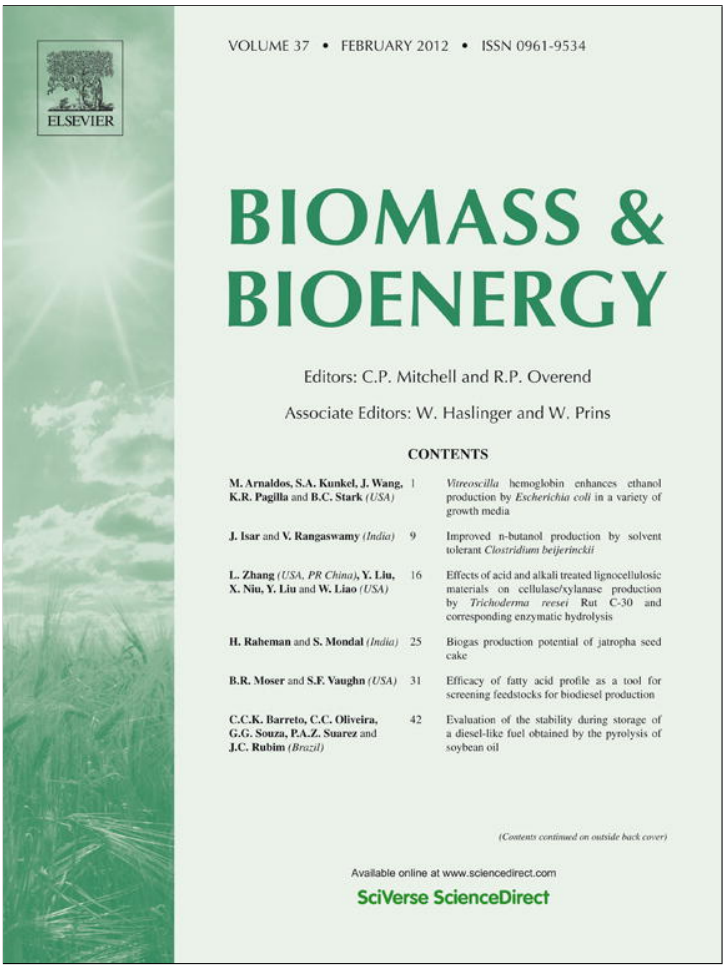

BIOMASS AND BIOENERGY 37 (2012) 60–66

Available online at www.sciencedirect.com

SciVerse ScienceDirect

http://www.elsevier.com/locate/biombioe

Influence of nutrient deprivations on lipid accumulation in a dominant indigenous microalga *Chlorella* sp., BUM11008: Evaluation for biodiesel production

Ramasamy Praveenkumar [a], *Kalifulla Shameera* [a], *Gopalakrishnan Mahalakshmi* [a], *Mohammad Abdulkader Akbarsha* [b,c], *Nooruddin Thajuddin* [a,c,*]

[a] *Department of Microbiology, School of Life Sciences, Bharathidasan University, Tiruchirappalli 620 024, Tamil Nadu, India*
[b] *Mahatma Gandhi-Doerenkamp Center (MGDC) for Alternatives to Use of Animals in Life Science Education, Bharathidasan University, Tiruchirappalli 620 024, India*
[c] *King Saud University, Riyadh, Saudi Arabia*

ARTICLE INFO

Article history:
Received 14 March 2011
Received in revised form
16 December 2011
Accepted 21 December 2011
Available online 9 January 2012

Keywords:
Chlorella sp.
FAME profiles
Microalgal biodiesel
Nutrient deprivation
Nitrogen deprivation

ABSTRACT

Microalgae are a potential source of biodiesel. The urgent need for an alternative and sustainable energy has created renewed interest to analyze the microalgae for biodiesel production. In this study, a dominant indigenous freshwater unicellular microalgal strain *Chlorella* sp., BUM11008, was examined for its efficiency towards biodiesel production. The organism was evaluated for ability to yield high of biomass and lipid productivity under normal and various nutrient-deprived conditions (nitrogen, phosphate-potassium, iron, and all three combined). Under normal conditions, after 20 days of cultivation in Chu10 medium, the organism yielded a biomass of 2.58 ± 0.07 g/L, with lipid content of 312.16 ± 2.38 mg/g. In a two-phase culturing system upon nutrition deprivation, the organism was able to respond with different levels of lipid accumulation. Among the various post-harvest treatments, nitrogen deprivation yielded the highest lipid productivity of 53.96 ± 0.63 mg/L d, followed by the combined deprivation condition (49.16 ± 1.36 mg/L d). FAME profiles of the isolate were found to meet the requirements of international standards for biodiesel. The study leads to the conclusion that the two-phase culturing system with nitrogen starvation as post-harvest treatment would be suitable for gaining maximum biomass productivity, and lipid content of high quality fatty acids. Thus, it is proposed that *Chlorella* sp., BUM11008, would be a promising candidate for sustainable biodiesel production.

1. Introduction

In the contemporary scenario, increase in mechanized transportation and large-scale industrialization have resulted in over-consumption of fossil fuel. Consequently, there is gradual decrease of world fossil fuel reserves, and the latter are projected to be exhausted within the next 50 years [1]. From the perspective of environment, combustion of fossil fuels is one of the major contributors to atmospheric pollution which in turn leads to global warming. Therefore, great interest has been evinced to find an alternative source of oil which would be eco-friendly. Bio-fuels, in general, and biodiesel in particular, being biomass energy, are looked at as a viable source to meet out the requirement. Bio-fuels are renewable, nontoxic, biodegradable

* *Corresponding author*. Department of Microbiology, School of Life Sciences, Bharathidasan University, Tiruchirappalli 620 024, Tamil Nadu, India. Tel.: +91 431 2407082; fax: +91 431 2407045.
E-mail address: nthaju2002@yahoo.com (N. Thajuddin).
0961-9534/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biombioe.2011.12.035

and eco-friendly in the sense that their combustion will produce little, if any, emission of harmful green house gases.

Even though oil crops such as sunflower, palm, jatropa, rape seed and soy bean fulfil a small fraction of the liquid fuel requirement, microalgae have gained importance from this perspective in view of their potential to provide suitable and sustainable biomass for biodiesel production since they are rapidly growing and can potentially yield very high volumes of oil [2–4]. The added advantage of these organisms is that they store excess energy as triacyl-glycerides (TAGs) most of which are suitable substrates for biodiesel production [5]. Upon transesterification a TAG molecule readily yields three fatty acid alkyl ester molecules, which form biodiesel, and a glycerol backbone, which will be a simple by-product.

In spite of such remarkable advantages, microalgal oil production still remains an expensive technology due to factors associated with algal cultivation. In this regard, identification of a suitable strain of microalga for mass cultivation and the response of this strain to different cultivation conditions are highly pertinent. While selecting a strain for biodiesel production, it is essential to find that the organism is capable of resisting the local invaders; otherwise, when grown in open ponds, they may be prone to contamination and often dominated by the native strains [6]. Hence, selection of a suitable, dominantly distributed indigenous strain of microalga with good biomass productivity and high lipid content will be a crucial step. Although obtaining a culture with high biomass yield and high lipid content remains a challenge under normal cultivation systems, it is possible to achieve the same under two-phase culturing system: (i) culturing the organism under optimized nutritional conditions with the aim to increase the biomass; and (ii) subjecting the organism to such of the stresses that would enhance lipid accumulation [1,7]. This method has been found to provide for increased biomass as well as high lipid yield. Here, the fatty acid composition of the algal lipids is a key factor for biodiesel production. Hence, it is also necessary to monitor the impact of culture condition on the fatty acid composition.

In this background, the present study was aimed at finding the response of a dominantly distributed indigenous strain of green microalga, *Chlorella* sp., BUM11008, isolated from a local freshwater pond, to nutrient deprivation (nitrate, potassium-phosphate and iron), with special reference to biomass and lipid productivity under two-phase culturing system. The effect of combination of nutrient deprivation on the organism was also investigated. The study involved the analysis of growth characteristics, fatty acid content during the entire course of cultivation and fatty acid profile of the cell cultivated under normal and nutrient-deprived conditions.

2. Methodology

2.1. Culture isolation and growth

Chlorella sp., BUM11008 (herein after *Chlorella* sp.) was isolated from a freshwater pond in the Bharathidasan University campus, Tiruchirappalli, India (Lat. 10°41′N/Lon. 78°44′15″E) and maintained in the microalgal repository of the Department of Microbiology, Bharathidasan University, Tiruchirappalli, India, and were grown in Chu10 medium. Other culture conditions included white photo-fluorescence at a rate of 300 μE m^{-2} s^{-1}, with 12/12 h light/dark photoperiod, at 24 °C throughout the experiment. In nutrient deprivation experiments, the cells were harvested on 16th day of incubation, washed with sterile distilled water and resuspended in the systems as mentioned below.

1. For nitrogen free treatment, the biomass was introduced into Chu10 medium that lacked calcium nitrate – $Ca(NO_3)_2$.
2. For potassium-phosphate free treatment, the biomass was introduced into Chu10 medium that lacked dipotassium hydrogen phosphate – K_2HPO_4.
3. For iron free treatment, the biomass was introduced into Chu10 medium that lacked ferric citrate.
4. For the combination of nitrogen, potassium-phosphate, and iron free treatment the biomass was introduced into Chu10 medium that lacked Ca $(NO_3)_2$, K_2HPO_4 and ferric citrate.

2.2. Measurement of growth rate

Growth rate of *Chlorella* sp. was determined by measuring the optical density. Briefly, 5 mL of thoroughly mixed culture was drawn at an interval of 4 days. During every sampling, 5 mL of fresh sterile Chu10 medium was added in order to compensate the volume. The optical density (OD) was used to monitor the algal growth. The OD of culture aliquot was measured at 600 nm using UV–vis spectrophotometer (Optizen 3220, Japan). Wherever necessary, the samples were diluted suitably so that their OD_{600} values fell between 0.2 and 0.8. The concentration of biomass was then calculated by multiplying the OD_{600} value with factor 0.71, derived from an earlier report [8]. A plot of OD_{600} versus dry cell weight (DCW) was made with samples of varying biomass concentrations.

2.3. Estimation of lipid

During the course of cultivation, the culture aliquots drawn for determining the growth rate were centrifuged at 10,000 rpm, at 4 °C for 4 min to collect the biomass and freeze-dried in a lyophilizer. The cell powder was extracted with methanol: chloroform mixture (2:1 v/v) for 20 min under continuous shaking. The mixture was filtered to separate cell-free organic phase, washed with water, vortexed and centrifuged to remove the upper aqueous phase. The lower organic phase was rinsed twice with equal volume of methanol: water (1:1 v/v). Finally, the extracted lipid was collected from the solvent phase, evaporated under vacuum and weighed.

2.4. Preparation of fatty acid methyl esters (FAME)

The lipid and methanolic sulphuric acid (2% H_2SO_4 in methanol) were refluxed for 4 h. The contents were mixed thoroughly with equal volume of distilled water in a separating funnel. The aqueous layer was extracted twice with ethyl acetate. The ethyl acetate extract containing the FAME was collected, dried over anhydrous sodium sulphate to remove any excess moisture, and concentrated under vacuum. The dried FAME samples of both normal and nitrogen-starved cultures were analyzed by gas chromatography.

2.5. Gas chromatography (GC) analysis

The FAME samples were analyzed by gas chromatograph (Shimadzu, GC2014, Japan) with flame ionization detector (FID). One microlitre of each sample was injected into FAME-WAX column (Restek, USA) (30 m×32 mm ID×25 μm film thickness). The temperature program was as follows: initial 140 °C with 5 min hold; ramp 2 °C/min to 230 °C with a 5 min hold. Column flow was set at 22.2 mL/min. The instrument condition was as follows: carrier gas nitrogen; FID set at 260 °C, and split ratio of 10:1. The run time for a single sample was 55 min. Each sample was analyzed in triplicates, and FAME identification was done by comparison with standard certificate, Supelco FAME mix C4 – C24 (Bellefonte, PA, USA).

3. Results

3.1. Growth and lipid characteristics of Chlorella sp.

Chlorella sp., was originally isolated from a freshwater pond in Bharathidasan University campus, Tiruchirappalli, Tamil Nadu, India. It was found to dominate the ecosystem with their luxuriant growth. It was grown in Chu10 medium, which was found to support higher biomass production in our earlier study. The growth curve of the isolate was deciphered from DCW values obtained every 4 days from the day of inoculation (Fig. 1). The plot clearly showed distinct phases of a typical growth curve where the growth reached a stationary phase on 12th day of incubation. The maximum biomass concentration of 2.6 ± 0.06 g/L was obtained on 16th day of cultivation. On 20th day of incubation a slight decrease (2.58 ± 0.07 g/L) in the biomass concentration was recorded and from then on it started to decrease rapidly (data not shown).

The time course of lipid accumulation in *Chlorella* sp., (Fig. 2) clearly revealed the capability of the organism to increasingly accumulate lipids with advanced age. Initially, on 4th day of incubation the lipid content of the cell was 145.67 ± 12.61 mg/g and it very slowly increased up to 12 days to reach 176.57 ± 6.12 mg/g. Upon further incubation, a rapid increase in lipid content was observed (262.89 ± 23.9 mg/g on 16th day) and it reached the maximum, 312.16 ± 2.38 mg/g, on 20th day of cultivation. This reveals that the present species

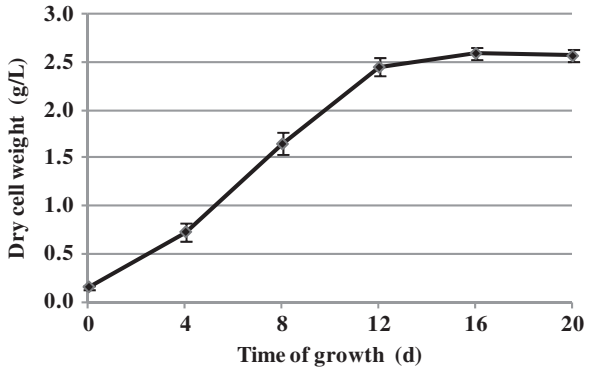

Fig. 1 – Growth curve of *Chlorella* sp. BUM011008, cultivated in the Chu10 medium based on DCW g/L values. Experiments were carried out in triplicates.

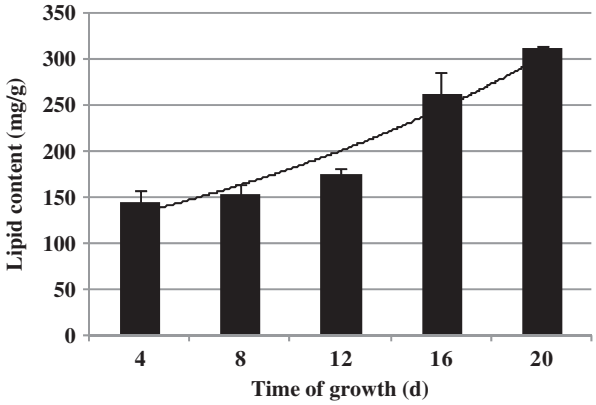

Fig. 2 – Time course of lipid content of the cells under cultivation with a trend line. Experiments were carried out in triplicates.

accumulates more lipids at its stationary phase. The fatty acid profile of *Chlorella* sp., expounded the presence of stearic (C18:0 – 25.94%), arachidic (C20:0 – 6.85%), lignoceric (C24:0 – 27.24%) and linoleic (C18:2 – 15.7%) acids as the major fatty acids. About 80.24% of total fatty acids were found to be either saturated or monounsaturated (Table 1).

3.2. Effect of nitrogen deprivation on biomass and lipid characteristics

After 16 days of normal growth, the organism was subjected to a second phase of nutrient deprivation for 4 days. Here, nitrogen deprivation affected the biomass slightly, resulting in a decreased biomass concentration of 2.52 ± 0.02 g/L (Fig. 3), whereas lipid content of the cell increased to 428.26 ± 12.61 mg/g (Fig. 4), which was significantly higher than at normal culture condition. Analysis of fatty acid profiles revealed the presence of stearic (36.45%), arachidic (16.78%), heneicosanoic (C21:0 – 7.02%) and linoleic (19.91%) acids as the major fatty acids. Thus, the fatty acid profile was dominated by saturated and monounsaturated fatty acids, comprising 75.6% of the entire pool (Table 1).

3.3. Effect of potassium-phosphate deprivation on biomass and lipid characteristics

Potassium-phosphate deprivation affected biomass concentration significantly, and it decreased to 2.46 ± 0.06 g/L (Fig. 3). Under this stress there was no significant increase in lipid content (319.89 ± 10.02 mg/g) (Fig. 4). Fatty acids such as stearic (15.38%), lignoceric (30.41%), oleic (C18:1 – 7.62%) and linoleic (9.88%) acid were found to dominate (Table 1). Increase in highly unsaturated fatty acids (presence of four or more double bonds) was noted, and they accounted for 4.64%, which is usually not a desirable feature in terms of biodiesel production.

3.4. Effect of iron deprivation on biomass and lipid characteristics

Under iron-deprived condition, there was a slight decrease in biomass (2.54 ± 0.03 g/L) (Fig. 3), with no significant increase in

Table 1 – FAME composition of extracted algal oil under normal and nutrients-deprived conditions.

S. No.	Fatty acid methyl esters		Nature	% Composition under different conditions (mean ± standard deviation)				
				A	B	C	D	E
1	Caproic	6:0	SFA	ND	ND	0.26 ± 0.08	ND	ND
2	Caprylic	8:0	SFA	0.22 ± 0.01	ND	0.25 ± 0.04	ND	ND
3	Capric	10:0	SFA	0.15 ± 0.02	0.09 ± 0.01	0.25 ± 0.01	0.40 ± 0.01	0.72 ± 0.01
4	Lauric	12:0	SFA	0.99 ± 0.01	0.43 ± 0.02	1.96 ± 0.01	1.25 ± 0.01	1.23 ± 0.02
5	Myristic	14:0	SFA	0.30 ± 0.03	0.26 ± 0.08	0.59 ± 0.02	0.81 ± 0.01	0.95 ± 0.04
6	Myristoleic	14:1	MUFA	0.42 ± 0.01	0.38 ± 0.04	0.99 ± 0.01	1.15 ± 0.05	1.06 ± 0.01
7	Pentadecanoic	15:0	SFA	1.43 ± 0.04	0.86 ± 0.12	2.23 ± 0.05	1.12 ± 0.04	1.37 ± 0.03
8	Cis-10-Pentadecanoic	15:1	MUFA	0.69 ± 0.07	0.14 ± 0.09	1.55 ± 0.04	ND	ND
9	Palmitic	16:0	SFA	0.41 ± 0.02	0.62 ± 0.04	0.72 ± 0.04	1.68 ± 0.02	1.40 ± 0.05
10	Palmitoleic	16:1	MUFA	2.41 ± 0.02	0.48 ± 0.01	6.30 ± 0.08	0.71 ± 0.04	0.68 ± 0.04
11	Heptadecanoic	17:1	SFA	ND	0.28 ± 0.02	ND	0.51 ± 0.04	0.60 ± 0.01
12	Cis-10-Heptadecanoic	17:1	MUFA	ND	0.10 ± 0.01	ND	0.28 ± 0.07	0.74 ± 0.03
13	Stearic	18:0	SFA	25.94 ± 0.05	36.45 ± 0.02	15.38 ± 0.01	28.40 ± 0.08	1.23 ± 0.08
14	Elaidic	18:1	MUFA	3.82 ± 0.10	3.43 ± 0.03	5.29 ± 0.08	6.84 ± 0.04	5.84 ± 0.04
15	Oleic	18:1	MUFA	3.03 ± 0.01	0.14 ± 0.08	7.62 ± 0.07	4.23 ± 0.11	4.06 ± 0.01
16	Linolelaidic	18:2	PUFA	0.47 ± 0.01	0.88 ± 0.06	0.57 ± 0.04	1.43 ± 0.09	1.01 ± 0.05
17	Linoleic	18:2	PUFA	15.70 ± 0.02	19.91 ± 0.04	9.88 ± 0.02	15.87 ± 0.01	28.42 ± 0.06
18	Arachidic	20:0	SFA	6.85 ± 0.02	16.78 ± 0.02	5.08 ± 0.03	15.57 ± 0.04	23.27 ± 0.01
19	Cis-11-Eicosenoic	20:1	MUFA	1.66 ± 0.06	3.22 ± 0.05	0.80 ± 0.06	2.52 ± 0.08	4.04 ± 0.01
20	Linolenic	18:3	PUFA	0.99 ± 0.04	2.14 ± 0.01	1.09 ± 0.01	3.26 ± 0.07	3.16 ± 0.02
21	Heneicosanoic	21:0	SFA	2.63 ± 0.01	7.02 ± 0.01	3.42 ± 0.05	9.35 ± 0.09	6.46 ± 0.06
22	Cis-11, 14-Eicosadienoic	20:2	PUFA	0.42 ± 0.01	0.59 ± 0.01	ND	0.36 ± 0.08	0.58 ± 0.04
23	Behenic	22:0	SFA	0.75 ± 0.01	0.80 ± 0.01	ND	0.42 ± 0.06	0.98 ± 0.07
24	Arachidonic	20:4	PUFA	0.43 ± 0.05	0.15 ± 0.02	0.59 ± 0.04	ND	0.52 ± 0.04
25	Tricosanoic	23:0	SFA	1.30 ± 0.08	2.05 ± 0.05	0.70 ± 0.05	1.27 ± 0.07	2.91 ± 0.01
26	Cis-13, 16-Docosadienoic	22:2	PUFA	ND	0.18 ± 0.01	ND	0.52 ± 0.05	ND
27	Lignoceric	24:0	SFA	27.24 ± 0.07	2.07 ± 0.10	30.41 ± 0.02	0.71 ± 0.01	7.60 ± 0.01
28	Cis-5,8,11,14,17-Eicosapentaenoic	20:5	PUFA	1.18 ± 0.01	ND	2.32 ± 0.01	ND	0.43 ± 0.08
29	Nervonic	24:1	MUFA	ND	ND	ND	0.52 ± 0.02	ND
30	Cis-4,7,10,13,16,19-Docosahexaenoic	22:6	PUFA	0.57 ± 0.01	0.55 ± 0.09	1.75 ± 0.01	0.82 ± 0.04	0.74 ± 0.09
			∑SFA	68.21 ± 0.03	67.71 ± 0.04	61.25 ± 0.03	61.49 ± 0.04	48.72 ± 0.03
			∑MUFA	12.03 ± 0.04	7.89 ± 0.04	22.55 ± 0.05	16.25 ± 0.05	16.42 ± 0.02
			∑PUFA	19.76 ± 0.02	24.40 ± 0.02	16.20 ± 0.02	22.26 ± 0.05	34.86 ± 0.05

A – Normal nutritional condition; B – Nitrogen-deprived condition; C – Potassium-phosphate deprived condition; D – Iron-deprived condition; E – Combined deprivation (nitrogen, potassium-phosphate and iron).
SFA – Saturated fatty acid; MUFA – Monounsaturated fatty acid; PUFA – Polyunsaturated fatty acid; ND – Not detected.

lipid content (314.62 ± 16.92 mg/g) (Fig. 4). Stearic (28.4%), arachidic (15.57%), heneicosanoic (9.35%) and linoleic (15.87%) acids were the dominant fatty acids under this condition. This was almost similar to the one that was found under nitrogen deprivation condition. Similarly, the overall fatty acid pool was dominated by saturated and monounsaturated fatty acids which together accounted for 77.74% (Table 1).

3.5. *Effect of combined deprivation on biomass and lipid characteristics*

Biomass concentration of *Chlorella* sp., was grossly affected by the combined deprivation of nitrogen, potassium-phosphate and iron. The final biomass concentration was 2.41 ± 0.06 g/L (Fig. 3), whereas a substantial increase in lipid content of the cell (407.94 ± 27.22 mg/g) was observed (Fig. 4). On analyzing the fatty acids, significant increase in linoleic acid content (28.42%) was observed, which formed the major fatty acid, along with arachidic acid that accounted for 23.27% (Table 1).

4. Discussion

The microalga *Chlorella* sp., investigated in this study, was found to be suitable for sustained outdoor mass cultivation, that too in open ponds. This was evident from their uniqueness in robust and luxuriantly growing nature and typically dominated all other microalgae in a local freshwater pond. Secondly, being an indigenous isolate, this organism would also have the flexibility to adapt to environmental changes.

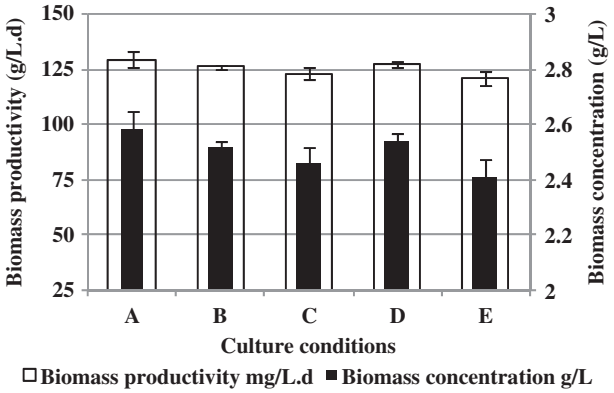

Fig. 3 – Biomass productivity and concentration of *Chlorella* sp. BUM011008, grown under normal and nutrients-deprived conditions. Experiments were carried out in triplicates. A – Normal nutritional condition, B – Nitrogen-deprived condition, C – Potassium-phosphate deprived condition, D – Iron-deprived condition, E – Combined deprivation (nitrogen, potassium-phosphate and iron).

When the *Chlorella* sp., was tested under laboratory conditions it grew luxuriously in Chu10 medium and accumulated lipids at moderate levels. Thus, it needs suitable biochemical engineering to selectively trigger lipid synthesis.

An earlier report suggests that under nutrient-deprived conditions microalgae can accumulate lipid making use of the available carbondioxide and solar energy [7]. The response towards the different stress conditions is highly dependent on the species investigated. When the biomass grown under normal conditions was transferred to nutrients-depleted medium (nitrogen, potassium-phosphate, iron and all three combined), *Chlorella* sp., was able to respond with different levels of lipid accumulation. It was interesting to infer from the present results that among the four approaches, nitrogen

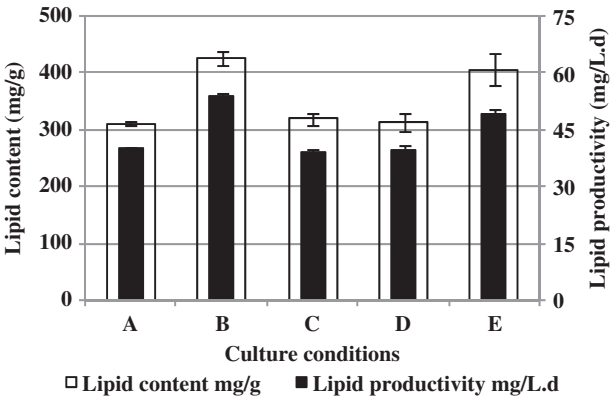

Fig. 4 – Lipid content and productivity of *Chlorella* sp. BUM011008 grown under normal and nutrients-deprived conditions. Experiments were carried out in triplicates. A – Normal nutritional condition, B – Nitrogen-deprived condition, C – Potassium-phosphate deprived condition, D – Iron-deprived condition, E – Combined deprivation (nitrogen, potassium-phosphate and iron).

deprivation was the most favourable post-harvest treatment in order to attain high biomass and high lipid content per cell. *Chlorella* sp., showed a profound increase in lipid content (42.8%) under this condition, with a minor loss in biomass which was very similar to the earlier reports (Table 2). This could be explained from the perspective that under nitrogen-deprived condition the cell would release nitrogen from the photosynthetic pigments and utilize the same for the metabolic processes. This would bring down the rate of photosynthesis leading to a cellular metabolic flux [9], and therefore, NADH would accumulate. This would inhibit the enzyme citrate synthase and stop acetyl CoA from entering the TCA cycle. The increase in the level of acetyl CoA would lead to activation of acetyl CoA carboxylase, which in turn would convert acetyl CoA in to malonyl CoA. As a result, lipid synthesis would get augmented [10], resulting in increased lipid accumulation.

Deprivation of nutrients, such as nitrogen, potassium-phosphate and iron, in a single case yielded 40.7% of lipid and it was significantly higher than under normal conditions. This is in agreement with the earlier reports where in the organism was tested against the combination of nitrogen and phosphate deficiency [11]. The enhanced lipid accumulation may be mainly due to the lack of nitrogen, rather than the absence of potassium-phosphate or iron. This inference is arrived at based on the outcome from the results of treatments 2 and 3 where, individually, under potassium-phosphate or iron deprivation conditions, there was no significant increase in lipid content. Upon deprivation of all the nutrients together (treatment 4), even though there was a slight decrease in the biomass, the overall lipid productivity was much higher when compared to normal condition and slightly lesser than under nitrogen-deprived condition. Earlier reports suggest that iron deprivation would decrease the cellular lipid content (Table 2). In contrast to this, in the present study iron deprivation did not produce effect on lipid content of the cell. This is in agreement with Menzyanova et al. [12], who showed that lowering of iron concentration did not change the lipid content. Similar result was observed under potassium-phosphate deprivation (Table 2). From the perspective of economics, in spite of the lesser lipid productivity, the combined nutrient deprivation can be considered as a suitable post-harvest treatment since it is not only very simple but involves only minimal and cost-effective nutrients and thereby has a great significance on the overall economics.

The important biodiesel properties such as cetane number, iodine number, heat of combustion, NO_x emission, oxidative stability, lubricity, viscosity and cold flow are solely dependent on the FAME profile [13,14]. Usually, the presence of unsaturated fatty acids leads to lowering of the cetane number of a biodiesel and increase in the NO_x emission. The unsaturated fatty acids are prone to oxidation which would also affect the lubricity of a biodiesel [14]. Hence, it is essential that the biodiesel must have high levels of saturated and monounsaturated fatty acids with low levels of polyunsaturated fatty acids. In the present study, it was interesting to find that different growth conditions significantly influence the fatty acid composition of *Chlorella* sp., which is clearly revealed in their FAME profiles. The FAME profiles

Table 2 – Comparison of biomass concentration, lipid content and lipid productivity of *Chlorella* sp. BUM011008 grown under normal and nutrient-deprived conditions compared with data from earlier reports.

S. No.	Organism	Particulars	Culture conditions					References
			Normal	Nitrogen deprivation	Phosphate deprivation	Iron deprivation	Combined deprivation	
1	*Chlorella* sp., BUM11008	BM	2.58 ± 0.07	2.52 ± 0.02	2.46 ± 0.06	2.54 ± 0.03	2.41 ± 0.06	Present work
		LC	31.2	42.8	31.9	31.4	40.7	
		LP	40.27 ± 0.12	53.96 ± 0.63	39.35 ± 0.5	39.96 ± 0.85	49.16 ± 1.36	
2	*Choricystis minor* (2 days starvation)	BM	1.45	NA	NA	NA	1.3	[11] [a]
		LC	25.2 ± 2.1	NA	NA	NA	34.2 ± 0.4	
		LP	NA	NA	NA	NA	NA	
3	*Choricystis minor* (5 days starvation)	BM	1.89	NA	NA	NA	1.86	[11] [a]
		LC	27.0 ± 0.8	NA	NA	NA	45.3 ± 3.0	
		LP	NA	NA	NA	NA	NA	
4	*Choricystis minor* (10 days starvation)	BM	1.85	NA	NA	NA	1.73	[11] [a]
		LC	26.0 ± 1.2	NA	NA	NA	59.5 ± 1.6	
		LP	NA	NA	NA	NA	NA	
5	*Chlorella vulgaris*	BM	0.86	NA	NA	NA	NA	[16]
		LC	29.53	1.8x	NA	NA	NA	
		LP	12.77	NA	NA	NA	NA	
6	*Botryococcus* sp., SK	BM	NA	NA	NA	NA	NA	[17]
		LC	15.8	20.7	NA	12	NA	
		LP	21.3	NA	NA	NA	NA	
7	*Botryococcus* sp., TRG	BM	NA	NA	NA	NA	NA	[17]
		LC	25.8	32.3	NA	14	NA	
		LP	46.9	NA	NA	NA	NA	
8	*Botryococcus* sp., PSU	BM	NA	NA	NA	NA	NA	[17]
		LC	5.7	14.3	NA	5	NA	
		LP	NA	NA	NA	NA	NA	
9	*Botryococcus* sp., KB	BM	NA	NA	NA	NA	NA	[17]
		LC	17.8	23.9	NA	12	NA	
		LP	39.7	NA	NA	NA	NA	
10	*Dunaliella tertiolecta*	BM	NA	NA	NA	NA	NA	[18]
		LC	1x	2.3x	0.9x	0.9x	NA	
		LP	NA	NA	NA	NA	NA	

BM – Biomass concentration in g/L; LC – Lipid content in percentage; LP – Lipid productivity in mg/L d; NA – Not available.
a The culture response towards combination of nitrogen and phosphate deprivation was studied.

under most of the conditions meet the requirement of European standard EN 14214 for biodiesel production [15]. According to EN 14214, the permissible level of C18:3 (linolenic acid) is ≤12%. In this study, the C18:3 content was less than 4% under all the experimental conditions. The level of highly unsaturated fatty acid (four or more double bonds should be ≤3%) was less than 3% except in the case of potassium-phosphate deprivation, where it reached up to 4.64%. In most of the cases, the saturated and monounsaturated fatty acid content together accounted for more than 75%. Combined deprivation state was the only condition which registered 65.14% of saturated and monounsaturated fatty acids. The FAME profiles under normal, nitrogen-deprived and iron-deprived conditions reflect the requirement expected of biodiesel.

Thus, this study suggests that the microalga *Chlorella* sp., is suitable for two-phase culturing system in order to obtain high yield of biomass and prolific lipid productivity. Under nitrogen-deprived condition this microalga accumulates high levels of lipids with the most appropriate fatty acid profile required of a biodiesel. With all these desirable properties, *Chlorella* sp. would be a highly suitable candidate for biodiesel production.

5. Conclusion

Chlorella sp., a dominant indigenous microalga, under two-stage cultivation processes, is shown to accumulate high level of lipids with different post-harvest conditions (nitrogen, potassium-phosphate, iron and all combined). Among the four different treatments, nitrogen deprivation was found to result in highly elevated total lipid content of the cell. Analysis of FAME profile under this condition revealed their superior quality with maximum levels of saturated and mono-unsaturated fatty acids. It is important that in the present case the slight decrease in the biomass, under experimental conditions, is only expected and it is more than compensated by the overall lipid productivity. The study leads to the conclusion that the two-phase culturing system, with nitrogen starvation as post-harvest treatment, would be the suitable moralities for gaining maximum biomass productivity and lipid content of high quality fatty acids. Thus, it is proposed that *Chlorella* sp., BUM11008 would be a promising candidate for sustainable biodiesel production.

The large-scale outdoor mass cultivation of *Chlorella* sp., BUM11008 in open raceway ponds, taking cues from all the

valuable outputs of the present study, will be taken up shortly. This report is rushed to demonstrate the technical feasibility of utilizing this organism for integrated and sustainable biodiesel production.

Acknowledgement

The financial support from the Department of Biotechnology (DBT), Government of India is gratefully acknowledged (Project reference: BT/PR11316/PBD/26/164/2008).

REFERENCES

[1] Rodolfi L, Chini Zittelli G, Bassi N, Padovani G, Biondi N, Bonini G, et al. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol Bioeng 2009;102(1):100–12.

[2] Moazami N, Ranjbar R, Ashori A, Tangestani M, Nejad AS. Biomass and lipid productivities of marine microalgae isolated from the Persian Gulf and the Qeshm Island. Biomass Bioenerg 2011;35:1935–9.

[3] Montes D'Oca MG, Viegas CV, Lemoes JS, Miyasaki EK, Moron-Villarreyes JA, Primel EG, et al. Production of FAMEs from several microalgal lipidic extracts and direct transesterification of the *Chlorella pyrenoidosa*. Biomass Bioenerg 2011;35:1533–8.

[4] Zemke PE, Wood BD, Dye DJ. Considerations for maximum production rates of triacylglycerol from microalgae. Biomass Bioenerg 2010;34:145–51.

[5] Chisti Y. Biodiesel from microalgae. Biotechnol Adv 2007;25: 294–306.

[6] Johnson MB, Wen Z. Production of biodiesel fuel from the microalga *Schizochytrium limacinum* by direct transesterification of algal biomass. Energ Fuel 2009;23: 5179–83.

[7] Xin L, Hong-ying H, Ke G, Ying-xue S. Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus* sp. Biores Technol 2010;101: 5494–500.

[8] Li Y, Horsman M, Wang B, Wu N, Lan CQ. Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans*. Appl Microbiol Biotechnol 2008;81: 629–36.

[9] Courchesne NMD, Parisien A, Wang B, Lan CQ. Enhancement of lipid production using biochemical, genetic and transcription factor engineering approaches. J Biotechnol 2009;141:31–41.

[10] Mandal S, Mallick N. Microalga *Scenedesmus obliquus* as a potential source for biodiesel production. Appl Microbiol Biotechnol 2009;84:281–91.

[11] Sobczuk TM, Chisti Y. Potential fuel oils from microalga *Choricystis minor*. J Chem Technol Biotechnol 2010;85:100–8.

[12] Menzyanova NG, Goltvyansky AV, Kuznetsova YA, Sysenko EI. Seasonal variability of iron effects on periodic culture of microalgae *Dunaliella viridis* Teod. Front Biol China 2009;4(3):328–36.

[13] Francisco EC, Neves DB, Jacob-Lopes E, Franco TT. Microalgae as feedstock for biodiesel production: carbondioxide sequestration, lipid production and biofuel quality. J Chem Technol Biotechnol 2010;85(3):395–403.

[14] Saraf S, Thomas B. Influence of feedstock and process chemistry on biodiesel quality. Process Saf Environ 2007;85: 360–4.

[15] European Standard EN14214. Automotive fuels – fatty acid methyl esters (FAME) for diesel engines – requirements and test methods. Brussels: European Committee for Standardization; 2008. pp. 1–15.

[16] Widjaja A, Chien CC, Ju YH. Study of increasing lipid production from freshwater microalgae *Chlorella vulgaris*. J Taiwan Inst Chem Eng 2009;40:13–20.

[17] Yeesang C, Cheirsilp B. Effect of nitrogen, salt and iron content in the growth medium and light intensity on lipid production by microalgae isolated from freshwater sources in Thailand. Biores Technol 2010;102(3):3034–40.

[18] Chen M, Tang H, Ma H, Holland TC, Simon Ng KY, Salley SO. Effect of nutrients on growth and lipid accumulation in the green algae *Dunaliella tertiolecta*. Biores Technol 2010;102(2): 1649–55.