GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

www.scienceandnature.org

ISOLATION AND PURIFICATION OF FILAMENTOUS ALGAE FROM NATURAL HABITAT FOR BIODISEL PRODUCTION

*R.A. Thakre, G.G. Shirnalli & M. Ashwini
College of Agriculture, Department of Agril. Microbiology, University of Agricultural Sciences, Dharwad (K. A.)
*Corresponding author email: riteshthakre23@gmail.com

ABSTRACT
Aim of the present study was collection, isolation and purification of native filamentous algal species and optimization of their growth conditions. During the present study, total 26 isolates were collected from 13 different locations of Dharwad district. Including river, ponds, Paddy field and damp soil places from the collected 26 samples 8 different algal samples are isolated and purified and grown on nitrogen based and nitrogen free media. It was observed that sample FA-7 grown on both nitrogen free and nitrogen based media remaining 7 grown on only nitrogen based media.

KEYWORDS: Isolation, purification, Filamentous, Isolate, Paddy, Field.

INTRODUCTION

With growing concerns surrounding the continued use of fossil fuels, renewable biofuels have received a large amount of recent attention. In addition to wastewater treatment applications, algae are also a potential source of feedstock for biofuels production. Biofuels produced using oil crops and waste oils cannot meet the existing demand for fuel, and algae appear to be a more promising feedstock option (Chisti, 2007, 2008). Algae could provide substantially more biodiesel than existing oilseed crops while using far less water and land (Sheehan *et al*., 1998). In addition to biodiesel, algal sludge may also be fed to an anaerobic digester for methane production (Golueke *et al*., 1957). Residual biomass from these processes can also be used as a fertilizer, soil amendment, or feed for fish or livestock (Roeselers *et al*., 2007). However, algal biofuels production has been handicapped by an inability to find a reliable and cost effective method of harvesting and processing the algae feedstock (Molina Grima *et al*., 2003). Hence the use of filamentous Algae is the reliable source for the biodiesel production due to easy , most convenient and less time consuming biomass harvesting due to filamentous nature.

MATERIALS &METHODS

Collection of water and soil sediment samples from various locations

Much excellent advice on the collection of algae is given by Lund (1961). Samples were collected from 13 different locations of Dharwad district. Including river, ponds, Paddy field and damp soil places. Samples were collected in glass jars, Plastic bags and bottles.

Sample Washing

All samples are washed by autoclaved double distilled water to prevent the bacterial growth in enrichment media and to remove other impurities.

Enrichment cultures

These are prepared by (Pringsheim, 1946) putting collected material into culture vessels together with a similar or greater volume of a suitable medium, depending upon the richness of the original sample. To check the maximum growth efficiency every and each algal culture was inoculated in Nitrogen based and nitrogen free medium .The algae are then allowed to grow under favorable conditions for one and half month.

Isolation and purification

This is a method whereby individual cells are picked up, washed, and inoculated into medium. The process can be carried out using an ordinary microscope with a x10 or lower-powered objective. Dry sterile slides stored in Petri dishes or foil is used, together with sterile needle. A drop of the alga-containing liquid is placed upon slide for examination under the microscope and is searched for single cell.

RESULTS & DISCUSSION

Collection of water and soil sediment samples from various locations

During the present study, total 26 samples (Table 1) were collected from 13 different locations of Dharwad district. Including river, ponds, Paddy field and damp soil places (Plate1). Samples were collected in glass jars, Plastic bags and bottles and then transferred to various nutrient media standardized during the present work using flasks, jars and plastic bottles.

At the time of sample collection GPS reading was taken through GPS machine and each sample is labeled like FA-1 (Filamentous alage-1) and so on.

Sample Washing

Clear and good sample free from turbidity and other contamination after 3 to 4 wash of double distilled water are obtained. These clear samples are used further for the isolation process.

Enrichment cultures

After the 15 days of inoculation of Algae culture, into enrichment media, 8 out of 26 sample were shown clear and filamentous type of growth with more biomass are FA-1, FA-2, FA-4, FA-7(N+), FA-7 (N-), FA11 and FA-

13.Out of 8 only 1, sample no. FA-7 shown growth on both nitrogen free and nitrogen based media.

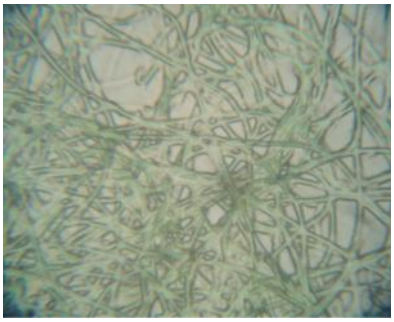
Isolation and purification

There are 8 out of 26 sample were isolate and purified successfully by capturing single cell under microscopic examination (Plate 2). FA-1, FA-2, FA-4, FA-7(N+), FA-7 (N-), FA-8, FA11 and FA-13 are the nomenclature given to isolated and purified Algae.

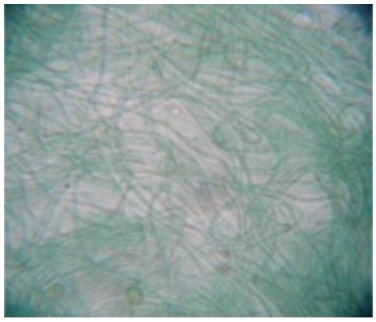
TABLE 1: List of collected water samples for isolation of filamentous algae from different location

Sample No.	GPS reading	Source	Isolate No.
1	N-15^{0}29.928' E-74^{0}59.137' Ele-683m	Hallikeri	FA-1
	----do-----		FA-2
	----do-----		FA-3
	----do-----		FA-4
2	N-15^{0}18.263' E-74^{0}43.905' Ele-510m	Halliyal	FA-5
3	15^{0}18.407' E74^{0}42.949' Ele-499m	Ajargaon	FA-6
	----do-----		FA-7
	----do-----		Other than filamentous algae
4	N-15^{0}18.458' E-74^{0}41.740' Ele-500m	Dangera	FA-8
	----do-----		FA-9
5	N-15^{0}14.251' E-74037.521' Ele-460m	Dandeli	FA-10
	….. do-----		FA-11
	----do-----		FA-12
6	N-15^{0}14.101' E-74^{0}37.451' Ele-459m	Ambikanagar	FA-13
7	N-15^{0}12.295' E-74^{0}34.067' Ele-567m	Pradanikere	FA-14
	----do-----		FA-15
	----do-----		Other than filamentous algae
8	N-15^{0}10.498' E-74^{0}32.971' Ele-548m	Joda(Nala)	FA-16
	----do-----		FA-17
9	N-15^{0}00.027' E-74^{0}30.718' Ele-577m	Joda	FA-18
	---do-----		FA-19
10	N-15^{0}00.889' E-74^{0}30.060' Ele-595m	Kogile cross	FA-20
11	N-15^{0}01.459' E-74^{0}29.586' Ele-567m	Kogilecross (Nala)	FA-21
	----do-----		FA-22
12	N-15^{0}00.060' E-74^{0}30.751' Ele-573m	Ulavi	FA-23
	----do-----		FA-24
13	N-15^{0}00.295' E-74^{0}30.588' Ele-593m	Sivapur	FA-25
	----do-----		FA-26
	----do-----		Chlorella

PLATE 1: Collection of water and soil sediment samples from various locations

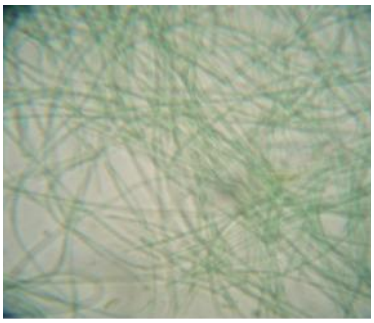


1N+



2N+

8N+



4N+

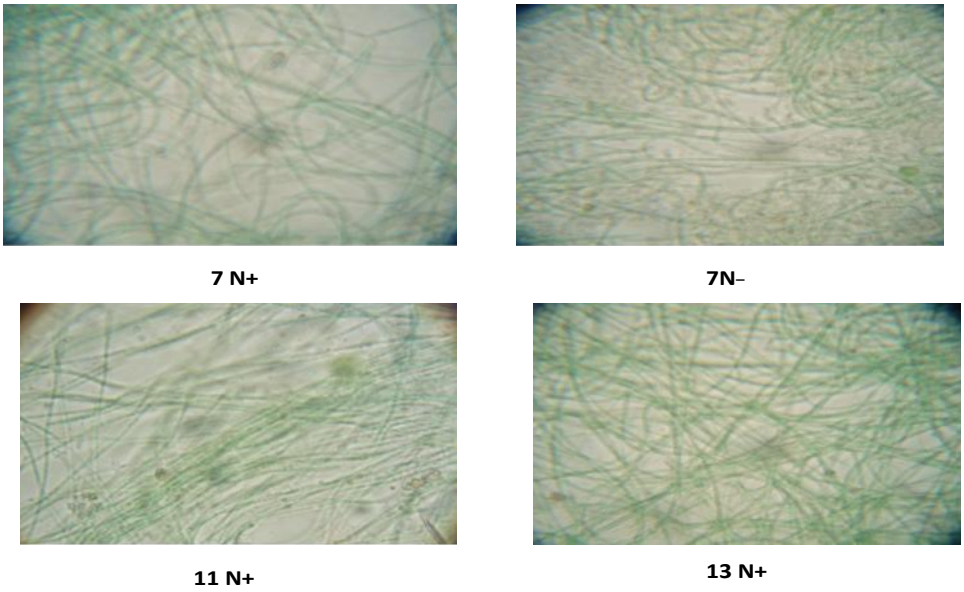


PLATE 2: Filamentous algae isolated for feasibility for biodiesel production

REFERENCES

Chisti, Y. (2007) Biodiesel from microalgae. *Biotechnol Adv*.,**25**: 294-306.

Chisti, Y. (2008) Biodiesel from microalgae beats. *Trends Biotechnol*.,**26**: 126-131.

Golueke, C.G., Oswald, W.J.,and Gotaas, H.B. (1957) Anaerobic digestion of algae. Appl Microbiol ., **5**: 47-55.

Molina, G.E., Belarbi. E.H., Acién, F.G., Robles, M.A. and Chisti, Y. (2003) Recovery of microalgal biomass and metabolites: process options and economics.,*Biotechnol Ad* ., **20**: 491-515.

Roeselers, G., Loosdrecht, M.V. and Muyzer, G. (2007) Phototrophic biofilms and their potential applications. *J Appl Phycol*., **20**: 227-235.

Sheehan, J., Dunahay, T., Benemann, J. and Roessler, P. (1998) A look back at the US department of energy's aquatic species program—biodiesel from algae. National Renewable Energy Laboratory (NREL) report: NREL. TP-580-24190. Golden, CO.

Pringsheim, E.G. (1946) Pure cultures of algae. Cambridge. Cambridge University Press.

Lund, *J.* W. G., 1961.,The algae of the Malham Tarn district. *Fld Stud.,* **1:** 85-119.