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RESEARCH ARTICLE

THE CHALLENGES OF BIODIESEL PRODUCTION FROM *OSCILLATORIA* SP.

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Abstract

Microalgae are the most promising sources for biodiesel. At constant light and temperature, four different *Oscillatoria* sp. were cultured in sterilized BG-11 medium for a long period. Nitrate concentration was measured each couple of days to conclude that *oscillatoria* sp. did not suffer from nitrogen starvation for short culture periods. Then *Osci.2* chosen for its highest dry cell weight; 450, 534, 1140, 2843, 2519 mg 100ml⁻¹ in days 3, 7, 22, 36 and 39, respectively. *Osci.2* cultivated for 21 days in BG-11 medium with different nitrate concentrations; (1500, 375, 186, 94, 47, 23 and 0.0 mg l⁻¹ NaNO₃. Biomass productivity (mg l⁻¹ d⁻¹), DCW (mg l⁻¹) and target fatty acids percent (%TFAs; (C14:0, C16:0, C18:0, C16:1, C18:1, C18:2, C18:3) for biodiesel production detected per seven-day's cultures. With different nitrogen concentrations, *Oscillatoria* sp. produced diverse of fatty acid patterns. Because of the main targets for biodiesel production are both biomass and TFAs, 375 mg l⁻¹ NaNO₃ is the suitable nitrate concentration to cultivate *Oscillatoria* for 21 days for biodiesel production.

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Introduction:-

The concept of microalgae biomass production for conversion to fuels (biogas) was first suggested in the early 1950s (Sheehan et al., 1998). In fact, algae are the highest yielding feedstock for biodiesel and it may be only the way to produce enough automotive fuel to replace current gasoline usage (Hossain and Salleh, 2008).

Cyanophyceae, Chlorophyceae, Chrysophyceae and Bacillariophyceae are extensively utilized for biodiesel production. Cyanophyceae received a considerable attention in last year's due to their high oil content and easily grown in non-arable lands (Vimalarasan et al., 2011).

Oscillatoria sp. is a blue green filamentous alga. According to Vimalarasan et al. (2011) *Oscillatoria annae* had interesting character for biodiesel production. Muthulakshmi and Meenatchisundaram (2015) revealed microalgae *Oscillatoria foreani* comparatively show effective production of biodiesel than *Spirogyra* sp. and *Chlorella pyrenoidosa* due to its high lipid content and biomass productivity. Therefore, *Oscillatoria* sp. were choice in this investigation.

Fatty acid profile of microalgae changed with changing culture conditions whether chemical or physical ones as nutrient starvation, salinity, and pH. Nitrogen depletion is the most investigated parameter for excessive lipid

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accumulation (Griffiths & Harrison, 2009; Gülyurt et al., 2016). The composition of nutrient media (Nitrogen, carbon, phosphorus and trace metals) is one of the most significant factors that affect growth parameter and biochemical composition of microalgae (Wang et al. 2014; Lin and Wu 2015). It is easy to induce oil content of 20–30% in several microalgal species (Chisti 2007).

At the lowest concentration of nitrogen and phosphorus, the microalgal cell tends to produce high lipid content (Hidayat, 2008). As described by many authors that under nutrient limitation, many algae stop growth and division to use all their energy to make lipids as storage products for survival (Hidayat, 2008).

Since the various fatty acid profiles influence biodiesel fuel properties, it is important to possess data on how the presence of nitrogen and the period of cultivation can influence the profile of produced fatty acids from algae. This study aimed to compare between the effect of nitrogen limitation and starvation on fatty acid profiles and biomass productivity of *Oscillatoria* sp. to enhance biodiesel production.

Materials and Methods: -

Isolation, Purification and Identification of *Oscillatoria* sp.: -

Under aseptic conditions, four *Oscillatoria* species were isolated from fresh water bodies at Port Said, Egypt. Then they were purified using streak method on solid BG-11 medium. Identification done by binocular light microscopy (SME-F4D, Rating: 85 V to 265 V, 50/60 Hz, Halogen lamp: 60 V 20 W, Delay-action fuse: 1 A) according to Smith (2010) (Fig. 1).

BG-11 medium composed of (g l^{-1}), NaNO_3 , 1.5; $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, 0.04; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.075; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.036; citric acid, 0.006, ferric ammonium citrate, 0.006; Na_2EDTA , 0.001; Na_2CO_3 , 0.02. In addition to 1 ml of trace metal solution (including H_3BO_3 , 2.86 g; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 1.81 g; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.222 g; $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.390 g; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 79 mg and $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 49.4 mg/l).

Experimental design:-

A touch having many filaments from the agar plates were transferred separately to standard BG-11 liquid medium and left to grow at $27 \pm 1^\circ\text{C}$ with continuous lighting $82.62 \mu\text{mol}^{-2}\text{sec}^{-1}$. These cultures used as a stock culture for next experiments.

Cultivation of algae in standard medium:-

Constant weights were transferred separately from each species to 100ml sterile BG-11 medium then left to grow for 39 days in the same culture conditions as previous. Biomass as dry cell weight (DCW) detected after 3, 7, 22, 36 and 39 days of cultivation.

Cultivation of alga (*Osci. 2*) in different N concentrations:-

Constant weights were transferred to 1l sterile BG-11 medium with different nitrogen concentrations; 1500, 375, 186, 94, 47, 23 and $0.0 \text{ mg l}^{-1} \text{ NaNO}_3$, separately then left to grow for 21 days in the same culture conditions as previous. Biomass as dry cell weight (DCW), Biomass productivity recorded weekly and fatty acid analysis after 7 days for all concentrations, 14 days for 186 and $375 \text{ mg l}^{-1} \text{ NaNO}_3$ and 21 days for $375 \text{ mg l}^{-1} \text{ NaNO}_3$.

Nitrate analysis:-

Nitrate residue for all sp. detected in medium each two-day using spectrophotometer (6405 UV/Visible Spectrophotometer, Jenway, England) according to Cataldo *et al.* (1975).

Dry cell weight: -

Certain volume of culture was filtered on pre-weighted glass fiber filter paper then dried at 60°C until constant to detect Dry cell weight (DCW).

Biomass productivity:-

Volumetric biomass productivity (PBiomass) calculated as follow:

$$\text{PBiomass (mg l}^{-1}\text{d}^{-1}) = (\text{X}_2 - \text{X}_1) / (\text{t}_2 - \text{t}_1) \quad \text{Eq. A.1}$$

Where X_1 and X_2 were the biomass dry weight concentrations (mg l^{-1}) on days t_1 (start point of cultivation) and t_2 (end of cultivation), respectively (Hempel *et al.*, 2012).

Oil extraction: -

A definite dry weight of *Oscillatoria* sp. was poured all night into hexane: ether (1:1, V: V) to extract oil, this step is repeated until the extract became hyaline. The extract evaporated in vacuum to release solvents using rotary evaporator (Diagonal Condenser-RE300, vacuum 1 mm Hg, made in U.K.) (Hossain & Salleh, 2008).

Fatty Acid Analysis:-

Extracted oil converted into fatty acid methyl ester according to Luddy *et al.* (1960) and measured using gas chromatography (Perkin Elmer Auto system XL) equipped with Flame Ionization Detector (FID), fused silica capillary column DB-5 (60 m × 0.32 mm i.d.). The oven temperature was maintained initially at 150 °C and programmed from 150 °C to 240 °C at rate 3 °C/min, then held at 240 °C for 30 min. The injector temperature was 230 °C. Detector temperature was 250 °C and carrier gas was Helium with flow rate of 1 ml/min.

Results:-

Since biodiesel properties affected by biomass and fatty acids extracted from cells; it is important to possess data on how nitrogen can influence the biomass and profile of target fatty acids (TFA) within algae.

Four *Oscillatoria* sp. (*Osci.1*, *Osci.2*, *Osci.3* and *Osci.4*) were cultured in sterilized 100ml BG-11 medium for a long period. Biomass as dry cell weight (DCW) detected after 3, 7, 22, 36 and 39 days of cultivation. The highest DCW values recorded for second species (*Osci.2*) which were 450, 534, 1140, 2843, 2519 mg 100ml⁻¹ in days 3, 7, 22, 36 and 39, respectively (Table 1).

Nitrate residue detected almost every 2 days, the results clarified that *Oscillatoria* sp. did not consume all nitrate in short period cultures and it will take more time to start starvation (Fig. 2).

Osci.2 was cultured for 21 days using BG-11 medium with different nitrogen concentrations (1500, 375, 185.7, 93.7, 46.8, 23.4 and 0.0 mg l⁻¹ NaNO₃). Dry cell weight (mg l⁻¹), biomass productivity (mg l⁻¹ d⁻¹) and fatty acid profiles detected weekly for all concentrations in addition at day 14 fatty acid profiles analyzed for cultures cultivated in 375 and 185.7 mg l⁻¹ NaNO₃. Also, after more 7 days, fatty acid profiles analyzed for culture that cultivated in 375 mg l⁻¹ NaNO₃.

With different nitrate concentration, *Osci.2* grown with different growth rates. The maximum dry weights (g l⁻¹); 3549 and 3232 mg l⁻¹ were recorded at 375 and 1500 mg l⁻¹ NaNO₃, respectively. The maximum biomass productivity (mg l⁻¹ d⁻¹); 131.68 and 122.11 mg l⁻¹ d⁻¹ were recorded at 375 and 1500 mg l⁻¹ NaNO₃, respectively. Then with the lower nitrate concentrations, the lower dry cell weights and biomass productivity were observed (Table 2).

Osci.2 tended to produce diverse of fatty acid patterns; saturated and unsaturated fatty acids with different amounts related to different nitrate concentrations that leads to the difference in the percent of target fatty acids; TFAs; (C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3) for biodiesel production. After first week of cultivation *Osci.2* produced the maximum percent (79.38) of target fatty acids at 186 mg l⁻¹ NaNO₃ that was decreased after more seven days to 43.95%. While at 375 mg l⁻¹ NaNO₃, TFAs increased gradually from minimum 9.3% to maximum 70.86% at day 21 (Table 3).

Table 1: - Dry cell weight (mg 100ml⁻¹) culture for a long period of different *Oscillatoria* sp.

	<i>Osci.1</i>	<i>Osci.2</i>	<i>Osci.3</i>	<i>Osci.4</i>
Day3	397	450	168	250
Day7	368	545	216	421
Day22	386	1140	329	1758
Day36	545	2843	1214	1216
Day39	1314	2519	1558	1847

Table 2: - Dry cell weight (mg 100ml⁻¹) and Biomass productivity (mg l⁻¹ d⁻¹) for long periods cultures of different *Osci.2*.

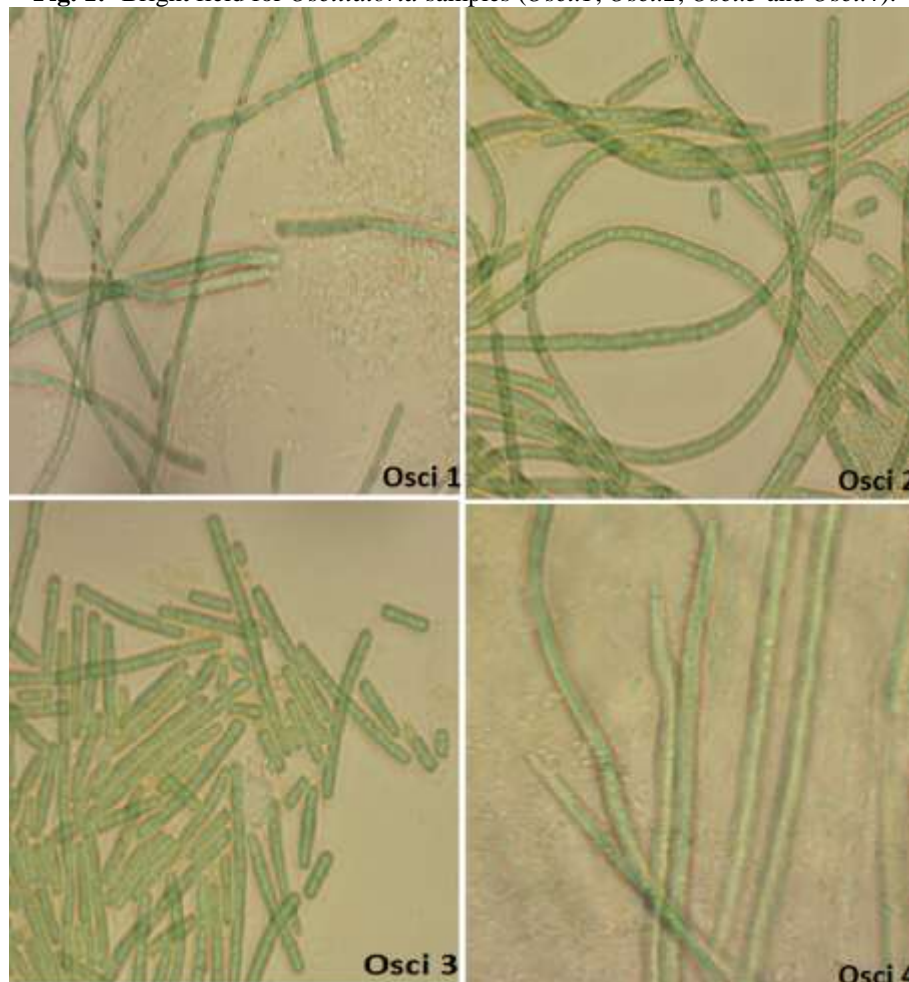
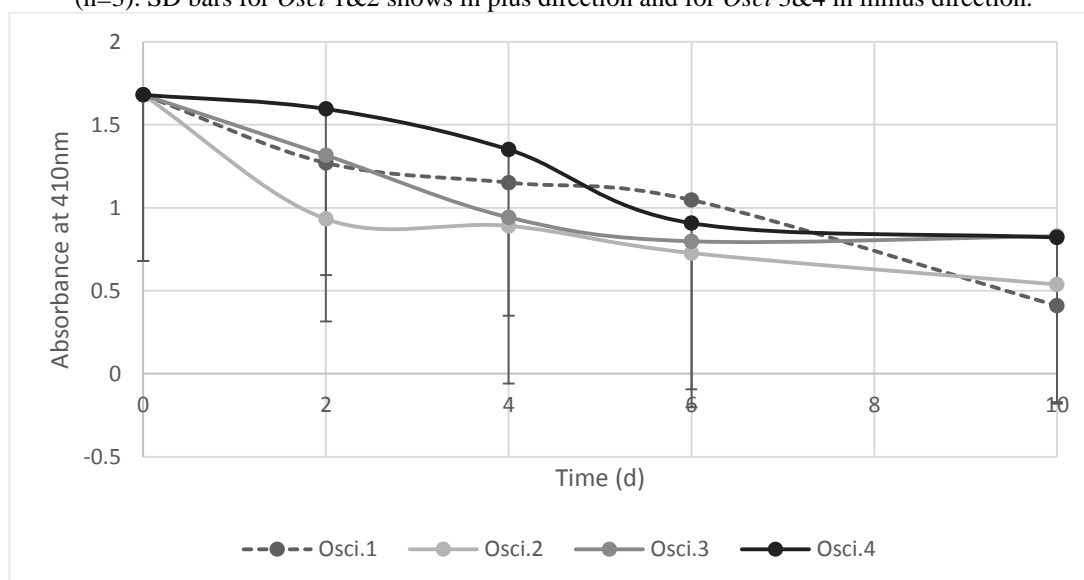
	Dry cell weight (mg 100ml ⁻¹)			Biomass productivity (mg l ⁻¹ d ⁻¹)		
NaNO ₃	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21

(mg ^l ⁻¹)						
1500	3232	5012	5006	461	358	238
375	3549	4973	5018	507	355	238
185	21	308	9	3	44	1.3
93	11	20	4	1.6	2.9	0.55
46	5	5	3	0.7	0.65	0.45
23	5	1	1	0.7	0.07	0.07
0.0	6.1	1	1	0.8	0.07	0.07

Table 3: - Fatty acids profiles of *Osci.2* grown in BG-11 medium with different nitrogen concentrations for 21 days.

- a) Target fatty acids for biodiesel production (% of total fatty acids).
 b) Saturated fatty acids (% of total fatty acids).
 c) Monounsaturated fatty acids (% of total fatty acids).
 d) Polyunsaturated fatty acids (% of total fatty acids).

Days	NaNO ₃ (mg ^l ⁻¹)	C14 :0	C16 :0	C17 :0	C18 :0	C18 :1	C18 :2	C18 :3	C20 :5	C22H 32	C22H 43	TF A ^a	SF A ^b	MUF A ^c	PUF A ^d
7	1500	11.42	14.0	29.72	3.05	12.66	8.24	2.10	18.74	NA	NA	51.47	58.19	12.66	29.08
	375	6.68	NA	NA	NA	NA	2.59	NA	77.52	3.56	9.43	9.27	6.68	0	93.1
	185	NA	40.44	NA	9.46	NA	29.48	NA	NA	NA	20.62	79.38	49.9	0	50.1
	93	2.56	3.17	2.93	0.49	NA	1.08	NA	0.57	NA	NA	7.3	97.9	0	1.65
	46	NA	NA	NA	NA	13.72	4.26	NA	55.25	NA	26.78	17.98	0	13.72	86.29
	23	36.21	NA	NA	NA	NA	3.40	NA	39.38	3.63	17.37	39.61	36.21	0	63.78
	0.0	NA	9.28	NA	19.31	10.70	5.84	2.85	15.48	21.94	15.58	47.98	28.59	10.7	61.69
14	375	NA	4.4	NA	NA	NA	20.2	NA	NA	NA	75.41	24.6	4.4	0	95.61
	185	NA	19.12	NA	7.71	NA	17.12	NA	5.81	NA	50.26	43.95	26.83	0	28.74
21	375	5.51	51.95	NA	7.89	NA	5.51	NA	7.76	NA	21.4	70.86	65.35	0	34.67

Fig. 1:- Bright field for *Oscillatoria* samples (*Osci.1*, *Osci.2*, *Osci.3* and *Osci.4*).**Fig.2: -** Average nitrate levels in the media \pm SD for *Oscillatoria* sp. cultured in standard condition; 1.5 g l^{-1} NaNO $_3$ (n=3). SD bars for *Osci* 1&2 shows in plus direction and for *Osci* 3&4 in minus direction.

Discussion:-

Many researchers believe that the choice of microalgae for biodiesel production needs a balance between species that grow quickly and produce oil in large quantities (Chisti, 2007). While others see that to choose microalgae for biodiesel, production needs to search about species that grow with large biomass and produce high concentration of target fatty acids because not all fatty acid types are suitable for biodiesel production.

Biodiesel quality and quantity produced from microalgae affected by biomass productivity, lipid productivity and target fatty acids percent.

Thomas *et al.* (1984) reported significant fatty acids for biodiesel production, like C14:0, C16:0, C16:1, C18:0, C18:1, C18:2 and C18:3 (Jena *et al.* 2012). Lee *et al.* (2011) stated that the saturated and unsaturated fatty acids as C16:0, C18:0, C18:1, C18:2 and C18:3 are common fatty acids used for biodiesel production. Knothe (2006) verified that fatty acid type's effect on biodiesel properties like Iodine number, Cetane number, Oxidative stability and Acid value.

BG-11 medium is a blue green medium. It is known as a good medium not only for blue green algae but also for some green algae. It contains nitrogen in a nitrate form. It is important to possess data about rate of nitrate consuming by all cultured species and when the starvation started. According to results of DCW and biomass productivity, there were no big differences between cultures cultivated in 1500 and 375 mg l⁻¹ NaNO₃. While with lower nitrate concentrations, the lower dry cell weights and biomass productivity were observed.

The fatty acid profiles of *Oscillatoria* sp. were detected in pure batch cultures experiments. *Osci.2* tended to produce diverse of fatty acid patterns; saturated (myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0)) and unsaturated fatty acids (oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3) and eicosapentaenoic acid (C20:5)) with different amounts related to nitrate occurrence that led to the difference in the percent of significant fatty acids for biodiesel production. So, the results show that the type and amount of fatty acids affected by the period of cultivation and mode of nitrogen supply.

The paper results accede with Jitha and Madhu (2016) that *Oscillatoria* sp. can be cultured in dairy effluent as a nutrient source for biodiesel production. Presence of palmitic (16:0), oleic (18:1), stearic (18:0), linoleic (18:2) and linolenic (18:3) acids strongly supports that the cultivated algae from the reactor can be used for the biofuel production.

Both growth and lipid content depend not only culture conditions like nutrients but also on algal strain and period of cultivation. Reviewers consistent with our results where; the fatty acids profile could be control not only by starvation but also by the level of limitation and biomass productivity is a good indicator of suitability for biodiesel production.

From presented results, twenty-one days of cultivation with NaNO₃ concentration of 375 mg l⁻¹ was the suitable condition to cultivate *Oscillatoria* sp. with maximum percent of biomass productivity and target fatty acids for biodiesel production.

References:-

1. Cataldo, Dominic & Maroon, M & Schrader, Lawrence & L. Youngs, V. (1975). Rapid Colorimetric Determination of Nitrate in Plant-Tissue by Nitration of Salicylic-Acid. Communications in Soil Science and Plant Analysis. 6. 71-80. 10.1080/00103627509366547.
2. Chisti Y. (2007): Biodiesel from Microalgae. Biotechnology Advances. 25:294–306.
3. Griffiths, M., and Harrison, S. (2009): Lipid productivity as a key characteristic for choosing algal species for biodiesel production. Journal of Applied Phycology. 21:493–507.
4. Gülyurt, M. O.; Özçimen, D. and İnan, B. (2016): Biodiesel Production from *Chlorella protothecoides* Oil by Microwave-Assisted Transesterification, International Journal of Molecular Sciences. 17: 579.
5. Hempel, N.; Petrick, I. and Behrendt, F. (2012). Biomass productivity and productivity of fatty acids and amino acids of microalgae strains as key characteristics of suitability for biodiesel production. J Appl Phycol. 24, 1407–141

6. Hidayat, S. (2008). Exploration of Indonesia's Biodiesel Producing Microalgae As Sustainable Energy Source, Alcoa Foundation's Conservation and sustainability Fellowship Program, Sustainability Institute: IUCN (International Union for Conservation of Nature).
7. Hossain, A.B.M. and Salleh, A. (2008). Biodiesel Fuel Production from Algae as Renewable Energy. Am. J. Bioch. & Biotech. 4, 250-254.
8. Jena, J.; Nayak, M.; Panda, H.S.; Pradhan, N.; Sarika, C.; Panda, P.K.; Rao, B.; Prasad, B.N. and Sukla, L.B. (2012). Microalgae of Odisha coast as a potential source for biodiesel production. World Environment. 2:11-16.
9. Jitha, G. and Madhu. (2016): cultivation of *Oscillatoria* sp in dairy waste water in two stage photo bioreactors for biodiesel production. GCivil Engineering and Urban Planning: An International Journal. 3 (2):87-96.
10. Knothe, G. (2006) Analyzing biodiesel Standards and other methods. Journal of the American Oil Chemist's Society, 83, 823-833. doi10.1007/s11746-006-5033-y.
11. Lee, S.J.; Go, S.; Jeong, G.T. and Kim, S.K. (2011). Oil production from five marine microalgae for the production of biodiesel. Biotechnology and Bioprocess Engineering. 16:561-566.
12. Lin, T. S. and Wu, J. Y. (2015): Effect of carbon sources on growth and lipid accumulation of newly isolated microalgae cultured under mixotrophic condition. Bioresource Technology. 184:100-107.
13. Luddy, F.E. Beerford, R.A. Riemenschneider, R.W. Direct conversion of lipid component to their fatty acid methyl ester. J. Am. Oil Chem. Soc. 37 (1960) 447-451.
14. Muthulakshmi, R. and Meenatchisundaram, K. (2015): biodiesel energy production from microalgae *Spirogyra* sp., *Oscillatoria foreani* and *Chlorella pyrenoidosa* , Life Science Archives. 1 (2):84-89.
15. Sheehan, J.; Dunahay, T.; Benemann, J. and Roessler, P. (1998). A look back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from algae. U.S. Report NREL/TP-580-24190. Golden CO: National Renewable Energy Laboratory, 323 p.
16. Smith, T. E. (2010): Revised list of algae from Arkansas, U.S.A. and new additions. – Int. J.Algae. 12: 230–256. doi: 10.1615/InterJAlgae.v12.i3.30
17. Thomas, W.H.; Tornabene, T.G. and Weissman, J. (1984): Screening for lipid yielding microalgae: Activities for 1983; Solar Energy Research Institute: Golden, Colorado, USA.
18. Vimalarasan A., Pratheeba N., Ashokkumar B., Sivakumar N. and Varalakshmi P. (2011) Production of biodiesel from cyanobacteria (*oscillatoria annae*) by alkali and enzyme mediated transesterification., Journal of scientific & industrial research, 70: 959-967.
19. Wang, W.; Han, F.; Li, Y. and et al. (2014). Medium screening and optimization for photoautotrophic culture of *Chlorella pyrenoidosa* with high lipid productivity indoors and outdoors. Bioresource Technology. 170:395-403.