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Cultivation of algae consortium in a dairy farm wastewater for biodiesel production

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ABSTRACT

Dairy farm wastewaters are potential resources for production of microalgae biofuels. A study was conducted to evaluate the capability of production of biodiesel from consortium of native microalgae culture in dairy farm treated wastewater. Native algal strains were isolated from dairy farm wastewaters collection tank (untreated wastewater) as well as from holding tank (treated wastewater). The consortium members were selected on the basis of fluorescence response after treating with Nile red reagent. Preliminary studies of two commercial and consortium of ten native strains of algae showed good growth in wastewaters. A consortium of native strains was found capable to remove more than 98% nutrients from treated wastewater. The biomass production and lipid content of consortium cultivated in treated wastewater were 153.54 t ha^{-1} $year^{-1}$ and 16.89%, respectively. 72.70% of algal lipid obtained from consortium could be converted into biodiesel.

1. Introduction

Exhausted water and petroleum resources have put the viability of algal biofuel into account. Although the idea of using microalgae as a source of biofuel is not new [4], in fact it has been known

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for more than five decades [23], production of biodiesel from rejected cooking oil is also very common [6,11] but indeed it is now being taken seriously because of escalating price of petroleum and well associated global warming with burning fossil fuels [14]. Algal biodiesel is the best option to decrease the intensity of radiative forcing in order to reduce the effects of global warming caused by burning fossil fuels. The water requirement for algae culture is very high especially in open pond system, but fortunately their capability to grow in industrial, farm, municipal, and agricultural wastewater [28] resolves this problem as well as contributes in treatment of wastewater by decreasing the value of COD, BOD and removal of heavy metals, the treated wastewater can be use in other purposes such as irrigation. In 2007, World Mapper Projects [32,33] had calculated the water used in domestic purpose and in industries was 990 billion m^3 and after used water turns into wastewater polluting the environment. Another source of nutrients containing wastewater is from livestock or cattle industries. The major problem with most cattle wastewaters is the high concentrations of nutrients, particularly total N and total P concentration, which require costly chemical-based treatments to remove them during wastewater treatment [13]. Total N and P concentrations can be found at values of 10–100 mg L^{-1} in municipal wastewater, $>$ 1000 mg L^{-1} in agricultural effluent and 500–600 mg L^{-1} in farm wastewater [2]. The ability of microalgae to effectively grow in nutrient-rich environments and to efficiently consume nutrients and accumulate metals from the wastewater, make them an extremely attractive means for sustainable and low cost wastewater treatment [5,26,22].

However, it has also long been proposed that wastewater-grown algae could be used for energy production [3,35]. Wastewater treatment usually involves additional cost thus, if the treatment itself produces income, prevents pollution and complies with environmental standards, it increases the profitability and enhances the sustainability of the industry. According to Chinnasamy et al. [3] almost 500 billion m^3 industrial wastewater could produce 37 million t of algal oil, with variation due to the difference in nutrients composition in wastewater as well as difference in potentiality to accumulate lipid in algal strain. In this study the undertaken aims were to isolate the algal strains which were present in wastewaters sites and the strains were selected on the basis of lipid productivity via using Nile red fluorescence analysis to form consortium. The final objective of this study was to evaluate treated dairy farm (Perlis, Malaysia) wastewater for biodiesel production by cultivating consortium.

The dairy farm generated almost 182.5 million L wastewater $year^{-1}$ which are capable to produce 15,700 t biomass of algal consortium and $\sim$3 million L algal oil $year^{-1}$.

2. Methods

2.1. Chemicals and reagents

Methylation catalysts, FAME standards, hexane and methanol were obtained from Sigma-Aldrich (Switzerland). All chemicals were analytical grade reagents.

2.2. Wastewater collection and analysis

The wastewater used in the study was collected from a moderate size commercial dairy farm located at Perlis, Malaysia and immediately stored at 4 °C to minimize substrate decomposition prior to analysis.

2.3. Isolation of microalgae from wastewater samples and their consortium

The untreated and treated wastewater samples were collected from the dairy farm wastewater collecting and holding tank sites Fig. 1. These samples were used as the sources of obtaining native algal strains. BG11 medium was used as for the enrichment of algal strains in wastewater prior to isolate them in different colonies. For strains enrichment, 50 mL of BG11 media were taken into 250 mL flask and added with 50 mL of treated and untreated wastewater sample in different flasks.

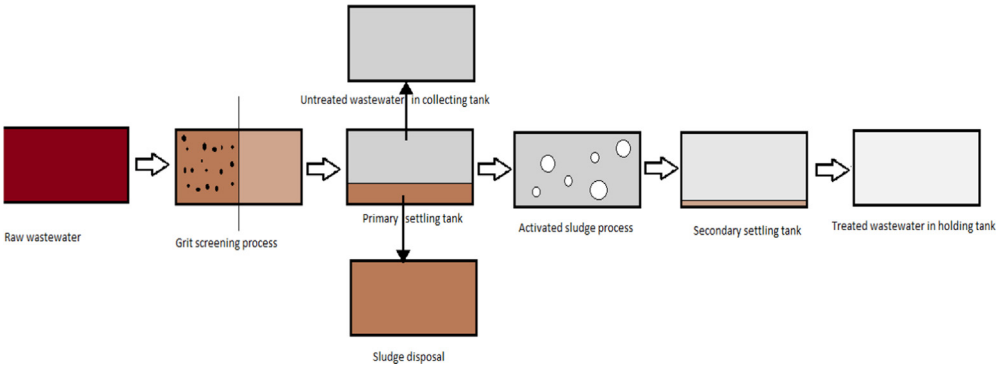

Fig. 1. Schematic diagram of dairy farm wastewater treatment plant.

The flasks were put under 80 μmol m^{-2} s^{-1} at 30 °C and *L*:*D* cycle of 12:12 h for two weeks and after that the cells were streaked with the help of loop onto 1.5% agar BG-11 medium plates to get as much as possible unialgal strains by selecting individual colonies for sequential sub-culturing. The purified strains were culture in conical flasks containing sterile BG-11 liquid medium in photo bioreactor. The consortium was prepared by mixing equal quantities of 10 wastewater isolated strains with a biomass concentration of ~0.1 g L^{-1} each.

2.4. Preliminary screening of microalgae

Four strains of microalgae *Chlorella saccharophila* UTEX 2911, *Chlamydomonas pseudococcum* UTEX 214, *Scenedesmus* sp UTEX1589 and *Neochloris oleoabundans* UTEX 1185 were obtained from UTEX and a consortium of wastewater isolates were screened in BG11, treated and untreated wastewaters to examine their growth response in terms of chlorophyll *a* content. The preliminary screening were conducted in universal bottle containing 15 mL of sterilized treated and untreated wastewater and with BG11 medium as control kept the incubation conditions same as mentioned in Section 2.3. On the basis of their growth response in screening two strains and consortium were selected to evaluate their feasibility to produce biomass and lipid in two different types of wastewaters from dairy farm.

2.5. Biomass production and nutrient removal of consortium from wastewater

To evaluate the biomass production of consortium and selected commercial strains of algae, the experiments were conducted under three different levels of CO_2 (ambient, 5% and 10%) in 1 L capacity flasks with 500 mL sterilized treated wastewater as growth medium in triplicates with an irradiance of 80 μmol m^{-2} s^{-1} and *L*:*D* cycle of 12:12 h for 10 days at 120 rpm in photo bioreactor. The CO_2 was bubbled through the medium with air mixture at the rate of 150 mL min^{-1}.

2.6. Upscale of algal biomass in high rate algae pond (HRAP)

The outdoor cultures of consortium were upscale in HRAP. The HRAP were of single loop raceway with the dimensions of 2.5 m × 0.7 m × 0.7 m with 600 L working capacity and mixed by paddle wheel (20 rpm). The transparent corrugated acrylic roofs were fixed at a height of approx 2.5 m above from the top of the pond to protect the algae from rain and to obtain the optimum intensities of sunlight (~80–120 μmol m^{-2} s^{-1}). The cultures were conducted at 10% CO_2–air mixture bubbled through the growth medium at a rate of 5 L min^{-1} and the tropical daylight–dark cycle was close to 12 h:12 h for 10 days. The recorded temperature for 10 days of culture growth medium varied from 27 to 32 °C.

2.7. Lipid extraction

To assess the feasibility of producing biodiesel from algal biomass, the consortium cultivated in treated wastewater in HRAP was harvested using a continuous flow centrifuge after 10 days. Harvested algae were dried at 60 °C for 24 h for extraction of lipids analysis. Lipids were extracted with hexane in a Soxhlet apparatus operated at 80 °C for 10 h. After Soxhlet extraction, hexane was evaporated at 50 °C and 100 mbar using a rotary evaporator. The crude lipid was then re-dissolved in hexane and stored into a sealed glass vial in the dark at 5 °C for not more than 15 days.

2.8. Production of biodiesel from extracted algal lipid

Biodiesel from crude algal lipid was obtained by two-step process. Crude lipid or oil containing more than 4% free fatty acids should go through an acid esterification process to increase the yield of biodiesel. The first step used an acid esterification. All reagents were added in stoichiometric excess. In acid esterification process methanol and 10% H_2SO_4 were added to the lipid in hexane solution stored in vial. The mixture was transferred into a flask, heated to 50 °C, and moderately agitated for 2 h. In the second step, 25% potassium methoxide (KCH_3O) in methanol was added drop wise until a pH value of 13 was attained [15], because of the following reasons [21].

(a) To neutralize acid added in acid esterification.
(b) To suppress interfering water contaminants.
(c) To transmethylate acylglycerols.
(d) To transmethylate phospholipids.

The mixture was evaporated at 60 °C to obtain fatty acid methyl ester (FAME).

2.9. Instrumental analysis of FAME

Analysis of the obtained FAME compositions were carried out using PerkinElmer Inc 600 GC-FID equipped with a SP™ 2340 fused silica column ($L \times$ I.D. 30 m $\times$ 0.32 mm, d_f 0.20 μm; Sigma Aldrich). Helium was used as the carrier gas at constant flow rate of 1.5 mL min^{-1} the oven temperature was raised from 150 °C at 2 °C min^{-1} to 200 °C and maintained for 10 min. Both the injector and the FID temperatures were 210 °C. One μL sample of FAME was injected into the column. Each sample was analyzed in triplicates. Individual FAME was identified by comparison of its retention time with that of the mixed standard FAME. Concentrations of different FAMEs in the sample were quantified by comparing their peak areas with those obtained from the mixed standard FAME of known concentration.

2.10. Quantification of common parameters

a) To determine the chlorophyll *a*, 10 mL of homogenized algal cells were centrifuged at 5000 rpm for 10 min. The algal pellet was exhaustively extracted with hot methanol until it was colorless. Chlorophyll *a* was determined using UV–vis spectrophotometer at 630 nm.
b) To calculate biomass, Whatman GF/C glass fiber filters were dried at 60 °C for 24 h.
c) Total carbohydrate was quantified by the method of Dubois et al. [9], using glucose as standard.
d) Protein content was determined by the method of Kjeldhal according to Hungria and Araújo [18].
e) Nile red fluorescence analysis was conducted as mentioned in Doan et al., [8].
f) Total lipid quantification was done as mentioned in Chinnasamy et al. [3].

2.11. Statistical analysis

Statistical analyses were performed using SPSS software. The screening data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Differences were considered significant at $p < 0.05$.

3. Result and discussion

3.1. Characteristics of dairy farm wastewater

The treated and untreated dairy farm wastewaters used in this study were analyzed for determining physico-chemical characteristics and their variation during the course of research. Many parameters such as biochemical oxygen demand (BOD) chemical oxygen demand (COD), total suspended solids (TSS), and total dissolved solids (TDS), total kjeldahl nitrogen (TKN) showed significant difference in treated and untreated wastewaters, listed in Table 1. Concentration of phosphorus was found sufficient to support algal growth in both treated and untreated wastewater. Hillebrand and Sommer [17] recommended 46.1:7.7:1 mass ratio of C:N:P for algal growth, whereas in this study the wastewater used had N:P ratio of 0.928:1 and 4.6:1 for treated and untreated wastewater, respectively, which indicated treated wastewater as N limitation media. The wastewaters used were considered as favorable for production of algal lipids as other researcher had also found that the stress of N deficiency increased the lipid content in algal cell [29]. Other parameters of wastewater for present study were found within safe range.

3.2. The microalgae community in dairy farm wastewater

The communities of algae found in wastewaters were continuously with exposure of same pollutants with what they had been cultivated in as medium. The composition of algal community in dairy farm wastewater were examined for one year, and listed in Table 2. Total 10 species of green algae, 7 species of cyanobacteria and 3 species of diatoms were observed in dairy farm wastewaters (treated and untreated). In terms of biovolume green algae was the major group of algae dominating both untreated and treated wastewater throughout the year followed by cyanobacteria and diatoms. *Chlorella* had the biggest % contribution in community of green algae followed by *Ankistrodesmus,*

Table 1
Physicochemical characteristics of dairy farm wastewater.

Parameter	Value (ppm)	
	Treated wastewater	Untreated wastewater
pH	7.01–8.79	6.46–8.01
Biochemical oxygen demand	87–91	500–540
Chemical oxygen demand	109.7–120.76	989–1100
Total suspended solid	21.45–50.77	156–213
Total dissolved solid	101.10–135.11	445.13–689.56
Total kjeldahl nitrogen	3.58–6.63	215.65–305.81
NH_4–N	0.62–4.01	162.76–201.14
NO_3–N	1.08–4.82	35.65–47.88
PO_4–P	3.26–6.11	46.77–54.78
Na	110.60–150.55	121.67–143.90
Fe	0.01–0.05	0.14–0.19
Cu	0.002–0.008	0.01–0.015
Zn	0.003–0.005	0.01–0.14

Table 2
% Bio-volume of algal community found in treated and untreated wastewater from dairy farm during a year.

Strains	% Bio-volume of algae during a year (Jan 2013–Dec 2013)							
	Treated wastewater				Untreated wastewater			
Chlorophyta	Jan–Feb	Mar–May	Jun–Jul	Aug–Dec	Jan–Feb	Mar–May	Jun–Jul	Aug–Dec
Ankistrodesmus sp.	17.46	21.14	13.09	27.67	2.19	2.88	1.03	2.85
Chlamydomonas sp	8.02	17.65	10.21	11.59	18.41	39.18	18.06	8.64
Chlorella sp.	20.77	33.88	16.93	30.73	20.03	43.03	26.80	44.11
Chlorella sorokiniana								
Chlorella vulgaris								
Chlorococcum humicola	4.64	3.89	4.09	5.02	18.08	2.81	23.12	41.68
Closterium sp.	1.36	0.78	1.10	1.12	0.08	NA	0.05	NA
Oedogonium sp.	NA	0.12	NA	0.78	NA	NA	NA	0.03
Scenedesmus quadricauda	5.21	11.43	7.47	15.75	2.43	10.02	1.91	1.20
Scenedesmus obliquus								
Total Chlorophyta	57.46	88.89	52.89	92.66	61.14	97.92	70.97	98.48
Cyanophyta								
Anabaena sp.	19.66	5.67	15.54	2.24	1.10	0.23	5.03	0.29
Chroococcaceae sp.	0.94	NA	0.45	NA	NA	0.02	3.60	NA
Limnothrix redekei	12.35	3.06	16.87	0.32	2.45	0.56	1.52	NA
Limnothrix sp								
Nostoc sp.	1.02	0.30	4.15	2.11	NA	NA	NA	NA
Oscillatoria sp	5.87	NA	2.21	1.21	32.97	1.08	13.79	0.95
Spirulina platensis	2.45	0.04	7.89	0.23	2.34	0.19	5.09	0.28
Total Cyanophyta	42.54	9.07	47.11	6.11	38.86	2.08	29.03	1.52
Diaoms								
Gyrosigma sp.	NA	0.17	NA	0.05	NA	NA	NA	NA
Nitzschia sp	NA	1.68	NA	1.06	NA	NA	NA	NA
Phaeodactylum tricornutum	NA	0.19	NA	0.12	NA	NA	NA	NA
Total diatoms	0	2.04	0	1.23	0	0	0	0

Chlamydomonas and *Scenedesmus* in treated wastewater while in untreated wastewater *Chlorella* was followed by *Chlamydomonas sp*. and *Chlorococcum humicola.* Very few diatoms appeared since March till May and August till December in treated wastewater only, while diatoms were not found in either of the wastewater since January through February and June through July, these seasons are with lower rainfall in Malaysia. The correlation between nutrients and biovolume of algal community is not clear since nutrients level did not show any constant effect on the biovolume of algal community. The variation in biomass of same strain of algae towards nutrients level established that other parameters such as seasonal variations, interaction between species, presence of zooplanktons or other organisms might play more important role rather than nutrients in determining species compositions. It is not surprising since previous researchers also carried the same point of view ([3,20]).

3.3. Isolation of native microalgal strains and their consortium

Total twelve isolates were obtained from wastewaters and analyzed for their lipid content via Nile red fluorescence analysis as discussed in Doan et al. [8] (Fig. 2). On the basis of lipid content ten isolates were selected to prepare inoculum of algal consortium to cultivate in wastewaters. 80% of the selected isolates were green algae and rest 20% were cyanobacteria. Each isolates were cultivated in BG11 as pure culture for 10 days and mixed $\sim$0.1 g L^{-1} each to prepare consortium. This consortium was used for further experiments considering it to be robust enough to withstand the harshness of open cultivation systems to cut down the cost of biodiesel production.

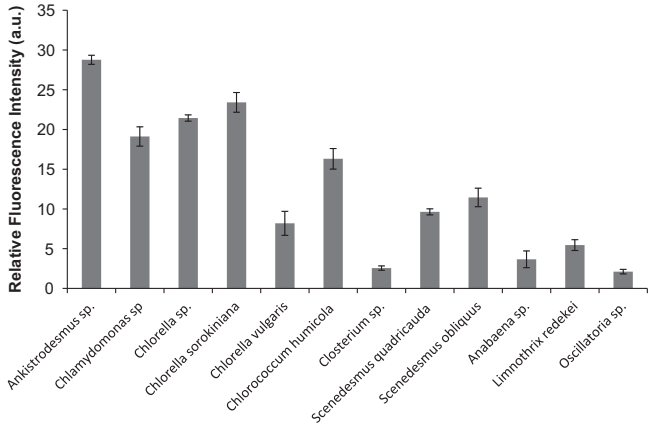

Fig. 2. NR fluorescence of isolated native strains after 10 days of cultivation in BG11 media.

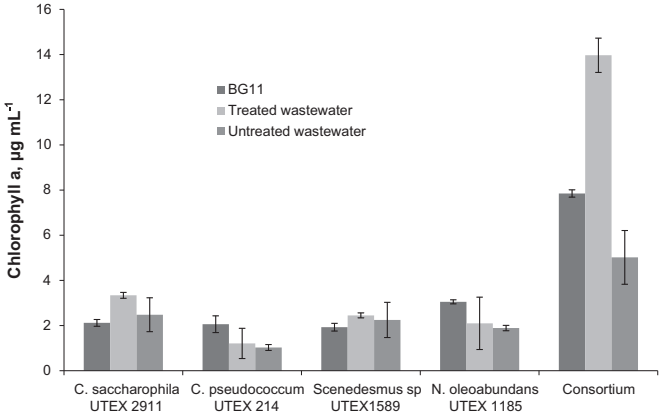

Fig. 3. Growth response of various algae and consortium cultivated in treated, untreated dairy farm wastewater and BG11 in term of obtaining chlorophyll *a*.

3.4. Preliminary screening of algae based on growth potential in wastewaters

Four algal strains namely *C. saccharophila* UTEX 2911, *C. pseudococcum* UTEX 214, *Scenedesmus sp* UTEX1589 and *N. oleoabundans* UTEX 1185, obtained from UTEX and consortium prepared from native algal strains obtained from wastewaters were cultivated in treated, untreated wastewaters and BG11 the standard growth medium to compare and evaluate their growth response, biomass production and lipid accumulation.

After cultivation of 10 days, the growth response of algae and consortium were estimated on the basis of percentage increase or decrease in chlorophyll *a* content, over standard growth medium BG11. Among four commercial tested strains; *C. saccharophila* UTEX 2911 and *Scenedesmus sp* UTEX1589 were shown 58% and 27% increase in chlorophyll *a* content in treated wastewater, respectively, in comparison of standard BG11 medium. The strain *C. pseudococcum* UTEX 214 and *N. oleoabundans* UTEX 1185 shown highest chlorophyll *a* in BG11 than in treated or untreated wastewaters. The *C. pseudococcum* UTEX 214 responded 41.2% and 50% less chlorophyll *a* in treated as well as in untreated wastewater, respectively, while *N. oleoabundans* UTEX 1185 acquired 31.14% and 38% less chlorophyll in treated and untreated wastewater, respectively, over BG11. The native algae consortium

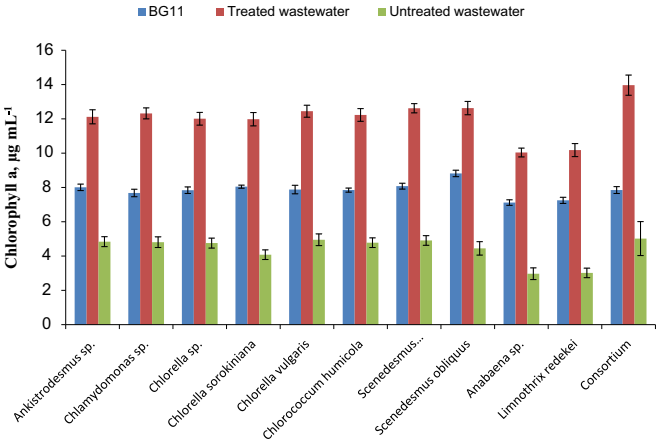

Fig. 4. Growth response of native strains of microalgae and consortium cultivated in treated, untreated dairy farm wastewater and BG11 in term of obtaining chlorophyll *a*.

recorded 78% increase chlorophyll *a* in treated wastewater comparing to BG11 while in untreated wastewater it was recorded as 36% less than BG11 (Fig. 3). Comparing growth response of consortium with the growth response of solo native strains in different culture media, it was found that consortium has shown best growth response and better stability than any of the pure microalgae strain cultured in regardless of any wastewater, nevertheless, BG11 clearly favor single native strains culture, as shown in Fig. 4.

On the basis of growth response of the pure strain and consortium in treated wastewater two pure strains and consortium were selected for further studies as they fulfilled the objective of this research, which was to evaluate the feasibility of producing biodiesel from algae grown in wastewater. The wastewaters had high turbidity due to high concentration of total suspended solid (Table 1) which hindered light to penetrate and thus it was probably not suitable for those algae to show good growth response which could not show facultative heterotrophic behavior.

It is not surprising that among the selected strains *C. saccharophila* and *Scenedesmus* sp. were identified as fit for biodiesel production in treated wastewater used as culture media. Previous researches [3,30,31,35] also reported that *Chlorella* sp. and *Scenedesmus* sp. can be used for treatment of different wastewater streams such as primary settled wastewater, settled and activated sewage, animal manure and industrial wastewater etc., suggesting such species could tolerate and survive different types of wastewater streams.

3.5. Biomass and lipid production potential of algae in treated wastewater

On the basis of growth potential of algae in treated wastewater two pure strains (*C. saccharophila* and *Scenedesmus* sp.) and consortium of native strains had been selected for further studies to evaluate their biomass and lipid production potential in treated wastewater with comparison of untreated wastewater and BG11. *C. saccharophila* and consortium showed their highest biomass in treated wastewater, while *Scenedesmus* sp. accumulate highest biomass in untreated wastewater followed by treated wastewater and BG11 (Table 3). This study finalized that among all cultures, consortium of native strains showed highest biomass production in treated wastewater. Consortium was considered to be as the most promising candidate that could produce 219.87 t of biomass and approximately 51.37 thousand L of algal oil ha^{-1} $year^{-1}$ (Table 3) in treated wastewater while 137.68 t of biomass and ~33.38 thousand L of algal oil ha^{-1} $year^{-1}$ in untreated wastewater. Data in Table 3 revealed that other optional cultures produced biomass and lipid far below than consortium in treated as well as untreated wastewaters. Biomass and algal oil production capacity of cultures were estimated on the

Table 3
Biomass and lipid production potential of *C. saccharophila, Scenedesmus sp* and consortium cultivated in treated, untreated dairy farm wastewater and BG11.

Algae	Culture medium	Biomass ($g\ L^{-1}\ d^{-1}$)	Lipid (%)	Estimated biomass productivity ($t\ ha^{-1}\ year^{-1}$)	Estimated lipid productivity ($L\ ha^{-1}\ year^{-1}$) $\times 10^3$
Chlorella saccharophila	BG11	0.198 ± 0.07	13.10 ± 1.09	142.73	21.24
	Treated wastewater	0.219 ± 0.04	18.70 ± 1.77	157.87	33.54
	Untreated wastewater	0.201 ± 0.03	21.82 ± 2.06	144.89	35.69
Scenedesmus sp	BG11	0.194 ± 0.09	11.50 ± 2.99	139.84	18.27
	Treated wastewater	0.208 ± 0.06	15.73 ± 1.52	149.94	26.80
	Untreated wastewater	0.211 ± 0.07	13.64 ± 0.84	152.10	23.57
Consortium	BG11	0.175 ± 0.05	20.33 ± 1.63	125.83	29.05
	Treated wastewater	0.276 ± 0.04	23.62 ± 1.19	219.87	51.37
	Untreated wastewater	0.191 ± 0.08	21.34 ± 1.26	137.68	33.38

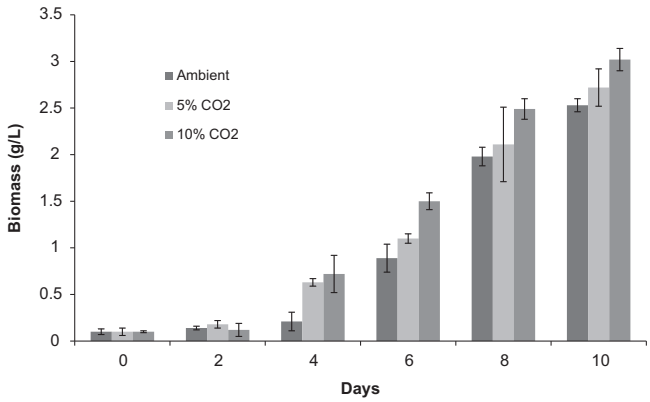

Fig. 5. Biomass accumulation of consortium at different dosage of CO_2 in treated wastewater.

basis of batch culture experiments conducted in 250 mL flask with ambient CO_2 under 80 μmol $m^{-2}\ s^{-1}$ of flux.

3.6. *Effect of CO_2 on biomass production and biovolume of strains in consortium*

Finally assigned consortium as the best candidate for biodiesel feedstock cultivated in treated wastewater was grown under three different concentration of CO_2 (ambient, 5% and 10%). The experiments showed a significant increase in biomass after 2 days of inoculation (Fig. 5). Initially (till 2nd day) 5% CO_2 appeared with highest biomass and it was followed by ambient and 10% CO_2. Accumulating least biomass initially in 10% CO_2 might be because of the less tolerance of algae cells towards high dosage of CO_2, which lead consortium strains to take little extra time to acclimatize in that particular atmosphere and thus appeared with slow growth. However, the highest productivity of biomass achieved with 10% CO_2 on 10th day which was 3.02 $g\ L^{-1}$ and almost 30 fold increases over the biomass of inoculums while ~1.11 and 1.18 fold more than final biomass achieved in 5% CO_2 and ambient, respectively.

Interestingly the consortium grown at different CO_2 dosage showed different biovolume of algal strains. The biovolume of the strains in consortium were examined on 5th and 10th day of culture with all three different concentrations of CO_2. The inoculum of consortium had equal amount of all the strains. For ambient and 5% CO_2 the bio-volume of strains did not show any change in the ratio (data not provided), however at 10% CO_2 *Scenedesmus sp* dominated in term of bio-volume and followed by *Chlorella sp*. The biovolume ratio of *Scenedesmus sp*. increased by 50.2% than inoculum and most of their cells shown 8 cells colony on 10th days. The morphological changes occurred in the presence of high dosage CO_2 and the formation of these colonies was not the result of lumping of free cells, because of the nicely arranged flat colonies, which strongly advocated that it was due to the reproduction. Previous research [27] also reported that *Scenedesmus* sp. are high CO_2 tolerant algae.

Mainly green algae of consortium community shown increase in their bio-volume ratio except *C. humicola*, while cyanobacteria reflected their weak credibility towards high CO_2 dosage (Fig. 6). This study revealed that the strains which can increase or withstand their bio-volume ratio during culture at high CO_2 dosage might be use as for CO_2 sequestration from flue gas.

The growth of algae in treated wastewater showed a good response despite of low N and P concentration with efficient removal of COD (98.8%). Zhou and researchers [35] removed 82.27–96.18% TOC from concentrated municipal wastewater, whereas Chinnasamy et al. [3] managed to remove $>96\%$ nutrients from carpet mill effluents. The nutrient consumption profile confirmed that

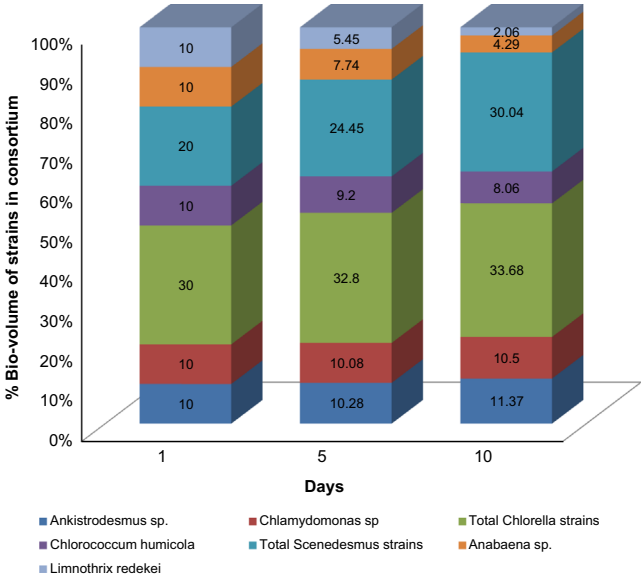

Fig. 6. % Bio-volume of strains in consortium cultured in treated wastewater with 10% CO_2 supplementation. *Total Chlorella strains includes *Chlorella sp., Chlorella sorokiniana, Chlorella vulgaris.* **Total Scenedesmus strains contents *Scenedesmus quadricauda, Scenedesmus obliquus*.

Table 4
COD and nutrients removal by consortium grown in dairy farm treated wastewater.

Parameters	Initial concentration (mg L^{-1})	Final concentration (mg L^{-1})	Reduction (%)
COD	112.57	1.37	98.8
PO_4–P	4.689	0.057	98.8
NO_3–N	3.342	0.021	99.4
NH_4–N	0.998	NIL	~100

PO_4–P, NO_3–N and NH_4–N in the culture media was depleted in 4 days, and reached up to 98.8% and 99.4%, respectively, whereas NH_4–N was found zero even after a short period of 18 h of culture (Table 4). Since the first day of culture the biomass and chlorophyll *a* increased with time but, after 4 days of incubation unexpectedly the biomass showed increasing trend although determination of chlorophyll *a* did not show any significant changes during culture (data not shown). However, the nitrogen in the medium was depleted within 4 days of incubation. Hecky and Kilham [16] and Chinnasamy et al. [3] had also noticed the same trends. The above observations clearly proved that growth in biomass after 4 days of incubation was due to increase in internal cellular nutrients rather than increase in number of algal cell. In nitrogen limiting medium, the carbon fixation during photosynthesis preferred synthesis of carbohydrate rather than to opt the path of protein synthesis [12,29].

3.7. Algal biomass production in high rate algae ponds (HRAP)

The consortium of native algal strains was cultivated in three HRAP of 600 L capacity each with 10% CO_2–air mixture for 10 days. The average productivity observed as 0.213 g L^{-1} d^{-1} or 153.54 t ha^{-1} $year^{-1}$. Obtained algal consortium biomass cultured in HRAP with treated wastewater was analyzed for its proximate biochemical composition before and after lipid extraction and the values are listed in Table 5. The stowed energy in algal consortium before and after lipid extraction were found as 25.57 kJ g^{-1} and 19.13 kJ g^{-1}, respectively, which were quite close to the values reported by previous researchers [19,1]. After lipid extraction the energy was reduced by 25.18%.

3.8. Biodiesel conversion

In order to obtain 100 g of algal oil from biomass of consortium cultured in treated wastewater, about 650 g of dry biomass were treated with dynamic hexane method. The energy content of the extracted algal oil was 38.12 kJ g^{-1} which was quite close to the fuel content energy of algal oil obtained by other researcher [[3]]. Generally algal oil contain high value of free fatty acids, which is an undesirable trait for biodiesel conversion process and this process is commonly resolved by acid catalyzed transesterification process [34]. It was necessary to go through an acid esterification process for free fatty acids present in algal oil to increase the yield of biodiesel. In present study acid value 69.98 mg of KOH/g indicated almost 35.2% free fatty acids, and these were converted into fatty acid methyl esters (FAMEs) via acid esterification process followed by alkaline catalyst process. The product yield after base transesterification was72.7% of the total algal oil with loses being mainly due to oil impurities, soap in oil, adhering to the glassware used. The algal oil was converted into FAME to examine the composition of fatty acids profile using GC. The profile showed subsequent series of fatty acids; 14:0, 15:0, 16:0, 16:1, 16:2, 18:0, 18:1, 18:2, 18:3, 20:0, 20:1, 20:4, 20:5, 22:5, 22:6 and 24:0 (Table 6). However, the main fatty acid components in algal oil obtained from consortium were composed of

Table 5
Compositional analysis of the native algal consortium before and after lipid extraction.

Parameters	Before lipid extraction	After lipid extraction
Proximate analysis (%)		
Moisture	6.24 ± 0.11	4.82 ± 0.23
Volatiles	72.53 ± 0.18	70.11 ± 0.73
Ashes	15.01 ± 0.16	17.99 ± 0.95
Biochemical composition (%)		
Lipids	16.89 ± 0.15	0.6 ± 0.04
Protein	45.09 ± 0.46	50.54 ± 0.89
Carbohydrates	10.13 ± 0.51	11.25 ± 0.86
Energy content	25.57 ± 0.39	19.13 ± 0.27

Table 6
Fatty acid composition of algal consortium cultivated in treated wastewater (% of total FAME, $n=3$).

Fatty Acids	Fatty Acid content (%) in crude algal oil obtained from consortium
Myristic acid (C14:0)	3.01 ± 0.55
Pentadecanoic acid (C15:0)	1.50 ± 0.72
Palmitic acid (C16:0)	30.28 ± 2.45
Palmitoleic acid (C16:1)	5.63 ± 1.10
Hexadecadienoic acid (16:2)	1.71 ± 0.25
Stearic acid (C18:0)	2.03 ± 0.76
Oleic acid (C18:1)	13.68 ± 2.88
Linoleic acid (C18:2)	12.41 ± 1.67
Linolenic acid (C18:3)	16.68 ± 3.54
Arachidic acid (C20:0)	0.12 ± 0.11
Gadoleic acid (C20:1)	1.02 ± 0.57
Arachidonic acid (C20:4)	4.44 ± 1.32
Eicosapentaenoic acid (C20:5)	7.18 ± 1.98
Docosapentaenoic acid (C22:5)	0.05 ± 0.03
Docosahexaenoic acid (C22:6)	0.11 ± 0.09
Lignoceric acid (C24:0)	0.15 ± 0.08
Saturated fatty acids	37.09 ± 2.06
Monoenoic fatty acids	20.33 ± 1.17
Polyenoic fatty acids	42.58 ± 2.27
FAME of major fatty acid (C16–C18)	82.42 ± 3.12

C16–C18 fatty acids which are suitable for biodiesel production [24,35]. The oil was mainly composed of 62.91% unsaturated fatty acid among the total known fatty acids (Table 6). European Standard EN 14214 [10] applied a condition on limit of 12% for C18:3 (linolenic) for quality vehicle biodiesel. However, the biodiesel produced by this study contained 16.68% of C18:3 (linolenic). In general the composition of many microalgal oils is not suitable to stand with the EN 14214 biodiesel standards, because of the extent of unsaturation of microalgal oil [4]. But this problem can be solved and the quality of biodiesel can be improved either by partial catalytic hydrogenation of the oil [7] or by blended with other sources of biodiesel obtained from non-food feed stocks [3].

It is interesting to note that the algal strains in consortium could also produce high-value fatty acids for human nutrition and food additives such as arachidonic acid (AA, C20:4) and eicosapentaenoic acid (EPA, C20:5) which account for 4.44% and 7.18% respectively of total fatty acid. EPA and AA are important PUFAs which play vital role in the prevention of various human diseases [25]. 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 ratio. AA and EPA of consortium can be extracted before the rest of oil can be converted to biodiesel. Based on the consortium ability to grow in treated dairy farm wastewater and high biomass and lipid productivity was considerably highly promising for sustainable algae biodiesel.

After the extraction of lipid the energy stored in the residual algal biomass could be recovered by anaerobic digestion into biogas.

4. Conclusion

In this study the screening process involved collecting algae samples from Dairy farm wastewater plant, and screening on the basis of NR fluorescence analysis provided successful way to select strains for determining microalgae cellular lipid content to form consortium. The dairy farm wastewaters could support the growth of consortium of native algae strains isolated from dairy farm wastewaters good for the production of biodiesel as well as possessed high wastewater nutrient removal efficiency. A consortium of 10 native strains produced maximum biomass and algal oil in treated wastewater at 10% CO_2 supplement. The bio-volume ratio of microalgae was regulated at high CO_2 concentration and

breakthrough a new research for Ankistrodesmus sp., Chlorella strains, and Scenedesmus strains to culture for CO_2 sequestration. Biomass and algal oil produced by consortium in HRAP was 153.54 t ha^{-1} $year^{-1}$ and 29.47 thousand L ha^{-1} $year^{-1}$, respectively.

This study revealed the capability of consortium of native strains supported by nutrients present in treated dairy farm wastewater to produce biodiesel and can remove $>98\%$ COD as well as nutrient very efficiently within 4–5 days of culture.

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