Coupling of Nutrient Removal and Biodiesel Production By *Asterarcys quadricellulare M*icroalga Grown in Municipal Wastewater

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Abstract

The present study focused on the feasibility of using municipal wastewater (WW) as culture medium for cultivation of microalgae. The study aimed to assess the efficiency of microalgae in nutrients removing capacity from wastewater and its biomass and lipid productivity for using as biodiesel feedstock. Based on that, the green microalga *Asterarcys quadricellulare* was isolated and grown for 24 days in Bold's Basal Medium as a control and at different concentration of secondary treated municipal wastewater (WW) diluted with distilled water (25%, 50%, 75% and 100%WW). Results of 75%WW treatment recorded 96.6%, 98.4%, and 89.9% removal efficiency for, nitrate, ammonia and total phosphorus, respectively. Also, it revealed high biomass productivity and biomass content, where it recorded 69.0 mgL^{-1} day^{-1}, and 1.44 g/L, respectively. Likewise, high lipid productivity 17.2 mg L^{-1} day^{-1} and 360.6 mg/L lipid content. Consequently, *Asterarcys quadricellulare* fatty acids profile estimation revealed an increase in Oleic, Palmitic and Linoleic acids levels. Most properties of biodiesel derived from the studied microalga meet with values established by the ASTM D6751 and EN 14214 biodiesel standards. According to this analysis, *A. quadricellulare* microalga could be used for wastewater bioremediation and biomass production with a suitable content of lipids proper as biodiesel feedstock. The predictive biodiesel properties pointed that it has a good quality compared with international standards.

Introduction

In many parts of the world water environment is beneath stress. For Egypt, water stress value (proportion between renewable water assets and water use) recorded 0.77 in 2004, which appears terrible circumstance agreeing to the United Nations classification [1]. Therefore, it is imperative to seek cost-effective methods and innovative techniques for treatment of wastewater to meet the freshwater demand for human and agriculture use. Long time ago, some researcher used microalgae to treat the effluents from secondary treatment processes to prevent eutrophication [2]. Right now, wastewater treatment using microalgae is an energy-efficient and capable for the removal of pollutants from wastewater as compared to the traditional chemical and physical treatment ways [3]. In wastewater, excess nutrients such as nitrate and phosphate contributes to eutrophication, thereby decreasing dissolved oxygen and increasing the growth of undesired vegetation in the aquatic environment. In addition, microalgae assimilate inorganic carbon from wastewater, which can increase the pH, resulting in ammonia volatilization and phosphorus precipitation [4]. Several earlier studies on nutrients removal efficiency of microalgae from diverse sorts of wastewater [5–8]. These studies suggest feasibility of wastewater treatment using microalgae, which can decrease nutrient cost and water demand while improving water quality.

Due to the broad use of fossil fuels, the world be faced with depletion of energy sources. In this regard, world considered biofuel as renewable alternative to fossil fuels, due to its sustainable features to overcome the global energy demand [9]. Nevertheless, because of their competition with food crops and agricultural lands, biofuel sustainability is often challenged [10]. So, microalgae as renewable source for sustainable biodiesel production is promising due to the ability to store high lipid content (20–75% wt.) than other oil crops [11], and it does not compete with other agricultural resources [12]. On other hand, although microalgae have great potential as biodiesel feedstock, there are many challenges for commercial application including the extensive freshwater need and the high cost of nutrients [13]. This trouble solved using microalgae cultivation in wastewater which will cut the cost of algae cultivation to make the process of producing biofuel from algae more economic, and environment friendly [14]. municipal wastewater used to substitute synthetic growth media for algal cultivation as an alternative and inexpensive source of nutrients [15]. In addition, the use of microalgae for municipal wastewater treatment and simultaneous lipid accumulation with nutrient removal reaching as high as 90% and lipid accumulation reaching 28.5% of dry algal biomass [16]. In order to diminish the reliance on fossil fuel. The study aimed to assess the efficiency of the green microalga purely isolated for nutrients removing capacity from wastewater and its biomass and lipid productivity Also, analyzing its fatty acids profile and assessing its physical-chemical properties for using as biodiesel feedstock.

Materials And Methods

Microalgae isolation and growth conditions

Water samples were picked up from different freshwater sources and municipal wastewater treatment plant located in Menoufia, Egypt. Samples were collected in sterilized plastic bottles and transferred to the laboratory shortly after collection. Then, the samples were enriched by using a mixture of 90% sample and 10% of cultural Bold's Basal Medium (BBM) [17]. The batch incubation process was carried out for one week, using continuous illumination under white light fluorescent lamps of 80 µmol $m^{-2}s^{-1}$, at 26 ± 2 °C and aerated using a sterile filtered air. After an enrichment period, microalgae growth was visually evaluated using an optical microscope to select the pure microalgae. The tested microalga was isolated, and raised to pure culture through plate technique. Images of the organism were captured through a light microscope. The isolate was propagated in BBM with the earlier growth conditions.

Pretreatment of wastewater

Secondary treated municipal wastewater samples were collected from the local wastewater plant. Large solid particles were removed by sedimentation and filtration.

Experiments design

The tested microalga was grown in a conical flask containing 150 mL of BBM growth medium and incubated under aforementioned growth conditions to be used as the inoculum for the following study experiments. Five mL were used for inoculation of the experimental flasks containing 150 mL of different treatments. In arrange to assess the capacity of the isolated microalga and use wastewater as the growth medium for biomass and lipid production, the microalga isolate was cultivated in some blends of wastewater with distilled water. The growth medium (BBM) used as a control, as well as four different concentrations of municipal wastewater diluted using distilled water (25%, 50%, 75%, and 100%WW) were inoculated with *Asterarcys quadricellulare* isolated microalga. All treatments were carried out in triplicate. The samples were withdrawn for analysis every three days' interval.

Microalga growth assessment

The study was carried over 24 days, microalga growth was determined by OD (optical density) measurement at 560 nm, and cell counting determined using haemocytometer., chlorophyll (a) was determined by the method adopted by [18]. In addition, cellular dry weight (CDW) was measured initially and after 24 days of incubation. Dry weight was determined according to [19]. Biomass and lipid productivities was calculated according to [20].

Biochemical composition assessment

Total lipid was determined according to the method described by [21] with modification according to [22]. Protein content determined using the Biuret reaction, adapted by [23]. The total carbohydrate content in the extract was determined by the phenol sulfuric acid method using D-glucose as standard [24].

Assessing of fatty acids profile using GC

The dry lipid extracts were transesterified to form fatty acid methyl esters (FAME) before being analyzed by gas chromatography [25]. FAMEs were subjected to analysis by gas chromatography using Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 μm film thickness).

Estimation of biodiesel properties

Fatty acids percentages of *Asterarcys quadricellulare* microalga were used to calculate biodiesel properties. Biodiesel properties were estimated according to [26] using "Biodiesel Analyzer© Ver. 2.2 (available on "http://www.brteam.ir/biodieselanalyzer")

Measurement of Nutrient Removal

The nutrient medium was separated from the biomass by centrifugation after being filtered through Whatman GF/C filter paper; consumption of ammonia, nitrite, nitrate, phosphate, the biological oxygen demand (BOD_5), Chemical oxygen demand (COD), electrical conductivity (EC), and total dissolved solid (TDS) by algae at the end of the 24-day growth period were determined. Total phosphate (TP) was measured according to [27], Ammonia nitrogen (NH_3–N), nitrate nitrogen (NO_3–N) and biological oxygen demand (BOD_5) were determined according to [19] and Chemical oxygen demand (COD) was determined according to [28]. Total dissolved solid (TDS) and electrical conductivity (EC) were measured using conductivity bench top meter. Based on the obtained result, the percentage removal efficiency (%) and removal rate (mg L^{-1} day^{-1}) were calculated according to [8].

Statistical analysis

All experimental trials were performed in triplicate and the results are presented as the means ± standard deviation(SD). Statistical analyses were performed using SPSS software 16.0. The comparisons of the mean values were conducted by one-way analysis of variance (ANOVA) followed by a Duncan's new multiple-range test for statistical significance. The differences were considered significant at (P) ≤0.05.

Results And Discussion

Identification of isolated microalga

The tested microalga was isolated through streaking on agar plates and sub-culturing in liquid media. Using microscopic characterization, a green microalga (chlorophyta) was morphologically identified as *Asterarcys quadricellulare* (Fig. 1). It has a unicellular spherical to ovoid form, up to 10 μm in diameter, containing a single parietal, sometimes lobed to fragmented chloroplast with a single prominent pyrenoid. Autosporangia containing 2-16 ovoid spores were seen, but no sexual or asexual flagellated stages were observed [29].

Growth of *Asterarcys quadricellulare* in municipal wastewater

A. quadricellulare was cultured on BBM as control and different concentrations of secondary treated municipal wastewater (25, 50, 75 and 100%WW) with its physical and chemical characteristics are presented in Table 1.

Growth was assessed using optical density (OD), chlorophyll (a) content and cells count. The results of microalga growth presented in fig. 2 showed that the least algal growth in 25%, 50% and 100%WW. Agreement with the study results, 22% concentration did not increase algal growth, that might be due to the relatively small increase in wastewater concentration. But when used 44% concentration, cell growth was almost two times higher than 22% wastewater concentration [30].

Indeed, it was noticed that algae grew faster in the 75%WW, where it recorded the highest growth as compared with control and the other concentrations. Using pre-treated municipal wastewater 77% concentration to grow *Scenedesmus acutus* in batch mode [5]. At 75%WW optical density, chlorophyll (a), cells count and dry weight recorded the highest values was 1.23, 11.12 mg L^{-1}, $38.9x10^4$ cells mL^{-1} and 1.44 gL^{-1}, respectively. Chlorophyll (a) content is sign of algal growth rate, which increased with time by increasing nutrients removal percent and algal growth biomass [31]. This indicating that 75%WW is suitable for applications requiring high-density microalga culture when grown in municipal wastewater. Generally, wastewater with high nutrient concentrations could inhibit algal growth, while on the other hand wastewater with low nutrient concentrations could insufficient for algal growth.

Biomass and biochemical composition

Results for biomass and biochemical composition presented in Table 2 and Fig. 3 showed that biomass productivity of *A. quadricellulare* recorded highest value 69 mgL^{-1} day^{-1} at 75%WW treatment. These results are consistent with those obtained for some other microalgae [32, 33]. Lipid accumulation by *Asterarcys quadricellulare* cultivated on different concentrations of municipal wastewater and BBM (control) are presented in Table 2 and Fig. 3. The results recorded high lipid content with value 360.6 mgL^{-1}, lipid productivity 17.2 $mgL^{-1}day^{-1}$ and lipid yield was 25.3% for 75%WW treatment. The highest lipid accumulation may be due to the highest accumulated biomass from this treatment at the end of growth period. In line with our results several studies were presented in Table 4, *Asterarcys quadricellulare* microalga produce 0.463 g/L lipid, 20% DW, with lipid productivity of 19.8 $mg/L^{-1}day^{-1}$ [34]. Also, *Asterarcys* sp. showed the greatest biomass productivity (80 $mgL^{-1}d^{-1}$) and higher lipid content (30.55%) [32]. lipid content was 19.4% after *Scenedesmus obliquus* cultivation using treated urban wastewater [6]. *Scenedesmus acutus* had a 28.3% lipid content on cultivation using pretreated municipal wastewater as the culture medium for 21 days [5].

Nutrients removal

The removal rates and efficiencies of some nutritional elements from secondary treated municipal wastewater were determined using *Asterarcys quadricellulare* as presented in Table 3. The result showed that *Asterarcys quadricellulare* had higher removal rate and efficiency to NH_3-N and NO_3-N, especially at concentration 75%WW. The highest NO_3-N removal rate in 75%WW was 0.37 mg L^{-1} day^{-1} with 4.6 times over control. It also recorded high NH_3-N removal rate as 0.04 mgL^{-1} day^{-1} indicating 2 times over control. High removal efficiencies (NH_3-N 98.41%, NO_3-N 96.61%) were recorded at 75%WW treatment. Removal of NH3-N from the wastewater by algae can be due to direct use as NH_3-N and/or NH_3 stripping [35]. Ammonium is the preferred form of nitrogen for microalgae growth due to its lower energy demand. Nutrient removal can also be increased by NH_3 drive out or phosphorus precipitation due to the increasing of the pH associated with photosynthesis [36].

Regarding phosphorus uptake, the recorded removal efficiency at concentration 75% WW with a value 89.9% (Table 3). was higher than satisfactory value (80%) established by European legislation [37]. Removal percentages of this study were closed to that of microalgae grown on mixed municipal and industrial wastewater [38]. The results revealed the ability of *Asterarcys quadricellulare* to assimilate high amounts of phosphorus and nitrogen for the synthesis of lipid, protein, and carbohydrates of microalga dry weight. The specific nutrient consumption values of NO_3-N, NH_3-N and TP by microalga were greater recorded in 75%, while 25%WW showed the lowest specific consumption (Fig. 4). This is attributed to the higher growth of *Asterarcys quadricellulare* on 75% in comparison to other wastewater concentrations and the control growth media.

Both COD and BOD are important in assessing water quality. As wastewater used for the growth of *Asterarcys quadricellulare* at concentration 75%WW, COD showed high removal efficiency 84.74% and removal rate 1.23 mgL^{-1} day^{-1} and BOD with removal efficiency 91.52% and removal rate 0.38 mgL^{-1} day^{-1} (Table 3). The higher COD and BOD values confirm the greater amount of organic matter. This result showed that *Asterarcys quadricellulare* prompted increasing the loosing in both BOD and COD values of the effluent and this could be attributed to the increasing of algal growth rate, which implied more photosynthesis happened producing more oxygen. Hence, oxidation of organic matter is improved by released oxygen. Using microalgae in wastewater treatment can increase the removal efficiency of COD [39]. Biological treatment of domestic wastewater using algae indicated 68.4% BOD and 67.2% COD removal, respectively [40].

Fatty acids profile

Lipid assessment results of *Asterarcys quadricellulare* microalga recorded its high lipid productivity at 75%WW concentration. Thus, its Fatty acids profile were analyzed using GC and compared to the control (BBM). It was revealed that *A. quadricellulare* fatty acids profile mainly consisting of monounsaturated fatty acid (MUFA), followed by polyunsaturated fatty acid (PUFA) and saturated fatty acid (SAF) shown in Fig. 5 and Table 5. The total MUFA content showed a significant increase in 75%WW treatment with 23.2% higher than control and a significant reduction in total PUFA content by 34.37% below control. The main fatty acids in *A. quadricellulare* microalga were 16-carbon and 18-carbon, a high proportion of Palmitic acid, Oleic acid and Linoleic acid were found in 75%WW and control treatments (Table 5). Microalgal lipids which have high proportion of C16:0 and C18:0 fatty acids are proper feedstock for biodiesel production [45, 46]. The dominant fatty acids in the microalgae were Palmitic, Stearic and Oleic acids [47]. Thus, this microalga strain is a promising candidate as feedstock for biodiesel production. The increase in the fatty acids (C16-C18) in 75%WW may refer to the composition balance of this medium.

Biodiesel Properties

Biodiesel properties of *Asterarcys quadricellulare* grown in BBM and 75%WW treatments were determined as shown in Table 6. Iodine value is an indicating characteristic of the degree of unsaturation of fatty acid which influences the viscosity and cold filter plugging point. Iodine value of control treatment is 84.09, and of 75%WW has 77.08. Because the melting point and oxidative stability are related to the degree of unsaturation, The greater the iodine value, the more unsaturation and the higher the susceptibility to oxidation. The lower the iodine value, the better the fuel will be as a biodiesel. Biodiesel with high amounts of saturated fatty acids will have a higher cetane number, while biodiesel with high amounts of unsaturated fatty acids will have a lower CN. Cetane number was 58.66 for control and 58.70 for 75%WW for *Asterarcys quadricellulare* biodiesel, it was found to be higher than both the standards ensuring good ignition quality low nitrous oxide emissions, less occurrence of knocking and easier engine start-up [48]. Our results are agreeing with the literature, which report that most properties of biodiesel derived from the studied microalga species already meet with the limit values established by the ASTM D6751 and EN 14214 biodiesel standards [47, 49]. Also, most of the biodiesel properties represented in Table 6 for *Asterarcys quadricellulare* in both studied treatments complied with those of another study [43].

Conclusion

Due to its relatively low organic content, light permeability and the existing mineral nutrients, secondary treated municipal wastewater could be used by microalgae as a low-cost growth medium to produce biomass-based biofuels. As a conclusion nitrogen and phosphorus were efficiently eliminated during the study experiment. The results of the current study indicated the highest percentage of C16-C18 fatty acids (54.92% from total lipids) at concentration containing a higher part of secondary treated wastewater, which could increase the economical production of the lipid-rich microalgae *Asterarcys quadricellulare* for biodiesel through saving water and nutrients. Our results offer a primary stage for isolation of microalga species convenient for local conditions, and further studies are needed to enhance the growth and lipid productivity of microalgae grown on wastewater as abundant cheap cultivation medium either in the lab scale and large-scale cultivation conditions.

Declarations

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Data availability: All data generated or analysed during this study are included in this published article.

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Tables

Table 1 Physico-chemical characteristics of secondary treated municipal wastewater, and obtained from a local municipal wastewater treatment plant used in this study

Parameters	Value
pH	7.6
Temperature	26
NO_3 mgL^{-1}	9.098
TP mgL^{-1}	1.390
NH_3 mgL^{-1}	0.870
BOD_5 (mgL^{-1})	12.167
COD (mgL^{-1})	44.670
TDS (mgL^{-1})	520.30
EC (μ mhos/cm)	1106

Table 2 Biomass and biochemical composition of *A. quadricellulare* grown for 24 days in BBM as control and different municipal wastewater concentrations (25%, 50%, 75% and 100%WW)

Parameters	BBM	25% WW	50% WW	75% WW	100% WW
Biomass (Dry weight gL^{-1})	$1.29±0.014^{B}$	$0.74±0.045^{D}$	$0.99±0.039^{C}$	$1.44±0.055^{A}$	$1.30±0.061^{B}$
Biomass productivity mgL^{-1} day^{-1}	$62±0.003^{B}$	$35±0.002^{D}$	$47±0.001^{C}$	$69±0.002^{A}$	$62±0.001^{B}$
Lipid content mgL^{-1}	$317.0±0.319^{B}$	$163.0±0.659^{D}$	$213.7±0.563^{C}$	$360.6±0.659^{A}$	$328.7±1.354^{B}$
Lipid productivity mgL^{-1} day^{-1}	$15.1±0.218^{A}$	$7.8±0.297^{D}$	$11.9±0.402^{C}$	$17.2±0.262^{B}$	$15.2±0.120^{AB}$
Lipid yield (% CDW)	$24.5±0.319^{A}$	$22.2±0.659^{B}$	$21.6±0.563^{B}$	$25.3±1.354^{A}$	$25.0±0.659^{A}$
Protein yield (% CDW)	$31.4±0.111^{A}$	$27.9±1.352^{B}$	$27.8±0.650^{B}$	$31.1±1.469^{A}$	$32.3±1.288^{A}$
Carbohydrate yield (% CDW)	$31.049±0.209^{A}$	$27.035±1.684^{B}$	$24.370±0.704^{B}$	$26.826±1.287^{B}$	$30.427±1.466^{A}$

Each value is the mean of three replicates ± SD

Values of the same raw with the same small letter showed insignificant differences (at $P \leq 0.05$)

Table 3 Nutrient removal rate (RR, mgL^{-1} day^{-1}) and removal efficiency (RE, %) of *Asterarcys quadricellulare* grown for 24 days in BBM and different municipal wastewater concentrations

parameters	Control (BBM)		25% WW		50% WW		75% WW		100% WW	
	RR	RE%	RR	RE%	RR	RE%	RR	RE%	RR	R
EC (μmhos)	7.48±0.13^{D}	61.37±1.63^{a}	3.34±0.35^{E}	27.16±2.50^{d}	8.36±0.18^{C}	37.11±0.89^{c}	16.73±0.22^{B}	45.37±1.15^{a}	19.22±0.54^{A}	4
TDS (mgL^{-1})	4.30±0.08^{D}	65.35±2.07^{a}	2.62±0.05^{E}	41.13±1.80^{d}	5.58±0.04^{C}	49.69±1.06^{c}	10.64±0.23^{B}	63.34±1.18^{a}	13.19±0.12^{A}	5
NO_3 (mgL^{-1})	0.08±0.00^{D}	92.96±1.20^{b}	0.09±0.01^{D}	76.76±2.88^{d}	0.19±0.00^{C}	87.18±1.48^{c}	0.37±0.01^{B}	96.61±3.05^{a}	0.31±0.00^{A}	9
NH_3 (mgL^{-1})	0.02±0.00^{E}	94.20±1.51^{b}	0.01±0.00^{D}	77.48±2.078^{d}	0.01±0.00^{C}	89.53±1.08^{c}	0.04±0.00^{B}	98.41±2.02^{a}	0.03±0.00^{A}	9
TP (mgL^{-1})	0.06±0.00^{A}	85.85±1.94^{a}	0.01±0.00^{D}	53.07±3.844^{c}	0.02±0.00^{C}	72.86±1.57^{b}	0.05±0.00^{B}	89.87±1.83^{a}	0.04±0.00^{A}	8
BOD_5 (mgL^{-1})	0.27±0.02^{C}	83.42±3.21^{a}	0.08±0.02^{E}	57.52±4.72^{c}	0.19±0.02^{D}	66.87±2.09^{b}	0.38±0.02^{B}	91.52±1.16^{a}	0.41±0.03^{A}	8
COD (mgL^{-1})	1.40±0.02^{B}	87.17±1.92^{a}	0.38±0.03^{E}	62.27±4.39^{b}	0.73±0.04^{D}	68.35±3.40^{b}	1.23±0.03^{C}	84.74±1.96^{a}	1.48±0.03^{A}	8

Table 4 Comparison of the results obtained in the present study with previously reported work for microalgae cultivated in different effluents of wastewater

Microalgae	Medium	Bp[a]	LP[b]	Nutrient removal efficiency (%)				Reference
				NO_3−N	NH_3−N	TP	COD	
Asterarcys quadricellulare	Municipal wastewater	69	17.2	96.6	98.4	89.9	84.7	This study
Asterarcys quadricellulare	BBM + 0.1 g/L glucose	-	19.8	(48)[TN]	-	50	12.4	[34]
Asterarcys quadricellulare	BBM	-	44	-	-	-	-	[41]
Asterarcys quadricellulare	Modified BBM	57.7	15	-	-	-	-	[32]
Asterarcys quadricellulare	BG-11 media	-	15.5	-	-	-	-	[42]
Asterarcys quadricellulare	Municipal wastewater	-	-	(52)[TN]	99.1	95.7	-	[43]
Chlorella sorokiniana	Municipal wastewater	73	16.2	74.2	83.3	78.0	61.9	[8]
Chlorella pyrenoidosa	Municipal wastewater	229	48.90	-	59.4	93.8	76.9	[44]

[TN] total nitrogen

[a] Biomass productivity calculated as mg L^{-1}day

[b] Lipid productivity calculated as mg L^{-1}day

Table 5 Fatty acids composition (% fatty acids) of *Asterarcys quadricellulare* grown for 24 days in control (BBM), and 75%WW treatments.

Fatty Acids	Control	75% WW
Saturated fatty acids (SFA)		
Palmitic acid (C16:0)	18.31	20.54
Stearic acid (C18:0)	0.64	0.59
Monounsaturated fatty acids (MUFA)		
Oleic acid (C18:1)	44.57	54.92
Polyunsaturated fatty acids (PUFA)		
Hexadecadienoic acid (C16:2)	3.92	2.22
Linoleic acid (C18:2)	20.29	13.38
Hexadecatrienoic acid (C16:3)	8.22	6.49
Linolenic acid (C18:3)	0.77	-
Hexadecatetraenoic acid (C16:4)	1.90	0.91
Stearidonic acid (C18:4)	1.40	0.94
SAT	18.95	21.13
MUFA	44.57	54.92
PUFA	36.49	23.95
Total	100.00	100.00

Table 6 Biodiesel properties based on fatty acid profile of *Asterarcys quadricellulare* grown in (BBM) and 75%WW for 24 days, compared to othr study and international standards

Biodiesel properties	*Asterarcys quadricellulare* This study		*Asterarcys quadricellulare* [43]	Biodiesel standards	
	Control	75%WW		ASTM	CEN
Degree of Unsaturation (DU)	89.49	83.56	102	-	-
Saponification Value (mg/g) (SV)	174.51	183.52	179	-	-
Iodine Value (gl/100g) (IV)	84.09	77.08	131	-	Less 120
Cetane number (CN)	58.66	58.70	44.7	Above 47	Above 51
Long Chain Saturated Factor (LCSF)	2.15	2.35	3.0	-	-
Cold Filter Plugging Point (°C) (CFPP)	-9.72	-9.10	-7.0	-5 to -13	5 to -20
Cloud Point (°C) (CP)	4.64	5.81	-		4
Pour Point (°C) (PP)	-1.79	-0.51	-	-15 to 10	
Allylic Position Equivalent (APE)	89.49	83.56	-	-	
Bis-Allylic Position Equivalent (BAPE)	21.83	13.38	-	-	
Oxidation Stability (h) (OS)	8.19	11.40	6.8	3	6
Higher Heating Value (HHV)	33.93	35.68	-	-	
Kinematic Viscosity (mm^2/s) (υ)	3.15	3.41	3.76	1.9 - 6	3.5 - 5
Density (g/cm^3) (ρ)	0.75	0.79	0.88	0.88	0.86 -0.90

Figures

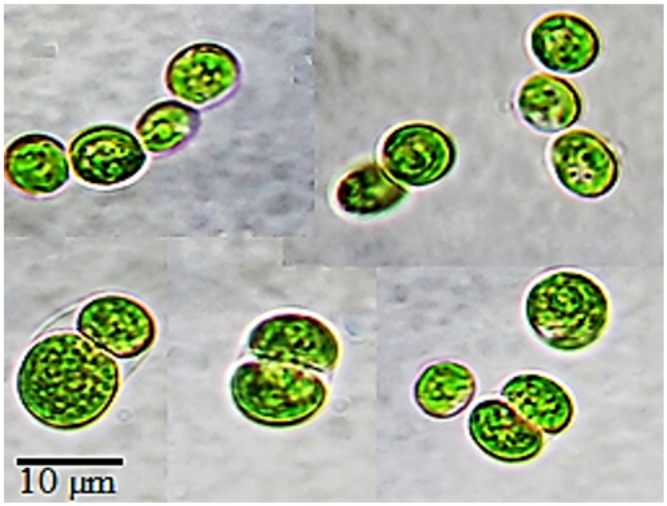

Figure 1

Photomicrograph (X400) of the green microalga Asterarcys quadricellulare

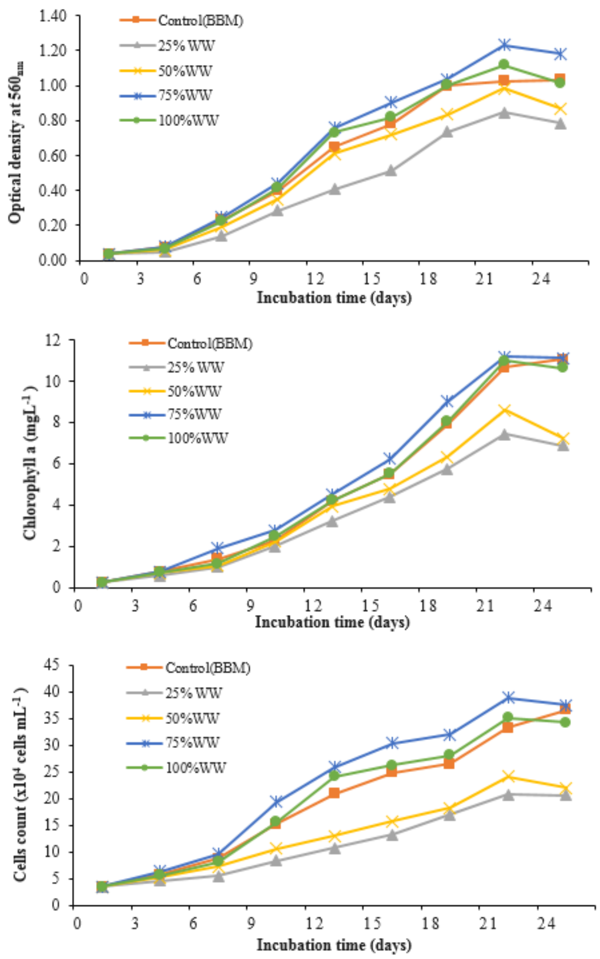

Figure 2

Growth curves of Asterarcys quadricellulare by monitoring of optical density, chlorophyll (a) and cells count grown for 24 days on control (BBM) and (25, 50, 75, 100%WW)

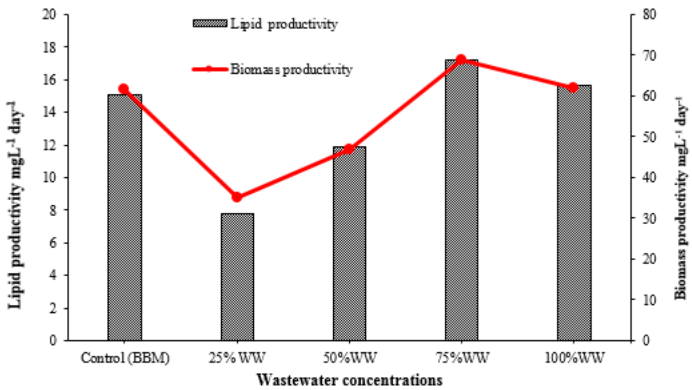

Figure 3

Biomass and lipid productivities of A. quadricellulare grown for 24 days on control and different municipal wastewater concentrations (25%, 50%, 75% and 100%WW)

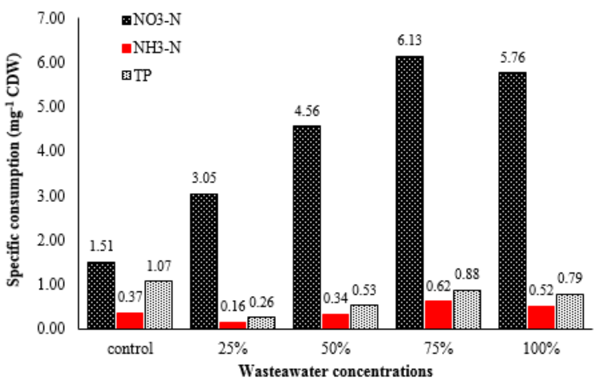

Figure 4

Specific consumption of nitrogen and phosphorous (mg g-1 CDW) by Asterarcys quadricellulare grown for 24 days on control and different concentrations of wastewater (25%, 50%, 75% and 100%WW)

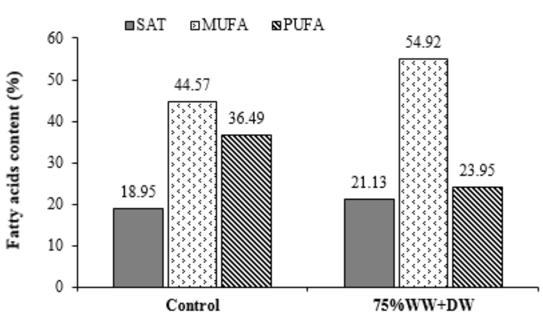

Figure 5

Fatty acids composition (SAT, MUFA, and PUFA) of Asterarcys quadricellulare grown in control BBM and 75%WW for 24 days