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Integrated microalgae cultivation, nutrient removal and product utilization bio-processes

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ABSTRACT

This research was done with different wastewater treatment plant effluent units (i.e. fine screen effluents (FSE), membrane bioreactor effluents (MBE) and final treatment effluent (FTE)). Microalgae that were grown in BG 11 medium were inoculated in the various wastewaters in indoor and outdoor culture system, and they were harvested for biodiesel production, production of bioethanol from the residue of biodiesel production, and finally, the protein content of the residue from bioethanol production was analysed for further co-products production. The cell number was highest in FSE outdoor microalgae with value of 15.95 cell × 10^4/L and least in MBE indoor microalgae with value of 3.04 cell × 10^4/L. FSE outdoor had initial nitrogen concentration of 105.91 mg N/L and gave nitrogen concentration after wastewater treatment with value of 1.03 mg N/L and FTE outdoor had the initial phosphorus concentration of 0.75 mg P/L and gave phosphorus concentration after wastewater treatment with value of 0.02 mg P/L. It was observed that FSE outdoor resulted in higher biodiesel yield (97.02%), and FSE indoor had the lowest biodiesel yield of 52.89%. The yield of ethanol got to 4.88% at 24 h fermentation period. The total protein analysis result was 0.53 $g_{protein}/g_{sample}$, i.e. approximately about 53% protein content of the total biomass content.

Keywords: Biodiesel; Bioethanol; Wastewater; Microalgae; Total protein; Biorefinery

1. Introduction

The necessity for clean energy is rising rapidly due to the increase in population and industrial development around the world, and as such renewable energy (solar, hydro and geothermal, etc.), carbon free transportation fuels and biofuels are being considered as the best alternatives to petrol fuels and other non-renewable sources such as natural gas, coal and nuclear [1]. Currently, one-fifth of the universe CO_2 emissions are from the transport sector, which thereby increase climate change due to the continuous use of fossil fuels that release these environmentally unfriendly gases to the atmosphere. The outlook for the reduction in emissions from this sector does not look hopeful as the number of motor vehicles on the roads globally is estimated to increase to over 2 billion vehicles by 2050 [1]. Also due to reduced supply, crude oil will continue to rise in cost, thereby making the production of fuels from alternate sources (such as biomass) more feasible. Biomass is one of the better

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sources of energy, and large-scale introduction of biomass energy could add to sustainable development environmentally and socio-economically, and its utilization could yield various end-products such as biofuels, bioenergy and bio-products (e.g. bioplastics, animal feed) [2]. Biodiesel produced from microalgal oil could thus substitute crude fossil petroleum by mass cultured biomass's microalgal oil for eco-sustainable biodiesel production in the near future. Biodiesel is a monoalkyl ester produced by the transesterification of triglycerides or free fatty acids with short-chain alcohols and has the ability to be used in conventional diesel engines with little or no modification [3]. Biodiesel has been experimentally shown to be less ecotoxic than petro-diesel. Lapinskiene et al. [4], reported from the study that diesel fuel at concentrations greater than 3% (w/w) is toxic to soil microorganisms. Biodiesel, however, is non-toxic at total soil saturation. Biodiesel contributes no net carbon dioxide or sulphur and overall less gaseous pollutants to the atmosphere than petro-diesel [4]. With growing concern for the environment, these factors play an important role in the acceptability of biodiesel, and to meet huge demands of this depleting energy in modern societies, bio-energy production based on photosynthesis will require tremendous amounts of water for cultivation of photosynthetic organisms such as microalgae. Fortunately, it is known that microalgae can use water of various sources such as wastewater, sea-water and freshwater [5]. However, the biomass and microalgae productivities in the wastewater would be re-estimated by the reuse efficiency of wastewater in cultivation of microalgae for biodiesel production. The production of biodiesel coupled with wastewater treatment is a promising solution to reduce the economic and environmental cost [6]. Hence, this research work aimed at the following objectives:

(1) To evaluate the nutrient removal efficiency by microalgae in the various components of wastewater treatment plant (WWTP) units.
(2) To produce biodiesel from the cultivated microalgae.
(3) To produce bioethanol from microalgae biomass residue from biodiesel production and
(4) To analyse the total protein content of microalgae biomass residue from bioethanol production.

2. Methodology

2.1. Microalgae sampling and inoculation

The microalgae used for this study were collected from the natural lagoon of the old Nicosia WWTP, and they were inoculated at 5% ($V_{\text{inoculation}}/V_{\text{BG 11 media}}$) in BG 11 (growth enrichment) culture medium. They were placed under Esco Class II Biosafety Cabinet photobioreactor in the laboratory, supplied with air blower (ADA AIR PUMP®, AP-2800) under continuous illumination of white fluorescent light of 45–50 μmol photon m^{-2} s^{-1} for two weeks before they were used for the wastewater treatment experiment. The microalgae growth parameters (such as optical density (OD), cell number and chlorophyll-*a* (Ch-*a*) and pH were analysed. The BG 11 (growth enrichment) medium contained these following chemical components:

Macro nutrients (g/L)	
$NaNO_3$	1.59
KH_2PO_4	0.40
$MgSO_4{\cdot}7H_2O$	0.08
Na_2CO_3	0.02
$Ca(NO_3)_2{\cdot}4H_2O$	0.02
EDTA	0.001
Citric acid	0.006

Micronutrients (g/L)	
H_3BO_4	1.43
$MnSO_4{\cdot}4H_2O$	0.91
$ZnSO_4{\cdot}7H_2O$	0.11
$CuSO_4{\cdot}5H_2O$	0.04
$Co(NO_3)_2{\cdot}6H_2O$	0.03

Three 2 L of BG 11 medium were produced and sterilized using autoclave at 1.5 MPa and 121 ℃ for 15 min [7].

2.2. Wastewater sampling and characterization

The wastewaters that were used in this study were collected from the New Nicosia Membrane Bioreactor (MBR) WWTP units in Nicosia, Turkish Republic of North Cyprus. The MBR treatment plant system consists of primary treatment where the solid part of the wastewater is being screened properly before feeding the water to the membrane treatment which consists of the biological phosphorus removal unit, aerobic unit, anaerobic unit, membrane filtration unit and finally the disinfection unit (Fig. 1). The wastewater samples were collected from fine screen effluent (FSE) unit, MBR unit and final treatment (FTE) unit as illustrated in Fig. 1.

All wastewaters were filtered using 0.2 μm nylon microfilters to remove fine suspended particles and were characterized for physicochemial and biological parameters according to standard methods [8] (Table 1) before they were applied for microalgae cultivation.

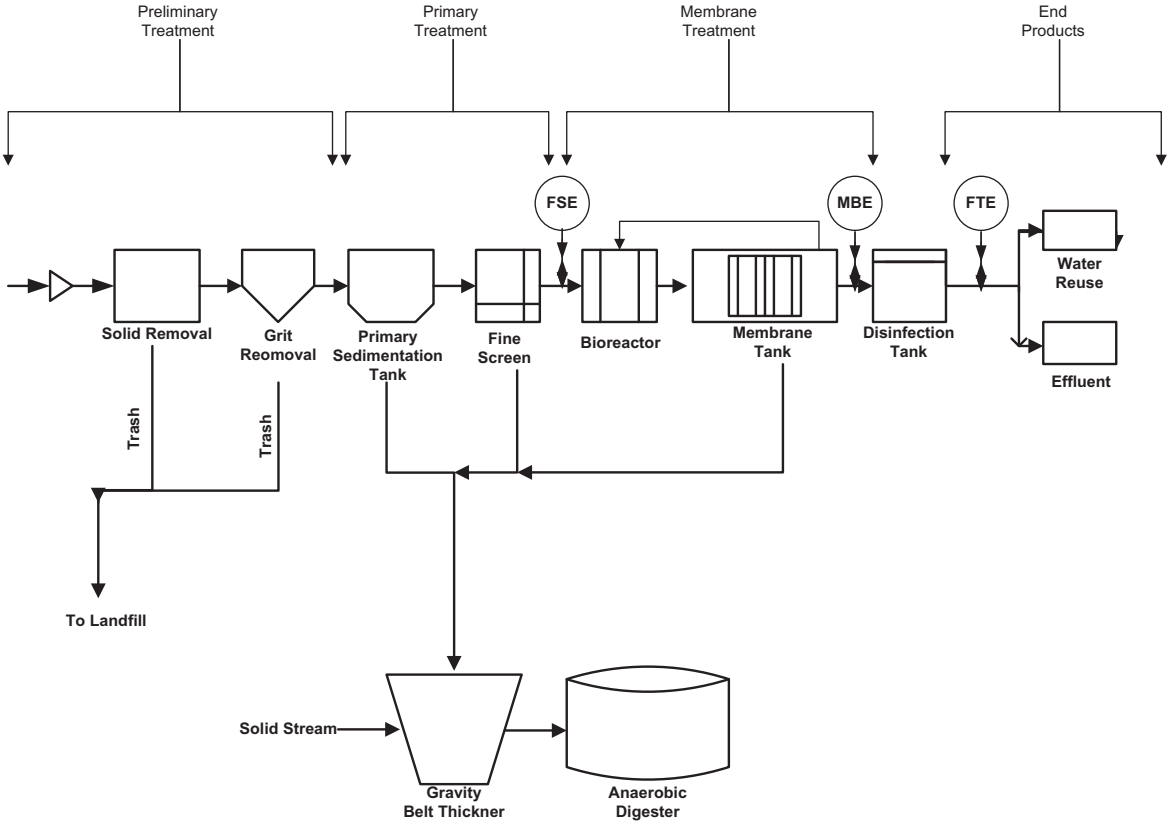

Fig. 1. New Nicosia MBR WWTP showing effluents sampling locations.

Table 1
Wastewater characterization from various WWTP units before microalgae inoculation

Parameters	FSE	MBE	FTE
pH	7.34	7.11	7.03
Temp (°C)	28.0	28.6	25.7
Turbidity (NTU)	46.1	0.65	0.17
COD (mg/L)	717	141	195
OD (680 nm)	0.077	0.001	0.001
EC (ms/cm)	2.10	2.01	2.02
TP (mg/L)	5.50	0.85	0.75
TN (mg/L)	105.91	12.91	11.86

2.3. Experimental set-up

The microalgae suspension in the BG 11 media were adjusted to an absorbance of 1.5 at an OD of 680 nm as measured using a spectrophotometer (UV-2450, UV–vis Spectrophotometer—Shimadzu) and were inoculated at 5% ($V_{inoculation}/V_{wastewater}$) into 1,000 mL of the various treatment media (FSE, MBR effluents (MBE) and FTE treatment media) in duplicate in both indoor (i.e. under Esco Class II Biosafety Cabinet photobioreactor in the laboratory and was supplied with air blower (ADA AIR PUMP®, AP-2800) under continuous illumination of white fluorescent light of 45–50 μmol photon $m^{-2}\,s^{-1}$) and in outdoor system (i.e. outside the laboratory, directly under the sunlight) for 5 d. The pH and temperature of the indoor treatment system were 8.3 ± 0.3 and 21 ± 1°C, respectively, while for the outdoor treatment system, the pH and temperature were 8.3 ± 0.5 and 21 ± 6°C, respectively. Batch treatment system was employed for this experiment, and samples for analysis were collected once everyday for 5 d from each treatment media.

2.4. Algae growth and nutrient removal analysis

2.4.1. Growth evaluation

Growth of all treatments were monitored both in indoor and outdoor culture media for 5 d. Growth of the microalgae were evaluated based on these parameters: OD, chlorophyll-*a* (Chl-*a*) content, cell number and specific growth rate (μ).

2.4.1.1. Chlorophyll-a (Chl-a) content. Chlorophyll-*a* was extracted and estimated using the procedure used by Chinnasamy et al. [9]. For this, 10 mL of algae biomass was suspended in the medium and centrifuged at 6,000 rpm for 30 min. Biomass collected after centrifugation was again suspended in 5 mL of methanol. The methanol and algae biomass suspension was then immersed in the water bath for 60 min at 60°C in order to extract the chlorophyll from the biomass. After the stipulated time, the chlorophyll-*a* concentration in the above suspension was spectrophotometrically determined using UV visible spectrophotometer (UV-2450, UV–vis Spectrophotometer—Shimadzu). The absorbance value was then substituted in Eq. (1) [9]:

$$\text{Chl}\,a\left(\frac{\mu\text{g}}{\text{mL}}\right) = 16.29\left(\text{A}^{665.2} - \text{A}^{750}\right) - 8.54\left(\text{A}^{652} - \text{A}^{750}\right) \quad (1)$$

where A750, A665.2, A652 are referred as the absorbance of algae biomass–chlorophyll suspension in methanol at 750, 665.2 and 652 nm, respectively.

2.4.1.2. Microscopic Cell Counting (cell × 10^4/L). One millilitres of algae biomass was collected using micro-pipette and was dropped in the haemocytometer slide and was viewed under the microscopy for cell count. The OD_{680} was converted to cell number (cell × 10^4/L), based on a linear relationship between the OD_{680} and cell number, which was obtained after an extensive data analysis and is given by Eq. (2):

$$\text{Cell number}\,(\text{cell} \times 10^4/\text{L}) = 18.412 \times \text{OD}_{680} + 0.4977\ \left(R^2 = 0.9812\right) \quad (2)$$

2.4.1.3. Specific growth rate ($\mu - d^{-1}$). Specific growth rate ($\mu - \text{d}^{-1}$) was calculated by fitting the microscopic cell number for the 5 d of cultivation to an exponential function, as shown in Eq. (3) [10]:

$$\mu = \frac{\ln(N_2/N_1)}{t_2 - t_1} \quad (3)$$

where N_1 and N_2 are defined as the microscopic cell number at times t_1 and t_2, respectively.

2.4.2. Nutrient monitoring and removal analysis

2.4.2.1. Total nitrogen (TN) analysis. Ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) were monitored daily using ion chromatography (IC—conductivity detector—Shimadzu (HIC-20A SUPER, conductivity detector) at 1 mL/min loading flow rate for anions and cation. AS9-SC (4 × 50 mm) column for anions and CS-12 (4 × 50 mm) column for cation) and they were added up to give the total nitrogen, as the algae are growing until the final culture period to estimate the rate of nutrient removal from the wastewater.

2.4.2.2. Total phosphorus (TP) analysis. Phosphorus was monitored daily spectrophotometrically using standard method [8]. The OD_{880} were converted to phosphorus (mg P/L), based on a linear relationship between the OD_{880} and phosphorus (mg P/L) which was obtained after an extensive data analysis and is given by Eq. (4):

$$\text{Phosphorus}\ \left(\text{mg P/L}\right) = 1.847 \times \text{OD}_{880} - 0.063 \quad \left(R^2 = 0.98\right) \quad (4)$$

2.4.2.3. Nutrient removal rate (N_{rx} mgL^{-1} d^{-1}). Nutrient removal rate (N_{rx} mgL^{-1} d^{-1}) was calculated using Eq. (5):

$$N_{\text{rx}}\left(\text{mg L}^{-1}\,\text{d}^{-1}\right) = \frac{N_{\text{f}} - N_{\text{i}}}{t_{\text{f}} - t_{\text{i}}} \quad (5)$$

where N_{f} and N_{i} are the final and initial nutrient concentration as TN or TP on t_{f} and t_{i}, respectively.

2.5. Biomass harvesting and treatment

Chemical precipitation was applied to separate biomass from the culture media. A solution of 2 M of NaOH was added in 20% v/v to the culture for precipitation of the biomass [11]. Mechanical mixing (40 rpm) allowed the suspension to be homogenized and was stopped 2 min after the end of NaOH addition to observe the behaviour of biomass [11]. The liquid phase was then removed using peristaltic pumping machine and the remaining precipitated biomass was dried overnight in an oven with forced air circulation set at 60°C. Afterwards, the weight of each dried sample was determined and recorded.

2.6. Microalgae oil extraction

Soxhlet extraction method using 250 mL of hexane as solvent was applied for the oil extraction. One gram

of dried algae was placed in the thimble (thimble is made from thick filter paper, which is loaded in the main chamber of Soxhlet extractor) of the Soxhlet apparatus. The Soxhlet extractor is placed onto a flask containing extraction solvent equipped with condenser. The solvent is heated to reflux at 56–60℃ for 8 h. The solvent phase was separated from oil phase by distillation after the extraction.

2.7. Transesterification and biodiesel production

Microalgae oils extract was mixed with a mixture of catalyst 1% NaOH (v/w) of microalgae oil and methanol: microalgae oil of 6:1. The mixture was kept for 4 h in magnetic stirrer at 125 rpm [12]. After shaking, the solution was kept for 16 h to separate the biodiesel and the sediment layers clearly. The biodiesel layer was separated by flask separator carefully. The percentage yield of biodiesel was calculated using Eq. (6):

$$\text{Yield of biodiesel}\,(\%) = \frac{\text{Grams of biodiesel produced}}{\text{Grams of extracted oil from algae}} \times 100 \quad (6)$$

2.7.1. Biodiesel analysis

The composition of algae biodiesel (fatty acid methyl esters) produced was determined using well-established GC analysis method as described by Nautiyal et al. [12]. Supelco™ 37 Component fame mix was used as a standard for the identification and quantification of the peaks obtained in the biodiesel sample on Shimadzu QP-2010 Plus with SP-2560 column (100 m × 0.25 mm × 0.20 μm) and flame ionization detector. A sample of 0.6 μL (0.5 mg of algae biodiesel in 1 mL of hexane) was injected under the split mode of 80:1, and injector temperature was maintained at 260℃ using nitrogen as a carrier gas. The oven was kept at 140℃ for 5 min and then heated up to 240℃ at the rate of 4℃/min with the holding time of 20 min. The detector temperature was set to 270℃. The fatty acid methyl ester peaks in the biodiesel sample were identified in comparison with the peaks obtained in the GC chromatogram for the above standard used.

2.8. Bioethanol production from microalgae biomass residue from biodiesel production

The algae biomass residues from biodiesel production were dried in the oven at 45℃ for 24 h and were further used for bioethanol production.

2.8.1. Biomass pretreatment

The dried biomass were all joined together, weighed and diluted in distilled water using 40 g/L raw material. 1% H_2SO_4 (v/v) was added and heated at 120℃ for 30 min for acidic pretreatment. Then, the pH was adjusted to 4.8 by adding 2 M NaOH, and finally, the total carbohydrate (TCH) was analyzed according to Rao and Pattabiraman [13] and total reducing sugar (TRS) was analyzed according to Miller [14].

2.8.2. Enzyme hydrolysis

A known volume of 25 U of cellulase per gram of substrate was added to the pretreated biomass sample in a 100-mL conical flask and was placed in a water bath device at 50℃ with little shaking at 125 rpm. The sample was withdrawn after 12 h contact time and was centrifuged for 30 min at 6,000 rpm. Finally, the supernatant was analysed for TRS and TCH.

2.8.3. Fermentation

Yeast medium which contains (*Saccharomyces cerevisiae*) was added to the sample at 7% (v/v), the conical flask was then sealed using cotton wool and paraffin and was placed in the water bath at temperature of 28℃ and pH of 4.25. Samples were collected from the medium at 0, 4, 8 and 24 h for TRS and ethanol analysis. Amount of ethanol produced from the fermentation process was analysed using the potassium dichromate method described by Adran [15].

2.9. Total protein analysis of microalgae residue from bioethanol production

The algae biomass residues from bioethanol production were dried in the oven at 45℃ for 24 h and were further used for protein content analysis. Bradford protein assay method was used for the analysis of the microalgae residue from bioethanol production [16]. The protein standard was done using bovine serum albumin (BSA), and absorbance was compared against a protein standard curve to calculate total protein content using Eq. (7):

$$\text{Protein content}\ (\text{g/g}) = 0.532 \times \text{OD}_{595} + 0.059 \quad (R^2 = 0.98) \quad (7)$$

3. Result and discussion

3.1. Microalgae growth and nutrient removal

3.1.1. Microalgae growth

Due to the possibility of contamination that may arise from the growth of heterotrophs [17] and feed wastewater of microalgae inoculant, the use of dry cell weight of microalgae for microalgae growth evaluation was not employed. Hence, the cell number and Chl-*a* content was used to evaluate microalgae growth.

From Figs. 2–4, the final cell number of FSE indoor microalgae was 12.28 cell × 10^4/L, FSE outdoor microalgae was 15.95 cell × 10^4/L, MBE indoor microalgae was 3.04 cell × 10^4/L, MBE outdoor microalgae was 4.41 cell × 10^4/L, FTE indoor microalgae was 4.24 cell × 10^4/L, and FTE outdoor microalgae was 4.57 cell × 10^4/L. From the result, FSE showed the highest growth rate which must have been due to the high nutrient content of the wastewater and presence of organic carbon can contribute to increase in biomass if other heterotrophs existed in the culture of microalgae, although FSE outdoor was higher maybe basically due to the high absorption of CO_2 from the natural environment and difference in light source and intensity. Growth of algae is affected by light intensity through its impact on photosynthesis [18]. Although rate of growth under increasing light intensity is a function of strain and culture temperature, the growth rate of algae is maximal at saturation intensity and decreases with both increase or decrease in light intensity [19].

The chlorophyll-*a* content of the microalgae cultivated in the various WWTP effluents in both indoor and outdoor was determined (Figs. 2–4). The final Chl-*a* content of FSE indoor microalgae was 0.70 μg/mL, FSE outdoor microalgae was 1.76 μg/mL, MBE indoor microalgae was 0.40 μg/mL, MBE outdoor microalgae was 0.46 μg/mL, FTE indoor microalgae was 0.42 μg/mL, and FTE outdoor microalgae was 0.53 μg/mL. From the result, FSE had the highest Chl-*a* content which must have been due to the high nutrient content of the wastewater although FSE outdoor was higher maybe basically due to the high absorption of CO_2 from the natural environment, light intensity and DO concentration. Chl-*a* content depends on light intensity, DO concentration and nutrient content of the culture media. Photoacclimation process in microalgae results in changes in properties of cell according to the availability of light and an increase in efficiency of photosynthesis [20]. This can occur through multiple mechanisms such as changes in quantities and types of pigments, growth rate, dark respiration rate or the availability of essential fatty acids [21]. Various microalgae have distinct responses depending on the source, availability and periodicity of inputs of dissolved nutrients [21]. According to Menendez et al.'s [22] study, the chlorophyll concentration of *C. linum* increased during the first 4 d of incubation in nitrogen treatments. After 10 d, this concentration decreased, but nitrogen content in the tissues remained stable. Their results indicate that *C. linum* used chlorophyll to store nitrogen when surplus nitrogen was available and that nitrogen was lost from the chlorophyll pool immediately after the removal of the external nitrogen supply.

The specific growth rates of the microalgae cultivated in the various WWTP effluents in both indoor and outdoor were determined using the exponential phase (which was day 2 and day 3 for FSE outdoor and day 2 and day 4 for FSE indoor, day 1 and day 5 for MBE indoor, day 1 and day 4 for MBE outdoor and day 1 and day 4 for FTE indoor and outdoor). The specific growth rate of microalgae in FSE indoor 0.97 d^{-1}, FSE outdoor was 1.61 d^{-1}, MBE indoor was 0.23 d^{-1}, MBE outdoor was 0.36 d^{-1}, FTE indoor

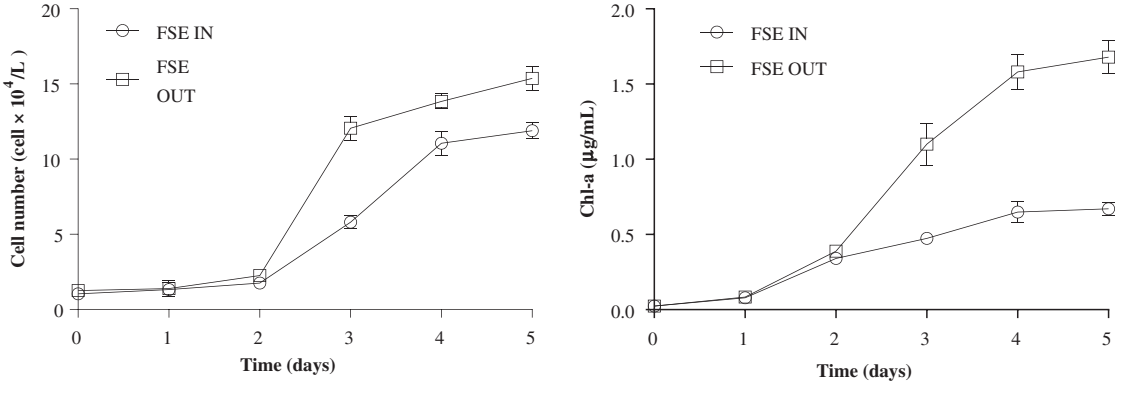

Fig. 2. Cell number and Chl-a content of microalgae in FSE indoor and outdoor.

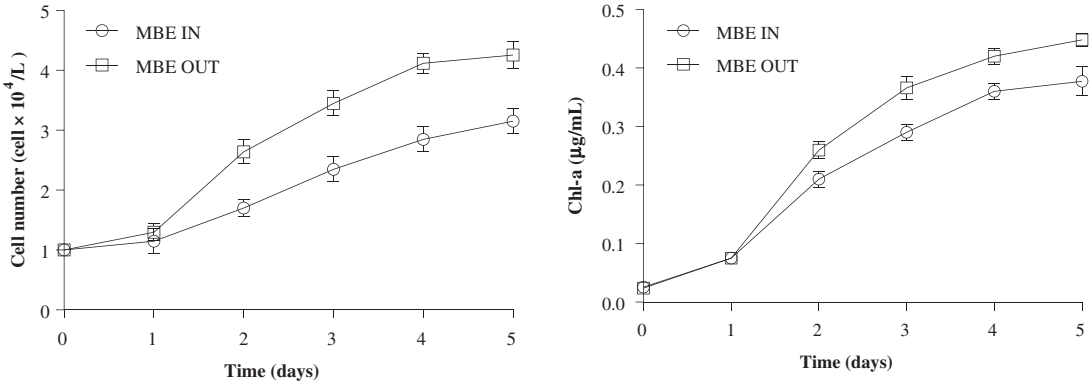

Fig. 3. Cell number and Chl-a content of microalgae in MBE indoor and outdoor.

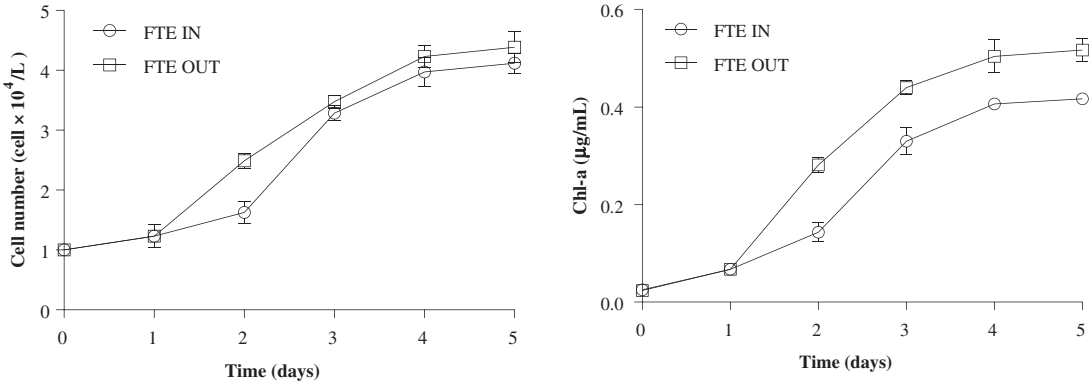

Fig. 4. Cell number and Chl-a content of microalgae in FTE indoor and outdoor.

microalgae was 0.37 d^{-1}, and FTE outdoor was 0.38 d^{-1} (Table 2). From the result, a higher μ was obtained in FSE outdoor which is in line with previous report such as Ji et al. [23] whose μ was higher in day 3 with 1.37 d^{-1}, and He et al. [24] reported higher μ of value 0.92 d^{-1} in N30 medium.

3.1.2. Nutrient removal

Treatments of wastewater with algae resulted in better removal of nutrient than the conventional activated sludge system [25]. Algae treatment is applied in some treatment processes, after an activated sludge system, as a tertiary treatment used to comply with discharged standards [26]. It may be beneficial to completely replace or simultaneously integrate the activated sludge system with algae-based treatment system for simultaneous nutrients and energy production rather than only being used as a tertiary treatment. Hence, this research tends to look at both possibilities.

Table 2
Specific growth rate (SGR) of microscopic cell number in various treatment media

Treatment media	SGR ($\mu - d^{-1}$)
FSE indoor	0.97
FSE outdoor	1.61
MBE indoor	0.23
MBE outdoor	0.36
FTE indoor	0.37
FTE outdoor	0.38

3.1.3. Nitrogen removal

The concentration of nitrogen and nutrient removal rate per day during different periods of time and treatments are shown in Figs. 5–7 and Table 3. The achieved concentrations after treatment in various wastewater treatment media are 1.85, 1.03, 1.20, 1.29, 1.05 and 1.48 mg N/L for FSE IN, FSE OUT, MBE IN, MBE OUT, FTE IN and FTE OUT, respectively. The nutrient removal rate per day for various wastewater treatment media are 26.01, 26.22, 2.72, 2.67, 2.65 and 2.54 mg N L^{-1} d^{-1} for FSE IN, FSE OUT, MBE IN,

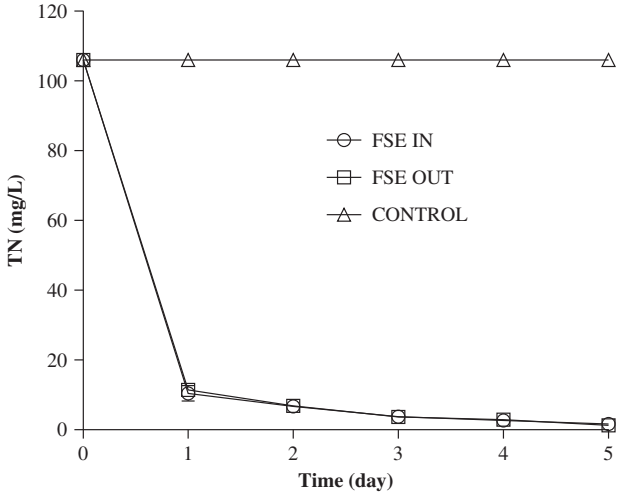

Fig. 5. Microalgae uptake of Total Nitrogen in FSE indoor and outdoor.

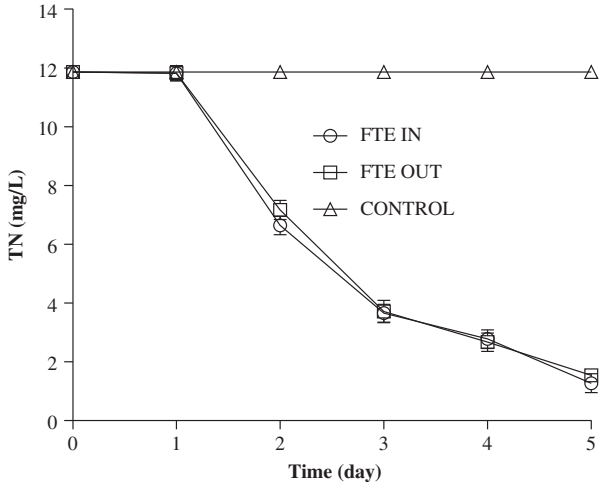

Fig. 7. Microalgae uptake of total nitrogen in FTE indoor and outdoor.

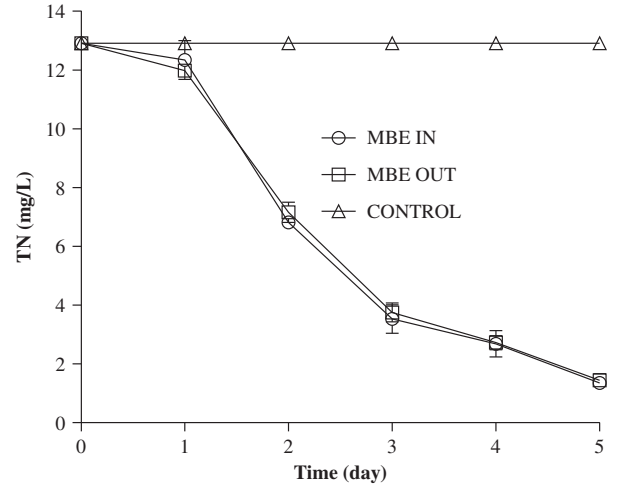

Fig. 6. Microalgae uptake of Total Nitrogen in MBE indoor and outdoor.

MBE OUT, FTE IN and FTE OUT, respectively. Rasoul-Amini et al. [26] reported 30.30 mg N/L as achieved concentration after treatment of wastewater using *Chlorella* sp. for 14 d. Ji et al. [23] reported that the maximum specific consumption rate of TN (16.8 mg-N g-cell^{-1}) was observed with *C. vulgaris*.

3.1.4. Phosphorus removal

The concentration of phosphorus and nutrient removal rate per day during different periods of time and treatments are shown in Figs. 8–10 and Table 3. The achieved concentrations after treatment in various wastewater treatment media are 0.93, 0.86, 0.14, 0.02, 0.1 and 0.2 mg P/L for FSE IN, FSE OUT, MBE IN, MBE OUT, FTE IN and FTE OUT, respectively. The nutrient removal rate per day for various wastewater treatment media are 1.12, 1.11, 0.17, 0.20, 0.16 and 0.17 mg P L^{-1} d^{-1} for FSE IN, FSE OUT, MBE IN, MBE OUT, FTE IN and FTE OUT, respectively. Rasoul-Amini et al. [26] reported 1.95 mg P/L as achieved concentration after treatment of wastewater using *Chlorella* sp. for 14 d. Ji et al. [23] reported that the maximum specific consumption rate of TP (3.1 mg P g-cell^{-1}) was observed with *S. obliquus*.

Table 3
Nutrient removal rate per day for all wastewater treatment media

Wastewater treatment media	TN (mg N L^{-1} d^{-1})	TP (mg P L^{-1} d^{-1})
FSE IN	26.01	1.12
FSE OUT	26.22	1.11
MBE IN	2.72	0.17
MBE OUT	2.67	0.20
FTE IN	2.65	0.16
FTE OUT	2.54	0.17

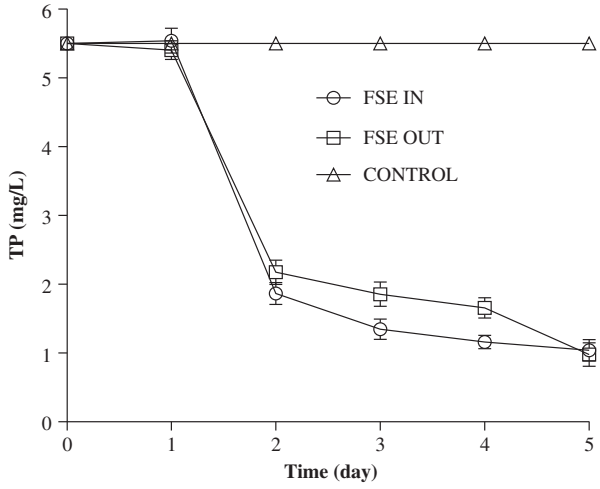

Fig. 8. Microalgae uptake of phosphorus in FSE indoor and outdoor.

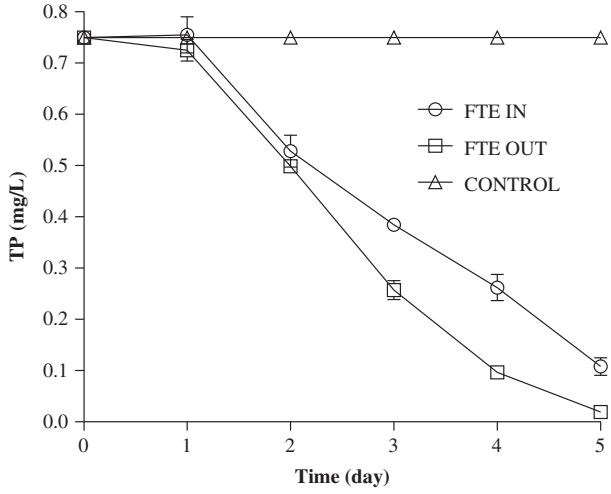

Fig. 10. Microalgae uptake of phosphorus in FTE indoor and outdoor.

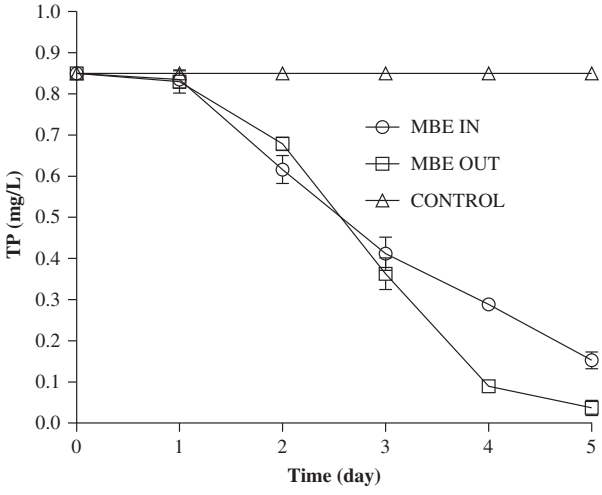

Fig. 9. Microalgae uptake of phosphorus in MBE indoor and outdoor.

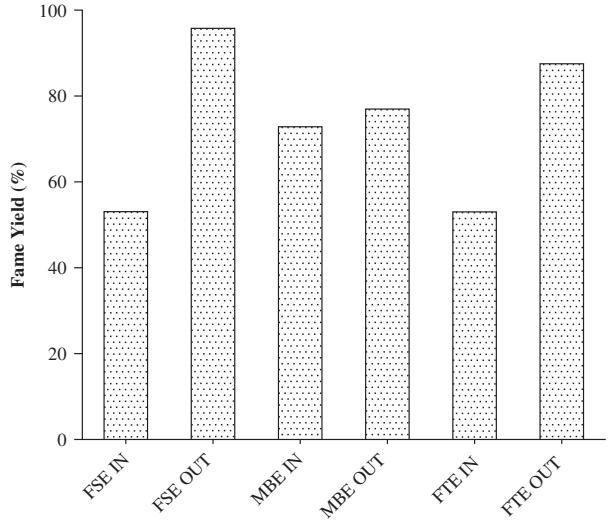

Fig. 11. FAME yield (% by weight of crude lipid) in all treatments.

3.2. Biodiesel production and analysis

3.2.1. Biodiesel production

The biodiesel yield obtained from various treatments microalgae is shown in Fig. 11. The biodiesel yield was expressed in terms of relative weights of biodiesel obtained to that of oil present in algae biomass. It was observed that FSE outdoor resulted in higher biodiesel yield (97.02%) compared to the other treatments (Fig. 11) and this may have been as a result of the higher growth rate observed in the same treatment. FSE indoor had the lowest biodiesel yield of 52.89%.

3.2.2. Biodiesel analysis

GC analysis was used to study the chemical composition of algae biodiesel produced from various wastewater treatment media. The major peaks were identified using the SupelcoTM 37 Component fame mix standard for all the biodiesel samples. The peaks in the chromatograms of biodiesel samples and the standard were compared, and their respective retention time was used to identify and quantify the peaks. The analysis majorly showed the presence of six saturated fatty acids (palmitic, caprylic, myristic, lauric, stearic and lignoceric) and five unsaturated fatty acids (linolenic, linoleic, oleic, palmitoleic and

Table 4
Fatty acid composition of algal biomass in various treatment samples

FAME	FORMULA	Fatty acids in various biodiesel (%)					
		FSE IN	FSE OUT	MBE IN	MBE OUT	FTE IN	FTE OUT
Caprylic acid	$C_8H_{16}O_2$	2.15	2.97	0.15	0.44	2.54	2.88
Lauric acid	$C_{12}H_{24}O_2$	1.94	2.07	4.35	3.47	1.96	2.50
Myristic acid	$C_{14}H_{28}O_2$	3.54	3.22	4.58	3.98	3.88	3.61
Pentadecanoic acid	$C_{15}H_{28}O_2$	2.21	1.44	3.79	3.45	2.94	2.19
Palmitic acid	$C_{16}H_{32}O_2$	32.15	28.44	14.56	15.54	22.54	25.44
Palmitoleic acid	$C_{16}H_{30}O_2$	12.57	14.46	5.45	6.66	11.54	12.40
Stearic acid	$C_{18}H_{36}O_2$	5.50	4.97	12.04	10.87	5.55	5.88
Oleic acid	$C_{18}H_{34}O_2$	10.05	10.99	15.48	14.77	9.98	10.05
Linoleic acid	$C_{18}H_{32}O_2$	5.88	9.45	11.48	10.88	12.55	10.54
Linolenic acid	$C_{18}H_{30}O_2$	15.48	13.22	14.56	16.78	14.4	15.01
Arachidonic acid	$C_{20}H_{32}O_2$	0.98	3.11	5.66	4.44	2.45	2.14
Eicosapentaenoic acid	$C_{20}H_{30}O_2$	4.11	3.15	2.45	3.34	4.58	4.90
Docosahexaenoic acid	$C_{22}H_{32}O_2$	0.66	1.15	1.99	1.77	1.4	1.25
Lignoceric acid	$C_{24}H_{48}O_2$	1.13	0.87	2.20	2.30	0.99	1.01
FAME of main fatty acid (C16–C18)		*81.63*	*81.53*	*73.57*	*75.5*	*76.56*	*79.32*

Note: The italic values are to distinctively specify the FAME content of main fatty acid (C16–C18) of the microalgae biodiesel produced from the study.

pentadecanoic) methyl esters in all the algae biodiesel chromatograms. However, three extra peaks of polyunsaturated fatty acid (PUFA) (eicosapentaenoic, docosahexaenoic and arachidonic) were observed for all samples' algae biodiesel. Table 4 shows C16–C18 fatty acids which are suitable for biodiesel production [12,27–29]. The oil was mainly composed of 46.19% unsaturated fatty acid among the total known fatty acids for FSE IN, 49.56% for FSE OUT, 50.76% for MBE IN, 52.54% for MBE OUT, 51.41% for FTE IN and 50.19% for FTE OUT (Table 4). European standard EN 14214 applied a condition on limit of 12% for C18:3 (linolenic) for quality vehicle biodiesel. However, the biodiesel produced by this study contained 15.48% of C18:3 (linolenic) for FSE IN, 13.22% for FSE IN which was much more closer to the EN 14214 linolenic acid limit, 14.56% for MBE IN, 16.78% for MBE OUT, 14.40% for FTE IN and 15.01% for FTE OUT. In general, the composition of many microalgal oils is not suitable to stand with the EN14214 biodiesel standards, because of the extent of unsaturation of microalgae oil [27,30]. But this problem can be solved and the quality of biodiesel can be improved either by partial catalytic hydrogenation of the oil [27,31] or by blending with other sources of biodiesel obtained from non-food feedstock [27,32]. It is interesting to note that the algal biomass in the various samples also produced high-value fatty acids for human nutrition and food additives such as arachidonic acid (C20:4), docosahexaenoic acid (C22:6) and eicosapentaenoic acid (C20:5) which account from 0.98 to 2.14%, 0.66 to 1.99% and 2.45 to 4.90%, respectively, of total fatty acid for all samples (Table 4). C20:4 and C20:5 are important PUFAs which play vital role in the prevention of various human diseases [27,33] while C22:6 are important PUFA which play vital role in the brain functionality [31]. Thus, it might be feasible to extract these high-value products to improve the overall economic viability and also in order to comply with biodiesel standard on the PUFA ra tio.

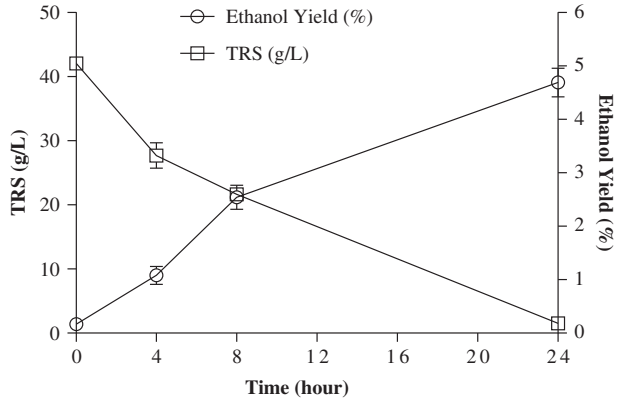

Fig. 12. Ethanol production yield and TRS consumption.

3.3. Bioethanol production

Fermentative conversion of hydrolysed microalgae biomass to ethanol was investigated by using the

ethanol-producing strain *S. cerevisiae* with separate hydrolysis and fermentation (SHF) processes. After pretreatment studies (acidic pretreatment and cellulosic hydrolysis), TRS amount was 27.09 g/L. When inoculated yeast medium was added to the pretreated sample, TRS rose up to 42.07 g/L at 0 h. Shortly after 4 h of inoculation, the TRS was reduced to 29.10 g/L, after 8 h decreased to 21.45 g/L, then after 24 h, it was significantly reduced to 1.47 g/L, which was accompanied by a sharp increase in ethanol concentration, achieving an ethanol yield of 4.88% (Fig. 12). This result was in agreement with that of Ho et al. [34] who observed high yield of ethanol concentration (11.7 g/L).

3.4. Total protein content of microalgae residue from bioethanol production

The total protein content of microalgae biomass was 0.53 g(protein)/g(sample), that is approximately about 53% protein content of the total biomass content. This result was in agreement with that of Lopez et al. [35] that reported total protein content of about 30–55% of dry weight. The value obtained from this study is higher than some protein sources such as soybean which is 48%, etc. therefore, it can be recommended that the residue could be further used for or as blends for animal, and fish feed production, cosmetics and food ingredients production, etc., since fish require diets containing 30–55% of crude protein and amino acid supply precisely adapted to meeting the needs for optimal growth [36] and animals such as birds need 15–20% of crude protein and amino acid supply precisely adapted to meeting the needs for optimal growth.

4. Conclusion

This study investigated nutrient removal from wastewater effluents and bio-refining of various products from the microalgae biomass used in this study. The various biorefinery products are biodiesel bioethanol and protein analysis for other co-products such as fish feed production and animal feed production.

Based on these experiments, it can be concluded also that

(1) Microalgae can grow in wastewater and can also be used to remove nutrients such as N and P from wastewater.
(2) Microalgae can reduce or remove nutrients better than activated sludge system.
(3) Wastewater from fine screening effluent chambers gave the best growth amount (15.95 cell × 10^4/L) and highest biodiesel yield (97.02%).
(4) The biodiesel residue was further used for bioethanol production of 4.88% at 24 h fermentation period.
(5) Finally, the residue from bioethanol production was analysed for protein content which gave about 53% protein content of the total biomass content which can be further used for co-products production such as animal feeds, condiments and drugs.

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List of symbols

OD_{680}	—	optical density at 680nm
R^2	—	regression coefficient
A^{750}	—	optical density at 750nm
$A^{665.2}$	—	optical density at 665.2nm
A^{652}	—	optical density at 652nm
N_1 and N_2	—	are defined as the microscopic cell number at times t_1 and t_2, respectively.
N_f	—	is the final nutrient concentration as total nitrogen (TN) or total phosphorus (TP)
N_t	—	is the initial nutrient concentration as total nitrogen (TN) or total phosphorus (TP)
t_i	—	is the corresponding nutrient concentration at "N_t"
t_f	—	is the corresponding nutrient concentration at "N_f"

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