Characterization of indigenous microalgae

Vivek P. Pankaj & Mamta Awasthi

Abstract— Global threats of fuel shortages in the near future and climate change due to green-house gas emissions are posing serious challenges and hence it is imperative to explore means for sustainable ways of prevention the consequences. The huge need for sustainable energy has led to an increased interest in new energy resources, such as production of microalgae, for use as biofuel and its other application. There are various advantages to using microalgae, for example, land use is much less than in terrestrial biofuel production, and several algae species can double their mass in 1 day under optimized conditions. In this study, eleven microalgae *sp* were isolated from different location of Hamirpur (31.68°N 76.52°E) district of Himachal Pradesh state in India, and characterized by using some techniques i.e. Microscopic images, Scanning Electron Microscopic (SEM), Atomic Force Microscope (AFM), for differentiate all microalgae *sp* morphologically and Fourier Transform Infrared spectroscopy (FTIR) techniques was used for potential of microalgae biofuel.

Index Terms— Microalgae, FTIR, SEM, AFM, Microscope.

1 INTRODUCTION

Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms that can grow rapidly and live in harsh conditions due to their unicellular or simple multicellular structure. Prokaryotic microorganisms are Cyanobacteria (Cyanophyceae) while eukaryotic microalgae are such as green algae (Chlorophyta) and diatoms (Bacillariophyta) [1-2] etc. Microalgae are present in all existing earth ecosystems, not just aquatic but also terrestrial, representing a big variety of species living in a wide range of environmental conditions. It is estimated that more than 50,000 species exist, but only a limited number, of around 30,000, has been studied and analyzed [3]. During the past decades, extensive collections of microalgae have been created by researchers in different countries. Microalgae are reproducing themselves using photosynthesis to convert sun energy into chemical energy, completing an entire growth cycle every few days [4]. Moreover, they can grow almost anywhere, requiring sunlight and some simple nutrients, although the growth rates can be accelerated by the addition of specific nutrients and sufficient aeration [5-6]. They have much higher growth rates and productivity when compared to conventional forestry, agricultural crops, and other aquatic plants, requiring much less land area than other biodiesel feedstock's of agricultural origin, up to 49 or 132 times less when compared to rapeseed or soybean crops, for a 30% (w/w) of oil content in algae biomass [7]. Therefore, the competition for arable soil with other crops, in particular for human consumption, is greatly reduced.

In this study, eleven microalgae *sp* were isolated from different location of Hamirpur (31.68°N 76.52°E) district of Himachal Pradesh state in India, and characterized by using some techniques i.e. Microscopic images, Scanning Electron Microscopic (SEM), Atomic Force Microscope (AFM), for differentiate all microalgae *sp* morphologically and Fourier Transform Infrared spectroscopy (FTIR) techniques was used for potential of microalgae biofuel.

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2 MATERIALS AND METHODS

This study was carried out in Hamirpur district, is one of the 12 districts of the state of Himachal Pradesh, India. This district occupies an area of 1,118 km². Microalgae were collected from wastewater as well as fresh water different location of Hamirpur which is situated between 31°25′N and 31°52′N and between 76°18′E and 76°44′E.

2.1 Microalgae collection and growth techniques

Microalgae were collected from waste water as well as freshwater from different locations of Hamirpur. The culture is collected by hand using of needle or knife, including part or all of the substrate (rock, plant, wood etc.) if possible. Search all habitats in the water body, including the edge of stones in fast-flowing water, aquatic plants, dam walls, and any floating debris. The UV Spectroscopic technique was used for a measured growth of algae, UV spectrophotometer was used in this study (Agilent carry -100) by measured OD (optical density) of the microalgae samples at 680 nm and incubation period was 21 day.

2.2 Isolation

The isolation process was done by the micromanipulation and some other conventional techniques; such as; streaking plate, serial dilution, pour plate and spreading plate techniques. These techniques were very useful to isolate single cell of microalgae.

2.3 Light Microscopy and Scanning Electron Microscopy

The algal cells were observed under light microscope for their morphological features and other cellular details, the cells were further studied using scanning electron microscope

(SEM) technique. The sample was screened by SEM for their absolute morphological studies. The basic steps for SEM sample preparations are fixing it with buffered aldehyde, post fixing it in glutaraldehyde, dehydrating it in ethanol, drying it with air dryer, mounting it on a specimen stub, coating it with gold and examining under the SEM.

2.4 Atomic Force Microscope (AFM)

In this approach, Atomic Force Microscope (AFM) studies were performed on different areas of the sensor surface, to characterize all isolated microalgae sp. cell. AFM is a powerful imaging tool that mechanically probes a surface with unprecedented resolution. It allows investigation of conductive and non-conductive samples in various environments (such as in ambient conditions, vacuum or liquid). It is recognized as a valuable tool for studying biological materials and can be used to image cells under physiological conditions in a non-destructive manner. This tool can generate 3-D images of surfaces (topographic imaging) and provides information about surface properties such as adhesion properties.

2.5 Fourier transforms infrared spectroscopy

FTIR Spectroscopy has been widely used to provide the information on the range of vibrational active functional groups (including O-H, N-H, C=O, =C-H, -CH2, -CH3, C-O-C, and >P=O) in biological specimens [8]. Although the technique has been largely used with isolated macromolecules and molecular complexes such as nucleic acid [9], Proteins [8], Lipids [10], Polysaccharides [11], studies carried out on the whole organism. The FTIR spectroscopy has successfully been established as a tool reliably, quickly and easily identifying microalgae [12]. The IR spectrum of dried algal biomass was recorded on Perkin IR spectrometer at room temperature. The dried algal powder was blended with potassium bromide (KBr) powder, and pressed into tablets before measurement. A region of 3500–500 cm-1 was used for scanning.

3 RESULTS AND DISCUSSION

Eleven microalgae *sp* were isolated from different location of Hamirpur (31.68°N 76.52°E) district of Himachal Pradesh state in India, such as 1. CEE-1: *Chlorophyceae sp*; 2. CEE-2: *Bracteacoccus sp*; 3. CEE-3: *Nannochloropsis sp*; 4. CEE-4: *Oedogonium sp*; 5. CEE-5: *Desmodesmus sp*; 6. CEE-6: *Oscillatoria sp*; 7. CEE-7: *Chlorella sp*; 8. CEE-8: *Microcystis sp*; 9. CEE-9: *Schroederia setigera*; 10.CEE-10: *Selenastrum sp*; 11. CEE-11: *Urospora sp*. These samples were grown in several environmental conditions (Optimized pH, temperature, light period and combined all optimized condition) using respected medium (Bold's basal medium and CHU#10 medium), at 21 days of time interval. These microalgae were primarily examined by microscopic image as shown in fig. 1.

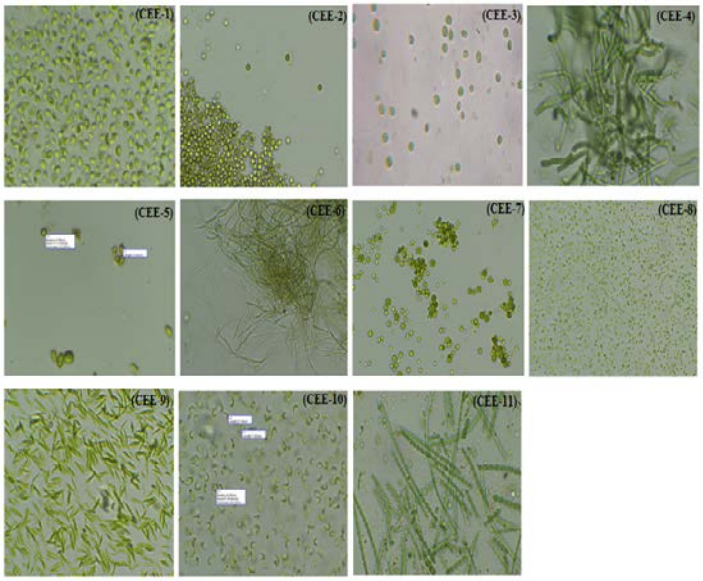

Fig. 1. Microscope image of all isolated microalgae *sp*

The morphological characteristics feature of these eleven isolated microalgae examined by microscopic and SEM images as shown in fig. 1 and 2 respectively and this shows that these samples have close similarity with genus of alga.

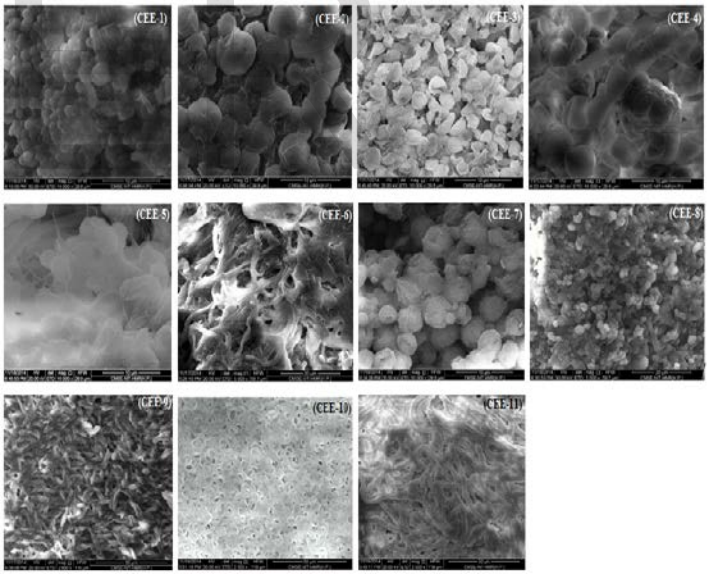

Fig. 2. Scanning Electron Microscope images of isolated microalgae *sp*

3-D images of surfaces (topographic imaging) and provides information about surface properties which differenciates all microalgae *sp* to each others, as shown in fig. 3.

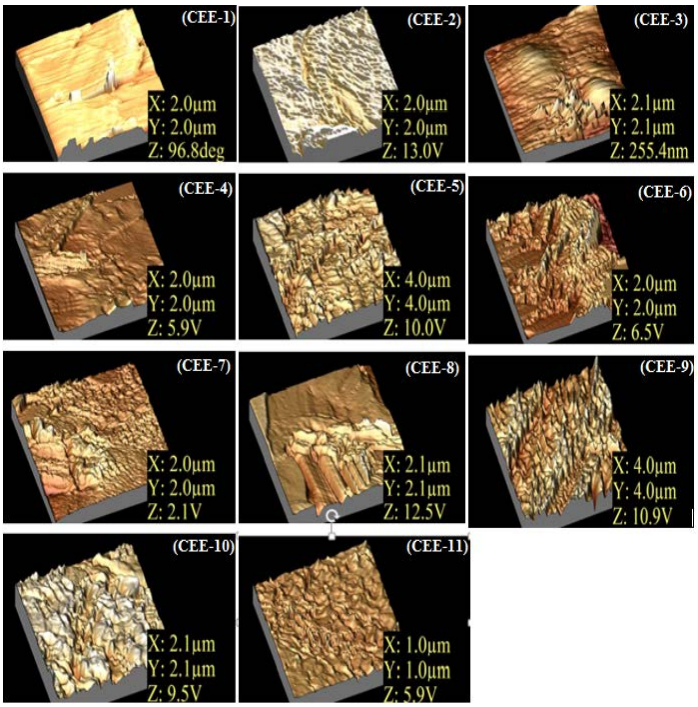

Fig. 3. Atomic Force Microscope (AFM) images of isolated microalgae *sp*

In FTIR spectra of all isolated microalgae *sp* as shown in Fig. 4 each peak assigned a functional group. The molecular assignments of the bands are based on published data phytoplankton, bacteria and other biological materials. Protein spectra characterized by strong peaks 1651 cm-1(amide I) and 1554 cm-1(amide II). These bands were due primarily to C=O stretching, vibration and a combination of N-H and C-H Stretching vibrations in amide complexes. Lipid and carbohydrates were characterized by strong vibrations the C-H 2922cm-1, while carbohydrates are the strongest absorbers between 1200 and 1000 cm-1. Several other classes of compounds, such as nucleic acids have functional groups with absorption bands in the same region of the spectrum. The strongest peaks 1554 shows that bending modes of methyl groups of protein [13]. The peak 1248 shows carboxylic acid present in the algae [14]. In this study ,the close correlation between the peaks and the existence of with band 2 suggested that lipid content very high and also carbohydrate, nucleic acid also present in these microalgae as shown in table 1. FTIR spectra shows that the isolated microalgae sp. having more potential of protein, lipid carbohydrates, etc.

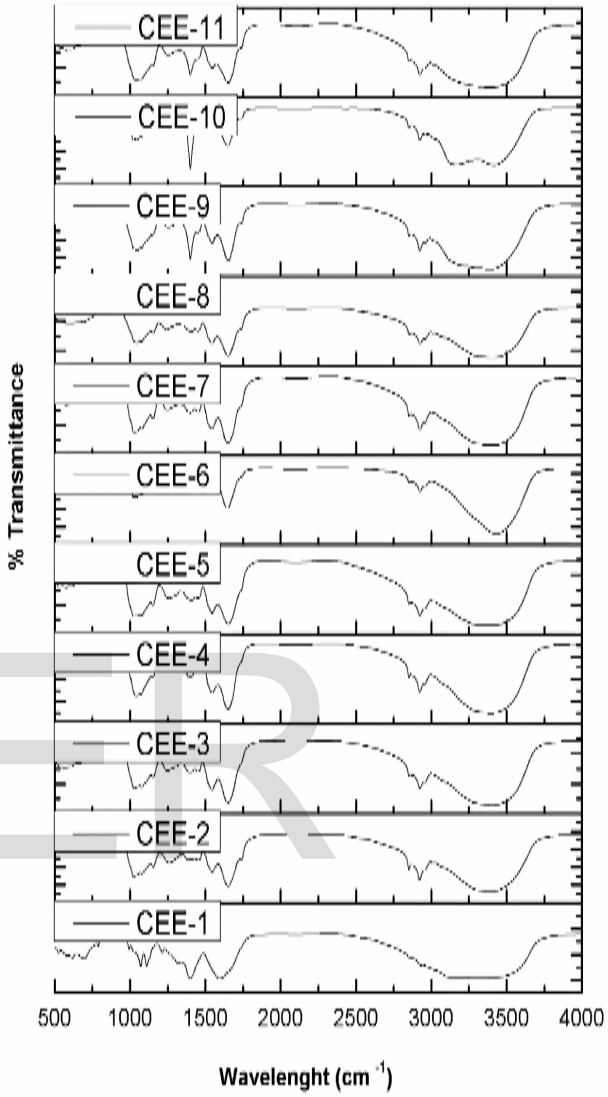

Fig. 4. FTIR spectra of all isolated microalgae *sp* (CEE-1 to 11)

TABLE 1
Shows the information on the microalgae group

Band	Main peak cm^{-1}	Typical band	Wave number ranges cm^{-1}
1	3429	Water V(O-H) stretching Protein V(N-H) stretching	3029-3639
2	2922	Lipid –carbohydrate mainly V_{as} (CH2) and $V_s(CH_2)$ stretching	2809-3012
3	1651	Protein amide I band mainly V(C=O) stretching	1583-1709
4	1554	Protein amide II band mainly σ(NH)bending V(C-N) stretching	1481-1585
5	1248	Nucleic acid (other phosphate containing compounds) V_{as}> P=0 stretching of phosphordiesters	1191-1356
6	1047	Carbohydrate V(C-O-C) of polysaccharides	980-1072

4 CONCLUSION

Current limitations to a more widespread utilization of this feedstock for biodiesel production concern the optimization of the microalgae harvesting, oil extraction processes, for a high efficiency of microalgae production. Also, light, nutrients, temperature, and O_2 levels need to be adjusted carefully to provide optimum conditions for oil content and biomass yield. It is therefore clear that a considerable investment in technological development and technical expertise is still needed before algal biodiesel is economically viable and can become a reality. This should be accomplished together with strategic planning and political and economic support. Further efforts on microalgae production should concentrate in reducing costs in small-scale and large-scale systems.

In this study, isolated eleven microalgae *sp* were characterized by using some techniques i.e. Microscopic images, Scanning Electron Microscopic (SEM), Atomic Force Microscope (AFM), for differentiate all microalgae *sp* morphologically and Fourier Transform Infrared spectroscopy (FTIR) techniques used for potential of microalgae biomolecules and these techniques are very useful to characterized microalgae, easily.

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