Simultaneous phycoremediation of petrochemical wastewater and lipid production by *Chlorella vulgaris*

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Abstract

A novel strategy of using microalgae *Chlorella vulgaris* for simultaneous bio-treatment of petrochemical wastewater and lipid production was developed in the present study. Phycoremediation was carried out in 30 days. The profile of fatty acids was identified, and the specifications of biodiesel including saponification value, iodine value, cetane number, long-chain saturated factor, cold filter plugging point, cloud point, allylic position equivalent and bis-allylic position equivalent were predicted by BiodieselAnalyzer® software. Besides, polycyclic aromatic hydrocarbons were determined in both wastewater samples and produced lipid. The observed data showed that biodiesel from *C. vulgaris* was superior to petrodiesel in terms of suitability in diesel engines. Moreover, contamination of petrochemical wastewater can influence the expression of a variety of genes in algae. To investigate the effectiveness of contamination on the expression of lipid synthesis as well as three photosynthesis genes, a real-time polymerase chain reaction assay was used to quantify transcript levels of *PsaB* (photosystem I reaction center protein subunit B), *psbC* (an integral membrane protein component of photosystem II), and *rbcl* (a large subunit of ribulose-1,5-bisphosphate carboxylase oxygenase). Furthermore, the gene expression level of *accD* (acetyl-coenzyme A carboxylase carboxyl transferase subunit beta, chloroplastic) was studied to discover the effect of wastewater on lipid production. The results showed that when diluted petrochemical wastewater (50%) was used as a media for *C. vulgaris* cultivation, these genes expression significantly increased. For 50% diluted wastewater, the maximum removal of BOD, COD, total nitrogen, and total phosphor has been 30.36%, 10.89%, 69.89%, and 92.59%, respectively.

Keywords Phycoremediation · Petrochemical wastewater treatment · Biodiesel · Real-time PCR · *Chlorella vulgaris*

1 Introduction

Most of the industrial sectors cause severe negative impacts on the environment. Petrochemical industries are one of the major polluting industries [1]. In the

phycoremediation method, algae are used to treat environmental contaminants [2]. Phycoremediation technology is economical, sustainable, energy-efficient, and highly proficient in removing pollutants from the water in comparison with the physical and chemical treatment

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methods [3]. In microalgae treatment, wastewater's nutrients and organics are converted to algae, which had raised interest over the last decades due to the sustainable production of lipid and other high-value biomolecules [4]. Microalgae are a large group of phototrophic microorganisms that play a significant role in the biofuel production field for several reasons, such as the ability to mitigate CO₂, reduce greenhouse gas emission, and not competing with the food supply [5, 6]. Algae have low area requirements, tolerance to harsh conditions, and the ability to grow in almost all types of water [7, 8]. More importantly, microalgae are proficient in removing nutrients from wastewater and simultaneously generate biomass for biofuel [9]. Biofuel produced by microalgae is known as a carbon-neutral energy resource, and its consumption could reduce up to 50% of CO₂ emissions compared with traditional petrodiesel [10]. Although traditional techniques are still in use, algae cultivation, as a novel approach, has been recently developed to treat wastewater with a high concentration of nutrients that have been developed in recent years [11]. *Chlorella vulgaris* is applied for treating wastewater as it can eliminate nitrogen, phosphorus, BOD, and COD successfully with diverse retention times in the range of 10 h to 42 days [12–14]. Salgueiro and co-authors (2016) studied the capacity of *C. vulgaris* for eliminating the phosphorous and organic matters from wastewater [15]. They proposed that *C. Vulgaris* can be utilized as a right candidate for reducing phosphorus level and chemical oxygen demand by 99% and 71%, respectively.

Biodiesel is composed of a combination of fatty acid alkyl esters [16]. Lipid content and the fatty acid profile are noticeable factors in algae cultivation [17]. Microalgae species contain proteins, pigments and vitamins, and lipids, which have applications in cosmeceuticals, nutraceuticals, fine chemicals, pharmaceuticals, and biodiesel [18, 19]. The fatty acid profile of algal extracts plays an essential role in predicting biodiesel's physicochemical properties [20, 21]. Biodiesel is highly potential to develop as one of the most important bioenergy for carbon capture and storage (BECCS) technologies.

Studying the expression of genes involved in the photosynthesis pathway and lipid production provides the opportunity to investigate algae grown in a wastewater environment at a molecular level [22]. The key genes involved in photosynthesis include the large subunit gene encoding the ribulose enzyme 1 and 5 bisphosphate-carboxylase/oxygenase and *psaB* and *psbC* genes [23]. The acetyl coenzyme A carboxylase is a crucial enzyme in lipid synthesis [24]. Based on the gene expression of the *accD* gene, the lipid accumulation in algae showed a significant correlation with this gene expression [25].

This study aimed to investigate the dual role of *C. vulgaris* for bioremediation of petrochemical wastewater and

lipid production as a feedstock for biodiesel and study the expression of the main genes involved in photosynthesis and lipid synthesis in the *C. vulgaris* cultivated in the petrochemical wastewater, including the large subunit of ribulose 1 and 5-bisphosphate carboxylase/oxygenase, *psbC*, *psaB*, and *accD* genes. The quality of produced lipid and biodiesel was also assessed in detail.

2 Material and methods

2.1 Algal cultivation

Microalgae *C. vulgaris* was purchased from the Scandinavian Culture Collection of Algae and Protozoa (CCAP). Following that, the microalgae was cultured in modified BG11 medium containing (g/L): NaNO₃ (1.5); K₂HPO₄ (0.04); MgSO₄·7H₂O (0.075); CaCl₂ (0.036); citric acid (0.006); Na₂CO₃ (0.02); Na₂EDTA (0.001); and ferric ammonium citrate (0.006).

Wastewater samples were obtained from a petrochemical complex in Asaluyeh, Iran [26]. The composition of petrochemical wastewater is shown in Table 1. Four diluted wastewater levels of 25%, 50%, 75%, and 100% were tested to explore the optimum concentration of wastewater and the nutrient removal efficiency for *C. vulgaris*. Two different wastewater levels (50%, 100%) were used to investigate the genes' expression (the large subunit of ribulose 1 and 5-bisphosphate carboxylase/oxygenase, *psbC*, *psaB*, and *accD* genes) in the *C. vulgaris* cultivated in the wastewater.

The experiments were carried out at room temperature in 250 ml Erlenmeyer flasks, each bioreactor containing 200 ml of BG11 and wastewater samples, for daily cycles of 16 h light and 8 h dark [12]. The algae were grown for 30 days in triplicate Erlenmeyer flasks.

Table 1 The specification wastewater samples

No	Parameter	mg /L
1	pH	5.5–8.0
2	TN	4.4–18
3	TP	2.5–3.7
4	TPH	50–150
5	COD	600–2300
6	BOD	200–1500
7	COD/BOD	1.62–3.43
8	pb	0.25–1.29
9	Cd	0.33–0.91
10	Ni	0.42–1.15
11	Zn	0.6–2.77
12	Fe	3.11–11.16
13	Total PAH	2.2–8.6 µg/L

2.2 Biomass production and lipid extraction

At the end of the experiments, algal biomass was harvested by centrifugation at 3500 rpm for 15 min, and in order to determine dry weight biomass, the samples were lyophilized at -60°C to reach a constant weight ($\text{g L}^{-1} \text{ day}^{-1}$). The method of Bligh and Dyer [27] was used for the extraction of total lipids. The lipid of algae was extracted by transferring the sample into a glass tube, and then methanol: chloroform 2:1 was added to that and mixed for 30 min. This process was followed by shaking and spinning. After that, the lower layer separated and dried under nitrogen flow, and the total lipid concentration was determined gravimetrically [27].

2.3 Chemical analysis

Chemical oxygen demand (COD), total nitrogen (TN), and total phosphorus (TP) were measured by a Hach DR 5000 Spectrophotometer (Hach Company, Loveland, CO, USA). QA/QC was carried out according to the analysis procedure. Moreover, five-day biochemical oxygen demand (BOD) was determined following the standard method [28]. The free fatty acid profile was analyzed using a Gas Chromatograph coupled with Flame Ionization Detector (GC-FID) (Agilent 6890; Agilent Technologies, Palo Alto, CA, USA). Polycyclic aromatic hydrocarbons (PAHs) were determined in both wastewater samples and the produced fatty acid. According to US-EPA (2018), samples were extracted by n-hexane. Determination of 16 US-EPA priority pollutant PAHs was performed by an Agilent 6890 gas chromatograph equipped with an Agilent 5970 mass selective detector. Besides, 48,953-U SUPELCO PAH Mix QA/QC was monitored as described by US-EPA procedures [26].

2.4 Characteristics of biofuel

Fatty acid content and composition were analyzed according to Breuer et al. [29]. As a gas carrier, helium was used at a constant flow rate of 1.1 mL/min. The temperature was programmed as follows: the initial temperature was held at 140°C for 1 min, 4°C per min ramp to 250°C , and was held for 5 min. The temperature of the detector and the injection port was kept at 260°C . Then, 1- μL of the sample was injected in spitless mode. SUPLCO standard of 18,919/18 was used to identify fatty acids.

BiodieselAnalyzer[®] software (version 2.2, BRTeam, Karaj-Iran) was employed to determine biodiesel characteristics empirically [25]. The software predicted some characteristics of biodiesel such as saturated fatty acids (SFA), mono unsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), degree of unsaturation (DU), saponification value (SV), iodine value (IV), cetane number (CN) for control

(BG11 medium), %50 diluted wastewater and pure wastewater media.

2.5 Statistical analysis

Statistical analyses were performed using SPSS 17.0 (SPSS, Chicago, IL, USA). In order to determine the statistical significance, a one-way analysis of variance (ANOVA) was used. When P was less than 0.05 or 0.01, the effects were significantly different. The Student's t -test was used for statistical significance between control and mean values of treated groups. Additionally, Duncan's Multiple Range tests were used to analyze the differences in relative expression levels of target genes.

2.6 Gene expression analysis

According to the manufacturer's instructions, total RNA was extracted from algae cells using TRIzol (Riboex GenAll, South Korea) and alcoholic deposition. Then, extracted RNA was verified by 1% agarose gel and measured via NanoDrop spectrophotometry (Thermo Scientific 2000c, USA) at 260, 280, and 230 nm. Afterward, extracted RNA was treated utilizing DNase I (Thermo Scientific, USA) to remove contamination of genomic DNA. Meanwhile, single-strand cDNA was synthesized by reverse transcriptase (Fast cDNA kit, Takara Japan) using 1 μg of total RNA. Herein, the oligo dT was performed as a primer to the synthesis of cDNA.

In this study, *accD*, *psaB*, *psbC*, *rbcl* were selected as target genes, and 18S rRNA (X13688) was selected as a housekeeping gene [30]. Sequences of these genes were collected from NCBI and aligned by Bioedit software [31]. The Oligo 7 software and Primer-BLAST tool were also utilized to design and check target primers. Primers designed for real-time PCR and annealing temperature are shown in Table 2.

Real-time PCR was carried out using the Stepone system (ABI stepone Real-Time PCR, USA), with the fluorescent dye SYBR[®] Green (Takara SYBR Green Kit). The real-time PCR was run at 95°C (10 min), 40 cycles at 94°C (30 s), 58°C (30 s), and 72°C (20 s). Afterward, obtained data was analyzed using Livak and Schmittgen's [32] method ($2^{-\Delta\Delta\text{Ct}}$) and normalized by multiplication efficiency. The mean comparison between different groups was tested based on the LSD test ($P < 0.05$).

To evaluate the cDNA quality, PCR was performed by primers of a housekeeping gene and query genes. Primers were designed based on exon-exon junction to avoid amplification of genomic DNA. According to Fig. 1, all of the studied genes illustrated the specificity of amplicons. Moreover, the melting curve analysis showed that all the primers are specific (Fig. 1, supplementary).

Table 2 Primers designed for real-time PCR and annealing temperature

Gene	Primer	Sequence	Sequence length	Ta
Accd	Acc-F	TTTAACTCGTACTACTCG TGTGC	123	56
	Acc-R	GGTGCCATTCCATCATCA AAGG		57
RbcL	Rbc-F	GCTACAGCAGAAGCA ATGATG	125	55
	Rbc-R	TCACGACAATAATGAGAT AAACTTG		53
PsaB	PsB-F	TTTCATCCTCAAACGCAA AGTCTATGG	129	60
	PsB-R	TTCTCGCATACTGTGACC AATACCG		61
PsbC	PsC-F	GATGGATGGATTGTCAGC GTAGATAAC	177	60
	PsC-R	AATGGCACCTAAACTGTA AGAAAGATAAGC		61
18S rRNA	18S rRNA -F	GGAGTATGGTCGCAAGGC TGAAAC	133	61
	18S rRNA -R	CTCAATCTGTCAATCCTC ACTATGTCTGG		61

Fig. 1 PCR gel electrophoresis of cDNA synthetic on 1% agarose gel. Lane M: DNA ladder, **a** *rbcL* gene, **b** 18S rRNA. **c** *psaB*, *accd*, *psbC* genes. Lane M: DNA ladder

3 Results and discussion

3.1 Phycoremediation

The ability of *C. vulgaris* for simultaneous growth in wastewater streams as well as total nitrogen and total phosphorus removal has been assessed. The highest percentage of total phosphorus removal was observed at 25% wastewater. The best performance of *C. vulgaris* for BOD removal was observed at 50% diluted wastewater. The highest percentage of COD removal was detected at 75% diluted wastewater, as presented in Tables 3, 4. According to the results of the t-test, all the variables examined (except BOD at 75% concentration) had statistically significant differences at 99% and 95% confidence intervals level with control. The results of the Student's t-test and the initial concentration of pollutants can be found in the supplementary data.

Cai and co-authors [33] found out a total nitrogen removal efficiency of 79–100% by microalgae *S. obliquus* from municipal wastewater. Lim et al. [34] attempted to treat textile wastewater samples using *C. vulgaris* and removed N and P by 45% and 33%, respectively. A wide range of N (55–88%) and P (12–100%) removal was reported in the use of municipal wastewater as the waste stream [35–38].

3.2 Lipid and biomass production

C. vulgaris has been widely applied in the lipid production process employed in producing biofuels in the last decade. Since the media utilized in this research is free and available media, this is a practical approach.

The algal lipid composition was analyzed by GC–MS chromatography to determine PAHs in algal oil. Since PAHs were not detectable in all the samples, the lipid was used as a raw material for biodiesel production without any health and environmental concerns. ANOVA indicated no significant differences between biomass productions in BG11 and various diluted wastewater levels to evaluate the effect of diluted wastewater on biomass and lipid production. Biomass production was in the range of 0.402–0.470 g/L.D. For comparison matters, Duncan's

Table 4 Removal efficiency of the pollutants by *Chlorella vulgaris* (%) in the petrochemical wastewater

Wastewater (%)	Removal%			
	TN	TP	COD	BOD
25	59.14	100.00	12.11	20.63
50	69.89	92.59	10.89	30.56
75	63.80	93.83	12.17	14.02
100	37.67	66.67	10.41	21.63

multiple range test was also used. The comparison of the mean of the lipid content and biomass production in *C. vulgaris* by Duncan's method has been shown in Table 5. According to the results, the highest lipid content was observed in 50% of wastewater. Then it was decreased by 75% and 100% of wastewater.

Zhou et al. [39] reported that *Chlorella* sp. produced biomass and lipid from municipal wastewater in the range of 231.40–241.70 mgL⁻¹d⁻¹ and 30.91–33.53 DW%. Chin-nasamy et al. [40] achieved 23.00 mgL⁻¹d⁻¹ biomass and 18.10 DW% lipids by *Chlorella saccharophila* from industrial wastewater. Ammonia nitrogen inhibited microalgae growth and reduced the utilization of wastewaters [41, 42]. The primary mechanism of ammonia was to inhibit the microalgae by poisoning their photosynthetic system [43, 44]. The higher algal lipid content for 50% diluted petrochemical wastewater conditions resulted from the existence of NH₄⁺ –N in pure wastewater or having lower toxicity than 75% and pure wastewater.

The lipid accumulation using *C. vulgaris* is essential due to reduced expenses while producing a lipid that is a useful source to be utilized in biodiesel production. This approach is economically significant and may be developed as an applicable BECCS. The same approach has been recently suggested by Rosli and co-authors [45].

3.3 Characteristics of biodiesel

The results of chromatography showed that since the fatty acid profile generated by *C. vulgaris* contained a significant amount of palmitic acid (C16:0), stearic acid (C18:0), and palmitoleic acid (C16:1), oleic acid (C18:1), it could be used as promising biodiesel feedstock. The biodiesel's predicted

Table 3 Removal nutrients and organic contaminants by *Chlorella vulgaris* (mg/L)

Wastewater (%)	Removal (mg/L)			
	TN (mg /L)	TP(mg /L)	COD (mg /L)	BOD(mg /L)
25	1.25 ± 0.02	0.891 ± 0.02	450.43 ± 0.14	146.17 ± 0.12
50	1.85 ± 0.42	0.132 ± 0.33	913.37 ± 0.26	116.88 ± 0.53
75	3.34 ± 0.53	0.165 ± 0.46	1350.39 ± 0.62	217.1 ± 0.45
100	7.67 ± 0.01	1.2 ± 0.26	1836.595 ± 0.03	287.39 ± 0.56

Table 5 Comparison of the mean of the examined *accD*, *rbcl*, *psaB*, *psbC* genes in *Chlorella vulgaris* by Duncan's method

	psbCct	psbCRE	psaBct	psaBRE	rbclCt	rbclRE	accDct	accDRE
Control (BG11)	36.99 a	1 c						
50%waste water	35.18 b	108.46 a	31.84 a	13.14 a	32.3 ab	1 c	30.86 a	1 b
100% waste-water	32.51 c	80.83 b	34.76 a	5.96 b	31.08 b	289.15 a	31.02a	81.055 a
			33.59 a		34.03 a	6.71 b	35.07 a	0.66c

properties from *C. vulgaris* showed that they reached the standards developed by ASTM (Table 6). *C. vulgaris* was introduced as a suitable candidate for biodiesel production [46, 47]. Ji et al. [48] used a piggery wastewater sample (TN: 56 ± 2 mg/L and TP: 13.5 ± 0.6 mg/L) as a medium for cultivation of *C. vulgaris* to integrate biofuel production and the treatment of piggery wastewater. They reported that *C. vulgaris* had good potential for *treating wastewater as well as producing high oil content* for lipid accumulation.

3.4 Quality control of produced biodiesel

PAHs were recognized as a mutagenic, carcinogenic, and teratogenic class of organic compounds, which was available in most of the wastewaters in the petroleum-related industries. Since the medium of algae was petrochemical wastewater in this study, PAH concentrations were determined in the produced lipid using gas chromatography/mass spectroscopy. Each time, 16 US-EPA priority pollutant PAHs were measured. The results indicated that the biodiesel had outstanding quality, and PAHs were not detectable in all biodiesel samples. Presumably, *C. vulgaris* used carbon to accumulate lipids mainly by the source of CO₂. Therefore, this type of produced biodiesel can be strongly proposed with no environmental concerns.

3.5 Gene expression

Since lipid composition and starch content vary among microalgae, not all are suitable for biofuel production. Some species can naturally biosynthesize and accumulate large amounts of starch and intracellular lipids, even further improved by genetic modifications, making them more useful in producing biofuels [49]. Statistical analysis of Duncan (Table 7) showed a significant difference in the relative expression of the *accD* gene in 50% and 100% wastewater treatments compared to the control group. Treatment with 50% of wastewater had the highest expression, as can be seen in Fig. 2a. The expression of *rbcl* and *psbC* also showed a significant difference between treatments, with high transcript abundance in 50% of effluent treatment (Fig. 2b, c). Based on the Ct value, this difference always exists. Likewise, the expression rate of *psaB* gene also illustrated the highest expression in 50% of wastewater compared to other treatments (Fig. 2d), while the Ct value showed no consistent difference as observed in the *rbcl* gene. According to the results of Sect. 3.2, increasing the expression of genes involved in the photosynthesis pathway at 50% wastewater leads to the lipid content increased at this concentration. Increasing the expression of genes involved in the lipid synthesis pathway is well analyzed by the data obtained from the optimization and the corresponding

Table 6 Predicted properties of biodiesel from *Chlorella vulgaris* by BiodieselAnalyzer® software

	BG 11	Wastewater (50%)	Wastewater (100%)
Saturated fatty acids (SFA)	32.4	29.0	26.6
Mono unsaturated fatty acid (MUFA)	14.1	16.2	16.3
Poly unsaturated fatty acid (PUFA)	20.5	22.1	26.8
Degree of unsaturation (DU)	55.1	60.3	69.9
Saponification value (SV)	135.796	136.755	141.500
Iodine value (IV)	77.036	77.897	92.134
Cetane number (CN)	69.160	68.684	64.143
Long chain saturated factor (LCSF)	23.42	21.50	20.65
Cold filter plugging point (CFPP) °C	57.02	51.07	48.39
Cloud point (CP) °C	− 1.205	− 2.362	− 2.625
Allylic position equivalent (APE)	41.0	44.1	54.4
Bis allylic position equivalents (BAPE)	71.8	67.4	75.3
Oxidation stability (OS) (hours)	11.525	10.263	8.547
Higher heating value (HHV)	26.423	26.515	27.453
Kinematic viscosity (U) (mm ² s)	2.415	2.410	2.437
Density (ρ) (g cm ³)	0.589	0.591	0.614
Pour point	− 8.129	− 9.385	− 9.671

mathematical models. According to the results of this study, nitrogen was found as a potential-limiting factor, which is confirmed by the statistical model. In addition, the affluent environment has no inhibitory effects on the expression of genes involved in the photosynthesis pathway as well as those involved in lipid synthesis.

Micro-algae stabilizes a considerable amount of carbon during photosynthesis; for example, the carbon fixation efficiency of *C. vulgaris* in the membrane photobioreactor is consistently 260 mg/ha [50]. Wawrik et al. showed that the expression of the ribulose 1 and 5 carboxylase/oxygenase (*rbcl*) bisphosphate in species of diatoms living in the shallow-water is less pronounced than those living in deep water [51]. In addition, Wan et al. reported the expression of large enzyme 1 and 5 carboxylase/oxygenase bisphosphate in different species of microalgae (*Dunaliella salina* and *Nannochloropsis oculata*, *sorokiniana*, *Chlorella*) under mixed culture conditions [52]. In this study, they realized that the expression of *rbcl* decreased in mixed culture conditions when glucose was added to the medium, which reduces the activity of *rbcl* and, consequently, reduces the process of photosynthesis. Yan et al. studied the expression of *rbcl*, *psaB*, and *psbC* genes involved in the photosynthesis process in *Chlorella vulgaris* species by adding glucose, atrazine, and diclofop-methyl herbicides, leading to decreased expression of *psbC* gene when these three herbicides are present, however, this expression was not significant. Nevertheless, the expression of *rbcl* and *psbC* genes decreased significantly. Moreover, they found real-time PCR as a reliable technique for assessing the toxicity of components in aqueous media [53].

According to Fan et al. [54] the expression of the *rbcl* gene in *C. pyrenoidosa* species in food deficiency conditions, including nitrogen, phosphorus, and iron, was inversely proportional to lipid content, proving the key role of this gene in photosynthesis and cell growth processes. However, the expression of the *accD* gene had a negative correlation between the expression of this gene and lipid content. Lee et al. examined the effect of nitrogen deficiency on the transcript abundance of *accD* gene and lipid content, and it was reported that the gene expression was directly related to lipid content [55].

4 Conclusion

Green algal *Chlorella vulgaris* was successfully employed for phytoremediation of petrochemical wastewater and lipid productivity at the same time. Biodiesel's physical and chemical characteristics demonstrate that biodiesel is a high-quality fuel and can easily adapt to diesel engines due to the sufficient similarities with petrodiesel, and it can be confidently recommended for diesel vehicles without a further engine change. PAHs were not detectable in all samples, which ensured the use of industrial wastewaters as an applicable source of alternative microalgae. The expression of *accD* in the wastewater environment was 40 times higher than that in the BG11 medium. In addition, the expression of this gene in 50% of effluent was significantly increased compared to 100% effluent and the BG11 environments. For 100% non-diluted wastewater (pure wastewater), the maximum removal of BOD, COD, total nitrogen, and total phosphorus has been 21.63%, 10.41%,

Table 7 Comparison of the mean of the examined *accD*, *rbcl*, *psaB*, *psbC* genes in *Chlorella vulgaris* by Duncan's method

	psbCct	psbCRE	psaBct	psaBRE	rbclCt	rbclRE	accDct	accDRE
Control (BG11)	36.99 a	1 c		31.84 a	32.3 ab	1 c	30.86 a	1 b
50%waste water	35.18 b	108.46 a		34.76 a	31.08 b	289.15 a	31.02a	81.055 a
100% waste-water	32.51 c	80.83 b		33.59 a	34.03 a	6.71 b	35.07 a	0.66c

Statistically differences are marked by various alphabets

Gene: *psaB*, Protein: Photosystem I P700 chlorophyll an apoprotein A2

Gene: *psbC*, Protein: Photosystem II CP43 reaction center protein

Gene: *rbcl*, Protein: Ribulose biphosphate carboxylase large chain

Gene: *accD*, Protein: Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta

Fig. 2 Relative expression of *accD*, *rbcl*, *psbC*, and *psaB* genes for various wastewater treatments

37.67%, and 66.67%, respectively. Since many pollutants are emitted in the petrochemical wastewater, its application as a media for algae cultivation may change its function as a biomass production and lipid accumulation, as well as its potential for remediation, lipid composition, and expression of photosynthesis genes functions.

Future studies should be directed toward applications of *C. vulgaris* for the treatment of wastewater from other industries, including food, beverage, agriculture, aquaculture. The biodiesel and petrodiesel blend should be developed for diesel consumer engines, which are presumably useful in capturing CO₂ from the atmosphere.

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Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

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