Environmental Science and Pollution Research (2023) 30:15808–15820
https://doi.org/10.1007/s11356-022-23332-x

RESEARCH ARTICLE

Production and use of *Scenedesmus acuminatus* biomass in synthetic municipal wastewater for integrated biorefineries

Melih Onay[1] · **Erdinc Aladag**[1]

Received: 14 April 2022 / Accepted: 24 September 2022 / Published online: 29 September 2022

Abstract

Bioethanol production from algal biomass is a promising alternative for sustainable biofuel production. Algae possess a high photosynthetic capacity and an adaptive ability to thrive under harsh environmental conditions. The potential properties of *Scenedesmus acuminatus* CCALA 436 were assessed in this research for its bioethanol efficiency, and the effects of growing the algae in wastewater and at different concentrations of mepiquat chloride were studied. Also, pre-treatment efficiencies of different concentrations of calcium oxide were carried out on microalgae biomass. Superoxide dismutase, catalase activity, glutathione, and malondialdehyde contents of microalgae were examined, and the changes in chlorophyll, photoprotective carotenoid contents, and protein concentrations were determined. The results revealed that the maximum sugar and ethanol contents of *Scenedesmus acuminatus* CCALA 436 were 44.7 ± 1.5% and 20.32 g/L, respectively, for 50% wastewater and mepiquat chloride (2.5 mg/L) after pre-treatment with calcium oxide (0.08%). Additionally, the levels of oxidative enzymes varied depending on the wastewater concentrations. These findings indicate *Scenedesmus acuminatus* CCALA 436 grown in wastewater and mepiquat chloride can be used for the treatment of wastewater and the production of ethanol and high-value products such as carotenoid.

Keywords Antioxidant enzymes · Bioethanol · Calcium oxide · Mepiquat chloride · Microalgae · Pigments · Synergistic effects

Introduction

Biofuels are renewable energy sources and contribute to the minimization of greenhouse gas emissions, environmental protection, and the agricultural economy. They exist in liquid and gaseous forms, and notable examples include biogas (biomethane and biohydrogen), biodiesel, biobutanol, and bioethanol. The chemical formula for ethanol is CH_3CH_2OH showing that it has hydrocarbon potential with higher octane rating compared to gasoline. Thus, they can be used as a blend in gasoline production to achieve a higher-octane rating (Staniszewski et al. 2007). Unlike fossil-fuel products, ethanol is less toxic and can easily decompose. In addition, ethanol releases a few toxic gases when they undergo combustion. Bioethanol has been used for many years. The first bioethanol was produced using sugar cane and sugar beet, and it is still produced in many countries such as America and Brazil. Later, some starch sources (rice and barley) were used for bioethanol production. First generation biofuels are produced from using food resources, and this consequently leads to their competition with food production. Therefore, the use of non-food resources was explored to generate second-generation biofuels (Maity and Mallick 2022). The second-generation bioethanol contains lignocellulosic plant sources such as woody plants. In this bioethanol production, the fermentation process can occur in a variety of ways. One of them is lignocellulosic yeast fermentation. It can be used for bioethanol production via pervaporation (Dadi et al. 2018). However, expensive equipment and a large amount of labor are required, which increase the capital costs (Maity and Mallick 2022).

Next, third-generation bioethanol production appeared, and the sources are microalgae. Compared with the first- and second-generation biofuel production, microalgae have

Responsible Editor: Ta Yeong Wu

✉ Melih Onay
melihonay@yyu.edu.tr

[1] Department of Environmental Engineering, Computational & Experimental Biochemistry Lab, Van Yuzuncu Yil University, 65080 Van, Turkey

higher growth rates and biomass productivities with large amounts of lipids and polysaccharides for biofuel production (Daroch et al. 2013). Importantly, microalgae require macro and micronutrients in order to biosynthesize carbohydrates.

The potential sugar content can be enhanced by the experimental manipulation of environmental factors (Onay 2020b). Major components in the medium can be adjusted to obtain higher carbohydrate productivity, and the obtained carbohydrate can be used for bioethanol production. The artificial culture medium containing C, H, O, P, S, and N is expensive, thus reducing the economic viability of biofuel production from algae. Moreover, the growth of microalgae requires more water in large-scale biofuel processes. Industrially, algae can be cultivated in various wastewaters, utilizing the nutrients in the wastewater for the biosynthesis of essential organelles for growth, which consequently leads to wastewater bioremediation (RoyChowdhury et al. 2019). Under stress conditions, microalgae are exposed to free radicals and oxidation reactions. When stress increases, the antioxidant enzyme and molecule concentrations reach higher levels to protect microalgae cells (Onay 2020b). The nutritional composition of wastewater is highly dependent on the source of the water. Thus, the lipid and carbohydrate contents of microalgae also vary with the nutrient content of the wastewater, for example, dairy, municipal, and industrial wastewater (Kumar et al. 2019).

In addition, microalgae have some metabolic properties, including stress effects. The effects of stress can sometimes be positive for the metabolism of microalgae. Stress-mediated molecules and extreme conditions can increase lipid, sugar, and protein concentrations (Onay 2020a). This situation likely depends on the nature of microalgae and their ability to adapt to harsh conditions. Microalgae typically reduce molecule toxicity by increasing antioxidant molecule concentrations. Antioxidant and toxic molecules can be measured by various systems, which can be enzymatic or non-enzymatic. For example, enzymatic systems include superoxide dismutase (SOD) and catalase (CAT). On the other hand, non-enzymatic systems involve molecules such as glutathione (GSH) and malondialdehyde (MDA) (Hamed et al. 2017). Growth regulators in these systems can be important for microalgae cultivation. For example, mepiquat chloride (MC) is a growth factor that mainly provides growth balance, used to control vegetative growth and yield of cotton crops. MC blocks gibberellic acid biosynthesis, which leads to the formation of short-statured plants, and stops cell elongation. Furthermore, MC decreases the photosynthesis rate in crops, probably because of stress formation (Tung et al. 2018a). Although MC accelerates ripening in plants, it has also been observed to reduce the yield of many plants (Yuan et al. 2021). Therefore, MC may cause a change in the metabolic contents of microalgae by creating stress that will slow down the growth.

Hydrolysis must be performed to extract the carbohydrates from microalgae. The hydrolysis process can be conducted using acid, base, or enzymes (Fetyan et al. 2022). However, using them directly in high concentrations can disrupt the carbohydrate structure. Before hydrolysis, pre-treatment methods can be used to enhance the effects (Nowicka et al. 2020; Onay 2022). The aim is to open the cell membranes of the microalgae, allowing more acid or base to enter the algae cells, thereby increasing the metabolic content. Calcium oxide (CaO) is an alkaline compound, which is relatively inexpensive. CaO has not been extensively used in the pre-treatment of microalgae, but it has been used for the pre-treatment of waste sludge (Xin et al. 2021). Since calcium peroxide is used in pre-treatment processes, CaO may be used in a more effective extraction of the metabolic contents of microalgae (Hung et al. 2022). For instance, CaO allows the use of lower acid or base concentrations in hydrolysis.

Carotenoids are commonly found in microalgae, and their amounts increase under stress conditions. Specifically, carotenoids are found in chloroplasts of microalgae which can be used as industrial colorants and additives to animal feed (Hadizadeh and Mehrgan 2020). Furthermore, carotenoids can be produced together with bioethanol during the biorefinery process (Ansari et al. 2021).

Briefly, the processes of bioethanol production consist of pre-treatment, saccharification, fermentation, and purification. *Zymomonas* and *Saccharomyces* are yeast species that affect bioethanol production in the fermentation process. *Saccharomyces* spp. uses cellulases and hemicellulases to enhance bioethanol production. On the other hand, *Z. mobilis* can produce higher amounts of ethanol in a wider pH range (3.5–7.5). Out of this range, *Z. mobilis* generally produces less biomass than *Saccharomyces* (Onay 2019). Also, *Z. mobilis* cannot metabolize sugars with 5C, and it has a lower tolerance to inhibitors or pollutants. In contrast, *Saccharomyces* has a higher tolerance and can metabolize sugars in the presence of oxygen. *Saccharomyces* has been certificated by GRAS and the FDA, conforming that it is safe for the use of human and animal feed, and it can be modified genetically. In conclusion, both can be used for industrial bioethanol production (Phwan et al. 2018). We used *S. cerevisiae* in the current study because this yeast has been used in our laboratory for many years.

In this study, *Sc*enedesmus *acuminatus* was chosen because these microalgae can be found in wastewater and grow rapidly when cultured (Devi et al. 2022). We investigated the effects of various concentrations of municipal wastewater on biomass, sugar, ethanol, and pigment yields from *Scenedesmus acuminatus* cultivated in a photobioreactor (PBR). The effects of MC and CaO on the carbohydrate contents of the microalgae were studied, and the enzymes affecting microalgal antioxidant systems were examined

under stress conditions. Their concentrations were measured, and their relationships were determined.

The goal of this study is to investigate the possibility of cultivating *Scenedesmus acuminatus* in wastewater with different concentrations of MC and convert the microalgae to bioethanol and pigments via fermentation of CaO-pretreated algae.

Materials and methods

Algal strain

Scenedesmus acuminatus CCALA 436 was obtained from CCALA in Czechia, and the municipal wastewater was prepared synthetically according to Zouboulis et al. (2017). Then, it was filtered and sterilized before the experiments. Each experiment was started with the same amount of wastewater content at the same time.

Setup of photobioreactor (PBR)

The flat photobioreactor (FPBR) used in this study has a volume of 1 L and an airflow rate of 0.6 L/min. Light intensity, temperature, and pH of the media were 130 µmol/m^2 s^1, 24 °C, and 7.4, respectively. These procedures were described in our previous study (Onay 2018).

In this study, different ratios of municipal wastewater were used to cultivate *Scenedesmus acuminatus CCALA 436* (0, 25, 50, 75, and 100%). Specifically, 100% of wastewater includes meat extract (100), $MgSO_47H_2O$ (3), NaCl (8), K_2HPO_4 (30), $CaCl_22H_2O$ (5), and peptone (150) mg/L. Then, it was autoclaved and mixed with ASM-1 medium before use. The ASM-1 medium was adjusted as the control (0% WW) and prepared according to Gorham et al. (1964). $NaNO_3$ (2000), K_2HPO_4 (100), Na_2HPO_4 (100), $MgCl_2$ (200), $MgSO_4$ (200), $CaCl_2$ (200), $FeCl_3$ (4), H_3BO_3 (40), $MnCl_2$ (7), $ZnCl_2$ (3.2), Na_2EDTA (20), $CoSO_4$ (0.08), $CuCl_2$ (0.0008) µmol/L are present in ASM-1 medium.

Also, 1, 2.5, 5, and 10 mg/L of MC were used for this study, and it was added to 50% wastewater. Microalgae were checked under a microscope for contamination. Cells were counted, and different cells were determined under a microscope using the Thoma chamber. In this study, the contamination was less than 2% per day. When living contaminants like bacteria and fungi were found, the medium had to be remade.

Monitoring of microalgae and the formation of dried biomass

In this study, the growth of *Scenedesmus acuminatus* CCALA 436 was monitored at 530, 600, 680, and 750 nm spectrophometrically. The r^2 value closest to 1 was found at 680 nm; therefore, we used this wavelength (data not shown). The dried biomass samples were gravimetrically weighed after being centrifuged at 3200g for 8 min at 4 °C (Onay 2020b). The following equations (Eqs. 1 and 2) were used to calculate the specific growth rates (μ) and doubling time (T_d) (Onay et al. 2014).

$$(\mu) : \mathrm{In}\left(X_1 - X_2\right)/\left(t_2 - t_1\right) \quad (1)$$

$$T_d = 0.693/\mu \quad (2)$$

X_1 is the final biomass concentration, X_2 is the first biomass concentration, t_2 is the final time, and t_1 is the start time.

Hydrolysis and fermentation procedures

Various concentrations (0.02–0.1%) of calcium oxide (CaO) were added to algal biomass for the investigation of the pretreatment effect. For this, the algae samples including CaO were incubated at 60 °C for 6 h with shaking (100 rpm). For hydrolysis, Miranda et al. (2012) used different hydrolysis methods for bioethanol production, and H_2SO_4 hydrolysis was used for hydrolysis. After incubation, algae samples were subjected to H_2SO_4 (Miranda et al. 2012). In our paper, *S. cerevisiae*, known as baker's yeast, was used for fermentation as reported by Onay (2019).

Analytical methods

Pigment concentrations were measured using a spectroscopic method (Onay 2020b). In this method, algae samples were incubated with absolute methanol in the dark at 45 °C. Then, they were centrifuged at 13,000 rpm for 5 min. The supernatant pigment contents were measured at 480, 652, 665, and 750 nm. The results were given as an average of three measurements. The pigment contents were calculated using Eqs. (3), (4), and (5) below.

$$\mathrm{Chl} - a(\mu\mathrm{g/ml}) = -8.0962 \times \mathrm{A}_{652} + 16.5169 \times \mathrm{A}_{665} \quad (3)$$

$$\mathrm{Chl} - \mathrm{b}(\mu\mathrm{g/ml}) = 27.4405 \times \mathrm{A}_{652} - 12.1688 \times \mathrm{A}_{665} \quad (4)$$

$$\mathrm{Carotenoid}(\mu\mathrm{g/ml}) = 4 \times \mathrm{A}_{480} \quad (5)$$

The total protein was extracted, and the protein concentrations were measured according to Onay (2020b). The total carbohydrate contents were calculated using the Anthrone method (Zhao et al. 2013). The total organic carbon (TOC) content of municipal wastewater was determined using the Albrektien method (Taylor et al. 2012). The total nitrogen (TN) and total phosphorus (TP) contents were determined by

spectrophotometry, performed according to Koistinen et al. (1980). The ethanol content in the microalgal slurry was determined according to Rizza et al. (2017).

Determination of antioxidant enzyme activities

The activities of CAT and SOD were measured using the nitroblue tetrazolium (NBT) method. The MDA content was measured using the thiobarbituric acid (TBA) method at 535 nm (Heath and Packer 1968; Onay 2020b). Also, the GSH content was determined using Anderson's formula (Anderson 1985).

Data analysis

The experimental groups utilized three parallel samples. All statistical analyses were performed with one-way analysis of variance (ANOVA) and Tukey's test, conducted using the MATLAB software package (MATLAB and SIMULINK, R2015a). The confidence level was higher than 95%. In this report, the results are expressed as mean ± standard error (SE).

Results and discussion

Effects of wastewater and mepiquat chloride

The growth rates of *Scenedesmus acuminatus* CCALA 436 can change under some environmental conditions such as light, pH, salt, nutrient limitation, and depletion. In our previous studies, *Scenedesmus sp ME02* was cultivated in various media such as BG-11, D medium, and Tris-Acetate-Phosphate (TAP) (Onay 2014). However, sufficient microalgal growth could not be achieved. Then, *Scenedesmus acuminatus* CCALA 436 was grown in municipal wastewater (100, 75, 50, 25, and 0%) and ASM-1 medium. The culture medium with 100% wastewater consisted completely of municipal wastewater, and the control group only had ASM-1 medium. Firstly, the contents of TOC, TN, and TP were calculated. The TOC, TN, and TP contents in 100% wastewater were 81.2, 12.5, and 5.2 mg/L, respectively. This result shows that *Scenedesmus acuminatus CCALA 436* can be grown in wastewater. In the literature, there are few studies related to municipal wastewater and *Scenedesmus acuminatus*. One report focused on *Synechocystis sp*, which was grown in municipal wastewater at an open raceway pond on a large scale (Ashokkumar et al. 2019). In their study, TOC (113), TN (21.5), and TP (5.5) mg/L values were enough for microalgae cultivation.

The growth curves of microalgae are given in Fig. 1. All microalgae were grown until their stationary phases. The culture containing 50% wastewater showed the fastest growth (1.90 ± 0.001) at 9 days. Like in the culture with 50% wastewater, after 9 days, the optical densities of the wastewater samples in the control, 25%, 75%, and 100% groups had all reached a stationary phase (1.44 ± 0.001, 1.61 ± 0.001, 1.28 ± 0.001, and 1.10 ± 0.003, respectively). The biomass concentrations (BCs) of the samples confirmed the absorbance results, and the maximum BC was found in the culture containing 50% wastewater (1711 ± 22 mg/L). In addition, the numbers of microalgae cells exhibited linear relationships with their BCs. The number of cells for the 50% wastewater culture was nearly 3.3×10^6 cells/mL at 9 days. Control, 25%, 75%, and 100% wastewater cultures had cell numbers of 2.6, 3, 2, and 1.9×10^6 cells/mL, respectively. According to the TOC (81.2), TN (12.5), and TP (5.2) mg/L analysis of wastewater, the cultures containing 75% and 100% wastewater contents in the culture medium include relatively low chemical contents compared to the ASM-1 medium content, and these conditions can limit microalgal biomass productivity (BP) (Wang et al. 2010). The cultures containing 25% and 50% wastewater had similar results. *Scenedesmus acuminatus* CCALA 436 grown in wastewater with fewer nutrients can integrate into culture conditions when enough organic compounds are available in the media (Brennan and Owende 2010).

Then, the effects of MC on the growth curves of microalgae were studied. The growth curves of microalgae including MC are given in Fig. 2. MC concentrations of 1, 2.5, 5, and 10 mg/L were used, and they were added to the cultures containing 50% wastewater since they produced the highest BC. According to our results, the control group (50% wastewater only) showed the highest growth curve values. Among MC concentrations, samples with 1 mg/L had the highest optical density (1.58), BC (1422 ± 11 mg/L), and cell number (2.8×10^6 cells/mL), and we did not see a drastic decrease in the growth curves of microalgae. These results were promising for high carbohydrate concentration expectations. However, as the concentration of MC increased, the growth curves of microalgae decreased. The BCs of samples with 2.5, 5, and 10 mg/L of MC were 1189 ± 11, 822 ± 11, and 689 ± 22 mg/L, respectively.

Kinetic parameters and determination of chlorophyll and carotenoid contents

Many stress factors, such as light, pH, temperature, and regulatory agents, can change the biochemical and physiologic properties of microalgae. To investigate the effects of stress factors, a FPBR was designed, and various concentrations of MC were added to the photobioreactor. After 9 days of cultivation, spectroscopic measurements were taken during the stationary growth phase, and the kinetic parameters of wastewater and MC are given in Table 1.

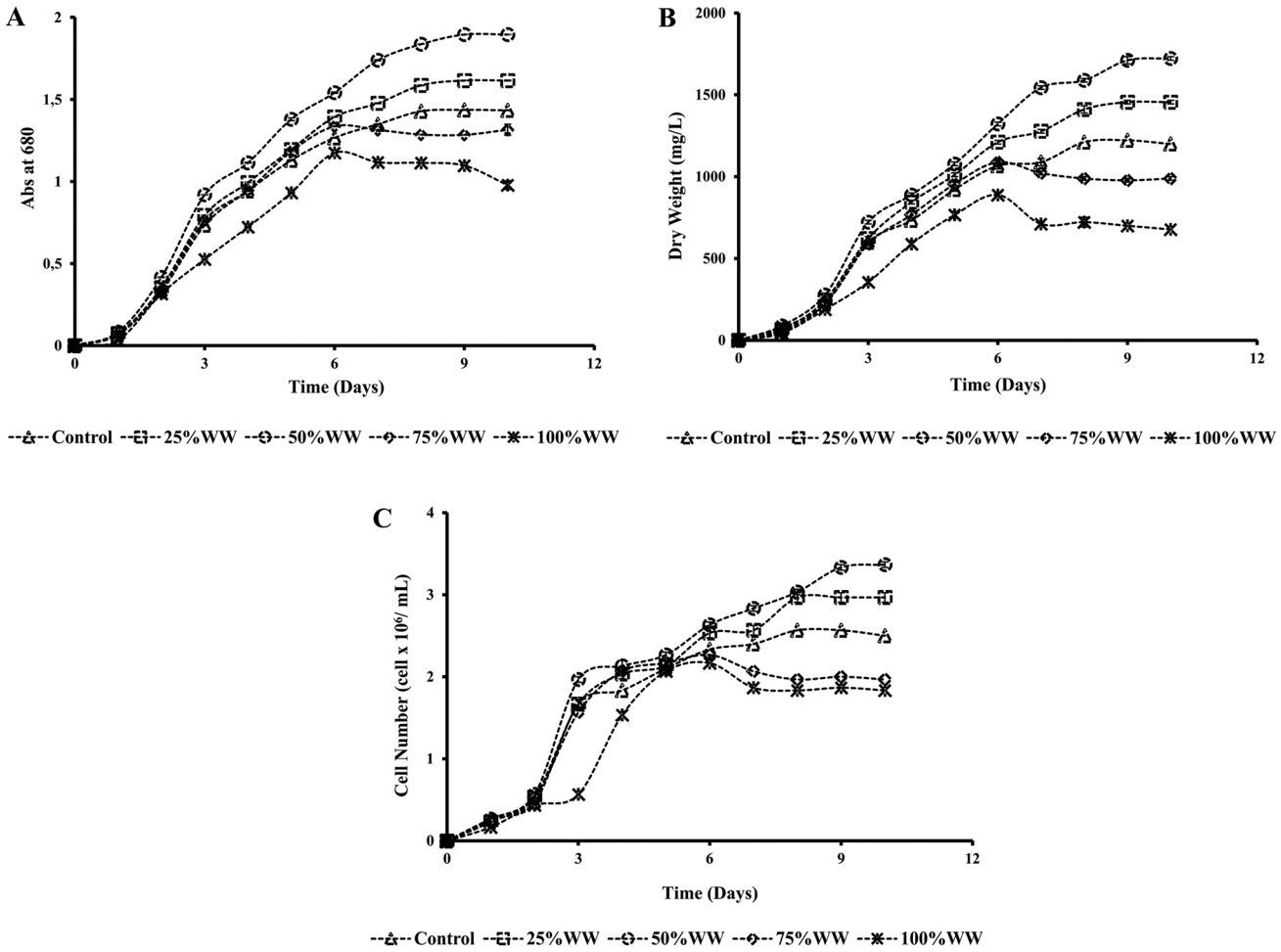

Fig. 1 Absorbance values (**A**), dry weight (mg/L) (**B**), and cell number (10^6/mL) (**C**) of *Scenedesmus acuminatus* CCALA 436 in wastewater

Among the wastewater samples, the culture containing 50% wastewater reached stationary phase with the maximum BP (0.190 ± 0.002 g/L/day) and SGR (5.39 ± 0.02 day^{-1}). The BP (0.162 ± 0.001 g/L/day) and SGR (5.27 ± 0.02 day^{-1}) in 25% wastewater were similar to those in 50% wastewater. The BP (0.109 ± 0.001 g/L/day) and SGR (4.85 ± 0.01 day^{-1}) were lower in the culture with 75% wastewater. Among the five groups, the medium containing 100% wastewater had the minimum BP (0.078 ± 0.002 g/L/day) and SGR (4.48 ± 0.07 day^{-1}). Inversely proportional to SGR, 50% wastewater samples showed the lowest T_d of 0.129 day. The T_ds in 25%, 75%, and 100% wastewater were 0.132, 0.143, and 0.155 day, respectively. Samples with 1 mg/L of MC had the highest BP (0.158 ± 0.001 g/L/day) and SGR (5.29 ± 0.02 day^{-1}). These results are slightly lower compared to those of the culture with 50% wastewater, which was an expected result. MC acted like a stress factor and slightly decreased the BP and SGR (Tung et al. 2018b). The main stress effects appeared in samples with 2.5, 5, and 10 mg/L of MC. The BP and SGR decreased while the amount of MC increased. Samples with 10 mg/L of MC had the most dramatic decline for the SGR (4.60 ± 0.06 day^{-1}) and BP (0.077 ± 0.002 g/L/day).

Previous studies have reported various kinetic parameters as functions of the stress conditions for microalgae in wastewater. The cultures with wastewater generally include microalgae and bacteria, which are called mixed systems. In a mixed system, microalgae and bacteria exhibit symbiosis. They maintain nutrients such as oxygen, carbon dioxide, and nitrogen for each other (Onay 2019; Driver et al. 2015). Moreover, some environmental factors affect photosynthetic synthetases. These enzyme systems cannot fully function, and microalgal growth decreases because of reactive oxygen species and free radicals that are produced. Their cell functions are impaired as the cells are exposed to stress (Ding et al. 2017; Wang et al. 2019). Furthermore, changes in antioxidant enzyme systems may also cause changes in the metabolic contents, such as carbohydrates, of microalgae.

According to the statistical analysis of SGR, the F value of the model was 76.63, which showed that the model was

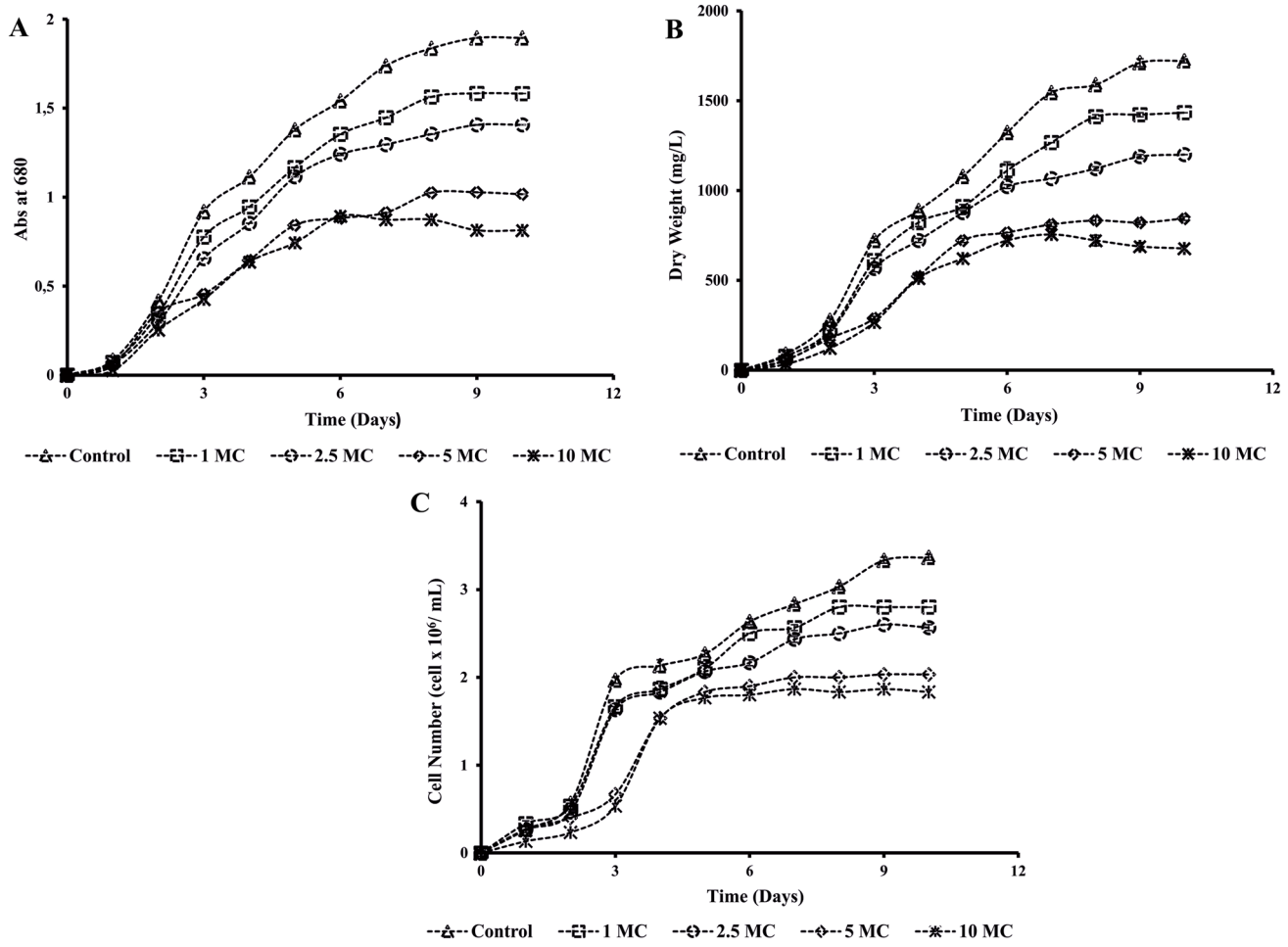

Fig. 2 Absorbance values (**A**), dry weight (mg/L) (**B**), and cell numbers (10^6/mL) (**C**) of *Scenedesmus acuminatus CCALA 436* in MC.

Table 1 Biomass concentrations (BC), biomass productivities (BP), specific growth rates (SGR), and doubling time (T_d) of microalgae in wastewater with MC. Different letters indicate a significant difference in the values of Prob > |*t*| less than 0.05

Contents	BC (g/L)	BP (g/L/d)	SGR (μ) (d^{-1})	Td (d)
Control	1.22 ± 0.01^{a}	0.136 ± 0.001^{a}	5.10 ± 0.01^{a}	0.136
25% WW	1.46 ± 0.01^{b}	0.162 ± 0.001^{b}	5.27 ± 0.02^{b}	0.132
50% WW	1.71 ± 0.02^{b}	0.190 ± 0.002^{b}	5.39 ± 0.02^{b}	0.129
75% WW	0.98 ± 0.01^{c}	0.109 ± 0.001^{c}	4.85 ± 0.01^{c}	0.143
100%WW	0.70 ± 0.02^{d}	0.078 ± 0.002^{d}	4.48 ± 0.07^{d}	0.155
MC1	1.42 ± 0.01^{b}	0.158 ± 0.001^{b}	5.29 ± 0.02^{b}	0.131
MC2.5	1.19 ± 0.01^{a}	0.132 ± 0.001^{a}	5.02 ± 0.01^{a}	0.138
MC5	0.82 ± 0.01^{e}	0.091 ± 0.001^{e}	4.69 ± 0.03^{e}	0.148
MC10	0.69 ± 0.02^{de}	0.077 ± 0.002^{de}	4.60 ± 0.06^{de}	0.151

significant. *p* values less than 0.05 indicated that the model terms were significant, while values greater than 0.10 indicated that the model terms are not significant. The predicted R^2 of 0.9358 was in reasonable agreement with the adjusted R^2 of 0.9588. Moreover, values of Prob > |*t*| less than 0.05 confirmed the difference between the two treatments. Compared with the control groups, all the MC concentrations we used can significantly affect BCs of the microalgae. The BP, SGR, and T_d were also affected because they are dependent on BC ($p < 0.05$). In addition, the wastewater concentrations affected the BC concentrations ($p < 0.05$).

Also, we studied the pigment concentrations of microalgae. Chlorophyll and carotenoid pigments play crucial roles as growth indicators in photosynthesis (Takyar et al. 2019). This phenomenon can indicate the impact of environmental stress on algae. In this study, we measured the chlorophyll-a (Chl-a) and chlorophyll-b (Chl-b) content of microalgae grown at various concentrations of municipal wastewater and MC, and the results are given in Fig. 3.

The culture in 50% wastewater had the highest Chl-a content (59.9 ± 3.5 mg/L). The results in 25% wastewater (53.2 ± 0.5 mg/L) were close to those in 50% wastewater. The culture with 100% wastewater had the lowest Chl-a content (33.9 ± 0.6 mg/L). Samples with MC content of 1 mg/L

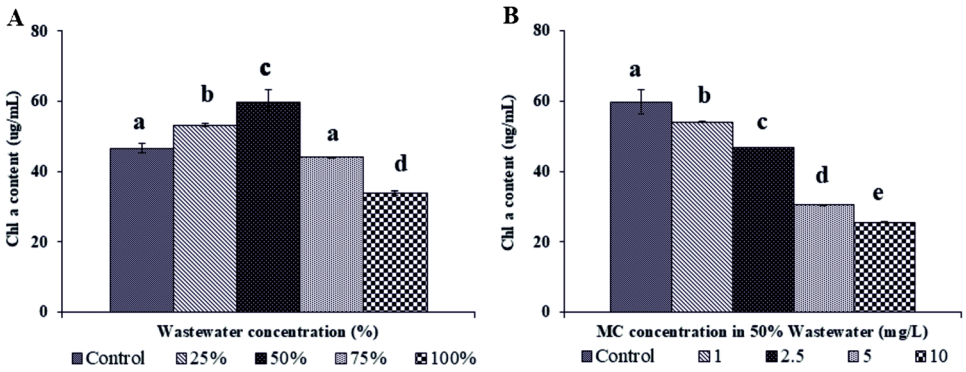

Fig. 3 *Scenedesmus acuminatus* CCALA 436 chlorophyll a (Chl-a) levels in wastewater (**A**) and MC (**B**). Different letters indicate a significant difference in the values of Prob > |*t*| less than 0.05

had notably higher Chl-a content (54.1 ± 1.4 mg/L). Then, microalgae cells showed dose effects across MC concentrations, and Chl-a content decreased fast while the concentration of MC (2.5, 5, and 10 mg/L) increased. Also, the Chl-b, PPC, SI, Chl-(a+b), and Chl-(a/b) contents of microalgae were calculated, and the results are given in Table 2.

The cultures in 75% and 100% wastewater had the highest carotenoid content, with 20.1 ± 0.4 mg/L and 19.8 ± 0.1 mg/L, respectively. The control group showed the lowest carotenoid content. This result was logical because the control group included enough nutrients for the growth of microalgae, and this situation did not have stress conditions. The stress index indirectly measures the carotenoid to Chl-a ratio and is negatively correlated with the carbon/nitrogen ratio of the cells (Heath and Packer 1968). The stress indexes of the cultures with 25% wastewater (0.94 ± 0.01), 50% wastewater (0.97 ± 0.01), and MC of 1 mg/L (1.09 ± 0.01) were very close to one another. Many studies have shown that environmental factors, such as temperature, CO_2 concentration, and pollutants, can change the pigment content by inhibiting the growth of microalgae and reducing the activity of photosynthetic pigment synthetases (Mohan et al. 2015; Mishra et al. 2008; Figueroa and Korbee 2005). This led to the enhancement of reactive oxygen species and cell stress. While microalgal cells were grown and doubled, chlorophyll content in the cells increased, and the carotenoid content and stress index decreased (Ding et al. 2017; Wang et al. 2019). When we evaluated Chl-a content in municipal wastewater by statistical analysis, the F value of the model was 64.82, indicating that the model was significant. The predicted R^2 of 0.8734 was reasonably consistent with the adjusted R^2 of 0.8980. For MC, the F value of the model was 179.10. Compared with control groups, MC can significantly inhibit Chl-a concentrations ($p < 0.05$). Moreover, the wastewater concentrations affected Chl-a concentrations ($p < 0.05$).

The carbohydrate and protein contents of *Scenedesmus acuminatus* CCALA 436

Enhancement of the sugar content by applying stress factors may be a logical option for bioethanol production (Cheng et al. 2017). We needed high BP to make this option viable. In this study, we tried to get high BP and sugar content via environmental stress conditions using various concentrations of wastewater and MC. After 9 days, the maximum sugar and protein contents were obtained during the late logarithmic phase. The results are shown in Fig. 4. According to this, the carbohydrate and protein contents increased proportionally over time. The carbohydrate contents changed with the concentrations of wastewater and MC. The cultures in 50, 75, and 100% wastewater had the highest sugar content, approximately 29%. The control group showed the lowest carbohydrate content, 21.7%. The carbohydrate content of

Table 2 Chl-a, Chl-b, carotenoid (PPC) contents, and stress index of *Scenedesmus acuminatus* CCALA 436

Contents	Chl-a (mg/L)	Chl-b (mg/L)	PPC (mg/L)	Stress index	Chl (a+b)	Chl (a/b)
Control	46.7 ± 1.4	13.4 ± 0.5	14.8 ± 0.1	0.94 ± 0.02	60.1 ± 1.7	3.5 ± 0.1
25% WW	53.2 ± 0.5	14.1± 1.1	16.6 ± 0.2	0.94 ± 0.01	67.3 ± 1.3	3.8 ± 0.3
50% WW	59.9 ± 3.5	18.4 ± 3.6	19.5 ± 0.5	0.97 ± 0.01	78.3 ± 6.0	3.5 ± 0.5
75% WW	44.1 ± 0.2	15.0 ± 0.7	20.1 ± 0.4	1.34 ± 0.03	59.0 ± 0.5	3.0 ± 0.2
100%WW	33.9 ± 0.6	14.2 ± 1.9	19.8 ± 0.1	1.68 ± 0.01	48.1 ± 1.4	2.5 ± 0.3
MC1	54.1 ± 1.4	18.1 ± 1.4	20.0 ± 0.1	1.09 ± 0.01	72.2 ± 0.3	3.0 ± 0.1
MC2.5	46.8 ± 0.1	18.0 ± 0.1	21.4 ± 0.1	1.33 ± 0.01	64.9 ± 0.1	2.6 ± 0.1
MC5	30.6 ± 0.2	16.9 ± 0.4	21.8 ± 0.1	1.98 ± 0.01	47.4 ± 0.3	1.8 ± 0.1
MC10	25.7 ± 0.1	14.9 ± 0.1	21.8 ± 0.1	2.34 ± 0.01	40.6 ± 0.1	1.7 ± 0.1

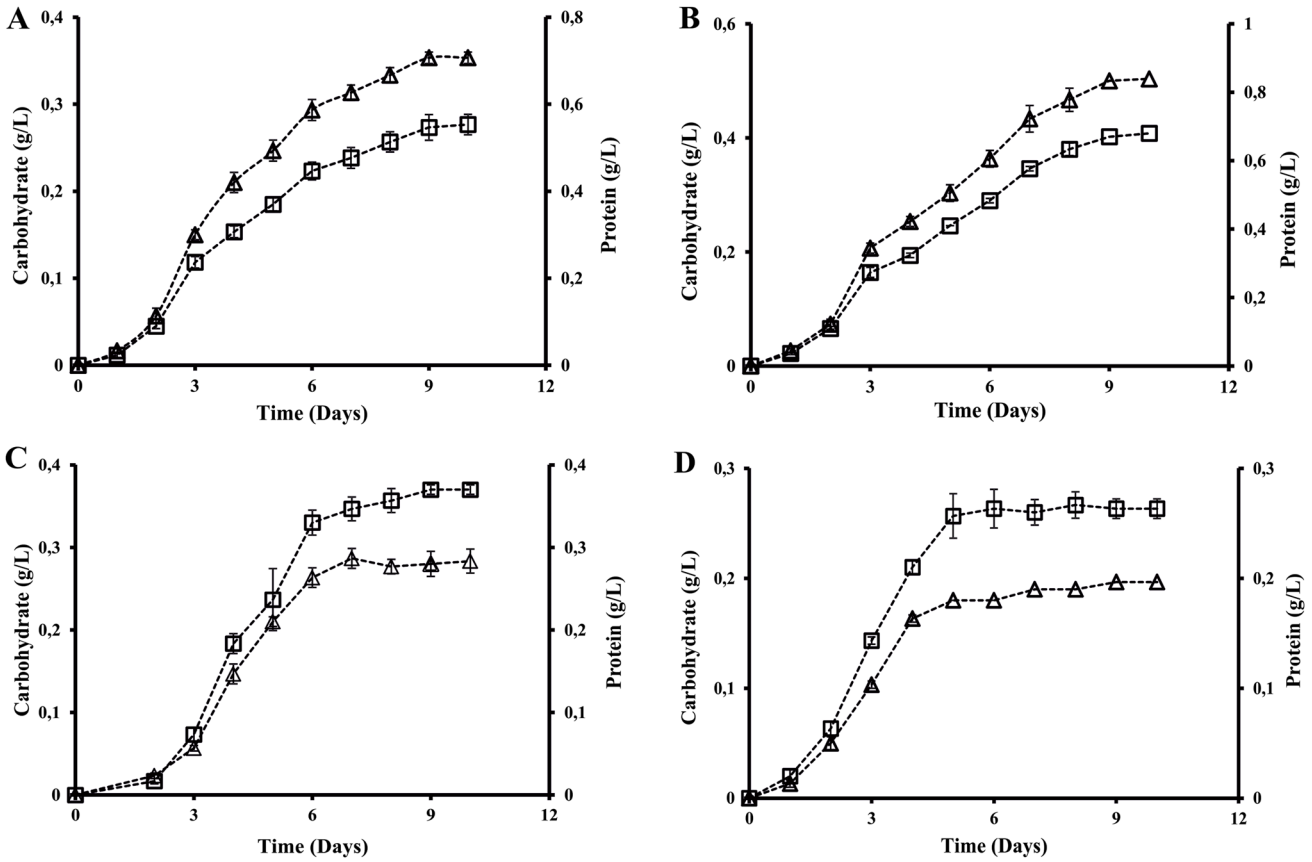

Fig. 4 Carbohydrate and protein contents of microalgae in (**A**) 25, (**B**) 50, (**C**) 75, and (**D**) 100% wastewater. The triangle represents carbohydrate concentration, while the square symbolizes protein concentration

the culture in 25% wastewater was 24.3%. Moreover, the protein content was nearly 39% in all wastewater concentrations. Therefore, the wastewater concentrations affected carbohydrate contents, but protein contents did not change.

MC had a positive effect on the carbohydrate content in microalgae. The carbohydrate content of 1 mgL^{-1} MC samples (32.1 ± 1.2%) was 10% higher than that of the control (21.7 ± 1.0%). When the amount of MC increased, the carbohydrate amount did not increase further and was nearly stable. Carbohydrate contents in samples with 2.5, 5, and 10 mg/L of MC were 39.2 ± 1.7, 32.4 ± 1.2, and 32.0 ± 1.3%, respectively. The protein contents in various concentrations of wastewater were similar. MC of 1 mg/L resulted in a lower protein concentration (36.8 ± 1.2%) compared to the control group (38.9 ± 1.6%). The carbohydrate and protein contents for the different MC concentrations are given in Fig. 5.

The carbohydrate contents increased up to 29.2 and 32.1% for 75 and 100% wastewater, respectively, from 21.7% at 50% wastewater and 1 mg/L MC. High levels of wastewater reduced photosynthetic capacity due to nutrient deprivation and increased the carbohydrate concentration (Onay 2019). Likewise, MC of 1 mg/L led to stress and affected carbohydrate metabolism positively by approximately 3%. The effects of CaO were also studied to obtain a higher carbohydrate content. Various concentrations (0.02–0.1%) of CaO were added to algal biomass to investigate the pretreatment effect. The results are shown in Fig. 6.

The CaO content of 0.08% exhibited the greatest concentration of carbohydrates, 0.64 ± 0.01 g/L. The control group (0% CaO) had the lowest amount of carbohydrates (0.46 ± 0.01 g/L). Compared with the control group, carbohydrate concentrations were notably promoted by the addition of CaO ($p < 0.05$). It is likely that CaO harshly disrupted algal cells and facilitated carbohydrate hydrolysis and saccharification (Khan et al. 2017).

Stress effects of wastewater and mepiquat chloride on *Scenedesmus acuminatus* CCALA 436

Microalgae produce a high amount of reactive oxygen species (ROS) under stress, like extreme environmental conditions. On the other hand, the metabolic systems of microalgae resist these ROS activities and form defense systems using antioxidant enzymes (Tripathi et al. 2006). One of them, SOD, catalyzes the conversion of the superoxide anion

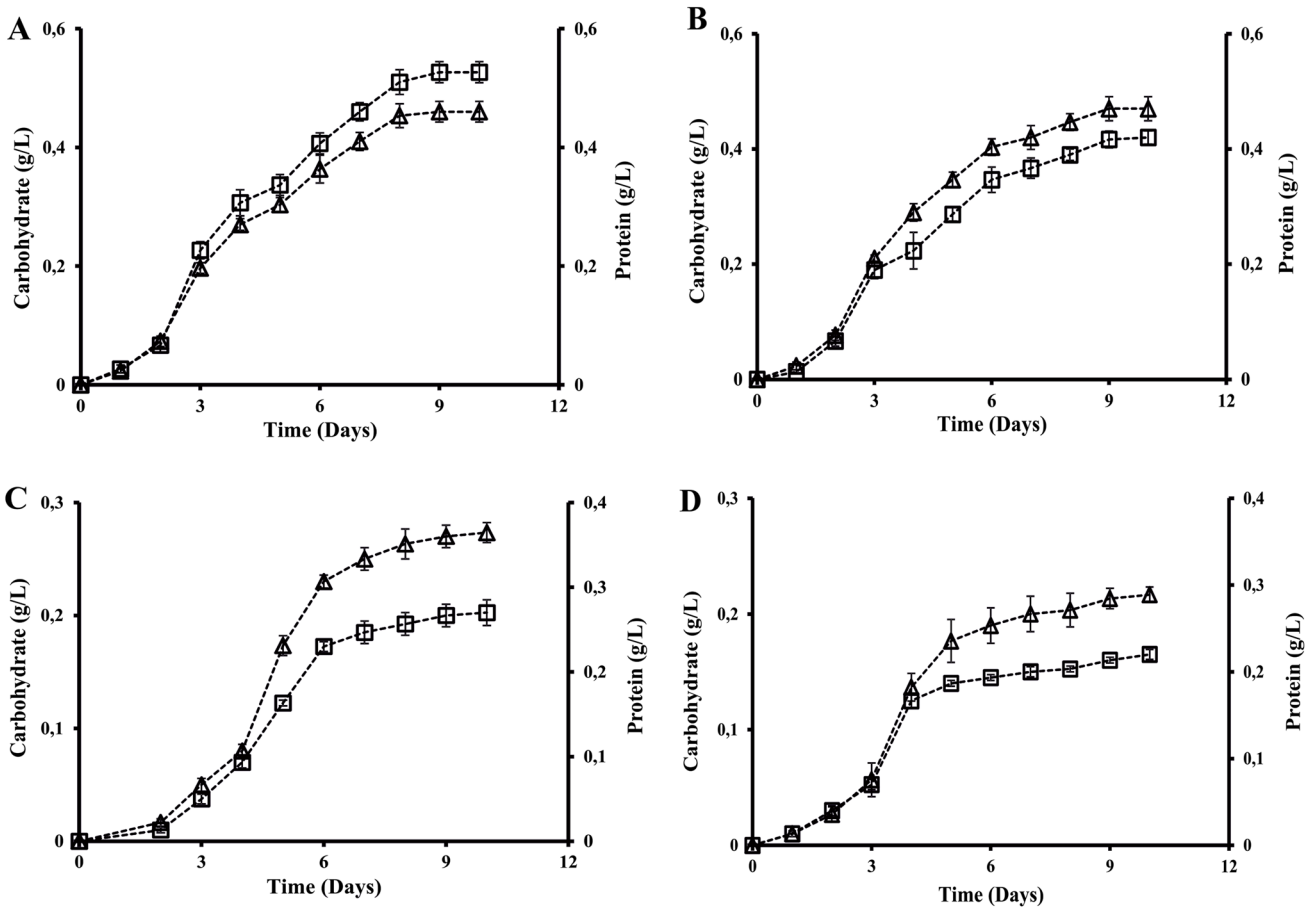

Fig. 5 Carbohydrate and protein contents of microalgae in samples with (**A**) 1, (**B**) 2.5, (**C**) 5, and (**D**) 10 mg/L MC. The triangle represents carbohydrate concentration, while the square symbolizes protein concentration

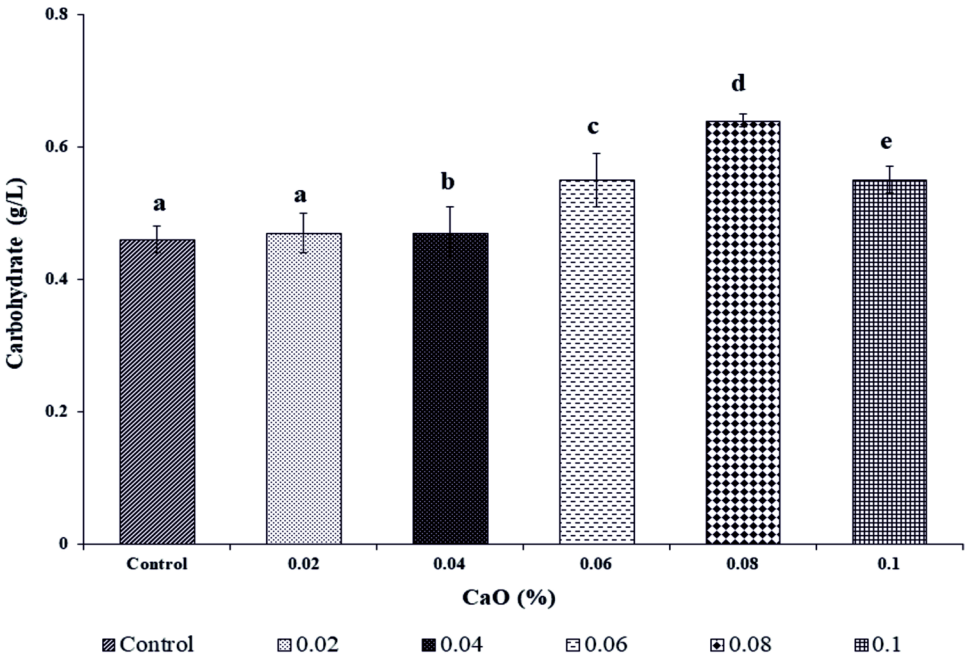

Fig. 6 Carbohydrate concentrations (g/L) of *Scenedesmus acuminatus* CCALA 436 pretreated with various concentrations of calcium oxide (CaO). Different letters indicate significant differences in the values of Prob > |*t*| less than 0.05

(O_2^-) to H_2O_2 and oxygen. Hydrogen peroxide is metabolized to oxygen and water by CAT to prevent its damaging effects. Like CAT, MDA is the result of membrane lipid peroxidation, and the MDA level is a crucial indicator of ROS activity (Cheng et al. 2018; Wan et al. 2014). GSH is another antioxidant molecule and is converted into GSSG to alleviate free radical damage. This is important to counteract ROS activity (Hong et al. 2008). High amounts of ROS induce damage to the membranes of microalgae and diminish their growth. In this study, we examined antioxidant enzymes and molecules in the cultures with wastewater and MC. The results are shown in Fig. 7.

The SOD activity increased initially and reached a peak of 45.33 ± 2.03 U/mL in the culture with 50% wastewater and then decreased in the cultures with 75% wastewater (26.0 ± 1.1.15 U/mL) and 100% wastewater (22.67 ± 1.20 U/mL). Similarly, when the wastewater concentration was increased to 50%, CAT activity reached its maximum (0.71 ± 0.03 U/mL). The culture in 100% wastewater showed the lowest CAT activity with 0.25 ± 0.02 U/mL. When *Scenedesmus acuminatus* CCALA 436 was subjected to wastewater with a 50% concentration, it accelerated antioxidant enzyme activities to discard ROS and decrease oxidative damage. Thus, the SOD and CAT activities increased. When the wastewater concentration is more than 50%, the antioxidant enzyme system is disrupted and leads to oxidative damage. Therefore, SOD and CAT activities were reduced due to the loss of defense mechanisms. Unlike SOD and CAT, MDA content initially decreased to 1.83 ± 0.19 nmol/mL in 50% of the wastewater and then increased to 2.30 ± 0.12 nmol/mL in the culture with 100% wastewater. This situation probably occurred from the enhancement of SOD and CAT activities in the culture with 50% wastewater. This phenomenon reduced ROS activity and diminished MDA content. Conversely, GSH content first increased to 2.73 ± 0.09 nmol/mL in the culture with 50% wastewater, then diminished to 2.33± 0.09 nmol/mL in 100% wastewater. The results showed that the formation of GSH was not intensely affected by various concentrations of wastewater. Next, we determined the effects of MC on antioxidant enzymes.

Tung et al. (2018a) studied MC for leaf photosynthesis and carbohydrate metabolism in cotton, and they showed that MC did not affect leaf photosynthesis and carbohydrate metabolism enzymes. In our study, the SOD activity had the highest value with 66.67 ± 2.60 U/mL for MC of 2.5 mg/L in the culture with 50% wastewater. The lowest SOD activity was 35.00 ± 2.31 U/mL for 10 mg/L MC. The CAT activity initially increased to 1.62 ± 0.13 U/mL for 1 mg/L

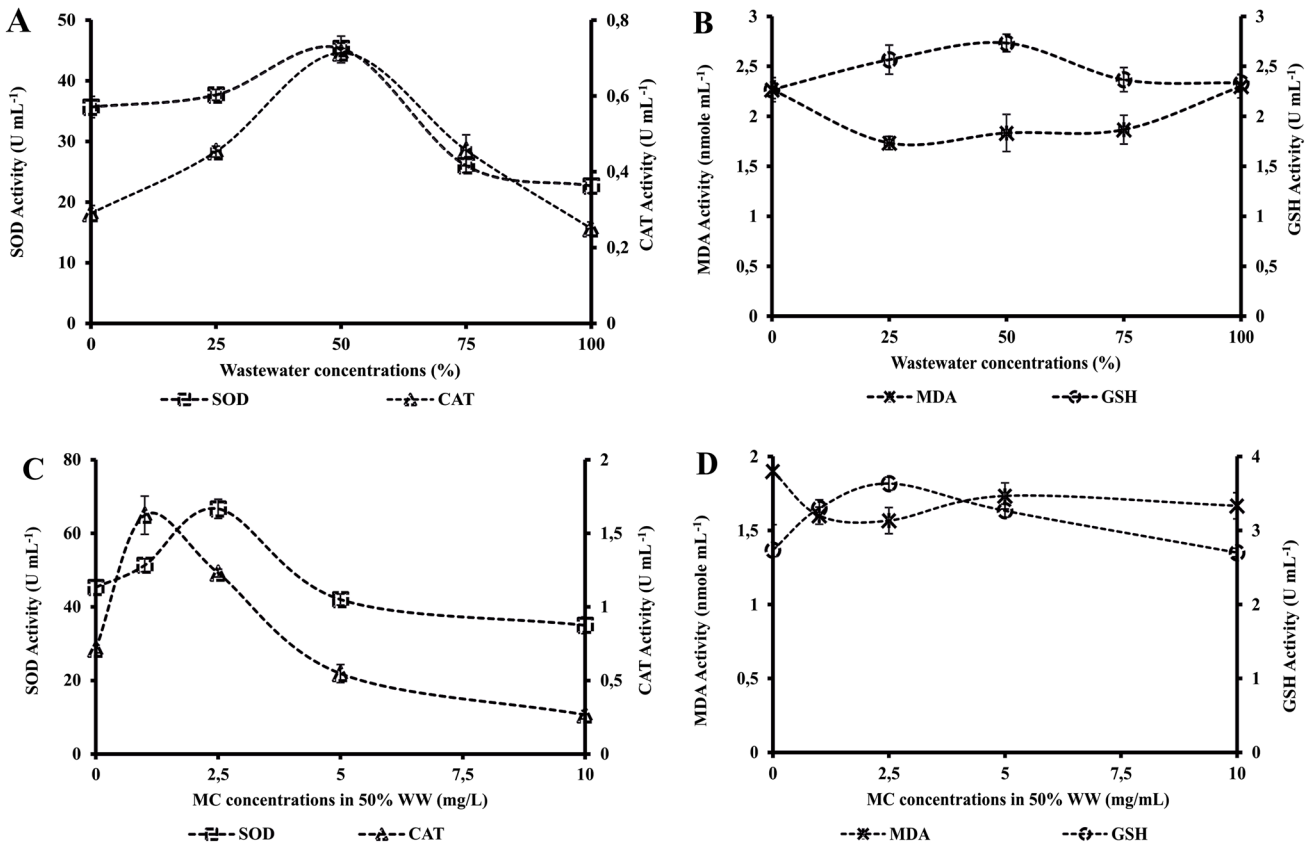

Fig. 7 Antioxidant activities of *Scenedesmus acuminatus* CCALA 436 in wastewater (**A**, **B**) and MC (**C**, **D**)

Table 3 Metabolic compositions of *Scenedesmus acuminatus* CCALA 436. Different letters indicate significant differences in the values of Prob > |*t*| less than 0.05

Contents	Carbohydrate (dwt %)	Protein (dwt %)	Lipid (dwt %)	BC (g/L)
Control	21.7 ± 1.0^{a}	38.9 ± 1.6^{a}	N.A.	1.22 ± 0.01^{a}
25% WW	24.3 ± 0.6^{a}	38.1 ± 1.8^{a}	N.A.	1.46 ± 0.01^{b}
50% WW	29.2 ± 1.4^{b}	39.5 ± 0.4^{b}	N.A.	1.71 ± 0.02^{b}
75% WW	28.7 ± 1.5^{b}	37.4 ± 1.0^{c}	N.A.	0.98 ± 0.01^{c}
100%WW	29.0 ± 0.5^{bc}	38.9 ± 1.6^{c}	N.A.	0.70 ± 0.02^{d}
MC1	32.1 ± 1.2^{bd}	36.8 ± 1.2^{cd}	N.A	1.42 ± 0.01^{b}
MC2.5	39.2 ± 1.7^{e}	35.0 ± 1.0^{d}	N.A.	1.19 ± 0.01^{a}
MC5	32.4 ± 1.2^{bd}	32.0 ± 1.7^{e}	N.A.	0.82 ± 0.01^{e}
MC10	32.0 ± 1.3^{bd}	32.5 ± 1.3^{e}	N.A.	0.69 ± 0.02^{de}

MC. Next, it decreased to 0.27 ± 0.03 U/mL for 10 mg/L MC. The MDA content had a minimum value of 1.57 ± 0.09 nmol/mL for 2.5 mg/L MC in the culture with 50% wastewater. Later, MDA content increased to 1.67± 0.09 nmol/mL for 10 mg/L MC. The GSH content varied slightly with MC content, ranging from 2.70 to 3.63 nmol/mL. These results showed that the cultures in 50% wastewater and MC generated stress factors for microalgal cells and changed the concentration of antioxidant enzymes. Also, they affected carbohydrate metabolism by increasing its content.

Bioethanol production

According to Table 3, *Scenedesmus acuminatus* CCALA 436 grown in culture with 50% wastewater and 2.5 mg/L MC had the maximum carbohydrate content (39.2 ± 1.7%) and SGR (5.02 ± 0.01 day^{-1}).

In addition, the sugar content was 44.7 ± 1.5% with the concentration of 50% wastewater, 2.5 mg/L MC, and 0.08% CaO. Therefore, this composition was chosen for bioethanol production, and the results are given in Table 4. For hydrolysis, we used H_2SO_4 (0.5 M), and its percent yield was nearly 86%. When *Scenedesmus acuminatus* CCALA 436 grown in the optimal wastewater composition was compared with the control, it had a higher bioethanol content (20.32 g/L and 0.20 g/g of biomass). Carbohydrate concentrations were noticeably promoted by increased MC concentrations compared with the control groups ($p < 0.05$), while the carbohydrate concentrations were slightly affected by wastewater concentrations.

Bioethanol values of the control group were 9.20 g/L and 0.09 g/g of biomass. In comparison to the control group, the amount of bioethanol produced by *Scenedesmus acuminatus* CCALA 436 was roughly 50% higher (20.32 g/L and 0.20 g/g of biomass). Based on the findings of the current research, it is possible to conclude that *Scenedesmus acuminatus* CCALA 436 cultivated in the culture in 50% wastewater with 2.5 mg/L MC and 0.08% CaO can generate significant quantities of bioethanol. Also, according to our results, while antioxidant enzyme activities change with stress factors, the carotenoid and carbohydrate amounts increase. These results show that carotenoid can be produced in addition to bioethanol, which can be integrated into the biorefinery process. Overall, biofuel production using wastewater and microalgae can be an attractive because it has a high safety factor, and algae is not needed as a food source or otherwise seen as a high-value product.

Conclusion

Zero waste is currently one of the most popular topics. Our research revealed that *Scenedesmus acuminatus* CCALA 436 grown using the culture with 50% wastewater, 2.5 mg/L MC, and 0.08% CaO produced significantly more ethanol than the control organisms that were grown in a PBR. The maximum amount of ethanol produced was 0.20 g per gram of microalgal biomass. In addition, compared to the control group, we were able to achieve a higher biomass concentration (1.71 0.02 g/L) as well as a higher carbohydrate content (44.7 ± 1.5%). Also, the concentrations of superoxide dismutase, catalase activity, glutathione, and malondialdehyde changed with the various concentrations of mepiquat chloride (MC) and wastewater. In conclusion, the use of 50% wastewater in bioethanol production reduces operating costs such as water and cultivation costs. *Scenedesmus acuminatus* CCALA 436 can be used for ethanol and carotenoid production and the treatment of wastewater with improvement via MC and CaO.

Table 4 Sugar and ethanol contents in the control group and culture with 50% wastewater and 2.5 mg/L MC

Samples	Biomass loaded (g/L)	Sugar content (g/L)	Ethanol content (g/L)	Ethanol content (g/g biomass)
Control	100	21.7	9.20	0.09
MC2.5 and 50% WW	100	39.2	17.91	0.18
MC2.5, 50%WW, and 0.08% CaO	100	44.7	20.32	0.20

Acknowledgements The authors would like to thank Van-YYU-Department of Environmental Engineering for technical support.

Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Author contribution Both authors contributed to all experiments, such as determining the carbohydrate, protein, and bioethanol concentrations. MO interpreted all the data in the manuscript and supervised this work. EA wrote the first draft which was further edited and reviewed by MO. Both authors approved the final paper.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

Anderson ME (1985) Determination of glutathione and glutathione disulfide in biological samples. Meth Enzymol 113:548–555. https://doi.org/10.1016/S0076-6879(85)13073-9

Ansari FA, Guldhe A, Gupta SK, Rawat I, Bux F (2021) Improving the feasibility of aquaculture feed by using microalgae. Environ Sci Pollut Res 28:43234–43257. https://doi.org/10.1007/s11356-021-14989-x

Ashokkumar V, Chen WH, Ngamcharussrivichai C, Agila E, Ani FN (2019) Potential of sustainable bioenergy production from *Synechocystis sp.* cultivated in wastewater at large scale-a low cost biorefinery approach. Energy Convers Manage 186:188–199. https://doi.org/10.1016/j.enconman.2019.02.056

Brennan L, Owende P (2010) Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sustain Energy Rev 14:557–577. https://doi.org/10.1016/j.rser.2009.10.009

Cheng J, Ye Q, Yang ZB, Yang WJ, Zhou JH, Cen KF (2017) Microstructure and antioxidative capacity of the microalgae mutant *Chlorella PY-ZU1* during tilmicosin removal from wastewater under 15% CO_2. J Hazard Mater 324:414–419. https://doi.org/10.1016/j.jhazmat.2016.11.006

Cheng J, Ye Q, Li K, Liu J, Zhou J (2018) Removing ethinylestradiol from wastewater by microalgae mutant *Chlorella PY-ZU1* with CO2 fixation. Bioresour Technol 249:284–289. https://doi.org/10.1016/j.biortech.2017.10.036

Dadi D, Beyene A, Simoens K, Soares M, Demeke MM, Thevelein JM, Bernaerts K (2018) Valorization of coffee byproducts for bioethanol production using lignocellulosic yeast fermentation and pervaporation. Int J Environ Sci Technol 15:821–832. https://doi.org/10.1007/s13762-017-1440-x

Daroch M, Geng S, Wang G (2013) Recent advances in liquid biofuel production from algal feedstocks. Appl Energy 102:1371–1381. https://doi.org/10.1016/j.apenergy.2012.07.031

Devi ND, Sun X, Ding L, Goud VV, Hu B (2022) Mixotrophic growth regime of novel strain *Scenedesmus sp*. DDVG I in municipal wastewater for concomitant bioremediation and valorization of biomass. J Clean Prod 365. https://doi.org/10.1016/j.jclepro.2022.132834

Ding T, Lin K, Yang B, Yang M, Li J, Li W, Gan J (2017) Biodegradation of naproxen by freshwater algae *cymbella sp*. and *Scenedesmus quadricauda* and the comparative toxicity. Bioresour Technol 238:164–173. https://doi.org/10.1016/j.biortech.2017.04.018

Driver T, Bajhaiya AK, Allwood JW, Goodacre R, Pittman JK Dean, AP (2015) Metabolic responses of eukaryotic microalgae to environmental stress limit the ability of FT IR spectroscopy for species identification. Algal Res 11:148-155. https://doi.org/10.1016/j.algal.2015.06.009.

Fetyan NAH, El-sayed AEB, Ibrahim FM, Attia YA (2022) Bioethanol production from defatted biomass of *Nannochloropsis oculata* microalgae grown under mixotrophic conditions. Environ Sci Pollut Res 29:2588–2597. https://doi.org/10.1007/s11356-021-15758-6

Figueroa L, Korbee N (2005) Effect of light quality on the accumulation of photosynthetic pigments, proteins and mycosporine-like amino acids in the red alga *Porphyra leucosticta* (Bangiales, Rhodophyta). J Photochem Photobiol B 80:71–78. https://doi.org/10.1016/j.jphotobiol.2005.03.002

Gorham PR, McLachlan J, Hammer UT, Kim WK (1964) Isolation and culture of toxic strains of Anabaena flos-aquae (Lyngb.) de Bréb, Internationale Vereinigung für theoretische und angewandte Limnologie. Verhandlungen 15(2):796–804. https://doi.org/10.1080/03680770.1962.11895606

Hadizadeh Z, Mehrgan MS (2020) The potential use of stickwater from a kilka fishmeal plant in Dunaliella salina cultivation. Environ Sci Pollut Res 27:2144–2154. https://doi.org/10.1007/s11356-019-06926-w

Hamed SM, Selim S, Klöck G, AbdElgawad H (2017) Sensitivity of two green microalgae to copper stress: growth, oxidative and antioxidants analyses. Ecotox Environ Safe 144:19–25. https://doi.org/10.1016/j.ecoenv.2017.05.048

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:189–198. https://doi.org/10.1016/0003-9861(68)90654-1

Hong Y, Hu HY, Xie X, Li FM (2008) Responses of enzymatic antioxidants and non-enzymatic antioxidants in the cyanobacterium *Microcystis aeruginosa* to the allelochemical ethyl 2-methyl acetoacetate (EMA) isolated from reed (*Phragmites communis*). J Plant Physiol 165:1264–1273. https://doi.org/10.1016/j.jplph.2007.10.007

Hung C, Chen C, Huang C, Shiung S, Yang Y (2022) Performance and bacterial community dynamics of lignin-based biochar-coupled calcium peroxide pretreatment of waste-activated sludge for the removal of 4-nonylphenol. Bioresour Technol 354:127166. https://doi.org/10.1016/j.biortech.2022.127166.

Khan MI, Lee MG, Shin JH, Kim JD (2017) Pretreatment optimization of the biomass of *Microcystis aeruginosa* for efficient bioethanol production. AMB Express 7(1):7–19. https://doi.org/10.1186/s13568-016-0320-y

Koistinen J, Sjöblom M, Spilling K (1980) Determining inorganic and organic phosphorus, in: K. Spilling, Biofuels from algae, methods and protocols, methods in molecular biology, Springer Science-Business Media, New York, 2020, pp 87-94. https://doi.org/10.1007/7651_2017_104

Kumar AK, Sharma S, Parikh BS, Dixit G, Gupta S, Divecha JM (2019) Cultivation of *Ascochloris sp . ADW007*-enriched microalga in raw dairy wastewater for enhanced biomass and lipid productivity. Int J Environ Sci Technol 16:943–954. https://doi.org/10.1007/s13762-018-1712-0

Maity S, Mallick N (2022) Trends and advances in sustainable bioethanol production by marine microalgae: a critical review. J Clean Prod 345:131153. https://doi.org/10.1016/j.jclepro.2022.131153

Miranda JR, Passarinho PC, Gouveia L (2012) Pre-treatment optimization of *Scenedesmus obliquus* microalga for bioethanol

production. Bioresour Technol 104:342–348. https://doi.org/10.1016/j.biortech.2011.10.059
Mishra V, Srivastava G, Mohan S, Abraham G (2008) Growth, photosynthetic pigments and photosynthetic activity during seedling stage of cowpea (*Vigna unguiculata*) in response to UV-B and dimethoate. Pestic Biochem Physiol 92:30–37. https://doi.org/10.1016/j.pestbp.2008.05.003
Mohan SV, Rohit MV, Chiranjeevi P, Chandra R, Navaneeth B (2015) Heterotrophic microalgae cultivation to synergize biodiesel production with waste remediation: progress and perspectives. Bioresour Technol 184:169–178. https://doi.org/10.1016/j.biortech.2014.10.056
Nowicka A, Zieliński M, Dębowski M (2020) Microwave support of the alcoholic fermentation process of cyanobacteria *Arthrospira platensis*. Environ Sci Pollut Res 27:118–124. https://doi.org/10.1007/s11356-019-05427-0
Onay M, Sonmez C, Oktem HA, Yucel AM (2014) Thermo-resistant green microalgae for effective biodiesel production: isolation and characterization of unialgal species from geothermal flora of Central Anatolia. Bioresour Technol 169:62–71. https://doi.org/10.1016/j.biortech.2014.06.078
Onay M (2018) Bioethanol production from *Nannochloropsis gaditana* in municipal wastewater. Energy Procedia 153:253–257. https://doi.org/10.1016/j.egypro.2018.10.032
Onay M (2019) Bioethanol production via different saccharification strategies from *H. tetrachotoma ME03* grown at various concentrations of municipal wastewater in a flat photobioreactor. Fuel 239:1315–1323. https://doi.org/10.1016/j.fuel.2018.11.126
Onay M (2020a) Enhancing carbohydrate productivity from *Nannochloropsis gaditana* for bio-butanol production. Energy Reports 6:63–67. https://doi.org/10.1016/j.egyr.2019.08.019
Onay M (2020b) The effects of indole-3-acetic acid and hydrogen peroxide on *Chlorella zofingiensis CCALA 944* for bio-butanol production. Fuel 273:117795. https://doi.org/10.1016/j.fuel.2020.117795
Onay M (2022) Sequential modelling for carbohydrate and bioethanol production from *Chlorella saccharophila* CCALA 258: a complementary experimental and theoretical approach for microalgal bioethanol production. Environ Sci Pollut Res 29:14316–14332. https://doi.org/10.1007/s11356-021-16831-w
Phwan CK, Ong HC, Chen WH, Ling TC, Ng EP, Show PL (2018) Overview: comparison of pretreatment technologies and fermentation processes of bioethanol from microalgae. Energy Convers Manag 173:81–94. https://doi.org/10.1016/j.enconman.2018.07.054
Rizza SL, Sanz Smachetti ME, Do Nascimento M, Salerno GL, Curatti L (2017) Bioprospecting for native microalgae as an alternative source of sugars for the production of bioethanol. Algal Res 22:140–147. https://doi.org/10.1016/j.algal.2016.12.021
RoyChowdhury A, Abraham J, Abimbola T et al (2019) Assessing oil content of microalgae grown in industrial energetic-laden wastewater. Environ Process 6:969–983. https://doi.org/10.1007/s40710-019-00396-5
Staniszewski M, Kujawski W, Lewandowska M (2007) Ethanol production from whey in bioreactor with co-immobilized enzyme and yeast cells followed by pervaporative recovery of product—kinetic model predictions. J Food Eng 82:618–625. https://doi.org/10.1016/j.jfoodeng.2007.03.031
Takyar MBT, Khajavi SH, Safari R (2019) Evaluation of antioxidant properties of *Chlorella vulgaris* and *Spirulina platensis* and their application in order to extend the shelf life of rainbow trout (*Oncorhynchus mykiss*) fillets during refrigerated storage. LWT 100:244–249. https://doi.org/10.1016/j.lwt.2018.10.079
Taylor P, Rimeika M, Zalieckien E, Šaulys V (2012) Determination of organic matter by UV absorption in the ground water. J Environ Eng Landsc Manag 20(1):163–167. https://doi.org/10.3846/16486897.2012.674039
Tripathi BN, Mehta SK, Amar A, Gaur JP (2006) Oxidative stress in *Scenedesmus sp.* during short- and long-term exposure to Cu^{2+} and Zn^{2+}. Chemosphere 62:538–544. https://doi.org/10.1016/j.chemosphere.2005.06.031
Tung SA, Huang Y, Ali S, Hafeez A, Shah AN, Song X, Ma X, Luo D, Yang G (2018a) Mepiquat chloride application does not favor leaf photosynthesis and carbohydrate metabolism as well as lint yield in late-planted cotton at high plant density. Field Crops Res 221:108–118. https://doi.org/10.1016/j.fcr.2017.09.032
Tung SA, Huang Y, Hafeez A, Ali S, Khan A, Souliyanonh B, Song X, Liu A, Guozheng Yang G (2018b) Mepiquat chloride effects on cotton yield and biomass accumulation under late sowing and high density. Field Crops Res 215:59–65. https://doi.org/10.1016/j.fcr.2017.09.032
Wan J, Guo P, Zhang S (2014) Response of the cyanobacterium Microcystis flos-aquae to levofloxacin. Environ Sci Pollut Res Int 21:3858–3865. https://doi.org/10.1007/s11356-013-2340-3
Wang L, Li Y, Chen P, Min M, Chen Y, Zhu J, Ruan RR (2010) Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae *Chlorella sp*. Bioresour Technol 10:2623–2628. https://doi.org/10.1016/j.biortech.2009.10.062
Wang X, Miao J, Pan L, Li Y, Lin Y, Wu J (2019) Toxicity effects of p-choroaniline on the growth, photosynthesis, respiration capacity and antioxidant enzyme activities of a diatom, *Phaeodactylum tricornutu*. Ecotoxicol Environ Saf 169:654–661. https://doi.org/10.1016/j.ecoenv.2018.11.015
Xin X, She Y, Hong J (2021) Insights into microbial interaction profiles contributing to volatile fatty acids production via acidogenic fermentation of waste activated sludge assisted by calcium oxide pretreatment. Bioresour Technol 320:124287. https://doi.org/10.1016/j.biortech.2020.124287
Yuan C, Zhen-yu L, Li H, Tambel L, Xiang Z, Yuan C et al (2021) Effects of plant density and mepiquat chloride application on cotton boll setting in wheat – cotton double cropping system. J Integr Agric 20:2372–2381. https://doi.org/10.1016/S2095-3119(20)63286-5
Zhao G, Chen X, Wang L, Zhou S, Feng H, Chen WN, Lau R (2013) Ultrasound assisted extraction of carbohydrates from microalgae as feedstock for yeast fermentation. Bioresour Technol 128:337–344. https://doi.org/10.1016/j.biortech.2012.10.038
Zouboulis AI, Gkotsis PK, Zamboulis DX, Mitrakas MG (2017) Application of powdered activated carbon (PAC) for membrane fouling control in a pilot-scale MBR system. Water Sci and Technol 75(10):2350–2357. https://doi.org/10.2166/wst.2017.108