Ultra Structural Studies of Macroalgae Collected from Coromandal Coast, India for Biofuel Production

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Abstract

Seven species of marine macro algae belonging to Chlorophyta, Phaeophyta and Rhodophyta were surveyed, collected from Coromandal coast, India and studied for their lipid composition and its accumulation for the future biodiesel production. Variability of chemical components and production of lipid granules are specific in macro algae and there is also an evidence for temporal variability in macro algal lipid composition. The lipids of algae have wide application in production of fuel. It was observed that lipid composition of macro algae in the sequence of members belonged to Phaeophyta>Chlorophyta>Rhodophyta. In this study we successfully localized the lipid bodies of seven macro algal strain by their ultra structural studies and Nile blue stain method. However, further research work should be carried out from different marine macro algae species for biofuel production to meet out the energy crisis globally in future.

1. Introduction

In Marine ecosystem, Macroalage are ecologically and biologically important which provide medical constituents, nutrition, reproduction and an accommodating environment for other living organisms. Because of these Properties they are most important organisms maintaining the ecosystem stability. Macroalgal Polysaccharides are used in the food, cosmetics, paints, crop, textile, paper, rubber, and building industries. In addition, they are used in energy sector, without any ecological disturbance and pollution.

Macro-algae are extensively grown and used as food in Asiatic Countries, or as source of chemicals. They are usually collected from natural water basins where they are seasonally available. Only recently they have been considered for energy (fuel) production, and the potential of some Pacific Ocean strains has been preliminarily studied (Gao et al., 1994). Variability in Chemical Components and production of lipid granules are specific in Macroalgae, there is also evidence for temporal variability in algal lipid composition.

Lipid levels in some Macroalgae increase in winter and decrease in summer (Rodriguez-Montesinos et al., 1991). The Lipids of algae have wide application in production of fuel in many industries. Looking in to the significance, the present work has been carried out to study the ultra structure of seven Macroalgae belonging to Chlorophyta, Phaeophyta and Rhodophyta, from Coromandal Coast, East coast of India for their Lipid accumulation and their significance pertaining to the Biodiesel Production.

2. Materials and Methods

2.1. Study site and macro algae sample collection

The experiment was carried out in the research lab of Department of Plant Biology and Biotechnology, Presidency College (Autonomous), Chennai, India. Seven different Algae were collected from the village Vadakadu, Rameshwaram Taluk, Ramanathapuram, Tamil Nadu, India. It is located at 9.28°N 79.3°E. It has an average elevation of 10 m (32 ft).

2.2. Anatomical studies of Algae

The Collected Macroalgae was fixed in FAA (Formalin-5 ml + Acetic acid-5 ml + 70% Ethanol-90 ml) for two hours. The materials were washed in distilled water and dehydrated through graded series of Tertiary butyl alcohol. Following dehydration, the materials were infiltrated with paraffin wax controlled temperature (55°C). After infiltration, the specimens were cast into paraffin blocks and the blocks were stored in refrigerator for sectioning. Serial sections to the thickness of 6-8 µm were prepared with the help of Rotary Microtome. The sections were dewaxed and stained with 0.05% Toluidine blue O (O'Brien et al., 1964) (dissolved in water) for general

anatomical studies. Since it is a Meta chromatic dye, it gave good results for studying gross anatomical features of the inner parts.

2.3. Nile Blue Staining

For localization of the Lipid Bodies in the Algae, sections were stained with Nile Blue Stain. The sample or frozen sections are fixated in formaldehyde, then immersed for 20 minutes in the Nile blue solution and rinsed with water. For better differentiation, it is dipped in 1% acetic acid for 10-20 minutes until the colors are pure. This might take only 1-2 minutes and then the sample is thoroughly rinsed in water (for one to two hours). Afterwards, the stained specimen is taken on a microscope slide and excess water is removed. The sample can be embedded in glycerol or glycerol gelatin. Both external and microtome sections were photographed with NIKON Coolpix-8400 Digital camera and NIKON Labphoto-2 microscopes. Magnifications of the micrographs are shown by the scale-bars.

3. Results and Discussion

In our present study, seven species of marine macro algae belonging to Chlorophyta, Phaeophyta and Rhodophyta were surveyed (Table 1), collected from coromandal coast, peninsular India and studied for their lipid composition and its accumulation for the future biofuel production.

3.1. Gelidiella acerosa

3.1.1. Structure of the plant body

The plant is roughly circular in outline measuring about 850µm thick. It consist of an epidermal layer of radially oblong, thick walled cells, followed by a wide cortical zone circular, compact thick walled cells and a central core of medulla of fairly large, thick walled polygonal cells.

3.1.2. Sections stained with Nile-Blue

All tissues exhibit light violet or purple color. Some dark spherical bodies, one each cell, are seen in the cells of the medulla. The cell wall of the cortical zone stain darker than the medullary cells (Figure 1.1 & 1. 2)

3.1.3. Sections stained with -Toludine blue O

The epidermis and cortical tissues stain dark purple. The medullary cells also stain deep. The cell walls appear thin.

Table 1: List of different species of Macroalgae analyzed in the study

Group	Name of Species
Gelidiella acerosa	Rhodophyta
Gelidium spp	Rhodophyta
Kapphaphycus alvarezii	Rhodophyta
Padina pavonica	Phaeophyta
Sargassum ilicifolium	Phaeophyta
Turbinaria ornata	Phaeophyta
Halimeda spp	Chlorophyta

No spherical bodies are evident in the cells of the medulla and cortex (Figure 2.1 & 2.2).

3.2. Padina pavonica

3.2.1. Structure of the plant body

The plant body is a flat thin thallus with two rows of cells. The marginal part of the thallus is folded abaxially and the extreme margin is slightly thinner than the middle part (Figure 3.1 & 3.2). The two rows of the cells are vertically elongated and thick walled, they are compact.

3.2.2. Sections stained with Nile Blue

The cell walls appear dark blue and the cytoplasm is purple. Some Lipid bodies in the cells are seen dark (Figure 3.1 & 3.2).

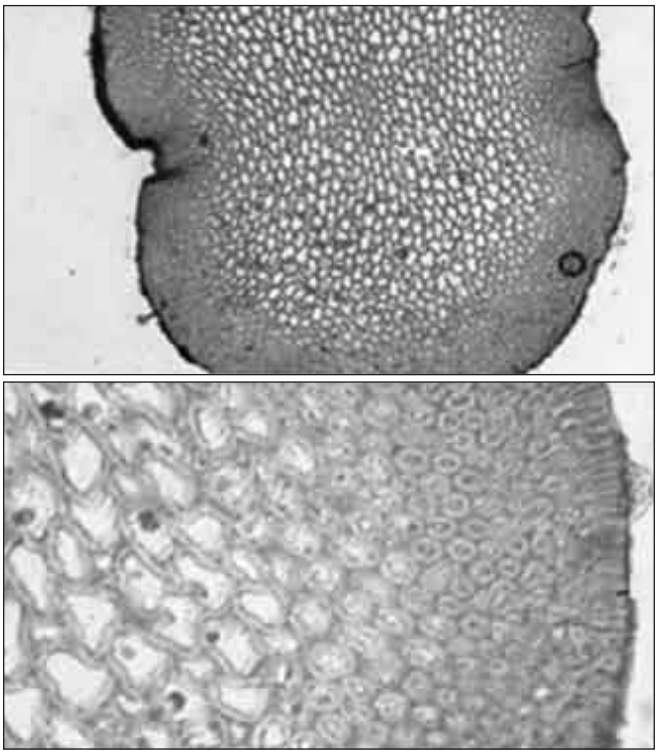

Figure 1.1 & 1.2: Nile Blue Stain of *Gelidiella acerosa*

3.2.3. Sections stained with Toludine Blue O

The cell walls are stained purple; the walls appear thinner. Dark green amorphous bodies are seen in some of the cells of both upper and lower layers (Figure 4.1 & 4.2).

3.3. Sargassum ilicifolium

3.3.1. Structure of the plant body

The plant body consist of flat leaf like lateral appendages central thick stipe. The stipe or stem has an epidermal layer of squarish cells, wide, thin walled, less compact cortical tissues and central core of thick walled angular medulla.

3.3.2. Sections stained with Nile Blue

Epidermal cells stain dark. Cortical cells and medullary cells stain purple. Darkly stained Lipid bodies are seen in the cells

of the medulla (Figure 5.1 & 5.2).

3.3.3. Sections stained with Toludine Blue O

The epidermal cells stain dark and the palisade (cortical) cells and the medulla stain dark violet. The spores stain dark (Figure 6.1 & 6.2).

3.4. Gelidium spp.

3.4.1. Structure of the plant body

The plant body consists of thin central axis which bears small lateral leaf like appendages. The lateral appendages are flat with single layer of epidermis and central medullary, four or five layers of large, parenchyma cells.

3.4.2. Nile Blue staining

The sections stained with Nile Blue show dark purple of all cells. Some minute granular bodies are wider in medullary cells (Figure 7.1 & 7.2).

3.4.3. Toludine Blue O

All the cells are uniformly purple or crimson violet. The cells inclusions are not stained (Figure 8.1 & 8.2).

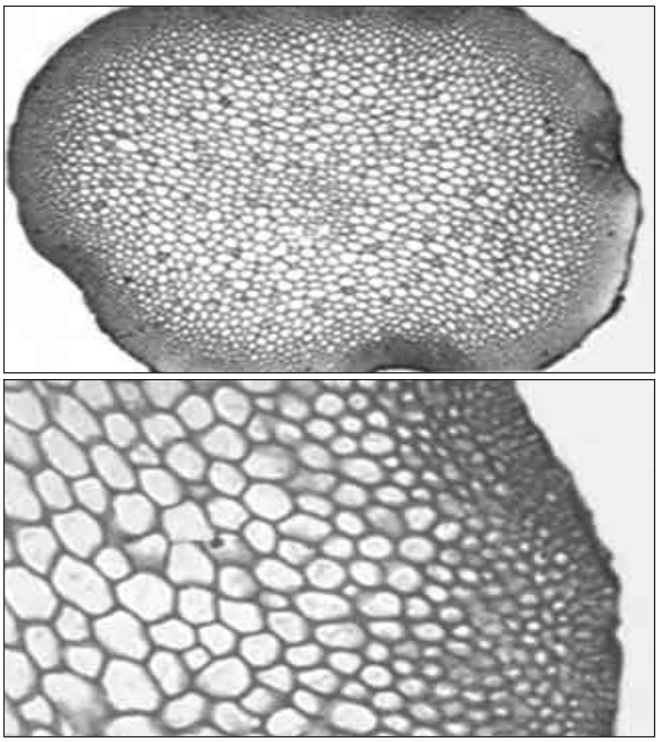

Figure 2.1 & 2.2: Toluidine-Blue O Stain of *Gelidiella acerosa*

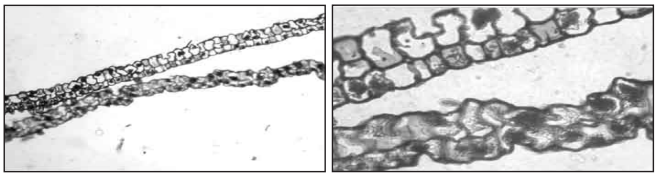

Figure 3.1 & 3.2: Nile Blue Stain of *Padina pavonica*

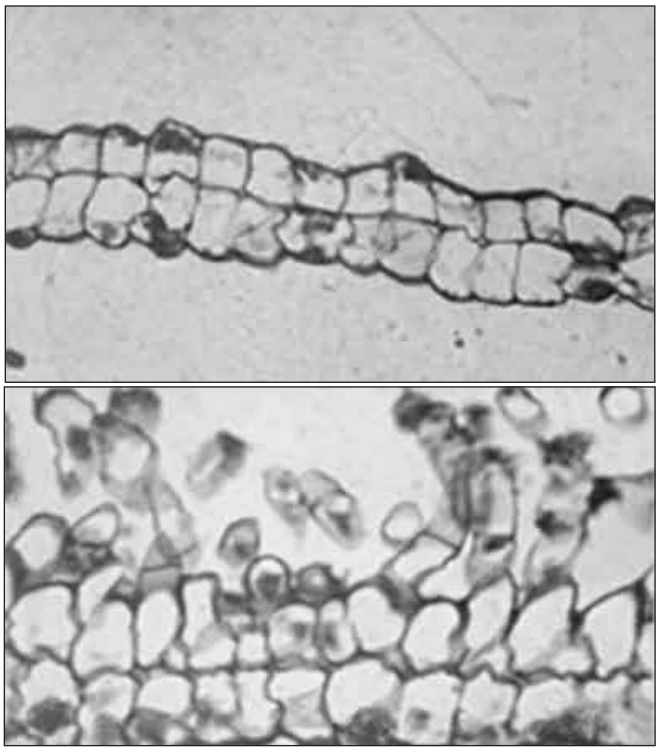

Figure 4.1 & 4.2: Toluidine-Blue O Stain of *Padina pavonia*

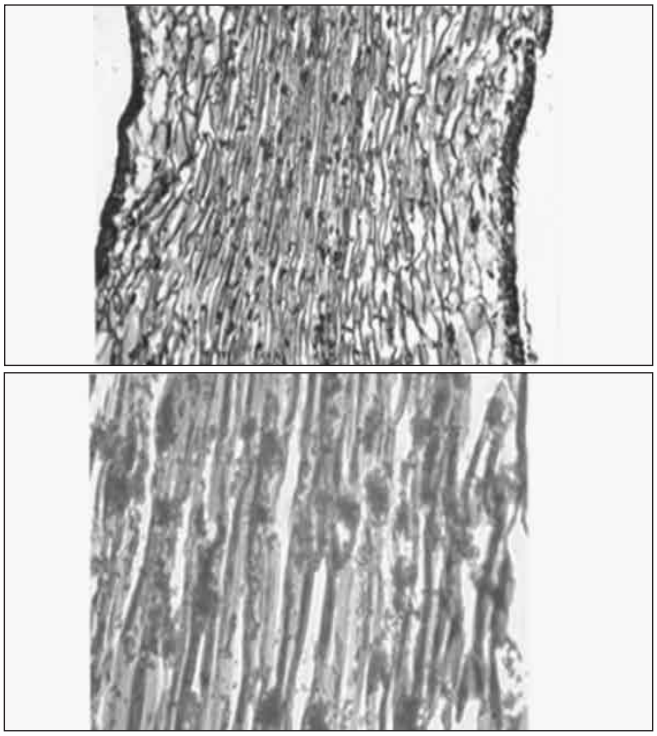

Figure 5.1 & 5.2: Nile Blue Stain of *Sargassum ilicifolium*

3.5. Halimeda Spp.

3.5.1. Structure of the plant body

The plant body comprises flat thick discoid part which is linked with each other by thin thread like structure. The thallus

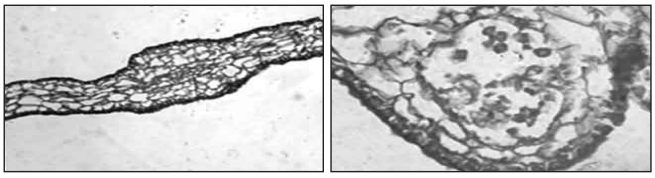

Figure 6.1 & 6.2: Toluidine-Blue O Stain *Sargassum ilicifolium*

consist of upper and lower epidermal layers; the mesophyll in between the epidermal layer is seen non cellular, granular, compact and many thin trabeculae arising from the epidermis and proliferating in the interior.

3.5.2 Nile Blue stain

The sections stained with NileBlue shows the epidermal cells and trabiculae which stained in dark and light purple respectively. Within the Trabiculae dense accumulation of lipid granules are seen like dark particles.(Figure 9.1 & 9.2)

3.5.3. Toludine Blue O

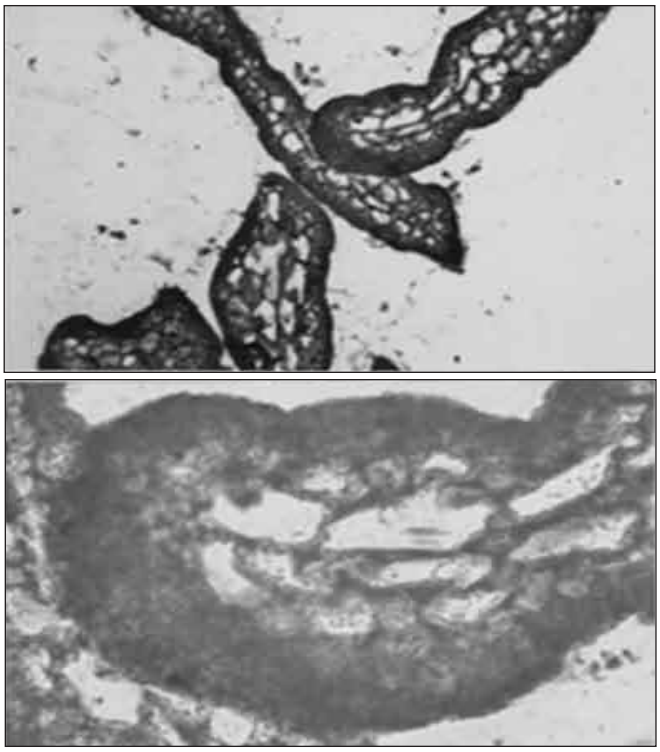

Figure 7.1 & 7.2: Nile Blue Stain of *Gelidium spp*

It shows fairly dense accumulation of dark, large spherical bodies within the trabiculae (Figure 10.1 & 10. 2). The cytoplasm and the cell walls are dark violet.

3.6. Turbinaria ornata

3.6.1. Structure of the plant body

The plant body is solid cylinder and appears lobed in transactional view. The epidermis is thin with small squarish cells. The cortical zone comprises fairly large, angular, thin wall compact cells. Medulla is wide and includes small, slightly thick walled compact cells.

3.6.2. Nile Blue Stain

Nile Blue stains cortex and medulla dark purple. The cell wall appears thick. Within the medullary cells are seen some granular or crystalline bodies (Figure 11.1 & 11.2).

3.6.3. Toludine Blue O

In Toludine blue staining, the epidermis appears dark. The cortex and medulla appears bright purple. The cells inclusions are not visible (Figure 12.1 & 12.2).

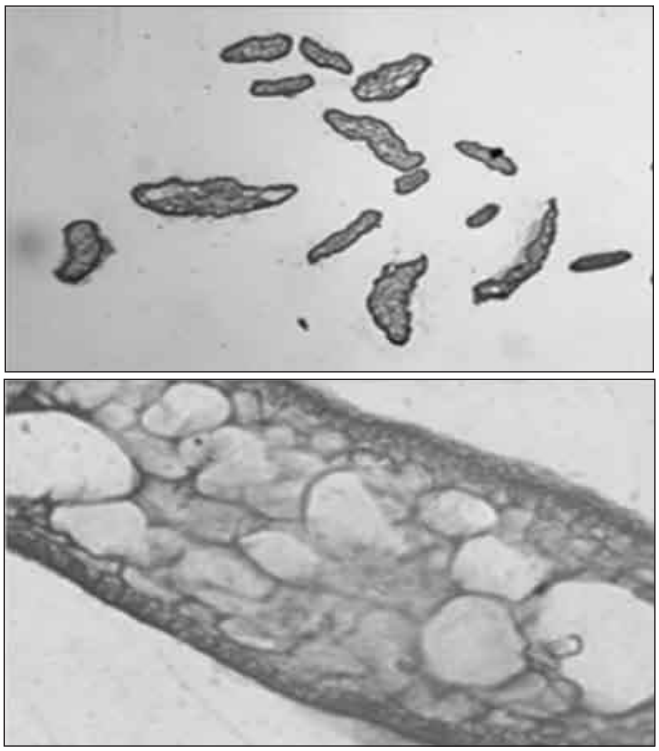

Figure 8.1 & 8.2: Toluidine-Blue O Stain of *Gelidium spp*

3.7. Kappaphycus alvarezii

3.7.1 Structure of the plant body

The thallus consists of a thick epidermal layer of radially elongated narrow cells and 2 or 3 layers of cylindrical palisade cells. The medulla consists of compact cells with thick wavy walls. The epidermis and the medullary cells stain dark purple with Nile Blue. A large quantity of mucilage seems to have formed in the cells which also stains dark purple.

3.7.2 Nile Blue Staining

Nile blue stains the epidermal cells as well as palisade cells. The cells of the medulla do not stain. Minute dark granular particles are seen in Medullary cells (Figure 13.1 & 13.2).

3.7.3 Toludine Blue O Staining

It shows uniform staining of purple color of all tissues (Figure 14.1 & 14.2). Variation of chemical components and production of lipid granules are specific in macro algae and there is also an

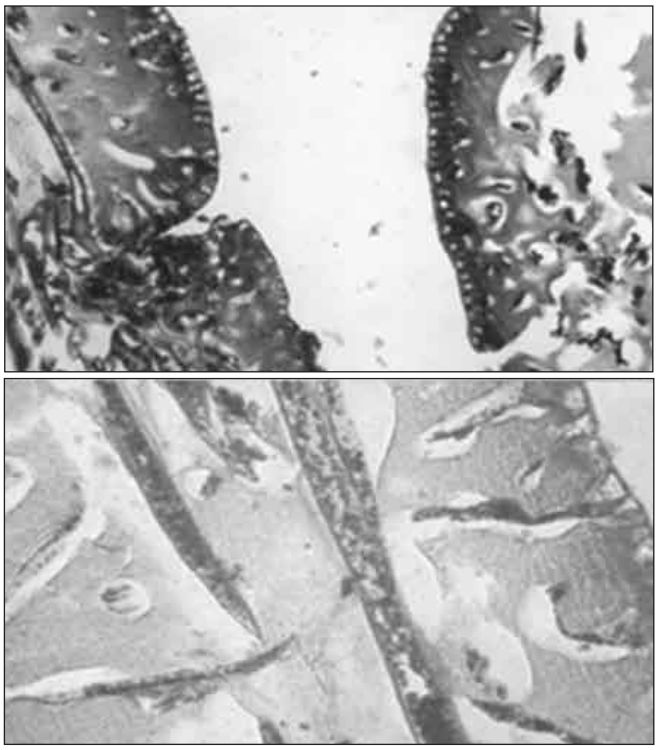

Figure 9.1 & 9.2: Nile Blue Stain of *Halimeda spp*

Figure 10.1 & 10.2: Toluidine-Blue O Stain of *Halimeda spp*

evidence for temporal variability in macro algal lipid composition. Sea weeds have been used since ancient times as food, fodder, fertilizer and as source of medicine today. Sea weeds

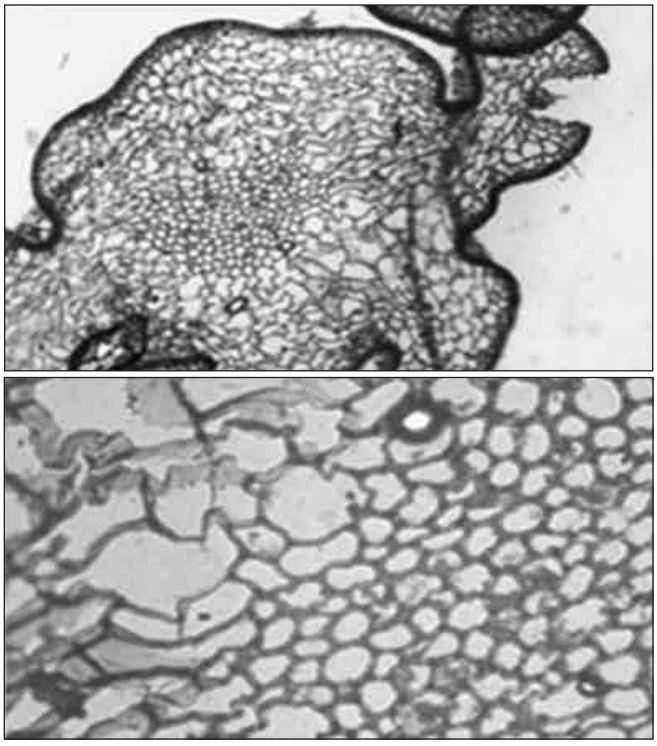

Figure 11.1 & 11.2: Nile Blue Stain of *Turbinaria ornata*

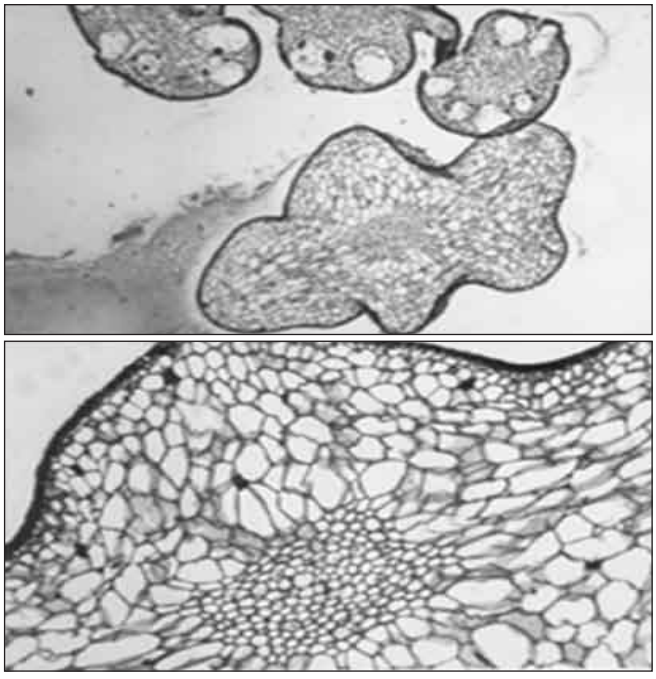

Figure 12.1 & 12.2: Toluidine-Blue O Stain *Turbinara ornata*

are the raw material for many Industrial productions like agar, algin and carrageenan but they continue to be widely consumed as food in Asian countries. (Manivannan et al., 2008).

Parekh et al., 1977 studied the chemical composition of 27 species of green seaweeds of Saurashtra coast, India. In general, sea weeds exhibit low lipid contents (Dave and

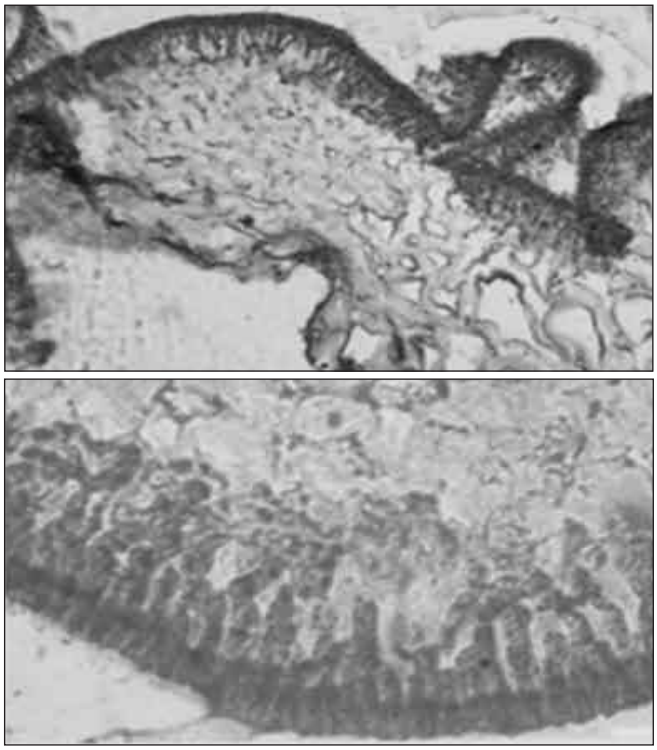

Figure 13.1 & 13.2: Nile Blue Stain of *Kapphaphycus alvarezii*

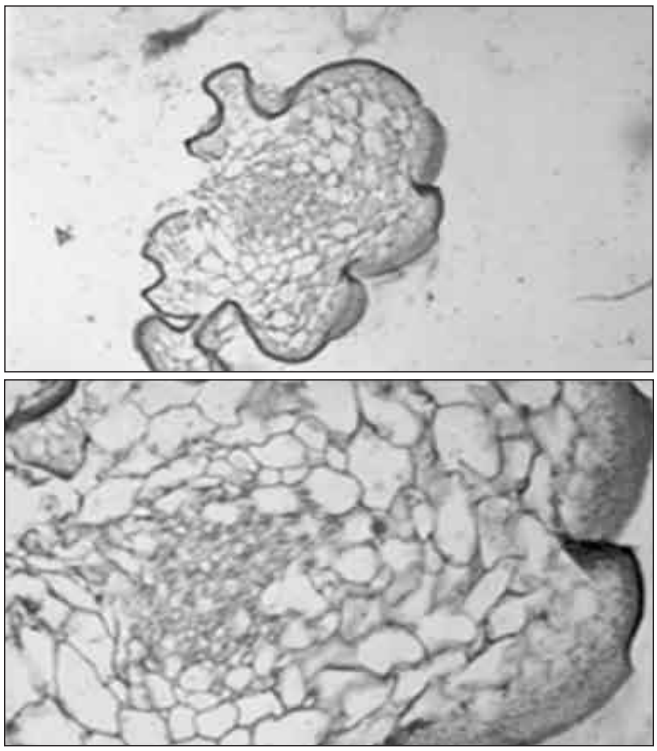

Figure 14.1 & 14.2: Toluidine-Blue O Stain of *Kapphaphycus*

Parekh, 1975). Seasonal lipid composition in macro algae of the Northeastern Pacific Ocean were studied by Nelson et al. (2002) and they reported that temporal variability in macro algal lipid composition. In our present study, out of seven macro algal species, the *Padina pavonica, Turbinaria ornata* and *Sargassum ilicifolium* showed the largest lipid storage in the cortex region of the macro algal body during unfavorable season. The least lipid granules recorded the *Gelidiella acerosa* and *Gelidium* spp whereas *Halimeda spp* shows the moderate accumulation of Lipid granules. In India, coromandal coast these are the major macro algal flora normally we recorded. The storage lipids that accumulate in macroalgae can be used for the biofuel production purpose and employment generation in a larger scale.

4. Conclusion

In conclusion, all these previous research and our present study suggests that the use of these species of macro algae in the coromandal coast India, may prove to be a very effective way of biofuel, glycerin and other essential nutrients, mineral production and creating large scale rural employment.

5. Acknowledgement

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