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Isolation, Identification, Morphological Studies and Lipid Granules Staining (Nile red) of Different Micro-Algae for Biodiesel Production from Fresh Water and Saline Water

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Abstract

In the present study, the algal samples were collected from temple tanks, saline water in Chennai, Mahapaliburam and Kovallam. Collections were carried out during the month of September 2010 and February 2011. Samples were studied in the laboratory and identified. In their surveyed the population, identification, morphological, and examined the Nile red method with microalgae genus of various classes. Various physical and chemical treatments were applied to the existing Nile red method to improve the effectiveness and efficiency. The following algae were present *Chlorella vulgaris, Nannnochloropsis, Dunaliella tertiolecta, Tetraselmis suecica, Chlorococcum humicolo, Scenedesmus acuminatus, Amphora coffeaformis,* and *Nitzschia longissima*. Algae are described with photographs.

Key Words: Micro-algae, Biodiesel, Nile red, *Chlorella vulgaris, Nannnochloropsis, Dunaliella tertiolecta, Tetraselmis suecica, Chlorococcum humicolo, Scenedesmus acuminatus, Amphora coffeaformis, Nitzschia longissima*

Introduction

Algae are microscopically small, unicellular organisms, some of these form colonies and reach size visible to naked eye as minute green particules. The organisms are finely dispersed throughout the water and may cause considerable turbidity showing the maximum algal bloom. The biofuel production from photosynthetic algae is considered as a process to produce renewable energy for Global Warming mitigation. Biodiesel is defined as the monoalkyl esters of long chain fatty acids, an alternative for fossil fuel. The most common biodiesel constituent used today is fatty acid methyl esters. Biodiesel is non toxic completely biodegradable fuel with reduced sulfur emission. Today over exploitation of fossil fuel for automobiles and industries leads to petroleum demand in high rate. Micro algae appear to be the only source of biodiesel that has the potential to completely displace fossil fuel. Depending on the seasons the algae appears and disappears.

Materials and Methods

Study area

The algae samples were collected from both saline in Kovalam, Mahapaliburam, and Temple tank in Pragatheswara temple, Parthasarathi temple, Triplicane, Chennai, India. The colour of the sample and water recorded as green. There is always intense sunlight so algal growth is found to be abundant.

Random sampling method has been applied in the algal collection procedure. Collection were carried out during the month of September 2010 and February 2011.The different types of algal forms were collected from lentic environment only. The soil is productive and climates are best suited for different class of algae. An initial examination of the living samples, the coarser material was removed by filtration through a mesh net. The algal samples were preserved in 4% formalin (aqueous solution of formaldehyde). The microalgae were stained Nile red. Glycerine was used for mounting the material. The centric organisms hs been photographed using a OLYMPUS CH20i microscope with attached SONY camera.

Culture Identification

The algae culture was identified through the manual, "Microalgae Identification for Aquaculture", by Barry H. Rosen (1990).

Preparation of Nile Red Solution

The 0.5g of Nile Red powder was suspended in one ml of acetone it used as stock solution. From this 0.05ml was mixed with 50ml of glycerol mixture (75:25, Glycerol and water). This solution was directly used for staining the lipid bodies of algal cells.

Nile red staining

Nile red (9-(Diethylamino) -5H benzo [α] phenoxazin- 5-one) staining was conducted to detect intracellular lipid droplets (Greenspan *et al.* 1985). Microalgal cells (0.5 ml) were collected by centrifugation at 1,500 rpm (Rotation per minute) for 10 min and washed with physiological saline solution (0.5 ml) several times. After the collected cells were re-suspended in the same solution (0.5 ml), the Nile red solution was added to cell suspensions (1:100 v/v) and incubated for 10 min. After washing once, stained microalgal

cells were observed by fluorescent microscopy (Tadashi Matsunaga *et al.* 2009).

Results and Discussion

Chlorella vulgaris Beijering (Fig. 1, 1a)

Algae free living, cells usually solitary or in small colonies, spherical and with a thin cell membrane. Chloroplast parietal, cup shaped and with a pyrenoid which is sometimes indistinct. Cells usually 25µm.

Occurrence: Tamil Nadu [Mahendraperumal and Anand, 2008].

Collected from: Pragatheswara temple and Parthasarathy temple, Triplicane, Chennai, India.

Nannochloropsis salina D. J. Hibberd (Fig. 2, 2a)

It is small, very simple ultra structure, non motile spheres which do not express any distinct morphological features. It is different from other related microalgae in that it lacks chlorophyll b and c. It is a high concentration of a range pigments. Such as astaxanthin, zeaxanthin and canthaxanthin. Cells usually 2 micrometers. It has high levels of polyunsaturated fatty acids.

Occurrence: Germany [Toepet et al., 2005].

Collected from: Kovalam and Mahapaliburam.

Dunaliella tertiolecta Butcher (Fig. 3, 3a)

It is marine green flagellate, motile, unicellular, rod to ovoid shaped. It is simple to cultivate and do not clump or form chain. The cell size of 10-12µm.

Occurrence: Norway [Naustvoll 2001].

Collected from: Kovalam and Mahapaliburam.

Tetraselmis suecica (Kylin) Butcher (Fig. 4, 4a)

It is marine green algae. It grows as single, motile cells. Cells more or less compressed, often slightly curved but never twisted, cells cordiform, elliptic or almost spherical. It is very high lipid level. Cells usually 35µm long × 14µm wide.

Occurrence: Sweden [Kylin 1949].

Collected from: Kovalam and Mahapaliburam.

Chlorococcum humicolo, (Naegeli) Rabenhorst (Fig. 5, 5a)

Cells spherical, solitary or number of cells crowded together to form a stratum. Chloroplast a hallow sphere with natural notch and a single pyrenoid. Cells 40 µm.

Occurrence: c. f. Tamil Nadu, [Anand, 1998].

Collected from: Pragatheswara temple and Parthasarathy temple, Triplicane, Chennai, India.

Scenedesmus acuminatus (Lagerhheim) Chodat (Fig. 6, 6a)

Colonies curved and of four to eight (usually four) fusiform cells with sharp pointed ends. All the cells in a colony lunate or interior cells forming a flat plate and the other cells lunate and at an angle to the plane of the interior cells; rarely, all the cells in the same plane. Cell wall smooth and without teeth or spines. Cells 25 µm, breadth 15 µm.

Occurrence: Assam [Biswas, 1934].

Collected from: Pragatheswara temple and Parthasarathy temple, Triplicane, Chennai, India.

Amphora coffeaformis Agardh (Fig. 7, 7a)

Frusules in gridle view elliptic lanceolate, truncate. Valves arcuate on the dorsal margin and straight or slightly concave on the ventral margin. End of the valves slightly protected and capitates. Straight delicate. Cells long 40 µm, breadth 20 µm.

Occurrence: Tamil Nadu [Venkataraman, 1939].

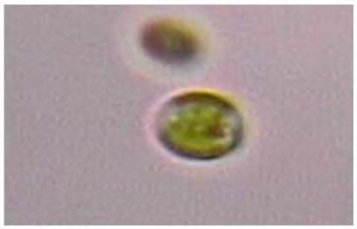
Collected from: Pragatheswara temple and Parthasarathy temple, Triplicane, Chennai, India.

Nitzschia longissima (Brebisson in kutzing) Ralfs (Fig. 8, 8a)

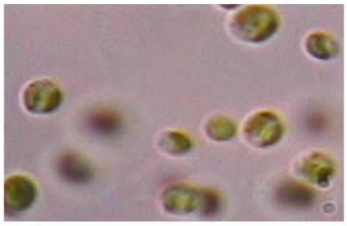
It is a common pinnate marine diatom. It found mostly in colder waters even at temperature between -4 and -6 degrees. Sometimes also extremophiles by dent of tolerance to high salinity. It is the mating system is heterothallic, experimental reduction of the cell size is possible by surgically cutting the cell endings with a razor blade. Cellus usually long 60 µm, breadth 12 µm.

Occurrence: North America [1861].

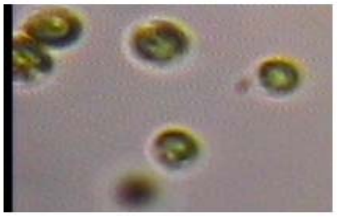
Collected from: Kovalam and Mahapaliburam.



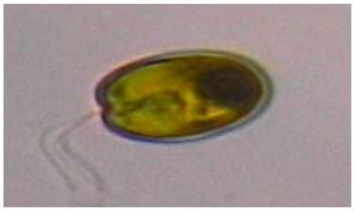
(Fig. 1) *Chlorella vulgaris* (Beijering) 25 µm



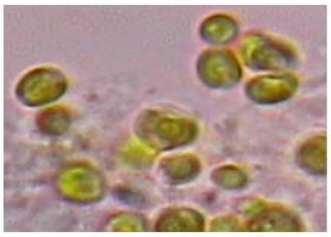
(Fig. 2) *Nannochloropsis salina* (Hibberd) 45 µm



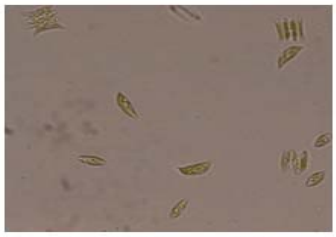
(Fig. 3) *Dunaliella tertiolecta* (Butcher) 12 µm



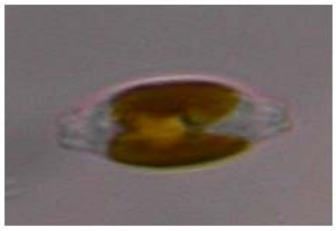
(Fig. 4) *Tetra selmis suecica* (Kylin) 35 µm



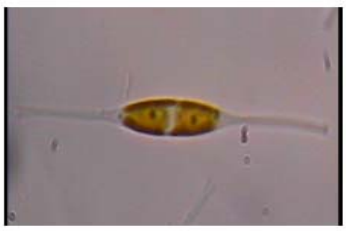
(Fig. 5) *Chlorococcum humicola* (Näegeli) Rabenhorst 40 µm



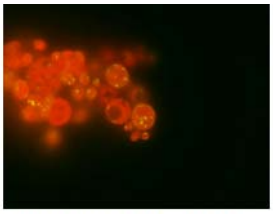
(Fig. 6) *Scenedesmus acuminatus* (Lagerheim) Chodat length 25 µm, Breadth 15 µm



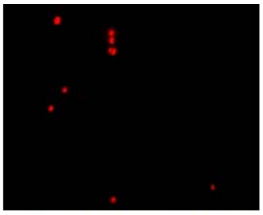
(Fig. 7) *Amphora coffeaformis* Agardh length 40 µm, breadth 20 µm



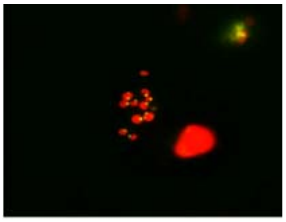
(Fig. 8) *Nitzshia longissima* long 60 um, breadth 12 um



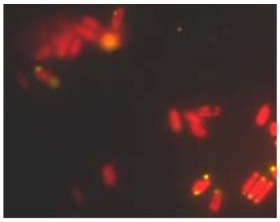
(Fig. 1a) Nile red staining of *Chlorella vulgaris*



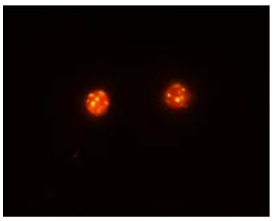
(Fig. 2a) Nile red staining of *Nannochloropsis salina*



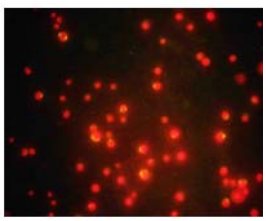
(Fig. 5a) Nile red staining of *Chlorococcum humicolo*



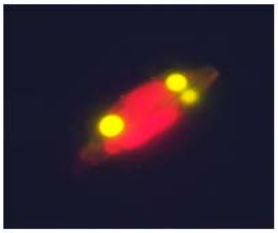
(Fig. 6a) Nile red staining of *Scenedesmus acuminatus*



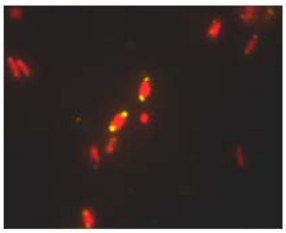
(Fig. 3a) Nile red staining of *Dunaliella tertiolecta*



(Fig. 4a) Nile red staining of *Tetraselmis suecica*



(Fig. 7a) Nile red staining of *Amphora coffeaformis*



(Fig. 8a) Nile red staining of *Nitzschia longissima*

Table: 1 List of fresh water and Saline water algal taxa recorded from temple tanks, saline water in Chennai, Mahapaliburam and Kovallam from September 2010 and February 2011.

S.NO	Name of the the species	Pragatheswara Temple		Parthasarathy Temple		Mahapaliburam		Kovallam	
		2010	2011	2010	2011	2010	2011	2010	2011
1.	*Chlorella vulgaris* Beijering	+	+	+	+	+	+	+	+
2.	*Nannnochloropsis salina* D. J. Hibberd	+	+	+	+	+	+	+	+
3.	*Dunaliella tertiolecta* Butcher	+	-	-	-	+	+	+	-
4.	*Tetraselmis suecica* (Kylin) Butcher	-	-	-	-	+	+	+	+
5.	*Chlorococcum humicolo*, (Naegeli) Rabenhorst	+	+	+	+	+	+	+	+
6.	*Scenedesmus acuminatus* (Lagerhheim) Chodat	+	+	+	+	+	+	+	+
7.	*Amphora coffeaformis* Agardh	-	-	-	-	+	+	+	+
8.	*Nitzschia longissima* (Brebisson in kutzing) Ralfs	-	-	-	-	+	+	+	+

(+) = Present, (-) = Absent; Maximum species occurrence of *Chlorella vulgaris, Nannochloropsis salina, Chlorococcum humicola, Scenedesmus acuminatus.*

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