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Extraction and Characterisation of lipids from some Diatoms collected from different water samples of Bihar region

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Abstract : *Diatoms are among the well-known water planktons. Diatoms were shown under appropriate conditions to produce up to 60% of their cellular mass as triacylglycerols (TAGs) under certain growth conditions. These TAGs can be easily converted into biodiesel through a transesterification reaction. In the present research work water sample collected from 10 different water bodies of Bihar region were examined. In which 14 diatom species under 09 genera were identified on the basis of microscopic observation as Cymbella sp., Fragilaria sp., Navicula sp., Synedra sp., Hantzschia sp., Melosira sp., Diploneis sp., Cylindrotheca sp., Nitzschia sp. were isolated from water sample of Patna region. Also water sample of Gundak, Begusarai, Synedra sp. and Cylindrotheca sp. were detected. It was found in microscopic analysis out of the 7 samples, 4 of them viz - samples A, B, C and D showed higher number of diatoms species. For culturing of diatoms, f/2 liquid Guillard media was prepared. Spectrophotometric analysis was done to detect the presence of oil in each sample. Then oil was extracted by Bligh and Dyer method Further analysis of oil was performed via HPLC in which out of 7 samples only 3 selected samples (A, C and D) were analysed which oozes more oil. Triacylglycerols (TAGs) peaks were identified. Erythritol, Myristic acid, and Fucoxanthin content were detected in sample A, Ethylene glycol, Capric acid, Palmitoleic acid, Lauric acid, Oleic acid and Stearic acid were identified in sample B while in sample C presence of Ethylene glycol, Caprylic acid, Fucoxanthine, Palmetoic acid, Linoleic acid, Oleic acid and Myristic acid were detected. Further work can be done for extraction and characterization of lipids from diatoms for biodiesel production.*

Key words:- *Diatom, Biodiesel, Triacylglycerols, water samples.*

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Introduction :

Diatoms are among the well-known water planktons. They are autotrophic and found in both marine and fresh waters (Pal et al. 2017). Diatoms are formally classified as belonging to the Division *Chrysophyta;* Class *Bacillariophyceae.* Diatoms are divided into two Orders. The Biddulphiales which have valve striae arranged basically in relation to a point, an annulus or a central areola and tend to appear radially symmetrical. The Pennales (Bacillariales), which have valve striae arranged in relation to a line and tend to appear bilaterally symmetrical; their silica-based skeletons do not readily decay and they can sometimes be detected even in heavily decomposed bodies. Diatoms are also used as biological indicators. They reproduce and respond rapidly to environmental change and provide early warnings of both pollution increases and habitat restoration success. It takes two to three weeks before changes are reflected to a measurable extent in the assemblage composition (Rotte 1991; Kelly et al. 1998). Diatoms were shown under appropriate conditions to produce up to 60% of their cellular mass as triacylglycerols (TAGs) under certain growth conditions. These TAGs can be easily converted into biodiesel through a transesterification reaction (Sheehan et al. 1998).

Materials and Methods :

Place of Work : This study was conducted in the laboratory of Department of Industrial Microbiology of Patna Women's College, Patna. All the water samples were collected from different water bodies of Bihar region during the month of July 2018.

Isolation and Characterisation of diatoms: Water samples from different water bodies with slime over it were collected in sterile bottle without any preservative. Diatoms were extracted from water samples, by the Nitric Acid Digestion method. The sample was digested by adding four drops of concentrated nitric acid and incubated overnight at room temperature. The samples were centrifuged at 4000 rpm for 10 minutes. The supernatant was decanted and replaced with distilled water. The sample was made transparent by vortexing it. This process of centrifuging and vortexing was repeated thrice to produce a pellet. The sediment were taken on slide and added one drop of formaline with glycerine and observed under compound microscope (Olympus CH-20i research binocular microscope) (magnification 10x). The suspected diatoms were identified and characterised on the basis of microscopic observations.

Culturing of Diatoms : For culturing of diatoms f/2 liquid Guillard media was prepared. (Guillard and Ryther 1962; Guillard 1975) 1000 ml of Guillrad media with the following components was taken. The trace elements (Sodium Dihydrogen Phosphate Heptahydrated, and Hydrated Sodium Silicate), main elements (Ethylene Diamine Tetraacetic Acid, Ferric Chloride, Mangenese Chloride, Zinc Suphate, Cobalt(II)Chloride, Copper Sulphate and Sodium Molybdate) and vitamins (Biotin, Cyanacobalamine and Thiamine HCl) were autoclaved separately and brought to the volume of 1L. Silica was autoclaved separately because it enhances precipitation when autoclaved. EDTA and $FeCl_3.6H_2O$ were added entirely and then other components were added.

All the three stock (main elements, trace element and vitamins) were autoclaved separately. Vitamins

were filter sterilized and kept in refrigerator and mixed the stock 1 and stock 2 aseptically. Then 10 ml of media in test tube was taken and 2ml of sample was added to see the growth of diatoms. After keeping the test tube for 5 days, the contents of the test tube was subjected to serial dilution upto 10^{-5} dilutions. (Vinayak et al, 2014) 5ml of serially diluted inoculants was aspetically added in flask containing 100ml media along with vitamins. It was treated with a single drop of Penicillin G (200 CH) to avoid bacterial contamination and incubated for 25 days provided with illuminator (CFL, Prompton, intensity 11 Watts at 25^{0}C). Each day the growth of the diatom were observed by preparation of slide under compound microscope.

Extraction and Analysis of Oil : After an incubation period of 25 days, spectrophotometric analysis was performed to detect the presence of oil in each samples at absorbance 630nm and 750nm (Sanjay et al. 2013). Then oil was extracted by Bligh and Dyer method by extracting lipids from homogenized cell suspension using 1:2 (v/v) Chloroform : Methanol (Bligh and Dyer,1959). Further analysis of oil was done via HPLC in which out of 7 samples only 3 selected samples (A, C and D) were analysed that were able to ooze more oil.

Results and Discussion :

Identification and Characterisation of Diatoms : In the present research work 14 diatom species under 9 genera were identified from 7 water bodies of Patna and Begusarai region of Bihar, India. (Figure 1) *Cymbella sp.* and *Fragilaria sp.* were collected from NIT Ghat riverside, Patna while *Navicula sp.* and *Synedra sp.* were collected from middle of NIT Ghat, Patna. From Ramlakhan Singh Talab, Anisabaad, Patna *Synedra sp., Hantzschia sp.* were isolated. *Melosira sp., Diploneis sp.* and *Cylindrotheca sp.* were detected in water sample of Raghopur Talab, Bansghat, Patna. Only one species of diatom i.e., *Nitzschia sp.* was isolated from Mangal Talab, Patna City, Patna and *Synedra sp.* from Gae Ghat, Patna. *Synedra sp.* was also detected in water sample of Gundak, Begusarai One more species like *Cylindrotheca sp.* was also present in this water sample. *Synedra sp.* was detected in water sample of middle of NIT Ghat, Patna as well as also in Ramlakhan Singh Talab, Anisabaad, Patna. *Synedra sp.* was found in three different water bodies like Ramlakhan Singh Talab, Anisabaad, Patna, Gae Ghat, Patna and Gundak, Begusarai. *Cylindrotheca sp.* was also present in water sample of two different region like Raghopur Talab, Bansghat, Patna and Gundak, Begusarai region.

Diatom taxa collected from different water bodies were observed under compound microscope which showed different features like *Cymbella sp.* had linear valves with broadly rounded ends, raphe thick folded with central pores prominent and terminal fissures thick and obliquely comma shaped. *Navicula sp.,* pinnate shaped, isopolar and had a prominent raphe and delicate rib-like structures. *Synedra sp.,* pinnate shaped, slightly bent with narrow and linear valves. Same characteristic of these diatoms were also identified (Sane et al. 2018). Frustules linear, loosely attached together to form short chains in girdle view. Valves were linear lanceolate with rounded ends. *Fragilaria sp.* were observed with frustules linear, loosely attached together to form short chains in girdle view. Valves were linear, lanceolate with rounded ends. *Hantzschia sp.* with broad, slender, linear Valves, Frustules short and

cylindrical shaped, spine like structure at the end was found in *Melosira sp., Nitzschia sp.* had linear to sigmoid type and striae along the other border. Similar features of these isolated diatoms were also observed (Rana and Bhandari, 2016). In *Diploneis sp.* valves were broadly elliptical. The axial area was occupied almost completely by a broad raphe sternum that encloses the raphe like similar features were also reported (Cleve, 1891). It was found in microscopic analysis that out of the 7 samples, 4 of them viz - samples A, B, C and D showed higher number of diatom species while the other 3 samples viz- E, F and G showed lesser number of diatom species.

Inoculation of Sample : After microscopic analysis, liquid media (f/2 Guillard media) was prepared to inoculate the samples in test tubes and flasks, to see the growth of the diatoms along with the oil production. 20ml of the sample was transferred into the tubes containing the media to check the growth of the diatoms culture and from this, 10ml of the sample was transferred to the flasks containing 100ml of the media, to incubate the cultures for 25 days to check the production of oil from each sample.

Extraction of Oil : Each day growth of the diatoms were monitored to check the growth along with slide preparation. After incubation period of 25 days spectrophotometric analysis was performed to detect the presence of oil in each samples at absorbance 630 and 750nm. (Table 1) (Sanjay et al. 2012). The dried biomass of the three samples were also estimated in which sample D showed the maximum amount of oil that is 0.008ml (Table 3). Further analysis of oil was performed via HPLC in which out of 7 samples only 3 selected samples (A, C and D) were analysed which oozes more oil. Triacylglycerols (TAGs) peaks were identified. (Figure 2) (Table 2(a), 2(b), 2(c)) Diatoms have been regarded as useful neutral lipid sources of liquid-fuel precursors (Renaud et al. 1994). Diatoms naturally oozing high lipid content would eliminate the expensive step in energy production of extracting oil out of the diatoms (Mercer and Armenta, 2011). They may prove to be key elements towards the goal of constructing biofuel generating diatom solar panels (Ramachandra et al. 2009).

Conclusion :

The marine diatoms hold promise as feedstock for biodiesel because of their ability to manufacture and store triacylglycerols (TAGs). These are the only renewable biofuels that can potentially relocate liquid fuels derived from gasoline. There is still more work that has to be done for extraction and characterization of lipids from diatoms for biodiesel production.

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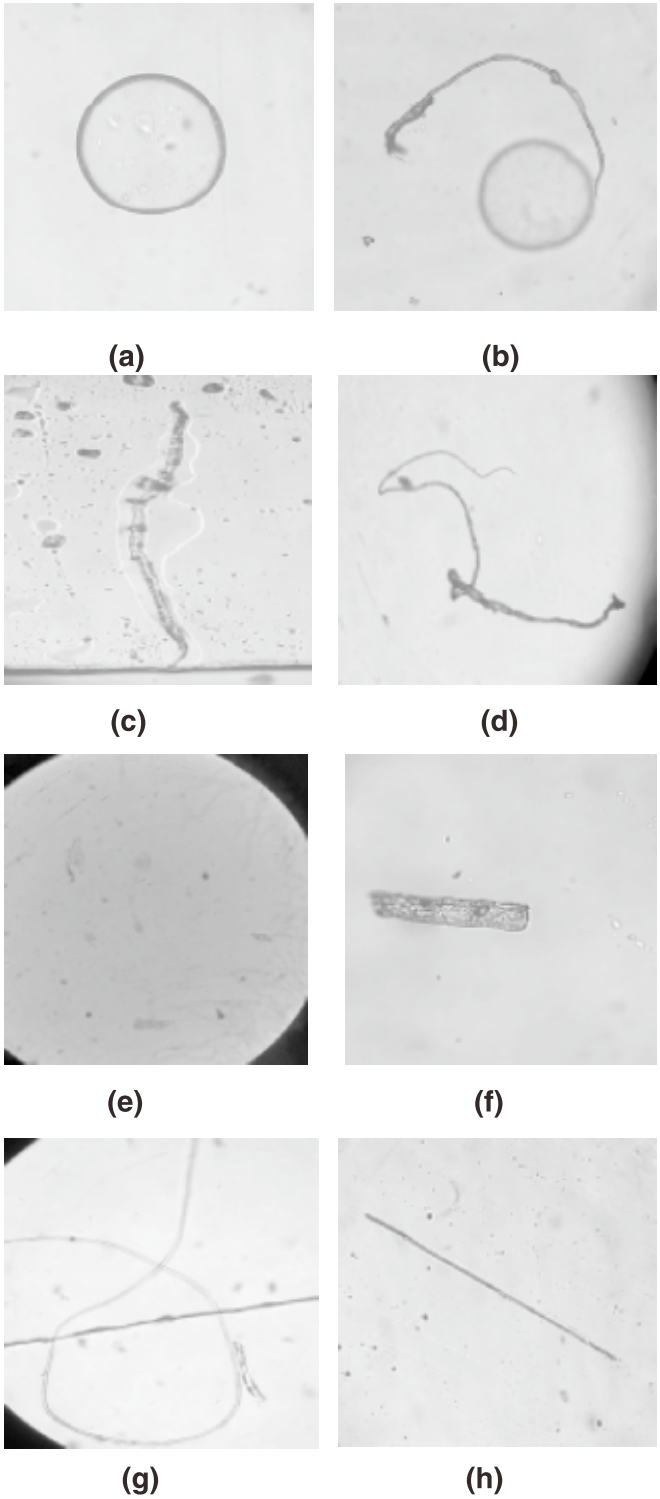

Fig. 1. Microscopic images of Diatoms isolated from different water samples

(a) Synedra sp. ; (b) Synedra sp. and Hantzschia sp. ; (c) Diploneis sp. ; (d) Nitzschia sp. (e) Cymbella sp. ; (f) Cylindrotheca sp. ; (g) Cylindrotheca sp. ; (h) Synedra sp.

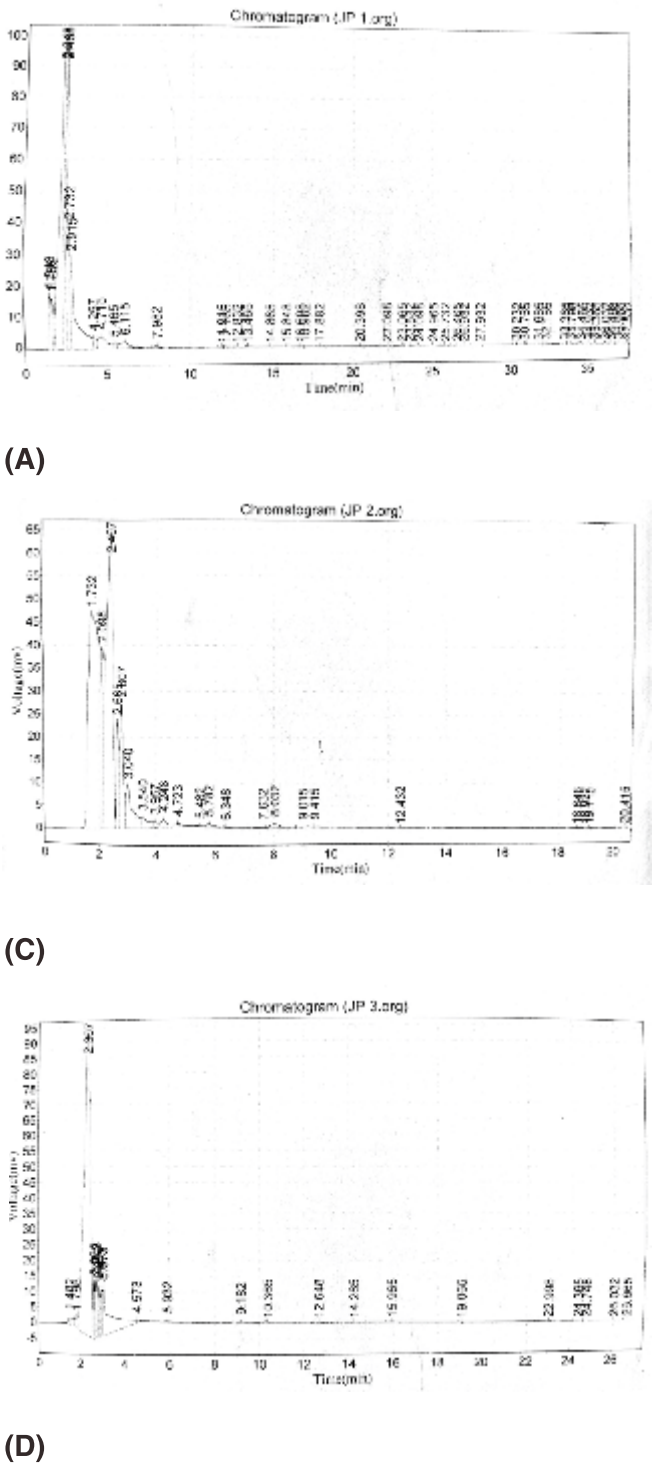

Fig. 2. Triacylglycerol (TAGs) peak identified by HPLC in different samples (A), (C) and (D)

Table 1. Absorbance for samples A to G by spectrophotometry

Sample	Absorbance at	
	630nm	750nm
A	0.266	0.241
B	0.077	0.081
C	0.124	0.121
D	0.109	0.110
E	0.077	0.086
F	0.084	0.088
G	0.065	0.076

Table 2(a) Components identified in Sample A by HPLC

Peak No.	Components	Retention time	Height	Area	Conc.
1	Ethylene glycol	2.732	38787.102	567363.313	12.5802
2	Caprylic acid	11.915	46.273	442.233	0.0150
3	Fucoxanthine	20.398	60.000	1510.500	0.0062
4	Palmetoic acid	22.098	19.000	431.200	0.0113
5	Linoleic acid	25.732	56.059	807.600	0.0114
6	Oleic acid	26.465	35.111	546.100	0.0168
7	Myristic acid	30.232	20.200	218.400	0.0082

Table 2(b) Components identified in Sample C by HPLC

Peak No.	Components	Retention Time	Height	Area	Conc.
1	Erythritol	3.540	2094.243	36421.660	0.9754
2	Myristic acid	18.648	39.000	503.000	0.0182
3	Fucoxanthin	20.415	108.516	1916.900	0.0505

Table 2(c) Components identified in Sample D by HPLC

Peak No.	Components	Retention Time	Height	Area	Conc.
1	Ethylene glycol	2.748	17707.908	99863.195	9.5882
2	Capric acid	9.182	16.692	258.700	0.0090
3	Palmitoleic acid	22.998	17.429	294.800	0.0094
4	Lauric acid	24.365	36.533	462.256	0.0198
5	Oleic acid	26.032	29.200	170.300	0.0158
6	Stearic acid	26.665	23.050	346.700	0.0125

Table 3. Lipid content of the samples A, C and D

Estimation of dried biomass

Sample	Amount of sample taken	Weight of plate without sample (g)	Weight of plate with sample (initial) (g)	Weight of dryed plate (final) (g)	Difference (initial - final) (ml)
A	4ml	27.78	62.18	57.25	0.005
C	4ml	29.12	68.26	60.78	0.006
D	4ml	28.55	64.17	58.38	0.008

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