resulted in poor biological MWW treatment (Vaz et al., 2023). This might be attributed to the lack of trace elements (TEs) in MWW. According to Ida and Eva (2021) who conducted research on more than 20 WWTP in Europe, the HMs level in MWW was relatively low (<2 mg/L) for microalgae cultivation.

Adding TEs into microalgae culture medium has been suggested as an efficient approach for improving microalgae growth and lipid content (Abdelfattah et al., 2023; Akbari et al., 2024). Among different TEs, iron (Fe) and zinc (Zn) are well-known as two main TEs for microalgae (Liu et al., 2021). Han et al. (2019) investigated the effect of Fe^{3+} addition on microalgae growth and lipid production. They reported that adding 6 mg/L of Fe^{3+} results in 29% and 20% on improvement in *Chlorella Sp.* growth and lipid content, respectively. In another attempt, Zhou et al. (2018) studied the effect of Zn ions addition on the growth, biochemical composition and photosynthetic performance of *Spirulina platensis*. Based on their achievement adding Zn at high dosage showed negative affect on microalgae growth but higher fatty acid profile. Ghafari et al. (2018) investigated the effect of macro/micronutrient on the lipid content of different species of microalgae. Based on their results, Fe (1.4 mg/L) and Zn (0.12 mg/L) addition increased 60% and 25% lipid content of *C. sorokiniana*, respectively.

Although, several research can be found in the literature which investigated the effect of different TEs on microalgae performance (Akbari et al., 2024; Fan et al., 2023; Liu et al., 2023; Wang et al., 2023; Zhang et al., 2023a), few studies have focused on the interaction effects of Fe and Zn on the microalgal biomass growth, nutrient uptake ability, bioproducts generation and biofuels production quality. In particular, the effect of TEs supplementation on the microalgae-based wastewater treatment has rarely been investigated because most of the existing works have added TEs to synthetic mediums to optimize the medium conditions rather than real wastewater. Moreover, beside the proven positive effect of TEs supplementation (at an appropriate dosage) on microalgae performance, the techno-economic investigation of adding TEs has been completely missed in the previous works. To the best of knowledge, no research has been published that comprehensively investigate the interaction effects of Fe and Zn supplementation on microalgal biomass growth, wastewater treatment capacity, bio-products generation and biofuel quality by using real municipal wastewater as culture medium as well as conducting economic feasibility study on the TEs supplementation.

Therefore, in this research, we tried to enhance microalgae performance by combine addition of Fe and Zn in MWW. *Chlorella sorokiniana. P91* was selected as the microalgae spices in this study. First, the effect of single Fe and Zn as well as their combination with different dosage on microalgae biomass was investigated and optimized. Then, the wastewater treatment ability under these optimum condition were evaluated by monitoring chemical oxygen demand (COD), ammonium (NH_4) and phosphate (PO_4^3) removal. The high value bioproducts including protein, carbohydrate and lipid were identified at the end of 10 days cultivation of *C. sorokiniana*. Ultimately, the quality of the biodiesel of the microalgae treated and untreated with TEs were compared together.

2. Materials and methods

2.1. Microalgae

Chlorella sorokinina Pa.91 which was obtained from Gela dairy WWTP, Sari, Iran (Taghavijeloudar et al., 2021b) was used as the microalgae spices in this study. First, the microalgae were pre-cultured in BG-11 medium (Yaqoubnejad et al., 2021) at room temperature (25 ± 2 °C) and 3000 lux light intensity (12L/12D). Then, the microalgae cells were passed through mesh filter sheet of 0.45 μm (Hebei, China) and three cycle purification were conducted before the cultivation in the actual wastewater.

Table 1
Characteristics of the MWW sample used in this study.

Parameter	Unit	Mean ± SD[a]
pH	–	7.6 ± 0.22
Turbidity	NTU	111 ± 13.7
Total suspended solid (TSS)	mg/L	166 ± 2.71
Dissolved oxygen (DO)	mg/L	1.52 ± 20.2
Biological oxygen demand (BOD5)	mg/L	152 ± 4.62
Chemical oxygen demand (COD)	mg/L	220 ± 8.29
Phosphate (PO_4^{-3})	mg/L	7.24 ± 0.87
Ammonium (NH_4^+)	mg/L	32 ± 0.92
Nitrite (NO_2^-)	mg/L	1.9 ± 0.2
Nitrate (NO_3^-)	mg/L	1.05 ± 0.09
Lead (Pb)	mg/L	0.005 ± 0.0001
Cupper (Cu)	mg/L	0.035 ± 0.015
Chromium (Cr)	mg/L	0.021 ± 0.021
Nickel (Ni)	mg/L	0.016 ± 0.007
Manganese (Mn)	mg/L	0.053 ± 0.024
Iron (Fe)	mg/L	0.56 ± 0.25
Zinc (Zn)	mg/L	0.11 ± 0.2

[a] SD: Standard deviation ($n > 3$).

2.2. Municipal wastewater

Actual municipal wastewater was collected from the first sedimentation tank of municipal wastewater treatment plant (WWTP) in Sari, Mazandaran, Iran. In order to generalize the results, samples were token at 5 different times and were mixed. To minimize the indigenous biological activates, the collected MWW were stored in a cold place (2 ± 1 °C). The characterization of the sample MWW is presented in Table 1.

2.3. Microalgal cultivation and experiments procedure

The microalgae cultivation experiments were conducted in 500 mL Erlenmeyer flasks filled with 300 mL of the MWW. The flasks were autoclaved at 120 °C for 60 min and placed in incubator. To investigate the effect of TEs on microalgae performance, different concentrations of $FeCl_3.6H_2O$ (3, 6, 9, 12 mg/L) and $ZnCl_2$ (0.5, 1, 1.5, 2 mg/L) were added to the MWW before starting cultivation test. The concentration range for Fe and Zn was adjusted based on the optimal concentrations of them for the best performance of microalgae which have been as previously reported in the literature (Han et al., 2019; Li et al., 2020; Rizwan et al., 2017; Zhou et al., 2018). As a results 17 cultivation experiments were performed under constant environmental conditions of 30 °C temperature and 2800 Lux light intensity with 12:12 h light to dark cycle. 10 mL suspension samples were taken from the medium every day during 10 days cultivation to measure the biomass concentration, nutrients concentration and other parameters. The bioproducts analyses were performed only for the biomass at the end of the cultivation period before stationary phase.

2.4. Analytical methods

The biomass concentration was measured using a UV spectrophotometer (2800 UV/VIS UNICO, China) at optical density at 680 nm (OD_{680}) (Fig. S1). The biomass concentration (BC) and productivity (BP) were calculated as follows:

$$BC = 1.824\ OD_{680} + 0.043\ \left(R^2 = 0.9889\right) \tag{1}$$

$$\mathrm{BP} = \frac{(\mathrm{BC}_{t1} - \mathrm{BC}_{t2})}{\mathrm{t1} - \mathrm{t2}} \times 100 \tag{2}$$

where, BC_{t1} and BC_{t2} are the biomass concentration at day $t1$ and $t2$ (day), respectively. Gompertz model was implemented to simulate the actual microalgae growth behavior before and after treatment with TEs. The Gompertz model is a mathematical model which has been extensively used to analyze the kinetics of microalgal and microbial growth rate (Çelekli et al., 2008; Wang and Guo, 2024). As presented in Eq. (3), Gomperts model contains two main fitting parameters of λ and μ which represent lag phase duration (d) and biomass specific growth rate (g/L/d).

$$BC_m = BC_f \exp\{-\exp[[(\mu e / BC_f)(\lambda - t)] + 1]\} \tag{3}$$

where, BC_m and BC_f were the biomass concentration predicted by Gompertz model and the maximum biomass concentration (g/L), respectively, μ is the maximum biomass growth rate (g/L/d), *e* is Napier number ~ 2.718 and ʎ is the lag phase duration (d). Errors of mean relative error (MRE), standard deviation (σ), sum of the squares of the deviation (SSD) and R-square (R^2) were used to evaluate the model accuracy (Taghavi et al., 2010). For each cases, the model fit was conducted using different values of fitting parameters (λ and μ) to achieve the minimum MRE and R^2.

To determine the nutrients removal efficiency during cultivation period, the concentrations of nitrate (NO_3^-), phosphate (PO_4^{-3}) and COD were measured daily following the standard protocols (SAP, 2012). The samples were centrifuged (at 4000 rpm for 15 min) and the final supernatant was used for measuring the nutrient concentrations as previously described (Taghavijeloudar et al., 2021a). The nutrient removal percentage was calculated as Eq. (4):

$$\text{Removal efficiency } (\%) = \frac{(C_0 - C_f)}{C_0} \times 100 \tag{4}$$

where C_0 and C_f (mg/L) are the initial and final nutrient concentrations at time t_f, respectively.

The concentration of carbohydrate, protein and lipid of *C. sorokinianana* cultivated in the two PBRs was measured to determine effect of TEs treatment on the bio-product compositions. The protocol used in this study for bioproducts concentration and content was exactly same as the one presented by (Akbari et al., 2024). Detailed information are provides in supplementary materials S.2). The total content of bio-products was calculated as percentage of cell dry weight (% CDW) using Eq. (5):

$$\text{bioproducts content } (\%\ \text{CDW}) = \frac{TC}{BC} \times 100 \tag{5}$$

where TC (mg/L) is the total concentration of extracted bioproducts and BC (mg/L) is the final biomass concentration. In addition, confocal laser scanning microscopy (CLSM) was conducted on *C. Sorokiniana* cells to determine the distribution of bioproducts inside the microalgae cells by using LSM510 (Carl Zeiss, Germany). HeNe laser (633 nm excitation wavelength) and Nile red (488 nm excitation wavelength) fluorescence intensities were applied for carbohydrate and lipid, respectively.

Regarding fatty acid profile analysis, the FAMEs yield was measured gravimetrically as described by Wang et al. (2019). To identify the fatty acid profile, the lower phase of the lipid samples were converted into fatty acid methyl esters using the protocol presented by Zhou et al. (2018). Finally, the fatty acid profile of *C. sorokiniana* before and after treatment were analyzed using GC-MC (GC, TG 2500, China).

To identify the biofuel quality of the *C. sorokiniana* before and after treatment, the value of saponification value (SV), degree of unsaturation (DU), long-chain saturated factor (LCSF), iodine value (IV), cetane number (CN), and cold filter plugging point (CFPP) were calculated from FAMEs compositions using Equations (6)–(11). The IV was determined using each fatty acid double bond number (D), fatty acid molecular weight (MWi), and each fatty acid percentage (*Ai*) (Eq (6)) and SV was calculated using M and each fatty acid percentage (*Ai*) (Eq (7)), The DU was determined by the polyunsaturated fatty acid (PUFA) and the monounsaturated fatty acid (MUFA) of microalgae (Eq (9)) and the LCSF and CFPP were calculated using Eqs. (10) and (11) (Rajabi Islami and Assareh, 2020)

$$IV = \sum (254 \times D \times Ai) \Big/ MWi \tag{6}$$

$$SV = \sum (560 \times Ai) \Big/ MWi \tag{7}$$

$$CN = 46.3 + \frac{5458}{SV} - (0.225 \times IV) \tag{8}$$

$$DU\ (wt.\%) = \%MUFA + 2 \times (\%PUFA) \tag{9}$$

$$LCSF\ (wt.\%) = (0.1 \times C16) + (0.5 \times C18) + (1 \times C20) + (2 \times C24) \tag{10}$$

$$\text{CFPP}\ (^\circ C) = (3.1417 \times LCSF) - 16.477 \tag{11}$$

2.5. Cost analysis

To investigate the economic feasibility of microalgae cultivation with TEs addition, the total cost (TC) for production of 1 kg microalgal biomass and bio-products from 1000 L wastewater with and without TEs treatment were calculated as follows:

$$TC_{MB}(€/kg) = OC_{MB}\ (€/kg) + C_{TE}\ (€/kg) \tag{12}$$

where TC_{MB}, OC_{MB} and C_{TE} are the total cost, operational costs and TEs costs for 1 kg biomass production from 1000 L real MWW, respectively. The OC_{MB} can be calculated from Eq. (13).

$$OC_{MB}\ (€/kg) = UP_{MB}(€/kg)/BC\ (kg/1000L) \tag{13}$$

where UP_{MB} is the unit price for producing l kg biomass from 1000 L medium and BC is the biomass concentration at the end of cultivation period with and without TEs supplementation. According to literature the operational cost for producing 1 kg microalgal biomass in small scale is around 2.5 €/kg. For example, Hoffman et al. (2017) and Branco--Vieira et al. (2020) reported 2.66 € ($673 tonne/gallon) and 2.01 € for 1 kg biomass production from small PBRs. Therefore, in this research the UP_{MB} for l kg biomass production from 1000 L medium were considered as 2.5 €/kg. And cost of TEs (C_{TE}) can be calculated using Eq. (14).

$$C_{TE}\ (€/kg) = UP_{TE}\ (€/kgTE) \times \text{TE optimal dosage } (g/L)/BC\ (g/L) \tag{14}$$

where UP_{TE} is the unit price of the TEs (€/kg). The UP_{TE} of $FeCl_3.6H_2O$ and $ZnCl_2$ were considered as 162 and 528 €/kg based on the local price. Ultimately, the total cost for 1 kg bio-products can be obtain using Eq. (15).

$$TC_{BP}\ (€/kg) = TC_{MB}\ (€/kg)/\ \text{Bioproduct content (CDW\%)} \tag{15}$$

where TC_{BP} is the total cost for 1 kg bio-product accident from biomass cultivated in 1000 L wastewater.

2.6. Statistical analysis

All measurement were performed at least three times (n > 3) to ensure the reliability of the results. The results were reported as mean value ± standard deviation (SD). Surfer software 25.0 were used for plotting 3D graphs. IMB SPSS Statistics 25 software and one-way analysis of variance (ANOVA) were used to identify the statistical significance of the various removal efficiencies obtained by the different systems. ns, *, ** and *** were used for not significant, p-value <0.05, 0.01 and 0.001 compared to the control group.

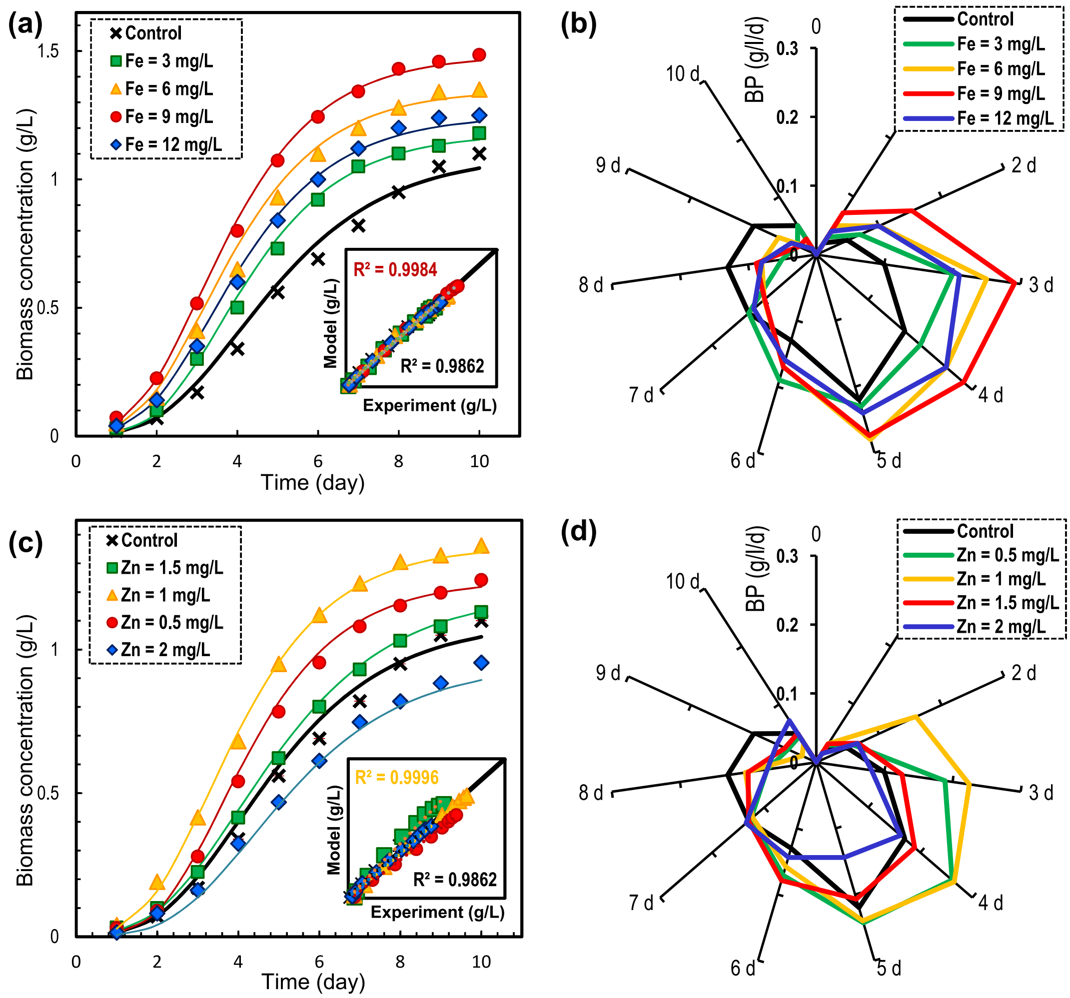

Fig. 1. Effect of $FeCl_3$ (a–b) and $ZnCl_2$ (c–d) dosage on microalgal growth rate (left) and the final biomass concentration (right).

3. Results and discussions

3.1. Effect of TE on microalgae growth

3.1.1. Effect of adding single TEs treatment on microalgae growth

Fig. 1 illustrates the effect of MWW pre-treatment with single TE (e.g. $FeCl_3$ or $ZnCl_2$) on the biomass production of *C. sorokiniana* during 10 days cultivation. In addition, Gompertz model was used to simulate and evaluate the microalgal growth behavior (detailed analyses are provides as Supplementary Materials). In the case of MWW without adding TE (control), the maximum biomass concentration (BC) of 1.1 g/L was achieved after 10 days of cultivation.

As shown in Fig. 1a, the optimum dose of Fe was 9 mg/L which resulted in 36.3% improvement of the maximum BC (from 1.1 to 1.5 g/L). Based on the simulation results (the small graph inside Fig. 1a), the experimental data of 9 mgFe/L showed better match with modeling data ($R^2 = 0.9983$) in comparison with the control ($R^2 = 0.9564$) which indicated microalgae had more homogeneous growth in the medium treated with appropriate dosage of Fe. Moreover, the lag phase was shorted from 2 to 1.4 days (30%) by treating the MWW with 9 mgFe/L (Table S3). It was observed that the maximum BP of the microalgae increased from 0.22 (at day 5) to 0.29 (at day 3) by adding 9 mgFe/L (Fig. 1b). The positive impact of Fe on growth is attributed to the role of iron on the promoting enzyme activity, electron transfer process and expression of some genes (e.g. rbcl and aacd) related to growth (Akbari et al., 2024). Moreover, Fe is the main component of chloroplasts (80%) and can form hydrogenate complex which improves photosynthesis (Hänsch and Mendel, 2009). Similar results were reported previously (Han et al., 2019, 2021; Polat et al., 2020; Sun and Huang, 2017).

According to Fig. 1c, adding 1 mg/L of $ZnCl_2$ to the MWW led to 23.6% improvement in the biomass concentration (from 1.1 to 1.36 g/L). This was attributed to the effect of Zn on metabolism activates of the microalgae cells (Ghafari et al., 2018). Zn is a part of carbonic anhydrase which acts as catalysis in transformation of CO_2 to HCO_3 before photosynthesis process (Dou et al., 2013). The simulation results (the small graph inside Fig. 1b) revealed that the accuracy of the Gompertz model was higher in the case of 1 mgZn/L ($R^2 = 0.9986$) in comparison to the control ($R^2 = 0.9564$). The lag phase was also reduced from 2 to 1.6 days (20%) (Table S1). As can be seen from Fig. 1d, the maximum BP also increased from 0.22 (at day 5) to 0.27 (at day 4) by adding 1 mgZn/L. However, adding more Zn showed negative effect on mucilage growth as the final biomass decrease 136. % (from 1.1 to 0.95 g/L). This might be due the toxicity effect of Zn on the microalgae cell's membrane which prevent the uptake of calcium (Li et al., 2011). Similar results were reported by Zhou et al. and Alam et al. when they investigated the effect of Zn treatment on growth rate of *Spirulina platensis* and *Chlorella vulgaris*, respectively (Alam et al., 2015; Zhou et al., 2018).

3.1.2. Effect of combined adding TEs on microalgae growth and optimization

The interaction effect of $FeCl_3$ and $ZnCl_2$ dosage on *C. sorokiniana* growth behavior was investigated by performing 25 cultivation tests with different dosage of Fe and Zn and the results are shown in Fig. 2. Based on the 3D graph in Fig. 2a, a synergistic effect between $FeCl_3$ and

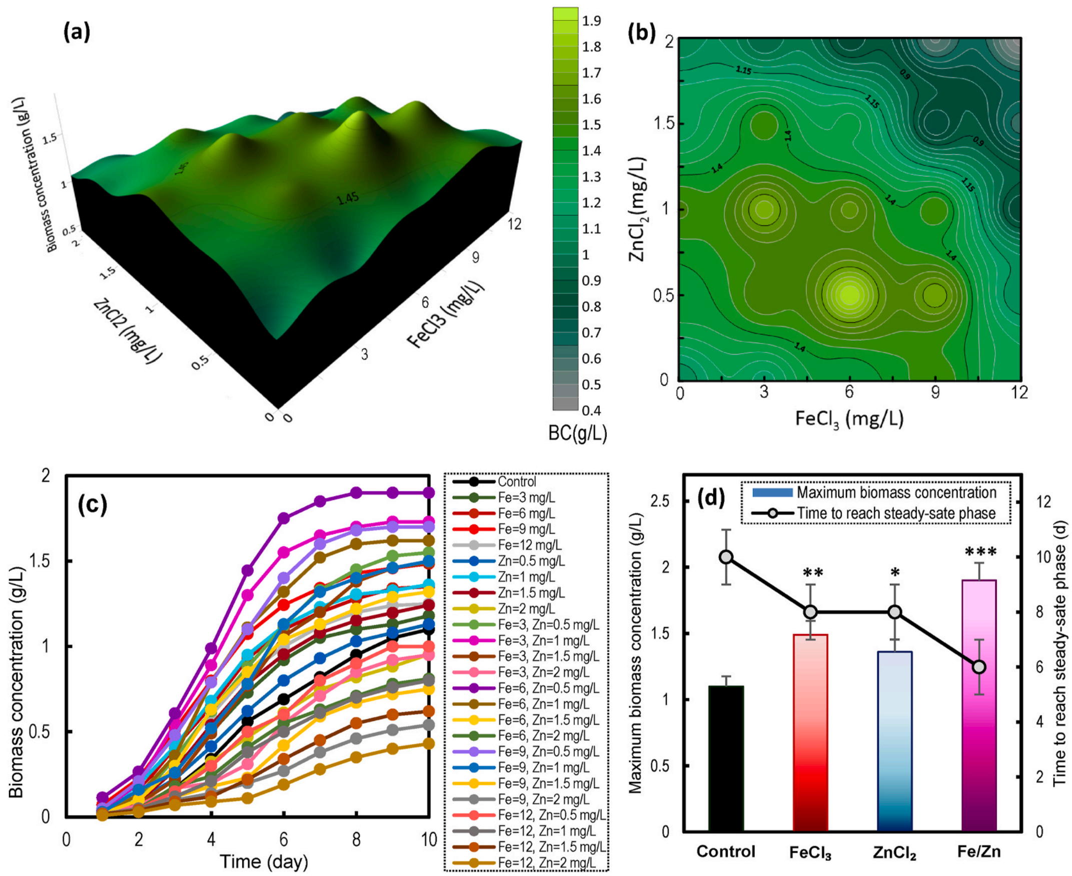

Fig. 2. Interaction effect of Fe and Zn on biomass concentration; (a) 3D and (b) contour, (c) biomass grow thing rate and (d) maximum BC and cultivation time.

$ZnCl_2$ on the microalgae growth was observed. The maximum biomass concentration of 1.9 g/L was achieved at dosages of 6 and 0.5 mg/L of Fe and Zn, respectively. In comparison with the single adding of Fe and Zn, the combined utilization of Fe/Zn could increase the maximum biomass concentration up to 27.5 % (from 1.49 to 1.9 g/L) and 39.7 % (from 1.36 to 1.9 g/L), respectively.

It can be observed from the contour graph (Fig. 2b) that the combination of $FeCl_3$ and $ZnCl_2$ not only improved the final biomass concentration but also reduced the optimal dosages of Fe (from 9 to 6 mg/L) and Zn (from 1 to 0.5 mg/L). The results reveled that, high dosages of TEs resulted in a deterioration in the microalgae growth. For example, combine adding Fe and Zn with dosages of 12 mgFe/L and 2 mgZn/L led to more than 50% reduction in the final biomass concentration. This might be attributed to the negative effect of high dosage of HMs on the cell membrane and protein structures (Zhou et al., 2018). Moreover, high dosage of HMs might increase ROS and oxidative stress in microalgae cells (Liu et al., 2023).

Fig. 2c shows the time courses of microalgae growth under different conditions of TEs. In all cases, the *C. sorokiniana* first grew slowly at the early stage due to adaptation phase (lag phase) and then grew quickly until days of 8 or 10 (exponential phase) and finally reached to a stable stage due to due to lack of nutrients in the medium (androgen phase). It was observed that the cultivation time to reach maximum biomass was also reduced from 10 days (control) to 6 days (40% faster) after the medium treatment with combined 6 mgFe/L and 0.5 mgZn/L.

Ultimately, Fig. 2d presents a comparison between the results of microalgae cultivation in the MWW without treatment and treated with $FeCl_3$ (9 mg/L), $ZnCl_2$ (1 mg/L) and Fe/Zn (6/0.5 mg/L) in terms of maximum biomass concentration and time to reach steady-state growth. The maximum biomass concentration of 1.1, 1.49, 1.54 and 1.9 g/L were archived after 10, 8, 8 and 6 days cultivation of *C. Sorokiniana* in the raw and treated MWW with $FeCl_3$, $ZnCl_2$ and Fe/Zn respectively. Our finding proved that MWW pretreatment with combined Fe/Zn, not only increased the maximum biomass concentration up to 72 % but also reduced the cultivation time up to 40 % (from 10 to 6 days). This might be due to the simultaneous improvement of photosynthesis (by promoting the enzyme activity and electron transfer process) and metabolism activity of cells induced by Fe and Zn, respectively (Akbari et al., 2024; Dou et al., 2013; Ghafari et al., 2018; Hänsch and Mendel, 2009).

3.2. Effect of TEs on biological wastewater treatment

In this research, the reduction of ammonium (NH_4), phosphate (PO_4) and COD in MWW was investigated as nutrients pollutant. Fig. 3 shows the effect of TEs supplementation on the biological treatment ability of the microalgae cells. As can be seen from Fig. 3a, adding TEs to MWW notably improved the removal rate of ammonium (NH_4). The concentration of NH_4 was reduced from 32 to 18.1, 11.5, 10.4, 0.5 mg/L after 10 days of *C. sorokiniana* cultivation in the untreated MWW (control) and the treatment MWW with Fe (9 mg/L), Zn (1 mg/L) and Fe/Zn (6/0.5 mg/L), respectively. It was observed that only in the case of Fe/Zn, the final concentration of NH_4 reached to below the standard level for agriculture reuse water (<6 mg NH_4/L according to EPA, 2020) within 5 days. According to Fig. 3b, the removal efficiency of NH_4 by microalgae was significantly improved from 46.9 % (control) to 67.2%, 60.3% and 98.4% after adding Fe, Zn and Fe/Zn to the MWW. The mass balance calculations revealed that 1 g of *C. sorokiniana* cultivated in the untreated MWW and treated with Fe, Zn and Fe/Zn could uptake 13.6, 14.5, 14.2 and 16.6 mg of NH_4 which indicates that the improvement in WWT is not only attributed to the higher biomass concentration after TE addition.

Similar results were also observed for phosphate (PO_4) removal

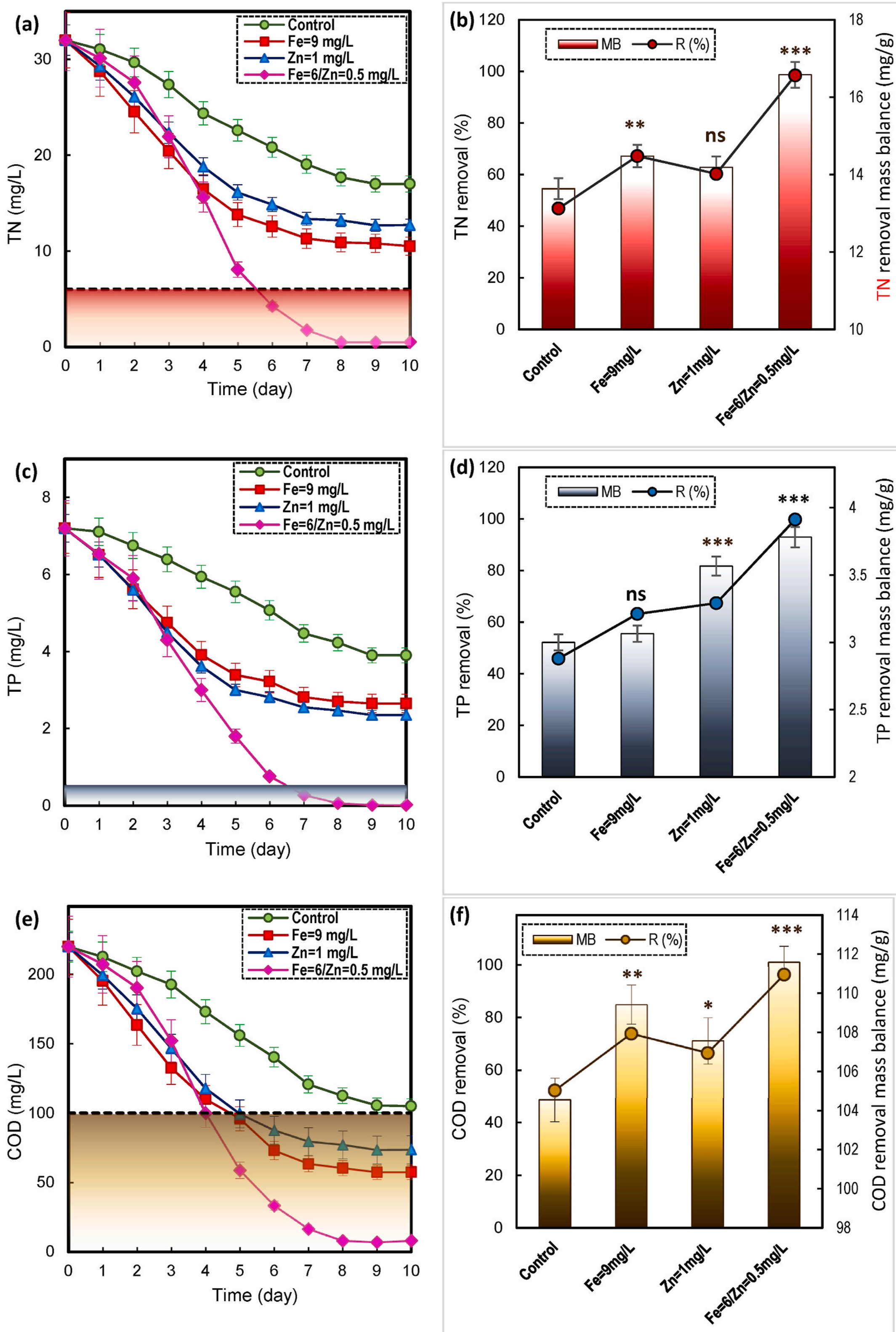

Fig. 3. Effect of TEs supplementation on microalgal nutrient xoncentration and mass balance, (a–b) TN, (c–d) TP and (e–f) COD removal.

(Fig. 3c). According to the results, the concentration of PO_4 was reduced from 7.2 (raw MWW) to 3.9, 2.65, 2.35 mg/L after 10 days of microalgae cultivation in the untreated MWW (control) and the treatment MWW with Fe (9 mg/L), Zn (1 mg/L), respectively. In the case of Fe/Zn (6/0.5 mg/L), the final concentration of PO_4 became almost zero. In contracts with the Fe/Zn The final concentration of PO_4 after 10 days cultivation in the MWW treated with single Fe and Zn did not meet the standard level for agriculture reuse water (<0.5 mgPO_4/L according to EPA, 2020). As can be seen from Figs. 3d and 1 g of *C. sorokiniana* cultivated in the untreated MWW and treated with Fe, Zn and Fe/Zn can uptake 3,

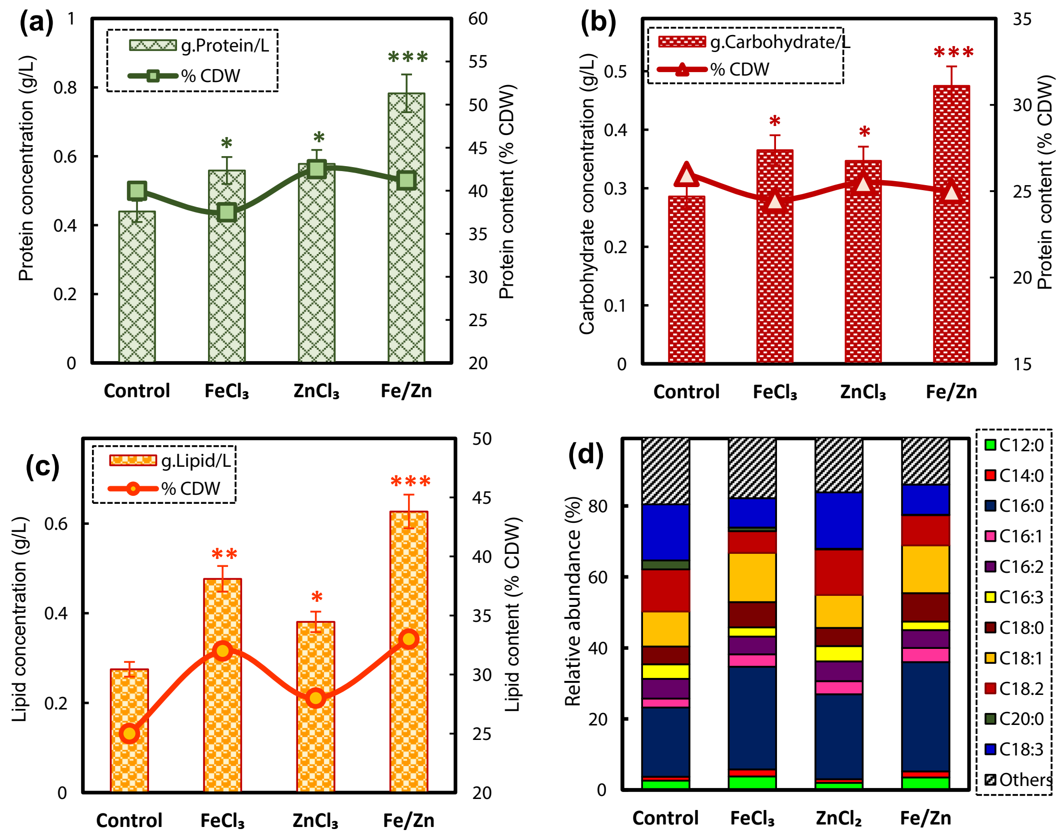

Fig. 4. Effect of TE supplementation of (a) protein, (b) Carbohydrate, (c) lipid generation and (d) fatty acid distribution.

3.06, 3.57 and 3.78 mg of PO_4, respectively. As a result, the removal efficiency of PO_4 increased from 45.8% (control) to 63.2%, 67.4% and 99.8% after adding Fe, Zn and Fe/Zn to the MWW.

Fig. 3e illustrates the COD reduction during 10 days of *C. sorokiniana* cultivation in the untreated MWW (control) and the treated MWW with Fe (9 mg/L), Zn (1 mg/L) and Fe/Zn (6/0.5 mg/L). The final concentrations of COD reached from 220 mg/L to 105, 57.5, 73.7, 8 mg/L after 10 days of *C. sorokiniana* cultivation in control, Fe, Zn and Fe/Zn, respectively. These number met the acceptable level COD for agriculture reuse water (<100 mg COD/L according to EPA, 2020). However, in the case of Fe/Zn the COD reached to the standard level with in within 4 days. Fig. 3f shows the results of COD efficiency and mass balance under different conditions. The COD final removal efficiencies of 52.3 %, 73.9%, 66.5% and 96.4% were achieved for control, Fe, Zn and Fe/Zn, respectively. According to the mass balance calculation, 1 g of *C. sorokiniana* cultivated in untreated MWW and treated with Fe, Zn and Fe/Zn could uptake 104.5, 109.4, 107.6.1 and 111.6 mg of COD.

The exact mechanism of the effect of Fe supplementation on nitrogen uptake capability of microalgal cells is not clear yet. However, the higher TN removal after Fe addition might be due to (i) stimulation of nitrogen adoption metabolism by enhancing photosynthesis efficiency (Polat et al., 2020), (ii) increasing the activity of some enzymes containing Fe (e.g., Crd1) in Calvin and Benson cycle which increase nitrogen consumption by microalgal cells (Vargas-Estrada et al., 2020). About the effect of Zn supplementation, Li et al. (2020) reported the TN removal efficiency did not changed significantly when the Zn dosage raged from 0 to 4 mg/L. Similarly in our research, the effect of Zn on TN removal was not statistically significant ($P < 0.05$). However, in contrast with Fe, Zn showed a remarkable effect of TP removal which due to two main mechanisms: (i) better phosphorus assimilation by microalgae cells and (2) phosphate (PO_4^3) reaction with Zn ions and sedimentation of $Zn_3(PO_4)_2.2H_2O$ (Li et al., 2020):

$$3Zn^{2+} + 2PO_4^3 + 2H_2O \rightarrow Zn_3(PO_4)_2.2H_2O \quad (16)$$

Overall, the results of MWW treatment by *C. sorokiniana* cultivation proved that combined utilization of Fe/Zn at an appropriate dosage significantly improve nutrients removal.

3.3. Effect of TEs on bioproducts accumulation

Fig. 4 illustrates the final concentration and content of protein, carbohydrate and lipid after 10 days *C. Sorokiniana* cultivation in the MWW before and after treatment with $FeCl_3$, $ZnCl_2$ and combined using Fe/Zn. Furthermore, the fatty acid distribution of the microalgae cells with and without treatment were determined and shown in Fig. 4d.

As shown in Fig. 4a, the protein content of *C. sorokiniana* decreased from 40 to 37.5 %CDW after treatment with 9 mgFe/L. This might be attributed to the stimulation of protease enzyme activity by the adsorbed Fe ions (e.g. Fe^{3+} and Fe^{2+}). It has been reported that protease enzyme breaks down proteins in microalgae cells resulting in lower protein content (Vargas-Estrada et al., 2020). However, the total protein concentration increased from 0.44 to 0.599 g/L by the addition of $FeCl_3$ which was due to the highest biomass production after Fe treatment. On the contrary, Zn supplementation showed a remarkable improvement on protein content (from 40 to 42.5 % CDW) resulted in 23% increment in the total protein concentration (from 0.44 to 0.578 g/L). The higher protein production after Zn treatment can be explain by the reaction of cells defense system to the toxicity of Zn (Ciurli et al., 2020). Under Zn toxicity stress, microalgae cells start to generate protective proteins such as Zn-binding proteins, peptide-disulfide and anti-oxidative resulting in higher protein content of the cells (Li et al., 2020). In the case of combined addition of Fe/Zn, the protein content increased from 40 to 41.2 % CDW (less than Zn but higher than Fe). Ultimately, the combined utilization of Fe/Zn at proper dosage resulted in significant improvement of protein concentration (from 0.44 to 0.783 g/L) mainly due to the higher

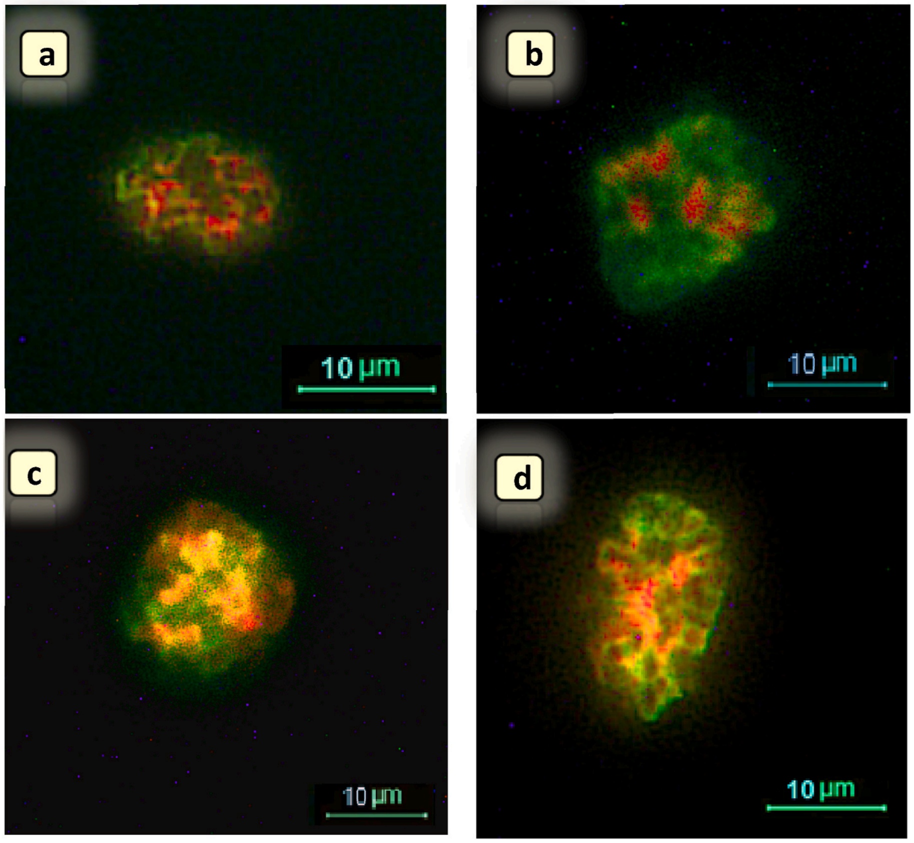

Fig. 5. CLSM images of C. sorokinian cell (a) without treatment and after treatment with (b) $ZnCl_2$, (c) $FeCl_3$ and (d) Fe/Zn (Color label: green, red and yellow color represent protein, carbohydrate and lipid respectively).

biomass production. Statistical analysis also proved the significant effect of TE supplementation on protein concentration (*P*-value <0.05).

In the case of carbohydrate (Fig. 4b), it was observed that $FeCl_3$ supplementation resulted in lower carbohydrate production as the carbohydrate content decreased from 26 to 24.5 %CDW after treatment with 9 mgFe/L. This might be attributed to the inhibitory effect of Fe ions on ADP-glucose pyrophosphorylase activities which led to lower starch synthesis (Dey et al., 2023). Similar results have been observed Polat et al. (2020) and Rizwan et al. (2017) when they were investigating the effect of different iron sources of *Auxenochlorella protothecoides* and *Dunaliella tertiolecta*, respectively. They reported that carbohydrate content decreased by increasing $FeCl_3$ concentration. Unlikely, in the case of Zn and combine Zn/Fe treatment no significant change (less than 1%) was observed on the carbohydrate content before and after treatment. However, the final carbohydrate concentration in the medium increased from 0.28 to 0.47 g/L after treatment with combined Fe/Zn supplementations due to improvement on biomass production.

According to Fig. 4c, adding $FeCl_3$ increased lipid content of *C. sorokiniana* from 25 % to 32 %CDW. The higher lipid production by the addition of Fe might be attributed to the following reasons; (i) iron involves in hydroxyl and superoxide radicals production leading to an oxidative stress in microalgae cells. As a result, microalgae increased lipids accumulation to overcome such oxidative stress (Rizwan et al., 2017). (ii) Fe also promotes the expression of genes such as accD and aaC1 which are key genes in lipid production (Akbari et al., 2024; Wan et al., 2014). (iii) Fe can modify the metabolic pathways related to lipid production in microalgae cells (Ren et al., 2014). Our finding was in consistent with previous studies in the literature (Che et al., 2015; Sun and Huang, 2017; Zhang et al., 2023b). In the case of $ZnCl_2$, it was observed that the lipid content increased from 25 to 28%CDW after treatment with 1gZn/L (corresponding to 44% improvement of lipid concentration). It has been reported that high Zn concentration induces a stress protein resulting in metabolic pathway turn from starch production to lipid generation (Ghafari et al., 2018; Sun and Huang, 2017). Moreover, higher Zn results in higher carbonic anhydrase (CA) which plays an important role in better photosynthesis and more lipid production (Wan et al., 2014). Consequently, in the case of combine Zn/Fe treatment, lipid content and lipid concentration increased from 25 to 33%CDW and from 0.27 to 0.62 g/L respectively. Statistical analysis also proved the significance of the effect of TE treatment on lipid production (*P*-value <0.05).

According to Fig. 4d, using TEs have a significant effect on fatty acids of microalgae *C. sorokiniana* ($p < 0.05$). Generally, for better biodiesel production the FAMEs composition of microalgal lipids should have high content of long-chain fatty acids such as *Palmitic acid* (C16:0) and *Stearic acid* (C18:0) (Lee et al., 2023). It was observed that by the addition of $ZnCl_2$, the amount of saturated fatty acids (SFAs) and polyunsaturated fatty acids (PUFAs) were increased from 30.70% to 32.51% and from 37.28% to 38.71%, respectively. This might be attributed to the activation of defense system or reparation mechanisms as a results of neutralize cellular damage (Vitali et al., 2023). It was observed that Zn did not affect much on the Mono-unsaturated fatty acids (MUFAs) even decreased in C18:1. Similar results of the effect of Zn on FAMEs composition were reported by of Zhou et al. (2018). However, $FeCl_3$ treatment mainly affected the SFA fatty acids such as *Lauric acid* (C12:0), *Myristoleic acid* (C14:0), *Palmitic acid* (C16:0) and *Stearic acid* (C18:0). Consequently, the SFAs increased from 30.70 % (control) to 43.01% ($FeCl_3$). Fe treatment could also increase the MUFAs from 12.45% (control) to 17.51% but decreased the amount of PUFAs from 37.28% to 23.5%. Our finding consist with the results of Sun and Huang (2017) and Akbari et al. (2024) as they reported the effect of Fe and Fe NPs on fatty

Table 2
Predicted biodiesel properties of *C. sorokiniana* before and after treatment with TEs.

Cultivation	SV	IV	CN	DU	LCSF	CFPP	C18:3
	(–)	(gI_2/100 oil)	(–)	(wt%)	(wt%)	(°C)	(wt%)
Control	160.50	94.85	58.96	87.01	7.01	5.36	15.82
$FeCl_3$	170.35	68.30	62.80	66.40	7.44	6.90	8.39
$ZnCl_2$	168.01	98.03	57.77	90.20	5.85	1.33	15.90
Fe/Zn	170.50	66.98	63.30	64.93	6.83	4.58	8.81
Standard EN 14214	NA	≤120.00	≥51.00	NA	NA	≤5.00	<12.00

* NA: not available.

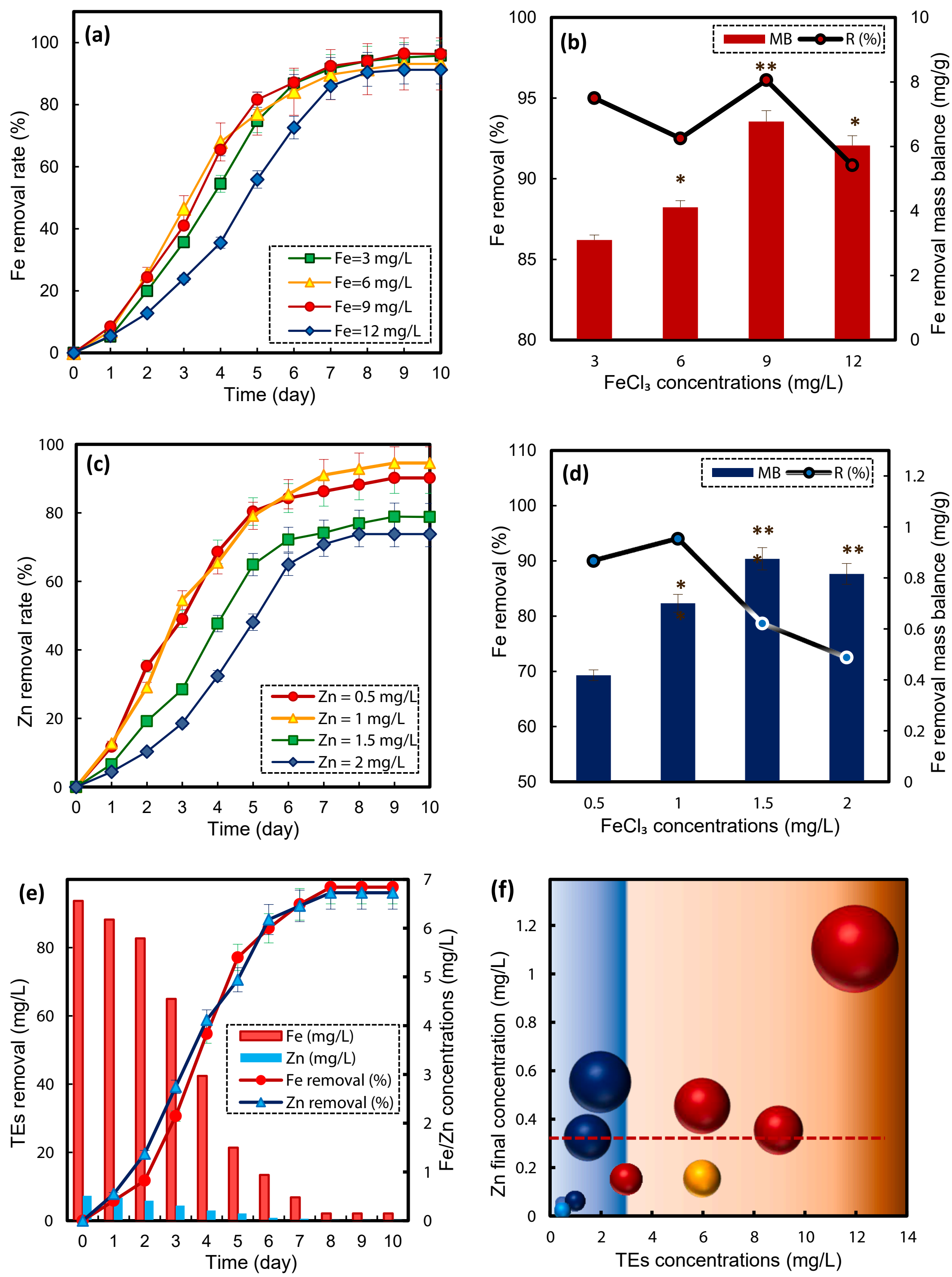

Fig. 6. The removal rate and mass balance of (a–b) iron from Fe supplemented medium (c–d) Zinc from Zn supplemented medium and (e) Zn and Fe from combined Zn/Fe supplemented medium and (f) final concentrations Zn of Fe and Zn under different condition.

acid distribution, respectively. In the case of combined FE/Zn, the fatty acids quality for biodiesel improved as the SFA increase from 30.70% to 43.93% which was attributed to the improving of *Palmitic acid* (C16:0) and *Stearic acid* (C18:0) up to 58% and 60%, respectively. It should be noted that higher SFA of microalgae indicates better oxidation satiability (Ahn et al., 2022). Moreover, the MUFA increased from 12.45% to 17.51% and the PUFA decreased from 37.28% to 23.91% after treatment with Fe/Zn respectively. In conclusion, the fatty acid distribution results revealed that the microalgae pre-treatment with combined addition of Fe/Zn at an appropriate dosage result in biodiesel production with better quality.

Fig. 5 shows the CLSM images of one *C. Sorokinana* cell before and after treatment with Fe, Zn and Fe/Zn. The green, red and yellow color represent protein, carbohydrate and lipid respectively. As can be seen from Fig. 5a–a *C. Sorokinana* cells without treatment showed slightly higher green (Protein) and red (carbohydrate) color, which is in consistent with the previous reports (Taghavijeloudar et al., 2022). It was observed that by adding Zn the green color increased remarkable which indicated more content of protein (Fig, 5b). On the contrary by adding Fe yellow part was significantly increased which indicated more lipid production (Fig, 5c). In the case of combined utilization of Fe/Zn higher lipid was notably observed while the amount of protein and carbohydrates didn't show significant changes (Fig, 5d). The CLSM images analysis confirmed the results obtained regarding the effect of TE on bioproducts content presented in previous section.

3.4. Effect of TEs on biodiesel properties

To evaluate the effect of TE supplementation on the biodiesel quality produced from *C. Sorokiniana*, SV, IV, CN, DU, LCSF and CFPP were calculated for untreated and treated microalgae with Fe, Zn and Fe/Zn using fatty acid profiles data (Fig. 4d) and as well as the European standards EN 14214 (Ahn et al., 2022) for biodiesel are shown in Table 2.

The SV is one of the common indicator representing biofuel quality which is related to the fatty acids chain length (Rajabi Islami and Assareh, 2020). According to Table 2, the SV of 160.23–167.21, 174.12 and 171.25 mgKOH/g were achieved for *C. sorokiniana* before and after treatment with Fe, Zn and Fe/Zn, respectively. European standards (EN 14214) has not defined any limitation for SV. However, our findings were in line with the results reported by Polat et al. (2020) and Rajabi Islami and Assareh (2019) about the effect of Fe addition on SV. The cetane number (CN) which represents the ignition ability of biofuel is an important factor for evaluation of diesel fuel quality. In other words, higher CN values indicate better cold start and performance of the engine (Sinha et al., 2016). According to Table 2, Zn treatment decreased the CN from 58.96 to 57.77, while Fe treatment increased the CN from 58.96 to 62.8. However, the highest CN of 63.3 (approximately to 10% more than the untreated microalgae) was achieved by the addition of combined Fe/Zn indicating improving the quality of biofuel. The iodine value (IV) which is calculated based on the total unsaturation within the FAMEs, is corresponding to deposition of engine lubricating oil biodiesel (Sinha et al., 2016). EN 14214 recommended 120 g I_2/100 g oil as the maximum value for IV. Based on our calculated the IV of *C. sorokiniana* before and after treatment with Fe, Zn and Fe/Zn were 94.85, 69.75, 86.48 and 67.32, respectively. It was observed that all the IV values met the standard. However, the lowest IV was achieved by Fe/Zn addition which suggesting better biodiesel quality. Degree of unsaturation (DU) is another biodiesel indicator which impacts the emission of Carbon monoxide (CO), nitrogen oxide (NO) and low hydrocarbons (Ahn et al., 2022). The DU amount of 87.01, 66.40, 90.2 and 64.93 were calculated for before control and treated with Fe, Zn and Fe/Zn, respectively. Although, EN 14214 did not specify limitation for DU, it is well accepted that lower DU indicates better diesel quality (Polat et al., 2020) which was achieved by Fe/Zn treatment. The cold filter plugging point (CFPP) represents the lowest temperature at which fuel still flow through a specific filter. According to the European standards (EN 14214), the CFPP should be less than 5 °C. Based on our finding the amount CFPP reduced to below standard level after treatment with FE/Zn. Long chain fatty saturated fatty acids (LCSF) is another parameter mainly depends on the CFPP. The LCSF decreased after treatment with Fe/Zn from 7.01 to 6.83 WT%. Linolencate (C18:3) is also important for biodiesel quality as the EN 14214 standard recommends it should be less than 12%. In our case, The amount of C18:3 for the Fe/Zn and Fe treatments were 8.1 and 10.8, respectively and it means unlike Zn and control samples they met standards limit.

3.5. Removal rate of TEs during microalgae cultivation

Zn and Fe which are toxic HMs at high dosage can be rapidly taken up by the microalgae cells through the metal binding groups on cell surfaces and be accumulated and utilized in the algal cell (Priya et al., 2022; Zhou et al., 2018). The removal rate of Fe and Zn and their final does at the end of *C. sorokiniana* cultivation under different concentrations of Fe and Zn supplementation were monitored to evaluate any secondary contamination (Fig. 6). As can be seen from Fig. 6a, microalgae could significantly uptake Fe from the medium as the final Fe removal efficiencies of 97.19%, 96.95%, 97.07%, 90.17% were achieved under medium containing initial Fe dosages of 3, 6, 9, 12 mg/L respectively. Generally, increasing the initial concentration of Fe decreased the removal efficiency performance (Fig. 6b). However, the maximum Fe removal efficiency of 96.1% was achieved in the case of 9 mg/L which was attributed to the better growth of microalgae and Fe consume as nutrient for growth. The mass balance analyzed also revealed that microalgae cultivated with 9 mg/L Fe could uptake more Fe than other cases (6.77 mg/g). Regarding Zn supplementation (Fig. 6c), microalgae cells could also efficiently remove Zn from the medium under different Zn initial concentrations. However, the removal of Zn was a little bit lower than Fe, as the maximum Zn removal efficiency of 94% was achieved when microalgae was supplemented with 1 mg/L of Zn (Fig. 6d). It is worth noting that the highest Zn uptake mass balance of 0.88 mg/g was observed under 1.5 mg/L Zn supplementation due to higher dose of Zn. Similar results for Fe and Zn removal by microalgae were reported by Yousefi et al. (2023) and Liu et al. (2023), respectively.

For the combine Fe and Zn supplementation (Fe/Zn), the concentration of residual of Fe and Zn in the medium was monitored only for the optimum case (initial concentrations of Fe = 6 mg/L and Zn = 0.5 mg/L) and the results are shown in Fig. 6e. It was observed that the Fe and Zn removal efficiency by the microalgal cells was significantly improved when Zn and Fe were combined used for MWW supplementation. According to the results, the final removal efficiencies of 98.3% (corresponding to 0.11 mgFe/L) and 96.8% (corresponding to 0.02 mgZn/L) were achieved for Fe and Zn, respectively after 10 days microalgae cultivation under Fe/Zn supplementation. These numbers were more than that in single Fe (98.3% vs. 93.14%) and Zn (96.8% vs. 90.2%) supplementations with 6 and 0.5 mg/L, respectively. The main reason for this improvement is the enhancing microalgae metabolisms by synergistic effect of Fe and Zn as previously discussed in Sections. Also, Zdzieblowska et al. (2024) reported that the addition of Fe in the medium improves the bioaccumulation of other TEs including Zn.

Fig. 6f shows the final concentrations of Fe and Zn under different concentration (the size of the bubble shows the amount of concentration). As can be seen the final concentration of Fe and Zn were under the standard limit for drinking water only in 2 cases of high dosage of Fe the final concentration was over the standard for drinking water that's about 0.3 mg/L. It is worth noting that standard level of Zn concentration for drinking water is 5 mg/L which was much higher than the supplemented dosage, however in the case of Fe the standard level is 0.3 mg/L and in the case of Fe with high dosage the final concentration of Fe was over the standard limit, which means it can cause secondary contamination for drinking water. Bud in the case of combined (Zn

Table 3
Tecno-economic analysis of using Fe and Zn for microalgae cultivation.

Case	TEs supplementation			Microalgae biomass			Total cost for 1 kg biomass/bio-products			
	Dosage (mg/L)	UP_{TE} (€/kg_{TE})	C_{TE} (€/kg_{MB})	BC (g/L)	UP_{MB} (€/kg)	OC_{MB} (€/kg)	Biomass (€/kg_{MB})	Lipid (€/kgL)	Protein (€/kgP)	Carbohydrate (€/kgC)
MWW	0.00	0.00	0.00	1.10	2.50	1.82	1.82	7.27	4.55	6.99
$FeCl_3$	9.00	162.00	0.98	1.49	2.50	1.35	2.33	7.28	6.21	9.50
$ZnCl_2$	0.50	528.00	0.39	1.36	2.50	1.47	1.86	6.64	4.37	7.29
Fe/Zn	6.0/0.5	_	0.65	1.90	2.50	1.05	1.70	5.16	4.13	6.81

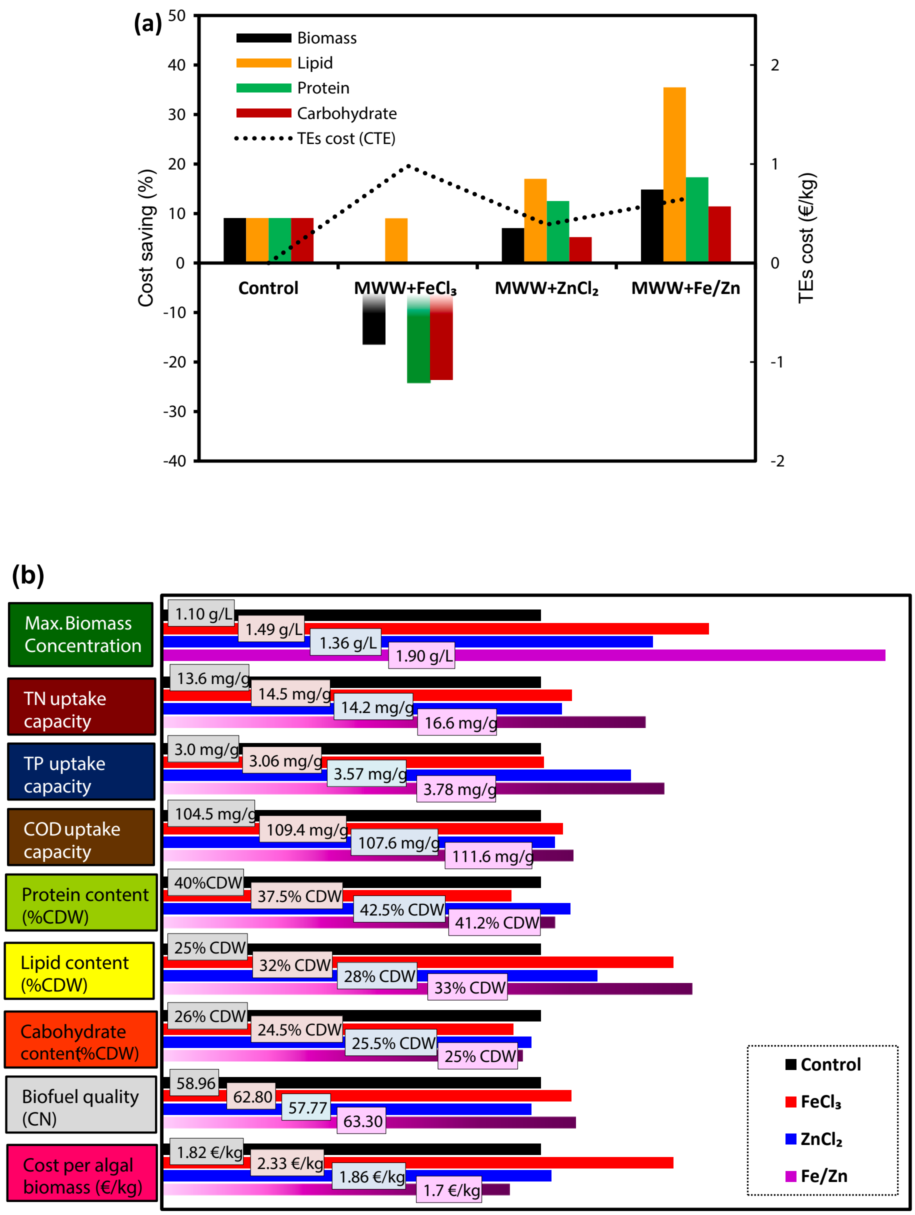

Fig. 7. Cost saving percentage (%) and TEs cost (C_{TE}) for 1 kg biomass and bio-products generation by microalgae cultivation in 1000 L MWW without (control) and with Fe, Zn and Fe/Zn supplementation (b) Comparison of the effect of TEs on microalgae performances.

yellow and Fe blue) each final concentration was lower than drinking water standard so there is no of secondary pollution was observed. This can because of the effect of Zn and Fe interaction in the optimum case which make both TEs removal much higher than the single cases.

3.6. *Techno-economic analysis of TEs supplementation*

Although MWW pre-treatment with combined Fe and Zn showed positive effect on microalgae performance, it may inject additional expense to the overall cost of microalgae cultivation at large scale. Table 3 presents a summary of techno-economic analysis of TEs supplementation for production of 1 kg microalgal biomass and bio-products from 1000 L MWW. Given that the unit price for 1 kg microalgal biomass production (UP_{MB}) is considered as 2.5 €/kg, the total operational cost for production of 1 kg biomass from 1000 L MWW (OC_{MB}) without TEs supplementation (control) was calculated as 1.82 €/kg. According to our fining, adding TEs resulted in higher biomass and bio products generation. Therefore, the OC_{MB} after treatment with Fe, Zn and Fe/Zn were reduced to 1.82, 1.35, 1.47 and 1.055 €/kg, respectively. However, adding TEs with their optimal dosage to 1000 L MWW injected additional cost (C_{TE}) which were calculated as 0.98, 0.39 and 0.65 €/kg for Fe, Zn and Fe/Zn, respectively. Ultimately, the total cost for 1 kg microalgal biomass production from 1000 L MWW (TC_{MB}) were obtained as 1.82, 2.33, 1.86 and 1.7 €/kg for Control. Fe, Zn and Fe/Zn, respectively. Similarly, the total cost for 1 kg bio-products generation from 1000 L MWW (TC_{BP}) were calculated using Eq. (15). As can be seen from Table 3, the total cost for lipid production significantly decreased after TEs supplementation which was attributed to the enhancing lipid content of the cells by the Fe, Zn and Fe/Zn treatment. In the case of protein and carbohydrate, Fe treatment led to higher cost which was due to the high TEs cost (C_{TE}) by using high dosage of Fe (0.98 €/kg) and the minimal effect of Fe on protein and carbohydrate content.

To get a better understanding of the economic feasibility of using TEs for microalgae cultivation, Fig. 7 illustrates the cost saving percentage (%) and TEs cost for 1 kg biomass and bio-products generation by microalgae cultivation in 1000 L MWW without (control) and with Fe, Zn and Fe/Zn supplementation. As can be seen from Fig. 7, microalgae cultivation in MWW without TEs addition resulted in 10% cost saving for biomass and bio-products generation with no need of additional cost for TEs addition. The results showed that using Fe for microalgae cultivation was not feasible (increase the overall cost up to 16%) due to high TEs cost (almost 1 €/kg). On the contrary, using Zn could reduce the total cost (up to 7% still less than control) as the optimal dosage of Zn was low (1 mg/L) which cause only 0.39 €/kg additional cost for TE supplementation. However, the best economic conditions were achieved by the combined addition of Zn and Fe which could save the overall cost up to 15%, 35%, 17 and 11.5% for biomass, lipid, protein and carbohydrate production from 1000 L MWW. The cost analysis revealed that adding single Fe and Zn to MWW for microalgae cultivation increased the overall cost and is not feasible economically. While, the combined addition of Zn and Fe could meaningfully reduce the overall cost of biomass and bio-products generation. Fig. 7b illustrates a comparison of the effect of TEs supplementation (at optimum conditions) on microalgae performances in terms of biomass production, nutrient uptake capacity, bio-products generation, biofuel quality and overall cost for production of 1 kg biomass from 1000 L MWW. Our results suggested that MWW supplementation with combined Fe/Zn could significantly improve microalgae performance in all aspects. While, pre-treatment with Fe showed negative impact and no effect on TP uptake, protein and carbohydrate content as well as overall cost. The Zn supplementation also showed no meaningful impact ($P < 0.05$) on TN uptake capacity, carbohydrate generation and overall cost for production of 1 kg biomass from 1000 MWW.

4. Conclusion

Present study explores enhancing *Chlorella sorokiniana* performance by synergistic effect of iron (Fe) and zinc (Zn) addition to real municipal wastewater (MWW). It was found that combined supplementation of Fe (6 mg/L) and Zn (0.5 mg/L) could increase biomass production up to 72% (from 1.1 to 1.9 g/L). In addition, the nutrient uptake capacity from wastewater was significantly increased as the removal efficiencies of COD, TN and TP increased from 52.3%, 45.8% and 46.9%–96.4%, 99.8% and 98.4%, respectively. It was observed that Fe supplementation resulted in more biomass productivity, while using high dosage of Zn had an inhibitory effect on microalgae biomass but increased lipid production at any dosage. According to the results, supplementation with Fe/Zn resulted in significant increase in lipid content (from 25% to 33%), while protein and carbohydrate content remained stable. The techno-economic analysis revealed that individual utilization of Fe and Zn is not economically feasible. However, combined Fe/Zn could save biomass production cost up to 15%. Our finding suggested that MWW pre-treatment with Fe and Zn can be promising approach to improve the microalgae industry and biodiesel quality. However, the synergistic effect of Fe and Zn addition to MWW should be further investigated for other microalgae spices. The possibility of secondary contamination by Fe and Zn should be checked when using them as TEs for improving microlagal biomass.

CRediT authorship contribution statement

Zahra Khodabakhshi Palandi: Writing – original draft, Software, Investigation, Formal analysis, Data curation. **Mohsen Taghavijeloudar:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Data curation, 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

Data will be made available on request.

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Appendix A. Supplementary data

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