International Journal for Environmental Rehabilitation and Conservation

ISSN: 0975 — 6272
X (1): 135 — 144
www.essence-journal.com

Review Article

A Review on Algal Biodiversity of North - Western Indian Himalayan Hillock - Himachal Pradesh and its Potential as an Attractive Feedstock for 3rd Generation Biofuels

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A R T I C L E I N F O

Received: 01 July 2020 | Accepted: 15 August 2020 | Published Online: 30 September 2020

EOI: 10.11208/essence.20.11.SP2.139

A B S T R A C T

The present study deals with exploring diversity and species composition of algal communities from different parts of north- western Indian Himalayan state - Himachal Pradesh, their cultivation and potential applications for bioconversion to alternative fuels. Algae represent one of the most diverse groups amongst living entities which are widely located in different habitats ranging from lakes, ponds, rivers and sea. Algae play an important role in maintaining aquatic ecosystem and form the base of food chain or food web. Algae classification pertains to rhodophyta, phaeophyta and chlorophyta *i.e.* red, brown and green algae based on their colour pigments. Algae have recently reemerged as promising organisms in the effort to develop sustainable options for production of food and fuel. Algae are divided into two categories: Microalgae and Macrolagae. Himalayan hillock – Himachal Pradesh situated in North West of Himalayas is a rich pocket of diverged algal species due to its favourable climatic conditions. In the present review, an attempt has been made to have a comprehensive documentation of the diversity of algal species that are present in Himachal Pradesh and explore cultivation techniques of these potential species as a rich feed stock mainly for 3rd generation biofuels.

K E Y W O R D S

Macro algae | Micro algae | Biodiversity | Biofuels | Feed stock

C I T A T I O N

Sharma, Nivedita; Sharma, Poonam and Sharma Nisha (2020): A Review on Algal Biodiversity of North - Western Indian Himalayan Hillock - Himachal Pradesh and its Potential as an Attractive Feedstock for 3rd Generation Biofuels. ESSENCE Int. J. Env. Rehab. Conserv. XI (SP2): 135 — 144. https://eoi.citefactor.org/10.11208/essence.20.11.SP2.139

Introduction

Biodiversity in the shape of living entities represent the ultimate building blocks of life on earth. Biodiversity refers to a the wide range of living organisms including plants, animals and microbes inhabiting the terrestrial, aquatic and other habitats. Till date, out of total only two million organisms have been identified on earth. This implies strongly that the full spectrum of global diversity has not been accounted in taxonomic description contained in flora and fauna. Though, it has become a need oh hour to document and conserve the diversity in order to maintain ecosystem and sustain life in this universe. But with a rapid increase in human population and over exploitation of conserved resources, the life supporting system of earth is becoming increasingly threatened. It has led to a growing strong realization throughout the world about the dine necessity to conserve the valuable biological diversity document it properly as well as exploit it rationally.

Algae are placed under category of lower plants because these lack leaves, roots and stems. Algae contain macroalgae and microalgae but also with well defined eukaryotic characteristics and these also include prokaryotic structures viz. cyanobacteria. The size of algae vary in range starting in diameter from 0.2 -2.0 mm (picoplankton), microalgae upto 50-60 m high fronds in macroalgae kelps. A successful cultivation of microalgae is possible through optimizing the process parameters in a system, while macroalgae adapted to natural habitats viz. sea weed. Microalgae are minute structures which are either aquatic or terrestrial in nature and have adapted to wider environmental conditions ranging from fresh water, marine water, salty water and waste water. Microalgae cell have main food reservoir as starch in cells with outer walls made up mainly of cellulose.

Microalgal communities comprise important taxonomic groups viz. diatoms (Bacillariophyceae), dinoflagellates (Dinophyceae), blue green algae (Cyanophyceae), green microalgae (Chlorophyceae), silicoflagellates, coccolithophores and the very small nanoplankters, which together determine primary production and various trophic level interactions. In this respect, the quantification of phytoplankton biomass and community composition is important for understanding the structure and dynamics of ecosystems. Microalgae are microscopic heterotrophic/ autotrophic photosynthesizing organisms that are able to use solar energy to combine water with carbon dioxide to create biomass. Microalgae are present in all existing earth ecosystems, both aquatic and terrestrial, and can flourish under a wide range of environmental conditions, including freshwater, brackish water, seawater, and even wastewater. The cell walls of green algae usually contain cellulose, and they store carbohydrate in the form of starch. In cyanobacteria (blue-green algae) and diatoms represent largest group and dominant life form of biomass producers on earth. Structure of ecosystem is ultimately an outcome of the quantification as well as composition of these communities as a whole. Algae plays a vital role in ecosystem viz. phytoplanktons being important primary producer in marine food web. It has been reported that there are 100,000 algal diatoms present on earth. Their cell wall are mainly having polymerised silica, and also oils and chrysolaminarin are present in them as inclusions. The photosynthetic nature of microalgae, faster growth and high lipid contents as well as capability to grow in waste water render them as an advantageous and attractive feedstock for 3rd generation biofuel i.e. biodiesel or bioethanol over first and second generation biofuels. Growth of microalgae is influenced by different biotic and abiotic factors in its vicinity. Generally it is observed that microalgae being free floating in nature acts as an important indicator to climate change also being sensitive to its environment.

Thus a shift in phytoplankton community directly becomes an assessing indicator to evaluate the level of climate change and its subsequent effect on structure and function of ecosystem. On the other hand macroalgae are fast growing reasonably tall in height reaching upto 60 m in length and mostly growing either in salt or fresh water. These are mainly classified into three broad categories based on their pigmentation: i) Brown macroalgae (Phaeophyceae) ii) Red Macroalgae (Rhodophyceae) iii) Green Macroalgae (Chlorophyceae). In a study, Rhizoclonium sp. algal biomass collected from various habitats of Himachal Pradesh (Sharma and Sharma, 2018).

Many researchers have studied the temperate environment around the world; however, in India especially the North Western Himalayas are still poorly understood regarding diversity of algae in different pockets. Himachal Pradesh despite of being a rich habitat of algae has not thoroughly been explored. Himachal Pradesh lies between 28°22 to 33 12 N latitude and 75 47 to 79 04 E longitude and has 12 districts namely Bilaspur, Chamba, Hamirpur, Kangra, Kinnaur, Kullu, Lahul &Spiti, Mandi, Shimla, Sirmaur, Solan and Una. Altitude ranges from 350 meters to 6975 meters above mean sea level. Himachal Pradesh is predominantly a mountainous. State has highly dissected mountain ranges interspersed with deep gorges and valleys. A very little information is available on the species composition and potential of algae occurring in water bodies of H.P.

As Himachal Pradesh has been characterized with diverse climate that ranges from semi tropical in lower hills, to semi arctic in the cold deserts areas of Spiti and Kinnaur that provide immense potential in algae diversity and could provide its use in different commercial applications. Algae represent a significant group of organisms for industrial exploitation, especially for valuable products, processes and services having high impact commercially and also for public health. Algae have potential use as single cell proteins *i.e.* food, animal feed and as bio-fertilizer. Biomass from micro-algae after drying in form of powders or pressed in the form of tablets are available in the market as powerful tonic/viability supplements. Micro-algal biomass food for human beings food belong to the groups of Spirulina, Chlorella. The popular example of Dunaliella, Nostoc and Aphanizomenon (Pulz and Gross, 2004). Besides ,algae have emerged as a strong alternative to 1st and 2nd generation biofuels due to several advantages thus 3rd generation biofuels are becoming a buzz word commercially.

According to different agroclimatic zones, Himachal pardesh has been divided into different agroclimatic zones as shown in Figure 1. Therefore diversity of algae has been also studied according to different agroclimatic zones and vast variations have been observed in different zones.

Diversity of algae in Himachal Pradesh according to different agroclimatic zones

Diversity of algae in Shivalik Hill Zone: Prevailing climate in this zone pertains to Sub Tropical and falls in foothills and valley area ranging from 350 to 650 meters above mean sea level. Bilaspur, Una, Hamirpur districts and some parts of Sirmaur, Solan Kangra and Chamba districts are covered in this zone. Different sites from this zone have been explored to study algal biodiversity Figure 2. The different Phyla of algal flora of this region studied by various researchers and many new algal species have been identified.

Dwivedi *et al*., (2008) studied the cyanophycean flora of different districts of Himachal Pradesh. 37 taxa, 18 genera, 32 species, 4 varieties and 1 forma were reported from their study. The genus Stichosiphon, Anabaenopsis reported from Mandi, Aphanocapsa and Fortiea from Una district, while genus Fortiea and Pseudanabaena from Hamirpur district and Cylindrocapsa from Sirmaur district only. These main taxa from study represented by Pseudanabaena, Cylindrospermum

Microcystis, Fortiea, Calothrix, Anabaenopsis. Oscillatoria, Lyngbya Chroococcus, Aphanocapsa, Aphanothece, Merismopedia, Coelosphaerium, Gomphosphaeria, Stichosiphon, Anabaena, Nostoc and Nodularia. Out of 37 taxa, 27 were report first time from the different parts of Himachal Pradesh as shown in table 1.

Singh and Sharma (2014) explored the diversity of microalgae Sheer Khad, a tributary of river Sutlej in Western Himalayas, plays a significant role in the ecology of river Sutlej and Gobindsagar reservoir. In the their study, three sample collection sites (S1to S3) were selected on Sheer Khad. Site S1 situated at Jaroh (31°38'19.64" N, 76°42'59.09"E), district . Site S2 situated on Sunail Khad at Mundkhar (31° 36'10.41" N, 76°41'38.27" E), district Hamirpur. Sunail Khad is a tributary of Sheer Khad and joins it at Jahu. Site S3 situated at Bhadrog (31°28'02.28" N, 76°41'25.29" E), district Bilaspur. In this study, 19 taxa were identified, which were Cyanophyta/Cyanoprokaryota (8 taxa), Chlorophyta (7 taxa), and Bacillariophyta (4 taxa) The taxa identified from study comprised, Hydrodictyon reticulatum, Oedogonium sp., Pediastrum simplex, Rhizoclonium sp., Zygnema sp., Spirogyra sp., Cosmarium sp.of phylum Chlorophyta. The phylum Cyanophyta comprised of Microcystis aeruginosa, Aphanothece castagnei, Anabaena sp., Nostoc spongiaforme, Oscillatoria sancta, Chroococcus minor, Lyngbya martensian and Phylum Bacillariophyta included Synedra sp., Navicula viridula, Gomphonema sp., Nitzschia sp. as shown in table 2.

Cholorophyceae algae of Kangra district of Himachal Pradesh has been explored by Kumar *et al.,* (2012). District Kangra of Himachal Pradesh is located in the Western Himalayas between 31°2 to 32°5 N and 75° to 77°45 E with the geographical area of 5,739 km. The district has remarkable diversity in its soils, and water bodies, which provide great potential for algal diversity. They had collected algae samples from different parts of district and explored many algal species which were new to Himachal Pradesh. In total, they identified 38 algal species of phylum Chlorophyceae. This includes Zygnema gangeticum, Zygnema gangeticum, Volvox aureus, Tetraedron minimum , Staurastrum pseudotetracerum, Scenedesmus bijugatus , Scenedesmus bijugatus, Scenedesmus arcuatus, Spirogyra reinhardii, Spirogyra regularis, Pediastrum tetras, Spirogyra punctiformis, Scenedesmus quadricauda, Oocystis elliptica, Oedogonium tapeinosporum, Micrasterias zeylanica, Dictyosphaerium ehrenbergianum and Chaetophora flagellifera. Despite of their tremendous importance green algae are still the least explored organisms in the state of Himachal Pradesh. There seems to be an urgent need to make a detailed study and survey of Chlorophycean members in the entire state of Himachal Pradesh and their proper documentationfor conservation, cultivation and commercial exploitation.Sharma *et al*., 2019(a) optimizing the process parameters to use this algal biomass for third generation biofuel production .

Diversity of algae in Mid Hill zone: This zone extends from 651 meters to 1,800 meters above mean sea level and represents mild temperate climate. Rampur of Shimla district, Palampur and Kangra Tehsil of Kangra district and parts of Mandi, Solan, Kullu, Chamba, A lot of praise worthy work has been done by researchers in this zone to explore the different sites for novel algal flora of this region (Fig. 2).

Kumar *et al*., (2015) conducted a research to study diversity of class Xanthophyceae and Euglenophyceae of algae from different parts of district Kangra. They isolated Xanthophyceae namely Botrydium granulatum, Vaucheria dichotoma, Vaucheria sessilis and Euglenophyceae namely Phacus longicauda and Phacus pleuronectes as shown in figure 4.

Mongra, (2012) studied algal diversity in hot water springs of Tattapani Distt. Mandi, Himachal Pradesh belonging to explore distribution pattern of cyanobacteria. Cyanobacteria (blue-green algae) are one of the most interesting groups among the algae from structural, functional, adaptation, distribution and economic point of view. Different blue green algae Mastigocludus, Chroococcus, Lyngbya, Phormidium, Microcystis, Oscillatoria and Synechococcus, Fortiea and Pseudanabaena sp. isolated from Mandi district by researchers as shown in figure 5.

Thakur *et al*., (2013) studied the algal diversity of three freshwater lakes of Distt. Mandi. The study was conducted on three lakes namely, Rewalsar Lake, Prashar Lake and Kuntbhyog Lake in the Mandi district (longitude, 76°37 20″–77°23′15″ E; latitude, 31°13′50″–32°04′30″ N) of Himachal Pradesh. Nine groups of algae having one hundred forty-eight species were studied over period of two years. Common species i.e. Cyclotella sp. and Cymbella ventricosa were found in Rewalsar and Kuntbhyog Lake in winters. Synedra sp. in high abundance was also common in both of these lakes. Post monsoon period showed cyanophytes in peak. In their study, similarly Microcystis aeruginosa was frequent heavily in Rewalsar and Kuntbhyog Lake all around the years. Spirulina sp. was very common observed at Rewalsar Lake having Spirulina gomontii representing maximum growth at Rewalsar and Kuntbhyog Lakes. Anabaena sp. was common in Rewalsar Lake during all seasons. Oscillatoria limosa, Oscillatoria princeps, and Oscillatoria stigonema were found present throught seasons in Rewalsar and Kuntbhyog Lakes. C. vulgaris was also marked in Kuntbhyog and Rewalsar Lakes, with luxurious growth. Volvocales like Chlamydomonas reinhardi and Chlorogonium sp. were present in Rewalsar Lake during the monsoon and post-monsoon seasons with C. reinhardi was having higher abundance. Only one species of Cryptophyceae, i.e., Cryptomonas erosa, was observed during the study. At the Prashar site in winter season species of Dinobryon sp. and Synura adamsii had been recorded, while these were found absent from the other two sites. Dinophycean members, Ceratium hirundinella and Peridinium inconspicuum. A similar pattern of appearance and seasonality was also followed by all the five Euglena sp. noticed during the study and were present at the Kuntbhyog and Rewalsar sites.

Jindal and Thakur (2012) studied the plankton diversity of Kuntbhyog lake. Kuntbhyog Lake (31° 37’ N; 76° 49’ 6” E) is situated at the beautiful hilltop of Rewalsar town at an altitude of 1750 m above msl (Figure 1). It is enclosed by hills from two sides. It is located in the north part of Mandi district of Himachal Pradesh at a distance of 12 km from Rewalsar town.Phytoplankton constituted the major portion of total plankton. Percentage composition revealed that Bacillariophyceae 55.98% (29.28–77.82%) and Chlorophyceae 27.68% (17.19–36.10%) formed the dominant component, whereas Cyanophyceae 13.49% (0.0–35.03) and Euglenophyceae 2.85% (0.0–13.32) constituted the subdominant components of phytoplankton. Bacillariophyceae showed abundance from September–October and February–March, Chlorophyceae during March-April, Cyanophyceae and Euglenophyceae during May-June. Total 48 species of plankton were recorded forms like Navicula sp., Nitzschia sp., Cymbella sp., Closterium sp., Scenedesmus spp., Cosmarium sp. and Oscillatoria spp., among phytoplankton; and Brachionus sp., Keratella tropica, Cylops sp. and Daphnia sp. among zooplankton were of common occurrence. Plankton showed abundance durings summer and post monsoon. Relatively higher values of temperature, intensity of light, hardness, pH and richness of nutrients were found to be favourable for the abundance of plankton, whereas high turbidity, cloudy weather and dilution in the

concentration of salts during monsoon were associated with the minimum number of plankton.

Phytoplankton dynamics and species diversity had been recorded in a shallow eutrophic, natural mid-altitude Rewalsar lake in Himachal Pradesh (Jindal *et al*., 2014) which is situated on a mountain spur in Mandi district (longitude, 76◦37_20__ to 77◦23_15__E; latitude, 31◦13_50__ to 32◦04_30__N), Himachal Pradesh, India. Forty-seven species had been identified from these lakes. Microcystis aeruginosa and Synedra ulna exhibited a perennial habit. Ankistrodesmus falcatus, Chlorella vulgaris, Scenedesmus bijugatus, Chlamydomonas reinhardi, Eudorina elegans, Navicula cuspidate, Synedra ulna, Euglena acus, Euglena oxyuris, Spirulina gomontii, Oscillatoria princeps and Arthrospira khannae were abundant, and Oscillatoria limosa and Microcystis aeruginosa were highly abundant.

On the same pattern diversity, Phytoplankton dynamics and water quality of Prashar Lake, Himachal Pradesh, India was studied by Jindal *et al*., (2014). Prashar Lake is located in Mandi district (longitude, 76_3702000–77_2301500 E; latitude, 31_1305000–32_0403000 N) of Himachal Pradesh, India A total of 67 phytoplankton species were identified belonging to eight groups of algae. Bacillariophyceae (18 species), Chlorococcales (12 species), Zygnematales (12 species), Cyanophyceae (11 species), and Volvocales (7 species) were the most diverse groups. Chlorophyceae exhibited highest species diversity (31 species), comprised of members of Zygnematales and Cladophorales (12 species), Chlorococcales (12 species), and Volvocales (7 species). Main algae species included Fragilaria capucina, Cocconeis sp., Gomphonema gracile, Navicula sp., Pinnularia gibba among Bacillariophyceae; Dimorphococcus lunatus, Chlamydomonas sp., Pteromonas *sp*., Closterium tumidium, Staurastrum unguiferum among Chlorophyceae; Dinobryon sp.. among Chrysophyceae; Ceratium hirundinell among Dinophyceae; Euglena tuba, Trachelomonas lacustris among Euglenophyceae; Calothrix castelli, Microcystis sp. and Oscillatoria amoena among Cyanophyceae were found abundant during the investigation period.

Jindal and Prajapat (2005) studied the productivity and trophic status of Renuka wetland (Distt. Sirmour). Renuka wetland located at an altitude of 645 m above msl is 173 km in south west of Shimla in Sirmour District. During their study they found that Phytoplankton(algae) constituted major portion of of total plankton. Bacillariophyceae(56.4%) constituted the dominat component, whereas Chlorophyceace (25.17%) and Cyanophyceae (14.2%) formed the subdominant components of phytoplankton. Bacillariophyceae showed abundance from October-November and March, and Cyanophyceae and Eugelophyceae during May-June. Chlorophyceae were abundant during March-April. Phytoplankton species like Ankistrodesmus, Closterium, Cymbella, Euglena, Fragilaria, Microcystis, Navicula, Nitzschia, Oscillatoria and Scendesmus were commoly found.

Diversity of algae in Cold dry zone: This region Comprises of Lahaul-Spiti and Kinnaur Districts and Pangi Tehsil of Chamba District lying about 2,200 meters above mean sea level. Four species of genus Lagerheimia Chod*et al*gae have been reported from Nako Lake (31°52′48.65″ N, and 78°37′42.42″ E) by Dwiwedi and Mishra, 2013. The lake is situated at an altitude of 3662 m. above mean sea level in Pooh subdivision of Kinnaur district of state Himachal Pradesh which is a part of Indo-western Himalaya. These freshwater algal genus included Lagerheimia Chodat namely L. ciliata (Lagerheim) Chodat, L. subsalsa Lemmernann, L. citriformis (Snow) Collins, L. chodatii C. Bernard (figure 6). All these taxa are the new the new additions to the Indian freshwater algal flora except L. chodatii C. Bernard.

Singh *et al*., (2014) studied the cyanobacterial diversity of high altitude lakes of lahul spiti in Himachal Pradesh. The four lakes which were studied Chandra Tal, Suraj Tal, Deepak Tal and Sissu Lake. These are located in the Lahaul-Spiti cold desert region of northwestern Himalayas, Himachal Pradesh. The various algal species isolated from this region included Limnothrix redekii, N. punctiforme, Nostoc linckia, , Nodularia sphaerocarpa, , Planktothrix agardhii, L. frigida and Geitlerinema acutissimum Plank. clathrata, , Gloeocapsopsis pleurocapsoides Leptolyngbya antarctica, , Pseudanabaena frigida and N. spongiaeforme, , L. benthonica, L. foveolarum, L. lurida, L. valderiana, Phormidium autumnale, P. chalybeum, Cyanobium parvum, and Synechocystis pevalekii (figure 7).

Algae Mass Cultivation Systems

Microalgae cultivation is important for their commercial exploitation and these require specific environmental conditions including temperature range, light intensities, mixing conditions, nutrient composition, and gas exchange. In this regard, different cultivation systems have been devised which are given below (figure 8).

Algae-Chemical Composition and cultivation systems

Commonly, algae have two prime properties i.e. capacity to perform photosynthesis and to live in abundance completely in an aquatic conditions in both fresh water as well as marine water. Cells of algae may be single or multicellular. The main components of algae biomass are proteins, carbohydrates, and lipids in the ratio of 40-60%, 20-30% and 10-20% respectively in addition to smaller amounts of nucleic acids and pigments such as carotenoids (Singh *et al*., 2011).

Lipids: Lipids present in algae are the major component for biofuel production due to rich source of energy density and easy upgrading to biodiesel. Lipids are mainly of two types: polar and neutral lipids (figure 9). The constituents of polar lipids mainly belong to free fatty acids i.e. glycolipids and phospholipids. Where as neutral lipids have mainly of triacylglycerol (TAG) with small amount of monoacylglycerol, diacylglycerol and sterols. In general, algae predominantly produce polar lipids at its optimum conditions and neutral lipids under stressed conditions. There is ~ 80% of the natural lipids present in biomass with 77% of DW (Chisti, 2007).

Proteins: Approximately one-half of the dry weight in microalgae cells are comprised of proteins and the level of proteins vary from species to species. It may range from 15-71% of dry biomass (figure 10). Due to enriched proteins, the algal proteins are considered an excellent source of animal feed. In the year 2007, 30% of the world's algae were estimated to be grown for animal protein source (Becker, 2007). Algal proteins are rich in amino acid contents, thus categorizing it as "well-balanced protein" as per definition of World Health Organization (WHO) thus proving algae as a rich source of protein (FAO/WHO, 1973).

Carbohydrates: The principal form of carbohydrates present in microalgae can be very diverse. Many green algae have carbohydrate level equal to the plants and mainly constitute cellulose and starch, while in diatomic algae the main components are laminaran, fucoidin and mannitol. Commonly the carbohydrate contents in algae may be high upto 75% dw of cells as well as the starch may be up to 60% DW (figure 11). Starch content in algae can be influenced by some macronutrient like sulfur, phosphorus and nitrogen. The fungicide Cyclohexamide has also been used to block protein synthesis and to increase carbohydrate levels of algae in some studies (Branyikova *et al*., 2011). Algal carbohydrates are of major interest for commercial applications mainly for two purposes i.e. as animal feed source and for sugar source in

an alcohol fermentation process for 3rd generation biofuel.

Potential as a feedstock for third generation biofuels

Keeping in view the high contents of fats and carbohydrates in most of the algal speci these have emerged as a potential feedstock for their bioconversion to third generation fuels i.e. biodiesel and bioethanol. Transformation of lipids present in oleogenous algae is becoming a successful commercial venture recently (Reference) attaining higher yield of biodiesel. On the other hand carbohydrate rich algal moitese have been demonstrated as an attractive feedstock for fuel grade ethanol production by enzymatic saccharification followed by fermentation. Sharma *et al*., 2019 (b) isolated the microalga Rhizoclonium sp.and further proceesed to bioethanol production by optimizing process parameters for high gravity ethanol production.

Thus, exploring new algal species analyzing their chemical composition, devising suitable cultivation techniques followed by exploiting them as possible feedstock to third generation biofuel can bring a commercial revolution amidst dire necessity to replace dwindling conventional fuel and rising pollution.

3rd generation fuels i.e. biodiesel and bioethanol etc. Tranformation of lipids present in algae is becoming a successful commercial model attaining upto 800-1600 ton / ha annual yield of biodiesel on an average (Awasthi and Singh, 2011). On the other hand carbohydrate rich algal species have been demonstrated as attractive feedstock for their enzymatic saccharification and fermentation to fuel grade ethanol. Thus exploration of new algal species, their chemical compositional analysis, suitable cultivation techniques followed by exploitation as a possible feedstock to 3rd generation biofuels can bring commercial revolution amidst of dwindling conventional fuel and rising pollution.

Conclusion

Himachal Pradesh situated in the Indian Himalayas is rich in algal diversity due to its unique geographical location. Algal diversity of North Western Himalayan state - Himachal Pradesh has not been studied intensively. Thus there is an urgent need to make detailed floristic survey of algae in the entire state of Himachal Pradesh to report unknown species for their conservation as well as possible commercial applications viz. 3rd generation biofuel. This investigation has provided necessary basic data on the rare diversity of algal flora of Himachal Pradesh.

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