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Treatment of wastewater from Azilal (Morocco) using microalgae: comparing indoor and outdoor cultures and biomass harvesting by coagulation–flocculation

Radouane El Amri[a,*], Reda Elkacmi[a], Belkacem Benadda[b], Mariem Kacem[c], Aziz Hasib[a], Otmane Boudouch[a]

[a]*Environmental and Agro-Industrial Process Team, Department of Chemistry and Environment, Faculty of Sciences and Technology, University Sultan Moulay Slimane, BP 523, Beni-Mellal, Morocco, emails: radouane.el.amri@gmail.com (R. El Amri), r.elkacmi@usms.ma (R. Elkacmi), azhasib@yahoo.fr (A. Hasib), oboudouch@gmail.com (O. Boudouch)*
[b]*Laboratory of Waste, Water, Environment, Pollution (DEEP), INSA, France, email: belkacem.benadda@insa-lyon.fr*
[c]*Laboratory of Tribology and System Dynamics (LTDS), ENISE Saint-Etienne, University Lyon, France, email: mariem.kacem@enise.fr*

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ABSTRACT

In this study, native microalgae cultivated in the batch mode were used to purify secondary municipal wastewater (MW) from Azilal (Morocco) at different concentrations; the treatment of the MW under laboratory conditions and natural conditions was compared. The biomass produced was harvested by coagulation–flocculation–sedimentation. The results showed that the maximum biomass was obtained in outdoor cultures by 1.11 vs. 0.79 g·L^{-1} in indoor cultures. Ammonium removal reached 99.8% in indoor cultures and 97.5% in outdoor cultures. The highest chemical oxygen demand removal percentage was 60.3% in indoor cultures and 68.9% in outdoor cultures. Regarding biomass harvesting, 140 mg·L^{-1} of ferric chloride resulted in 97.4% of the biomass recovered in 20 min of sedimentation and improved the final effluent quality. Finally, these native microalgae showed a promising alternative for MW treatment combined with valuable biomass production.

Keywords: Ammonium removal; Chemical oxygen demand removal; Coagulation–flocculation; Municipal wastewater; Native microalgae

1. Introduction

Water pollution has a significant danger to society and the environment. Industrial, domestic and agricultural discharges are the main sources of water pollution; they are overloaded with hazardous substances (heavy metals, organic matter, and nutrients). The discharge of these effluents into natural ecosystems without any treatment contributes to ecosystem destruction, affects public health, and alters water supplies [1,2]. For this reason, wastewater treatment before discharge has become imperative to ensure the protection of the environment and the preservation of water resources.

Despite the efforts made in terms of wastewater treatment in Morocco, it is confronted with a significant delay due to population growth and economic development. The techniques currently used in wastewater treatment are mainly based on conventional biological treatment, including the following treatment steps: pretreatment, primary treatment, secondary treatment (biological treatment), and tertiary treatment. Tertiary treatment can be combined with other physical or chemical processes such as reverse osmosis, ultrafiltration, adsorption on activated carbon, disinfection by chlorine, ozone or UV radiation [3], electrocoagulation [4]. Nevertheless, these treatment techniques

* Corresponding author.

have several drawbacks: high operating cost, variable efficiency depending on the elements to be eliminated, secondary pollution, loss of valuable potential nutrients [5], hence the need to look for other, more efficient, and less expensive alternatives.

Several studies and research projects on microalgae cultivation to purify wastewater and produce biodiesel from the biomass generated have been conducted over recent years [6–11]. These studies have shown the considerable potential of microalgae in the elimination of nutrients, especially ammonium, phosphorous, and organic matter [4,12,13]; the absorption of heavy metals [14,15] and synthetic dyes [4,16–18]. Other recent studies have reported microalgal-bacterial consortia potential in several applications, such as algal biomass production and nutrient removal [6,19]. A symbiotic interaction was found in microalgae-bacteria systems. The bacteria use the oxygen produced by the microalgae during photosynthesis to oxidize organic matter; in exchange, the bacteria release the CO_2 necessary for photosynthetic reactions [2].

However, these microalgae treatment systems suffer from some limitations, especially the harvesting of the biomass produced [20]. Current methods of harvesting algal biomass include chemical operations (coagulation, flocculation), mechanical operations (centrifugation, filtration), and electrical operations (electrophoresis). Mechanical and electrical techniques require high operating costs [21]. Meanwhile, the use of coagulation/flocculation is an ideal process, recommended to produce clean wastewater; it can be applied to a wide variety of microalgae, allows percentages of biomass recovery greater than 95% and it does not require high energy [22,23]. Besides, the use of an immobilized algae system [24] is another alternative. Still, the immobilization matrix's high costs and inefficiency over a long operating period may be limiting factors if the objective is to purify large quantities of wastewater. However, another solution to overcome this harvesting problem is using a settleable microalgal-bacterial system [25], where microalgae spontaneously form flocs that can be quickly settled.

Algal biomass is a natural source that has attracted the interest of biologists and research organizations worldwide. Recently, with the development of novel technologies, this algal biomass can be converted into products of significant biotechnological value (e.g., fatty acids, proteins, minerals, antioxidants, and pigments) that can be used in the future in several fields, such as energy, agriculture and chemistry [26–30]. However, algal biomass produced using wastewater as a growing medium will rarely be suitable for producing foodstuffs or even high value-added chemicals due to health risks [31]. Similarly, the production of fertilizer products should only be carried out if the biomass does not contain heavy metals or toxic compounds [32].

In previous studies on the purification of wastewater by microalgae, the authors opted for filtration and sterilization of the wastewater studied or, in some cases, for the use of synthetic wastewater to avoid the presence of suspended particles and indigenous bacteria that could inhibit the microalgae growth and affect the treatment efficiency [33–38]. In this study, the secondary municipal wastewater (MW), collected from Azilal WWTP (Morocco), was not filtered or sterilized; it contained bacteria and high levels of suspended solids. The objective was to evaluate the ability of native microalgae to treat the MW at different concentrations in batch mode photo-bioreactors. A comparison of the treatment under laboratory conditions (indoor cultures) and natural conditions (outdoor cultures) was carried out to determine the difference between indoor and outdoor cultures concerning the removal of nutrients, especially ammonium and organic matter. The biomass produced was harvested by coagulation–flocculation–sedimentation.

2. Materials and methods

2.1. Microalgae

The microalgae used were collected from the maturation pond of Azilal Wastewater Treatment Plant (WWTP), (31° 56′ 09″ N 6° 37′ 08″ W Azilal, Morocco). The WWTP uses natural lagoon as a purification process. The microalgae collected were subjected to pre-culture in a 1 L covered beaker under continuous illumination (provided by 4 LED lamps of 9 W installed above the beaker) at 22°C ± 1°C; the air was injected through the beaker's bottom using an air compressor. Diluted secondary wastewater collected from the outlet of the facultative pond of the same WWTP was used as a culture medium; the effluent was first left to settle overnight and then filtered through a cloth to remove large particles. The biomass was harvested during the exponential growth phase and used as inoculums for batch experiments. Observation under a light microscope showed that the native microalgae were *Chlorella* sp. (Fig. 1).

2.2. Wastewater characteristics

The secondary municipal wastewater (MW) used in this study was collected from the facultative pond of Azilal WWTP, Morocco. It was transported to the laboratory in 5 L plastic bottles and left to settle overnight before use. The main physico-chemical characteristics of the MW are given in (Table 1).

2.3. Experimental protocol

Two series of experiments were carried out to compare MW treatment with native microalgae, under different culture conditions, in outdoor batch culture and indoor batch culture. All experiments were performed under non-sterile conditions.

2.3.1. Experiment series I: indoor batch culture

The experiments were conducted under laboratory conditions, in glass cylindrical photobioreactors (PBRs) of 2 L working volume, containing 200 mL of microalgae (inoculums of 240 ± 2 $mg{\cdot}L^{-1}$) and 1,800 mL of MW, at a temperature of 22°C ± 1°C, continuous illumination was provided by 4 LED lamps of 9 W installed above the PBRs. The air was injected into the bottom of the PBRs using an air compressor to supply the CO_2 required for microalgae growth and keep the microalgae in suspension (Fig. 2). Three MW were tested: 25% MW, 50% MW, and 75% MW prepared by diluting the wastewater with distilled water.

2.3.2. Experiment series II: outdoor batch culture

The experiments were conducted under natural outdoor conditions, in glass cylindrical PBRs of 2 L working volume, containing 200 mL of microalgae (inoculum of 240 ± 2 mg·L^{-1}) and 1,800 mL of MW, under sunlight (approximately 14/10 h light/dark period). The air was injected into the bottom of the PBRs using an air compressor to supply the CO_2 required for microalgae growth and keep the microalgae in suspension. The temperature varied between 24°C and 28°C during the day and between 12°C and 15°C at night (Fig. 3). Three municipal wastewaters were tested: 25% MW, 50% MW, and 75% MW prepared by diluting the MW with distilled water.

2.3.3. Biomass harvesting

The biomass produced was collected by coagulation–flocculation; two coagulants were tested on microalgae samples collected from the stationary growth phase. The coagulants used were ferric chloride and aluminum sulfate with polyelectrolyte. Jar tests were conducted to find out the optimal coagulant doses, allowing the best biomass removal efficiency. The polyelectrolyte was used as a flocculant with aluminum sulfate to obtain comparable results (using aluminum sulfate without flocculant for water with a high level of total suspended solids (TSS) does not give good results). Three flasks containing 100 mL of algal biomass (601 mg·L^{-1} concentration) were placed on magnetic stirrers; after injection of the coagulant, the flasks were stirred at 350 rpm for 2 min to ensure coagulation (neutralization and destabilization of the particles to form flocs). Following this, the agitation

Table 1
Physico-chemical characteristics of the secondary municipal wastewater

Parameters	Value
pH	7.77 ± 0.10
Dissolved oxygen O_2 (mg·L^{-1})	2.80 ± 0.12
Electrical conductivity (mS·cm^{-1})	1.79 ± 0.01
TSS (mg·L^{-1})	372 ± 5.0
COD (mg·L^{-1})	108.00 ± 1.20
NH_4^+ (mg·L^{-1})	104.00 ± 0.40
NO_2^- (mg·L^{-1})	0.80 ± 0.02

Mean ± Standard deviation

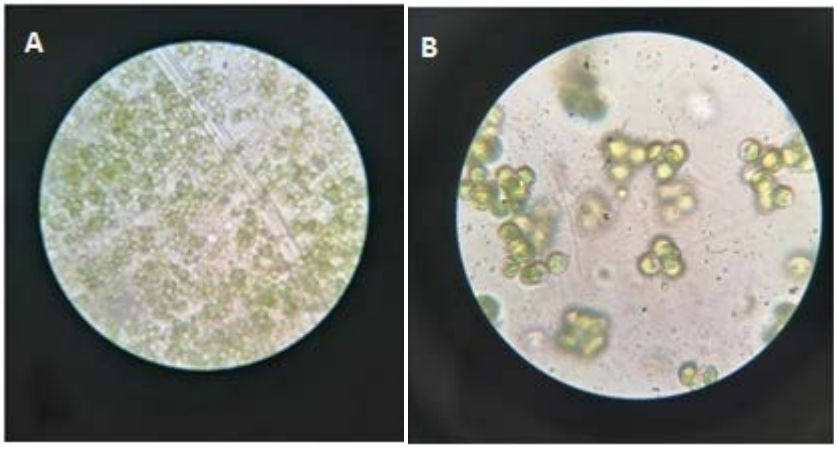

Fig. 1. Micrographs of microalgae: (A) light microscope 40X and (B) light microscope 100X.

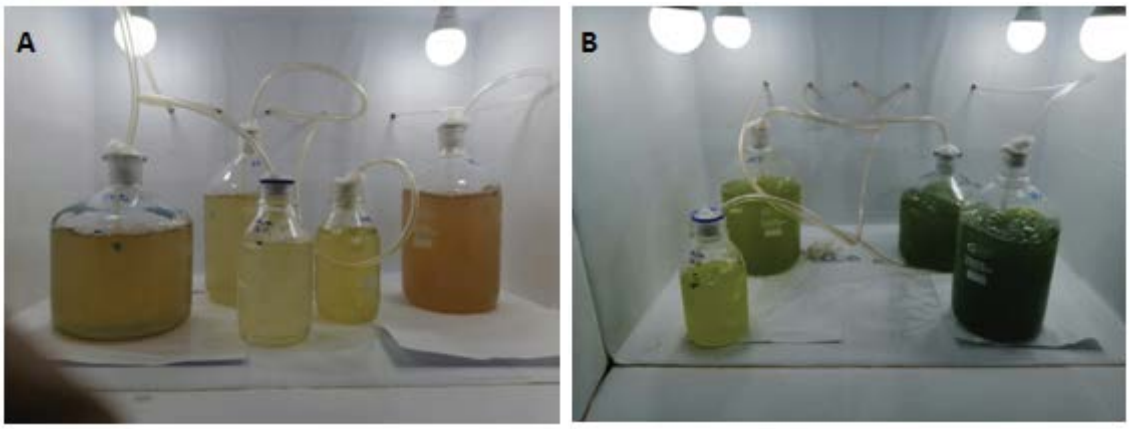

Fig. 2. Appearance of indoor cultures: (A) on day 0 and (B) after 4 d.

Fig. 3. Appearance of outdoor cultures: (A) on day 0 and (B) after 7 d.

was reduced to 50 rpm for 20 min to ensure flocculation (the agglomeration of the flocs formed to have more voluminous flocs). The flocculant was injected after reducing the agitation to give the flocs weight and facilitate their settling. Three doses of each coagulant were tested, 60, 100 and 140 mg·L^{-1} for ferric chloride and 100, 140 and 180 mg·L^{-1} with 2 mg·L^{-1} of polyelectrolyte for aluminum sulfate.

2.4. Analytical methods

Samples of approximately 100 mL of the microalgae culture were taken every day from each photo-bioreactor to analyze the physico-chemical parameters and evaluate wastewater treatment and quality during the experiments. The parameters measured were: pH, dissolved oxygen, total suspended solids (TSS), electrical conductivity (EC), chemical oxygen demand (COD), ammonium (NH_4^+), and nitrite (NO_2^-).

The pH was measured using a pH meter (pH 1100 L, VWR) with a pH electrode (pHenomenal 111, 662–1157, VWR). Dissolved oxygen was measured using an oximeter (Oxi 1970i, WTW, Germany) with an O_2 sensor (CellOx 325, WTW, Germany). EC was measured using a conductivity meter (cond 7310, WTW, Germany) with a standard EC measuring cell (TetraCon 325). TSS were determined by the standard gravimetric method (filtration of the suspension and heating the solid at 105°C). Soluble COD, NH_4^+, and NO_2^- were measured using a UV-visible spectrophotometer (HACH LANGE DR 500). For NH_4^+ and NO_2^- analysis, the samples were first passed through a 0.45 µ membrane filter and diluted. The same analysis methodology was used for the analysis of the wastewaters.

The algal biomass produced was expressed as TSS (g·L^{-1}). Specific growth rate (µ) and algal biomass productivity (P_B) were calculated in the exponential growth phase using Eqs. (1) and (2).

$$\mu = \frac{\ln\left(X_t - X_0\right)}{t_t - t_0} \quad (1)$$

$$P_B = \frac{X_t - X_0}{t_t - t_0} \quad (2)$$

where X_t and X_0 are the algal biomass concentration (g·L^{-1}) at times t_t and t_0, representing the beginning and end of the exponential growth phase.

3. Results and discussion

3.1. Algal growth

The evolution of algae growth is shown in Fig. 4. It could be observed that there was an absence of the lag phase for all MW (25% MW, 50% MW, and 75% MW) in indoor and outdoor cultures. This showed that the microalgae were already adapted to MW as both microalgae and MW were collected from the same WWTP. In the indoor cultures (Fig. 4A), the exponential phase lasted 3, 4, and 5 d for 25% MW, 50% MW, and 75% MW, respectively. In the outdoor cultures (Fig. 4B), the exponential phase lasted 9 d for 25% MW and 10 d for 50% MW and 75% MW before entering the stationary phase. The experiments lasted 13 d in outdoor cultures and 7 d in indoor cultures; this difference was due to the illumination cycle, in the indoor culture, the illumination was continuous, photosynthesis took place 24 h a day, and as a result, the nutrients were consumed quickly compared to the outdoor experiments. The illumination cycle in outdoor cultures was approximately 14/10 h light/dark, and photosynthesis took place only during the day.

As shown in Table 2, the maximum biomass concentration recorded was 0.37, 0.59 and 0.79 g·L^{-1} in the indoor cultures and 0.74, 1.09 and 1.11 g·L^{-1} in the outdoor cultures for, 25% MW, 50% MW and 75% MW, respectively. It could be noticed that the concentration of the algal biomass increased by increasing the concentrations of the wastewaters; the highest biomass concentration was obtained at 75% MW because it contained more nutrients compared to 25% MW and 50% MW.

The biomass concentrations found in this work were lower than those found by Lu et al. [39], who cultivated *Chlorella* sp. in raw dairy wastewater 2.25–3.05 g·L^{-1} in the indoor bench-scale and 0.7–1.6 g·L^{-1} in the outdoor pilot-scale. This difference could be due to the presence of suspended solids and colloidal particles in the MW used in this study (92–280 mg·L^{-1}) compared to the dairy wastewater used by Lu et al. [39] (1.74 mg·L^{-1}), which could inhibit

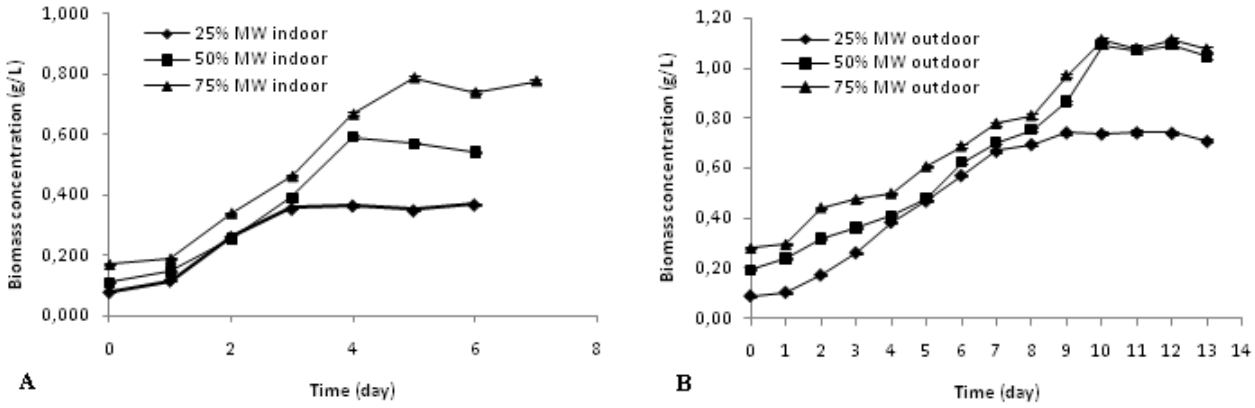

Fig. 4. Algae growth in indoor cultures (A) and outdoor cultures (B), for different wastewater concentrations.

Table 2
Max. biomass concentration, specific growth rate, and biomass productivity of indoor and outdoor cultures

Treatments		Biomass ($g \cdot L^{-1}$)	μ_{max} (d–1)	P_B ($mg \cdot L^{-1} \cdot d^{-1}$)
Indour culture	25% MW	0.37 ± 0.004	0.498 ± 0.012	92.00 ± 2.00
	50% MW	0.59 ± 0.004	0.418 ± 0.009	120.00 ± 1.75
	75% MW	0.79 ± 0.005	0.334 ± 0.007	124.00 ± 1.40
Outdoor culture	25% MW	0.74 ± 0.007	0.372 ± 0.012	91.00 ± 2.25
	50% MW	1.09 ± 0.011	0.191 ± 0.006	77.09 ± 1.36
	75% MW	1.11 ± 0.010	0.179 ± 0.006	77.50 ± 2.50

Mean ± Standard deviation

algal growth by preventing the penetration of light [40,42]. The abiotic loss of nutrients at high pH also influenced the biomass concentration in this study, notably the loss of NH_4^+ by volatilisation as NH_3 and the loss of phosphates by precipitation. Ji et al. [42] reported that the biomass concentration observed in *Chlorella vulgaris* cultivated in tertiary treated domestic wastewater supplemented with 15% of carbon dioxide (CO_2) was 0.29 $mg \cdot L^{-1}$, lower than those found in this study, and this could be attributed to the low nutrient levels in the tertiary wastewater used by Ji et al. [42] (NH_4^+ = 0.4 $mg \cdot L^{-1}$, PO_4^{3-} = 1.69 $mg \cdot L^{-1}$). The biomass concentrations produced in outdoor cultures were higher than those obtained in indoor cultures. This could be explained by the fact that there was more abiotic loss of nutrients in indoor cultures than in outdoor cultures (see the section on nutrient removal).

It should also be noted that the concentration of algal biomass could be influenced by the N:P ratio, which is a very important factor influencing the efficiency of microalgae treatment. The lack of essential nutrients for microalgae growth or their low bioavailability in the wastewater could negatively affect the nutrient removal efficiency and the biomass concentration [43,44].

The specific growth rates and the biomass productivities obtained in indoor cultures were 0.5, 0.42 and 0.33 d^{-1} and 92, 120 and 124 $mg \cdot L^{-1} \cdot d^{-1}$ for 25% MW, 50% MW and 75% MW, respectively, higher than, 0.37, 0.2 and 0.18 d^{-1} and 91, 77.1 and 77.5 $mg \cdot L^{-1} \cdot d^{-1}$ found in outdoor cultures for 25% MW, 50% MW, and 75% MW, respectively, showing that the microalgae grew faster under indoor conditions than outdoor conditions. This could be explained by the parameters controlled in the indoor cultures, especially temperature and light intensity, which were constant during the experiment. In contrast, they varied under natural conditions (temperature varied between 12°C and 28°C, and sometimes it was cloudy).

The specific growth rates in the indoor cultures were comparable to those found by Mennaa et al. [45] (0.44, 0.38, and 0.37 d^{-1}) for *Chlorella kessleri*, *Chlorella vulgaris*, and *Chlorella sorokiniana* grown in batch mode in urban wastewater and by Wang et al. [46] (0.41, 0.43, and 0.34 d^{-1}) for *Chlorella* sp. grown in wastewaters before and after primary settling and effluent from aeration tank, but were lower than the growth rate of *Chlorella* sp. grown in the centrate from sludge centrifuge (0.98 d^{-1}). Regarding biomass productivity, the values obtained in this study (101, 124.5 and 129.4 $mg \cdot L^{-1} \cdot d^{-1}$ for 25% MW, 50% MW and 75% MW, respectively) were lower than those found by Lu et al. [39] (260 and 338.8 $mg \cdot L^{-1} \cdot d^{-1}$ for two different inoculums concentrations); This could be explained by the high levels of TSS in the effluent tested in this study, which caused a problem in the use of light in photosynthesis [40] compared to that used by Lu et al. [39].

In the outdoor cultures, the specific growth rates and biomass productivities obtained in this work for 25% MW, 50% MW, and 75% MW were 0.37, 0.19, and 0.18 d^{-1} and 91, 77.1, and 77.5 $mg \cdot L^{-1} \cdot d^{-1}$, respectively, which were comparable to those found by Lu et al. [39] who cultivated *Chlorella* sp. in dairy wastewater (DW) at different concentrations in outdoor pilot-scale and obtained the values of 0.098, 0.2 and 0.16 d^{-1} and 47.5, 160, and 110 $mg \cdot L^{-1} \cdot d^{-1}$ for 5% DW, 10% DW and 25% DW, respectively. The specific growth rates of this study were also similar to those of *Chlorella zofingiensis* cultivated in 60 L flat photobioreactors under outdoor conditions in autumn (0.16–0.45 d^{-1}) and lower than those obtained in spring (0.32–0.99 d^{-1}) reported by Feng et al. [47], the biomass productivities were higher than those found by Feng et al. [47] (19.4–58.4 $mg \cdot L^{-1} \cdot d^{-1}$).

3.2. Nutrients removal

The evolution of ammonium concentration is shown in Fig. 5. It could be observed that the ammonium concentration

was significantly reduced at the end of the experiments for all MW tested. In the indoor cultures, ammonium removal percentages reached 99.4%, 99.7%, and 99.8%, respectively for 25% MW, 50% MW, and 75% MW; More than 95% of NH_4^+ was removed after 4 d of microalgae cultivation. While in the outdoor cultures, ammonium removal percentages reached 94.3%, 97.2%, and 97.5%, respectively for 25% MW, 50% MW, and 75% MW; More than 95% of NH_4^+ was removed after 4 d of microalgae cultivation. These percentages of ammonium removal obtained in this study of both indoor and outdoor cultures were higher than those found in other studies on *Chlorella* grown in different wastewaters [38,46,48,49]. It could also be noted that although almost all of the ammonium was consumed by day 4/5 (Fig. 5), the growth curves in Fig. 4B show that the microalgae continued to grow until day 9/10, indicating that other sources of nitrogen were present in the MW (particularly nitrate).

The maximum ammonium removal rates obtained (Table 3) in indoor cultures were 6.2, 12.72, and 17.7 $mg{\cdot}L^{-1}{\cdot}d^{-1}$ for 25% MW, 50% MW, and 75% MW, respectively, higher than those found in outdoor cultures which were 4.46, 8.22, and 11.68 $mg{\cdot}L^{-1}{\cdot}d^{-1}$ for 25% MW, 50% MW, and 75% MW, respectively, showing that NH_4^+ removal was faster under laboratory conditions, and this could be explained by the controlled parameters in the indoor cultures (continuous illumination and constant temperature). It could also be noticed that the removal rate increased with increasing wastewater concentration, as concentrated wastewater contained more ammonium to be removed.

Ammonium could be removed by different processes, either by microalgae absorption, by oxidation by nitrifying bacteria, or by NH_3 stripping at high pH values (alkaline medium) [9,49,50]. In this study, the ammonium removal could be attributed mainly to microalgae uptake and NH_3 stripping since the algal biomass concentration increased, and the pH exceeded 10 in all cultures. The loss of ammonium by NH_3 stripping reduced algal growth, which could explain the low biomass concentrations obtained compared to those obtained in previous studies (Table 4). The evolution of nitrites concentration for indoor and outdoor cultures showed no significant variation of NO_2^- during the experiment (Fig. 6), which indicated the absence of nitrifying bacteria activity. The non-consumption of nitrite also noted that it was not the preferred source of nitrogen for the native microalgae.

Regarding COD removal, similar COD concentration variation patterns were observed for all MW in indoor and outdoor cultures (Fig. 7). The COD concentration decreased in the first few days (2 to 3 d) in both indoor and outdoor cultures, and then it increased at the end of the experiments. In the indoor cultures, an important reduction of COD concentration was observed for 50% MW and 75% MW, while for the 25% MW, no significant COD reduction was recorded (Fig. 7A). After 3 d, the maximum COD removal

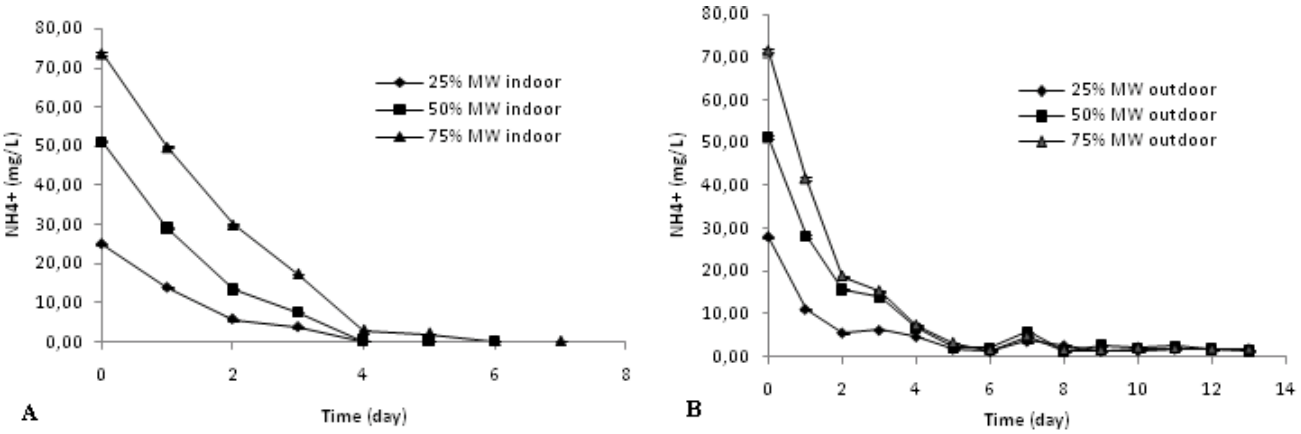

Fig. 5. Variation of ammonium concentration in indoor cultures (A) and outdoor cultures (B).

Table 3
Nutrients removal percentage and removal rate indoor and outdoor cultures

Treatments		NH_4^+ removal percentage (%)	NH_4^+ removal rate ($mg{\cdot}L^{-1}{\cdot}d^{-1}$)	COD removal percentage (%)	COD removal rate ($mg{\cdot}L^{-1}{\cdot}d^{-1}$)
Indour culture	25% MW	99.40 ± 0.28	6.205 ± 0.077	32.30 ± 1.64	3.940 ± 0.400
	50% MW	99.73 ± 0.14	12.718 ± 0.102	57.02 ± 1.31	11.593 ± 0.533
	75% MW	99.77 ± 0.11	17.698 ± 0.102	60.32 ± 1.05	15.280 ± 0.487
Outdoor culture	25% MW	94.29 ± 0.25	4.457 ± 0.065	65.64 ± 1.03	12.800 ± 0.500
	50% MW	97.19 ± 0.14	8.225 ± 0.072	67.50 ± 0.94	21.600 ± 0.620
	75% MW	97.51 ± 0.10	11.677 ± 0.072	68.89 ± 0.97	24.800 ± 0.680

Mean ± Standard deviation

Table 4
Comparison of some studies on ammonium removal by microalgae

Microalgae	Effluent	Mode and operating condition	Parameter	Value	Time (d)	References
Desmodesmus sp.	Mixture landfill leachate-municipal wastewater	- Batch mode - V reactor: 4 L - Manual shaking - Without aeration - Illumination: 53 μmol·m^{-2}·s^{-1} (12:12 h L/D)	Algal biomass NH_4^+ removal	1.95 g·L^{-1} 82%	28	[9]
Mixed cultures of *Euglena gracilis* and *Selenastrum*	Aquaculture wastewater	- Batch mode - V reactor: 2 L - Aeration: 0.5 L·min^{-1} - Illumination: 250 μmol·m^{-2}·s^{-1} (16:8 h L/D)	Algal biomass NH_4^+ removal	1.5 g·L^{-1} 98.9%–99.5%	14	[11]
Chlorella sp.	Municipal wastewater	- Batch mode - V reactor: 250 mL - Agitation: 100 rpm - Without aeration - Continuous illumination 200 μmol·m^{-2}·s^{-1}	Algal biomass NH_4^+ removal	NR 74.7%–82.4%	9	[46]
Chlorella sp.	Concentrated municipal wastewater	- Batch mode - V reactor: 250 mL - Agitation: 100 rpm - Without aeration - Illumination: 50 μmol·m^{-2}·s^{-1}	Algal biomass NH_4^+ removal	1.02 g·L^{-1} 93.9%	14	[49]
Euglena sp.	Raw domestic wastewater	- Batch mode - V reactor: 10 L - Periodic agitation - Without aeration - Under sunlight (12:12 h L/D)	Algal biomass NH_4^+ removal	98% 1.19 g·L^{-1}	9	[57]
Chlorella sp.	Municipal wastewater	- Batch mode - V reactor: 2 L - Aeration - Outdoor culture: under sunlight - Indoor culture: continuous illumination	Outdoor culture: - Algal biomass - NH_4^+ removal Outdoor culture: - Algal biomass - NH_4^+ removal	1.11 g·L^{-1} 97 5% 0.79 g·L^{-1} 99.8%	13 7	This study

V reactor: volume of reactor; L/D: light dark cycle; NR: not reported.

was achieved by 32.3%, 57%, and 60.3% for 25% MW, 50% MW, and 75% MW, respectively. From the fourth day, COD started to increase; the percentages of COD removal ranged from 17.7% to 49.4% on the last day of the experiments. COD was significantly reduced in the outdoor cultures after 2 d for the three MW tested (Fig. 7B). The maximum COD removal achieved was 65.6%, 67.5% and 68.9% for 25% MW, 50% MW and 75% MW, respectively. The final percentages of COD removal ranged from 27.7% to 40.8%. The increase of the COD concentration at the end of the experiments could be attributed to the excretion by microalgae of some molecular organic compounds in the medium, such as glycolic acid produced during the photosynthetic carbon reduction cycle [46,51], or to the hydrolysis of the biomass at high pH values [52]. To a lesser extent, this increase in COD was also reported in other studies [12,39,52,53].

The maximum COD removal percentages and removal rates obtained in the outdoor cultures were somewhat higher than those obtained in indoor cultures (Table 3); this might be due to the different illumination nature. Lee and Lee [54] studied the effect of the light-dark cycle on wastewater treatment by *Chlorella kessleri* and found that COD removal under light/dark lighting (86.5%) was higher than under continuous illumination (83.3%), coincided with the results of this study. Lu et al. [39], who cultivated *Chlorella* sp. in raw dairy wastewater, found opposite results; the best COD removal was recorded in indoor cultures with continuous illumination by percentages ranging from 81.2% to 84.3%, higher than those obtained in the indoor cultures of this study which did not exceed 60.3%. Whereas in the outdoors cultures, the maximum COD removal found in this research was higher than that obtained by Lu et al. [39] (22%

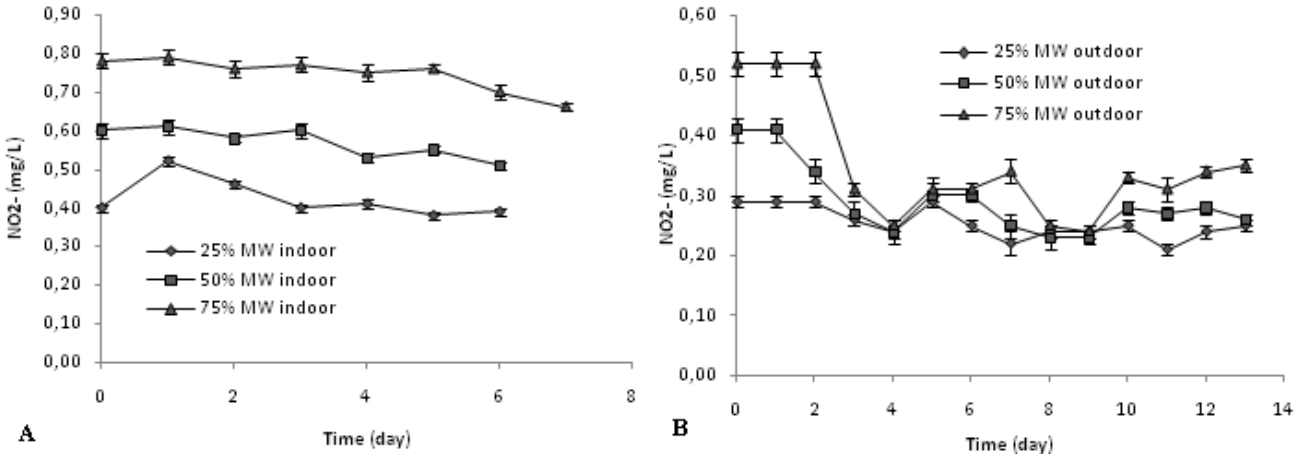

Fig. 6. Variation of nitrites concentration in indoor cultures (A) and outdoor cultures (B).

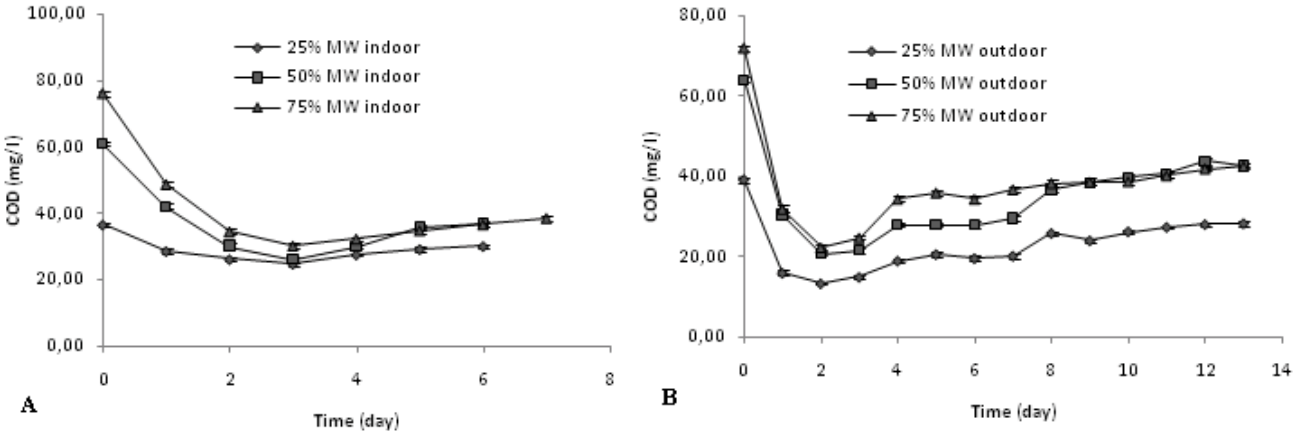

Fig. 7. Variation of COD in indoor cultures (A) and outdoor cultures (B).

to 54.8%), especially in the first 2 d, showing that the native microalgae used in this study were more efficient in the elimination of COD under natural conditions.

In general, the COD removal percentages obtained at the end of the experiments were low compared to other studies on *Chlorella* cultivated in different wastewaters. 66% to 75.6% of COD removed found by Zhao et al. [52] in concentrated synthetic wastewater, 50.9% to 83% found by Wang et al. [46] in different municipal wastewaters, and 90.3% to 90.8% by Li et al. [49] in concentrated municipal wastewater. This difference might be primarily due to the increase of COD concentration at the end of the cultures and the low initial COD concentration of the tested municipal wastewater compared to the wastewaters used in the other studies.

The COD removal mechanisms could be attributed to microalgae's absorption and the degradation of organic carbon by the indigenous bacteria since the wastewaters were not sterilized. According to several studies, some microalgae species are mixotrophic such as *Chlorella* sp. They can satisfy their carbon needs from organic or inorganic matter [46,49,52,55]. Similarly, they can satisfy their energy needs from organic carbon or light source [55]. In this study, the use of organic carbon and CO_2 by the native microalgae for their mixotrophic growth contributed to COD reduction. Heterotrophic bacteria in the wastewater also contributed to COD removal through the degradation of organic matter, using oxygen produced by photosynthesis and oxygen provided by aeration.

Fig. 8 shows the evolution of the EC in the three MW. At the end of the experiments, the EC decreased in all photobioreactors. A significant decrease was observed for 50% MW and 75% MW in indoor and outdoor cultures, showing that these microalgae could remove the different dissolved matter from the wastewaters during their growth. Other authors also reported this; Ramos et al. [48] found that *Chlorella sorokiniana* and *Scenedesmus* sp., grown in synthetic wastewater, reduced the concentration of some elements such as potassium K^+, sodium Na^+, sulfur S, and phosphorus P, by biosorption when the pH was above 9 (an alkaline stress condition) or by metabolic processes. Wang et al. [46] reported that *Chlorella* sp. could remove Al, Fe, Mg, Mn, Zn, and Ca^{2+} from all wastewaters tested efficiently. The heavy metal removal mechanism from wastewater was related to the large surface area of microalgae and their high binding affinity. Calcium was removed by precipitation as calcium phosphates at pH > 9.

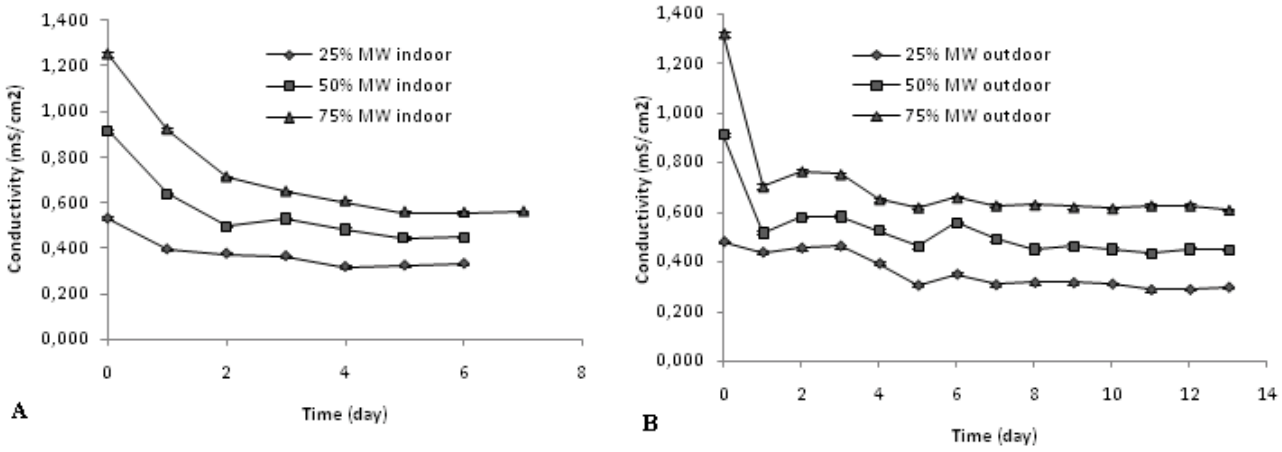

Fig. 8. Variation of conductivity in indoor cultures (A) and outdoor cultures (B).

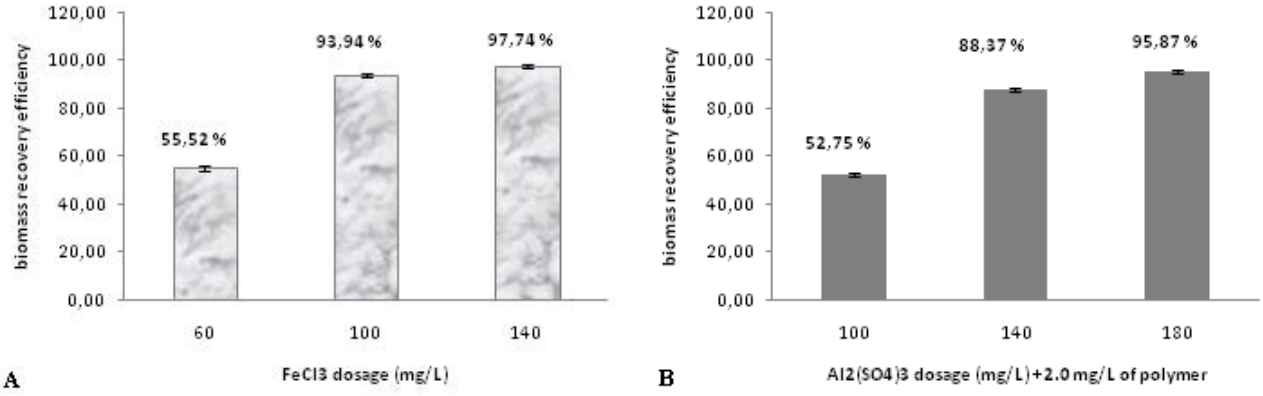

Fig. 9. Biomass recovery efficiency by coagulation–flocculation for two coagulants, (A) ferric chloride and (B) aluminum sulfate.

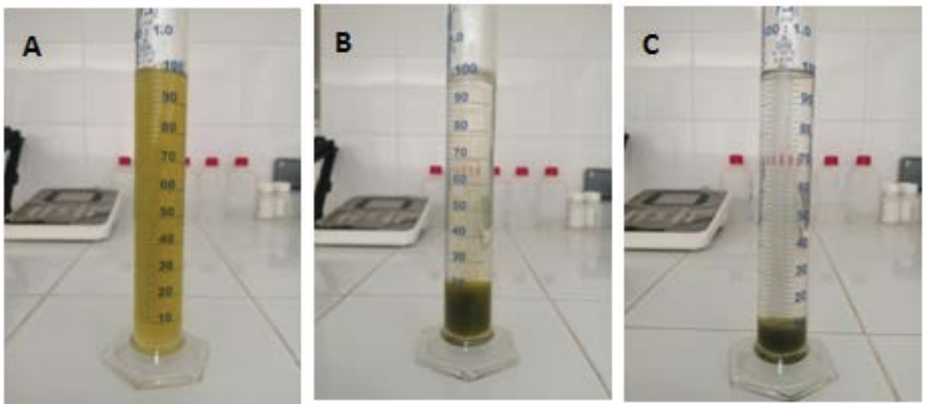

Fig. 10. Sedimentation of the algal biomass after coagulation by ferric chloride (dose of 140 mg·L^{-1}), (A) initial sample, (B) after 5 min of sedimentation, and (C) after 20 min of sedimentation.

3.3. Biomass harvesting

Fig. 9 illustrates the biomass removal efficiency (%BR) as a function of coagulant doses. The doses of 100, 140 and 180 mg·L^{-1} of aluminum sulfate used with 2.0 mg·L^{-1} of polyelectrolyte allowed the recovery of 52.7%, 88.4% and 95.9% of the biomass, respectively (Fig. 9A). While the use of 60, 100, and 140 mg·L^{-1} of ferric chloride allowed the recovery of 55.5%, 93.9%, and 97.4% of the biomass, respectively, in 20 min of sedimentation (Fig. 9B), which showed that ferric chloride performed better than aluminum sulfate, even with

the use of a polymer with aluminum sulfate. The optimal dose of ferric chloride was 140 mg·L^{-1}; it allowed the recovery of 97.4% of the biomass produced (Fig. 10). Different results were found in other studies on algal biomass harvesting by coagulation–flocculation using ferric chloride as a coagulant. The removal efficiency of 95% was obtained for *Scenedesmus obliquus* using 100 mg·L^{-1} $FeCl_3$ [56], and more than 90% of the algal biomass was recovered in 20 min of sedimentation for different algal species (*Ankistrodesmus falcatus*, *Scenedesmus obliquus*, *Chlorella* sp., *Botryococcus braunii*, *Neocloris oleabundans* and Natural algal bloom) using 60 mg·L^{-1} $FeCl_3$ [45]. The differences that could be found in the results might be due to the different species of algae to be harvested, the dose of coagulant, the initial biomass concentration, the different components of the wastewater, or the Jar test parameters (speed and time of stirring, type of stirrers, etc.).

4. Conclusion

In this study, the native microalgae showed considerable potential in removing nutrients from secondary municipal wastewater at different concentrations in both indoor and outdoor cultures. Ammonium removal percentages reached 99.8% in indoor cultures and 97.5% in outdoor cultures. For COD, maximum removal was obtained in outdoor cultures, with removal percentages ranging from 65.6% to 68.9%. The microalgae's capacity to remove COD was not well evaluated, given that the municipal wastewater studied contained a low concentration of organic matter (low COD). Furthermore, the coagulation–flocculation process demonstrated exemplary performance in biomass harvesting, recovering 97.4% of the biomass produced in 20 min of sedimentation, using 140 mg·L^{-1} of ferric chloride. From these results, it could be concluded that the studied microalgae can be successfully used for MW treatment combined with the production of valuable biomass that can be used in different fields such as biodiesel and fertilizer production. However, further experiments in the continuous mode under natural conditions should be performed to optimize the culture parameters and scale up this biotechnology.

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Conflicts of interest

The authors declare that there is no conflict of interest.

References

[1] R. Elkacmi, M. Bennajah, Advanced oxidation technologies for the treatment and detoxification of olive mill wastewater: a general review, J. Water Reuse Desal., 9 (2019) 463–505.
[2] A.L. Gonçalves, J.C.M. Pires, M. Simões, A review on the use of microalgal consortia for wastewater treatment, Algal Res., 24 (2016) 403–415.
[3] O. Fawzi, S. Khasawneh, P. Palaniandy, Occurrence and removal of pharmaceuticals in wastewater treatment plants, Process Saf. Environ. Prot., 150 (2021) 532–556.
[4] R. Elkacmi, O. Boudouch, A. Hasib, M. Bouzaid, M. Bennajah, Photovoltaic electrocoagulation treatment of olive mill wastewater using an external-loop airlift reactor, Sustainable Chem. Pharm., 17 (2020) 100274, doi: 10.1016/j.scp.2020.100274.
[5] N. Abdel-Raouf, Microalgae and wastewater treatment, Saudi J. Biol. Sci., 19 (2012) 257–275.
[6] F. Bélanger-lépine, A. Tremblay, Y. Huot, S. Barnabé, Cultivation of an algae-bacteria consortium in wastewater from and industrial park: effect of environmental stress and nutrient deficiency on lipid production, Bioresour. Technol., 267 (2018) 657–665.
[7] X. Chen, Z. Li, N. He, Y. Zheng, H. Li, H. Wang, Y. Wang, Y. Lu, Nitrogen and phosphorus removal from anaerobically digested wastewater by microalgae cultured in a novel membrane photobioreactor, Biotechnol. Biofuels, 190 (2018) 1–11, doi: 10.1186/s13068-018-1190-0.
[8] J.L. Salgueiro, L. Perez, R. Maceiras, A. Sànchez, A. Cancela, Semicontinuous culture of *Chlorella vulgaris* microalgae for wastewater treatment, Int. J. Environ. Res., 12 (2018) 765–772.
[9] A. Hernández-García, S.B. Velásquez-Orta, E. Novelo, I. Yáñez-Noguez, I. Monje-Ramírez, M.T. Orta, Wastewater-leachate treatment by microalgae: biomass, carbohydrate and lipid production, Ecotoxicol. Environ. Saf., 174 (2019) 435–444.
[10] M.T. Nguyen, C. Lin, C. Lay, Microalgae cultivation using biogas and digestate carbon sources, Biomass Bioenergy, 122 (2019) 426–432.
[11] M. Tossavainen, K. Lahti, M. Edelmann, R. Eskola, A. Lampi, Integrated utilization of microalgae cultured in aquaculture wastewater: wastewater treatment and production of valuable fatty acids and tocopherols, J. Appl. Phycol., 31 (2018) 1753–1763.
[12] J. Fito, K. Alemu, Microalgae – bacteria consortium treatment technology for municipal wastewater management, Nanotechnol. Environ. Eng., 2 (2019) 1–9.
[13] H. Fan, K. Wang, C. Wang, F. Yu, X. He, J. Ma, X. Li, A comparative study on growth characters and nutrients removal from wastewater by two microalgae under optimized light regimes, Environ. Technol. Innov., 19 (2020) 100849, doi: 10.1016/j.eti.2020.100849.
[14] O. Spain, M. Plöhn, C. Funk, The cell wall of green microalgae and its role in heavy metal removal, Physiol. Plant., 173 (2021) 526–535.
[15] Z.S. Birungi, E.M.N. Chirwa, Bioremediation of toxic metals and recovery of target metals from actual wastewater using algal sorbents, Ital. Assoc. Chem. Eng., 64 (2018) 535–540.
[16] R.M. Moghazy, Activated biomass of the green microalga *Chlamydomonas variabilis* as an efficient biosorbent to remove methylene blue dye from aqueous solutions, Water SA, 45 (2019) 20–28.
[17] A. Lúcia, E. Carissimi, G.L. Dotto, L.A. Feris, Biosorption of Rhodamine B dye from dyeing stones effluents using the green microalgae *Chlorella pyrenoidosa*, J. Cleaner Prod., 198 (2018) 1302–1310.
[18] J.Y. Chin, L.M. Chng, S.S. Leong, S.P. Yeap, N.H.M. Yasin, P.Y. Toh, Removal of synthetic dye by *Chlorella vulgaris* microalgae as natural adsorbent, Arabian J. Sci. Eng., 45 (2020) 7385–7395.
[19] L. Delgadillo-Mirquez, F. Lopes, B. Taidi, D. Pareau, Nitrogen and phosphate removal from wastewater with a mixed microalgae and bacteria culture, Biotechnol. Rep., 11 (2016) 18–26.
[20] B. Molinuevo-salces, B. Riaño, D. Hernández, Microalgae and Wastewater Treatment: Advantages and Disadvantages, M. Alam, Z. Wang, Eds., Microalgae Biotechnology for Development of Biofuel and Wastewater Treatment, 2019, pp. 505–533. Available at: https://doi.org/10.1007/978-981-13-2264-8_20
[21] S. Abinandan, S. Shanthakumar, Challenges and opportunities in application of microalgae (Chlorophyta) for wastewater treatment: a review, Renewable Sustainable Energy Rev., 52 (2015) 123–132.
[22] F.G. Acién, Wastewater treatment using microalgae: how realistic a contribution might it be to significant urban wastewater treatment?, Appl. Microbiol. Biotechnol., 100 (2016) 9013–9022.

[23] S.K. Gupta, K. Dhandayuthapani, F.A. Ansari, Chapter 19 – Techno-Economic Perspectives of Bioremediation of Wastewater, Dewatering, and Biofuel Production From Microalgae: An overview, V.C. Pandey, K. Bauddh, Eds., Phytomanagement of Polluted Sites: Market Opportunities in Sustainable Phytoremediation, Elsevier Inc., Amsterdam, 2019, pp. 471–499. Available at: https://doi.org/10.1016/B978-0-12-813912-7.00019-3.
[24] N. Malik, Biotechnological potential of immobilised algae for wastewater N, P and metal removal: a review, BioMetals, 15 (2002) 377–390.
[25] Y. Su, A. Mennerich, B. Urban, Municipal wastewater treatment and biomass accumulation with a wastewater-born and settleable algal-bacterial culture, Water Res., 5 (2011) 2–9.
[26] G.K. Sharma, S.A. Khan, M. Shrivastava, R. Bhattacharyya, A. Sharma, D.K. Gupta, P. Kishore, N. Gupta, Circular economy fertilization: phycoremediated algal biomass as biofertilizers for sustainable crop production, J. Environ. Manage., 287 (2021) 112295, doi: 10.1016/j.jenvman.2021.112295.
[27] A.S.A. de P. Pereira, J. de S. Castro, V.J. Ribeiro, M.L. Calijuri, Organomineral fertilizers pastilles from microalgae grown in wastewater: ammonia volatilization and plant growth, Sci. Total Environ., 779 (2021) 146205, doi: 10.1016/j.scitotenv.2021.146205.
[28] A. Aslam, A. Bahadar, R. Liaquat, M. Saleem, A. Waqas, M. Zwawi, Algae as an attractive source for cosmetics to counter environmental stress, Sci. Total Environ., 772 (2021) 144905, doi: 10.1016/j.scitotenv.2020.144905.
[29] J. Liu, I. Obaidi, S. Nagar, G. Scalabrino, H. Sheridan, The antiviral potential of algal-derived macromolecules, Curr. Res. Biotechnol., 3 (2021) 120–134.
[30] S.V. Vassilev, C.G. Vassileva, Composition, properties and challenges of algae biomass for biofuel application: an overview, Fuel, 181 (2016) 1–33.
[31] K.R. Hakeem, M. Jawaid, U. Rashid, Eds., Biomass and Bioenergy: Processing and Properties, Springer, Cham, 2014, pp. 307–326, doi: 10.1007/978-3-319-07641-6.
[32] K. Chojnacka, K. Moustakas, A. Witek-Krowiak, Bio-based fertilizers: a practical approach towards circular economy, Bioresour. Technol., 295 (2020) 122223, doi: 10.1016/j.biortech.2019.122223.
[33] R. Tripathi, A. Gupta, I.S. Thakur, An integrated approach for phycoremediation of wastewater and sustainable biodiesel production by green microalgae, *Scenedesmus* sp. ISTGA1, Renewable Energy, 135 (2019) 617–625.
[34] K. Katam, D. Bhattacharyya, Simultaneous treatment of domestic wastewater and bio-lipid synthesis using immobilized and suspended cultures of microalgae and activated sludge, J. Ind. Eng. Chem., 69 (2019) 295–303.
[35] F. Gao, Z. Yang, Q. Zhao, D. Chen, C. Li, M. Liu, J. Yang, J. Liu, Y. Ge, Mixotrophic cultivation of microalgae coupled with anaerobic hydrolysis for sustainable treatment of municipal wastewater in a hybrid system of anaerobic membrane bioreactor and membrane photobioreactor, Bioresour. Technol., 337 (2021) 125457, doi: 10.1016/j.biortech.2021.125457.
[36] W. Kong, J. Kong, H. Lyu, P. Yuan, Z. Wang, B. Shen, S. Feng, Integrating municipal wastewater treatment with CO_2 fixation and fatty acid production by cultivating *Tetradesmus obliquus*, J. Cleaner Prod., 320 (2021) 128916, doi: 10.1016/j.jclepro.2021.128916.
[37] E. Amini, A. Babaei, M. Reza, J. Shayegan, Municipal wastewater treatment by semi-continuous and membrane algal-bacterial photo-bioreactors, J. Water Process. Eng., 36 (2020) 101274, doi: 10.1016/j.jwpe.2020.101274.
[38] H. Beydeş, I. Karapinar, Algal nutrient removal from wastewater in fed-batch operated photobioreactor, Int. J. Environ. Res., 12 (2018) 303–311.
[39] W. Lu, Z. Wang, X. Wang, Z. Yuan, Cultivation of *Chlorella* sp. using raw diary wastewater for nutrient removal and biodiesel production: characteristics comparison of indoor bench-scale and outdoor pilot-scale cultures, Bioresour. Technol., 192 (2015) 382–388.
[40] S. Cho, T. Thao, D. Lee, Y. Oh, T. Lee, Reuse of effluent water from a municipal wastewater treatment plant in microalgae cultivation for biofuel production, Bioresour. Technol., 102 (2011) 8639–8645.
[41] R. Chen, R. Li, L. Deitz, Y. Liu, R.J. Stevenson, W. Liao, Freshwater algal cultivation with animal waste for nutrient removal and biomass production, Biomass Bioenergy, 39 (2012) 128–138.
[42] M-K. Ji, R.A.I. Abou-Shanab, S.-H. Kim, E.-S. Salama, S.-H. Lee, A.N. Kabra, Y.-S. Lee, S. Hong, B.-H. Jeon, Cultivation of microalgae species in tertiary municipal wastewater supplemented with CO_2 for nutrient removal and biomass production, Ecol. Eng., 58 (2013) 142–148.
[43] G. Markou, D. Vandamme, K. Muylaert, Microalgal and cyanobacterial cultivation: the supply of nutrients, Water Res., 65 (2014) 186–202.
[44] E. Posadas, A. Soltau, I. Díaz, R. Muñoz, Carbon and nutrient removal from centrates and domestic wastewater using algal–bacterial biofilm bioreactors, Bioresour. Technol., 139 (2013) 50–58.
[45] F.Z. Mennaa, Z. Arbib, J.A. Perales, Urban wastewater treatment by seven species of microalgae and an algal bloom: biomass production, N and P removal kinetics and harvestability, Water Res., 83 (2015) 42–51.
[46] L. Wang, M. Min, Y. Li, P. Chen, Y. Chen, Y. Liu, Y. Wang, R. Ruan, Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant, Appl. Biochem. Biotechnol., 162 (2010) 1174–1186.
[47] P. Feng, Z. Deng, Z. Hu, L. Fan, Lipid accumulation and growth of *Chlorella zofingiensis* in flat plate photobioreactors outdoors, Bioresour. Technol., 102 (2011) 10577–10584.
[48] A. Ramos, S. Regan, P.J. McGinn, P. Champagne, Feasibility of a microalgal wastewater treatment for the removal of nutrients under non sterile conditions and carbon limitation, Can. J. Chem. Eng., 97 (2018) 1289–1298.
[49] Y. Li, Y. Chen, P. Chen, M. Min, W. Zhou, B. Martinez, J. Zhu, R. Ruan, Characterization of a microalga *Chlorella* sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production, Bioresour. Technol., 102 (2011) 5138–5144.
[50] J. Liu, Y. Wu, C. Wu, K. Muylaert, W. Vyverman, H.Q. Yu, R. Muñoz, B. Rittmann, Advanced nutrient removal from surface water by a consortium of attached microalgae and bacteria: a review, Bioresour. Technol., 241 (2017) 1127–1137.
[51] R. Amiri, M. Ahmadi, Treatment of wastewater in sewer by *Spirogyra* sp. green algae: effects of light and carbon sources, Water Environ. J., 34 (2014) 311–321.
[52] Y. Zhao, Z. Ge, H. Lui, S. Sun, Ability of different microalgae species in synthetic high-strength wastewater treatment and potential lipid production, J. Chem. Technol. Biotechnol., 91 (2016) 2888–2895.
[53] S. Zhu, L. Qin, P. Feng, C. Shang, Z. Wang, Z. Yuan, Treatment of low C/N ratio wastewater and biomass production using co-culture of *Chlorella vulgaris* and activated sludge in a batch photobioreactor, Bioresour. Technol., 274 (2018) 313–320.
[54] K. Lee, C. Lee, Effect of light/dark cycles on wastewater treatments by microalgae cell growth under different light conditions, Biotechnol. Bioprocess Eng., 6 (2001) 194–199.
[55] N. Nirmalakhandan, T. Selvaratnam, D. Tchinda, I.S.A. Abeysiriwardana-Arachchige, H.M.K. Delanka-pedige, S.P. Munasinghe-arachchige, Algal wastewater treatment: photoautotrophic vs . mixotrophic processes, Algal Res., 41 (2019) 101569, doi: 10.1016/j.algal.2019.101569.
[56] I. De. Godos, H.O. Guzman, R. Soto, P.A. García-encina, E. Becares, R. Muñoz, V.A. Vargas, Coagulation/flocculation-based removal of algal – bacterial biomass from piggery wastewater treatment, Bioresour. Technol., 102 (2011) 923–927.
[57] D.M. Mahapatra, H.N. Chanakya, *Euglena* sp. as a suitable source of lipids for potential use as biofuel and sustainable wastewater treatment, J. Appl. Phycol., 25 (2013) 855–865.