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Application of NaCl for Biodiesel Components Production by *Botryococcus braunii*

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Abstract

Objectives: To reveal the potential use of NaCl for cultivation of *Botryococcus braunii* on commercial basis for bioenergy production. **Methods/Analysis:** The experiments were performed with different amounts of NaCl, two *Botryococcus braunii* strains and *Anopheles* mosquito larvae under controlled conditions. In order to find any role of hydrocarbons or lipids for killing of larvae, experiments with the algal extracts prepared in two solvents i.e., n-hexane and chloroform:methanol (2:1), were conducted. Hydrocarbons and lipids were analyzed by Gas Chromatography-Mass Spectrometry. **Findings:** Oil and hydrocarbon content in *Botryococcus braunii* were enhanced in Chu-13 medium supplemented with 0.5 M NaCl and growth was also not much affected. Relative ratios of different kinds of lipids and hydrocarbons produced by the algal strains were also affected significantly. Additionally, medium supplemented with NaCl was found to be fatal to *Anopheles* mosquito larvae which cause severe losses of algal biomass particularly during mosquito breeding season during outdoor algal cultivation. **Novelty/Improvement:** Finding of killing of *Anopheles* mosquito larvae and improvement in the quantity and quality of hydrocarbons and lipids by NaCl in nutrient medium can be probably exploited for the cultivation of *Botryococcus braunii* for biodiesel production.

Key Words: Algae, *Anopheles*, Biodiesel, NaCl, Lipids

1. Introduction

Biofuels are good substitutes of fossil fuels which are the main cause of greenhouse gas emissions and other related concerns[1]. Biodiesel is mostly obtained from the oils and hydrocarbons produced by the higher plants[2]. Algae are the most promising and sustainable resource of oil and hydrocarbons for biodiesel production when it comes to food security, environmental issues[3] and other advantages like they have faster growth rate and can help in CO_2 sequestration and can be cultivated all year round on wasteland unsuitable for any other use and requirement of comparatively less water for their cultivation. In addition to this, the yield of algal oil is much greater comparative to the land-based crops. The residual algal biomass remained after the extraction of oil can possibly be used as feed for animals, manure for crops or for the production of ethanol or methane by fermentation[4-7].

Green alga *Botryococcus braunii* has been observed to produce 30-70% hydrocarbons as the important biodiesel components under various environmental conditions. Sea water and nutritional stress conditions have been reported to enhance the quantity and quality of the desirable oils and hydrocarbons with substantial growth in *Botryococcus braunii*[8,9]. Higher cost of generation of algal biomass is one of the major limitations in commercialization of algal biodiesel technology. For large scale cultivation of *Botryococcus braunii*, the medium with very few nutrients and that too in considerably reduced concentration can be exploited for the generation of biomass and biofuel components[10,11].

The present study concentrates on the potential application of NaCl, the main component of sea water, for enhancing the biofuel components and controlling the mosquito larvae which cause the major loss to the algal biomass during their outdoor cultivation.

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2. Materials and Methods

2.1 Test Organisms and Growth Conditions

Two *Botryococcus braunii* strains were isolated, one from Loktak Lake, Manipur (24.55° N, 93.78° E) and the other from Udaiagar Lake, Udaipur, Rajasthan (24.60° N, 73.67° E), respectively, by the method of serial dilution and plating on the solidified Chu-13 nutrient medium[12]. Individual colonies of the two strains were isolated separately and then transferred into liquid nutrient medium. Cultures were incubated under the controlled conditions of temperature 27 ± 1°C, light intensity 1.2 ± 0.2 Klux and 16:8 hrs. light:dark cycle. For determining the survival of *Anopheles* mosquito larvae, 2-days old larvae were incubated separately with the two strains of *Botryococcus braunii* and their survival was observed at different time intervals.

2.2 Chlorophyll Measurement

Method of Lichtenthaler[13] was used for the measurement of chlorophyll. Cells were harvested from a known volume of algal culture, suspended in 90% methanol and then heated at 60°C for 30 minutes.

2.3 *Botryococcus braunii* Extract Preparation

For preparation of *Botryococcus braunii* extracts (0.1 g/ml), dried algal powder was homogenised separately in chloroform:methanol (2:1) and n-hexane. For the measurement of survival of *Anopheles* larvae, 400 µl of extract was added in 100 ml of Chu-13 medium containing *Anopheles* larvae and different concentrations of NaCl.

2.4 Gravimetric Measurement of Hydrocarbons and Oils

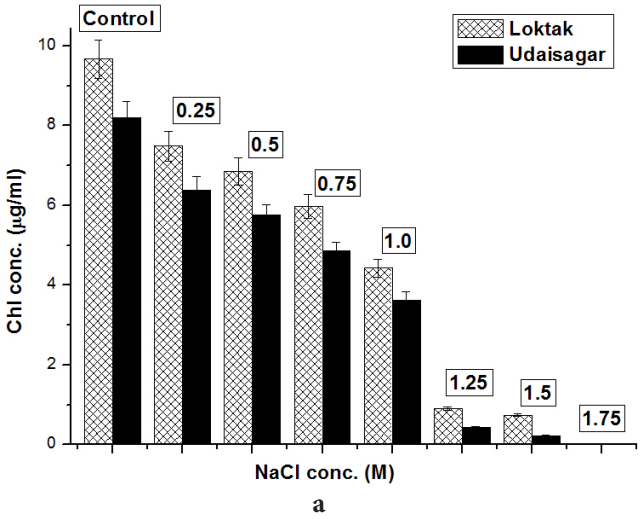
Botryococcus braunii cells were freeze dried and homogenized in *n*-hexane and chloroform:methanol (2:1) separately. Hydrocarbons and oils were estimated by weighing the dried supernatants thereof[14].

2.5 Analysis of Hydrocarbon and Fatty Acid by GC-MS

Fatty Acid Methyl Esters (FAME) were prepared by the method of Christie[15]. Identification of FAME in chloroform:methanol (2:1) and hydrocarbons in n-hexane was done by GC-MS (Shimadzu QP 2010 Plus). Their separation patterns were also compared with the standard peaks (Sigma) and the NIST library[14].

3. Results and Discussion

Hydrocarbon (26.48% for Udaisagar strain and 30.65% for Loktak strain in NaCl-supplemented medium with the control values of just 17.67% and 20.98%, respectively) and oil contents (43.06% for Udaisagar strain and 45.97% for Loktak strain in NaCl-supplemented medium with the control values of 22.46% and 25.67%, respectively) in both the algal strains increased significantly when Chu-13 medium was supplemented with 0.5 M NaCl, which was lethal to *Anopheles* larvae, without much affecting the growth is shown in Figure 1.



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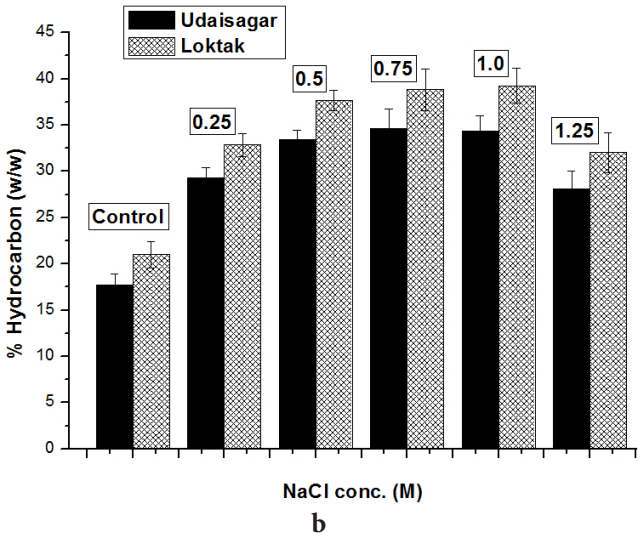

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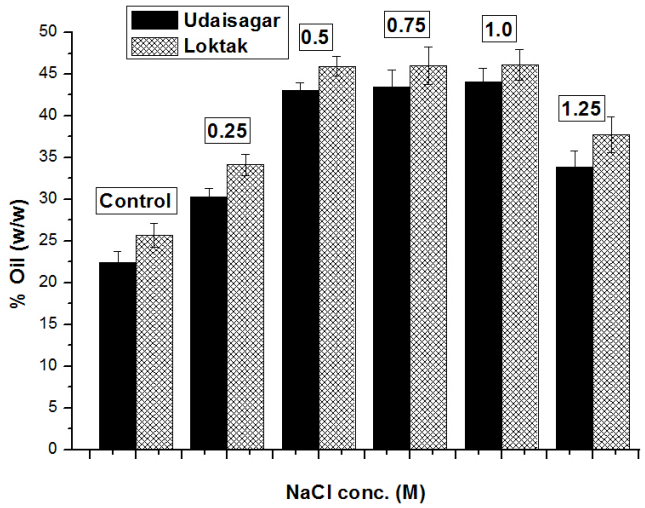

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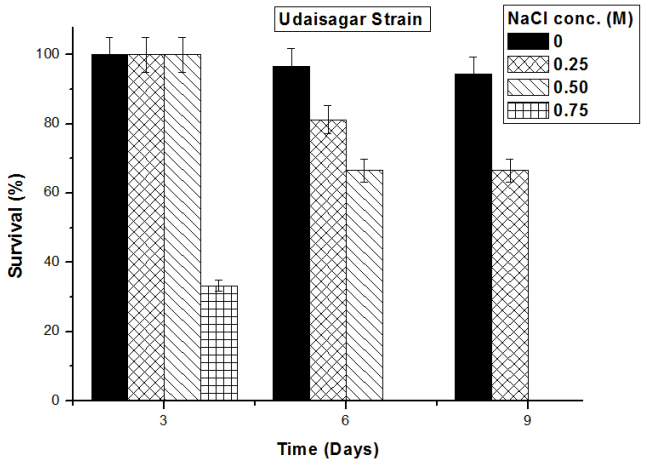

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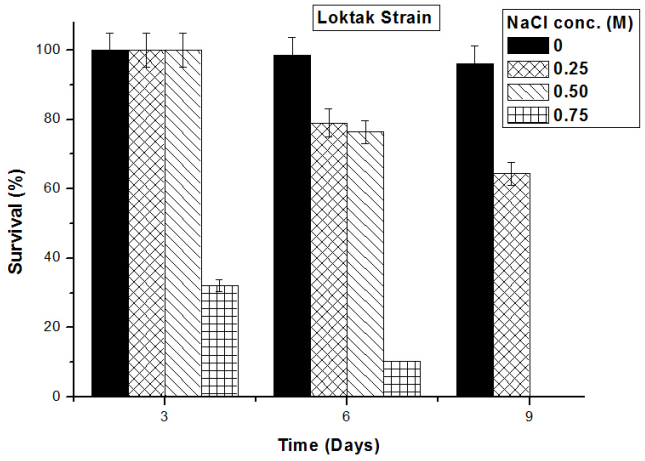
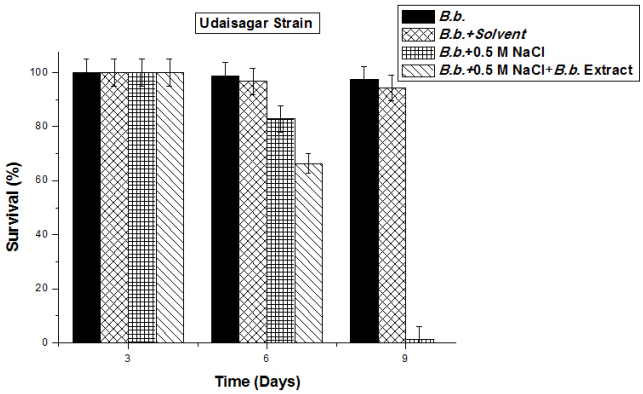


e

Figure 1. **(a)** Growth, **(b)** hydrocarbon and **(c)** oil production by *Botryococcus braunii strains* on 21st day and **(d)** *Anopheles* mosquito larvae survival with Udaisagar and **(e)** Loktak strains, respectively, in Chu-13 medium on supplementation with 0.5 M NaCl.



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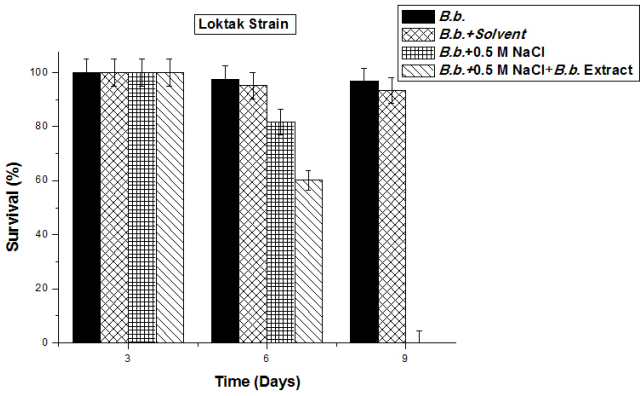

b

Figure 2. Survival of *Anopheles* mosquito larvae with **(a)** Udaisagar and **(b)** Loktak strains of *Botryococcus braunii* in (i) Chu-13 medium (*B.b.* (ii) Chloroform:methanol (2:1) solvent (*B.b.*+Solvent), (iii) 0.5 M NaCl (*B.b.*+0.5 M NaCl) and (iv) 0.5 M NaCl and chloroform:methanol (2:1) extract of *Botryococcus braunii* (*B.b.*+0.5 M NaCl+*B.b.* Extract). Transformation of mosquito larvae into adults took place on 9th day, if survived.

Many insect larvae, including *Anopheles* mosquito larvae, have been observed to cause severe losses to algal biomass by ingestion during their outdoor cultivation.

Experiments on the survival of larvae using extracts of *Botryococcus braunii* grown in 0.5 M NaCl supplemented medium in solvents n-hexane and chloroform:methanol (2:1), that preferably solubilised hydrocarbons and oils, respectively, confirmed that some biologically active metabolites produced due to presence of NaCl in the medium were lethal for larvae in addition to the death occurring due to salinity of the medium is shown in Figure 2.

Both the algal strains were observed to produce mainly saturated hydrocarbons ranging between C_{15} to C_{35}, major ones were $C_{17}H_{34}$, $C_{19}H_{38}$, and $C_{21}H_{44,}$ as revealed on identi-

Table 1. Comparative account of hydrocarbon profile of two strains of *Botryococcus braunii* in Chu-13 medium (control) and Chu-13 medium on supplementation with 0.5 M NaCl

Compound	Peak No.	Relative %			
		Udaisagar Strain		Loktak Strain	
		Control	NaCl	Control	NaCl
$C_{15}H_{30}$ 1-Pentadecene	1	6.38	-	6.58	-
$C_{15}H_{32}$ Pentadecane	2	-	2.34	-	1.87
$C_{16}H_{34}$ Hexadecane	3	-	1.31	-	1.68
$C_{17}H_{34}$ 1-Heptadecene	4	16.033	5.23	10.62	4.68
$C_{17}H_{36}$ Heptadecane	5	-	9.47	-	6.94
$C_{18}H_{38}$ Octadecane	6	-	0.82	-	0.28
$C_{19}H_{38}$ 1-Nonadecene	7	16.43	16.63	17.23	16.36
$C_{20}H_{42}$ Eicosane	8	-	2.12	-	0.85
$C_{21}H_{44}$ Heneicosane	9	18.78	2.04	22.43	2.23
$C_{22}H_{46}$ Docosane	10	-	14.11	-	18.61
$C_{23}H_{46}$ 9-Tricosene	11	-	6.85	17.68	8.27
$C_{24}H_{50}$ Tetracosane	12	18.57	11.88	-	13.51
$C_{27}H_{54}$ 1-Heptacosene	13	12.85	3.93	12.07	0.40
$C_{27}H_{56}$ Heptacosane	14	8.35	8.57	7.77	9.03
$C_{28}H_{58}$ Octacosane	15	-	0.67	-	1.12
$C_{29}H_{60}$ Nonacosane	16	-	6.18	-	6.79
$C_{30}H_{62}$ Triacontane	17	-	1.12	-	1.58
$C_{35}H_{70}$ 17-Pentatriacontene	18	1.28	4.15	2.08	4.33
$C_{35}H_{72}$ Pentatriacontane	19	0.85	0.90	2.04	1.12
$C_{44}H_{90}$ Tetratetracontane	20	0.43	1.68	1.50	0.35
		100	100	100	100

fication by GC-MS. These hydrocarbons were significantly affected by the supplementation of 0.5 M NaCl in Chu-13 is shown in Table 1. In *Botryococcus braunii,* mainly saturated (C_{20} to C_{31}) and branched-chain (C_{14} to C_{28}) hydrocarbon have been observed by various workers[16,17].

Major fatty acids found in both the strains were palmitic, stearic and oleic acids shows in Table 2 which are in agreement to that reported by [18] and [19]. On supplementation of Chu-13 medium with 0.5 M NaCl, both the algal strains showed a significant increase in relative proportion of palmitic acid (16:0) and of stearic acid (18:0) in Loktak strain showing their possible contribution in killing of *Anopheles* larvae along with the salinity of medium. Antimicrobial activity has also been reported to be possessed by some fatty acids in the extracts of a few microalgae[20]. Application of 0.5 M NaCl in the culture medium can enhance the production of biologically active lipids which have potential biofuel application in *Botryococcus braunii* in addition to its role in controlling the mosquito larvae during outdoor cultivation of algae.

Table 2. Comparative account of lipids produced by two strains of *Botryococcus braunii* in Chu-13 medium (control) and Chu-13 medium on supplementation with 0.5 M NaCl

Fatty Acids	Peak No.	Relative %			
		Udaisagar Strain		Loktak Strain	
		Control	NaCl	Control	NaCl
14:0 Tetradecanoic acid	1	-	3.51	-	0.69
15:0 Pentadecanoic acid	2	-	2.00	-	1.08
16:1 Palmitoleic acid	3	-	5.99	7.77	6.78
16:0 Palmitic acid	4	26.78	39.3	20.59	34.12
17:0 Margaric acid	5	-	1.02	0.66	1.14
18:2 Linoleic acid	6	17.39	4.87	0.92	6.44
18:1 Oleic acid	7	31.29	16.95	52.81	28.23
18:0 Stearic acid	8	13.29	12.03	7.17	11.34
20:0 Arachidic acid	9	0.65	-	1.66	2.54
22:1 Erucic acid	10	7.33	-	0.29	-
22:0 Behenic acid	11	1.40	4.97	1.23	1.38
24:0 Lignoceric acid	12	-	1.47	0.61	1.63
26:0 Hexacosanoic acid	13	-	-	2.08	1.69
28:0 Octacosanoic acid	14	1.87	7.89	4.21	2.94
		100	100	100	100

4. Conclusion

NaCl supplementation in culture medium and sea water can be applied for biofuel production with additional advantage of controlling mosquito breeding during outdoor growth of *Botryococcus braunii* for biodiesel production.

5. Acknowledgements

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