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Characterization of microalgal biomass through fourier transforms infrared (FT-IR) spectroscopy

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Abstract
The microalgal species have the tendency to accumulate significant amounts of biomass which can be utilized for the production of wide range of biofuels, an alternative to fossil fuels. Microalgae are viewed as one of the potential feedstock for biomass production. The production of different biofuels depends on composition of biomass. In the present investigation, five microalgal isolates were identified using algal identification manuals. While Fourier transform infrared techniques were employed to analyze the functional groups in bio molecules. The microalgal isolates were identified as *Scenedesmus* sp., *Chlorella* sp., *Monorapidium* sp., *Actodesmus* sp. and *Cyclidium* sp. based on their surface morphology under microscope. Fourier infrared spectroscopy examination of algal biomass revealed the presence of –OH, –COOH, NH_2, and C=O organic compound groups. The results suggest that the biomasses of all the isolates are very much sustainable for biofuel production.

Keywords: microalgae, biomass, fourier transform infrared spectroscopy

Introduction
Microalgae species could be a source of biomass; it can further used to generate alternative fuel to fossil fuels. Microalgae have the efficiency to accumulate significant amounts of biomass that can be converted into a wide range of biofuels, including bio ethanol, biogas, jet fuel and biodiesel. In this concern, microalgae have been truly considered as a probable biomass source for biofuel production [1]. Based on photosynthetic light efficiency the algal biomass yield in the Indian condition has been estimated and it was found to be 74 $g/m^2/day$ [2]. Isolation and identification of culture is the first step towards the selection of potential microalgae for any application. Isolation of cells on agar media is a common method, it is ideal for most of the microalgae, purity of the culture was ensured by repetitive streaking on agar plate and conventional microscopic observation [3].
Fourier transform infrared (FTIR) spectroscopy is a method for whole organism analysis using intact cells, which involves the measurement of infrared absorption in relation to a range of molecular vibrational modes [4]. Especially it can be used to identify the functional groups. The use of FTIR spectroscopy in algal biomass analysis has been useful in characterizing and monitoring the composition of a chemically complex substance [5], which allows the detailed biofuel feedstock characterization. FTIR spectroscopy has been shown to enhance the biological and ecological analysis of algae and to assess quantitative changes in the intracellular macromolecular pools of microalgae [6].

Material and methods
Isolation and identification of microalgae
The microalgae sample was collected from five lakes of Bengaluru, namely Yelahanka lake, Hebbal lake, Agara lake, Dore lake and Nagavara lake. Pure cultures were isolated by centrifugation washing and streak plate method prescribed by [7]. In brief, 15 mL enrichment culture in BG 11 media was taken in centrifuge tubes and centrifuged at 3000 rpm for 15 minutes. The cells (pellet) were suspended in fresh sterile water using vortex mixer. Washed microalgae allowed to streak on BG 11agar media and kept for eight days to grow under light. Isolates were selected on the basis of colony morphology, colour, size, and morphological differences. The morphology based identifications of the microalgae species were conducted using binocular compound microscope (CX41RF, Olympus Corporation, Tokyo, Japan) under 100X magnification. The algal isolates were identified up to species, under a microscope by following the keys of Prescott [8] and also comparing with "Algae identification lab guide" by Agriculture and Agri-Food Canada [9]. The identified single colonies are allowed to grow in BG 11 broth media.

Fourier Transform Infrared (FTIR) spectroscopy analysis of microalgal biomass
The microalgae biomass was extracted by centrifugation form two weeks old culture. The biomass were freeze dried and analysed by Perkin Elmer spectrum one FTIR. The dried algae samples of approximately 1.5 mg were ground with 100 mg of spectroscopic grade potassium bromide (KBr) powder in an agate mortar [10].
The instrument was equipped with mercury cadmium telluride (MCT) detector and the spectra were recorded in the frequency range of 450 to 4000 cm^{-1} at a resolution of 4 cm^{-1}.

Results and discussion
Isolation and Identification of isolates
The five microalgal isolates were isolated from five lakes of Bengaluru, all the isolates are photosynthetic, because of their chlorophyll content. The microscopic observations are shown in fig. 1.

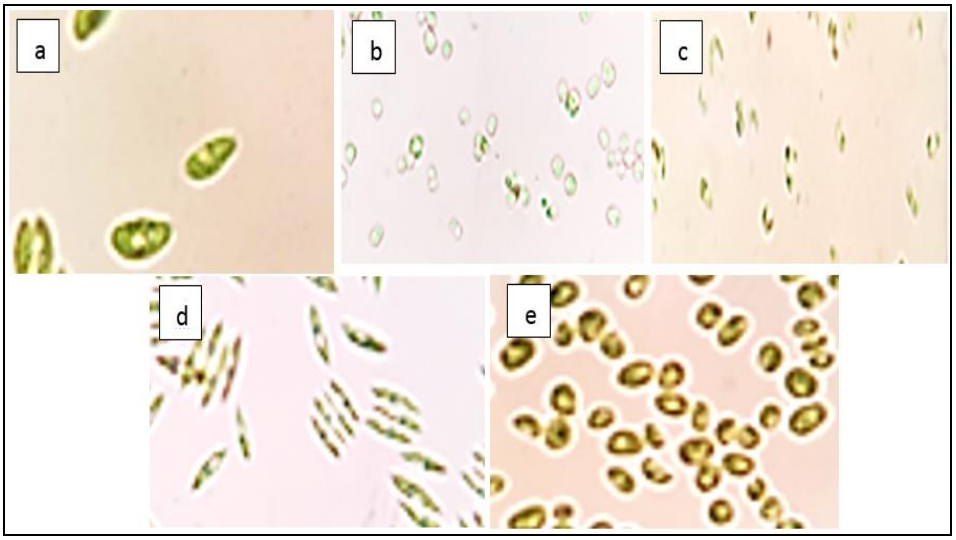

Fig 1: Microscopic views of isolates a) *Scenedesmus* sp. b) *Chlorella* sp. c) *Monorapidium* sp. d) *Actodesmus* sp. e) *Cyclidium* sp. under 100X magnification.

The cells of isolate 1 is identified as *Scenedesmus* sp. on the basis of cylindrical, with round cells, which is confirmed by the recorded observation of [11]. Tripathi *et al* [12] also reported the same observations. Microscopic observations of isolate 2 in present work are in agreement with those recorded for *Chlorella* sp. in the studies [13], Rendón-Castrillón *et al* [14] who characterized spherical, small and unicellular cells. The cells of isolate 3 are identified as *Monorapidium* sp. on the basis of crescent shape cells which is confirmed by the previous report of [15, 16].

Present result concords with this. Isolate 4 showed the characters of cylindrical cells with pointed ends and identified as *Actodesmus* sp. similar observations are also recorded by [17- 19]. Fifth isolate is identified as *Cyclidium* sp. on the basis of its shape: ovoid, ellipsoidal with a conspicuous apical plate As reported by [20, 21].

FTIR spectra of microalgal isolates

The composition and molecular functional groups can be determined by analysing the position, width, and intensity of infrared light absorption [22].The algal samples were studied using Fourier Transform Infrared (FTIR) technique. FTIR spectroscopy has proven and accepted to be a powerful tool for the study of biological samples during the last decade [23]. The application of FTIR spectroscopy in algal biomass examination has been useful in characterizing and monitoring the chemical composition of a complex substance, such as microalgae [5].

The results of FTIR transmittance of algal species were represented in fig. 2, reveals the presence of organic compound groups.

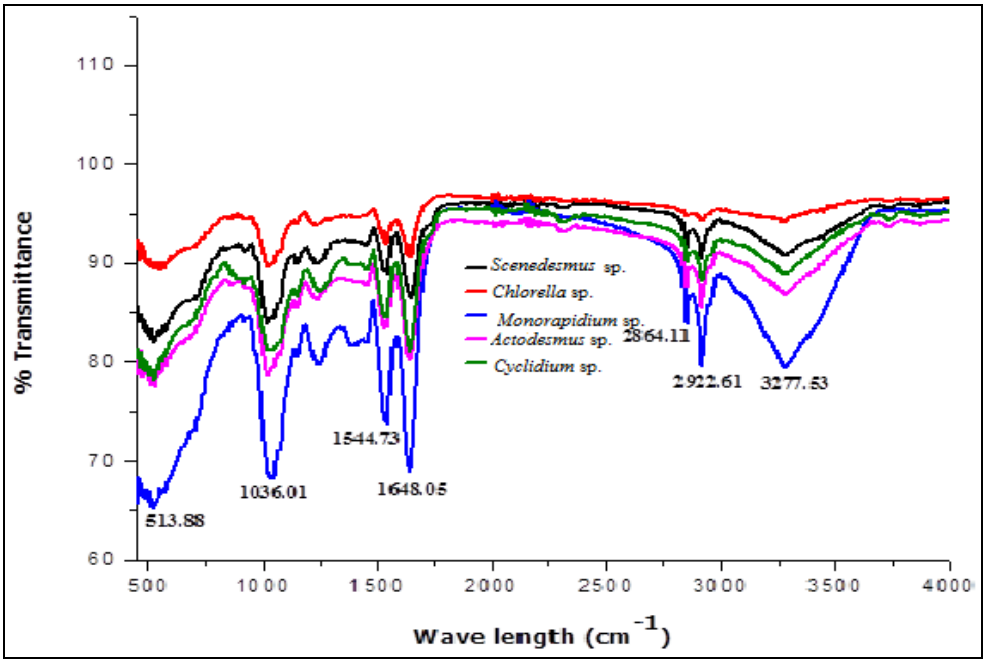

Fig 2: FTIR spectra of microalgal biomass.

The absorption spectra showed distinct absorption bands which corresponding to diverse bio molecules such as carbohydrates, proteins and lipids over the wave number range 4000-500 cm^{-1}. Infrared spectrum examinations of all the 5 isolates showed the seven distinct bands at 513, 1036, 1544, 1648, 2864, 2922 and 3277 cm^{-1}, which reveals that

all the algal isolates have similar organic groups, but there is variations in their contents among the isolates.

The bands observed in the region 3500 to 2800 cm^{-1} corresponds to CH_3 and CH_2 asymmetric and symmetric stretches of hydrocarbons from lipids and the band at 1700 to 1500 cm^{-1} was due to carbonyl stretches from esters of fatty acids (triacylglycerides). The bands at 3277 cm^{-1} reveals the presence of hydroxyl group. This result is in agreement with [10, 24]. As an increase in the peaks at 1700–1500 cm^{-1} was observed at hence it was conformed that there is greater concentrations of fatty acids C=O are due to the presence of proteins, and this region is specific for proteins and carbohydrates [25]. It was clear from the FTIR spectrum of *Monorapidium* sp. that distinct fingerprints for triglycerides and lipids exist in the biomass. Compared to other isolates, the biomass of isolate 3 there was a general increase in the protein and carbohydrate content which indicated due to the presence of high carbohydrates and proteins.

The band at 1036 cm^{-1} confirms the presence of C-O-C polysaccharides from carbohydrates. The aliphatic groups confirm a significant increase in the amount of hydrocarbons the region of aliphatic group present at 513cm^{-1}. This reveals that the presences of hydrocarbons.

FTIR spectroscopy offers rapid screening of microalgal cells and thus seems to be a viable analytical approach to determine biofuel potential from algae. The FTIR analysis of microalgae clearly indicated the presence of lipid groups, alcoholic groups, and carboxyl groups. From the present. Investigation it can be concluded that all the microalgal isolates can be a beneficial raw material for sustainable biofuel production.

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