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Simultaneous nutrients removal and biodiesel production by green microalgae cultivated in Yellow River water

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ABSTRACT

Microalgae provide an effective and sustainable biological approach for polluted water treatment with simultaneous lipid accumulation for biodiesel production. In this study, three microalgae (*Chlorella sorokiniana* GEEL-01, *Parachlorella kessleri* GEEL-02, and *Desmodesmus asymmetricus* GEEL-05) were selected and cultivated in Yellow River (YR) water for coupled nutrients removal and biomass/lipid accumulation. *P. kessleri* GEEL-02 showed highest specific growth rate of 0.963, 0.843, and 0.909 d^{-1} in filtrated, autoclaved, and untreated YR water, respectively. The removal efficiency of total nitrogen (TN) and total phosphorus (TP) was >90% and >95%, respectively. While, *D. asymmetricus* GEEL-05 exhibited high removal efficiency of heavy metals (HMs) such as Zn^{2+} (98.1%) and Cu^{2+} (93.9%) when cultivated in autoclaved YR water. The proportion of saturated fatty acids (FAs), mono-unsaturated FAs, and poly-unsaturated FAs content in harvested microalgal biomass were in the range of 39.14%–74.72%, 12.06%–29.06%, and 10.40%–35.13%, respectively. FAs accumulated in microalgal biomass were appropriate to produce biodiesel.

Keywords: Microalgae; Heavy metals; Yellow River water; Nutrients removal; Fatty acids; Biodiesel quality

1. Introduction

Water pollution with organic and inorganic compounds is an environmental hazard and public health risk all over the world [1]. Conventional water treatment methods (such as chemical removal of phosphorus, nitrification, and denitrification) are complex and do not meet the strict nutrients emission load standards [2–4]. Recently, coupling of biological waste treatments with biofuels generation have gained much attention [5]. One potential approach to treat(waste)water is microalgae-based, in which the harvested biomass is used to generate biodiesel [5,6]. This approach can be improved by screening and selection of microalgal species tolerant to pollutants, appropriate cultivation media, and pretreatment methods for successful biological water treatment [7,8].

Microalgae are an attractive approach for treatment of different kind of waters (such as rivers, industrial, agricultural, and sewage wastewater) due to their versatile metabolic nature (e.g., autotrophic, heterotrophic, and mixotrophic) [9,10]. Beside their ability to uptake nutrients (nitrogen and phosphorus) from water, microalgae show high tolerance to environmental toxicity and can also accumulate several pollutants such as heavy metals (HMs) [7,8]. HMs uptake and accumulation in microalgae involves two mechanisms; an initial rapid (passive) cell surface adsorption of metal ions within a relatively short time span, and a much slower (active) metabolism-dependent uptake

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causing the transport of metal ions across the cell membrane into the cytoplasm [11,12]. HMs accumulation in cell leads to photosynthesis inhibition, reduced microalgal growth, loss of cell solutes due to enhanced permeability in plasmalemma, disrupted membrane integrity due to damaged protein structure, abnormal morphological development, inhibition of enzyme activity due to essential metal ions displacement, and loss of flagella in various microalgae [13].

Microalgae overcome HMs toxicity through intracellular and extracellular metal binding strategies (including ion exchanges, chelation, physical adsorption, and complexation) and transform the toxic HMs to nontoxic form [14,15]. The harvested microalgal biomass after water treatment can be utilized as an important feedstock in biodiesel production [16]. Such treatment of water is considered to be an efficient and promising mean not only for complete nutrient removal from water, but also for production of higher amount of biomass which can be utilized for several valuable products (biodiesel, fodder, and biofertilizer) [17,18]. Various approaches have been utilized for wastewater treatment including primary settling, biological treatment (secondary treatment), and finally tertiary treatment (UV treatment or filtration). However, these approaches can't be implemented for the treatment of polluted river water. Yellow river (YR) is the longest river passing through Lanzhou city and considered as the main water source. Petrochemical, textile, and steel industries are the major source of pollutant discharge in YR [19,20]. Coagulation/flocculation, filtration, and chlorination are the main reported treatments for YR water [21,22]. However, removal of pollutants such as TN, TP, and HMs is intricate by these techniques.

In this study, biological treatment of Yellow River water was performed using microalgae to prompt biomass/lipids accumulation for subsequent production of biodiesel. Three microalgae species were selected and cultivated in the Yellow River water to evaluate their capacity for coupling of biomass production with nutrients removal. The kinetic assessment of microalgal growth rate and nutrient elimination (including total nitrogen, total phosphorus, and heavy metals uptake) were evaluated. The fatty acids composition was also explored followed by biodiesel quality estimation.

2. Materials and methods

2.1. Microalgae strain and culture condition

Chlorella sorokiniana GEEL-01, *Parachlorella kessleri* GEEL-02, and *Desmodesmus asymmetricus* GEEL-05 were selected to evaluate their capability for simultaneous treatment of Yellow River (YR) water and microalgal biomass/lipid accumulation (Fig. 1). These microalgal strains showed high growth and nutrients removal from synthetic medium in our previous investigation [23], as well, they are recommended for coupling of high biomass/lipid accumulation with biological wastewater treatment as they are tolerant to polluted waters [24,25]. YR water at Lanzhou city, was collected (~8 L) and used as culture medium for microalgal cultivation. The physicochemical composition of untreated and treated YR water was analyzed and the major nutrients in YR water including conductivity, total dissolved solid (TDS), salinity, dissolved oxygen (DO), total suspended solid (TSS), total nitrogen (TN), and total phosphorus (TP)

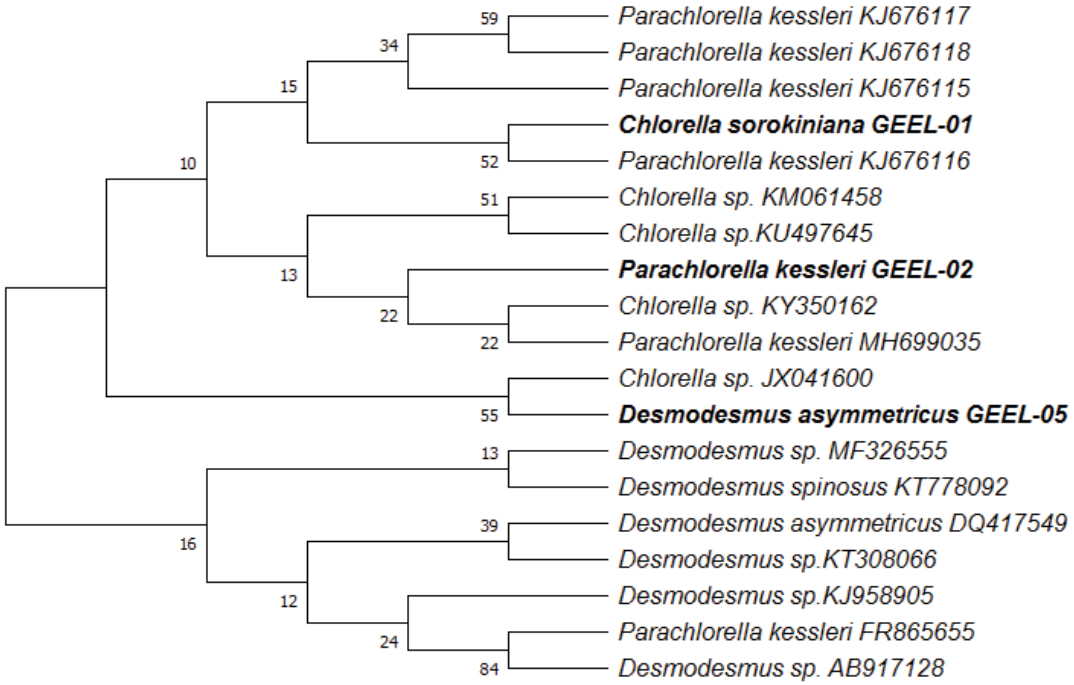

Fig. 1. Phylogenetic tree showing the relationship of the sequences of the isolates GEEL-01, GEEL-02, GEEL-05, and the most similar sequences retrieved from the NCEI nucleotide database.

were detected (Table 1). Two different pretreatment including autoclaving and filtration were applied in the current experiment and the microalgal growth was compared with untreated YR water (control). For sterilization of YR, autoclave (Shenan, LDZF-75L-II, China) was used at 121°C for 15 min. YR water was filtered through 0.22 μm cellulose filter paper attached to a device with circulating water vacuum pump (Yiheng, BSH-3A, China) for the removal of microalgae and other organisms. A volume of 180 mL from the YR water (including autoclaved, filtrated, and untreated) was added in 250 mL Erlenmeyer flasks. Then, microalgal inoculum (absorbance 0.2 at 680 nm) obtained at 10th day of active culture (log phase) was added. The flasks were incubated at 25°C ± 2°C, 150 rpm for 8 d under continuous illumination with an intensity of 40 μmol m^{-2}/s.

2.2. Determination of microalgal growth and nutrients removal

The microalgal growth was evaluated every 2 d by measuring the optical density (OD) at 680 nm using a spectrophotometer (UV 5500, Metash, China). The specific growth rate (μ) were calculated using the following equation:

$$\mu = \frac{\ln N_2 - \ln N_1}{t_2 - t_1} \quad (1)$$

where N_1 and N_2 are defined as biomass concentration at optical density (OD_{680nm}) at times t_1 and t_2, respectively.

TN and TP were measured according to the instruction of LH-NT 100 and LH-TP100 kit (Lianhua, Tech-Co., China) by water-quality auto-analyzer LH-3BN and 5b-3b (V8) (LianHua Tech-Co., China) [26]. Heavy metals were analyzed by ICP-OES (Perkin Elmer, PE Avio 500, America), where 5 mL water sample was filtered through 0.22 μm membrane filter, followed by adding nitric acid to the filtrate until the pH was <2. The pH of the culture solution was analyzed with a PHS-3C$^+$ pH-meter (REX Instruments, PHS-3C, Shanghai, China) [27].

2.3. Lipid and fatty acid composition analyses

After 8 d of cultivation, the microalgal biomass was harvested and used for lipid extraction following the method described by Bligh and Dyer [28]. In brief, 40 mg of microalgal biomass was mixed with 1.25 mL chloroform and 2.5 mL methanol (1:2, v/v). The mixture was sonicated for 30 min at frequency of 40 KHz (ultra sonicator Kunshan-KQ-300DB, China). The tubes were incubated for 12 h at 25°C ± 2°C with shaking at 150 rpm, followed by adding 1.25 mL chloroform in the tubes. The mixture was sonicated for 30 min, till the chloroform and aqueous methanol layers were separated and 1.25 mL deionized water was added. The chloroform layer was gently removed from the bottom after centrifugation at 3,900 rpm for 10 min and the remaining suspension was re-extracted using 2.5 mL chloroform. The chloroform portions were collected from the bottom of the tube gently and washed using 5 mL of NaCl solution (5%).

Fatty acids (FAs) were analyzed by modifying the method of Lepage and Roy [29], where 1 mL of sample was taken from the crude lipid layer, and 1 mL of methanol and 0.3 mL of H_2SO_4 were added. The mixture was vortexed for 3–5 min and incubated at 100°C for 10 min. After cooling at room temperature, 1 mL of distilled H_2O was added and vortexed for 3–5 min, followed by centrifugation at 4,000 rpm for 10 min. Then the lower phase (chloroform) was transferred to another tube. Fatty acids methyl esters (FAMEs) were evaluated using gas chromatograph equipped with a flame ionization detector GC-(FID) (Foli instruments, Model number, China) and a KB-FFAP (30 m × 0.32 mm) column. For FAMEs, the conditions of the inlet and detector were 240°C and 260°C, respectively, and the oven was set at 100°C (2 min), raised by 4°C/min to 180°C (0 min), and raised by 5°C 8/min to 235°C (9 min). Then, 1 μL sample was injected using nitrogen as the carrier gas. The mixture of FAMEs standard, Supelco® 37-component (Sigma-Aldrich, USA), was used to identify FAs composition present in the extracted microalgal lipid [30]. Other reagents used were of analytical grade.

2.4. Biodiesel properties based on FAME profiles

Biodiesel quality inculding unsaturation degree (UD), iodine value (IV), cetane number (CN), saponification value (SV), cold filter plugging point (CFPP), and long chain saturated factor (LCSF) were determined based on microalgal species fatty acid content using the following equations:

$$UD = \sum\left[MUFA + \left(2 \times PUFA\right)\right] \quad (2)$$

Table 1
Physiochemical characteristics of untreated and treated Yellow River (YR) water used in this study

Parameter	Untreated YR	Filtrated YR	Autoclaved YR
pH[a]	8.07	8.29	8.35
Conductivity (μS/cm)	472.00 ± 1.73	469.33 ± 1.15	342.33 ± 0.58
Total dissolved solid (TDS, mg/L)	335.00 ± 0.82	332.67 ± 0.47	243.67 ± 0.47
Salinity (mg/L)	227.33 ± 1.15	226.33 ± 0.58	166.33 ± 0.58
Dissolved oxygen (DO, μmol/L)	6.50 ± 0.10	3.77 ± 0.15	3.60 ± 0.10
Total nitrogen (TN, mg/L)	4.73 ± 0.27	3.10 ± 0.54	4.00 ± 1.40
Total phosphate (TP, mg/L)	0.69 ± 0.20	0.12 ± 0.07	0.60 ± 0.19
Total suspended solid (TSS, mg/L)	272.67 ± 19.14	ND	360.00 ± 12.96

[a]no unit, ND = not detected.

$$SV = \sum\left[\frac{560 \times N\%}{M}\right] \quad (3)$$

$$IV = \sum\left[\frac{254 \times N\% \times D}{M}\right] \quad (4)$$

$$CN = 46.3 + \left(\frac{5,458}{SV}\right) - \left(0.225 \times IV\right) \quad (5)$$

$$LCSF = \left(0.1 \times C16{:}0\right) + \left(0.5 \times C18{:}0\right) + \left(1 \times C20{:}0\right) + \left(1.5 \times C22{:}0\right) + \left(2 \times C24{:}0\right) \quad (6)$$

$$CFPP = \left(3.1417 \times LCSF\right) - 16.477 \quad (7)$$

where UD is the unsaturation degree (%), MUFA is monounsaturated fatty acids, PUFA is polyunsaturated fatty acids, *N*% is the percentage of each fatty acid, *M* represents the molecular weight of the fatty acid, *D* is the number of double bonds, SV is saponification value (mg KOH g^{-1}), IV is iodine value (g I_2/100 g oil), CN is the cetane number, LCSF is long-chain saturation factor, and C16:0, C18:0, C20:0, C22:0, and C24:0 represent the weight percentage of the corresponding fatty acids. Wt.% and cold filter plugging point (CFPP) (°C) were estimated [31].

2.5. Statistical analysis

All the experiments and analyses were carried out in triplicate and data are expressed as mean ± standard deviation (SD). SPSS 22.0 (IBM SPSS statistics) was used to perform all statistical analysis in this study. The difference between microalgal strains and variables (including growth kinetics, TN, and TP removal) were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests (at $p < 0.05$).

3. Results and discussion

3.1. Characterization of sampled water

In this study, Yellow River water was used as a culture medium for microalgal biomass growth due to the presense of major nutrients (Table 1). The concentrations of macronutient were total nitrogen (4.73 mg/L), and total phosphorus (0.69 mg/L), crucial for microalgal growth and biomass enhancement (Table 1). Microalgal growth is affected by various parameters such as pH, macro-micro-nutrients concentration temperature, and inoculum volume [32]. Yellow river water also showed presence of some HMs such as zinc (0.050 mg/L), copper (0.008 mg/L), cobalt (0.002 mg/L), nickel (0.013 mg/L), cadmium (0.003 mg/L), chromium (0.002 mg/L), and lead (0.001 mg/L), some of which play a vital role in cell functioning, catalytic cofactors for numerous metallo-enzymes, and can be removed by microalgae [33].

3.2. Microalgal growth assessment

Microalgal growth in the collected water sample (filtered, autoclaved, and untreated) was examined by optical density (OD_{680nm}) values and specific growth rate was calculated successively for 8 d (Fig. 2). *P. kessleri* GEEL-02 showed highest specific growth rate in both pretreatments and untreated sample ($p < 0.05$). The specific growth rate was highest at 2nd day for all microalgal strains under provided conditions. *P. kessleri* GEEL-02 had highest specific growth rate in filterated YR water (0.963 d^{-1}), autoclaved (0.843 d^{-1}), and untreated YR water (0.909 d^{-1}) (Fig. 2). Such high specific growth during the lag phase of the cultivation time might be due to the active inoculum and the optimal cultivation conditions (such as temperture, light, nutrients, and pH), which accelerate the microalgal growth [34]. The variations observed in specific growth rate under varying pretreatments might be also due to the species-specific proficiency of microlagal strains to endure YR toxicity. At 8th day, a slow

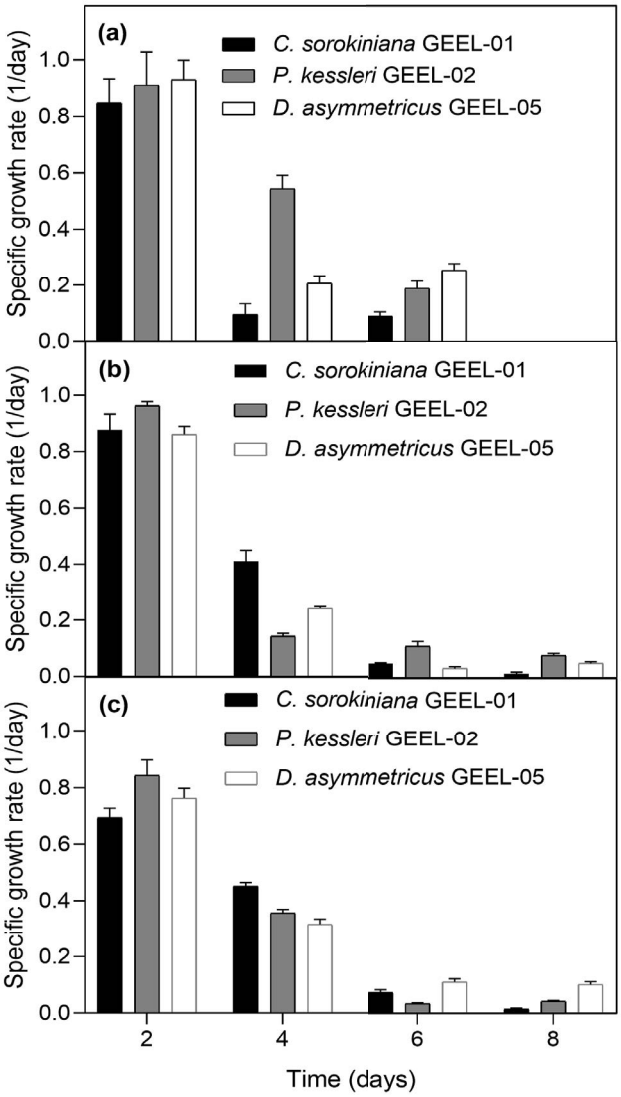

Fig. 2. Variation in specific growth rate of microalgae under different pretreatment: untreated (a), filtration (b), and autoclaved (c). Statistical differences among the microalgae of each group for specific growth rate were analyzed by one-way (ANOVA) followed by Duncan's multiple comparisons. The differences are marked by various alphabets.

growth was observed in both filtered group and autoclaved group, whereas no growth in untreated group. Microalgal growth were supported by macronutrients (including TC, TN, and TP) in all groups, however, the growth inhibition after 8 d might be due to the exhaustion of nutrients [35].

Microalgae has the capability to uptake CO_2 from medium and use Ci transporters to transport the CO_2 into the form of HCO_3^- inside the chloroplasts. The conversion of HCO_3^- to CO_2 is carried out by utilizing proton (H^+), during photosynthesis Rubisco fix the CO_2 and release OH^- inside cell. The neutralization of released OH^- require H^+ intake from outside culture medium which subsequently increase the pH [36]. An increase in the pH of the medium was observed in the beginning when microalgae started propagating and photosynthetic activity rises, but then declined in all groups. The pH for *D. asymmetricus* GEEL-05 showed highest increase and reached at 11.35 at 2nd day of untreated group. Minute variations in pH during lag phase were observed by Arif et al. [23], whereas, a differential increase during log phase and 9.4–10.5 at last day due to enhanced photosynthetic activity of microalgal species. The pH variation in medium depends on the photosynthetic activity and the growth status of different microalgae species [37].

3.3. Elimination of total nitrogen and phosphorus during microalgal growth

The approach of advanced wastewater treatment coupled with biomass/fatty acids accumulation by microalgal cultivation has been received much attention [38]. In this study, all three microalgae strains demonstrated a positive outcome in TN and TP elimination from the YR water. The average range of TN removal for all species was 88.88%–98.14% under different pretreatments (Figs. 3a–c). Nitrogen is among the eminent nutrient assisting biomass accumulation process in microalgae, as it contributes to 1%–10% of the total biomass content, and play an important factor in synchronizing the lipid content inside microalgal cells [39,40].

Furthermore, phosphorus utilization in energy metabolism of algae is a key factor that can promote algal growth. However, external parameters such as high pH and dissolved oxygen concentration in culture media can cause phosphorus precipitation [41]. *D. asymmetricus* GEEL-05 showed the highest removal rate of TP under all pretreatment conditions (Figs. 3a–c). TP removal was significant at 2nd day for all strains, while the highest removal of TP > 95% was observed at the end of cultivation (8th day). A significant difference was observed among three microalgae strains ($p < 0.05$) for TN and TP removal (Fig. 3).

A comparison of TN and TP removal efficiency of several microalgal strains cultivated in pretreated wastewaters is presented in Table 2. It was observed that, pretreatment of wastewater prior to microalgal cultivation resulted in 65%–100% TN and TP removal from anaerobic digestor, municipal, and piggery/swine wastewater (Table 2). In most cases, the performance efficiency of microalgae is higher after autoclaving and filtration of wastewater, as these approaches helps in reducing the load of suspended solids and other competing microorganisms (such as bacteria and fungi) [42]. Therefore, the availability of TN and TP for microalgal growth can be ensured by coupling microalgal cultivation with suitable pretreatment prior to cultivation.

3.4. Removal of cations during microalgal growth

The metal removal efficiency of microalgal strains for copper (Cu^{2+}), cobalt (Co^{2+}), and zinc (Zn^{2+}) is shown in Fig. 4. The main mechanisms employed in elimination of metal cations are biosorption and bioaccumulation by the cell membrane, and cation transport during cultivation of microalgae [43]. The highest removal rate of Zn^{2+} was

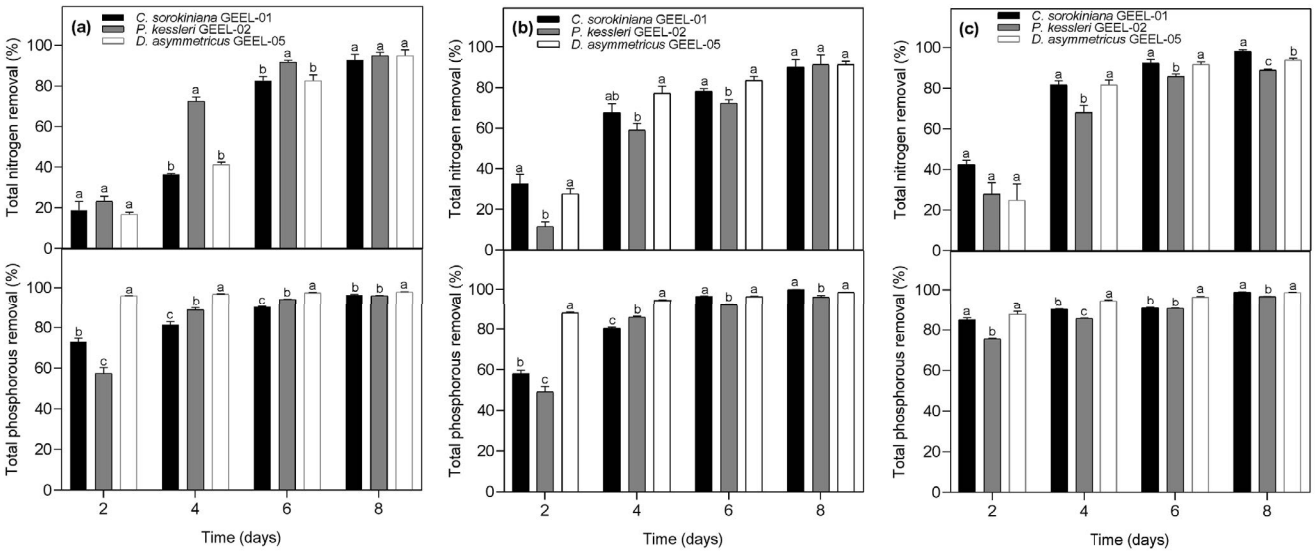

Fig. 3. Removal of total nitrogen and phosphorus from Yellow River water using various microalgal strains under different pretreatment. While panels (a–c) represent untreated, filtrated, and autoclaved Yellow River water. Statistical differences among the microalgal strains of each treatment for nutrients removal were analyzed by one-way (ANOVA) followed by Duncan's multiple comparisons. The differences are marked by various alphabets.

Table 2
Removal of TN and TP from various wastewater by different microalgae in this study and previous reported work

Cultivation media	Microalgal species	Cultivation period (days)	Pretreatment used	Nutrient removal (%)	Reference
Anaerobic digestate	*C. sorokiniana* UTEXI230	4	Autoclaved	65 TN, NM TP	[57]
Anaerobic digestate	*C. sorokiniana* UTEXI230	4	Dilution, filtration	95.3 TN, 78.3 TP	
Municipal wastewater	*Chlorella vulgaris, Scenedesmus obliquus*	10	Filtration	>99 TN, >99 TP	[38]
Piggery wastewater	*Chlorella zofingiensis*	10	Sedimentation, filtration and autoclave	68.9–82.7 TN, 85–100 TP	[58]
Municipal wastewaters	*M. reisseri*	8	Filtration	86 TN, 95 TP	[59]
Municipal wastewaters	*Ourococcus, Multisporus*	7	Filtration	100 TN, 100 TP	[60]
Swine wastewater	*Chlorella vulgaris*	16	Filtration	95 TN, 95 TP	[61]
Municipal wastewater	*Chlorella* sp.	14	Sedimentation and filtration	89 TN, 79–81 TP	[62]
Yellow River water	*C. sorokiniana*, *P. kessleri*, and *D. asymmetricus*	8	Filtration and autoclave	>90 TN, >95 TP	This study

NA = not mentioned.

98.1% by *D. asymmetricus* GEEL-05 in autoclaved group and 96.0% by *P. kessleri* GEEL-02 in filtration group. In autoclaved group, 93.9% of Cu^{2+} was removed by *D. asymmetricus* GEEL-05 after 8 d. Altogether, *D. asymmetricus* GEEL-05 showed a significant removal efficiency for Cu^{2+} and Zn^{2+} in pretreated samples. Metal adsorption depends on the structure and composition of cell-wall in microenvironment [43]. Multi-metal biosorption capacity of microalgae was lower than single-metal biosorption, when used to treat a mixture of metal solution [44,45]. Zn^{2+} is a crucial element for microalgae growth, it plays an eminent role in multiple metabolic pathway such as photosynthesis, energy storage, and activation of multiple enzymes [46]. The removal of Zn^{2+} by three strains was efficient in present study, which is in accordance with Yang et al. [38]. High accumulation of Cu^{2+} due to inability to pass through algal membranes can cause toxicity to microalgae and growth inhibition [47,48]. Microorganisms opt diverse methods to remove metals such as chelation, complexation, microprecipitation, and ion exchange [49,50].

3.5. Fatty acids composition of microalgae species

Microalgal FAs composition has been used as a potential indicator of biodiesel quality determination. The C16 and C18 contents (as % of the total FAME) of microalgae have been used to assess biodiesel/oil productivity [51]. Microalgal FAs profile generally depends on the microalgal physiology, cultivation media, nutrients level, temperature, light spectra, and intensity [52]. FAs composition of the microalgal species is represented in Table 3 and Fig. 5. The highest SFAs accumulation (74.72% and 74.48%) was observed in autoclaved YR water group for *C. sorokiniana* GEEL-01 and *P. kessleri* GEEL-02, respectively. Moreover, it was 71.84% in filtered YR water for *D. asymmetricus* GEEL-05 (Table 3). SFAs of *C. sorokiniana* GEEL-01 and *P. kessleri*

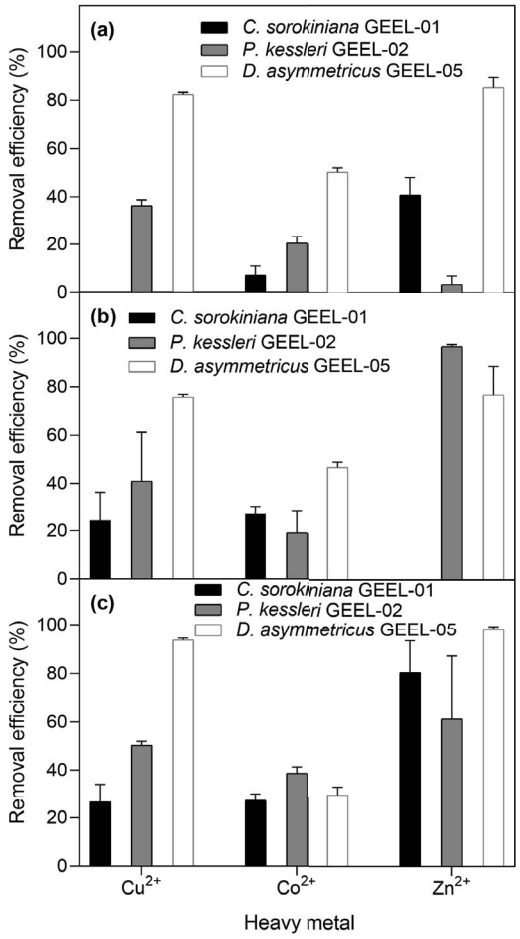

Fig. 4. Heavy metals (Cu^{2+}, Co^{2+}, and Zn^{2+}) removal efficiency of different microalgae under different pretreatment: untreated (a), filtration (b), and autoclaved (c).

Table 3
Fatty acids saturation degree (%) of microalgal lipids after cultivated for 8 d under different pretreatment

FAME (%, W/W)	Untreated YR			Filtrated YR			Autoclaved YR		
	C. sorokiniana GEEL-01	*P. kessleri* GEEL-02	*D. asymmetricus* GEEL-05	*C. sorokiniana* GEEL-01	*P. kessleri* GEEL-02	*D. asymmetricus* GEEL-05	*C. sorokiniana* GEEL-01	*P. kessleri* GEEL-02	*D. asymmetricus* GEEL-05
Saturated fatty acids	39.76 ± 6.64	39.14 ± 8.79	57.64 ± 15.55	69.09 ± 3.88	58.33 ± 0.72	71.84 ± 2.83	74.72 ± 3.26	74.48 ± 0.76	64.21 ± 2.88
Monounsaturated fatty acids	29.06 ± 16.07	25.72 ± 11.43	15.12 ± 2.05	12.06 ± 0.37	26.69 ± 2.33	15.11 ± 1.43	14.87 ± 1.29	13.52 ± 1.61	13.56 ± 3.18
Polyunsaturated fatty acids	31.17 ± 9.63	35.13 ± 4.72	27.24 ± 13.69	18.84 ± 4.25	14.96 ± 2.98	13.05 ± 2.23	10.40 ± 2.03	11.99 ± 1.22	22.22 ± 5.72
Σ*C*12–*C*18	63.65	72.05	71.14	87.92	90.52	80.28	53.38	63.92	65.02

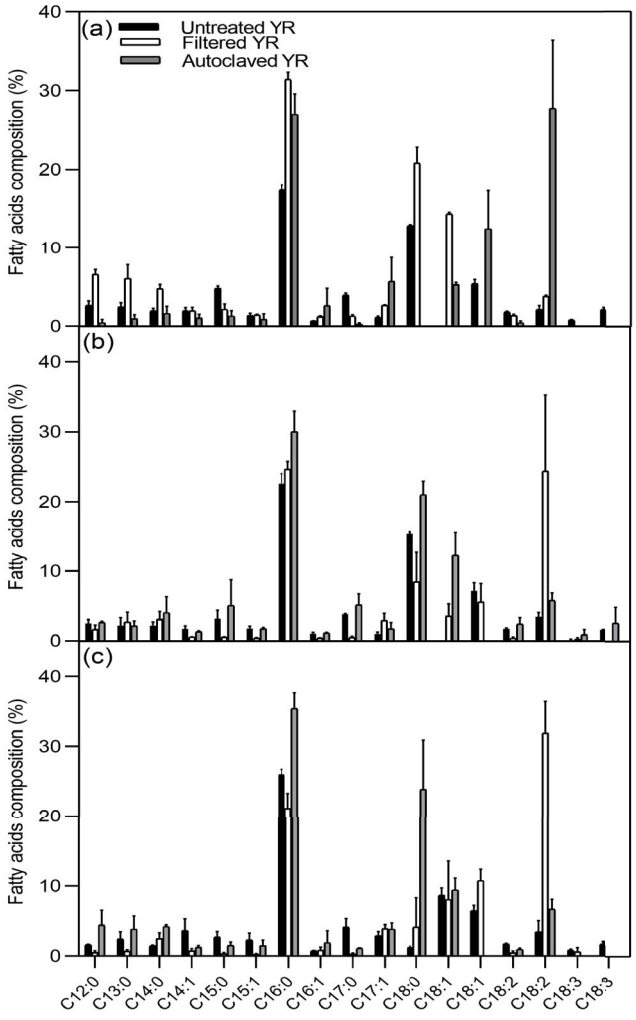

Fig. 5. Fatty acids composition (%) of *C. sorokiniana* GEEL-01 (a), *P. kessleri* GEEL-02 (b), and *D. asymmetricus* GEEL-05 (c) after 8 d cultivation in treated and untreated YR water.

GEEL-02 were increased in comparison to the FAs profile of same strains cultivated in synthetic medium previously [23].

Among the SFAs, palmitic acid (C16:0) was the major FAs which accounted for 32.43%, 30.04%, and 35.41% in *C. sorokiniana* GEEL-01, *P. kessleri* GEEL-02, and *D. asymmetricus* GEEL-05 cultivated in pretreated YR water (Fig. 5). Microalgal lipids with high C16:0 have been reported to contribute higher oxidative stability, high cetane number, and lower NO× emissions [53]. Pretreatments may decrease nutrients availability, especially *N* (Table 1), that would be the reason for increase in lipids/FAs in the current study, as it has been reported that, low *N* concertation reduces protein synthesis which leads to high lipids/FAs production [54].

The major unsaturated fatty acids (USFAs) were C18:1 and C18:2. C18:2 was found to be highly influenced by treatment of the cultivation medium. Autoclaved pretreatment of YR improved C18:2 in *C. sorokiniana* GEEL-01. While, filtration enhanced the accumulation of C18:2 in *P. kessleri* GEEL-02 and *D. asymmetricus* GEEL-05

(Fig. 5). High USFAs (such as C18:1 and C18:2) content in oils offers a reasonable balance of fuel properties (including combustion heat, ignition quality, oxidative stability, cold filter plugging point, lubricity, and viscosity). Other FAs existed in smaller amounts (Fig. 5).

3.6. Biodiesel characteristics estimation according to microalgal FAME

The significant biodiesel characteristics that are influenced by the fatty acid composition, and subsequently by the structural features of the several fatty esters are the unsaturation degree (DU), saponification value (SV), cold filter plugging point (CFPP), long-chain saturated factor (LCSF), iodine value (IV), and cetane number (CN). Tri-acylglycerols and phospholipids assimilated these fatty acids and eventually converts them to biodiesel in a procedure identified as transesterification [38]. Ignition quality of a diesel fuel is described by CN value [55]. For all microalgal species, CN value was ranged in between 52.94–65.26 which are higher than the suggested value (≥45) of biodiesel standards (including ASTM D6751, EN 14214, Australian standard, and Brazilian standard ANP255) [38,56]. The estimated biodiesel properties were in accordance with biodiesel derived from microalgae and crops (Table 4). IV describes the content of unsaturated FAs. The assessed IV in this study was ≤120 for *C. sorokiniana* GEEL-01, *P. kessleri* GEEL-02, and *D. asymmetricus* GEEL-05. A maximum value of 120 g I_2 100 g^{-1} is necessary as heating of higher unsaturated fatty acids (i.e., –C=C–) results in glycerides polymerization, causing deposition and lubricating oil deterioration, which is increased by the presence high double bonds content in microalgal FAs chain. Hence, it is suggested to reduce the USFAs accumulation. In the current study, IV values in the estimated microalgal biodiesel were lower than the maximum limits approved by EN 14214. The estimated biodiesel had high LCSF (wt.%) and CFPP (°C) due to high SFAs of microalgal lipids. Biodiesel with high LCSF and CFPP can be used in hot regions only. High SFAs lower the IV value which prevents oil oxidation and deterioration, however, it limits the use of biodiesel in colder region [23,56].

4. Conclusions

Microalgal strains cultured in Yellow River water were found to be promising for nutrients (N, P) removal, heavy metal remediation, and biodiesel production. *P. kessleri* showed relatively higher specific growth rate when cultured in different groups of pretreated and unpretreated water sample. In addition, *D. asymmetricus* exhibited high nutrients removal efficiency under both pretreated and untreated water sample. Fatty acid composition of microalgae species displayed high concentration of major fatty acids (C12/C18). High long chain fatty acids content in the microalgal lipids improved the biodiesel properties. The significant nutrients removal, and fatty acid profile of *D. asymmetricus* and *P. kessleri* makes these two strains feasible alternative feedstocks for biodiesel production coupled with nutrients removal.

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Table 4
Properties of biodiesel produced from microalgal species used in this study compared with previous studies and international standards

Treatment used	Microalgal species	UD	SV	IV	CN	LCSF	CFPP	References
Untreated YR	*C. sorokiniana* GEEL-01	91.40	203.75	52.82	61.20	16.49	35.33	This study
	P. kessleri GEEL-02	95.98	201.26	39.74	64.47	19.39	45.55	
	D. asymmetricus GEEL-05	69.59	200.87	55.38	61.00	18.79	44.45	
Filtrated YR	*C. sorokiniana* GEEL-01	49.75	200.55	81.26	55.23	4.99	–0.81	This study
	P. kessleri GEEL-02	56.52	208.54	86.78	59.42	7.53	7.21	
	D. asymmetricus GEEL-05	41.21	206.59	59.07	52.94	13.48	25.87	
Autoclaved YR	*C. sorokiniana* GEEL-01	35.68	215.89	37.42	63.16	15.65	32.70	This study
	P. kessleri GEEL-02	37.51	198.77	61.94	59.82	19.74	33.92	
	D. asymmetricus GEEL-05	58.01	206.41	33.21	65.26	19.39	36.48	
Amphora sp.		55.00	57.56	57.56	62.33	9.19	12.41	[56]
Chlamydomonas reinhardtii		87.02	170.56	102.35	55.27	4.58	–2.09	
Chlorella sp.		74.1	217.8	65	56.7	6.7	4.5	[63]
Parachlorella hussii		–	184.06	57.03	63.12	–	–	[64]
Soybean		112	204	139.5	–	3.1	–6.74	[65]
Palm		–	205	59	64.5	–	–	
EN 14214				≤120	≥51		≤5/≤–20	[38]
ASTM 6751-03					≥47			

UD = Unsaturation degree; CN = cetane number; IV = iodine value as g I_2/100 g oil; SV = saponification value as mg $KOHg^{-1}$; LCSF = long chain saturation factor as wt.%; CFPP = Cold filter plugging point as °C.

References

[1] X. Liao, Y. Tian, Y. Gan, J. Ji, Quantifying urban wastewater treatment sector's greenhouse gas emissions using a hybrid life cycle analysis method - an application on Shenzhen city in China, Sci. Total Environ., 745 (2020) 141176, doi: 10.1016/j.scitotenv.2020.141176.

[2] R. Kumar, P. Pal, Assessing the feasibility of N and P recovery by struvite precipitation from nutrient-rich wastewater: a review, Environ. Sci. Pollut. Res. Int., 22 (2015) 17453–17464.

[3] K. Li, Q. Liu, F. Fang, R. Luo, Q. Lu, W. Zhou, S. Huo, P. Cheng, J. Liu, M. Addy, P. Chen, D. Chen, R. Ruan, Microalgae-based wastewater treatment for nutrients recovery: a review, Bioresour. Technol., 291 (2019) 121934, doi: 10.1016/j.biortech.2019.121934.

[4] R. Whitton, F. Ometto, M. Pidou, P. Jarvis, R. Villa, B. Jefferson, Microalgae for municipal wastewater nutrient remediation: mechanisms, reactors and outlook for tertiary treatment, Environ. Technol. Rev., 4 (2015) 133–148.

[5] F. Polesel, H.R. Andersen, S. Trapp, B.G. Plósz, Removal of antibiotics in biological wastewater treatment systems a critical assessment using the activated sludge modeling framework for xenobiotics (ASM-X), Environ. Sci. Technol., 50 (2016) 10316–10334.

[6] L. Zhu, S. Li, T. Hu, Y.K. Nugroho, Z. Yin, D. Hu, R. Chu, F. Mo, C. Liu, E. Hiltunen, Effects of nitrogen source heterogeneity on nutrient removal and biodiesel production of mono- and mix-cultured microalgae, Energy Convers. Manage., 201 (2019) 112144, doi: 10.1016/j.enconman.2019.112144.

[7] E.-S. Salama, M.B. Kurade, R.A.I. Abou-Shanab, M.M. El-Dalatony, I.-S. Yang, B. Min, B.-H. Jeon, Recent progress in microalgal biomass production coupled with wastewater treatment for biofuel generation, Renewable Sustainable Energy Rev., 79 (2017) 1189–1211.

[8] A.E.-F. Abomohra, W. Jin, R. Tu, S.-F. Han, M. Eid, H. Eladel, Microalgal biomass production as a sustainable feedstock for biodiesel: current status and perspectives, Renewable Sustainable Energy Rev., 64 (2016) 596–606.

[9] K. Suresh Kumar, H.-U. Dahms, E.-J. Won, J.-S. Lee, K.-H. Shin, Microalgae – a promising tool for heavy metal remediation, Ecotoxicol. Environ. Saf., 113 (2015) 329–352.

[10] A.K. Zeraatkar, H. Ahmadzadeh, A.F. Talebi, N.R. Moheimani, M.P. McHenry, Potential use of algae for heavy metal bioremediation, a critical review, J. Environ. Manage., 181 (2016) 817–831.

[11] S.K. Mehta, J.P. Gaur, Use of algae for removing heavy metal ions from wastewater: progress and prospects, Crit. Rev. Biotechnol., 25 (2005) 113–152.

[12] D. Kaplan, Absorption and adsorption of heavy metals by microalgae, Appl. Phycol. Biotechnol., 2 (2013) 602–611.

[13] E.-S. Salama, H.-S. Roh, S. Dev, M.A. Khan, R.A.I. Abou-Shanab, S.W. Chang, B.-H. Jeon, Algae as a green technology for heavy metals removal from various wastewater, World J. Microbiol. Biotechnol., 35 (2019) 1–19, doi: 10.1007/s11274-019-2648-3.

[14] E. Priyadarshini, S.S. Priyadarshini, N. Pradhan, Heavy metal resistance in algae and its application for metal nanoparticle synthesis, Appl. Microbiol. Biotechnol., 103 (2019) 3297–3316.

[15] A. Mantzorou, E. Navakoudis, K. Paschalidis, F. Ververidis, Microalgae: a potential tool for remediating aquatic environments from toxic metals, Int. J. Environ. Technol., 15 (2018) 1815–1830.

[16] Y. Ling, L.-p. Sun, S.-y. Wang, C.S.K. Lin, Z. Sun, Z.-g. Zhou, Cultivation of oleaginous microalga *Scenedesmus obliquus* coupled with wastewater treatment for enhanced biomass and lipid production, Biochem. Eng. J., 148 (2019) 162–169.

[17] C.M. Beal, L.N. Gerber, D.L. Sills, M.E. Huntley, S.C. Machesky, M.J. Walsh, J.W. Tester, I. Archibald, J. Granados, C.H. Greene, Algal biofuel production for fuels and feed in a 100-ha facility: a comprehensive techno-economic analysis and life cycle assessment, Algal Res., 10 (2015) 266–279.

[18] G. Huang, F. Chen, D. Wei, X. Zhang, G. Chen, Biodiesel production by microalgal biotechnology, Appl. Energy, 87 (2010) 38–46.

[19] Y. Jiang, U.J. Yves, H. Sun, X. Hu, H. Zhan, Y. Wu, Distribution, compositional pattern and sources of polycyclic aromatic hydrocarbons in urban soils of an industrial city, Lanzhou, China, Ecotoxicol. Environ. Saf., 126 (2016) 154–162.

[20] H. Tong, P. Shi, City profile Lanzhou, Cities, 45 (2015) 51–59.

[21] Y. Hou, W. Chu, M. Ma, Carbonaceous and nitrogenous disinfection by-product formation in the surface and ground water treatment plants using Yellow River as water source, J. Environ. Sci., 24 (2012) 1204–1209.

[22] S. Zhao, B. Gao, Q. Yue, S. Sun, Y. Wang, Q. Li, Determination of active ingredients of a new coagulant aid-Enteromorpha by floc characteristics on-line monitoring in Yellow River water treatment, Chem. Eng. J., 232 (2013) 310–318.

[23] M. Arif, L. Wang, E.-S. Salama, M.S. Hussain, X. Li, M. Jalalah, M.S. Al-Assiri, F.A. Harraz, M.-K. Ji, P. Liu, Microalgae isolation for nutrient removal assessment and biodiesel production, Bioenergy Res. (2020) 1247–1259, doi: 10.1007/s12155–020–10136–5.

[24] G. Gatidou, P. Anastopoulou, M. Aloupi, A.S. Stasinakis, Growth inhibition and fate of benzotriazoles in *Chlorella sorokiniana* cultures, Sci. Total Environ., 663 (2019) 580–586.

[25] S.K. Gupta, F.A. Ansari, A. Shriwastav, N.K. Sahoo, I. Rawat, F. Bux, Dual role of *Chlorella sorokiniana* and *Scenedesmus obliquus* for comprehensive wastewater treatment and biomass production for bio-fuels. J. Cleaner Prod., 115 (2016) 255–264.

[26] F. Ndayisenga, Z. Yu, Y. Yu, C.H. Lay, D. Zhou, Bioelectricity generation using microalgal biomass as electron donor in a bio-anode microbial fuel cell, Bioresour. Technol., 270 (2018) 286–293.

[27] G. Chen, R. Shan, S. Li, J. Shi, A biomimetic silicification approach to synthesize CaO–SiO_2 catalyst for the transesterification of palm oil into biodiesel, Fuel, 153 (2015) 48–55.

[28] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol., 37 (1959) 911–917, doi: 10.1139/y59-099.

[29] G. Lepage, C.C. Roy, Improved recovery of fatty acid through direct transesterification without prior extraction or purification, J. Lipid Res., 25 (1984) 1391–1396.

[30] R.A.I. Abou-Shanab, I.A. Matter, S.-N. Kim, Y.-K. Oh, J. Choi, B.-H. Jeon, Characterization and identification of lipid-producing microalgae species isolated from a freshwater lake, Biomass Bioenergy, 35 (2011) 3079–3085.

[31] L.F. Ramírez-Verduzco, J.E. Rodríguez-Rodríguez, A.d.R. Jaramillo-Jacob, Predicting cetane number, kinematic viscosity, density and higher heating value of biodiesel from its fatty acid methyl ester composition, Fuel, 91 (2012) 102–111.

[32] E. Daneshvar, C. Santhosh, E. Antikainen, A. Bhatnagar, Microalgal growth and nitrate removal efficiency in different cultivation conditions: effect of macro and micronutrients and salinity, J. Environ. Chem. Eng., 6 (2018) 1848–1854.

[33] H. Alishah Aratboni, N. Rafiei, R. Garcia-Granados, A. Alemzadeh, J.R. Morones-Ramirez, Biomass and lipid induction strategies in microalgae for biofuel production and other applications, Microb. Cell Fact., 18 (2019) 178, doi: 10.1186/s12934-019-1228-4.

[34] W. Lu, Z. Wang, X. Wang, Z. Yuan, Cultivation of *Chlorella* sp. using raw dairy wastewater for nutrient removal and biodiesel production: characteristics comparison of indoor bench-scale and outdoor pilot-scale cultures, Bioresour. Technol., 192 (2015) 382–388.

[35] W.N.A. Kadir, M.K. Lam, Y. Uemura, J.W. Lim, K.T. Lee, Harvesting and pre-treatment of microalgae cultivated in wastewater for biodiesel production: a review, Energy Convers. Manage., 171 (2018) 1416–1429.

[36] Z. Chi, J.V. O'Fallon, S. Chen, Bicarbonate produced from carbon capture for algae culture, Trends Biotechnol., 29 (2011) 537–541.

[37] C. Zhu, X. Zhai, Y. Xi, J. Wang, F. Kong, Y. Zhao, Z. Chi, Efficient CO_2 capture from the air for high microalgal biomass production by a bicarbonate Pool, J. CO_2 Util., 37 (2020) 320–327.

[38] I.-S. Yang, E.-S. Salama, J.-O. Kim, S.P. Govindwar, M.B. Kurade, M. Lee, H.-S. Roh, B.-H. Jeon, Cultivation and harvesting of

microalgae in photobioreactor for biodiesel production and simultaneous nutrient removal, Energy Convers. Manage., 117 (2016) 54–62.
[39] E.M. Fakhry, D.M. El Maghraby, Lipid accumulation in response to nitrogen limitation and variation of temperature in *Nannochloropsis salina*, Bot Stud., 56 (2015) 1–8, doi: 10.1186/s40529-015-0085-7.
[40] F. Qie, J. Zhu, J. Rong, B. Zong, Biological removal of nitrogen oxides by microalgae, a promising strategy from nitrogen oxides to protein production, Bioresour. Technol., 292 (2019) 122037, doi: 10.1016/j.biortech.2019.122037.
[41] T. Cai, S.Y. Park, Y. Li, Nutrient recovery from wastewater streams by microalgae: status and prospects, Renewable Sustainable Energy Rev., 19 (2013) 360–369.
[42] L. Yang, H. Li, Q. Wang, A novel one-step method for oil-rich biomass production and harvesting by co-cultivating microalgae with filamentous fungi in molasses wastewater, Bioresour. Technol., 275 (2019) 35–43.
[43] Y.K. Leong, J.S. Chang, Bioremediation of heavy metals using microalgae: recent advances and mechanisms, Bioresour. Technol., 303 (2020) 122886, doi: 10.1016/j.biortech.2020.122886.
[44] P.I. Gomes, T. Asaeda, Phycoremediation of chromium(VI) by *Nitella a*nd impact of calcium encrustation, J. Hazard. Mater., 166 (2009) 1332–1338.
[45] P.I. Gomes, T. Asaeda, Phytoremediation of heavy metals by calcifying macro-algae (*Nitella pseudoflabellata*): implications of redox insensitive end products, Chemosphere, 92 (2013) 1328–1334.
[46] M. Chen, H. Tang, H. Ma, T.C. Holland, K.Y. Ng, S.O. Salley, Effect of nutrients on growth and lipid accumulation in the green algae *Dunaliella tertiolecta*, Bioresour. Technol., 102 (2011) 1649–1655.
[47] L. Rugnini, G. Costa, R. Congestri, L. Bruno, Testing of two different strains of green microalgae for Cu and Ni removal from aqueous media, Sci. Total Environ., 601–602 (2017) 959–967.
[48] W. Shi, X. Fang, X. Wu, G. Zhang, W. Que, F. Li, Alteration of bioaccumulation mechanisms of Cu by microalgae in the presence of natural fulvic acids, Chemosphere, 211 (2018) 717–725.
[49] D.M. Park, D.W. Reed, M.C. Yung, A. Eslamimanesh, M.M. Lencka, A. Anderko, Y. Fujita, R.E. Riman, A. Navrotsky, Y.Q. Jiao, Bioadsorption of rare earth elements through cell surface display of lanthanide binding tags, Environ. Sci. Technol., 50 (2016) 2735–2742.
[50] K.S. Kumar, H.-U. Dahms, E.-J. Won, J.-S. Lee, K.-H. Shin, Microalgae-a promising tool for heavy metal remediation, Ecotoxicol. Environ. Saf., 113 (2015) 329–352.
[51] Z.L. Poh, W.N. Amalina Kadir, M.K. Lam, Y. Uemura, U. Suparmaniam, J.W. Lim, P.L. Show, K.T. Lee, The effect of stress environment towards lipid accumulation in microalgae after harvesting, Renewable Energy, 154 (2020) 1083–1091.
[52] F. Gao, H.L. Yang, C. Li, Y.Y. Peng, M.M. Lu, W.H. Jin, J.J. Bao, Y.M. Guo, Effect of organic carbon to nitrogen ratio in wastewater on growth, nutrient uptake and lipid accumulation of a mixotrophic microalgae *Chlorella* sp., Bioresour. Technol., 282 (2019) 118–124.
[53] W.P. Sandani, G.K.S.H. Nishshanka, R.G.M.M. Premaratne, S.C. Nanayakkara Wijayasekera, T.U. Ariyadasa, J.K. Prema-chandra, Comparative assessment of pretreatment strategies for production of microalgae-based biodiesel from locally isolated *Chlorella homosphaera*, J. Biosci. Bioeng., 130 (2020) 295–305.
[54] J.K. Suastes-Rivas, R. Hernández-Altamirano, V.Y. Mena-Cervantes, E.J. Barrios Gómez, I. Chairez, Biodiesel production, through intensification and profitable distribution of fatty acid methyl esters by a microalgae-yeast co-culture, isolated from wastewater as a function of the nutrients' composition of the culture media, Fuel, 280 (2020) 118633, doi: 10.1016/j.fuel.2020.118633.
[55] M.A. Islam, G.A. Ayoko, R. Brown, D. Stuart, K. Heimann, Influence of fatty acid structure on fuel properties of algae derived biodiesel, Procedia Eng., 56 (2013) 591–596.
[56] É.C. Francisco, D.B. Neves, E. Jacob-Lopes, T.T. Franco, Microalgae as feedstock for biodiesel production: carbon dioxide sequestration, lipid production and biofuel quality, J. Chem. Technol. Biotechnol., 85 (2010) 395–403.
[57] F. Sayedin, A. Kermanshahi-pour, Q.S. He, S.M. Tibbetts, C.G. Lalonde, S.K.J.A.R. Brar, Microalgae cultivation in thin stillage anaerobic digestate for nutrient recovery and bioproduct production, Algal Res., 47 (2020) 101867, doi: 10.1016/j.algal.2020.101867.
[58] L. Zhu, Z. Wang, Q. Shu, J. Takala, E. Hiltunen, P. Feng, Z. Yuan, Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment, Water Res., 47 (2013) 4294–4302.
[59] R.A.I. Abou-Shanab, M.M. El-Dalatony, M.M. El-Sheekh, M.-K. Ji, E.-S. Salama, A.N. Kabra, B.-H. Jeon, Cultivation of a new microalga, *Micractinium reisseri*, in municipal wastewater for nutrient removal, biomass, lipid, and fatty acid production, Biotechnol. Bioprocess Eng., 19 (2014) 510–518.
[60] 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.
[61] K. Nam, H. Lee, S.-W. Heo, Y.K. Chang, J.-I. Han, Cultivation of *Chlorella vulgaris* with swine wastewater and potential for algal biodiesel production, J. Appl. Phycol., 29 (2016) 1171–1178.
[62] Y. Li, Y.-F. 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.
[63] A.F. Talebi, S.K. Mohtashami, M. Tabatabaei, M. Tohidfar, A. Bagheri, M. Zeinalabedini, H. Hadavand Mirzaei, M. Mirzajanzadeh, S. Malekzadeh Shafaroudi, S. Bakhtiari, Fatty acids profiling: a selective criterion for screening microalgae strains for biodiesel production, Algal Res., 2 (2013) 258–267.
[64] R.K. Bharti, D.W. Dhar, R. Prasanna, A.K. Saxena, Assessment of biomass and lipid productivity and biodiesel quality of an indigenous microalga *Chlorella sorokiniana* MIC-G5, Int. J. Green Energy, 15 (2017) 45–52.
[65] A. Gopinath, S. Puhan, G. Nagarajan, Theoretical modeling of iodine value and saponification value of biodiesel fuels from their fatty acid composition, Renewable Energy, 34 (2009) 1806–1811.